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From: Chief, Production Branch

Subj: Attached "Arthropod-borne Virus Information Exchange"

To: Recipients of the "Arthropod-borne Virus Information Exchange"

Attached is your copy of the most recent "Arthropod-borne Virus Information Exchange."

Every effort was made to mail this as soon as possible since it contains abstracts of the arbovirus papers to be presented at the annual meeting of the American Society of Tropical Medicine and Hygiene in San Antonio, Texas. You may wish to read these abstracts before the meeting begins on December 4.

Your attention is called to an excellent book review on page 2 by Dr. Roy Chamberlain. You are invited to submit reviews of books of interest to arbovirologists.

Address all communications to the undersigned.

W. ADRIAN CHAPPELL, Ph.D
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Biological Products Program
Center for Infectious Diseases
Centers for Disease Control
Atlanta, Georgia 30333, U.S.A.

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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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# GUIDE FOR AUTHORS

The Arthropod-borne Virus Information Exchange is issued for the purpose of timely exchange of information among investigators of arthropod-borne viruses. It contains reports, summaries, observations, and comments submitted voluntarily by qualified investigators. The appearance of any information, data, opinions, or views in this publication does not constitute formal publication. Any reference to or quotation of any part of this publication must be authorized directly by the person or agency submitting the article. The editor of the "Information Exchange" cannot authorize references and quotations.

Deadlines for articles to be published are March 1 and September 1.

The following format should be used for all articles submitted:

1. Heading

   The heading should be typed with capital letters, including name of laboratory and address. For example:

   REPORT FROM THE BIOLOGICAL PRODUCTS PRODUCTION BRANCH, CENTER FOR INFECTIOUS DISEASES, CENTERS FOR DISEASE CONTROL, ATLANTA, GA. 30333

2. Body of Report

   The text of the report should be as brief as possible to convey the intended message and should make reference to tables and figures included in the report. The text should be single spaced with double spacing between paragraphs.

3. Authors' Names

   The names of authors should be in parentheses following the text.

4. Tables and Figures

   Tables and figures should be numbered and titled if appropriate. Tables and figures should not be submitted without some description or explanation.

Reports should be typed only on one side of each page since they have to be photographed for reproduction. Each page should be numbered. Only the original typed report should be submitted.

## Book Review

California Serogroup Viruses: Proceedings of an International Symposium, Cleveland, Ohio, November 12 and 13, 1982, 399 pp. Editors, Charles H. Calisher and Wayne H. Thompson. Alan R. Liss, Inc., 150 Fifth Avenue, New York, New York, ISBN 0-8451-0123-4; Library of Congress No. 83-7936.

The editors of this volume, Charles Calisher and Wayne Thompson, and the nine other members of the symposium's organizing committee, are to be congratulated on a job well done. By planning the symposium to immediately follow the annual meeting of the American Society of Tropical Medicine and Hygiene, they were able to assemble a notable group of contributors and registrants, comprising a large share of the world's experts on this group of viruses. The locale was ideal also, being essentially the geographic hub of much of the research over the last 10-15 years on LaCrosse virus, a leading cause of arboviral encephalitis in the United States.

In two full days of sessions 32 formal reports were given, followed by a final summary and recommendations supplemented by discussion from the floor by many of the 148 registered participants. These reports are reproduced in the present volume. A rapid photocopy process was used, which permitted rapid publication and availability only a month after the symposium.

The stage was set by an informative review by Charles Calisher of the taxonomy, classification and geographic distribution of the California serogroup bunyaviruses. It is clearly written and easy to follow. This is true of the many reports in the proceedings; despite the complexity of some of the topics covered, the reports are generally presented in such manner as to be understandable, even by persons unintitiated in the the field of arbovirology. This I judge to be a considerable attribute and an example of clear thinking as well as good writing.

The reports are grouped in seven sections:

Section I. Ecology and Epidemiology (7 reports).

Section II. Virus Variation, Genetic Reassortment and Gene Structure-Function Relationships (6 reports).

Section III. Clinical Aspects of LaCrosse Encephalitis (4 reports).

Section IV. Prevalence of California Serogroup Viruses and the Infections they Cause (8 reports).

Section V. Practical Problems in Medical Aspects of California Serogroup Viruses (3 reports).

Section VI. Biology and Control of California Serogroup Viruses (4 reports).

Section VII. Summary and Recommendations (a compilation and summary of recommendations derived from the symposium by open discussion between the audience and a panel of experts comprised of William C. Reeves (Chairman), George B. Craig, Jr., Karl M. Johnson, Thomas P. Monath, Neal Nathanson, Phillip K. Russell and Leslie Spence).

One criticism of many of the reports is that their introductions are repetitive. A large share of the authors laid a brief background of the classification and importance of the California serogroup viruses before launching into the main part of their report. Calisher's excellent review made much of this unnecessary. However, the system used does have an obvious advantage; each report stands on its own feet, so to speak, and is not dependent on other reports in the Proceedings for complete understanding.

My advice is to read the volume from cover to cover. The organizers of the symposium planned subjects to follow in logical sequence. If, however, you are given to skipping around, by all means catch Calisher's review of taxonomy, classification and distribution, Thompson's vector-virus relationships, DeFoliart's vector biology in relating to persistence of LaCrosse virus, Bishop et al, Coding assignments of the RNA genome, Beaty et al, Potential for RNA reassortment in the vector, Gundersen's and Brown's clinical aspects, Chun's neurological and psychological sequellae, Thompson's and Gundersen's LaCrosse disease and control in a suburban area, and Craig's factors affecting control of Aedes triseriatus. Then by all means go back and read the other reports as well, or you will find yourself short of the complete picture.

I guarantee that by the time you finish this volume you will be greatly impressed by the tremendous progress that has been made in the study of this important and fascinating group of arboviruses. Much of the knowledge gained also helps in our understanding of other kinds of arboviruses as well.

Roy W. Chamberlain

## Publications

Dr. Goro Kuno informed me about the availability of a recent publication entitled "Bibliography of Dengue Fever and Dengue-like Illnesses, 1780-1981". This bibliography was compiled and edited by Goro Kuno and Bess Flores with the technical assistance of Malia Elaseto and Conrad Hopman. It was published by the South Pacific Commission, Noumea, New Caledonia. The cost is approximately $35.00 (US). Anyone interested in obtaining copies should contact the South Pacific Commission, Post Box D5, Noumea Cedex, New Caledonia for exact price and availability.

## A Request for Dengue Virus Isolates

The Immunochemistry Branch at the Centers for Disease Control (CDC), Fort Collins, Colorado has begun a molecular-epidemiological study of world-wide dengue virus isolates. Work in our laboratory has previously shown that oligonucleotide fingerprinting of dengue 2 virus genome RNA can be correlated with the geographical site of isolation of these viruses and is a useful epidemiological tool. We are now expanding this study to other dengue serotypes and to the use of an imaging computer to analyze the laboratory date. We are interested in obtaining a variety of low passage dengue isolates from both recent and old outbreaks for comparative analysis. We invite interested persons to contribute viruses to this study through Dr. Thomas P. Monath at the CDC laboratory, P.O. Box 2087, Fort Collins, Colorado 80522-2087. Contributors will be provided with results and given appropriate recognition.

## Position Wanted

Position wanted in virology by a man with M.D. and Dr. P.H. degrees and who has worked in arbovirology for about 15 years. Relocation is needed. Please write to the editor of this publication for further details.

## Western Equine Encephalitis Educational Materials Available

The following information was received from Dr. Roy A. Ellis, Prairie Entomoligical Research Consultants, 36 East Lake Drive, Winnipeg, Manitoba, R3T 4V5, Canada (Telephone 204-261-0241)

Outbreaks of Western equine encephalitis have occurred in Manitoba during 1975, 1977, 1981 and this year. PERC is offering several items for sale which document the 1983 outbreak. The various educational items described below are designed for classroom, lecture and library use and for independent study.

1. Western Equine Encephalitis Surveillance in Manitoba
Twenty 35 mm slide transparencies with accompanying text describing the methods and materials used in Manitoba's WEE surveillance program. Packaged in protective slide holder.
WEE Slide Set 83-1 @ $29.95

2. Western Equine Encephalitis Control in Manitoba
Twenty 35 mm slide transparencies with accompanying text describing Manitoba's 1983 emergency mosquito control operations. Packaged in protective slide holder.
WEE Slide Set 83-2 @ $29.95

3. Public Perceptions of Emergency Mosquito Control Programs
A set of about 130 newspaper articles documenting the 1983 health emergency and the events leading up to it, assembled in chronological order in a soft-covered binder. Contents provide an insight into the media's and public's perception of this recurring and complex issue.
WEE Information Package 83-1 @ $24.95

Terms: All orders must be prepaid. Please allow 30 days for handling and shipping. All items are protected by copyright. No item may be duplicated without permission. Prices include handling and shipping. Offer in effect until October 31st, 1983. Guarantee: If not totally satisfied, return item postpaid and in good condition to receive a full refund. Freebie: Order all 3 items ($84.95) and receive them attractively assembled in a woodgrain-finish, 3-ring binder at no extra cost.

ABSTRACT. IN VITRO VIRUS SPECIFIC ANTIBODY SYNTHESIS BY LEUKOCYTES OBTAINED FROM BLOOD AND CEREBROSPINAL FLUID OF PATIENTS WITH ACUTE JAPANESE ENCEPHALITIS. D.S. Burke, A. Nisalak, M.A. Ussery, W. Lorsomrudee, T. Laorakpongse. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Kampangphet Provincial Hospital, Kampangphet, Thailand.

During the July 1982 epidemic of Japanese encephalitis (JE) at the Kampangphet (Thailand) Provincial Hospital, leukocytes from 40 patients with admitting clinical diagnoses of meningitis or meningo-encephalitis were tested for spontaneous *in vitro* synthesis of antibodies to JE. Acute specimens of CSF and heparinized blood were obtained within 24 hours of hospital admission and a second specimen of heparinized blood was obtained 5-10 days later. Peripheral blood mononuclear leukocytes (PBL) were purified by density flotation, exhaustively washed, and incubated at $10^6$ cells per milliliter for 72 hours; unfractionated CSF leukocytes (CSFL) were washed and tested at concentrations determined by the number obtained (always $< 10^6$ per milliliter). No mitogens or specific viral antigens were added to the cultures. JE reactive IgM and IgG antibodies were detected in 72 hour cell culture supernatant fluids with a sensitive "antibody capture" type radioimmunoassay. Fifteen patients were proven to be acute flavivirus infected as shown by a four fold or greater rise in serum JE HAI antibodies. JE IgM and IgG antibody synthesis was detectable in "acute" PBL cultures from 9 and 13 of these patients, respectively. JE IgM and IgG antibody synthesis was detectable in "acute" CSFL cultures from 0 and 4 of these patients, respectively. All four CSFL cultures which synthesized JE IgG antibodies were obtained from patients whose PBL also showed active JE IgG synthesis. In every case parallel control cultures of PBL and CSFL were incubated with cycloheximide (4 micrograms per milliliter) in the culture fluid; all control cultures showed a marked reduction in synthesis (control titer $< 25\%$ of test titer). Nineteen additional patients were shown by serology not to be acutely infected with a flavivirus; none of the PBL or CSFL cultures from these non-infected patients showed either JE IgM or IgG antibody synthesis. Of six patients with equivocal serologic evidence for infection, PBL from two synthesized both JE IgM and IgG. We conclude that specific antibody producing cells are present in the blood and CSF early in the clinical course of acute meningo-encephalitis due to JE virus. The full implications of this observation for the rapid diagnosis of viral infections of the nervous system remain to be explored.

ABSTRACT. ARTIFICIAL VIREMIA IN MICE, A PRACTICAL *IN VIVO* METHOD TO INFECT MOSQUITOES WITH ARBOVIRUSES. B. E. Dutary and C. G. Hayes, Gorgas Memorial Laboratory, Republic of Panama.

Vector competence studies sometimes require that field populations and colonized mosquitoes be infected with virus *per os*. Some virus replicate well in laboratory animal models and infected blood meals are easily provided. Other mosquito-arbovirus studies must depend on the use of hanging drop, pledgets or membrane impregnated with blood-virus suspensions as source of virus meal. These last methods of infecting mosquitoes have the disadvantage that for most species only a low percentage (10-30%) of females feed and then rarely to repletion. Artifical viremia can be produced in the adult male mouse by inoculation into the tail vein of 0.5 ml of the virus dilution selected. The technique is now in use in this laboratory with yellow fever (YF) virus. When the virus injection contained 8 x $10^7$ pfu/0.1 ml, virus circulated in the mouse blood for five hours, and the blood titer measured every 30 minutes by eye puncture was $\geq 10^5$ $TCID_{50}$/0.1 ml. These results were reproducible. Mosquitoes feeding during the first hour were incubated for 14 days at their optimal temperature, humidity and light/dark cycle. This method was first tested with 100 female *Haemagogus equinus* from the Maje '75 colony. Ninety-nine females engorged in 60 minutes and 87 were positive for virus. Mean mosquito titer in this group was $log_{10}$ 3.48 + 0.83 pfu/per mosquito. Complete data and data with dengue virus tested with this method will be presented. The mosquitoes feeding on the virus-inoculated adult mouse engorged readily to repletion and had infectivity titer, when tested individually in Vero YF plaque assay, similar to the titers observed in mosquitoes infected by oral feeding on infected *Aotus trivirgatus* (night monkey).

ABSTRACT. TRANSOVARIAL TRANSMISSION OF DENGUE VIRUSES BY AEDES MEDIOVITTATUS MOSQUITOES. Jerome E. Freier and Leon Rosen. Arbovirus Program, Pacific Biomedical Research Center, University of Hawaii and the Laboratory for Arbovirus Research and Surveillance, University of Notre Dame.

The ability of Aedes mediovittatus mosquitoes to transmit dengue viruses transovarially was tested in a strain of this species obtained from Puerto Rico. Aedes (Gymnometopa) mediovittatus is found throughout most of the Caribbean region. Immatures are associated with a wide variety of aquatic habitats including natural and artificial containers. Also, since the females are known to bite humans and are highly susceptible to oral infection with dengue virus, it is likely that this species may be significant epidemiologically as a vector.

The results of our experiments have shown that Ae. mediovittatus is capable of transmitting dengue virus transovarially at the highest rates ever demonstrated for these viruses in any species of mosquito. Minimum infection rates among larval progeny from individual females infected with strains of dengue type 2 virus from Bangkok and Puerto Rico were 19.2% and 15.2% respectively. In addition, Ae. mediovittatus females have been shown capable of transmitting transovarially all 4 dengue serotypes and transmission of dengue type 2 virus to the $F_1$ adult has been demonstrated. Transovarial transmission of dengue type 2 virus has also been shown for strains of virus from Asia, Africa, and the Caribbean.

Since the transovarial transmission rates that we observed approach those cited for experimental infections of mosquitoes with bunyaviruses, it is possible that Ae. mediovittatus may have an important role in the maintenance of dengue viruses in nature.

ABSTRACT. A CASE OF NATURALLY OCCURRING CONCURRENT HUMAN INFECTION WITH TWO DENGUE VIRUSES. D.J. Gubler, G. Kuno, G.E. Sather, S.H. Waterman, A. Oliver, M. Velez, I. Rios, Dengue Branch, San Juan Laboratories, Division of Vector-Borne Viral Diseases Center for Infectious Diseases, CDC, San Juan, Puerto Rico.

Concurrent transmission of two or more dengue virus serotypes is common in large urban cities of the tropics and it is likely that on occasion, concurrent infections of man also occur. It has been suggested that this phenomenon may be responsible for the more severe form of the disease, dengue hemorrhagic fever. Here we describe the first documented case of concurrent human infection with 2 dengue serotypes. The patient, a 16 year old Puerto Rican male, was identified during routine virologic surveillance in Puerto Rico. The illness was mild and generally unremarkable, with no hemorrhagic manifestations. Virus isolation was made in the C6/36 clone of *Aedes albopictus* cells. Identification of both dengue 1 and 4 virus infection in the cell cultures was by the monoclonal antibody indirect fluorescent antibody test. The presence of both viruses was confirmed by reisolation from the original human serum. The acute serum from this patient was inoculated into mosquitoes which were then used to prepare antigen for CF identification. CF results were compatible with infection by both dengue 1 and 4 viruses, with titers of 512, 256, 8 and 128 for the 4 dengue serotypes respectively. Neutralization experiments were carried out using monotypic dengue 1 and 4 human antisera. After incubation, the neutralized virus suspensions were inoculated in mosquito cell cultures and reidentified by monoclonal antibody. Virus suspensions incubated with dengue 1 immune serum still showed a small number of cells infected with this serotype, but dengue 4 was the predominant virus whereas virus suspensions incubated with dengue 4 immune serum showed only dengue 1 infection. Pure cultures of both dengue 1 and dengue 4 viruses were obtained by the plaque picking technique. This report confirms the occurrence of concurrent infection of humans by 2 dengue viruses, but does not support the hypothesis that dual infection may lead to more severe disease.

ABSTRACT. AEDES (GYMNOMETOPA) MEDIOVITTATUS, A POTENTIAL NEW VECTOR FOR DENGUE VIRUSES IN THE CARIBBEAN. D.J. Gubler, R.J. Novak, N.A. Colon, E. Vergne, M. Velez and J. Fowler, Dengue Branch, San Juan Laboratories, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, CDC, San Juan, Puerto Rico.

An interesting and puzzling aspect of dengue ecology in Puerto Rico is the confirmed persistence of dengue virus transmission during interepidemic periods in rural or semi-rural communities, where human population densities are low. A characteristic common to all of these areas is the presence of a forest species of Aedes belonging to the subgenus Gymnometopa. This species, Aedes mediovittatus, has moved into the peridomestic environment and shares many breeding sites with the known principal vector of dengue, Ae. aegypti. Field and laboratory studies were initiated to investigate the possibility that Ae. mediovittatus was contributing to maintenance and transmission of dengue viruses in these areas. First, field studies on the biting behavior of this species showed that it fed readily on man and that its feeding behavior was similar to Ae. aegypti with 2 peaks of activity, one in the morning right after daybreak and another in the late afternoon. Comparative studies with Ae. mediovittatus and a Puerto Rican strain of Ae. aegypti showed that both species were equally susceptible to parenteral infection with dengue 2 virus and the rate and extent of viral replication was similar. However, Ae. mediovittatus was significantly more susceptible to oral infection with dengue 2 virus, with infection rates of 74 and 38% for Ae. mediovittatus and Ae. aegypti respectively. Fluorescent antibody studies have demonstrated extensive salivary gland infection in Ae. mediovittatus and dengue virus was successfully transmitted to both mice and to hanging drop blood suspensions. Finally, transovarial transmission of dengue virus by this species was demonstrated after parenteral infection. The minimum filial infection rate was 1:334 in the first 3 gonotrophic cycles. To date, however, virus has not been isolated from 1244 Ae. mediovittatus larvae collected in a rural area in western Puerto Rico where sporadic dengue transmission was occurring. Collectively, the data suggest that Ae. mediovittatus is a more efficient host than Ae. aegypti for dengue viruses and that this species may play an important role in dengue virus maintenance in Puerto Rico during interepidemic periods.

ABSTRACT. FACTORS AFFECTING THE RESPONSE TO MEASLES VACCINE IN HAITIAN INFANTS.
N.A. Halsey, R. Boulos, F. Mode, F. Andre, S. Touro, R. Yaeger, J. Rohde. Departments of Tropical Medicine and Pediatrics, Tulane University, New Orleans; Medical-Social Complex of Cite Simone; Division of Nutrition, Ministry of Health; and Management Sciences for Health, Port-au-Prince, Haiti.

In order to identify factors influencing the response to measles vaccine, 628 infants in a crowded, lower socioeconomic status suburb of Port-au-Prince, Haiti were studied. 33% of the infants had serologic evidence of natural measles infections by 11 months of age. The nutritional status of children who had developed natural measles was significantly lower as measured by weight for age ($p = 0.0004$) and weight for height ($p = 0.007$) than for children who had not developed measles. For children without HI antibody at the time of vaccination, the seroconversion rates by age were: 6 months - 49%, 7 months - 71%, 8 months - 77%, 9 months - 85%, 10 months - 94%, 11 months - 95%, and 12 months - 100%. The response rates by age for the 20% of children with second and third degree malnutrition (less than 75% of the NCHS Standard mean weight-for-age) were not different from normally nourished children. Similarly, the response for the 38% of children who were less than 90% of the NCHS Standard mean weight-for-height was not different from well nourished children. The presence of acute respiratory infections (cough, fever, or rhinorrhea) did not influence the response to vaccine. The mean convalescent titer for males (1:61) was significantly ($p < .05$) higher than for females (1:40). Seroconversion rates for infants 6-9 months of age born to women with low HI titers ( $\leq$ 1:40) were significantly ($p < 0.01$) higher than for infants born to mothers with higher titers. The mothers' HI antibody titers were not influenced by their nutritional status.

Natural measles contributes to the high rate of malnutrition for infants in developing countries. Measles vaccine should be given to all infants in developing countries as soon after 9 months of age as possible, regardless of nutritional status or the presence of acute illnesses.

ABSTRACT. EPITOPIC ANALYSIS OF DENGUE VIRUS ANTIGENS USING MONOCLONAL ANTIBODIES. E.A. Henchal, W.E. Brandt, D.S. Burke, AND M.K. Gentry. Walter Reed Army Institute of Research, Washington, D.C. 20307; Armed Forces Research Institute for Medical Sciences, Bangkok, Thailand.

Monoclonal antibodies (mAb) prepared against the four dengue virus serotypes were used in competitive binding assays to identify distinct antigenic domains on the E(V3) protein of dengue type 2 (DEN-2) virus. Monoclonal antibodies, chosen on the basis of their serological characteristics, were partially purified by either Sephadex-Staph A column chromatography or ammonium sulfate salt fractionation and subsequently radiolabeled with $I^{125}$. Dilutions of unlabeled monoclonal ascitic fluids were incubated for 12 hours at room temperature with gradient-purified DEN-2 virus adsorbed to wells of flexible microtiter plates. Radiolabeled mAb was added and allowed to compete with the unlabeled mAb for 8 to 12 additional hours at room temperature. The use of mAb allowed the identification of the following unique epitopes: type specific (TSa, TSb, TSc), subcomplex-specific (DSCa, DSCb), complex-specific (DCa, DCb), and flavivirus group reactive (FGR). The FGR epitope was positionally distinct from the dengue type-, subcomplex-, and complex-specific domains. However, some type-specific epitopes were contiguous with subcomplex- or complex-specific domains. In some cases the binding of one type of antibody was promoted by a second. The binding of FGR antibodies was promoted by TSc, DSCa, and DCb antibodies. Similarly, TSc antibody binding was promoted by FGR and DSCa antibodies. The interaction of two mutually promoting antibodies (TSc and FGR) was shown to induce protection similar to that of polyclonal DEN-2 hyperimmune fluid in mouse passive protection studies.

ABSTRACT: MECHANICAL TRANSMISSION OF RIFT VALLEY FEVER VIRUS BY HEMATOPHAGOUS DIPTERA. A.L. Hoch and C.L. Bailey. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland.

Various characteristics of Rift Valley fever (RVF) virus, such as (1) high viral titers in susceptible animals, (2) long-term virus stability at ambient temperatures, (3) the explosive nature of epizootics and epidemics, and (4) the broad spectrum of insect taxa from which the virus has been isolated, make mechanical transmission a probable means of disseminating this agent. In an attempt to elucidate the rapid onset and spread of numerous RVF cases observed during epizootics and epidemics, we designed a series of experiments to qualitatively and quantitatively evaluate mechanical transmission of RVF virus by hematophagous diptera. Golden Syrian hamsters, inoculated intraperitoneally with approximately 10,000 plaque forming units (PFU) of the Zagazig Hospital 501 strain of RVF virus, were used as the infectious blood source for all transmission attempts. Experimental insects were exposed to the virus donor hamsters at either 16 or 24 hours postinoculation, at which time the virus titers ranged between $10^{5-7}$ and $10^{8-10}$ PFU/ml of blood, respectively. Transmission of virus to hamsters and sheep by partially fed insects was attempted at 0, 2, 6, and 24 hours post-virus exposure to the insects. RVF virus was mechanically transmitted to hamsters by 6 of the 7 species of diptera evaluated. *Culex pipiens*, Egypt, 9/70 (number of virus transmissions to hamsters/number of transmission attempts); *Aedes taeniorhynchus*, USA, 13/24; *Lutzomyia longipalpis*, Brazil, 5/34; *Culicoides variipennis*, USA, 2/35; *Stomoxys calcitrans*, USA, 4/34; and *Glossina morsitans*, Africa, 6/10. *Culex pipiens* transmitted virus to 3/10 (30%), 3/10 (30%), 1/10 (10%), and 2/40 (5%) at 0, 2, 6, and 24 hours postexposure to the viremic hamster, respectively. With several species, individual flies successfully transmitted virus to more than one host. However, none of 30 *Aedes aegypti*, Africa, successfully transmitted virus following interrupted feedings. Virus was successfully transmitted to 4 lambs by *Culex pipiens* (2/2) and *Glossina morsitans* (2/2) at 0 hour. These studies document that RVF virus can be transmitted following the interrupted feeding of insects on viremic animals, and may help to explain the epidemiology of RVF virus.

ABSTRACT .JAPANESE ENCEPHALITIS IN THAILAND: AN ESTABLISHED PATTERN OF RECURRENT ANNUAL EPIDEMICS. Charles H. Hoke, Jr, M.D., Sujarti Jatanesen, M.D., and Donald S. Burke, M.D. Department of Virology, Armed Forces Research Institute of Medical Sciences and Department of Epidemiology, Ministry of Public Health, Bangkok, Thailand.

Since 1969 encephalitis has been a leading cause of death due to infectious disease in Thailand. Encephalitis data collected by the Ministry of Health of Thailand was reviewed and compared with data obtained in a detailed clinical study of encephalitis due to Japanese encephalitis virus JEV. Between 1970 and 1981, an annual average of 1598 cases (annual rates of 2.9 to 5.1/100,000) and 370 deaths were reported. In one independently studied province, the national surveillance program identified 88% of cases, of which 89% were due to JEV. Encephalitis peaked each July: 62% of all cases occurred during that month in the 48 most heavily affected districts. Cases were concentrated in 7 northern provinces and one northeastern province, and a north-south gradient in attack rates occurred [log (Attack rate) = 2.6 - .75 log(Kilometers from the northern border). ($r = -.58$, $p << .01$)]. A major increase occurred in 1980 when approximately 2300 cases were reported (overall attack rate in Thailand = 5.1/100,000). Increasing refinement of denominators revealed high risks of over 150/100,000 among 5-9 year olds in some districts. In 1980, but not during the other years reviewed, rates of reported encephalitis by province were inversely related to those for dengue hemorrhagic fever (DHF) ($p = .001$, Fisher's exact test). Encephalitis occurred primarily in the northern provinces and DHF in the central and northeastern provinces. The focality of encephalitis in Thailand and high proportion of cases due to JEV suggests that control measures should be evaluated. Recommendations for immunization of travellers to Thailand should be adjusted according to surveillance data. Immunization against JE for visitors to Thailand should be recommended only for those visiting northern and northeastern provinces during the months of June, July, and August.

ABSTRACT. EXPERIMENTAL EBOLA HEMORRHAGIC FEVER (EHF) IN RHESUS MACAQUES.

E.D. Johnson, N. Jaax, T.M. Cosgriff, K.T. McKee, Jr. USAMRIID, Ft. Detrick, MD 21701.

Ebola virus (EV), the newest member of the proposed *Filoviridae* family, causes a severe and often fatal hemorrhagic fever in humans. Since its isolation in 1976, EV has caused sporadic infection and large epidemics in central Africa with mortality as high as 60-70%. Studies of disease pathogenesis, prophylaxis, and therapy have been hindered by the absence of a well-characterized primate model. In an effort to define the clinical and clinicopathologic features of EHF, we have successfully infected Rhesus macaques with EV. The resultant clinical disease was uniformly fatal within 10 days, with evidence for widespread disturbance of physiologic function. Adult macaques were inoculated intramuscularly with 1-10 $TCID_{50}$ of Mayinga strain EV. Animals were active and healthy until 6 days post-inoculation, when anorexia, depression, and fever appeared. Clinical symptoms increased in severity, and a petechial rash involving the trunk and proximal limbs was observed on the seventh day. No overt hemorrhage from nasopharynx or gastrointestinal tract was noted. By day 8, a sudden drop in arterial blood pressure occurred, and death ensued in all animals within 24 hours. White blood counts rose on day 6 due to a neutrophilia with left shift, while platelet numbers remained generally unaffected. Coagulation studies showed prolongation of prothrombin and activated partial thromboplastin times on the 2 days prior to death. Biochemical abnormalities included marked elevation of SGOT, LDH, and X-glutamyl transferase on the 2 days prior to death; alkaline phosphatase was affected to a lesser degree. In addition, serum BUN and creatinine values rose dramatically on the day of death. Serum virus and interferon levels were maximal on the day of death. Our findings indicate that Rhesus macaques are highly susceptible to infection with the Mayinga strain of EV. Gross alterations of hepatic and possibly renal function, together with abnormalities of the coagulation system imply overwhelming multisystem dysfunction. Our observations provide insight into human EHF pathogenesis, and offer a potential primate model for future evaluation of prophylactic and therapeutic modalities.

