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

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COMMENTS FROM THE EDITOR

Every effort was made to deliver this issue of the Arthropod-borne Virus Information Exchange to you as soon as possible after the September 1 deadline. If your article was received after September 8, it could not be included in this issue. Late articles will appear in issue Number 44, March 1983.

I hope this issue reaches you before the 31st Annual Meeting of The American Society of Tropical Medicine and Hygiene in Cleveland, Ohio, November 7-11, since it contains abstracts of the arbovirus papers to be presented at that time.

Please let us hear from you concerning items of general interest to arbovirologists such as information on retirements, changes in employment, deaths, honors, meetings, publications, etc.

If you are considering submitting an article for the next issue, the deadline is March 1, 1983. Please refer to "Guide for Authors" in this issue before typing your next report.

Communications should be addressed to:

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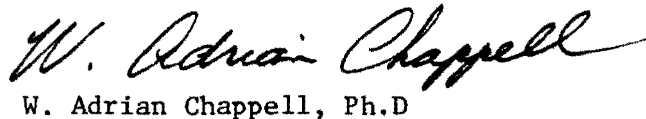
Any suggestions for improving the "Information Exchange" will be appreciated.



W. Adrian Chappell, Ph.D

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.

Dr. William Franklin Scherer 1925-1982

Dr. William F. Scherer died on 12 May 1982. Bill was one of the group that realized the need for an organization of arbovirologists that developed into the ACAV. He served on the Board of Directors from 1961-65 (Chairman 1963-65) and from 1978 to May 1982. He served on the subcommittees for Information Exchange and Interrelationships among Catalogued Arboviruses, and he chaired the Subcommittee on Laboratory Safety. The report of the latter was of major importance in the development of national standards of laboratory safety for arboviruses by the NIH.

Bill's early work pioneered the propagation of polio and later other viruses in tissue culture systems; his studies with Ed Buescher on the ecology of Japanese encephalitis virus set new standards in field/laboratory research. In 1963 with co-workers, he isolated Venezuelan encephalitis virus in Mexico concurrently with its isolation by CDC researchers in Florida; he also serendipitously discovered, and then refined the use of hamsters as sentinels and in the laboratory and in the field for the study of VE virus. The use of hamsters as sentinels revolutionized field research for VE virus. His subsequent studies elucidated the ecology of the enzootic strain and mapped its range in northern Middle America. Field work in Mexico and Guatemala 1963-1968 provided a baseline for comparisons of the epidemiology and range expansion of the explosive 1969 epizootic of VE virus that reached Texas in 1971. Field research in Middle America 1970-80 sought to determine whether the epizootic strain had become established in enzootic foci and if not, why. His discovery with co-worker

Eddie Cupp that the vector of the enzootic strain of VE virus was imcompetent to act as a vector of the epizootic strain was a revolutionary contribution.

In the laboratory Bill and his students made major contributions to the characterization of geographical isolates of VE virus. With co-worker Michael Wiebe, he defined the number of envelope polypeptides among strains of VE virus and, utilizing hydroxylapatite column techniques refined by his former student Peter Jahrling, they searched for epidemic strain virions among isolates of enzootic strains from Middle America and Colombia.

Bill's leadership, including the Presidency of the American Society of Tropical Medicine and Hygiene (1980-81) and service to the fields of microbiology, virology, and especially arbovirology, are legend. His participation on councils, advisory bodies, peer review panels and editorial review boards has been detailed elsewhere (Tropical Medicine and Hygiene News).

Among his other contributions, two must be mentioned. First, Bill was a crusader for open information exchange and cooperative research among laboratories- if it was good for our science, it would be good for all the individuals involved. He organized a multilaboratory comparison of serological methods used in identifying infections caused by dengue viruses, and a four-laboratory cooperative study of dengue infections in chimpanzees. At the 1969 ACAV meeting Bill spearheaded a working session of workers involved in the past summer's chaotic efforts to document the explosive VE outbreak in Middle America. That sharing of information led to organized non-conflicting efforts in the subsequent two years of the epizootic. Although largely uncredited, Bill was the moving force behind the 1972 PAHO Workshop/Symposium on VE virus that yielded the greatest compilation of information on any single arbovirus up until that time.