ABSTRACT. HANTAAN VIRUS (HV) INFECTION IN SUCKLING MICE. G.R.Kim, K.T.McKee,Jr., J.M. Dalrymple, Ph.D.
USAMRIID, Ft. Detrick, MD 21701

Studies of HV pathogenesis in murid rodents have been hampered by difficulties associated with colonization of natural reservoir species. In an attempt to devise a model for rodent infection, we have produced lethal disease in outbred suckling laboratory mice using 2 non-mouse adapted HV strains. Fatal outcome is age and inoculation route related. Preliminary evidence suggests that the 2 virus strains are similar to each other in their biologic behavior in suckling mice. The 76-118 (prototype) and 79/90HUB (a putative human blood isolate) strains of HV, isolated in Apodemus lung, were propagated in E6 Vero cells to titers of $1.3 \times 10^7$ and $1.0 \times 10^8$ PFU/ml, respectively. Outbred suckling ICR mice of varying post-natal age were inoculated by intracerebral (IC), intraperitoneal (IP), intramuscular (IM), and subcutaneous (SC) routes. IC inoculated animals became hyperactive from 11-13 days post-inoculation, lost weight by 13 days, developed limb paralysis by 15 days, and began to die at 16 days. IC $LD_{50}$ values were calculated at $10^{-4.70}$ and $10^{-5.71}$ for 76-118 and 79/90HUB pools, respectively, but were essentially identical when corrected for PFU in inoculum. Using 10 IC $LD_{50}$ in 0.02 ml, all animals inoculated IC from birth to 72 hours died (MTD=19 days); lethality progressively diminished to 50% by 7 days of age. IC and IP routes were 100% fatal in mice $\leq$ 24 hours of age, while IM inoculation killed 75% and 88%, and SC 41% and 69% with 76-118 and 79/90HUB, respectively. Peak serum viremia ($\sim 10^5$PFU/ml) was delayed by 1 week in SC inoculated animals when compared with other routes. Immunofluorescent (IF) antibody appeared by 12-16 days in all groups. Examination of brain, thymus, heart, lung, liver, spleen, and kidney by direct IF and plaquing revealed largest amounts of antigen and virus in brain, lung, and kidney. SC inoculated animals had large amounts of antigen in spleen as well. All findings were similar for both 76-118 and 79/90HUB strains. Suckling outbred ICR mice are susceptible to lethal infection with non-mouse adapted HV. With inocula of 10 IC $LD_{50}$, animals are highly sensitive to IC and IP challenge but less so by IM and SC routes. Both HV strains tested behave similarly in this system, suggesting biologic relatedness. The suckling mouse provides a sensitive lethal model for HV investigation. While differing from natural reservoir infection in its fatal outcome, much information can be gained by continued exploitation of this model system.

ABSTRACT SELECTIVE INHIBITION OF ANTIBODY-MEDIATED DENGUE VIRUS INFECTION BY CHLOROQUINE. S. Kliks, D.S. Burke, and W.E. Brandt. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Walter Reed Army Institute of Research, Washington, D.C.

Dengue virus can infect macrophages via a virus receptor on the cell surface, or via an Fc receptor when infection is carried out in the presence of antibodies at subneutralizing concentrations. In the latter case, the number of infected cells and virus production is enhanced up to 100 fold, and this may play a role in the development of dengue hemorrhagic fever by dengue-2 virus. It is not known if the post-penetration pathway of infection is the same following attachment to the two different receptors. Fc receptor-mediated entry is known to be associated with endocytosis. Since lysosomal vesicles are involved in endocytosis, and since chloroquine is known to concentrate in lysosomal vesicles and raise the pH within those vesicles, we determined whether or not chloroquine would selectively inhibit virus infection initiated via the the Fc receptor. Fresh suspension culture human monocytes obtained by elutriation, and the P388D1 mouse macrophage cell line were pretreated for 30 minutes with varying concentrations of chloroquine, and infected and cultured for three days in the presence of the same chloroquine conentrations. The cells were infected with the 16681 strain of dengue-2 virus via the Fc receptor by mixing the inoculum with dengue-2 antiserum diluted beyond the neutralization titer, or with a monoclonal antibody preparation directed against a flavivirus-common epitope. Normal (virus receptor) infection was accomplished by mixing the inoculum with the same dilutions of normal serum. Virus infection was measured by counting the cells reactive in an indirect immunofluorescence assay. In human monocytes, chloroquine at 0.01 mM inhibited infection via the Fc receptor by 85% whereas there was no inhibition (and even an increase) in the number of monocytes infected via the virus receptor. Both Fc and virus receptor routes of infection were inhibited (92% and 85% respectively) using 0.02 mM chloroquine; 0.05mM chloroquine killed the cells. Similar results were obtained with the P388D1 mouse macrophage cell line, but both virus and Fc receptor routes of infection were easier to demonstrate in the human cells. The selective inhibition by chloroquine suggests that the pathway of infection is associated with lysosomal vesicles following attachment of virus-antibody complexes to Fc receptors.

ABSTRACT. COMPARATIVE VECTOR COMPETENCE OF AEDES MOSQUITOES FOR WEE VIRUS. L.D. Kramer, J.L. Hardy, E.J. Houk, and S.B. Presser. Naval Biosciences Laboratory and Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720.

Aedes dorsalis females infected after ingestion of a high concentration of western equine encephalomyelitis (WEE) virus are efficient oral transmitters of virus after only 7 days incubation at 26°C; whereas Aedes taeniorhynchus females are not efficient transmitters until 21 days or longer after infection. This difference in vector competence is related to differences observed in viral multiplication within and/or dissemination from the mesenteron. In female Ae. dorsalis, viral titers in mesenterons rapidly increased to peak titers within 1 day after ingestion of virus. Dissemination of virus to extra-mesenteronal tissues was detected in 100% of females by 2 days after infection. In contrast, viral titers in mesenterons of female Ae. taeniorhynchus decreased rapidly within 1 day after infection and, in most females, remained at relatively low levels throughout the experiment. Disseminated infections were detected in only 20% of the females at 14 days, at which time a high proportion of mosquitoes had mesenteronal escape barriers. The percent of disseminated infections increased to 50% by 21 days and 70% by 28 days.

In a subsequent study, immunofluorescent assay indicated peak concentration of WEE viral antigens in mesenterons at 2 days in infected Ae. dorsalis females and at 6 days in Ae. taeniorhynchus females. Thus, delay in dissemination of WEE virus from mesenterons of infected Ae. taeniorhynchus females appears to be related to their ability to rapidly modulate viral titers to low levels.

ABSTRACT. THE EFFECT OF NORMAL LARVAL TISSUES OF CULEX TARSALIS ON THE DETECTABILITY OF TURLOCK AND HART PARK VIRUSES IN POOLS OF 4TH INSTAR LARVAE. T. G. KSIAZEK, J. L. HARDY, W. C. REEVES. DEPARTMENT OF BIOENVIRONMENTAL HEALTH SCIENCES, SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF CALIFORNIA, BERKELEY.

During investigations on the role of transovarial transmission in the maintenance of Turlock (TUR) and Hart Park (HP) viruses, it was observed that the titer of stock virus was reduced to an undetectable level when virus was mixed with a triturated suspension of uninfected (normal) 4th instar larvae of *Culex tarsalis*. Usual test methods included trituration of larvae in 2.0 ml of 20% fetal bovine serum in Dulbecco's PBS and assay on monolayer cultures of duck embryonic cells for TUR virus and Vero cells for HP virus. A linear relationship existed between number of larvae in the pool and titer of virus recoverable. No virus was detectable when 1000-10000 PFU of virus was added to pools containing 25 or more larvae. Suspensions of up to 25 adult male or female *Cx. tarsalis* had little effect on detectable viral titers while pupal suspensions had an intermediate effect. The viral reducing effect of normal larval tissues could be counteracted for both TUR and HP viruses by use of polycations or a high pH buffer. Further investigation demonstrated a similar reduction in titers of these viruses with increasing numbers of larvae in pools of two other mosquito species, *Aedes melanimon* and *Anopheles franciscanus*. In addition to TUR and HP viruses, larval tissues of *Cx. tarsalis* similarly reduced titers of California and St. Louis encephalitis viruses. However, titers of western equine encephalomyelitis virus were not affected by larval tissues. These findings may have significant bearing on the interpretation of transovarial transmission attempts in which pooled larvae are being assayed for the presence of virus.

ABSTRACT. CHANGES IN HYDROXY AND CARBOXYLIC ACID METABOLISM IN MOSQUITO CELL CULTURES FOLLOWING INFECTION WITH DENGUE VIRUSES. G. Kuno, J.B. Brooks, and B.J. Wycoff. Dengue Branch, San Juan Laboratories Division of Vector-Borne Viral Diseases and Biotechnology Branch, Division of Bacterial Diseases, Center for Infectious Disease, Centers for Disease Control.

The supernatant fluids of dengue virus-infected mosquito cell cultures were analyzed for hydroxy and carboxylic acids by means of frequency-pulsed electron capture gas-liquid chromatography. The hydroxy acid profiles of normal and virus-infected cell cultures differed qualitatively and quantitatively. The profiles of hydroxy acids in the DEN 1- and DEN 4-infected cultures were different from each other and from those of DEN 2 or DEN 3-infected cultures. Although quantitative differences of a few peaks could be found between the hydroxy acid profiles of DEN 2 and DEN 3-infected cultures, in the absence of clear qualitative difference, the two profiles were considered essentially indistiguishable. The carboxylic acid profiles of virus-infected cultures were different from that of normal cell culture, but none of the 4 serotypes of DEN viruses induced type-specific profiles. Thus, these findings contrasted to previous results with rhesus monkey kidney cell cultures (LLC-$MK_2$) in which serotype-specific sets of hydroxy acids and a DEN 1-specific set of carboxlic acids were released in the supernatant fluids by the infection with dengue viruses.

ABSTRACT. EXPANDING HORIZONS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME: SEROLOGIC EVIDENCE OF HANTAAN-LIKE VIRUS ACTIVITY BEYOND ITS TRADITIONALLY RECOGNIZED BOUNDARIES. J.W. LeDuc, G.A. Smith and K.M. Johnson. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md 21701.

Hantaan (HTN) virus, the causative agent of hemorrhagic fever with renal syndrome (HFRS), and other antigenically similar HTN-like viruses, have traditionally been recognized from North Asia, including Japan, Korea and Manchuria, across the USSR and into Northern Europe and Scandinavia. We recently reported evidence of the presence of a HTN-like virus in port cities of the US, well beyond this traditionally recognized range. In the present report, we further describe the characteristics of the HTN-like virus which we recovered from wharf rats (*Rattus norvegicus*) captured in the US and present serological evidence for the existence of HTN-like viruses in other areas well outside the traditionally recognized boundaries of HFRS. Sera from domestic and peridomestic rats from Burma, Thailand, Hong Kong, the Philippines, Argentina, Brazil and Egypt were examined. Anti-HTN virus antibody was found by both immunofluorescent antibody (IFA) assay and plaque reduction neutralization tests using the 76-118 strain of HTN virus in some individuals from virtually every area sampled. Antibody prevalence rates were generally about 5-10%; however, distribution of antibody was highly focal in most areas sampled and prevalence rates within some foci of infection reached 30% or greater. Serosurveys of resident human populations have not been attempted for all areas where anti-HTN virus antibody was found in rats, but in the few which have been examined, low prevalence rates, generally less than 5% by IFA, have been encountered. These results suggest that HTN-like viruses may be distributed much more widely than is presently recognized. The search for associated human illness is in progress, but as yet no cases have been identified.

ABSTRACT. ARGENTINE HEMORRHAGIC FEVER (AHF) IN RHESUS MACAQUES: VIRUS STRAIN DIFFERENCES AND CLINICAL DISEASE. K.T.McKee,Jr., W.B.Mahlandt, L.R.Bagley. USAMRIID, Ft. Detrick, MD 21701

AHF is a severe, often fatal human infection caused by Junin virus (JV), a member of the family Arenaviridae. There currently exists limited understanding of the spectrum of clinical disease observed in AHF, due in part to absence of a realistic primate model. We have now demonstrated that the Rhesus macaque is susceptible to JV infection, with a resultant clinical illness that closely mimics human AHF. Moreover, susceptibility to illness and pattern of disease appear to be virus strain dependent. Nineteen macaques were inoculated under maximum containment (P-4) conditions with $\sim 10^4$ PFU of 3 JV strains of human origin (Romero, Espindola, and Ledesma). Romero (from a non-fatal case) caused no significant illness in any animal, induced no viremia or virus shedding in oral secretions detectable by plaque assay, but produced a brisk immunofluorescent (IF) and neutralizing (NT) antibody response detectable by 14-17 days post-infection. Espindola (from a hemorrhagic fatal case) caused a predominantly hemorrhagic syndrome that was fatal in 8/8 animals (MTD=33 days). Virus was detected in serum by day 7, peaked on day 17(GMT=1.0 x $10^5$PFU/ml), and persisted until death in most animals. Virus was detected in oral secretions by day 7-10, reached peak GMT$>10^4$PFU/ml by 21 days, and usually persisted to death. IF antibody was detected by 14-17 days, but NT antibody did not appear until after day 21. Ledesma (from a fatal case dominated by neurologic signs) produced minimal bleeding but recognizable neurologic changes in some animals, and was fatal to 5/7 (MTD=35 days). Serum and throat swab virus titers were lower than those seen in Espindola-infected animals (GMT=3.7 x $10^1$ and 1.5 x $10^3$PFU/ml, respectively); virus was detectable on fewer days as well. IF antibody appeared by 10-14 days, while NT antibody was observed by day 14. Hematologic alterations included depression of white blood count to 1/2-2/3 of pre-inoculation values by day 7 in all virus groups, and depression of mean platelet counts to $<100,000/mm^3$ by day 21 in Espindola and Ledesma infected animals; Romero produced no significant platelet changes. Infection of Rhesus macaques with JV results in disease clinically similar to human AHF. The nature and degree of symptomatic clinical infection, as well as the virologic events and serologic response to infection, vary with JV strain. This model holds promise for more realistic studies of AHF pathogenesis than were possible previously, and will allow investigation of such problems as the nature of virus strain variation, disease prophylaxis, and therapy.

ABSTRACT. SNOWSHOE HARE VIRUS PERSISTENCE THROUGHOUT THE YUKON TERRITORY 1972-1983. D.M. McLean. Medical Microbiology, University of British Columbia, Vancouver, Canada.

Attempts to define the geographic distribution and maintenance in nature from year to year of snowshoe hare (SSH) virus (California serogroup of Bunyavirus) have required collection of unengorged female mosquitoes and larvae each summer at boreal forest locations throughout the Yukon Territory. After speciation, mosquito pools were assayed for virus content in suckling mice 1972-83, and also in baby hamster kidney tissue cultures 1981-1983. From 1972-1982, 53 SSH isolates were achieved from 132,428 adult mosquitoes of 7 species including 32 of 84,153 *Aedes communis*. East of Whitehorse at latitude 61°N, SSH virus has been isolated from adult mosquitoes during every week of June and July in 1972-73 and 1978-82 and virus assays on mosquitoes collected on 29 April and in June and July 1983 will be reported. Larvae collected east and west of Whitehorse have yielded SSH virus, suggesting transovarial transfer. Near the Arctic Circle, SSH virus has been isolated during 5 of 8 weeks in June and July and in open woodland at 69°N during early July. *Ae. communis* mosquitoes have transmitted SSH virus (1974 topotype) after incubation at 4°C and 13°C both after oral feeding and after intrathoracic injection and the 1975, 1978, 1980 topotypes have multiplied in their salivary glands after injection of 0.1-3 PFU virus. These results demonstrate clearly the persistence of SSH virus throughout the Yukon Territory and point towards *Ae. communis* as a major natural vector.

A VARIANT OF LACROSSE VIRUS ATTENUATED FOR *AEDES TRISERIATUS* MOSQUITOES. Barry R. Miller, University of Illinois at Urbana-Champaign.

A plaque-purified variant was cloned from prototype LaCrosse (LAC) virus. This variant (PP-31) was lethal to suckling mice by the intracerebral route, produced "wild-type" plaques in Vero and BHK-21 cells and grew to high titers (>$10^7$ PFU/ml) in suckling mice and in cell culture. The variant was able to orally infect the vector, *Aedes triseriatus*; however it was unable to escape infected midgut cells and disseminate to secondary target organs. Large, atypical, focal accumulations of viral antigen were detected in these midguts by immunofluorescence. Orally infected mosquitoes were unable to transmit virus by bite to suckling mice or vertically to their progeny. Even after inoculation of the variant virus into mosquitoes, there appeared to be a restriction on cell to cell virus movement. The role such variants may play in the modulation of infection in an arthropod vector is discussed.

ABSTRACT. DETECTION OF YELLOW FEVER IN VIREMIC SERA BY ANTIGEN CAPTURE ELISA. T. P. Monath and R. R. Nystrom. Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado.

An antigen capture ELISA was developed for detection of yellow fever virus. Gradient purified yellow fever 17D virus diluted in buffer or pooled normal human serum was used as antigen to test the relative sensivitity of various assay procedures. Capture antibodies were coated either directly to polystyrene plates or indirectly by use of appropriate antiglobulins. Capture antibodies tested included human IgM yellow fever antibody; four monoclonal mouse type-specific anti-yellow fever antibodies (alone and as mixtures); flavivirus group-reactive monoclonal antibody; hyperimmune mouse ascitic fluid and hyperimmune rabbit serum. In a system employing human IgM antibody for capture, detecting antibodies investigated included monoclonal type-specific and group-reactive antibodies, hyperimmune mouse ascitic fluid, and hyperimmune rabit serum, either linked directly to enzyme (alkaline phosphatase) or followed by appropriate enzyme-conjugated antiglobulins. The most sensitive assay was a 4-step procedure employing the following sequence of reagents: 1. human IgM antibody or any of the three type-specific anti-E glycoprotein $IgG_{2a}$ monoclonal antibodies; 2. virus; 3. flavivirus group-reactive monoclonal antibody conjugated to enzyme; 4. substrate (p-nitrophenyl phosphate). Maximal sensitivity was 0.014 micrograms viral protein or $10^{3.3}$ plaque-forming units/.05 ml; similar results were obtained for virus suspended in buffer and normal serum. Viremic sera from cynomologus and rhesus monkeys infected with wild YF virus were tested by this technique. By the antigen capture ELISA, virus was detectable in these sera, however, the sensitivity of the assay was less (up to 50-fold) than for purified virus suspended in normal serum. For identification of yellow fever viremic sera with low concentration of virus, a rapid amplification step in mosquito cell culture followed by antigen detection ELISA on cell culture fluid was shown to be useful.

ABSTRACT. DENGUE TRANSMISSION 1972-1978 IN AREAS OF HIGH AND LOW INCIDENCE OF DHF/DSS. RETROSPECTIVE DETERMINATIONS BASED UPON PRELIMINARY EXAMINATION OF AGE-STRATIFIED SERUM SPECIMENS. D Morens, N Sangkawibha, S Rojanasuphot, A Karyadi, N Panjaitan, K Larsen, R Watanabe, S Halstead. University of Hawaii School of Medicine; Virus Research Institute, Ministry of Health, Bangkok, Thailand; Epidemiology Unit, Ministry of Health, and Medan Provincial Health Office, Indonesia.

Using PRN tests of antibody to dengue 1-4 we reconstructed transmission patterns of dengue in areas of high and low DHF/DSS incidence by examining age-stratified serum specimens collected in 1980-1981 from exposed children. The study areas included a part of Rayong Province, Thailand (reported 1980 DHF/DSS incidence per 100,000 persons of 240), and Medan Timur Kecamatan, Indonesia (reported 1980 incidence of 3). The Rayong study population included 2,750 children identified by a random >25%-sampling of all schoolchildren in grades K-8 (2,329 children), or by household cluster-sampling of preschoolers (421 children); and the Medan Timur Kecamatan population 5,006 (6% of) children aged <1-15, identified by random household selection. 1,441 sera tested so far include 881 from Rayong children aged <1-8, and 560 from Medan Timur children aged <1-9. Monotypic antibody (MA) was defined by >50% plaque reduction to a single type at a 1:30 serum dilution, and heterotypic antibody (HTA) by >90% reduction to 2 or more types at a 1:30 dilution.

In both areas HTA varied directly with age, from <5% (born 1980) to >25% (born 1972); and MA to each of dengue types 1,3,& 4 was found in 5-10% of children born before 1978 only. The Rayong study population differed in greater population prevalence of MA to types 1,3,& 4 combined in children born before 1976 only (22% v 12%); high dengue 2 MA prevalence (15%) regardless of age; and, relatively high dengue 4 MA prevalence (10%) in children born before 1976 only.

The serologic data suggest that Rayong and Medan Timur may differ in that: 1) there has been greater dengue circulation in Rayong; 2) there is greater MA immunity to dengue types 1,3,& 4 combined in Rayong children born before 1976; and 3) dengue 2 probably circulated widely in Rayong at 1 or more times between 1977-1980, but did not circulate widely in Medan Timur. Differences in DHF/DSS incidence were not explained by different infection rates. These inferences will be discussed in reference to the 1980 Rayong DHF/DSS epidemic, and the DHF/DSS age-specific incidence peaks in Rayong children born before 1976 noted during that epidemic.

ABSTRACT. GENETIC ANALYSIS OF 3 URBAN POPULATIONS OF AEDES AEGYPTI AND ITS RELATIONSHIP TO DENGUE VIRUS SUSCEPTIBILITY/ R.J. Novak, D.J. Gubler, W.L. Hartberg and J. Fowler, Dengue Branch, San Juan Labs, DVBVD, CID, CDC, San Juan, Puerto Rico, Dept. of Biology, Georgia Southern College, Statesboro, GA.

Several studies have examined genetic variation of *Aedes aegypti* populations based primarily on geographic distribution, employing either analysis of isoenzymes or characterization of morphological traits. The purpose of this report is to determine whether genetic variations of this mosquito may correlate with dengue virus susecptibility. *Aedes aegypti* populations were from San Antonio, Texas, New Orleans, Louisiana, and Miami, Florida. Larval samples were collected from several different locales in each city and maintained as subpopulation lines. Each subpopulation was characterized by: (1) electrophoretic analysis of 18 enzymes (2) morphological type ("Morphotype") and (3) oral susceptibility to dengue 1 virus. Electrophoretic analysis has revealed that only 6 of the 18 enzymes assayed were polymorphic. Variation based on Chi-square analysis at one or more of these loci occurred not only between populations but within populations. For example at the Idh (Isocitrate dehydrogenase) loci the Magazine subpopulation from New Orleans exhibited electromorph frequencies of 0.465 at 100 and 0.535 at 108. The Almonaster subpopulation, also from New Orleans, was monomorphic with only a single electromorph at 100 (1.000). Population affinities based on Nei's Index of genetic distance reveals that the closest similarity exists between the San Antonio-New Orleans populations, whereas the most distant were San Antonio-Miami. Morphotype analysis has revealed variation in classes (class 0=*Ae. aegypti formosus*, class 1= *Ae. aegypti* classes 2-7= *Ae. aegypti queenslandensis*) within and between these populations. For example in the New Orleans, Chef Menteur subpopulation, 23 of 100 adult females examined were *Ae. aegypti formosus* as compared to only 2 of 100 from the Almonaster subpopulation. In San Antonio the Zaramoras subpopulaton consisted of 35 of 100 *Ae. aegypti aegypti* in contrast to Balcones where 75 of 100 were in this class. Susceptibility to dengue type 1 virus also exhibited variation within these populations, ranging from 12 to 31 percent in New Orleans, 15 to 26 percent in Miami, and 12 to 23 percent in San Antonio. To date no distinct correlation has been observed between enzyme or morphotype variation and virus susceptibility.

ABSTRACT. ORIGINAL ANTIGENIC SIN IN DENGUE FEVER PATIENTS. J.G.Olson and T.L. Thirkill. Yale Arbovirus Research Unit, New Haven, CT. 06510

Acute and convalescent phase blood specimens were examined from 10 Indonesian children with uncomplicated dengue fever. Dengue virus was recovered from the acute phase serum specimen of each patient and the virus identified by complement-fixation test. Acute phase sera were tested by hemagglutination-inhibition (HI) and virus neutralization (NT) to determine if patients had been previously infected with dengue virus. Patients who had dengue HI or NT antibody in their acute phase sera were classified as secondary infections. Convalescent phase sera were assayed for dengue antibodies by immunofluorescence (IFA), NT and enzyme immunoassay (EIA). Patients experiencing reinfection developed high NT antibody titers in the convalescent phase of their illnesses to dengue serotypes other than the one which had been isolated. Quantitation of the IgM antibody response by EIA revealed highest titers for the isolated serotypes in most instances. This observation documents the existence of original antigenic sin among patients experiencing a mild form of disease due to dengue viral infection. This finding supplements those of previous studies of patients with the more serious forms of disease (dengue hemorrhagic fever and dengue shock syndrome).

ABSTRACT. ISOLATION AND IDENTIFICATION OF LA CROSSE VIRUS INTRACELLULAR RNAS. H.L.Regnery, J.F.Obijeski, and Guang-jer Wu. Molecular Virology Branch, Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia 30333; Vaccine Development, Genentech Inc., South San Francisco, California 94080; Department of Microbiology and Immunology, Emory University School of Medicine, Emory University, Atlanta, Georgia 30322

La Crosse virus specific RNAs from infected cell cytoplasmic extracts were analyzed to determine which RNAs represent either replicative forms or messenger RNAs. Separation of these two RNA populations was accomplished by fractionation on cesium chloride density gradients. The replicative RNAs were identified by electrophoresis of duplex molecules on polyacrylamide gels. Three messenger RNAs with approximate molecular weights of $2.3 \times 10^6$, $1.5 \times 10^6$, and $0.27 \times 10^6$ were identified by acid-urea agarose gel electrophoresis, duplex formation, and *in vitro* translation. Three polypeptides were translated *in vitro* from La Crosse virus messenger RNAs. A large polypeptide was synthesized in minimal amounts and represented a possible precusor for the glycoproteins of La Crosse virus. La Crosse virus nucleocapsid protein and a nonstructural protein (approximately 10,000 daltons) was translated from the smallest messenger RNA that was purified by hybridization-selection.

ABSTRACT. ANALYSIS OF 17D YELLOW FEVER VIRUS NEUTRALIZING (N) EPITOPES WITH MONOCLONAL ANTIBODIES. J.J.Schlesinger, E.E.Walsh, and M.W.Brandriss. U. Rochester and Rochester General Hospital, Rochester, N.Y.

Thirteen monoclonal antibodies (MAB) against the envelope glycoprotein of yellow fever virus (YF, Virology 125:8,1983) and two previously described non-N flavivirus group reactive dengue 2 MAB (Gentry et al, Am J Trop Med & Hyg 31:548,1982) were used in a competitive binding assay to map antigenic sites on the 17D vaccine strain of YF. Results are summarized in the Table. Binding of radioiodinated YF type specific N MAB (4E3, 2C9, 2E10) was blocked by each YF type specific N MAB but not by a MAB (8A3) which neutralizes only 17D YF. Two non-N YF type specific MAB (2B8,3A3) as well as three non-N flavivirus group reactive MAB (5E6; dengue 2 4G2, 1B8) also blocked the binding of YF type specific N MAB. In some cases enhancement of N MAB binding by a non-N MAB was observed. Non-N dengue 2 MAB enhanced neutralization of 17D YF by 17D N MAB. These results support the concept of separate 17D YF strain and YF type specific epitopes and suggest close proximity between the YF type specific N determinant and at least some regions of the YF type and flavivirus cross reactive regions of the virus surface glycoprotein. Enhanced binding of some N MAB may reflect an increase in the availability of antigenic sites and/or binding affinity due to allosteric changes.

| | Unlabeled Competitor MAB | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | | | C | | | | | D | | | | E | Dengue | |
| 125I N MAB | 8A3 | 4E3 | 2C9 | 2E10 | 2B8 | 5E3 | 2D12 | 3A3 | 4E1 | 4E11 | 5E5 | 5E6 | 5H3 | 3E9 | 4G2 | 1B8 |
| 4E3 | -[a] | +[b] | + | + | + | - | - | + | - | - | - | + | - | - | + | + |
| 2C9 | - | + | + | + | + | - | - | + | - | - | - | + | - | - | + | + |
| 2E10 | - | + | + | + | + | - | - | + | - | - | - | + | - | - | + | + |

(a) no competition
(b) ≫90% competition
A = YF strain specific: neutralizes 17D YF only
B = YF type specific: neutralizes 17D YF and Asibi YF
C = YF type specific: neutralizes Asibi YF only
D = flavivirus group reactive: neutralizes Asibi YF
E = YF type specific: non-N

ABSTRACT. NEUTRALIZATION SITES ON SINDBIS VIRUS: ANTIGENIC, TOPOGRAPHIC, AND BIOLOGIC RELATIONSHIPS. A.L. Schmaljohn, K.M. Kokubun, D.S. Stec, and G.A. Cole
Dept. of Microbiology, U. of Maryland School of Medicine, Baltimore, Md. 21201

We previously showed, using monoclonal (MC) antibodies to Sindbis virus (SIN), that humoral immunity to alphaviruses can be mediated either by neutralizing (NT) antibodies or by antibodies that lack detectable NT activity and act instead on infected cells. The former antibodies and the antigenic sites to which they bind are described in this report. SIN contains two envelope glycoproteins, E1 and E2. Most MC antibodies with NT activity reacted with E2, and a competitive-inhibition enzyme-linked immunosorbent assay (CI-ELISA) suggested that all E2-specific MC antibodies thus far obtained reacted with a single topographical region of E2; nevertheless, E2-specific MC antibodies exhibited diverse idiotypes, isotypes, and (when tested on SIN variants) different reaction patterns. The E1 glycoprotein, which together with E2 forms the virion spikes, was more antigenically complex, having at least five topographically distinct antigenic sites demonstrable by CI-ELISA. Although four of the E1 sites were undetectable on virion surfaces, a MC antibody to the fifth site had NT activity comparable to that of E2-specific NT antibodies. Complement (C') augmented NT activity of antibodies to either site, but neither E2- nor E1-specific NT antibodies required C'. Consistent with the known conservation of E1 among alphaviruses, the E1 NT site was shared with western equine encephalitis virus. When detergent-disrupted SIN was used as solid-phase antigen in the CI-ELISA, the E1 and E2 NT sites appeared spatially unrelated. A similar CI-ELISA performed with intact virions, immobilized to the solid-phase by antigen-capture, revealed a small but significant degree of reciprocal inhibition between E2- and E1-specific NT antibodies. Thus, while it is clear that antibodies to either glycoprotein can effect neutralization, the question of whether E2- and E1-specific NT antibodies function via the same mechanism(s) remains unresolved. Both NT sites were present on infected-cell surfaces as well as on virion surfaces; however, only the E1-specific NT antibody inhibited the pH-dependent cell fusion and polykaryon formation mediated by SIN. The latter observation raises the possibility that E1-specific NT antibodies may act, not by inhibiting attachment of virions to cell surfaces, but by inhibiting the pH-dependent fusion of internalized virions with endosomal membranes thought to be required for SIN entry into host-cell cytoplasm. (Supported by the U.S. Army Research and Development Command and USPHS grant NS17741)

ABSTRACT. DENGUE-4 VACCINE: ORAL INFECTION, TRANSMISSION, LACK OF REVERSION, AND THE EFFECT OF EXTRINSIC INCUBATION TEMPERATURE ON VECTOR-VIRUS INTERACTIONS IN THE MOSQUITOES, AEDES ALBOPICTUS AND AEDES AEGYPTI.

R. J. Schoepp, B. J. Beaty, and K. H. Eckels. Department of Microbiology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523; Walter Reed Army Institute of Research, Washington D.C. 10012.

The dengue-4 candidate vaccine virus (PDK-35TD3 FrhL-3) and its parent virus (H-241) were compared for their ability to orally infect, to replicate in, and to be transmitted by Aedes albopictus and Aedes aegypti mosquitoes. The vaccine virus was attenuated in its ability to infect and replicate in Aedes albopictus mosquitoes. After ingesting infectious blood meals containing $\geq 7.0\ \log_{10} TCID_{50}/ml$ of the respective virus, 80% (91/114) of the mosquitoes became infected with parent virus, in contrast to only 20% (23/116) of the mosquitoes ingesting the same amount of vaccine virus. Of the mosquitoes with disseminated infections that fed successfully, 1/3 (33%) transmitted vaccine virus, while 7/38 (18%) transmitted parent virus. Extrinsic incubation of Aedes albopictus mosquitoes at temperatures higher than 27°C resulted in higher virus titers in mosquitoes and greater infection of secondary target organs by disseminated parent virus. The vaccine virus remained temperature-sensitive (restrictive temperature 39°C) after infecting and replicating in Aedes albopictus mosquitoes. Similar experiments are being conducted with Aedes aegypti mosquitoes.

ABSTRACT. SPECIFIC IgM AND IgG RESPONSES TO DENGUE-2 (DEN-2) PR-159-S-1 VACCINE.

R.McN. Scott, P.L. Summers, D.S. Burke, K.H. Eckels, M.A. Ruiz and W.H. Bancroft. The Walter Reed Army Institute of Research, Washington, D.C., U.S.A.; The Armed Forces Research Institute of the Medical Sciences, Bangkok, Thailand.

In dose response studies of the DEN-2 vaccine, yellow fever immune (YFI) and yellow fever non-immune (YFN) recipients have shown different responses to the DEN-2 PR-159/S-1 vaccine. Neutralizing (N) antibody developed in 52% (11/21) of YFI vaccinees. This N antibody had a geometric mean titer (GMT) of 235 at 30 days and persisted for at least three years. Although a similar proportion (47%, 9/19) of YFN vaccinees developed N antibody, the GMT at 30 days in this group was only 49 and 5 of these subjects lost antibody by six months after immunization. Specific IgM antibody was measured for seven subjects from each group using IgM antibody capture immunoassays. DEN-specific IgM developed as early as two weeks after inoculation in one representative of each group and was detected by one month in all N antibody positive subjects tested. While IgM persisted for a year or longer in one subject in each group, it disappeared by one year in the majority of subjects. Specific IgM antibody capture immunoassays appeared to be more sensitive than N antibody assays for identifying the immune responses to DEN-2 vaccine. Specific IgM was detectable after N antibody had waned in four out of five YFNs in whom N antibody was undetectable by six months; in one case specific IgM activity was detected in the absence of N antibody. Sucrose gradient (SG) separations were performed on serum selected for the presence of DEN-2 IgM from each of the twelve YFI and YFN vaccinees. IgM-capture assays showed specific IgM only in the 19S portion of the SG in all 12 sera tested. An IgG antibody capture assay showed specific IgG activity in the 7S portion of the SG in five of the six YFI sera but in none of the YFN sera. N antibody activity was found in the IgG region of all six YFIs and in three of the five YFNs tested. Two of the three YFN volunteers with DEN-specific IgG, maintained N antibody activity for longer than six months. These data suggest that the transient N antibody activity detected in the YFNs may have resulted from limited IgM responses which did not progress in the usual fashion to IgG.

ABSTRACT. A FIELD STUDY ON THE EFFECTS OF FORT MORGAN VIRUS ON THE REPRODUCTIVE SUCCESS OF SYMBIOTIC CLIFF SWALLOWS AND HOUSE SPARROWS IN MORGAN COUNTY, COLORADO. T.W. Scott, G.S. Bowen, and T.P. Monath. YARU, Yale University, New Haven, Connecticut; New Jersey State Department of Health, Trenton, New Jersey; Centers for Disease Control, Fort Collins, Colorado.

We studied the transmission of Fort Morgan (FM) virus within colonies of nesting cliff swallows and house sparrows under three bridges in Morgan County, Colorado during 1976. Nests were examined and blood or brain specimens were collected from nestlings once or twice a week. Flying birds and small mammals were also studied. We analyzed nesting activity, virus isolations from nestlings of both species, fledging successes, multiple infections within a brood of nestlings, infection frequency by age of nestlings, nestling mortality, and infection frequencies by avian species and bridge site. Fort Morgan virus was isolated from 7% (80/1,156) of the blood and brain samples collected from nestlings. The duration of viremia for nestling house sparrows was at least 3-4 days based on virus isolation from sequential blood samples. Fort Morgan virus viremia of nestling cliff swallows and house sparrows did not reduce their fledging successes nor were young nestling sparrows viremic more frequently than older nestling sparrows. Nests falling down were a more important cause of nestling mortality than FM virus infection. Even though all age groups of nestling sparrows were viremic with FM virus at equal rates, younger nestlings ( $\leq$7 days old) were more likely than older nestlings ( >7 days old) to develop an encephalitic infection. Among nestling house sparrows, FM virus infections were clustered in time and space. Nestling house sparrows with a FM virus infected nest-mate were virus infected more often than conspecifics whose nest-mates lacked a FM virus infection. We concluded that nestling cliff swallows and symbiotic house sparrows that reside in swallow nesting colonies are the principle vertebrate hosts for the maintenance and amplification of FM virus. Foci of FM virus transmission provide a unique opportunity to investigate the ecology of vertebrate hosts in an arboviral transmission system because spacial and temporal dimensions of a focus can be easily defined, an overwintering mechanism for the virus is known, and large numbers of vectors and hosts from an endemic focus can be examined and experimentally manipulated.

ABSTRACT. ANTIBODY TO ALASKAN ARBOVIRUSES IN A POPULATION AT OCCUPATIONAL RISK OF INFECTION. S.K. Stansfield, C.H. Calisher, A.R. Hunt, W.G. Winkler. Division of Viral Diseases and Vector-Borne Disease Division, Center for Infectious Diseases, Centers for Disease Control.

A total of 435 U.S. Geological Survey and Forest Service workers were studied for serological evidence of previous infection with four Alaskan arboviruses which are known or suspected human pathogens. Neutralizing antibody to Jamestown Canyon (JC) virus was present in 89 (20.5%) of the agencies' personnel, while 59 (13.6%) had antibody to Snowshoe hare (SSH) virus, 17 (3.9%) to Northway (NOR) virus, and 15 (3.4%) to Klamath (KLA) virus. The most significant correlates with seropositivity to the antigenically related JC and SSH viruses include the indices of occupational exposure, including history of fieldwork activities (P=0.0001) and duration of employment by the agencies (p=0.0001 for JC and 0.004 for SSH). Seropositivity to the four arboviruses also correlated with a history of travel in certain remote or wilderness areas in Alaska.

ABSTRACT. IG-G ANTIBODIES TO RUBELLA AND CYTOMEGALOVIRUS IN ZAMBIAN MATERNAL AND CORD SERA. M. Stek, Jr., I. Kakoma*, C. DeiSanti, and J.F. Duncan, Jr., Dept. of Prev. Med., Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814; *Ndola Tropical Disease Research Center, Ndola, Zambia.

Since rubella and cytomegalovirus (CMV) infections are hyperendemic in the tropics, it may be surprising that greater morbidity and mortality associated with these diseases are not seen during the fetal-newborn-infant period. A possible explanation for this is that prevalence rates in the tropics are so high that most females of childbearing age possess anti-rubella and anti-CMV antibodies; and these antibodies could be transferred via the placenta to the fetus. A precarious balance may exist whereby these viral infections, potentially so devastating to the fetus and newborn, are prevented in children until maternal antibody wanes. Infection at that point would tend to elicit less deleterious consequences and establish host immunity. This immunity could be transferred subsequently by the immune pregnant female to her offspring. We analyzed 20 matched maternal and cord serum samples from patients at the Ndola Central Hospital, Ndola, Zambia by enzyme immune assay (EIA) for anti-rubella and anti-CMV IgG antibodies. The results confirmed that the majority of pregnant females in this tropical environment have protective antibody. Ninety percent of females had antibody against rubella. Adequate levels of anti-rubella IgG antibodies were detected in 94% of cord blood samples of babies with seropositive mothers. However, 15% of the total cord collection did not have anti-rubella antibody. Ninety percent of the females also had antibody against CMV. CMV seronegative mothers were not the same as those seronegative for rubella. Of the cord samples, from matched seropositive mothers, 89% had anti-CMV IgG. Anti-CMV antibody was not detected in 10% of the matched cord samples. CMV seronegative newborns were not the same as those seronegative for rubella. Our data indicate that a small percentage of the population of this tropical area are susceptible to rubella and CMV. Seronegative individuals, especially females, should be identified. Vaccine is available for rubella and in research use for CMV. As development in tropical countries progresses, the prevalence of these infections may decline altering their epidemiology sufficiently to tip the current precarious balance. This will necessitate an expansion of immunization because the pool of naturally developed seropositives would decline with increasing risks to the fetus and newborn.

ABSTRACT. IMMUNE RESPONSE TO DENGUE-2 (PR-159/S-1) VACCINE MEASURED BY THREE DIFFERENT IMMUNOASSAYS. P.L. Summers, K.H. Eckels, R. McN. Scott, and S.M. Lemon. Walter Reed Army Institute of Research, Washington, D.C., U.S.A.

Sera from human volunteers inoculated with an attenuated dengue-2 (DEN-2) vaccine (PR-159/S-1) contained antibodies that were detected by 3 different immunoassays. An indirect radioimmunoassay (RIA) required purified DEN-2 virions as an antigen for optimal reactivity while an antibody capture RIA or enzyme-linked immunosorbent assay (ELISA) were able to utilize crude antigens from either DEN-infected mouse brains or *Aedes albopictus* cell culture supernatants. When the 2 RIA techniques were compared, the indirect test was a better assay for IgG while the antibody capture test was more sensitive for IgM detection. Using the antibody capture technique, the ELISA was as sensitive for detection of DEN-specific IgM as the RIA. Selected human sera were examined for IgG, IgM, and IgA responses using indirect and antibody capture techniques at various intervals following immunization. Although there were differences in magnitude, yellow fever immune as well as yellow fever non-immune volunteers responded to DEN-2 vaccination by demonstrating IgG, IgM, and IgA antibody responses. In the yellow fever non-immune group, the most abundant immunoglobulin detected was IgM, while in the yellow fever immune group, the predominant post-DEN-2 vaccine immunoglobulin was IgG. All classes of immunoglobulins attained maximum levels between 21 and 60 days post vaccination. In the majority of volunteers, IgM responses were relatively transient and could not be detected 6 months after immunization while IgG and IgA antibodies were still detectable after this period. These immunoassays are sensitive immunological tests that can be used to measure specific immunoglobulin responses after DEN-2 vaccination. Immunoglobulin levels can be measured directly eliminating the need to purify these immunoglobulins prior to assay.

ABSTRACT. ELECTRON MICROSCOPIC EVALUATION OF LA CROSSE VIRUS INFECTION IN *AEDES TRISERIATUS* AND BHK-21 CELLS. D.R.Sundin[1], B.J.Beaty[1], M.L. Hegedus[1], W.A.Rowley[2]. Department of Microbiology and Environmental Health, Colorado State University, Fort Collins, Colorado[1]. Department of Entomology, Iowa State University, Ames, Iowa[2].

Cultured BHK-21 and *Aedes triseriatus* (La Crosse) cells infected with La Crosse virus were examined using electron microscopy. Fewer virions were seen in *A. triseriatus* cells compared to BHK-21 cells at equivalent times post-infection. In BHK-21 cells virions were usually seen in the Golgi complex or in intracytoplasmic vesicles associated with the Golgi complex. In contrast, virions in infected *A. triseriatus* cells were more frequently found in larger smooth-membraned vesicles which also contained fibrillar material and other cell debris. These findings may have important implications for explaining the relative permissiveness of BHK-21 cells for La Crosse virus replication compared to *A. triseriatus* cells. The relatively non-cytolytic virus-mosquito cell interaction is important for survival of infected vectors in nature.

ABSTRACT: A BACULOVIRUS IN THE YELLOW FEVER MOSQUITO, AEDES AEGYPTI.

W. J. Tabachnick and D. L. Knudson. Loyola University of Chicago; Yale University.

As part of a study to analyze mitochondrial DNA sequence variation among populations of Aedes aegypti we have observed an actively replicating baculovirus infection in several A. aegypti strains. Restriction endonuclease enzymes have been used to compare the A. aegypti baculovirus DNA with other known baculoviruses. The A. aegypti viral DNA is ca. 120 Kb in length. Comparisons among several viral isolates have been performed in order to determine viral polymorphism. Electron microscopy of infected adult mosquitoes show viral particles in a variety of cells, tissues, and organs including the head and particularly the trachioles. As typical of baculovirus, virus can also be observed within cell nucleii as well. The morphology of the A. aegypti baculovirus will be described.

ABSTRACT: EFFECT OF RIFT VALLEY FEVER VIRAL INFECTION IN LAMBS ON THEIR ATTRACTIVENESS TO CULEX PIPIENS.

M.J. Turell, C.L. Bailey, and C.A. Rossi. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland.

The difference in attractiveness to mosquitoes between infected and uninfected animals needs to be considered in any model of the natural history of an arbovirus. Animals infected with a virus often exhibit both increased body temperature and $CO_2$ release. Because both of these factors attract host-seeking mosquitoes, it is possible that a viremic animal may be more attractive to potential mosquito vectors than an uninfected animal. This hypothesis was tested by inoculating one of each of 5 pairs of twin lambs (aged 3 days to 8 weeks) with the Zagazig Hospital 501 strain of Rift Valley fever (RVF) virus. Experiments were carried out in a "mosquito-proof" room in a high containment (P-3½) suite. Female Culex pipiens were released into this room 28, 48, and 52 hours after inoculation of the lamb, and all mosquitoes were collected approximately 3 hours later. Engorged mosquitoes were removed after cold anesthesia, and stored at -70°C until triturated and assayed for virus. By our definition, mosquitoes from which virus was recovered fed on the viremic lamb, while those from which no virus was recovered fed on the nonviremic lamb. In the 2 experiments with 3-day-old lambs, peak viremias were $10^{6.2}$ and $10^{7.0}$ plaque forming units (PFU)/ml, while in the 3 experiments with 6- to 8-week-old lambs peak viremias were $10^{4.7}$, $10^{5.0}$, and $10^{5.3}$ PFU/ml. Peak temperatures ranged from 1.1 to 1.9°C higher in the viremic lambs than in their uninfected twin. Both of the 3-day-old lambs died within 72 hours after infection, while none of the 6-week or older lambs died as a result of their infection. Overall, 66% (728/1100) of the engorged females contained virus. This was a significance preference for the viremic lamb (p-value <.001). Preference for the viremic lambs was similar for 3-day and 6- to 8-week-old lambs, 65 and 67%, respectively; and for 28, 48, or 52 hours after infection, 66, 64, and 68%, respectively. In this study, infection with RVF virus increased the likelihood of that animal providing a blood source for a potential vector by a factor of 2 to 1, as compared to a similar, uninfected animal. This preference for viremic animals needs to be accounted for in any model of the natural transmission cycle.

ABSTRACT: INCREASED VECTOR COMPETENCE FOR RIFT VALLEY FEVER VIRUS IN AEDES TAENIORHYNCHUS ASSOCIATED WITH CONCURRENT INFECTION WITH BRUGIA MALAYI. M.J. Turell, P.A. Rossignol, A. Spielman, C.A. Rossi, and C.L. Bailey. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, and Department of Tropical Medicine, Harvard School of Public Health

Mosquitoes are approximately 1,000-fold more sensitive to Rift Valley fever (RVF) virus by intrathoracic inoculation as compared to ingestion. Because microfilariae ingested in a blood meal bore through the mesenteron into the hemocoel, it is possible that ingestion of a viremic blood meal from a host with a concurrent microfilaremia may permit inoculation of virus from the blood meal directly into the hemocoel, thus greatly increasing vector competence. A pair of gerbils (Meriones unguiculatus), one of which was infected with Brugia malayi, was used to test this hypothesis. Both gerbils were inoculated intraperitoneally with $10^{7.7}$ plaque forming units (PFU) of RVF virus, and anesthetized 24 and 30 hours after infection to provide a blood meal for female Aedes taeniorhynchus. Assay of freshly engorged females demonstrated that mosquitoes ingested similar doses of virus from gerbils with and without a microfilaremia, $10^{3.2}$ and $10^{3.3}$ PFU, respectively. Engorged females were maintained at 26°C, allowed to oviposit, and subsequently allowed to feed on susceptible hamsters to determine the ability of the mosquitoes to transmit virus. Following the transmission attempt, the legs and body of each female were triturated and assayed separately to determine the infection/dissemination status for that female. Because infection with Egyptian strains of RVF virus is uniformly lethal for hamsters, hamster death was used as the criterion for viral transmission. Of mosquitoes ingesting blood from the Brugia-infected gerbil, 89% (54/61) became infected as compared to 59% (50/85) of those feeding on the gerbil without a microfilaremia. The difference between these 2 groups was even more pronounced for the percentage of females with disseminated infections between 4 and 13 days after the infectious blood meal: 62% (31/50) and 15% (9/62) for the microfilaremic and nonmicrofilaremic groups, respectively. Transmission rates for refeeding females were 33% (12/36) and 6% (3/50) for the same 2 groups, respectively. Thus, concurrent infection with B. malayi and RVF virus in the viremic host was associated with >4-fold increase in disseminated infections and >5-fold increase in transmission rates.

ABSTRACT. ISOLATION OF JAPANESE ENCEPHALITIS VIRUS STRAINS FROM PATIENTS, PIGS, AND MOSQUITOES IN KAMPANGPHET PROVINCE DURING THE 1982 EPIDEMIC SEASON. M. A. Ussery, D.S. Burke, A. Nisalak, R.G. Andre, C. Leake, M.R. Elwell, and T. Laorakpongse, Armed Forces Res. Inst. Med. Sci., Bangkok; Kampangphet Provincial Hospital, Thailand.

Japanese encephalitis virus (JEV) strains were isolated during the epidemic period (15 June to 15 August 1982) in Kampangphet, Thailand. This province has been shown to have a consistently high encephalitis rate attributable to JEV. CSF and serum from hospital encephalitis cases were tested for anti-JEV IgM antibodies by MAC-ELISA. Fifteen patients with evidence of recent flavivirus infections were identified. An isolate of JEV was obtained from the needle brain biopsy from 1 of 4 fatal cases. Sentinel pigs placed at the houses of JE patients from the current or previous seasons were bled every 2 to 3 days. 10/21 pigs showed JEV HAI seroconversion. Pigs seroconverted 14.4 days after placement and 4 JEV strains were isolated 5.5 days before seroconversion. Mosquitoes were collected near the pigpens using CDC light traps with and without $CO_2$. Over 112,605 mosquitoes have been processed and 22 viruses have been isolated, all but one from $CO_2$ traps, resulting in an overall isolation frequency of 0.02%. *Cx. tritaeniorynchus* comprised 88% of the total catch and all 11 JEV strains as well as 4 other flaviviruses and 2 non-togaviruses have come from this species. The overall isolation rate from *C.t.* is 0.03% (0.02% for JEV, 0.007% for the other flaviviruses). Non-JEV flaviviruses represent the only isolates from other mosquitoes; *Cx. vishnui* 4(0.06%) and *Cx. gelidus* 1 (0.07%). JEV isolates were made from mosquitoes trapped 8.6 days before pig seroconversion. Viruses were isolated principally on *albopictus* C6/36 cells with plaque assay on LLC-Mk2 cells. ELISA screening of the mosquito pools identified a high proportion of positive pools. Viruses were typed by immunofluorescence on *pseudoscutellaris* AP-61 cells using polyvalent grouping reagents, and flavivirus group reactive and JEV specific monoclonal antibodies. Typing was confirmed by monoclonal ELISA and plaque neutralization on LLC-Mk2 cells.

ABSTRACT. DENGUE TRANSMISSION IN TWO PUERTO RICAN COMMUNITIES, 1982. S.H. Waterman, D.J. Gubler, R.J. Novak, G.E. Sather, R.E. Bailey, and I. Rios. Dengue Branch, San Juan Laboratories, Division of Vector-Borne Viral Diseases, Center for Infectious Disseases, CDC, San Juan, Puerto Rico.

Two Puerto Rican municipalities, Manati and Salinas, were studied in an attempt to identify enviromental/ecologic risk factors for dengue transmission during a dengue 4 outbreak in summer-fall, 1982. Initial serosurveys were performed in the two municipalities in August, and the sample populations were rebled in December. At the same time environmental characteristics and socioeconomic levels of the sample households were recorded. An entomologic survey was done in each of the study areas in the interval between bleedings. Serologic data indicated that significant transmission took place in both municipalities with many cases occuring before the first bleeding. Overall recent dengue infection rates were 35% (151/434) in Salinas and 26% (84/323) in Manati. Recent infections did not vary significantly by age or sex. Dengue cases tended to cluster in the sampled members of individual households. Among environmental variables which were significantly associated with both a higher rate of recent infection and higher prevalence of dengue antibody in the combined Salinas-Manati data were low socioeconomic level, slum housing, and the absence of screened doors or windows. Tree height of 20-100 ft was significantly associated with a higher prevalence of antibody ($p < .001$). Neighborhood infection rates varied from 22-45% and 22-35% in Salinas and Manati, respectively. Indices of larval abundance were relatively high in both communities with neighborhood Breteau indices ranging between 62 and 172 in Salinas and between 43 and 94 in Manati. Neighborhood recent infection rates did not correlate with the Breteau index in either municipality but did correlate in Salinas with the potential container index, the total number of potential breeding containers per 100 houses ($r = .89$). Although rates of dengue infection were high throughout both communities, this study suggests that environmental/ecologic risk factors for dengue transmission can be identified, thus allowing for more effective prevention and control of epidemic dengue.

ABSENCE OF DENGUE 2 INFECTION ENHANCEMENT IN HUMAN SERA CONTAINING JAPANESE ENCEPHALITIS ANTIBODIES.

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A pilot survey was conducted to determine the flavivirus infection status of young adults in a rural area of North Thailand. The sera were studied for both plaque reduction neutralizing antibodies to dengue (DEN) 1-4 and Japanese encephalitis (JE) viruses and for infection-enhancement of DEN 2 virus in the human monocyte cell line, U-937.

In 52 sera tested there were 23 with antibodies to one or more DEN viruses with or without anti-JE; 16 sera demonstrated antibodies only to JE and 13 had no detectable flavivirus antibodies. All but two DEN antibody-containing sera enhanced DEN 2 infection in U-937 cells, often to titers of 1:10,000 or greater. By contrast, just one of 16 JE-immune sera enhanced, and that only at a dilution of 1:100. None of the flavivirus-negative sera had DEN-2 enhancing activity.

Until the present study, the general finding has been that polyclonal antibodies to any flavivirus enhance DEN 2 infection, *in vitro*. The failure of naturally acquired human anti-JE to enhance correlates with the absence of recorded instances of dengue shock syndrome in individuals sequentially infected with JE then a DEN virus. Similarly, clinically enhanced dengue illness has not been observed in yellow fever immunes. Our results may provide an *in vitro* correlate of these phenomena, offering an opportunity to study the mechanisms involved.

REPORT FROM THE ARBOVIRUS PROGRAM, PACIFIC BIOMEDICAL RESEARCH CENTER, UNIVERSITY OF HAWAII, HONOLULU, HAWAII 96822

Transovarial transmission of dengue viruses by *Aedes mediovittatus* mosquitoes

Recent studies of the ability of mosquitoes to transmit dengue viruses have provided additional evidence that some *Aedes* species in addition to those included in the subgenus *Stegomyia* can serve as vectors of those viruses under laboratory conditions. These observations prompted us to carry out experiments with certain of such *Aedes* to determine if they were also capable of transmitting dengue viruses transovarially - and thus potentially serving as reservoirs of the viruses in nature. Among the species tested was *Aedes* (*Gymnometopa*) *mediovittatus*. This species was chosen for study because, 1) it is widespread in the Caribbean area where dengue is endemic, 2) it occurs in domestic habitats and, 3) it feeds readily on man. Feeding experiments with dengue types 1 and 2 showed that *Ae. mediovittatus* was highly susceptible to oral infection with those viruses.

Inseminated unfed females of a Puerto Rican strain of *Ae. mediovittatus* were infected with dengue viruses by intrathoracic inoculation and held at 27°C for 5 days before they were allowed to take a blood meal on a normal mouse. Eggs were collected either en masse from a group of females or from individual females held in separate containers. Initially, 4th instar $F_1$ larvae were tested for the presence of virus in pools of 50, but in later experiments the pool size was reduced to 5 or 10 larvae. Viral assay was carried out by inoculating aliquots of triturated suspensions of larvae into *Toxorhynchites amboinensis* mosquitoes. The latter were examined for the presence of dengue virus by the indirect fluorescent antibody technique applied to head squashes. Rates of infection for the $F_1$ larvae were estimated by the method of Le (Am. J. Epid. 114:132-136).

Our experiments demonstrated that *Ae. mediovittatus* was capable of transmitting all 4 dengue serotypes transovarially. In experiments in which eggs were collected en masse and $F_1$ larvae were examined in pools of from 10 to 50, estimated infection rates for $F_1$ larvae were highest for dengue type 1 (7.4%) and type 2 (7.0%) and lowest for dengue type 3 (0.7%) and type 4 (0.3%). These initial experiments employed dengue type 1, 2, 3, and 4 strains from Fiji, Bangkok, Burma, and Medan, respectively.

Since studies with other arboviruses have shown great variation in transovarial transmission among individual females of the same mosquito strain and species, we next examined progeny from individual females in a series of "family" experiments. These experiments were carried out with *Ae. mediovittatus* females infected with strains of dengue type 2 from Africa, Asia, and the Caribbean. $F_1$ larvae were tested in pools of 5 or less and the results are shown in Table 1. For each of the viral strains employed, at least some females transmitted virus to their progeny at high filial infection rates. The highest rates were observed with the virus strain from Puerto Rico where one family had an estimated filial infection rate of 25.7%. This is the highest rate reported thus far for any flavivirus in any species of mosquito. Table 1 also shows that less than two-thirds of infected females transmitted detectable virus to their progeny.

This observation may explain why highly variable filial infection rates are observed from one experiment to another when $F_1$ eggs are collected en masse from a group of females. Data from the experiment with the Puerto Rican strain are shown in greater detail in Table 2.

(Jerome E. Freier and Leon Rosen)*

*Part of the work reported here was carried out in facilities of the Department of Biology, University of Notre Dame, Notre Dame, Indiana.

Table 1. Transovarial transmission of dengue type 2 viruses by individual <u>Aedes mediovittatus</u> females.

| Virus strain | Families: No. pos./No. test. | Families: (% pos.) | Range of estimated filial infection rates in percent |
|---|---|---|---|
| Africa | 3/8 | (37.5) | 4.2 - 17.4 |
| Bangkok | 3/10 | (30.0) | 2.6 - 19.2 |
| Puerto Rico | 8/13 | (61.5) | 3.5 - 25.7 |

Table 2. Estimated filial infection rates by family for *Aedes mediovittatus* mosquitoes infected with a Puerto Rican strain of dengue type 2

| Family No. | No. $F_1$ progeny tested | Estimated infection rate in percent |
|---|---|---|
| 1 | 61 | 3.5 |
| 2 | 22 | 0.0 |
| 3 | 46 | 25.7 |
| 5 | 14 | 0.0 |
| 6 | 53 | 7.4 |
| 8 | 16 | 0.0 |
| 9 | 16 | 7.2 |
| 10 | 8 | 0.0 |
| 11 | 11 | 18.2 |
| 14 | 40 | 13.8 |
| 15 | 59 | 4.4 |
| 16 | 49 | 4.5 |
| 18 | 35 | 0.0 |

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

### Dengue

During the year 1982, the arbovirus laboratory received 138 paired sera and 121 single blood specimens from New Caledonia, Fiji, Vanuatu, Wallis and Horne Islands.

#### Virus isolations

Sera collected in the acute phase of the disease, from 136 patients, have been inoculated by using two methods :

1/ Intra-thoracic inoculation (IT) to *Toxorhynchites* mosquitoes of patients sera (0.2 µl) diluted 1:5 in PBS supplemented by 20 p. 100 normal rabbit serum.

2/ Inoculation to tissue culture (TC) , *Aedes albopictus* cells, Igarashi's clone C6/36,of diluted sera (1:10) in Leibovitz L 15 medium : 0.1 ml per 2 $cm^2$.

In both methods, the viral infection was demonstrated by indirect fluorescent antibody staining, using grouping ascitic fluids A and B and specific antibodies of dengue 1, 2, 3, 4 (NIAID) in the first step and a fluorescein conjugated goat serum anti-mouse IgG - H + L (IPP).

In two imported cases, dengue type 4 virus was identified by using both IT and TC. In a third case, the results of *Toxorhynchites* neurocytes staining were as follows.

| Group A | Group B | DEN 1 | DEN 4 |
|---|---|---|---|
| negative | positive (2+) | ± | positive (1+) |

C6/36 cell inoculations and passages failed to demonstrate the virus in the same early serum.

#### Serology

Inhibition hemagglutination tests (IH) were performed on 138 paired sera and 11 single specimens from patients with fever, headache and painful joints. Only two cases showed a significant rise of antibody titer, in relation to an isolation of dengue type 4 virus.

Antibody titers (IH) from 10 to 160 without a significant rise were found in 60.3 % of the other patients.

### Epidemic polyarthritis (Ross River virus)

No RR virus strain has been isolated from 136 human sera nor from 226 mosquito pools.Serological examination of 138 paired sera showed a rise of RR antibodies from <10 to 80, in one of the two dengue cases observed. Attempts to isolate RR virus from the early serum failed, only dengue type 4 virus was obtained. The immune response of the patient to RR antigen was probably anamnestic.

## Vector surveillance

5.912 mosquitoes have been collected, mainly in the vicinity of Noumea - La Tontouta international airport, on human bait or by miniature light-traps. To research a viral infection, the specimens have been pooled by species, processed and inoculated to *Toxorhynchites* and/or C6/36 cells.

The species collected were : 10 *Aedes aegypti*, 82 *Ae. alternans*, 32 *Ae. notoscriptus*, 175 *Ae. vexans*, 3.725 *Ae. vigilax*, 1.308 *Culex annulirostris*, 7 *C. iyengari*, 280 *C. quinquefasciatus*, 159 *C. sitiens*, 134 *Coquillettidia xanthogaster*.

Positive immunofluorescent reactions on *Toxorhynchites* head squashes were given by two pools, as follows :

| | Group A | RR | SIN | Group B | DEN 1 | DEN 4 |
|---|---|---|---|---|---|---|
| Pool 1100<br>87 *annulirostris* | 2 + | N | N | N | N | N |
| Pool 1101<br>7 *quinquefasciatus* | N | N | | 1 + | N | N |

Attempts to pass or to re-isolate the presumptive viruses failed.

## Epidemiological data

Only two imported cases of dengue, confirmed by both serology and isolation of type 4 virus have been observed. The patients became sick during a stop-over in Wallis Island in September 1982. The third positive result, obtained by IT inoculation appears more dubious. The patient was a Wallisian recruit examined in Noumea in October 1982. No case was seen from Vanuatu and Horne Islands. On twenty serum pairs sent from Suva, Fiji, by Dr. Mataika, no dengue nor RR fever were diagnosed but eight patients showed an immune response to influenza B antigen.

(P. Fauran G. Le Gonidec)

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## COMPARISON OF DENGUE VIRUSES AND SOME OTHER FLAVIVIRUSES BY cDNA - RNA HYBRIDIZATION ANALYSIS

The flaviviruses used were the prototype strains of the four dengue virus serotypes: DEN-1 (Haw), DEN-2 (NGC), DEN-3 (H87) and DEN-4 (H241); two DEN-2 viruses isolated in Thailand in 1980 designated T80G and H (provided by Dr D.S. Burke, Armed Forces Research Institute of Medical Sciences, Bangkok); and three viruses isolated in Australia: Edge Hill (C281), Stratford (C338) and Kunjin (MRM16). The viruses were grown in the C6/36 clone of *Aedes albopictus* cells and virus extruded from the cells was precipitated with polyethylene glycol. RNA was extracted from the viral pellet with a phenol/chloroform mixture and precipitated three times with ethanol before use in cDNA synthesis. The synthesis was carried out using AMV reverse transcriptase, the primer pd($T_{12-18}$) and [$^{32}$P]-dATP.

The cDNAs produced were separated on a polyacrylamide gel. The prototype strains for DEN-1 and DEN-2 produced a larger amount of cDNA compared with the other flaviviruses tested, even though the same concentration of RNA was used for each virus isolate. The level of cDNA produced by Kunjin and Stratford RNA was similar to that of Edge Hill which was about 19 times less than that of DEN-1 and DEN-2 (NGC) according to laser densitometer tracings. The major cDNA band was very similar in electrophoretic mobility to the RNA band from which it was produced, indicating that it is close to a full-length cDNA copy. The ability of all of the flavivirus RNAs tested to produce a certain amount of cDNA was not due to self priming since no cDNA bands were produced in the absence of the primer. The ability to make near full-length cDNA in the presence of an oligo(dT) primer provides evidence for the presence of a poly(A) tract, however short, at the 3′ end of the viral RNA.

Since DEN-1 and DEN-2 (NGC) RNA produced a substantial amount of cDNA, they were used to investigate further the relationship of some flavivirus RNAs by cDNA-RNA hybridization. cDNA treated with 0.5M NaOH/0.2% SDS was electrophoresed on a 3 mm thick 3% polyacrylamide gel containing 7M urea. The radioactive band representing the largest molecular weight cDNA was cut out, electroeluted from the gel, extracted with phenol and precipitated with cold ethanol twice before being used in hybridization experiments. cDNA-RNA hybrids were formed at 65°C for 20 h and were assayed with 100 units/ml S1 nuclease. The formula used to calculate the percentage S1 nuclease resistance of the homologous and heterologous hybrids was:

$$\text{Corrected percentage S1 nuclease resistance} = 100 \times \frac{\text{S1 nuclease resistance of hybird (\%)} - \text{S1 nuclease resistance of cDNA (\%)}}{100 - \text{S1 nuclease resistance of cDNA (\%)}}$$

This percentage S1 nuclease resistance reflects the sequence homology of the cDNA-RNA hybrids. The hybrids formed between homologous cDNAs and RNAs were 90-110% S1 nuclease resistant. These homologous hybrids were adjusted to 100% and all of the heterologous hybrids were then adjusted accordingly.

The results of the heterologous hybridization experiments (Table 1) reveal that:
(1) the cDNA-RNA hybridization method cannot distinguish between RNAs from the same serotype since the DEN-2 (NGC) probe hybridized to the same extent with the strains T80G and T80H of serotype 2 as it did with its homologous RNA. We have shown previously that RNA from these three isolates of dengue serotype 2 exhibit different ribonuclease T1 fingerprints.
(2) the background of non-homologous RNA (in this case yeast RNA) was between 10 and 17% while the average minimum sequence homology observed for the flavivirus RNAs was 25%.
(3) most of the viral RNAs showed between 25 and 30% sequence homology with DEN-1 and DEN-2 cDNA probes while there were two pairs which exhibited a much greater sequence homology. These were DEN-1 and DEN-4, and DEN-2 (NGC) and Edge Hill. Edge Hill RNA protected the DEN-2 cDNA probe from nuclease digestion to a large extent. The nuclease resistance of a high-molecular weight hybrid was confirmed by electrophoresis of the S1 nuclease-treated hybrids on a polyacrylamide gel followed by autoradiography.
The hybridization experiments described were carried out under stringent conditions (0.18M NaCl during the hybridization reaction and 0.08M during the S1 nuclease assay). The S1 nuclease resistance of the cDNA probe increased 2-fold when hybridization reactions were carried out at 0.56M NaCl and 4-fold when the S1 nuclease assay conditions were raised to 0.56M NaCl. The percentage S1 nuclease resistance obtained in Table 1 therefore represents the lowest percentage of sequence homology for the hybrids formed, since increasing the NaCl concentration will allow more mismatching of basepairing regions to occur in these hybrids.

The flaviviruses are grouped by their reactions in serological tests. Each virus contains antigenic determinants common to the group, others which specify a complex of viruses, and those which determine serotype specificity. The four dengue virus serotypes form a discrete complex of flaviviruses and there is some published evidence of a subcomplex of dengue virus serotypes 1 and 3. The hybridization results suggest a closer relationship between dengue serotypes 1 and 4 (73% homology) and a relationship between dengue 1 and other dengue viruses only slightly greater than that between other flaviviruses tested. There need not be a correlation between serological tests and hybridization since the antigenic determinants are located on the major virus glycoprotein (V3) which is encoded in less than 15% of the virus genome. The closer genetic relationship of types 1 and 4 implies that evolutionary patterns may not be common to all four serotypes.

The hybridization test revealed an unexpected genetic relationship between Edge Hill virus and dengue virus type 2. Edge Hill virus forms part of an antigenic complex distinct from other flavivirus complexes and is not considered closely related to dengue viruses. There are also distinct ecological differences between the viruses. Dengue viruses infect man and are transmitted by *Aedes* spp of the subgenus *Stegomyia*. The vertebrate hosts for Edge Hill virus are marsupials and the virus has been isolated in eastern Australia from *Anopheles amictus, Culex annulirostris* and *Aedes* spp of the subgenus *Ochlerotatus*.

Hybridization of flavivirus RNA with complementary DNA of dengue viruses has demonstrated the presence of poly(A) in the genomes and provided estimates of relationships between complete genomes instead of portions encoding antigenic determinants. The results suggest that such analyses would be valuable in the

study of the evolutionary origins of flaviviruses. (J. Blok and B.M.Gorman).

Table 1

Estimated Percentage Sequence Homology Among Flavivirus RNAs*

| RNA used for cDNA synthesis | RNA used in hybridization reaction | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DEN-1 (Haw) | DEN-2 (NGC) | DEN-2 (T80G) | DEN-2 (T80H) | DEN-3 (H87) | DEN-4 (H241) | Edge Hill | Kunjin | Strat ford | Yeast |
| DEN-1 (Haw) | 100 | 42+ | 36 | 38 | 38 | 73 | 27 | 34 | 26 | 17 |
| DEN-2 (NGC) | 36 | 100 | 106 | 101 | 24 | 23 | 71 | 30 | 36 | 10 |

* cDNA-RNA hybrids were formed and assayed with S1 nuclease and the estimated percentage sequence homology was calculated as described in the text.

\+ each percentage represents an average of 6 experiments.

## REPORT FROM CSIRO LONG POCKET LABORATORIES
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In the course of a study of insects and arboviruses associated with livestock at Beatrice Hill (12° 39'S, 131° 21'E) in the Northern Territory of Australia, eighteen arboviruses were isolated. The viruses were identified at this laboratory or at the Queensland Institute of Medical Research or at the Yale Arbovirus Research Unit. Nine of the viruses recovered in the study have been isolated from both *Culicoides* (Biting midges) and mosquitoes collected in the field. Details of virus, insect species and locality are listed in the table.

Prior to processing for virus isolation, the insects were sorted to species under a dissecting microscope and those insects containing blood discarded. It is unlikely that the isolates originated from an undigested blood meal. Arthropods were collected by means of light traps, truck traps, magoon taps, manitoba traps and animal bait collections. In all cases the traps were operated close to grazing livestock. Frequently, large marsupials (*Macropus agilis agilis*) grazed in company with cattle and water buffalo (*Bubalus bubalis*). The insects listed in the table fed mainly at dusk and dawn and were frequently observed feeding on the same host animals at the same time.

The viruses listed in the table have been shown to infect cattle or marsupials or both (Doherty 1972, 1977), while the arthropods listed are known to feed readily on a range of hosts including cattle, buffalo and marsupials (Muller *et al*., 1981). Thus the conditions under which collections were made and the procedures followed in the project were more likely to reveal broad host ranges of viruses than studies concentrating on one group of insects or vertebrates.

The isolation of the same virus from two different families of haematophagous diptera is not an extraordinary event. There is no more reason to expect a virus to be confined to one insect family than there is to expect it to be restricted to one vertebrate family. Viruses should not be categorised as "mosquito viruses" or "*Culicoides* viruses" on the evidence of field collections alone, as in many instances these are restricted to one or other of the insect groups or to insects attacking one species of vertebrate. In some cases the apparent narrow host range of the virus is a reflection of inadequate sampling or the specialization of the collector.

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(T.D. St.George, H.A. Standfast, A.L. Dyce, M.J. Muller and D.H. Cybinski).

**ARBOVIRUSES RECORDED AT BEATRICE HILL, NORTHERN TERRITORY, AUSTRALIA WHICH HAVE BEEN ISOLATED FROM MORE THAN ONE FAMILY OF DIPTERA**

| Serogroup | Virus | *Culicoides* spp. | Locality | Mosquito spp. | Locality |
|---|---|---|---|---|---|
| *Bunyavirus* | | | | | |
| | Murweh | *C. marksi* | Beatrice Hill, NT* | *Cx annulirostris* | Charleville, Qld. (3) |
| | | | | *Ae normanensis* | Charleville, Qld. (3) |
| | Belmont | *C. marksi* | Beatrice Hill, NT* | *Cx annulirostris* | Rockhampton, Qld. (2) |
| Simbu | Faceys Paddock | Mixed spp. | Beatrice Hill, NT* | *Cx annulirostris* | Charleville, Qld. (3) |
| *Rhadbovirus* | | | | | |
| | Bovine ephemeral fever | Mixed spp. | Kenya (1) | *An bancroftii* | Beatrice Hill, NT. (4) |
| | | | | Mixed spp. | Rockhampton, Qld. (4) |
| *Orbivirus* | | | | | |
| | Eubenangee | *C. marksi* | Beatrice Hill, NT* | *Cx annulirostris* | Beatrice Hill, NT.* |
| | | | | Mixed spp. | Innisfail, Qld. (5) |
| | Warrego | *C. marksi* | Beatrice Hill, NT* | *An meraukensis* | Kowanyama, Qld. (3) |
| | | *C. marksi* | Charleville, Qld(2) | *Cx annulirostris* | Charleville, Qld. (2) |
| | | *C. dycei* | Charleville, Qld(2) | | |
| Ungrouped | | | | | |
| | Leanyer | *C. marksi* | Beatrice Hill, NT* | *An meraukensis* | Darwin, NT. (6) |
| | Parkers Farm | *C. marksi* | Beatrice Hill, NT* | *Cx annulirostris* | Charleville, Qld. (3) |
| | Wongorr | *C. pallidothorax* | Beatrice Hill, NT* | *Cx annulirostris* | Beatrice Hill, NT.* |
| | | | | *Ae lineatopennis* | Kowanyama, Qld. (2) |
| | | | | *Cx annulirostris* | Kowanyama, Qld. (3) |

* Beatrice Hill study in preparation.


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Report from the Institute of Virology, Chinese Academy of Medical Sciences, Beijing, 100052, China

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Studies of Monoclonal Antibodies Specific for Epidemic Hemorrhagic Fever

Two monoclonal antibodies against Epidemic Hemorrhagic Fever (EHF) virus were obtained by fusion of SP2/o myoloma cells with spleen cells from BALB/C mice immunized with inactivated $A_9$ strain of EHF virus isolated from *Apodemus agrarius* treated by ultraviolet radiation.

The titer of monoclonal antibody against EHF virus was around 1/5120-1/81920 when tested by indirect fluorescent antibody technique.

Monoclonal antibody "25" showed the best neutralizing antibody with a neutralizing index up to 2.5 $Log_{10}$. It is interesting to find that monoclonal antibody "25" and "32" can differentiate the classical EHF virus ($A_9$ strain) isolated from *Apodemus agrarius* and virus of the mild form of EHF virus ($R_{27}$ strain) isolated from *R. Norvegicus*.

We succeeded to conjugate monoclonal antibody with fluorescein isothiocyanate isomer and made direct immunofluorescent test of EHF antigen. The titer of monoclonal antibody conjugated is 1/16384, but none with Arbovirus, Poliovirus type III and Reovirus type I, II, III. Obvious blocking effect of EHF antiserum to immunofluorescence elicited by 25-I monoclonal antibody was observed.

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## EPIDEMIC JAPANESE ENCEPHALITIS IN KAMPANGPHET PROVINCE, THAILAND.

FROM THE DEPARTMENT OF VIRUS DISEASES, ARMED FORCES RESEARCH INSTITUTE OF MEDICAL SCIENCES, BANGKOK, AND KAMPANGPHET PROVINCIAL HOSPITAL, KAMPANGPHET, THAILAND.

For the third consecutive year, (1981, 82, and 83) we conducted epidemiological and clinical studies of Japanese encephalitis in Kampangphet Province, Thailand. During the three years of the study, 40, 41, and 66 cases occurred, respectively, marking 1983 as a year of particularly intense JE activity in that province. The 1983 epidemic began abruptly in mid June and rose to a sharp peak during the week of 13-19 July. In that week alone, 14 encephalitis cases, of which 11 were confirmed as due to JEV, were admitted to the hospital requiring the dedication of special hospital ward exclusively for the care of encephalitis patients. By the third week in August, admissions due to encephalitis were decreasing.

Of the 66 cases in 1983, Japanese encephalitis was established as the etiology of 49 by detection of IgM anti-JE antibody in the admission cerebrospinal fluid with a JE MAC ELISA test (1). Twelve confirmed cases were fatal, for a case fatality ratio of 24%. Post-mortem brain biopsies or autopsies were performed in 10 of the 18 fatal encephalitis cases, and JE virus was isolated from brain tissue in at least 8 of these cases. Cultures of CSF, performed by inoculating AP-61 (Aedes pseudoscutellaris) cells at the bedside, yielded JE virus in 5 out of 47 attempts from confirmed cases.

There are approximately 560,000 people in Kampangphet Province, thus the attack rate for JE was 7 cases per 100,000 population. Age specific attack rates were highest in 6-10 (22/100,000) and 1-5 year olds (21/100,000). Within the province, the geographic distribution of the 1983 cases extended approximately 50km further south cases in 1981 or 1982, suggesting some southward movement of the than it did for disease.

We conclude that:

1) Japanese encephalitis epidemics continue to reccur on an annual basis in Thailand.
2) 82% of cases of encephalitis were confirmed as due to JEV.
3) Age specific attack rates are such that any immunization programs for JE must be targeted at pre-school age, as well as school age children.
4) The diagnosis of JEV encephalitis can be confirmed by post-mortem needle brain biopsy in a high percent of fatal cases.
5) The technique of bedside inoculation of CSF into AP-61 cells yields a virus isolate in a surprising percentage of cases.

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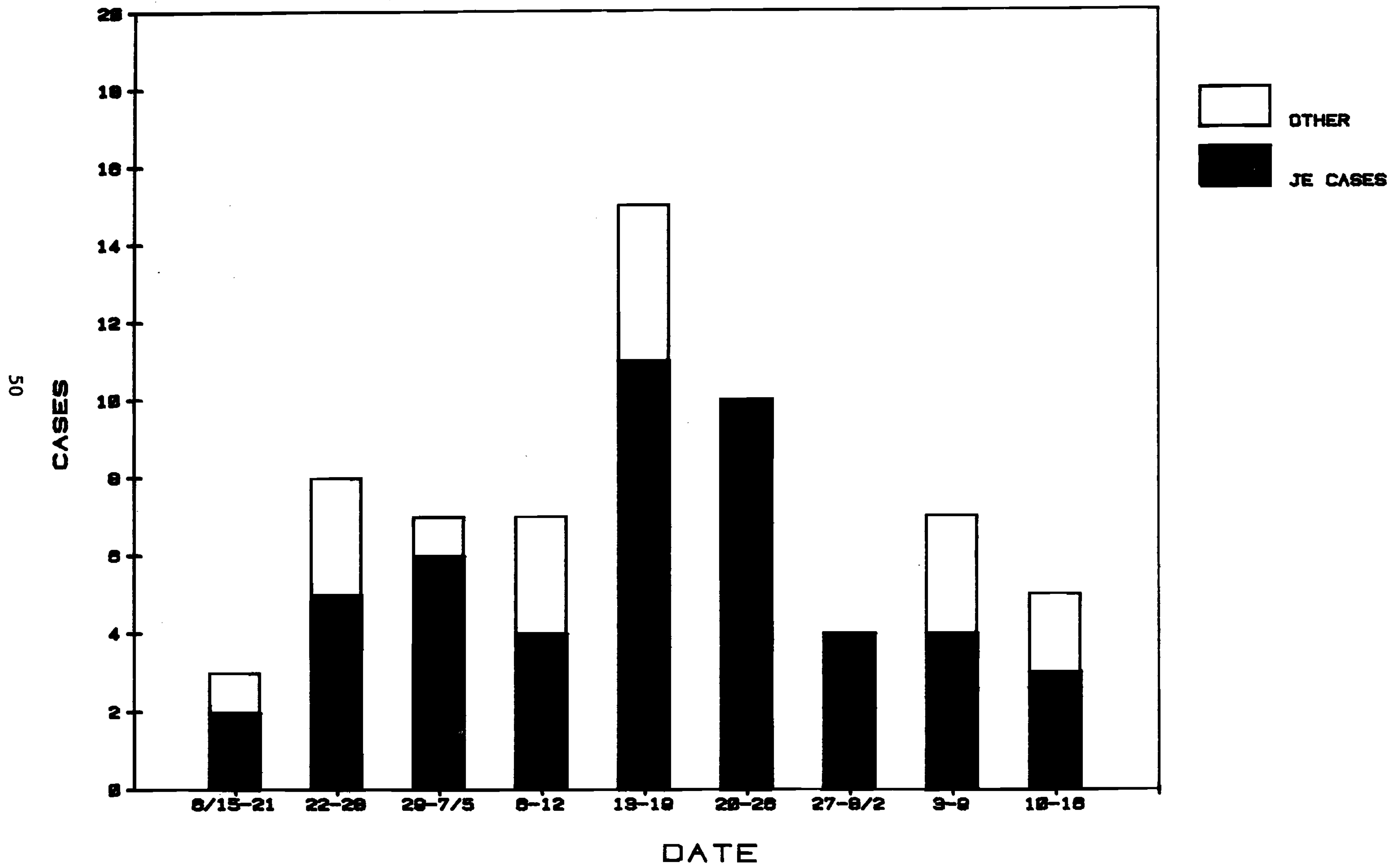
(CHARLES H. HOKE, JR., COLIN J. LEAKE, WANIDA LORSOMRUDEE, ANANDA NISALAK, THANOM LAORAKPONGSE AND DONALD S. BURKE)

# ENCEPHALITIS EPIDEMIC
# KAMPANGPHET, THAILAND
# JUNE 15 – AUGUST 15, 1983



REPORT FROM NATIONAL INSTITUTE OF VIROLOGY, PUNE, INDIA.

## TRANSOVARIAL TRANSMISSION OF JAPANESE ENCEPHALITIS VIRUS IN *AEDES AEGYPTI*

Transovarial transmission (TOT) of Japanese encephalitis (JE) virus has recently been demonstrated in *Aedes albopictus*, *A. togoi* and *Culex tritaeniorhynchus* mosquitoes[1,2]. Evidence of TOT in *Aedes aegypti* which is one of the suspected vectors of JE virus in some areas[3], has not been reported so far. Therefore, experiments were carried out to determine the capability of this mosquito for TOT of JE virus.

Mosquitoes used in the present study were obtained from the laboratory colony being maintained at the NIV, Pune. The JE virus strain (P-20778) used in this study was originally isolated from human brain from an autopsied fatal case of JE at Vellore, Tamil Nadu, India in 1958 which had undergone 13 passages in infant mice. One hundred and fifty *Aedes aegypti* females were infected with JE virus by intrathoracic inoculation. They were fed on 1 to 2 days old normal chickens on 5th and 10th post-inoculation days. The viraemia was detected in chickens on 2nd and 3rd post feeding days. The eggs laid by the mosquitoes were collected after each blood meal. The heads of all surviving mosquitoes were found positive for the virus by indirect immunofluorescence test (IFT) when tested on 21st post inoculation day. The eggs were conditioned at 28° to 30°C for 36 to 48 hours at 80% to 90% relative humidity (R.H.) and reared to adults at the same temperature and R.H. Four to five days after emergence, the females were fed on one week old normal chickens.

From the progeny of the first gonotropic cycle, 5 pools consisting of 466 females after 5 to 6 days of the blood meal and 9 pools consisting of 833 males were inoculated intrathoracially in *Aedes aegypti* females. The head squashes of the inoculated mosquitoes were tested for virus by IFT on 10th PI day. Likewise, from the progeny of the second gonotropic cycle, 5 pools consisting of 479 females and 4 pools consisting of 360 males were tested.

JE virus was detected in only two pools of male mosquitoes from the progeny of the first gonotropic cycle, indicating transovarial transmission of the virus.

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(M. A. ILKAL, D. T. MOURYA, M. S. MAVALE and V. DHANDA)

REPORT FROM NATIONAL INSTITUTE OF VIROLOGY, PUNE, INDIA

## SUSCEPTIBILITY OF DELHI STRAIN AND LABORATORY ADAPTED STRAIN OF AEDES AEGYPTI TO DENGUE-1 VIRUS STRAINS

Delhi is known to be endemic for dengue viruses. The city experienced extensive dengue epidemics in 1967, 1970 and 1982. During the present study Delhi strain of *Aedes aegypti* collected during the 1982 epidemic was tested for its susceptibility to dengue 1 virus strains and a comparison made with the mosquitoes obtained from the established laboratory colony. The Delhi strain of mosquitoes used in the study consisted only of $F_1$ generation adults obtained from the field collected larvae. The established laboratory colony of *Aedes aegypti* used for comparison, is being maintained at this Institute since 1960 from material collected in Vellore in Tamil Nadu state. The viruses used were: (1) Dengue 1, Delhi strain, isolated from acute-phase serum collected from a febrile patient during the 1982 epidemic, isolated in *Aedes aegypti* mosquitoes by intrathoracic (IT) inoculation. (2) Laboratory adapted strain of dengue 1 virus (Hawaian strain) which had undergone more than 90 mouse brain passages. The virus pools for both the strains were prepared in infant mouse brain after intracerebral (IC) inoculation.

The mosquitoes were infected by feeding through a chicken skin membrane on defibrinated chicken blood mixed with virus suspension. Soon after feeding, the blood-virus mixture was titrated in infant mouse by IC inoculation as well as in *Aedes aegypti* mosquitoes by IT inoculation. The titres of the Delhi strain of virus varied from 3.7 to 5.1 dex LD50/0.02 ml in infant mice and 4.1 to 5.2 dex ID50/0.2 µl in mosquitoes. The titres of the laboratory adapted strain varied from 5.2 to 5.7 dex in infant mice and from 5.2 to 5.6 dex in mosquitoes.

From the results presented in Table 1, it appears that both the strains of mosquitoes were more susceptible to the Delhi strain of virus than to the laboratory adapted strain. Among the two strains of mosquitoes, the Delhi strain appears more susceptible than the laboratory adapted strain to the Delhi strain of virus. On the contrary, with the laboratory adapted strain of virus, the laboratory colony mosquitoes appear to be more susceptible than the Delhi strain of mosquitoes.

Table-1. Susceptibility of Delhi strain and laboratory adapted strain of *Aedes aegypti* to dengue 1 virus strains.

| Dengue 1 strain | *Aedes aegypti* strain: Delhi | *Aedes aegypti* strain: Laboratory colony |
|---|---|---|
| Delhi strain | 20/75 (27%) | 11/92* (12%) |
| Laboratory adapted | 0/39 ( 0%) | 3/90 ( 3%) |

* Number positive/Number tested. (Figures in parenthesis show the percentages of positive mosquitoes.)

( V. DHANDA, M. A. ILKAL, D. T. MOURYA AND M. S. MAVALE )

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

A microtiter serum neutralization test with tick-borne encephalitis, West Nile and Tahyna viruses

We developed a rapid method for microculture titration and microculture neutralization tests with some flaviviruses (tick-borne encephalitis and West Nile) and with bunyavirus (Tahyna). The susceptibility of a small-cellular clone, derived from "PS" line of porcine kidney epithelial cells for the study of group B arboviruses was already described. Therefore, these cells were used in microtitration with some flaviviruses and bunyavirus isolated in Czechoslovakia.

Viruses: All stock viruses were prepared in suckling mice. Infected brains were removed before death, prepared as 10% suspensions in Eagle's basal essential medium (BEM), clarified at 10,000 rpm and stored at $-70^{o}$ C.

Antisera: West Nile and Tahyna antisera were prepared in mice and TBE antiserum in goats.

Tissue cultures: PS cells, small cellular clone, derived from PS line of porcine kidney epithelial cells were used in all experiments. PS cells were cultured in BEM with 10% heat inactivated foetal calf serum (FCS) and antibodies.

Virus and antibody assays: Titrations were performed in sterile disposable flat-bottomed micro-tissue-cultures plates. Each well was seeded with $1 \times 10^5$ PS cells in 0.1 ml of BEM with 10% heat inactivated FCS. The plates were incubated for 24 hrs at $+37^{o}$ C in $CO_2$ atmosphere. Then the medium was removed and the cells were infected with 0.1 ml of TBE, WN, Tahyna viruses, respectively.

The antiserum potency was determined by virus-neutralization tests. The individual dilutions of inactivated antisera were mixed with homologous viruses, incubated for 18 hrs at $+4^{o}$ C and the mixtures were added to all monolayers. The plates were incubated at $37^{o}$ C for 3-4 days until 100% CPE was observed. The cell monolayers were fixed with 4% formaldehyde and then stained with 0.1 ml of 0.5% crystal violet for 1 minute. After the crystal violet was decanted, the plates were rinsed with water and dried in air. The 50% endpoints were determined by visual examination of monolayer with and without the aid of microscope.

The cytopathic titres of TBE virus could be observed on 3rd-4th days p.i.; $CPD_{50}$ reached the value from $10^7$ $CPD_{50}$/ml to $10^{10}$ $CPD_{50}$/ml.

The cytopathic activity of West Nile virus reached the value of $10^5$ $CPD_{50}$/ml on the third day p.i..

The Tahyna virus attained cytopathic endpoints on the 4th day p.i.; the cytopathic activity reached the value of $10^6$ $CPD_{50}$ per ml.

The distinctness of the cytopathic activity allows the use of microtiter serum neutralization (N) tests.

No attempts were made so far to test tick-borne encephalitis, West Nile and Tahyna viruses in microtitration test based on the reduction of cytopathic effect. Our method was specific, reproducible and sparing of reagents and therefore it could be recommended for routine laboratory arbovirus work.

(M. Gresikova, O. Kozuch)

REPORT FROM THE ARBOVIRUS DEPARTMENT,
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D.I.IVANOVSKY INSTITUTE OF VIROLOGY,WHO COLLABORATING
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## SANDFLY FEVER IN THE REPUBLIC BANGLADESH

Little information is available on the distribution of phleboviruses in the Republic Bangladesh.In this study serological survey for some phleboviruses was carried out.Blood samples were collected from febrile patients admitted to one of Dacca hospitals on filter paper discs. The sera were tested against SFS,SFN and Karimabad viruses by HI,inhibition of revers passive hemagglutination (IRPH) and radial hemolisis (RG) tests.

Sucrose acetone antigens from suckling mouse brain were used for all tests. Diagnosticum for IRPH test were prepared from formalinazed and tannic acid treated sheep erythrocytes with immune gammaglobulin to corresponding viruses (see S.Ya. Gaidamovich,G.A.Klisenko,N.K.Shanoyan,Am.J.trop.med.hyg.,1974, v.23,p.526-529).The RG procedure was the same as discribed previously for togaviruses (S.Y.Gaidamovich,E.E.Melnikova,Intervirology,1979,v.13,p.16-20). All sera were screened at dilution 1:10 in IRPH and only positive samples were titrated.

From 160 sera tested in IRPH antibodies were found in 18 to Karimabad virus, in 10 and 2 samples to SFS and SFN viruses correspondently.

The results of this study provides new information on the geographical distribution of Karimabad virus. Previous serological studies (R.Tesh et all,1976) revealed antibodies to SFS and SFN viruses in population of Bangladesh. The occurence of antibodies to Karimabad virus was shown for the first time.

The comparison of sensitivity of three tests used for the detection of antibodies presumed the preference of IRPH. As it is shown on the table,18 samples were positive by IRPH,15 by HI and 13 by RG. The number of positive sera by three tests became 24. RG was positive when the IRPH titre was 1:40 or higher In spite of less sensitivity RG may be recommended for serological investigation with Karimabad virus due to its simplisity and possibility to process hundreds of samples during short period of time

(S.Y.Gaidamovich,M.A.Baten)

The titres of antibodies to Karimabad virus in IRPH,RG and HI.

| № of serum | methods | | | № of serum | methods | | |
|---|---|---|---|---|---|---|---|
| | IRPH | RG | HI | | IRPH | RG | HI |
| 81 | 0 | 7 | 20 | 133 | 10 | 0 | 0 |
| 89 | 80 | 9 | 40 | 138 | 0 | 0 | 40 |
| 93 | 40 | 11 | 80 | 140 | 80 | 7 | 80 |
| 94 | 40 | 7 | 0 | 141 | 40 | 7 | 0 |
| 98 | 0 | 0 | 20 | 143 | 10 | 0 | 0 |
| 101 | 80 | 7 | 20 | 147 | 20 | 0 | 0 |
| 102 | 20 | 0 | 0 | 148 | 80 | 7 | 20 |
| 103 | 0 | 0 | 10 | 152 | 10 | 7 | 20 |
| 115 | 0 | 0 | 10 | 153 | 10 | 0 | 0 |
| 122 | 0 | 12 | 80 | 156 | 80 | 7 | 40 |
| 131 | 80 | 7 | 20 | 96 | 80 | 7 | 20 |
| 132 | 20 | 0 | 0 | 142 | 10 | 0 | 0 |

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

# *Visualization of Soldado virus by TEM in the tissues of a naturally infected* Ornithodoros (Alectorobius) maritimus *tick*

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Different virus-like particles were previously described in the salivary glands or other tissues of infected ticks by means of transmission electron microscopy (TEM) [Nosek *et al.*, 1972 - Sixl-Vogt *et al.*, 1973 - Ciampor and Nosek, 1976 - Megaw, 1978 - Diehl *et al.*, 1980].

When the ticks were experimentally allowed to engorge on viremic mice inoculated with a known arbovirus such as TBE virus [Ciampor and Nosek, 1976], there was no difficulty to identify the virus-like particles. But the actual classification of certain particles accidentally found by TEM in naturally infected ticks was frequently difficult to establish.

By means of TEM, we were able to visualize typical virus particles of Soldado virus (Bunyaviridae, *Nairovirus*) in the tissues of a *Ornithodoros (Alectorobius) maritimus* tick naturally infected by this virus.

## *MATERIAL AND METHODS*

During June 1981, many specimens of *O. (A.) maritimus* ticks were collected at "Belle-Ile-en-Mer", South-Brittany, France, in the nests of gulls and shags *(Larus argentatus* Pontoppidan, *Larus marinus* L. and *Phalacrocorax aristotelis)*.

Some of the specimens were pooled by stage and sex, made suitable for and inoculated in suckling mice for virus isolation, while other specimens were preserved in insectarium (25°C, relative humidity 75%) for six months.

The specimens tested for virus were found heavily infected by Soldado virus: 18 positive from 39 inoculated pools or 46 per cent.

So there was a high probability that each preserved individual specimen would be infected. Four ticks (3 ♀ + 1 ♂) from insectarium were then selected for a simultaneous virological and ultrastructural study.

The ticks were dissected, their salivary glands were removed and prepared for TEM: post-fixation in buffered osmium tetroxide, dehydration in an ethanol series and embedding in an Araldite-Epon mixture. Sections were cut using a LKB ultrotome III with diamond and they were stained with uranyl acetate and lead citrate. Grids were examined and photographed in a Zeiss EM9-S microscope at 60 kv.

The remaining tissues of each tick were triturated in a chilled mortar in such a way used for virus isolation attemps from tick pools. Suspensions of tissues were clarified and inoculated in suckling mice by IC route.

*RESULTS*

Only one tick suspension (T772) corresponding to a ♀ was positive for virus and the isolate was identified by CF tests as a "Old World" variant of Soldado virus [Chastel *et al.*, 1982-1983]. The three other suspensions were negative.

By TEM examination, clusters of virus particles were found only in T722 tissues and these particles were found typical for *Bunyaviridae* family and identical in morphology to those previously described for Soldado virus infesting suckling mice brains [Chastel *et al.*, 1979 - Chastel and Le Lay-Roguès, 1980].

Clusters of virus were unfrequent and found in pseudo-vacuoles located in an amorphous material bordering the plasma membrane of unidentified cells, possibly belonging to a type II salivary gland acinus (Figure 1).

Virus particles were easily identified. They were round, pleomorphic, with a mean diameter of 102 nm (88 to 120 nm) and limited by a trilamellar envelope without obvious "spikes". The internal density of particles was reinforced beneath the envelope. These characters are currently found in *Bunyaviridae* family virions [Murphy *et al.*, 1968]. However, typical maturation processes by budding through the Golgi apparatus membranes were not seen in tick tissues.

So the demonstration in the same tick of the virulence of tissues and the presence of virus particles typical for *Bunyaviridae* family made very probable these particles represent in fact the Soldado virions *in situ*.

The main difficulty encountered during this study was in identifying the actual cells (salivary or not) supporting the Soldado virus replication. At time, electron microscopic documents concerning *Argasidae* tissues are very scarce [Roshdy and Coons, 1975 - El Shoura, 1979 - Coons and Roshy, 1981], particulary for *Ornithodoros* genus.

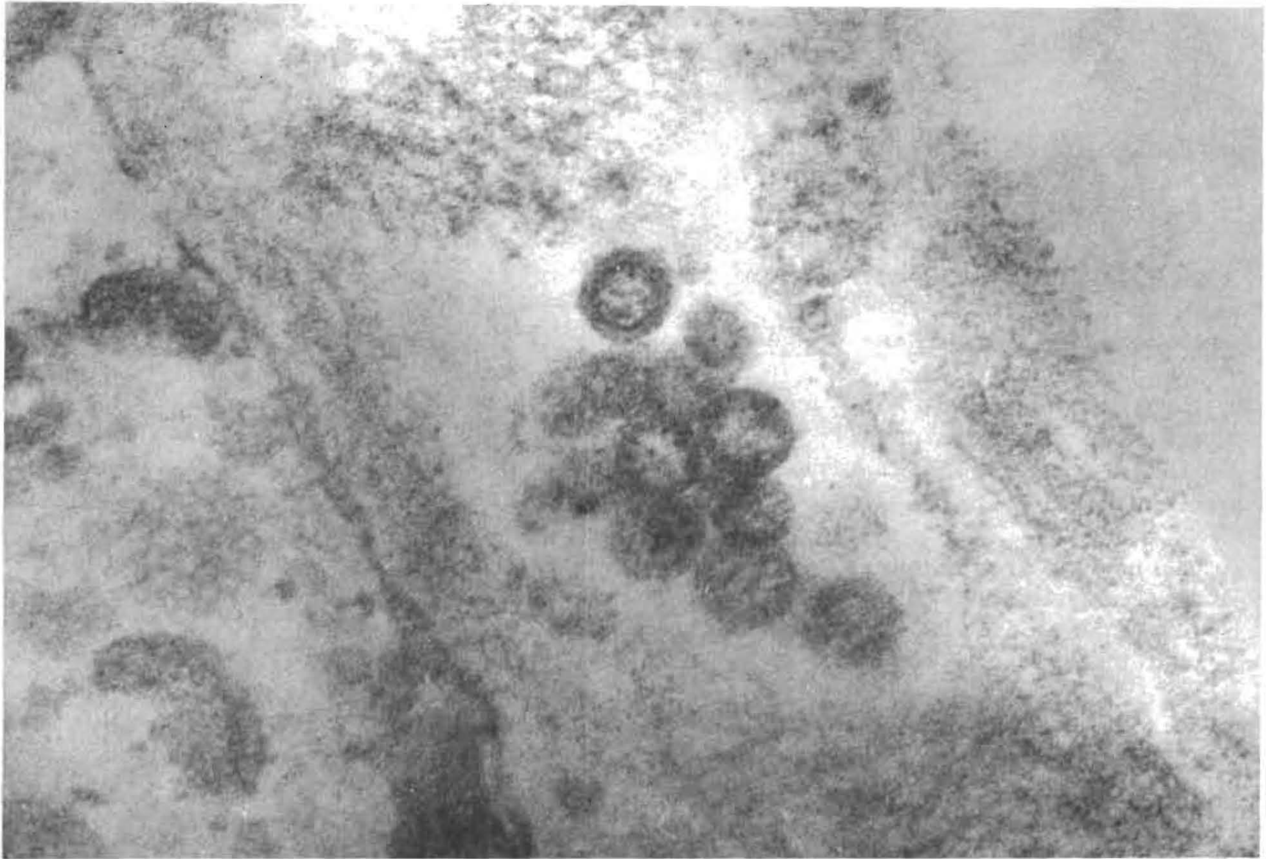

*Figure 1.* Ornithodoros (Alectorobius) maritimus *tissues naturally infected by Soldado virus. Clusters of virus particles with typical characters of* Bunyaviridae *family. Note the pleomorphism of enveloped particles* (120 000 x).

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To be published in details In "Annales de Parasitologie Humaine et Comparée", Paris, 1983.

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Report from the Arbovirus Section
Laboratory of Virology
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## ISOLATION OF AN AGENT ANTIGENICALLY RELATED TO THOGOTO VIRUS IN PORTUGAL

In June and July, 1978, 79 ticks were collected at the slaughterhouse of Lisbon, from goats arriving from the Vila Vicosa region, approximately 150 km east of Lisbon (Lat. 38' 47' N; Long. 7' 25' W). The ticks were identified as *Rhipicephalus sanguineus sanguineus* (Latreille, 1806).

Pools of ticks were processed as usual for arbovirus isolation. From these ticks, two agents (PO Ti 503 and PO Ti 509), virulent for mice, were isolated. One was from ticks collected June 6, 1978, and the other from ticks collected July 4, 1978.

After several passages, these agents killed suckling mice 3 to 4 days after intracerebral inoculation. These viruses were then compared with the other viruses in the reference collection of our laboratory, but no antigenic relationships were found.

In 1982, strains PO Ti 503 and PO Ti 509 were sent to Dr. Charles H. Calisher, Division of Vector-Borne Viral Diseases, Centers for Disease Control, Ft. Collins, Colorado, U.S.A., where they were compared with more than 200 other arboviruses. These strains were found to be isolates of the same virus, and both are closely related to Thogoto virus, an *Orthomyxovirus*. Prior to obtaining these isolates, we did not have this virus in our collection of arboviruses in Lisbon.

It is interesting to note that another orthomyxovirus, Dhori virus, was isolated in Portugal from *Hyalomma marginatum* ticks collected in 1971 in the Vidigueira region, about 70 km south of Vila Vicosa.

We are indebted to Dr. Charles H. Calisher, CDC, Ft. Collins, for his cooperation in identifying these strains. Further comparative studies of Thogoto virus strains from a variety of geographical areas are being carried out in collaboration between this laboratory and CDC, Ft. Collins.

(Armindo R. Filipe)

REPORT FROM THE NERC INSTITUTE OF VIROLOGY, MANSFIELD ROAD, OXFORD, OX1 3SR, UNITED KINGDOM

## VIRUSES ISOLATED FROM SEABIRD TICKS

Two viruses were isolated from three female Ixodes uriae ticks found on a dead puffin (Fratercula arctica) on a beach at Arbroath, Scotland. The viruses were isolated by inoculating the tick pool homogenate into chick embryo fibroblast and liver cell cultures. The isolated viruses were separated by plaque purification in African Green Monkey (virus 1) and Xenopus laevis cell cultures (virus 2). Only virus 1 showed evidence of replication in mice, and this only occurred after passage of the virus through cell cultures.

The viruses were characterised using electron microscopy, serological and physicochemical tests. In complement fixation tests positive reactions were observed with the Kemerovo serogroup of orbiviruses. There were no positive results with any other virus groups (see Table). In neutralization tests no positive results were obtained with Kemerovo group viruses isolated at the Institute of Virology (see Table). Positive results were obtained with a bunyavirus of the Uukuniemi serogroup isolated from St Abbs Head, Scotland (see Table).

The results indicate that virus 1 is a member of the Kemerovo serogroup although it appears to be of a different serotype to other Kemerovo group viruses isolated at the Institute of Virology. Virus 2 can be placed in the Uukuniemi serogroup of the Uukuvirus genus of the family Bunyaviridae and is of the same serotype as a virus isolated from St Abbs Head, Scotland.

(S.R. Moss, P.A. Nuttall)

Results of complement fixation and neutralization tests

| Immune ascitic fluid (serogroup) | Complement fixation titre of immune ascitic fluid, against uncloned antigen | Neutralization titre of immune ascitic fluid with | |
|---|---|---|---|
| | | Virus 1 | Virus 2 |
| Uncloned Arbroath | 1/64 | >1/256 < 1/512 | <1/16 |
| Arbroath 1 | 1/64 | >1/256 < 1/512 | <1/16 |
| Arbroath 2 | <1/8 | <1/16 | 1/64 |
| St Abbs FT363 (KEM) | 1/32 | <1/8 | ND |
| Shiant Islands (KEM) | 1/16 | <1/8 | ND |
| Farne Islands (KEM) | 1/64 | <1/8 | ND |
| Cape Wrath (KEM) | 1/32 | ND | ND |
| Kemerovo | 1/16 | ND | ND |
| St Abbs M349 (UUK) | <1/8 | ND | >1/64 < 1/128 |
| Clo Mor (SAK) | <1/8 | ND | ND |
| Avalon (SAK) | <1/8 | ND | ND |
| Hughes group | <1/8 | ND | ND |
| Gt. Saltee Island (GS80-3) (HUG) | <1/8 | ND | ND |

The antigen used in complement fixation tests was prepared from infected mouse brains and used at a dilution of 1:8.

KEM - Kemerovo
UUK - Uukuniemi
SAK - Sakhalin
HUG - Hughes
ND - Not done

REPORT FROM THE PASTEUR INSTITUTE, DEPARTMENT OF

VIROLOGY, DAKAR - SENEGAL

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## COMPARATIVE BIOCHEMICAL ANALYSES OF YELLOW FEVER VIRUSES ISOLATED FROM DIFFERENT GEOGRAPHIC REGIONS IN SENEGAL SINCE 1976.

A series of 14 yellow fever virus (YFV) isolated from mosquitoes, monkey and humans in different epidemiological contexts (figure 1) were analysed by RNase T1 oligonucleotide fingerprints of the genomic $^{32}P$ labeled RNA, by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of the intracellular virus-specified polypeptides and by peptide mapping of the envelope protein E54. These strains had no passage on suckling mouse, one to two passages on *Aedes aegypti* mosquitoes and 7 passages on *Aedes aegypti* C17 cultured cells.

Table 1 summarized the differences recorded in the long oligonucleotides of each virus RNA and corresponding to about 10 % of the genome. The first unique strain isolated from *Aedes furcifer taylori* in Kedougou in 1976 (ARD 24553) was taken as a reference. The results suggested a 94 to 98 % similarity (without the PM 27078 strain which shared only 86 % similarity) in the long oligonucleotides with the reference genome, indicating that these viruses were quite similar to the PM 24553 and to each other. No correlation can be made either between the severity of infection in man and the genetic study of the virus, or between the strain isolated from wild or urban mosquitoes.

In order to show whether a heterogeneity existed in the envelope structural protein from these different isolates, it was isolated from purified $^{35}S$ methionine labeled virions and subjected to SDS-PAGE : the electrophoretic profiles of envelope protein E showed identical mobility.

This protein was then subjected to peptide mapping by limited proteolysis using either V8 protease from *S. aureus* or α chymotrypsin and the fragments separated by electrophoresis on SDS-PAGE. All the virus studies produced identical V8 or α chymotrypsin maps.

A study of the intracellular virus-specified polypeptides of the YF viruses was performed by SDS-PAGE. These proteins were isolated from $^{35}S$ labeled cell lysates by immunoprecipitation using hyperimmune ascitic fluids as antibodies to YF virus. Ten proteins could be easily identified without any background and showed identical profiles for each virus.

If the shift of the YF virus can be explained from Kedougou to the West by the receptivity of the population and the abundance of monkeys and mosquitoes, its translation to the North was probably due to the migration of monkeys. One case of seroconversion was enregistred in Bandia (figure 1) suggesting that the virus passed through in this area. The low amount of monkeys and mosquitoes in savana permitted only a low-noise circulation of the virus. The outbreak occuring in Meckhe in 1981 has been due to a receptivity of non vaccinated children.
At this time, no correlation can be made between genetic or biochemical markers and phenotypic changes, but we can say that the evolutionary changes in the primary nucleotide sequence of the YFV genome is very low in a limited time interval and in a specific area, suggesting that the YF virus is very stable.
The use of monoclonal antibodies may be necessary to follow antigenic variations among these wild strains.

(V. DEUBEL, J.P. PAILLEZ, M. CORNET and J.P. DIGOUTTE).

Table 1

| Area | N° | Year | Origin | Homology with the reference strain | Missing oligonucleotides | New oligonucleo tides. |
|---|---|---|---|---|---|---|
| KEDOUGOU | PM 24553 | 1976 | Ae. furc. tayl. | – | – | – |
| | PM 25697 | 1977 | Ae. furc. tayl. | 49/50 (98 %) | 19 | – |
| | PM 25112 | 1977 | Ae. luteocephalus | 47/50 (94 %) | 8, 22 | a |
| | PM 27340 | 1978 | Ae. furc. tayl. | 48/50 (96 %) | 8 | a |
| | PM 27078 | 1978 | Ae. neafricanus | 43/50 (86 %) | 9, 21, 46 | b, c, d, e |
| | RV 26923 | 1978 | Erythrocebus patas | 49/50 (98 %) | 37 | – |
| GAMBIE | PM 27797 | 1/1979 | Ae. aegypti | 47/50 (94 %) | 16, 37 | f |
| | 79 H 327 | 1/1979 | Human | 49/50 (98 %) | – | g |
| SINE SALOUM | Pa H 791 | 10/1979 | Human | 48/50 (96 %) | 37 | g |
| | SH 29174 | 11/1979 | Human | 47/50 (94 %) | 37 | g,h |
| GAMBIE | SH 33880 | 10/1981 | Human | 47/50 (94 %) | 22, 37 | f |
| MEKHE | PM 34410 | 10/1981 | Ae. furc. | 48/50 (96 %) | 22 | i |
| | SH 33812 | 10/1981 | Human | 48/50 (96 %) | 22 | i |
| | SH 33836 | 10/1981 | Human | 48/50 (98 %) | 22 | i |

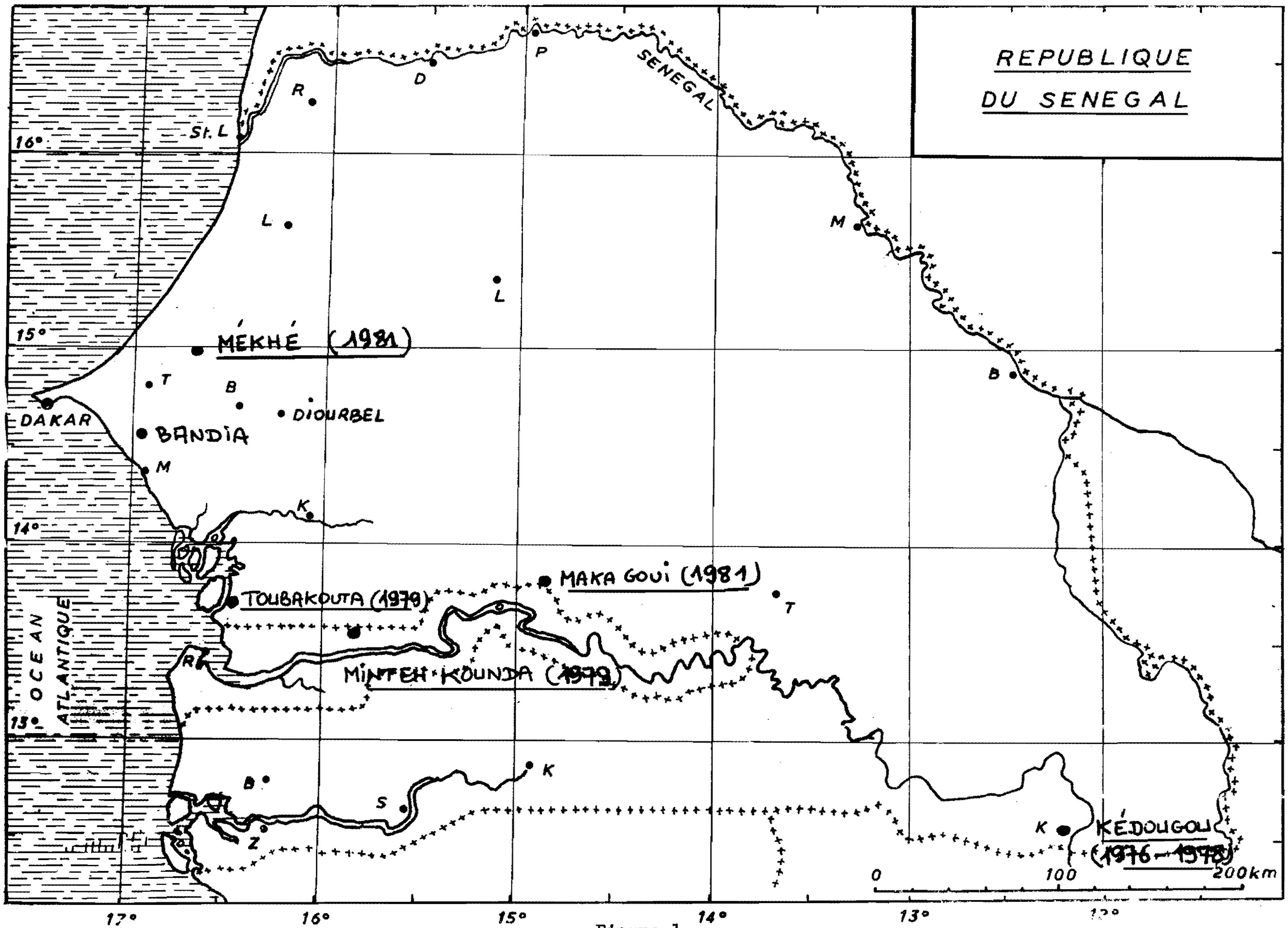


Figure 1

REPORT FROM THE ARBOVIRUSES LABORATORY

INSTITUT PASTEUR & O.R.S.T.O.M.
01 B.P. 490
ABIDJAN - IVORY COAST

## ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF SPECIFIC IgM IN YELLOW FEVER DIAGNOSIS AND SURVEILLANCE

During a yellow fever rural epidemic, we had the opportunity to isolate 27 human strains and to follow the titer of the IgM antibodies in these cases.

The ELISA technique is conducted in polystyrene microtiter plates treated with $\gamma$ rays. Wells are first coated with goat IgG anti-human $\mu$ chain. Following steps of the ELISA test are realised by successive additions of the reagents alternating with triple plate washing :

- patients sera dilutions for immunological capture of IgM antibodies,
- yellow fever antigene purified by CHROM-ELISA[1] technique as described previously (A.-B.V. Information Exchange n°42)
- mouse anti-yellow fever ascitic fluid,
- sheep anti-mouse IgG labeled with horse-radish peroxidase.

The chromogene substrate used to reveal the positive reaction is ortho-Tolidine in presence of hydrogene peroxide. Blue coloration enables a first reading with the naked eye. The reaction is stopped after 10 minutes by adding 4N sulfuric acid. A second reading is done with a spectrophotometer at 450 nm.

ELISA titer represents the reciprocal value of the highest serum dilution which gives an absorbance at least twice the background of negative sera.

Kinetics has shown that in all cases anti-yellow fever IgM were detectable at the latest 8 days after viremia. Three subjects even had IgM while viremic. We had evidence of the decrease in IgM titers after 30 and 60 days.

During the icteric phase, this method is also very useful as we found 16 out of 18 icteric subjects with specific IgM at their first blood sampling (Table 1).

In 1982, 121 yellow fever cases were accounted in Ivory Coast. Diagnosis was confirmed 120 times by IgM evidence, 28 times by virus isolation, and only once by histopathology (1 positive out of 3 tested). In one case even, histopathological conclusion was impossible while yellow fever virus was isolated from the same liver.

Table 1 : Yellow Fever outbreak May 1982, M'Bahiakro, Ivory Coast

| | | |
|---|---|---|
| Number of patients studied at Day : 0, 8, 30, 60. | 216 | |
| Number of patients with virological and serological evidence of infestation | 27 | |
| | YF IgM - | YF IgM + |
| at Day 0 | 24 | 3 |
| at Day : 8, 30, 60 | 0 | 27 |

## SELVATIC CIRCULATION OF YELLOW FEVER VIRUS IN IVORY COAST

Thanks to a regular surveillance of the populations of selvatic potential vectors, we could emphasize the gradual advance of yellow fever virus from the epidemic area of M'BAHIAKRO (7°40'N), in the preforest zone, north to the sub-soudanian savana zones, near SIKOLO (9°30'N).

During the second half of April, yellow fever virus was most probably present in selvatic zone, during the outbreak. It was found in DABAKALA (8°25'N) only at the end of June and SIKOLO only the 20th of November, and thus needed 5 to 6 months to cover the 200 km during the rainy season.

In December, no selvatic vector was found near KONG (9°10'N) and SIKOLO. Therefore virus transmission had been stopped.

These observations, which do not exclude other circulation processes, strongly support the hypothesis of a seasonal outspread of yellow fever virus from epizootic focus in guinean region, onwards.

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## REPORT FROM THE VETERINARY RESEARCH LABORATORY

## P.O. KABETE, KENYA

### Rift Valley Fever Research:

Mosquito collections made during and following the short rainy season of 1981, and the long rains of 1982 were processed for RVF virus isolation by the inoculation of hamsters. No virus isolates were made.

The short rains of 1982 were unusually heavy and prolonged. A computer analysis of the rainfall data in relation to previous epizootics of RVF indicated that the disease might be expected in November and December 1982.

The first clinical case of RVF was not detected until January 1983, virus was isolated from a calf which died in the early post natal period. However RVF virus was isolated in two known epizootic areas from mosquitoes collected in November and December. The isolations are summarised below:

### Site 1:

An area of grassland which is adjacent to indigenous forest at approximately 1700 metres. All pools were made from $Co_2$ baited light trap collections:

| Species | Date |
|---|---|
| *An. christyi* | 23.11.82 |
| *An. coustani* | 1.12.82 |
| *Cx. vansomerini* | 1.12.82 |
| *Cx. antennatus* | 1.12.82 |
| " " | " |

### Site 2:

An area of moist bushed grassland at 1525 m, some 25 km NE of Nairobi. Collections were made from $Co_2$ baited light traps & human bait; larvae were taken to the laboratory and processed after maturing to the adult stage.

Light trap collections:

| Species | Date |
|---|---|
| *Ae. cumminsii* | 7.12.82 |
| *Cx. antenatus* | 31.12.82 |
| *Cx. zombaensis* | " |
| *An. pharoensis* | " |

Human bait:

Ae. lineatopennis 29.12.82

Larval collections:

Ae. lineatopennis 20.11.82
Ae. " "
Ae. lineatopennis proved to be in far greater numbers than any other species.

Clinical Disease:

Virus has been isolated from a single clinical case, a calf which died in the early post natal period. Serological results will be reported later.

It must be emphasised that this RVF virus activity was predicted by a computer analysis of epidemiological data.

Orbiviruses:

Three virus strains were isolated from sheep during outbreaks of bluetongue, which were not bluetongue strains. They were shown to be orbiviruses in their general characterics and were members of the Palyam group. Their relationship to other Palyam virus strains isolated from cattle and Culicoides in Kenya is being investigated.

During pathogenicity studies in sheep where pyrexia and viraemia were produced by these strains, one sheep died during the viraemic period.

Some 70-80 isolates of bluetongue virus have been made and initial typing has been carried out by plaque inhibition tests. Quantiation of serological relationships with the type strains is now being attempted.

Bunya - viruses:

Nairobi sheep disease virus can be transmitted by Rhipicephalus appendiculatus (Ixodidae) from one instar to another after feeding upon immune, susceptible or non-susceptible hosts. An infective feed appeared to be necessary however for transovarian transmission ot occur.

Rhipicephalus pulchellus and Rh. evertsi were unable to transmit the virus either trans-stadially or transovarially. It must be pointed out however that the experiments were carried out with Kenya races of these tick species.

# REPORT FROM NATIONAL INSTITUTE FOR VIROLOGY, SANDRINGHAM, 2131, SOUTH AFRICA.

Culex zombaensis and Aedes circumluteolus as vectors of Rift Valley Fever virus in South Africa.

RVF virus infection of cattle in the subtropical, coastal region of Natal province, is widespread, enzootic, and periodically epizootic. The bovine disease in this region has not been a significant problem, much of it is clinically unrecognized, while these few outbreaks identified have remained localized. Furthermore, as could be expected from the low incidence of bovine infection, the disease in humans has rarely been diagnosed. This situation is in contrast to the severe, widespread epizootics in sheep and cattle, accompanied by significant secondary involvement of humans, which have intermittantly occurred on the temperate inland plateau over the past 30 years.

Past studies on the plateau, together with transmission tests, indicated that Culex theileri was the main epizootic vector on the plateau. However, this is a scarce species on the Natal coast, and on this account, could not be involved significantly in this region.

Despite recurring activity by RVF virus in the coastal region, tests on over 400,000 mosquitoes from 1955 to 1980 produced only 2 isolations from Aedes circumluteolus in 1955 and one isolation from Eretmapodites quinquevittatus in 1971, making firm conclusions on vectors in this region virtually impossible.

However, in 1981 several isolations were obtained from Cx. zombaensis during an isolated outbreak in a herd of 300 cattle in coastal Natal. The outbreak started in September, following heavy rain, peaked in November, when the diagnosis was made and mosquitoes were collected for virus isolation. There were 4 fatalities among adult cattle and 11 abortions. Antibody tests on 246 cattle and 33 human residents after the outbreak suggested that 40% of the cattle and one human had been infected.

A total of 14,227 mosquitoes, belonging to some 14 species was collected at the peak of the outbreak and tested for virus. Seven isolations were obtained from 6,621 Cx. zombaensis, the most prevalent species, one isolation from 1953, of the primarily ornithophilic, Cx. neavei, and one isolation from 962 Ae. circumluteolus. Some of the pools of the latter probably contained a small number of a related species, Ae. luteolateralis.

After the outbreak Cx. zombaensis and Ae. circumluteolus were tested for vector capability and the results are shown in the table. Both species were readily infected at the higher virus doses, but showed either reduced susceptibility (zombaensis) or non-susceptibility (circumluteolus) at the low dose. Both transmitted after infection at the higher doses, although optimum transmission by zombaensis took longer, indicating a rather lengthy extrinsic incubation period. But, when this had been reached, its transmitting ability was marginally superior to that of the other species. With all factors considered, zombaensis would evidently be the better field vector.

These findings thus implicate a third species of the subgenus Culex as epizootic vector: the others being Cx. theileri on the South African plateau and a member of the pipiens complex in Egypt. They also provide

additional evidence implicating a Neomelaniconion species in the coastal region and this augments the list of those South African Aedes previously implicated on the plateau, viz: Ae. (Neomelaniconion) lineatopennis as well as Ae. (Ochlerotatus) juppi and possibly Ae. (Ochlerotatus) caballus.

The role of mosquitoes, therefore, in at least the incidental transmission cycles involving domestic ungulates would appear to rest on firm foundations. Furthermore, if it were true that domestic ungulates are the successors to the herds of vanished feral ungulates amongst which the erstwhile primary vertebrate hosts possibly resided, it is likely that among the above-mentioned mosquito species are to be found the present primary vectors.

(B.M. McINTOSH, P.G. JUPP)

Experimental infection of Cx. zombaensis and Ae. circumluteolus with RVF virus and their transmission of the virus between hamsters.

| Species | Virus dose $log_{10}$/ml | Infection rate | | Transmission rate | |
|---|---|---|---|---|---|
| | | Day | Ratio[a] (%) | Day | Ratio[b] (%) |
| Cx. zombaensis | 8,5-9,6 | 16 | 40/42 (95) | 14 | 1/40 ( 3) |
| " | 7,3-9,9 | 24 | 37/42 (88) | 22 | 15/37 (41) |
| " | " | 31 | 12/22 (55) | 29 | 5/12 (42) |
| " | 8,0 | 28 | 34/37 (92) | | Not done |
| " | 5,2 | 28 | 17/49 (35) | 26 | 3/17 (18) |
| Ae. circumluteolus | 9,3-10,2 | 16-17 | 27/38 (71) | 16 | 8/23 (35) |
| " | " | 27 | 22/23 (96) | 25 | 7/22 (32) |
| " | " | 33 | 8/8 | 31 | 3/8 (38) |
| " | 6,0 | 30-38 | 0/34 | 21-36 | 0/13[c] |

a, number infected/total tested; b, number transmitting/number infected feeding; c, in these transmission experiments hamsters were exposed to groups of mosquitoes: in all 13 mosquitoes bit 7 hamsters.

REPORT FROM THE SPECIAL PATHOGENS UNIT
NATIONAL INSTITUTE FOR VIROLOGY, SANDRINGHAM 2131
REPUBLIC OF SOUTH AFRICA

## 1. CRIMEAN-CONGO HAEMORRHAGIC FEVER (CCHF)

We reported previously (Arbovirus Info. Exchange 41, September 1981) that CCHF virus was isolated from a boy who died in February 1981 after attending a nature study camp (Veldschool) near Bloemhof in the west of the Transvaal province of South Africa. It was soon established that there was a high prevalence of CCHF virus in questing ticks (mainly *Hyalomma* spp. adults) in the nature reserve concerned, that there was a high prevalence of antibodies in the sera of sheep and cattle from neighbouring farms and that antibodies were present in hare sera collected at various sites in South Africa between 1977 and 1979 (Swanepoel *et al.*, 1983b). Deaths which subsequently occurred in antelope at the nature reserve in mid 1981 were ascribed to harsh winter conditions, but CCHF antibodies were found in the sera of 3 eland antelope (*Taurotragus oryx*) tested (Swanepoel *et al.*, 1981b).

Following these initial investigations we undertook more extensive antibody surveys, using mainly the reversed passive haemagglutination technique (Swanepoel *et al.*, 1983a), but making some comparisons with indirect immunofluorescence and immunodiffusion. Total results obtained to date are as follows:-

| Sera from Zimbabwe | Positive/tested |
|---|---|
| Staff, 33 rural hospitals and clinics | 5/830 |
| Cattle, 40 sites | 347/763 |
| Dogs, 16 sites | 59/655 |
| Wild animals 24 spp., mainly large herbivores | 115/918 |
| **Sera from South Africa** | |
| Staff, Veldschool and nature reserve | 5/74 |
| Staff, Veterinary Research Institute | 0/164 |
| Staff, rural hospital | 0/79 |
| Cattle, 70 sites | 2512/3770 |
| Sheep, 4 sites | 74/270 |
| Dogs | 31/629 |
| Antelope, 2 sites | 3/61 |
| Hares, 21 sites | 32/210 |
| Small mammals, mainly rodents | 20/1263 |

The survey has not yet been completed, but the results already confirm the initial impression that the virus is widely prevalent in Southern Africa. In particular, it is planned to test certain population groups, such as veterinarians, slaughtermen and rural hospital staff, more extensively to try and determine the medical

significance of the virus. Pathogenicity tests in sheep and cattle will be undertaken shortly as will transmission trials with the more important species of tick.

## 2. COMPARISON OF RVF AND OTHER AFRICAN PHLEBOVIRUSES IN SHEEP.

These experiments were undertaken to: a) standardise and compare serological techniques for the diagnosis of RVF; b) determine whether known African phleboviruses, other than RVF, are likely to cause veterinary problems and c) to determine whether the cross-reactivity of antibodies to RVF and the other phleboviruses is likely to cause diagnostic problems.

Antibody response to RVF infection was monitored for six months in three sheep by the following methods: reversed passive haemagglutination inhibition (RPHI) haemagglutinatin inhibition (HAI), complement fixation (CF), indirect immunofluorescence (IF), neutralisation (NT), immunodiffusion (ID) and enzyme-linked immunosorbent assay (ELISA). Radio-immunoassay tests have yet to be done. ELISA, NT and IF tests produced the highest titres, but all techniques would be of diagnostic value in particular circumstances. CF titres were most transient and ID antibodies tended to disappear by six months, but all other techniques demonstrated antibody at a fairly constant plateau level after the initial rise and slight decline. The NT test based on cytopathic effect in micro-cultures (CPE NT) produced slightly lower titres than did the plaque reduction test (PR NT), but it is highly convenient for routine or survey use.

Non-fatal RVF infection in sheep produced fever, viraemia and transient raised levels of GLDH, gamma GT and SDH liver enzymes. Arumowot, Gordil, Saint Floris and Gabek Forest phleboviruses were non-pathogenic.

Antibodies following RVF infection cross-reacted to some extent with the other viruses, but primary responses to the other African phleboviruses are unlikely to cause confusion in testing for antibodies to RVF. Even homologous antibody responses are minimal.

Infection with the other phleboviruses failed to render the sheep immune to challenge with RVF virus. However, such sheep appeared to be sensitized and had exaggerated antibody responses to RVF following challenge. This could account for the variation in level of antibody response to RVF noted in animals from the field since the earliest days of research with the virus.

Full results will be presented in due course.

3. RIFT VALLEY FEVER VIRUS (RVFV) PROTEIN SYNTHESIS IN VERO CELLS.

RVFV appears to be unique among the bunyaviruses in producing filamentous, eosinophilic intranuclear inclusions which have been shown to fluoresce specifically with antiserum to the virus (Swanepoel and Blackburn, 1977). We recently reported finding a major non-structural protein (NS1, mol. wt. 34K) in RVFV-infected cells (Struthers and Swanepoel, 1982) and have now made further observations on protein synthesis in such cells. Five virus-induced polypeptides can be detected in the cells (Fig. 1): the nucleocapsid protein (N, 25K), NS1, the two glycoproteins, G1 and G2 (60K and 58K in our determinations) and an 80K protein. G1 and G2 labelled with ($^3$H) glucosamine and ($^3$H) mannose. The 80K protein labelled poorly with carbohydrate precursors, but its migration rate, like that of G1 and G2, was clearly increased following Tunicamycin treatment of cultures, identifying it as a glycoprotein. NS1 was found to be the only virus-induced protein which is phosphorylated.

Sequential synthesis of viral polypeptides in Vero cells is shown in Fig. 2. Inhibition of synthesis of host polypeptides was gradual and only four virus-induced polypeptides could be detected against the host background in whole cell extracts (Fig. 2a). The N and NS1 polypeptides were detected from 4h post-infection and although the initial level of their synthesis varied in different experiments, they were never detectable at earlier time points. The glycoproteins band was detectable against the background of host polypeptides from 14h post-infection.

Examination of immunoprecipitates of partitioned cytoplasms confirmed that the N polypeptide became detectable at 4h post-infection and indicated that initial synthesis of the glycoproteins in fact, occurred at 6h post infection (Fig. 2b). Prolonged exposure of X-ray film failed to render these proteins detectable at earlier time points. The 80K polypeptide could be detected from 8h post-infection in immunoprecipitates.

Examination of extracts of whole partitioned nuclei indicated that high concentrations of NS1 occurred in the nuclei from 4 hours post-infection (Fig. 2c), the same time at which it first became detectable in whole cell extracts. Apart from trace amounts of N protein, probably due to contamination of nuclear extracts with cytoplasmic material, no other virus-induced polypeptides were detected in nuclear fractions. As NS1 is the only RVFV-induced protein which specifically associates with

the nucleus, it seems probable that it is responsible for the formation of the intranuclear inclusions seen in infected cells, and it is also implied that it has a nuclear role in the replication of the virus.

R. SWANEPOEL, J.K. STRUTHERS, A.J. SHEPHERD, G.M. McGILLIVRAY, S.P. SHEPHERD and M.J. ERASMUS.

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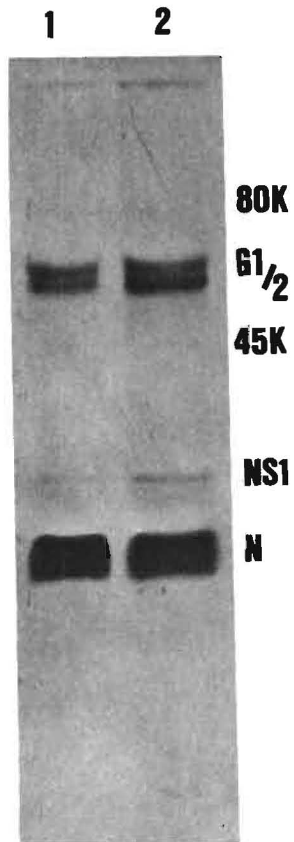
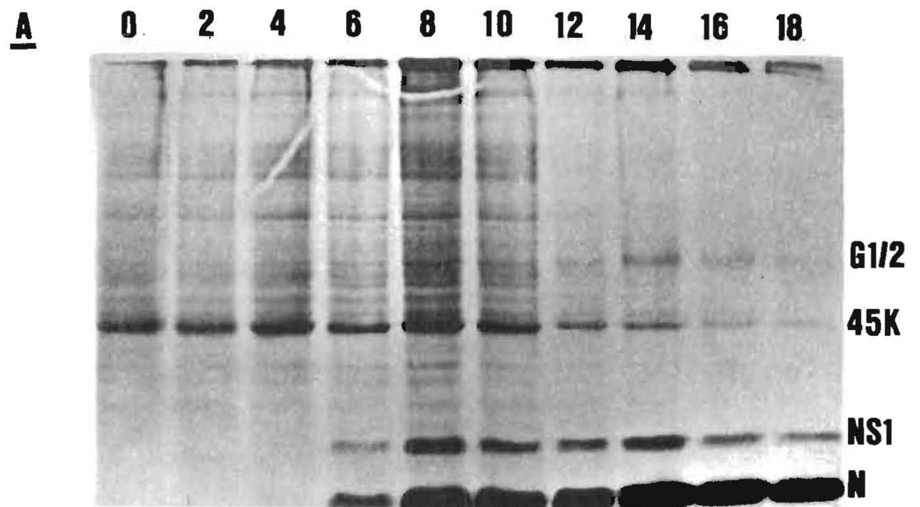
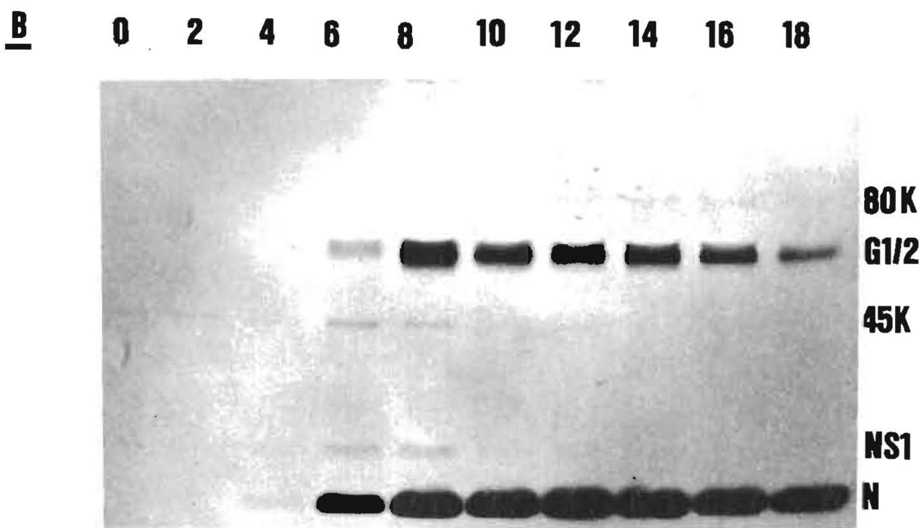



Fig. 1. RVFV polypeptides induced in Vero cells. $5 \times 10^5$ cells/ 35 mm petri dish were infected at a m.o.i. of 20 and were labelled for 1 h with ($^{35}$S) methionine at 12 h post-infection. Cytoplasmic fractions were prepared and virus-induced proteins obtained by immunoprecipitation with immune sheep serum (Lane 1) or with mouse ascitic fluid (Lane 2). RVFV polypeptides are identified as 80K, two glycoproteins, G1 and G2, NS1 and nucleocapsid protein N. A host protein (45K) was also immunoprecipitated.



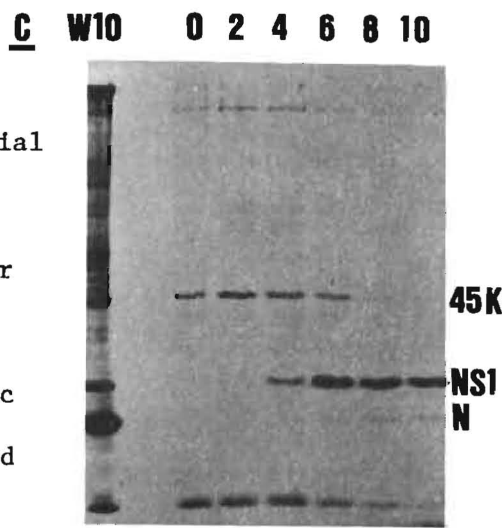




Fig. 2: Fluorogram showing sequential RVFV polypeptide synthesis in Vero cells. Cells were infected as in Fig. 1, and at the indicated times (h post-infection) were labelled for 1 h with ($^{35}$S) methionine. Following the pulse, samples were prepared as follows: (A)whole cells; (B)immunoprecipitates of cytoplasmic fractions; (C)whole nuclei. W10: whole infected-cell extract labelled for 1 h from 10 h post-infection with ($^{35}$S) methionine.

REPORT FROM TANDIL VIRUS RESEARCH LABORATORY, FACULTY OF VETERINARY SCIENCES, NATIONAL UNIVERSITY OF CENTRAL BUENOS AIRES PROVINCE (UNCPBA), ARGENTINA

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*"Infections in humans and horses by Flavivirus at Tandil county, Buenos Aires Province"*

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Three hundred thirty six human sera collected at Tandil county (from April 24, 1979 to October 26, 1981) and 282 equine sera from the same county and surrounding (taken between March 20, 1979 and October 25, 1980) were investigated searching for IH, CF and NT antibodies against three flavivirus: Ilheus, Saint Louis Encephalitis and Yellow Fever (ILH, SLE, and YF respectivally).

Seventeen out of 336 human sera were positive for one and/or more test given a prevalence of 5.06% (females 4.83%, males 5.24%) as shown on tables. Under 20 years old females were all negatives. The activity of SLE and ILH was established in humans. No activity for YF was found in spite of having people 80 years old and older in the sample. The broad reacting human serum (#17) is a lab worker vaccinated with 17D in 1960.

Forty nine equine sera reacted with one or more flavivirus giving a prevalence of 17.38% for this antigenic complex. Twenty two of them (7.80%) neutralized ILH, 4 SLE (1.42%) and 2 both of them (0.71%) and 21 could not be endorse to ILH or SLE but to some other/s flavivirus present in the environment and not in the test. Other 12 sera with no IH or CF antibodies for any of the three viruses did not neutralize ILH or SLE.

(Mettler N.E., Pardo D.A., Di Santo M.I., Schettino A.M and Fernandez A.S.)

RESULTS OF 282 EQUINE SERA FROM TANDIL AND SURROUNDING AGAINTS SLE, ILH and YF (17D)

| # sera with similar pattern | I L H | | | S L E | | | Y F | % |
|---|---|---|---|---|---|---|---|---|
| | IH | CF | NT | IH | CF | NT | IH | |
| 18 | 20-160 | 4-32 | Pos** | 0* | 0* | ND | 0 | (22/282) 7.80% I L H |
| 3 | 20-320 | 8-16 | " | 20-160 | 8 | Neg | 0 | |
| 1 | 40 | 4 | " | 20 | 0 | " | 0 | |
| 2 | 40 | 4-32 | Pos | 20 | 4-8 | Pos | 0 | (2/282) 0.71% SLE + ILH |
| 1 | 0 | 0 | ND | 0 | 8 | Pos | 0 | (4/282) 1.42% S L E |
| 1 | 0 | 0 | " | 20 | 0 | " | 0 | |
| 1 | 0 | 0 | " | 20 | 4 | " | 0 | |
| 1 | 0 | 8 | Neg | 20 | 4 | " | 0 | |
| 2 | 0 | 0 | Neg | 0 | 0 | Neg | 20-40 | (21/282) 7.45% Other/s Flavivirus |
| 2 | 0 | 0 | " | 20-80 | 0 | " | 0 | |
| 1 | 40 | 0 | " | 20 | 16 | " | 0 | |
| 6 | 0 | 4-8 | " | 0 | 0 | " | 0 | |
| 3 | 0 | 0 | " | 20 | 4-16 | " | 0 | |
| 7 | 0 | 0 | " | 0 | 8-16 | " | 0 | |
| 12 | 0 | 0 | Neg | 0 | 0 | Neg | 0 | Negative 82.62% (233/282) |
| 221 | 0 | 0 | ND | 0 | 0 | ND | 0 | |

0* means negative in 1:20 for IH or 1:4 for CF, lowest dilutions used.

** neutralize 100 LD50 i.c, adult mice, undiluted serum.

HUMAN SERUM SAMPLE BY SEX AND GENERATION

ANALIZED AGAINST THREE FLAVIVIRUS

| Sex | Age | | | All |
|---|---|---|---|---|
| | ≲20 years | 21 to 40 | 41 or more | |
| Females | 0*/26 | 1/54 | 6/65 | 7/145 |
| Males | 5/100 | 2/45 | 3/46 | 10/191 |
| Both | 5/126 | 3/99 | 9/111 | 17/336 |

* Numerator give number of positive and denominator number of sera analized.

RESULTS WITH 17 HUMAN SERA REACTING WITH ANY OF THE ANTIGEN TESTED

| Conc. # | SLE | | | ILH | | | YF | Age | Sex | Group |
|---|---|---|---|---|---|---|---|---|---|---|
| | IH | CF | NT | IH | CF | NT | IH | | | |
| 1 | 40 | 4 | Pos | 0 | 0 | ND | 0 | 54 | M | Pat* |
| 2 | 80 | 0 | " | 0 | 0 | " | 0 | 50 | M | " |
| 3 | 20 | 0 | " | 0 | 0 | " | 0 | 26 | M | " |
| 4 | 40 | 0 | Neg | 0 | 0 | " | 0 | 19 | M | " |
| 5 | 20 | 0 | " | 0 | 0 | " | 0 | 52 | F | " |
| 6 | 20 | 0 | " | 0 | 0 | " | 0 | 74 | F | " |
| 7 | 0 | 0 | - | 40 | 8 | Neg | 0 | 33 | M | Lab.W. |
| 8 | 0 | 0 | - | 40 | 64 | Pos | 0 | 15 | M | Healthy |
| 9 | 0 | 0 | - | 20 | 16 | " | 0 | 17 | M | " |
| 10 | 0 | 0 | - | 20 | 16 | " | 0 | 14 | M | " |
| 11 | 0 | 0 | - | 20 | 8 | Neg | 0 | 52 | F | Pat |
| 12 | 0 | 0 | - | 20 | 8 | " | 0 | 60 | M | " |
| 13 | 0 | 0 | - | 20 | 4 | " | 0 | 57 | F | " |
| 14 | 0 | 0 | - | 20 | 8 | Pos | 0 | 9 | M | " |
| 15 | 0 | 0 | - | 20 | 128 | Neg | 0 | 40 | F | " |
| 16 | 0 | 0 | - | 20 | 16 | Pos | 0 | 65 | F | " |
| 17 | 40 | 0 | Neg | 80 | 16 | Neg | 40 | 47 | F | Lab.W. |

* Pat, Lab.W, and Healthy means: Patient, Laboratory Worker and Healthy person respectivally.

REPORT FROM ARBOVIRUS LABORATORY

INSTITUT PASTEUR

B.P. 304

97305 - CAYENNE CEDEX - FRENCH GUIANA

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## DENGUE VIRUS SURVEILLANCE IN FRENCH GUIANA, GUADELOUPE AND MARTINIQUE DURING 1982

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From january to december 1982, human sera from French Guiana, Guadeloupe and Martinique were studied for Flavivirus antibodies using Hemagglutination inbibition and CF tests.

Serums samples were collected from patients with fever or unknown origin and were tested with Yellow fever, Saint-Louis Encephalitis, dengue 2, dengue 3 and Ilheus antigens.

687 serum specimens were studied in French Guiana. A presumptive diagnosis of dengue infection was obtained from 107 patients (15.6 %) The percentage positive was at the maximum in July (30,8 %) and subsequently decreased to 5.9 in december.

11 serum samples only were received from Guadeloupe : they were all low titered.

From Martinique , 108 serums specimens were received and studied and a presumptive diagnosis of dengue infection was obtained from 19 patients (17.6 %). The maximum was reached in November (29.4 %).

89 blood specimens were submitted for virus isolation. All were from patients in French Guiana : one dengue 4 virus was isolated from a patient in march. Most of the serum samples studied had high titer of Flavivirus antibodies.

(Y. ROBIN)

## REPORT FROM THE GORGAS MEMORIAL LABORATORY

Panama City, Republic of Panama

St. Louis Encephalitis in Altos de Majé Island, Bayano, Panama.

Gorgas Memorial Laboratory initiated arbovirus surveillance at Altos de Majé Island in the Bayano River Basin (70 Km East of Panama City, R.P.) in 1973 when construction of a hydroelectric dam begun. The first research extended from 1973 to 1979. Its objective was to describe the effects of flooding and lake formation on population dynamics of vectors and hosts and the resulting changes in arboviral cycles. A second project at Majé designed specifically to follow St. Louis Encephalitis (SLE) virus continued surveillance activities from 1980 to 1983.

Brief periods of SLE activity were detected at Majé in 1973, 1974, 1976, 1978, 1980, 1981 and 1982. In 1977 SLE was isolated sporadically between March and October. This year surveillance has detected an outbreak of SLE virus activity which is apparently more intense than any since 1977. Sentinel chickens at ten of 39 sites have seroconverted between May 31 and August 15, 1983. In addition, SLE was isolated from a sentinel chicken which died on July 12 and from a pool of 30 *Haemagogus equinus* mosquitoes collected with human bait on June 30.

We have initiated a detailed study to determine whether SLE virus is enzootic in the Bayano River Basin or periodically reintroduced from elsewhere. The more information that can be gained about the ecology of SLE virus in tropical areas such as Panama, the greater the probability of understanding the relationship between the tropical and temperate cycle of this virus.

The number of sentinels and stations was increased at the beginning of August, in the hope of providing even more precise data on the type(s) of habitat(s) associated with SLE virus activity in Majé. Arthropod collections, by a number of different methods besides human bait and bleeding of wild birds (both local and migratory) were begun in early August. Stations on the 1,000 m long North-South transect which begins at the Wetmore bridge are emerging as sites of most intense SLE activity in the current outbreak. The human population in nearby areas will be monitored for acute fever of short duration, compatible with arbovirus etiology.

Two tables are included to illustrate the SLE surveillance results from July, 1973 to August 15, 1983. Table 1 includes the sentinel animals that seroconverted and the totals observed each year, also the wild animals tested for SLE antibodies and the mosquitoes processed each year with their virus isolations. Table 2 shows the sites monitored and the type of sentinel exposed in each area.

(B. E. Dutary, P. H. Peralta, A. Adames, P. Galindo and W. C. Reeves)

TABLE 1.

SLE Surveillance Program at Altos de Majé Island, 1973 - 1983

| Year | 1973* | 1974* | 1975* | 1976* | 1977* | 1978* | 1979* | 1980** | 1981** | 1982** | 1983 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SENTINEL ANIMALS | | | | | | | | | | | |
| Monkeys | 2/5*** | 0/4 | 0/10 | 0/6 | 3/5 | 0/3 | | 0/7 | 0/6 | 0/9 | 0/2 |
| Sloths: | | | | | | | | | | | |
| Bradypus | 0/1 | | | | | | | | | | |
| Choloepus | 0/1 | 0/1 | | | | | | | | | |
| Hamsters | 0/39 | 0/39 | 0/167 | 3/135 | 14/237 | 1/487 | 0/60 | 0/71 | 0/108 | 0/90 | 0/62 |
| Chickens | 0/14 | 1/16 | 0/15 | 0/6 | 4/10 | 0/10 | | 4/55 | 3/30 | 1/28 | 10/41 |
| WILD ANIMALS | | | | | | | | | | | |
| Monkeys | | | | | 2/83 | | | | | | |
| Sloths: | | | | | | | | | | | |
| Bradypus | | 1/2 | 5/12 | 4/46 | | | | | | | |
| Choloepus | | 3/7 | 6/13 | 15/32 | | | | | | | |
| Birds | | | 1/40 | 1/51 | 22/199 | 3/178 | | | | | |
| MOSQUITOES | | | | | | | | | | | |
| Mosquitoes | 1/**** 4,620 | 0/ 3,583 | 0/ 8,321 | 0/ 31,456 | 9/***** 318,143 | 0/ 169,689 | | | | | 1/****** 399 |

* Included in: Galindo, P., Adames, A., Perlata, P., Johnson, C.M., and Read, R. Impacto de la hidroeléctrica de Bayano en la transmisión de Arbovirus. Rev. Med. Panamá 8,89-134, 1983.

** Data provided by Dr. C. G. Hayes.

*** Positives/Total tested.

**** *Haemagogus lucifer*

***** *Mansonia dyari*

****** *Haemagogus equinus*

TABLE 2.

Sentinal Animals Exposure Sites at Altos de Majé Island, 1973 - 1983

| Area (Sites) | 1973 | 1974 | 1975 | 1976 | 1977 | 1978 | 1979 | 1980 | 1981 | 1982 | 1983 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Entrance Trail (51,52) | M-H-Ch* | M-H-Ch | M-H-Ch | M-H-Ch | M-H-Ch | M-H-Ch | H | H | H-Ch | H-Ch | H-Ch |
| Dock Area (53,54,55) | H | H | H | H | H | H | H | H-Ch | H | H | H |
| Camp (13,15,17,18,19) | H-Ch | H-Ch | H-Ch | H-Ch | H-Ch | H-Ch | H | H-Ch | H-Ch | H-Ch | H-Ch |
| Main Trail (27,28,29) | H | H | H | H | H | H | H | H | H | H | H |
| Tower A (21,23,25) | H | H | H | H | H | H | H | H-Ch | H-Ch | H-Ch | H-Ch |
| Tall Tree (81,82) | | | | | | | | M | M | M | M |
| Tower B (41,42,45) | H-Ch | H | H-Ch | H-Ch | H-Ch | H-Ch | H | H-Ch | H-Ch | H-Ch | H-Ch |
| Tower C (71,75) | M | M | M-Ch | M-H-Ch | M-H-Ch | M-H-Ch | H | H-Ch | H-Ch | H-Ch | H-Ch |
| Tower D (97,99) | | | H | H | H | H | H | Ch | Ch | Ch | Ch |
| Tall Tree (91,95) | M | M | M | M | M | M | | H-Ch | H-Ch | H-Ch | H-Ch |
| Wetmore Bridge (31,32,34,37) | M-H S | M-H-Ch S | M-H-Ch | M-H-Ch | M-H-Ch | M-H-Ch | H | M-H-Ch | M-H-Ch | M-H-Ch | M-H-Ch |
| Transect (61,62,63,64, 65,66,67,68,69) | | | | | | | | Ch | Ch | Ch | Ch |
| Tall Tree (73,74) | | | | | | | | Ch | Ch | Ch | Ch |

\- SLE Positive sentinel by site, data not available for 1976, 77, 78.

* Monkey = M
Hamster = H
Chicken = Ch
Sloth = S

REPORT FROM SAN JUAN LABORATORIES
DIVISION OF VECTOR-BORNE VIRAL DISEASES, CENTER FOR INFECTIOUS DISEASES
CENTERS FOR DISEASE CONTROL, GPO BOX 4532,
SAN JUAN, PUERTO RICO 00936

## A CASE OF NATURAL CONCURRENT HUMAN INFECTION WITH TWO DENGUE VIRUSES

The first known documented case of concurrent human infection with 2 dengue serotypes is reported. The patient, a 16 year old Puerto Rican male, was identified during routine virologic surveillance in Puerto Rico. The illness was mild and generally unremarkable, with no hemorrhagic manifestations. Nonspecific symptoms included fever, headache, nausea and vomiting.

Virus isolation was made in the C6/36 clone of *Aedes albopictus* cells. Detection from the acute phase serum taken on day of onset of both dengue 1 and 4 viruses in the cell culture was made by the monoclonal antibody IFAT. In that test, the majority of infected cells reacted to the dengue 1 monoclonal antibody, but a smaller number of cells reacted to the dengue 4 monoclonal antibody as well. The acute serum was also inoculated into mosquitoes to confirm the isolation. These mosquitoes were then used to prepare antigen for identification using the CF test. The results are presented in Table 1 and are compatible with infection by both dengue 1 and dengue 4. CF titers for dengue 1, 2, 3 and 4 antisera were 512, 256, 8 and 128 respectively. It should be noted that the antigen control patterns for the 4 serotypes are quite distinct, and there is little crossing between dengue 1 and 4 (Table 1).

This is, to our knowledge, the first case of documented concurrent human infection with 2 dengue viruses although the phenomenon is probably not uncommon in highly endemic areas where 2 or more serotypes are being transmitted simultaneously.

Table 1

Identification of the Dual Infection Isolate by Complement Fixation Test

| Antigen | Antisera DEN 1 | DEN 2 | DEN 3 | DEN 4 | Interpretation |
|---|---|---|---|---|---|
| DEN 1 Control | <u>512</u> | 64 | 16 | 8 | DEN 1 |
| DEN 2 Control | 128 | <u>1024</u> | 16 | 32 | DEN 2 |
| DEN 3 Control | 64 | 64 | <u>128</u> | 8 | DEN 3 |
| DEN 4 Control | 16 | 32 | 16 | <u>512</u> | DEN 4 |
| 66043* | 512 | 256 | 8 | 128 | DEN 1 & DEN 4 |

*Isolate from dual infection patient.

## COMPARATIVE SENSITIVITY OF MOSQUITO INOCULATION AND MOSQUITO CELL CULTURES FOR DENGUE VIRUS ISOLATION

Dengue viruses are among the most difficult arboviruses to isolate and propagate in the laboratory. The mosquito inoculation technique provided for the first time, a highly sensitive and relatively simple method for isolating these viruses. However, it is time consuming and many virology laboratories do not have the facilities to maintain mosquito colonies for this purpose.

In recent years, several mosquito cell lines have been selected which are relatively easy to handle and which are susceptible to dengue virus infection. Many laboratories use these cell cultures exclusively for dengue virus isolation and claims have been made by some that they are as sensitive as mosquito inoculation. This study was carried out to compare the sensitivity of mosquito inoculation and mosquito cell cultures for dengue virus isolation from human sera during a one-year period.

The cell cultures used in these experiments were the C6/36 clone of *Ae. albopictus* and the TRA-284-SF cell line from *Toxorynchites amboinensis*. The former was used early in the year and the latter during the last half of the year. Procedures used for virus isolation using cell cultures have been described previously. Briefly, 4 day-old tube cultures of cells were inoculated with 0.05 ml undiluted serum without removing the growth medium. After one hour adsorption, another 1.0 ml medium was added and the tubes incubated for 10 days at 28°C. Cells were harvested, spotted on slides and the presence or absence of virus determined by the direct fluorescent antibody test (DFAT) using a conjugate prepared from pooled human sera.

The same sera were inoculated undiluted into mosquitoes, either *Tx. amboinensis* or *Ae. aegypti*, which were incubated for 14 days at 30°C. Mosquitoes were killed by freezing, and the presence or absence of viral antigen in brain tissue was determined on head squashes by DFAT. Virus identification was by CF or by the monoclonal antibody IFAT.

During 1982, 173 acute sera from suspected dengue patients were processed for virus isolation by the two systems. The results are presented in Table 2. Using mosquito cell cultures, there were 93 isolates (53.8%). All of these same viruses were also isolated by mosquito inoculation; additionally 13 more virus strains that were negative in tissue culture were isolated by this technique. Thus, 106 isolates (61.3%) were made using the mosquito inoculation technique. There were no isolates made in tissue culture which were negative by mosquito inoculation. The virus isolates by both isolation systems represented all 4 dengue serotypes.

The difference in isolation rates between the 2 systems is statistically significant ($X^2 = 11.08$, $P < 0.001$) (McNemar matched pair test). The data reinforce the policy of this laboratory that although the mosquito cell culture system is adequate for routine virologic surveillance, the more sensitive mosquito inoculation technique should be used for more important specimens such as those from fatal and hemorrhagic fever cases.

DENGUE VIRUS ISOLATIONS FROM HUMAN SERA* BY
MOSQUITO INOCULATION AND BY MOSQUITO CELL CULTURE

| | | Mosquito Inoculation | | | |
|---|---|---|---|---|---|
| | | Positive | Negative | Totals | % |
| Mosquito Cell Culture | Positive | 93 | 0 | 93 | 53.8 |
| | Negative | 13 | 67 | 80 | 46.2 |
| | Totals | 106 | 67 | 173 | |
| | % | 61.3 | 38.7 | | |

$X^2 = 11.07$; $P < 0.001$ (McNemar matched pair test).

*Isolation attempts made from the same human sera by both isolation systems.

(D.J. Gubler, G. Kuno, R. Novak, G. Sather, S.H. Waterman, M. Velez, A. Oliver, and I. Rios.)

REPORT FROM INSTITUTO DE MEDICINA TROPICAL "PEDRO KOURI", INSTITUTO SUPERIOR DE CIENCIAS MEDICAS DE LA HABANA, MINISTERIO DE SALUD PUBLICA, SIBONEY, CIUDAD DE LA HABANA, CUBA

Current Research on Dengue in Cuba

The Dengue Haemorrhagic Fever/Dengue Shock Syndrome/DHF/DSS epidemic due to dengue 2 virus which occurred in Cuba in 1981 was preceded by a dengue 1 epidemic in 1977.

There had been no dengue activity prior to 1977 and now since the 1981 epidemic ended, because of the present low *Aedes aegypti* index. This has provided an ideal situation for several retrospective epidemiological studies which have been started by the Institute to have a better understanding of the etiopathogenicity of DHF/DSS.

The following are being investigated:

1. A random sample of 3000 persons in an area of more than 100,000 persons is being studied for immunity to dengue viruses. The results will be correlated to age, sex, and ethnic origin to determine whether any of these parameters is a risk factor in DHF/DSS.

2. A study of 200 adults hospitalized during the epidemic to correlate the clinical picture with the immune status of the individuals to dengue viruses (primary and secondary infections).

3. Serological studies on 100 children admitted to hospital with DHF/DSS to determine the relationship of the haemorrhagic syndrome to antibodies to dengue 1 and dengue 2 viruses.

4. The influence of race on dengue infections. This will be investigated by comparing the replication of the 1981 epidemic strain in macrophage cultures from groups of black and white natives. Similar studies will be done with male and female groups.

5. Comparison of several D-2 strains isolated during the epidemic from classical dengue and DHF/DSS cases and from different parts of the Country, with another Caribbean classical D-2 strain for infectivity to different cell culture systems.

All of these studies are part of a research program on Dengue Haemorrhagic Fever carried out by the "Pedro Kouri" Institute of Tropical Medicine, Havana City, with financial support from the International Development and Research Centre (IDRC), Canada.

(G. P. Kouri, Ma. G. Guzman, J. Bravo, L. Morier, M. Soler, S. Vazquez.)

## The application of the ELISA technique in the serologic diagnosis of Dengue

Studies have begun for the use of the ELISA technique for the serologic diagnosis of the four serotypes of Dengue virus through antibody detection. The results obtained are compared to the hemagglutination inhibition test as well as the complement fixation test. The preliminary results found are promising.

At the same time, different methods for antigen purification for the ELISA method are being tested. Satisfactory results were encountered using gel chromatography of a sucrose acetone extract from suckling mouse brain infected with Dengue virus. This partially purified antigen is adequate and avoids the inconveniences of density gradient ultracentrifugation.

Experiments are under way in our laboratory to identify isolated viruses through the double sandwich technique using ascitic fluid and monoclonal antibodies.

(De la Cruz, F.; Fernandez, R. J.; Kouri, G.)

## ELISA method in Dengue 1 antibody detection

Conditions and criteria were determined for the detection of Dengue 1 antibody using the ELISA technique.

The conditions of the test were set up with known negative, mildly positive and positive sera against antigen through a checkerboard titration design. For positivity criteria, 18 serum samples were used as controls (9 negative and 9 positive).

The technique was further tested with sera of healthy persons as well as Dengue 1 patients. The sera of these patients was collected when only Dengue 1 serotype was in circulation in the country.

At all times, results obtained with the ELISA technique were compared to the IHA method. A satisfactory correlation was found between both methods.

The antigen used in these tests was Den-1 (Hawaii strain) extracted by the sucrose acetone technique. Working dilution of the sera was 1:100 while the congugate used was a peroxidase labelled anti-human Ig (Cappell) at a dilution of 1:4000.

The results obtained demonstrate the usefulness of the ELISA technique for the detection of anti-Dengue 1 antibody as well as its feasability in serological surveys and for diagnostic purposes.

(Fernandez, R.J.; de la Cruz, F.; Kouri, G).

## Infection of a Poikilothermic cell line (XL-2) with Eastern Equine Encephalitis and Western Equine Encephalitis viruses

The presence of Eastern Equine Encephalitis has been detected in Cuba since before the 40s. Varied epizootics have occurred in the country from which viral isolations have been accomplished from horses, birds and rodents.

Isolations have also occurred in inter epizootic periods. The only isolation of Western Equine Encephalitis was achieved from a sick pigeon found in the vicinity of Havana University. As a result of this finding, sensitive systems are required for the isolation and identification of these agents.

The task of this work was to compare the sensitivity of continuous cell lines obtained from the toad, *Xenopus laevis* (XL-2) Pudney, et al (1973) with the primary chicken embryo culture (CEC) usually used for isolation purposes as well as for other assays.

For this study, EEE virus, C-110 strain isolated from horse brain and WEE virus, UPA strain, isolated from a sick pigeon were inoculated into both cell systems. A clear cytopathic effect (CPE) consisting of rounding and detachment of cells was observed in both cell culture systems infected with EEE and WEE viruses. By 18 hours post-infection, there was a partial destruction of the cell monolayer and by 24 hours the CPE was total.

The infection titers of EEE and WEE in XL-2 and CEC were similar. Both viruses produced small plaques in XL-2 (0.5-1.0 mm in diameter).

At present we are studying the sensitivity of the XL-2 cellular system for direct isolation from field samples and for the detection of several Flaviviruses by the immunofluorescence (FA) technique.

(Morier, L., Solar, M., Instituto Medicina Tropical "Pedro Kouri". (IPK). La Habana, Cuba)

## REPORT FROM THE CENTER FOR TROPICAL ANIMAL HEALTH COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF FLORIDA, GAINESVILLE, FLORIDA, USA.

### Epidemiology of bluetongue in Florida and the Caribbean Region.

In collaboration with the Arthropod-borne Animal Diseases Laboratory, Denver, the Plum Island Animal Disease Center, Plum Island and the Animal Virus Research Institute Pirbright, England, a serotype of bluetongue virus (BTV) hitherto unrecognized in the Western Hemisphere was isolated from cattle in the US. No clinical disease was seen in the cattle which were part of a sentinel herd system in Florida and the Caribbean designed for studying the epidemiology of BTV. The isolation of serotype 2 is the first recovery of a different serotype of BTV in the US since 1967. At least 21 serotypes of BTV have been reported worldwide; the 5 serotypes of BTV now recognized in the US are 2, 10, 11, 13 and 17.

A serological survey organized through the Inter-American Institute for Co-operation on Agriculture of 6,250 sera from cattle, sheep and goats in 7 Caribbean countries and 2 in South America showed that antibody to bluetongue virus was widely distributed in each species throughout the survey area. Overall prevalences of antibody were 70% in cattle, 66% in sheep and 76% in goats as assessed by an immunodiffusion test. Within countries the prevalences were Jamaica 77%, St. Kitts/Nevis 70%, Antigua 76%, St. Lucia 82%, Barbados 61%, Grenada 88%, Trinidad and Tobago 79%, Guyana 52% and Suriname 84%. No clinical cases of bluetongue have been confirmed in the area surveyed and there are no virus isolates yet available to indicate which serotype(s) of virus is/are causing the infection(s).

(E. Paul J. Gibbs & Ellis C. Greiner)

REPORT FROM DEPARTMENT OF EPIDEMIOLOGY, MEDICAL DIVISION, U.S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES, FREDERICK, MD 21701

DETECTION OF IgM AND IgG ANTIBODIES TO VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS BY ELISA

Enzyme-linked immunosorbent assays (ELISAs) were developed for detection of IgG and IgM antibodies to Venezuelan equine encephalomyelitis (VEE) virus using the attenuated strain, TC-83 as antigen. Virus was grown in Vero cells and purified on sucrose density gradients (titer $1 \times 10^9$ pfu/ml). The IgG test was a direct ELISA (antigen adsorbed to plate) while the IgM test was an antibody capture system in which affinity purified anti-human IgM was attached to the plate. Sera in both tests were considered positive for anti-VEE antibody if absorbance readings were $\geq$2.1 times the mean of the negative serum control.

To determine the specificity of the IgG assay, sera from 100 plaque reduction neutralization (PRNT 80%) antibody negative individuals were tested for antibody at a 1:20 screening dilution. Two were positive; when all 100 were retested two different sera gave positive results, indicating a 2% false positive rate.

The sensitivity of the IgG assay was compared to that of the PRNT using a second set of 100 coded human sera from laboratory personnel receiving alphavirus vaccines. Sixty-two positives and 38 negatives were found by ELISA; 58 of the 62 positives were also positive by PRNT. No PRNT positive ELISA negative sera were found. Immunization histories of the 4 ELISA positive/PRNT negative sera indicated 2 had been immunized with VEE vaccine more than 8 years earlier; the other 2 had had no known exposure to VEE virus. Forty-three positive sera were titrated by both ELISA and PRNT, and titers in 38 (88%) varied randomly by four-fold or less between tests; in the remaining 5, ELISA titers were eight-fold higher than PRNT titers.

To investigate the appearance and persistence of IgG and IgM antibody, thirteen VEE TC-83 vaccinees were bled weekly for 4 weeks and intermittently thereafter. Three individuals failed to produce detectable IgG or IgM antibodies by either ELISA or PRNT and were excluded from further studies. IgM antibody was detected on the second week post-immunization (PI) in 8 of the remaining vaccinees and on the third week in the ninth. The tenth subject did not have blood drawn until the fourth week PI, at which time he was IgM positive. The peak titers of IgM which occurred by weeks 2-3 ranged from 1:80 to 1:5120. IgM antibody persisted 7-9 weeks in 7 vaccinees and 15-24 weeks in the other 3. One hundred sera from non-immunized PRNT negative individuals screened for non-specific IgM reactions were all negative.

The IgG ELISA demonstrated the appearance of low titer antibodies in 6/9 samples tested at 2 weeks PI. Titers peaked in the 4th week for all 10 vaccines, and decayed slightly but remained positive in all cases at the 15th week. All 3 samples tested at week 24, contained high titers of IgG antibody.

To study the ability of the IgG ELISA to detect antibodies in field collected samples, a group of 220 sera from soldiers stationed in Panama were tested by PRNT and ELISA for IgG antibody. Ten were positive by PRNT (1:10 dilutions) but only 9 by ELISA (1:20 dilutions). The false negative serum

that was initially not detected by ELISA had a PRNT titer of 1:10; when retested several times border line positive ELISA reactions were obtained. The 10 PRNT positive sera came from individuals who had recent clinical illnesses compatible with VEE infection. Blood samples obtained 8-10 days after onset of illness were also positive for IgM antibody by ELISA. In serial samples from one patient, IgM antibody was still present at 7 weeks, the last date tested.

These results suggest that VEE-IgG and -IgM antibody ELISA systems are both rapid and highly sensitive measurements of antibody status in vaccinated and naturally infected individuals.

(F.F. Macasaet, R.R. Rosato, J.A. Mangiafico, P.B. Jahrling, J.W. LeDuc)

## RAPID DIAGNOSIS STUDIES

Studies are in progress to develop ELISAs for IgM and IgG antibodies resulting from infections with a number of arthropod-borne viruses including Punta Toro, Charges, sandfly fever (Naples and Sicilian), West Nile, and Sindbis viruses. Such studies would be facillitated if large numbers of human or monkey sera containing specific antibodies were available. We would greatly appreciate the opportunity to collaborate with colleagues having such sera, or desiring to establish such tests in their laboratories. Additionally, we have developed a number of antigen detection ELISA systems utilizing monoclonal antibodies. We are attemping to collect for future studies published and unpublished data on monoclonal antibodies to arthropod-borne viruses and viruses causing hemorrhagic fevers. Any such data would also be greatly appreciated. Please address correspondence to: Chief, Department of Epidemiology, Medical Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701.

REPORT FROM THE VIROLOGY PROGRAM
STATE OF NEW JERSEY DEPARTMENT OF HEALTH
TRENTON, NEW JERSEY

## Arbovirus Surveillance in New Jersey, 1983

During the 1983 surveillance period, from May through September, 1232 mosquito pools containing up to 100 mosquitoes each, were tested for viruses in chicks. There have been to date five (5) mosquito pools positive for Eastern equine encephalitis (EEE) and Western equine encephalitis was isolated from four (4).

EEE activity began with late July collections and has continued into August. There were four (4) isolates from *Culiseta melanura* from two sites and one (1) from *Coquillettidia perturbans*.

The WEE isolates were from August collections at two sites and were from *Culiseta melanura* pools.

There have been four (4) horse cases of EEE, three (3) isolations from brain material and one (1) seroconversion, in late July and August.

Sentinel chicken flocks of ten (10) cockerals were placed at four sites in June. The flocks are bled bi-weekly on a rotating schedule and St. Louis encephalitis hemagglutination inhibition tests were conducted. There were no conversions observed in the 170 sera tested.

(David Kirsh, Bernard Taylor and Wayne Pizzuti)

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

Update on Eastern Equine Encephalitis in Upstate New York

Equines: A total of 15 sick equines from the Syracuse region of New York State were investigated for evidence of EEE virus infection by serology (14 cases) and/or by virus isolation (7). Fourteen horses were residents of three contiguous counties in upstate New York where previous outbreaks of EEE had occurred in 1971-72 and 1976-77: Onondaga (7 horses), Oneida (5), and Oswego (2). One burro from neighboring Madison County had been relocated from California. Onset of illness in these animals occurred from 7.5 to 8.11.83; eleven exhibited signs of central nervous system (CNS) disturbance including ataxia, circling, tooth grinding, paralysis, stupor, depression and fever ranging from 98.8 to 106°F. Three horses died and 6 were euthanized, due to rapidly deteriorating conditions, within 1 to 4 days of onset. According to the veterinarians' notes, 11 of the 15 animals had never received EEE vaccination; 2 equines (1 horse, 1 burro) were vaccinated in 1982, another animal had a questionable history of EEE vaccination in March 1983 and no information was available for the remaining horse.

An EEE virus infection was confirmed in 2 adult unvaccinated horses by 4-fold or greater rises of HI antibody to EEE virus. The foal of 1 serologically confirmed case, which was delivered by cesarian section 1 week prematurely, died 2 days later; EEE virus was isolated from post-mortem brain tissue of this infant. The 3 confirmed cases were all residents of Oneida County. Results of HI tests for EEE and WEE in 10 of the remaining 11 animals studied revealed levels of antibody to EEE compatible with prior vaccination in 2 cases, no evidence of infection in 4 equines, and high titers (160-5120) suggestive of recent infection in 4 horses, 1 from Oswego County and 3 from Onondaga County. Tests are still pending on the serum from 1 horse.

Mosquitoes: A focus of EEE virus infection was detected in mosquitoes of Onondaga County where 3 presumptive equine cases, with onset of illness from 7.25 to 8.3.83, were diagnosed. To date, 20 pools comprising 1,539 adult specimens taken from this county during the period 7.5 5o 7.21.83 have been analyzed; 4 pools of 393 females captured from 7.12 to 7.21.83 yielded isolations of EEE virus, 2 each from *Culiseta melanura* and *Culiseta morsitans*. EEE virus was not recovered from 336 pools of 22,488 mosquitoes captured between 6.7 and 8.3.83 from 5 other counties in 4 regions of the state.

Humans: Results of HI tests of convalescent sera from 45 CNS patients examined as of August 26, 1983 failed to yield evidence of human infections with EEE virus in New York State.

(Margaret A. Grayson and Rudolf Deibel)

Department Microbiology, Cornell Univ. Medical College
and
Department Entomology, Cornell University

Three foci from which enzootic strains of Venezuelan encephalitis virus had been isolated in northern Colombia were studied for two week periods each by personnel from Cornell University Medical College and the Instituto de Salud (Bogata). The three areas were forests near Guacahca and Don Diego rivers (about 45 and 59 kilometers east of Santa Marta respectively); Rio de Oro (along the Venezuelan border about 91 kilometers north of Tibu) and Boquillas (about 35 kilometers northeast of Magangue in the lower Rio Madgelena valley). The principal goal was to obtain more isolates of subtype I-D for studies of variation within that complex.

None of 56 Hamsters (515 hamster nights) died at Guacahca and Don Diego forests. Culex (Melanoconion) crybda was collected de "de-vac" and human bait at Guachaca but Culex (Mel.) pedroi, the probable vector in northern Colombia was not found at Guachaca, and was present in very low numbers at Don Diego. Those forests should be followed at other periods of the year and through several years to determine if the virus is still present. The disappearance of VE virus in the absence of radical habitat changes has not been documented.

In contrast, 16 of 28 hamsters (231 hamster nights) died at Boquillas and 35 of 35 (254 hamster nights) died at Rio de Oro! Culex (Mel.) pedroi and Culex (Mel.) spissipes were abundant in the forest at Rio de Oro. The first three hamsters tested from Rio de Oro yielded VE virus, other isolations are in progress. Sera from eleven of twelve equines and seven of thirty humans collected in the Boquillas region contained N antibodies to subtype I-D VE virus.

A total of 10530 mosquitoes were collected for virus isolations at the INS, however 82% are from diurnal biting collections. Plasmas collected from bats and wild rodents are under study at the INS.

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# Trends in Arbovirus Encephalitis in the United States, January-August, 1983

Report from the Division of Vector-Borne Viral Diseases
CID, CDC, Fort Collins, Colorado

In the United States, encephalitis caused by arboviruses is seasonal, with most cases occurring from June to September. The following is a mid-seasonal report on the occurrence of arbovirus encephalitis in 1983 from data collected through August 30 (Figure).

Meteorologic conditions which preceded the summer were expected to favor the epidemic spread of St. Louis (SLE) and Eastern (EEE) and Western (WEE) equine encephalitis viruses this year: An unusually mild winter, heavy early spring rainfall, and hot, dry summer were factors previously associated with epidemic spread of St. Louis encephalitis in the Ohio and Mississippi valleys; in the west, heavy snowpack, and a wet spring have been associated with past epidemic WEE and SLE in California; and unusually heavy rainfall in two consecutive years is a climatic pattern associated with outbreaks of EEE in the northeastern United States.

The predictions of increased EEE transmission in the northeast and WEE and SLE in the west, have been largely fulfilled but the absence of SLE transmission in the midwest has been a striking phenomenon contrary to expectations.

## St. Louis Encephalitis

Climatic conditions this season resemble closely those experienced in 1933 and 1975 when the largest documented SLE outbreaks in the United States occurred. This year, however, minimal SLE transmission is evident in the central states, where most past *Cx. pipiens*-borne epidemics have occurred. The proportion of antibody positive birds, particularly juvenile birds, in cross-sectional surveys has been an effective indicator of SLE transmission and small incremental changes in these proportions have signaled the advent of human outbreaks. This year, in specimens from 11,770 adult and 9,652 juvenile birds from Ohio, Illnois, Indiana, Tennessee, Texas, and Kentucky, where weekly cross-sectional surveys have been undertaken since May, only 49 (0.4%) adult and 45 (0.5%) juvenile birds were SLE (HI) seroreactors. In epidemic years, seropositivity rates 10-50 fold greater have prevailed. The low proportion of birds infected with SLE in these states this year has been paralleled by the absence of human cases in the corresponding period.

In the West, the predictive value of excessive snowpack with consequent runoff providing water for spring breeding of *Culex tarsalis* and transmission of SLE and WEE was previously established. Local flooding this year from heavy spring rains and melting snow, created conditions favorable for *Cx. tarsalis* breeding in large areas of Utah, Arizona, and California. Surveillance in early summer indicated large populations of *Cx. tarsalis* and subsequent collections yielded large numbers of WEE and SLE virus isolates with MIR's of up to 5/1000 for SLE virus. The only two cases of SLE in the

United States so far this year occurred in residents of areas adjacent to flooded sections of the Colorado River. One case was in a 22 year-old Yuma, Arizona woman and the other in a 3 year-old Bard, Cali- fornia boy; both recovered.

Western Equine Encephalitis

The same conditions which favor transmission of SLE by Cx. tarsalis in the West also generally apply to transmission of WEE virus. Mosquito collections in Imperial County in southeastern California and the adjacent Arizona counties on the Colorado River, initially yielded large numbers of Cx. tarsalis, averaging 1,400 per trap/night. However, seroconversion rates of sentinel chickens in the Imperial Valley of California have not been excessive and only three confirmed equine cases (two in Arizona, one in California) and no human cases have been reported.

The apparent low rate of WEE transmission to horses and humans in the presence of large vector populations and high mosquito infection rates is unexplained. Factors which may have mitigated the epidemic spread of WEE include the early widespread application of larvicides in flooded areas and unusually cool nighttime temperatures in midsummer.

In the Red River valley, which divides Minnesota and North Dakota, heavy early summer rains led to development of large Cx. tarsalis populations. Surveillance showed mosquito minimum infection rates as high as 5/1000 in counties scattered across the breadth of North Dakota and in central Minnesota. The early occurrence of an equine case in the Red River valley and the presence of seroreacting chickens in commerical flocks was further evi- dence of WEE transmission. Acting on this data, Minnesota initiated a 31 county aerial spraying adulticiding program covering over 30,000 square miles. Active surveillance in both states has not uncovered any confirmed human cases.

Eastern Equine Encephalitis

An unusually warm winter in the northeast and two consecutive years of heavy rainfall led to expectations that EEE might be epidemic this year. Populations of C. melanura, however, have been low to normal in areas of the northeast where longitudinal data of vector populations are available.

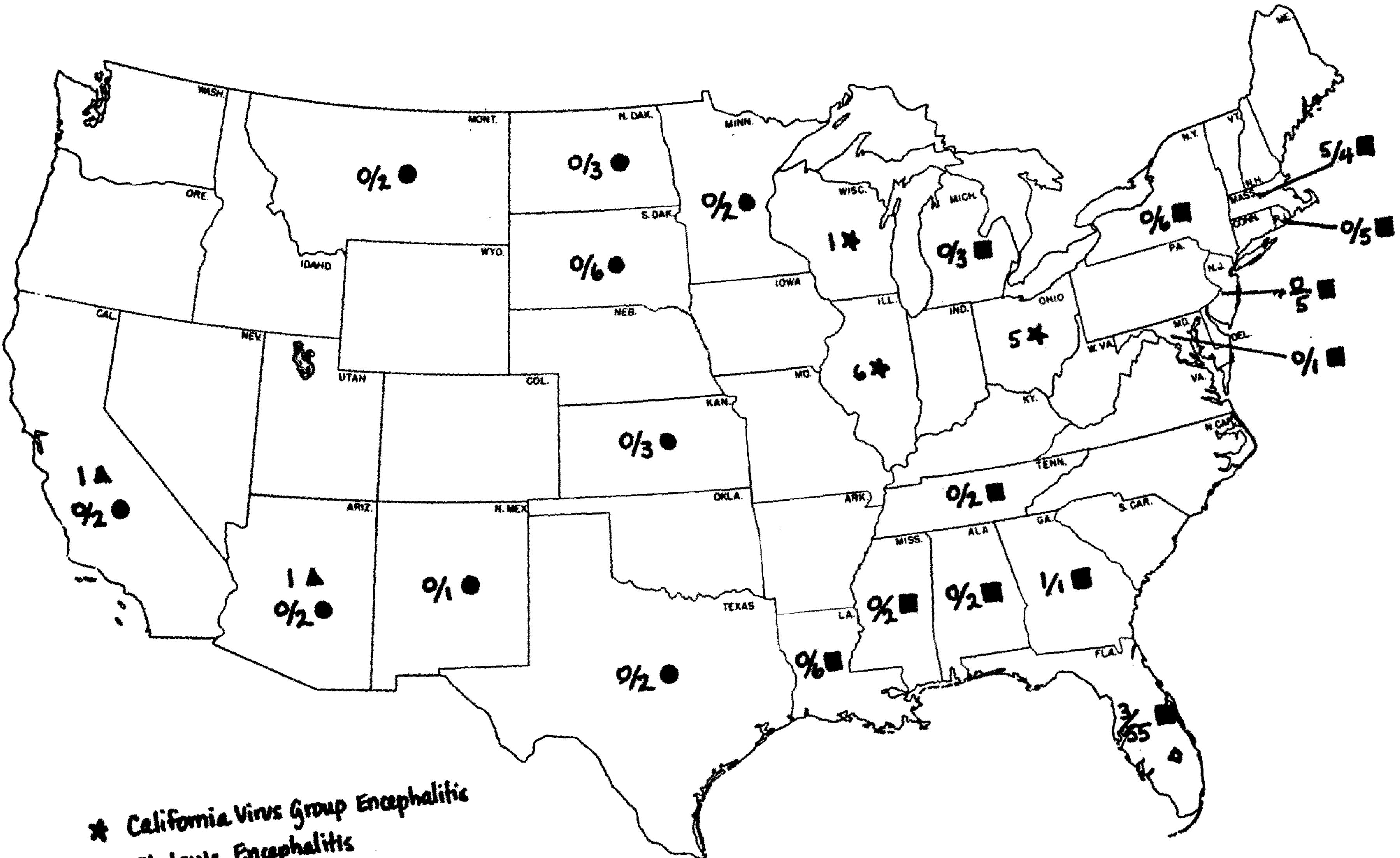
The number of horse and human cases has not been excessive although the unusually early appearance of an equine case in New Jersey and the first occurrence of equine cases in Rhode Island in ten years suggests that, at least in some locales, greater than normal transmission of EEE is occurring. EEE virus was isolated from Culiseta melanura in southern Rhode Island, but populations of potential epidemic/epizootic vectors were very low. Hyper- enzootic transmission in Florida, Massachusetts, and New York and sporadic equine cases in other eastern states is occurring in areas that historically experience EEE. The observation that equines are invalid sentinels in areas where high vaccination rates prevail was made again in Massachusetts this year where the expected ratio of human to equine cases was reversed.

## California Virus Group Encephalitis

Unusually hot, dry weather in the midwest where most cases of LaCrosse encephalitis occur, might be expected to reduce vector populations of *Aedes triseriatus*. However, even in areas where there has been no rainfall for three weeks, larvae have been found in tree holes and other water reservoirs in locales where cases may have been exposed. Most cases of LaCrosse encephalitis are reported in late summer; the number of cases reported to date seems typical of most years.

(T. Tsai, C.G. Moore, D.B. Francy, T.P. Monath)

ARBOVIRUS ENCEPHALITIS, UNITED STATES
JAN. - AUG., 1983



# Detection of Yellow Fever Virus in Serum by Enzyme Immunoassay

Yellow fever (YF) virus is present in patient's blood during the acute phase of illness. Virus isolation and identification provides a potential method of early diagnosis, but available techniques are slow and require specialized materials and equipment. An alternative approach is direct detection of YF antigen in serum by means of enzyme-linked immunosorbent assay (ELISA). An antigen-capture ELISA was developed, which used anti-YF antibodies, immobilized on a solid phase (polystyrene plates), to capture YF virus from serum samples. After addition of the virus-containing sample, anti-YF detecting antibody conjugated to alkaline phosphatase was added to detect viral antigen. Trials with various capture and detecting antibodies in systems employing purified YF 17D virus, led to the selection of (1) two capture antibodies (pooled human serum containing high titer YF IgM antibodies and a type-specific YF monoclonal antibody developed by Schlesinger et al., Virology, 125:8-17, 1983) and (2) a detecting antibody conjugate consisting of monoclonal antibody broadly cross-reactive with all flaviviruses, purified by affinity chromatography, and conjugated to alkaline phosphatase. The limit of sensitivity in tests against purified YF 17D virus diluted in buffer or normal human serum was $10^{3.0}$ - $10^{3.6}$ PFU/.05 ml or .007-.029 µg viral protein/.05 ml. Sera obtained at intervals from rhesus and cynomolgus monkeys after infection with a wild YF virus strain were tested. The limit of sensitivity of the assay applied to viremic monkey serum was similar (approximately 3.5 $\log_{10}$ PFU/.05 ml). Monoclonal capture antibody was more potent than human IgM for antigen capture from viremic sera containing high virus titers, whereas IgM provided a more sensitive assay for sera with low titers. Use of monoclonal capture antibody provided a specific virus identification. The procedure offers promise for use as a diagnostic test in human cases.

The diagnosis of yellow fever (YF) presently depends upon histopathologic examination of liver from fatal cases, virus isolation from blood, or serological tests on appropriately-timed specimens. Virus isolation provides a potential means of early diagnosis, but the available techniques, though sensitive, are slow and require specialized facilities or equipment. An alternative approach to early YF diagnosis is the direct demonstration of viral antigen in the patient's serum by immunoassay. Theoretical advantages include rapidity, since the test can be performed in a few hours, and applicability under field conditions. These considerations are important in the improvement of surveillance, investigation of outbreaks, and management of patients. In this paper, we describe use of the enzyme-linked immunosorbent assay (ELISA) for detection of YF virus in sera of experimentally-infected monkeys. The results indicate that the technique may also be useful for diagnosis of human YF.

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## Japanese Encephalitis Vaccine - Preliminary Results as an Investigational New Drug in the United States

Following the occurrence in 1981 of Japanese Encephalitis (JE) in five expatriates in Beijing including the fatality of an American student, considerable interest was generated to make available an effective JE vaccine to United States citizens travelling to JE endemic areas.

A vaccine produced by Biken Laboratories, Osaka, Japan has been extensively used in Japan; since no candidate vaccine produced in the U.S. was available, we selected the Biken vaccine for evaluation. The vaccine is prepared from adult mouse brains infected with the Nakayama strain of JE virus and is partially purifed by ultracentrifugation and protamine sulfate precipitation.. Favoring selection of the Biken vaccine are the following factors: (1) the vaccine has been used in its present form since in 1966 and in excess of 80,000,000 doses have been used to immunize school children in Japan; (2) Japan's requirements for efficacy and safety of biological products are similar to standards of the United States, FDA; (3) Published reports and Biken's records support the vaccine's safety and efficacy.

On May 17, 1983 the Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control received approval from BOB/FDA to proceed with preliminary evaluation of the Biken JE vaccine as an investigational new drug (IND). This report summarizes information on the initial 25 participants in the IND evaluation.

### Serologic Responses to JE Vaccine

Eleven participants were recruited from the Washington, D.C. area and 15 from Fort Collins, Colorado. Five participants were excluded from analysis of serological response because they either were seropositive to JE virus prior to receiving the first vaccine dose (4 participants) or failed to complete the course of two vaccine doses (1 participant). Sera of vaccines were tested at serial twofold dilutions in a plaque reduction neutralization test in Vero cells.

Table 1 shows the results of immunization of the 21 seronegative participants: 18 (86%) had a fourfold or greater antibody response to the Nakayama JE virus strain; 11 (52%) developed titers $\geq 32$ and 16 (76%) developed titers of $\geq 8$. The post immunization sera of 2 (10%) remained seronegative ( $<2$) after 2 doses of the Biken vaccine (1 each in age range 30-39 and 40-49).

Table 2 compares serologic responses of 14 participants to two JE virus strains - Nakayama and strain G8924 isolated in the 1950's in India. Four (33%) of the twelve persons seroconverting to Nakayama remained seronegative to the Indian strain. Most of those who seroconverted to the Indian strain developed titers comparable to those against the Nakayama strain.

A history of prior vaccination with another flavivirus (yellow fever) had no evident effect on the serological response to the JE vaccine (Table 3).

<u>Clinical Reactions to JE Vaccine</u>

Of 25 participants who completed the 2 dose primary vaccination series, none had febrile responses or changes in blood pressure or pulse at followup examination 2 to 3 days after each vaccine dose. Eleven (44%) reported local tenderness, lasting 1 to 3 days, and included 1 who reported an episode of shooting pain down the arm; 3 (12%) had redness at the inoculation site and 4 complained of other symptoms including sore throat (1), stiff neck (1), diarrhea (1), transient nausea, dizziness, myalgia, and feeling feverish (1). The latter respondent thought her symptoms may have related to a chemical she had worked with on the day of vaccination. One of the initial 26 volunteers had an anaphylactic reaction while engaged in an aerobics session about 7-1/2 hours after her first vaccine dose. It appeared that her reaction was not causally related to the vaccine and she was referred to an allergist for an evaluation, who concluded that she suffers from exercise induced anaphylaxis. She had a prior history of two similar episodes encurred while swimming and, 19 days after being entered into this study, reported a repeat occurrence during another aerobics session. Although we concluded her post vaccination symptoms were not vaccine induced we elected not to administer the scheduled second dose of vaccine. This experience illustrates the utility in excluding from pilot studies such as this persons who have histories of severe allergic episodes since a coincidence of such an episode with administration of an experimental product confuses evaluation of the product's effects. In fact, if a second dose had been administered to this participant 14 days after the first dose (as was done with several of her co-participants) we would have had a reported repeat allergic reaction occurring within 5 days to further confound the evaluation of this product.

In summary, although the number of participants evaluated to date is small, our experience is consistant with published accounts of the effects of the JE (Biken) vaccine: 86% of persons initially seronegative to JE had a fourfold increase in antibody to the vaccine; local tenderness was reported in slightly less than half of vaccinees and redness in about 1/3 of those reporting tenderness. No severe reactions attributable to the vaccine was detected.

Of some concern in regards to the efficacy of this vaccine is the possible failure to provide protection to JE strains currently in circulation in Asia. Further evaluation of N antibody responses to a variety of JE strains are underway.

(J. D. Poland, T. P. Monath, C. B. Cropp)

Table 1. Serologic responses to 2 doses of JE vaccine of 21 seronegative participants - August 1983.

| Age Group | No Vaccinated | Post Immunization Neutralizing Antibody Titers | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | <2 | 2 | 4 | 8 | 16 | 32 | ≥64 |
| 20-29 | 9 | | | 1 | 1 | 2 | 2 | 3 |
| 30-39 | 4 | 1 | 1 | 1 | | | 1 | |
| 40-49 | 7 | 1 | | | 2 | | | 4 |
| 50-59 | 1 | | | | | | 1 | |
| Totals | 21 | 2 | 1 | 2 | 3 | 2 | 4 | 7 |

Table 2. Comparison of serological response following 2 doses of Biken JE vaccine Nakayama vs. Indian strain G8924.

| Indian JE G8924 | Neutralizing Antibody Titers Nakayama strain | | | | | | | Totals |
|---|---|---|---|---|---|---|---|---|
| | <2 | 2 | 4 | 8 | 16 | 32 | ≥64 | |
| <2 | 2 | 1 | 2 | 1 | | | | 6 |
| 2 | | | | | | | | |
| 4 | | | | | | | | |
| 8 | | | | | | 1 | | 1 |
| 16 | | | | | 2 | | 1 | 3 |
| 32 | | | | | | | | |
| ≥64 | | | | | | 2 | 2 | 4 |
| Totals | 2 | 1 | 2 | 1 | 2 | 3 | 3 | 14 |

Table 3. Correlation of prior yellow fever vaccination and neutralizing antibody response to Japanese encephalitis vaccine.

A. Number of respondents by JE titer:

| History of prior YF vaccination | Number vaccinated | Post JE immunization titer | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $<2$ | 2 | 4 | 8 | 16 | 32 | $\geq 64$ |
| Yes | 13 | 2 | 1 | 0 | 2 | 1 | 3 | 4 |
| No | 7 | 0 | 0 | 2 | 1 | 1 | 1 | 2 |
| Totals | 20* | 2 | 1 | 2 | 3 | 2 | 4 | 6 |

B. 2 x 2 Comparison

| Post JE vaccination titer | History of prior YF vaccination Yes | No | Totals |
|---|---|---|---|
| $\leq 4$ | 3 (23%) | 2 (29%) | 5 (25%) |
| $\leq 8$ | 10 (77%) | 5 (71%) | 15 (75%) |
| Totals | 13 | 7 | 20 |

* Vaccination history for 1 individual unknown.

Experimental Transmission of Rocio Encephalitis Virus by
*Aedes scapularis* (Diptera: Culicidae)
from the Epidemic Zone in Brazil

Rocio encephalitis is an epidemic flaviviral infection of man first described in Sao Paulo, Brazil, in 1975. Rocio virus has been isolated from arthropods collected in nature only once, and the vector relationships of the virus in Brazil are essentially unknown. We have undertaken vector competence studies with mosquito strains from the epidemic zone. *Aedes scapularis* $F_1$ progeny from females collected during May, November, December, January, and February at Pariquera-Acu, Brazil, were susceptible to per os infection with Rocio virus. The $ID_{50}$ [ca. $10^{3.5}$ Vero cell plaque-forming units (PFU)] is well within the range of a competent vector. Infection and transmission rates of 100% were achieved when *Ae. scapularis* fed on a chick circulating $10^{7.5}$ PFU/ml of virus; however, infection rates generally were lower (Table 1). The susceptibility of *Ae. scapularis* to oral infection with Rocio virus, its ability to transmit virus experimentally, and its abundance and close association with man in the epidemic zone make this species a prime suspect as a vector during the 1975 and 1976 epidemics in Sao Paulo State.

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Table 1. Susceptibility of *Aedes scapularis* from Pariquera-Acu, Sao Paulo State, Brazil, to per os infection with Rocio virus and transmission efficiency to chicks.

| Date parent mosq. coll. | Virus titer of infective meal* | Days extrinsic incub. | Infection | | Transmission | |
|---|---|---|---|---|---|---|
| | | | Proportion | % | Proportion*** | % |
| 7 May 1981 | 5.6 | 20 | 3/18 | 16.7 | -+ | - |
| | 5.9 | 20 | 7/19 | 36.8 | - | - |
| | 6.9 | 20 | 18/21 | 85.7 | - | - |
| 12 Nov. 1981 | 5.8 | 21 | 21/37 | 56.8 | - | - |
| | 6.0 | 21 | 28/34 | 82.4 | - | - |
| | 6.4 | 21 | 39/42 | 92.9 | - | - |
| 7 Jan. 1982 | 4.6 | 21 | 1/16 | 6.3 | - | - |
| | 5.7 | 21 | 2/6 | 33.3 | - | - |
| 10 Dec. 1981 & 21 Jan. 1982 | 5.4 | 17 | 3/16 | 18.8 | - | - |
| | 6.8 | 19 | 18/29 | 62.1 | 6/6 | 100 |
| | 7.7 | 19 | 12/15 | 80.0 | 12/12 | 100 |
| | 7.9 | 19 | 34/43 | 79.1 | 15/16 | 93.8 |
| 4 & 18 Feb. 1982 | 7.5 | 19 | 11/11 | 100 | 11/11 | 100 |
| | 7.9 | 19 | 11/13 | 84.6 | 8/10 | 80 |

* Postfeeding titer expressed as $\log_{10}$ Vero PFU/ml.

** No. mosquitoes that contained virus/no. mosquitoes tested.

*** No. mosquitoes that transmitted virus/no. of infected mosquitoes that refed.

\+ Not done.

REPORT FROM THE INFECTIOUS DISEASES RESEARCH LABORATORY, IZAAK WALTON KILLAM HOSPITAL FOR CHILDREN, HALIFAX, NOVA SCOTIA AND THE NATIONAL ARBOVIRUS SERVICE, DEPARTMENT OF MEDICAL MICROBIOLOGY, UNIVERSITY OF TORONTO, TORONTO, ONTARIO, CANADA.

## California Group Arbovirus Activity in the Maritime Provinces of Canada, and an Imported Case of Powassan Encephalitis.

Evidence of California group (CAL) arbovirus activity has been found among wild and domestic animal populations in the Maritime Provinces of Canada. These findings are the first reported evidence of CAL activity in this area of Canada.

### Nova Scotia

Hemagglutination-inhibition (HI) tests have shown that 113 of 1003 (11.3%) snowshoe hare (*Lepus americanus*) sera collected from 1975 to 1977 were positive to the snowshoe hare (SSH) strain of CAL [1]. In horse sera collected in 1976 and 1977, 106 of 861 (12.3%) samples tested by HI were positive for SSH antigen; neutralization tests (NT) confirmed this finding in 72 of the 106 HI-positive samples [2]. In moose (*Alces alces americana* Clinton) sera collected in 1977 and 1978, 107 of 289 (37%) samples were positive for SSH by HI and 189 of 280 (67.5%) were SSH positive by NT [3]. Six sera samples had neutralizing antibodies to Jamestown Canyon (JC) virus.

In 1981, the first reported case of human disease in the Maritime Provinces to be attributed to a CAL virus occurred in Halifax County. HI tests of the patient's sera showed a diagnostic change in antibody titre [4]. Two of 5 sentinel rabbits placed one year later in the backyard of the patient's residence seroconverted as tested by HI, NT and complement fixation tests. These findings correspond to a high prevalence (20.4%) of CAL activity in wild hares sampled in the same county [1]. This case indicates the potential threat of CAL to the human population in this region.

Diagnostic changes in titres to Powassan virus antigen were found in a boy from New York State who, while travelling in the province, became ill with an encephalitis that mimicked herpes simplex encephalitis [5]. Epidemiological evidence strongly indicates that the boy was infected in his home state.

### New Brunswick

Neutralizing antibodies to SSH were found in 6 of 129 (4.7%) deer (*Odocoileus virginianus*) sera collected during 1976. Horse sera collected

during 1977 showed antibodies to SSH by HI in 54 of 204 (26.5%) samples. Of these 54, 36 also had neutralizing antibodies to SSH. In 127 samples of moose serum collected in 1979 and tested by NT, 94 (74.6%) had antibodies to SSH, 4 had antibodies to JC, and 17 had equal antibody titres to SSH and JC [6].

### Prince Edward Island

Sera collected from 1976 to 1979 showed HI and neutralizing antibodies to SSH in 33 of 215 (15.4%) snowshoe hares, in 48 of 248 (19.4%) horses and in 1 of 40 (2.5%) cattle [7].

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(J. A. EMBIL, B. L. MCFARLANE and H. ARTSOB)

REPORT OF THE ARBOVIRUSES AND SPECIAL PATHOGENS UNIT
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## 1. RISKS OF IMPORTING PRIMATES; A POXVIRUS EPISODE IN MARMOSETS

Dangerous exotic diseases can be introduced to disease free areas by travellers, legally or illicitly imported animals or pets. Naturally-acquired smallpox has been eradicated since 1977, but 110 cases of human monkeypox have been acquired in Africa since 1970. This presentation describes an outbreak of skin lesions caused by a poxvirus in marmosets and shows the potential risks in importing primates. Contrary to the Marburg tragedy this experience fortunately ended without sequelae.

Eighty marmosets (Callithrix jacchus), were captured in Paraguay, shipped via Spain to Florida, held for six weeks and then delivered to Toronto. Two marmosets had mild papular lesions upon arrival; three weeks later an outbreak occurred that involved 29 animals over a period of six months. Thirteen animals died, five without and eight with a history of skin lesions.

Electron microscopy of skin scrapings revealed a poxvirus. This finding was confirmed by the Centres for Disease Control in Atlanta (CDC). Attempts for virus isolation and laboratory tests which could have involved live virus were then conducted in CDC. Measures of response to an episode with a potentially dangerous exotic agent were undertaken.
Material from the lesions of a total of five animals were positive for the same characteristic poxvirus with tubular ridges and an outer envelope. Lack of growth on chorioallantoic membrane of embryonated eggs, absence of precipitation against anti-vaccinia antiserum in agar-gels, lack of antibodies by the haemagglutination inhibition test against vaccinia antigen in two animals with lesions, and histopathological and electron microscopic resemblance to Tanapox ruled out an orthopoxvirus. The agent was not morphologically related to the parapox group.

Separate colonies of rhesus monkeys and rabbits in the facility did not develop any lesions. Sera were obtained for reference from 21 subjects who were considered exposed to the monkeys or to clinical specimens, but none became ill.

ɑree animals received from the same supplier by the .S. Army Medical Research Institute for Infectious )iseases, Fort Detrick, Maryland were free from lesions.

Exposure of the marmosets to Old World monkeys could not be established. The origin of this agent could not be traced and it remains as an unidentified poxvirus either of or transmitted to this species of primate.

The animals were released to other institutions when the following criteria were satisfied: a) the marmosets were individually examined and were free from any lesions, and from exposure to any animals which had lesions, for six weeks at least, b) they were maintained under quarantine by the recipient institution(s) which should have facilities for strict isolation and for protecting handlers. The animals were accordingly released,did not develop any lesions and have been well.

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## 2. CONTINUING RECOGNITION OF CALIFORNIA GROUP VIRUSES (CGV) AS NEUROPATHOGENS IN ONTARIO

Association of the snowshoe hare serotype of CGV with human disease was documented in three cases in Quebec in 1978 (1) and in Ontario since then (2). In this report we describe two confirmed cases of CGV disease, further evidence on the emergence of members of these viruses as neuropathogens.

A 29 year old man with ten day history of mild illness developed headache, shaking chills, vomiting and photophobia. He was admitted to hospital, found feverish but with normal neurological examination. Profile of his cereberospinal fluid suggested however viral or partially treated meningitis. He received intravenous penicillin, had no fever or headache after three days and was discharged without sequalae a week later.

Sera obtained three and nine days post admission had haemagglutination inhibition(HAI) titres of 160 and 80 to CGV antigens but showed significant rise to Jamestown Canyon(JC) serotype by neutralization tests.

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(1) Fauvel, M. et al., Can. Med. Assoc. J. 122:60-3, 1980

(2) Mahdy, M. S. et al., Can. Dis. Wk. Rep. 8:185-192, 1982

Recent infection with other viruses could not be established.

Jamestown Canyon virus of the CGV was isolated in 1979 and 81 in Ontario and frequency of JC antibody in patients' sera has been rare. This is the only case of JC human disease so far detected in Canada; eleven cases have been documented in the U.S.A.

A 14 year old girl was admitted to hospital about four days post illness. She had a history of fever, sore throat and headache and presented with acute encephalopathy with high levels of blood ammonia. She lapsed into coma and died after two days; her case was diagnosed as Reye's syndrome(RS). Histopathological examination of samples from patient's brain and liver is in progress.

Recent infection with viruses usually associated with RS could not be demonstrated. The patient had however significant titres to the California group by the HAI (160,320), complement fixation(4,4) and neutralization (80,160). Serum obtained five days post onset was also positive for the snowshoe hare serotype of CGV by ELISA IgM capture. We have confirmed another case of snowshoe hare disease, but without sequalae, in the patient's area.

There are no previous reports on the possible association of CGV infections with Reye's syndrome. To our knowledge, this case would be the first record of snowshoe hare disease which appears to have caused fatality and presented as Reye's syndrome.

Since 1976 we have had a monitoring program to forewarn against activity of arboviruses and to assess their role in acute human disease of the central nervous system. In 1983, we confirmed three cases of CGV, the only cases detected till August in Canada.

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3. <u>WESTERN EQUINE ENCEPHALITIS(WEE); IS THERE ACTIVITY AND POTENTIAL FOR SPREAD IN NORTHWESTERN(NW) ONTARIO?</u>

Epizooticsand epidemics of WEE in midwestern Canada and north central states of the U.S. have repeatedly occurred and been reported for fifty years, records of virus activity in Ontario, are however, scarce. During the 1977 Manitoba outbreak, we identified WEE infection in a horse 20 miles from Kenora in NW Ontario. Single acute serum had antibody titres of 640 and 4 by the haemagglutination inhibition(HAI) and complement fixation(CF) tests. The animal had been destroyed by its owner; further samples of sera or brain material could not be obtained. The horse had no history of either vaccination or travel outside Ontario.

Patients' sera (355) submitted to the Thunder Bay Public Health Laboratory and to four area hospitals for other than viral infections were tested therefore against arbovirus antigens. Twenty from 355(5.6%) were seropositive; 14 to California group viruses, 3 to flaviviruses and three to WEE antigens. The three last patients had HAI and CF titres of 160, 80, 40 and 16, 4, 4 respectively; two of them had repeated history of travel to Winnipeg, Manitoba. All Ontario cases of St. Louis encephalitis (SLE) which occurred during the 1975 major outbreak of North America and the subsequent activity of 1976 occurred in the southwestern strip of the province. Furthermore the three flavivirus reactors had no detectable neutralizing antibodies to SLE and history of travel to flavivirus-endemic areas was not known.

During the 1981 Manitoba epidemic, a horse case of WEE in Fort Francis, Ontario and another in Dryden, Ontario were confirmed. Neither animal had a history of vaccination or travel outside this province. Sera from 174 chickens in four locations-excluding Dryden had no detectable HAI antibodies to the Manitoba, Highland J and Flemming antigens of the WEE complex. Adult mosquitoes of the primary vector(<u>Culex tarsalis</u>) were scarce and the density of the possible secondary vectors (<u>Culex restuans</u> and <u>Culiceta innornata</u>) was low. So far, there has been no record of human disease.

The potential exists for low grade WEE activity in NW Ontario. Investigations to assess the ecology for such potential are warranted.

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REPORT FROM THE NATIONAL ARBOVIRUS REFERENCE SERVICE

DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF TORONTO

TORONTO, ONTARIO, CANADA

## Mouse Neurovirulence of California group Viruses Isolated in Canada

A study was undertaken of the virulence of 17 snowshoe hare, SSH, and 11 Jamestown Canyon, JC, topotypes isolated from different parts of Canada. Tenfold dilutions of virus were inoculated by the intracerebral, i.c., and intraperitoneal, i.p., routes into 7 day old Swiss white mice. Two mice were inoculated per virus dilution. Prototype strains of SSH and JC virus were also inoculated. Mice were monitored for up to 14 days post inoculation.

The results, presented in Table 1, show $LD_{50}$'s of the different California, CAL, group strains after i.c. and i.p. inoculation as well as the log difference in mouse virulence between i.c. and i.p. challenge. It was assumed that the smaller the log difference between i.c. and i.p. challenge, the greater the neurovirulence of the CAL group strains.

The log differences in virulence between i.c. and i.p. challenge of SSH strains varied from 1.0 to 4.0 with most strains showing log virulence differences of 2.0 to 2.5. The JC strains exhibited a wider virulence difference between i.c. and i.p. challenge, i.e. 0.5 to 4.5, but, on average, were slightly more mouse neurovirulent than the SSH strains.

Differences in mouse neurovirulence could not be directly related to SSH or JC topotypes isolated from any particular part of Canada. However it was of interest to note that the SSH topotype exhibiting the greatest amount of mouse neurovirulence, isolate 134A12, was recovered from Entrelacs, Quebec at the residence of two boys who suffered from meningoencephalitis in 1978 (1).

## Reference

1. Fauvel, M., H. Artsob, C.H. Calisher, L. Davignon, A. Chagnon, R. Skvorc-Ranko and S. Belloncik. 1980. California group virus encephalitis in three children from Quebec. Clinical and serologic findings. Can. Med. Assoc. J. 122:60-64.

(H. Artsob, L. Spence and C. Th'ng)

Table 1. Comparative virulence of California group viruses in 7 day old Swiss white mice.

A. Snowshoe hare Strains

| Strain Number | Passage level | Place of Isolation | $LD_{50}$/0.02 ml i.c. | $LD_{50}$/0.02 ml i.p. | Log Difference in i.c. & i.p. virulence |
|---|---|---|---|---|---|
| R2929 | 4 | Ontario | $10^{-5.0}$ | $10^{-1.0}$ | 4.0 |
| 84-75 | 3 | Ontario | $10^{-5.0}$ | $10^{-2.0}$ | 3.0 |
| 72-Y-121 | 3 | Yukon | $10^{-6.0}$ | $10^{-3.0}$ | 3.0 |
| 76-Y-286 | ? | Yukon | $10^{-5.5}$ | $10^{-3.0}$ | 2.5 |
| 27B1B2 | 3 | Quebec | $10^{-4.5}$ | $10^{-2.0}$ | 2.5 |
| 25A3A4 | 3 | Quebec | $10^{-5.0}$ | $10^{-2.5}$ | 2.5 |
| 9-312 | 3 | Ontario | $10^{-5.5}$ | $10^{-3.0}$ | 2.5 |
| 185-82 | 2 | Newfoundland | $10^{-5.0}$ | $10^{-2.5}$ | 2.5 |
| Burgdorfer[a] | 21 | Montana | $10^{-5.5}$ | $10^{-3.5}$ | 2.0 |
| 9-180 | 2 | Ontario | $10^{-6.0}$ | $10^{-4.0}$ | 2.0 |
| 9-192 | 2 | Ontario | $10^{-5.5}$ | $10^{-3.5}$ | 2.0 |
| 852-854-76 | 3 | Ontario | $10^{-4.5}$ | $10^{-2.5}$ | 2.0 |
| 136B2 | 4 | Quebec | $10^{-5.0}$ | $10^{-3.0}$ | 2.0 |
| WM55-59-75 | 4 | Saskatchewan | $10^{-5.0}$ | $10^{-3.0}$ | 2.0 |
| 436-454-76 | 4 | Ontario | $10^{-5.5}$ | $10^{-4.0}$ | 1.5 |
| 74-31162/3 | 4 | Quebec | $10^{-5.0}$ | $10^{-3.5}$ | 1.5 |
| 78-Y-120 | 2 | Yukon | $10^{-4.5}$ | $10^{-3.0}$ | 1.5 |
| 134A12 | 4 | Quebec | $10^{-2.5}$ | $10^{-1.5}$ | 1.0 |
| **B. Jamestown Canyon Strains** | | | | | |
| 61V-2235[a] | 10 | United States | $10^{-5.0}$ | $10^{-0.5}$ | 4.5 |
| 78-80 | 4 | Newfoundland | $10^{-5.5}$ | $10^{-2.0}$ | 3.5 |
| 1-237-81 | 1 | Ontario | $10^{-6.0}$ | $10^{-3.0}$ | 3.0 |
| 9-71 | 2 | Ontario | $10^{-4.0}$ | $10^{-1.5}$ | 2.5 |
| 9-237 | 2 | Ontario | $10^{-5.5}$ | $10^{-3.5}$ | 2.0 |
| 136A3 | 3 | Quebec | $10^{-5.0}$ | $10^{-3.0}$ | 2.0 |
| 9-68 | 2 | Ontario | $10^{-3.5}$ | $10^{-2.0}$ | 1.5 |
| 9-178 | 2 | Ontario | $10^{-6.0}$ | $10^{-4.5}$ | 1.5 |
| Mn256-260 | 2 | Manitoba | $10^{-5.0}$ | $10^{-3.5}$ | 1.5 |
| WMC216-82 | 2 | Saskatchewan | $10^{-4.0}$ | $10^{-3.0}$ | 1.0 |
| 137A17 | 3 | Quebec | $10^{-5.0}$ | $10^{-4.0}$ | 1.0 |
| 9-54 | 1 | Ontario | $10^{-4.5}$ | $10^{-4.0}$ | 0.5 |

a = prototype strain