Secondly, Bill had a deep-seated interest in Korean hemorrhagic fever and worked actively to catalyze the exchange of scientific materials and ideas.

Finally, Bill was a leader in the training of graduate students in the field and laboratory (and he considered the field a wonderful wall-less laboratory) - teaching by example. In spite of his administrative duties as Chairman of the Department of Microbiology at Cornell University Medical College, he spent from 20 to 40 hours a week at the laboratory bench with his students and staff. His energy in the field, his attention to detail, his constant search for new questions (and their answers) and concepts, set standards that his students and associates will never forget.

(Submitted by Robert W. Dickerman)

Prof.Dr.Dr.h.c.Manfred Mussgay 1927-1982

Professor M.Mussgay died very unexpectedly on 6. July 1982. He was Director of the Federal Research Institute for Animal Virus Diseases. Manfred Mussgay spent much of his professional career on research of the arboviruses. Because of this keen interest in tropical virus diseases he spent two years at the 'Instituto Venezolano de Investigaciones Cientificas' at Caracas where he worked in close collaboration with Professor Bergold.

Besides quite a number of publications concerning the structure of arboviruses his sound knowledge of arboviruses led to him writing two monographs. Both of these dealt with the replication of arboviruses. In addition he investigated the antigenic relationships between arboviruses and also took an active part in the development of arbovirus vaccines.

In recent years his research activities were directed towards the study of virus induced mouse tumours as a model for the origin and development of tumours of animal virus diseases.

In the last years he took an active part in the development of a synthetic vaccine for foot and mouth disease by means of gene technology.

Professor Mussgay always retained a very keen interest in arbovirology which is documented by the support he gave quite a number of his collaborators working in that field.

Only last year he was an active participant at a WHO Meeting on Rift Valley fever.

He will be remembered by many as a man of high scientific standing as well as a friend.

G. Wittmann

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

A booklet collection is being edited by our University to help spanish-speaking students and profesionals interested in the field of human and veterinarian medical Virology.

Each booklet contains the current knowledges on a whole virus family except the first one having a general information. Up to now nine booklets are out and one is in press. They can be adquired at cost prices since the University is a nonprofit organization, or otherwise could be exchange by other publications among libraries.

Any one interested in this publication can get more information at the following mailing address:

Departamento de Publicaciones y Canje
Pinto 399 - UNCPBA - Tandil (7000)
Pcia. Buenos Aires - ARGENTINA

The booklet already available are:

#1: Generalidades (42 pgs.)
#2: Familia Rhabdoviridae (46 pgs.)
#3: Familia Iridoviridae (12 pgs.)
#4: Familia Retroviridae (34 pgs.)
#5: Familia Togaviridae (68 pgs.)
#6: Familia Herpetoviridae (48 pgs.)
#7: Familia Arenaviridae (56 pgs.)
#8: Familia Paramyxoviridae (40 pgs.)
#9: Familia Picornaviridae (104 pgs.)
#10: Familia Bunyaviridae (68 pgs.)

Norma E. Mettler

Position Change

Associate Professor S. K. Lam of the Department of Medical Microbiology, University of Malaya, Kuala Lumpur, Malaysia, will be seconded to the Virus Disease Unit, World Health Organization, Geneva for a period of two years with effect from May 1982. During his absence, Dr. T. Pang will be heading the Dengue Research Programme which was established in 1980 and funded by the W.H.O. Regional Office in Manila.

Dr. Lam, who has been with the University of Malaya for 16 years, is primarily interested in the Rapid Diagnosis of Viral Infections and in 1979 the Department was designated a W.H.O. National Centre for the rapid diagnosis of viral infections. One of the areas of research being undertaken in his department is to establish a quicker method for the diagnosis of dengue and Japenese Encephalitis infections.

Among the many duties that Dr. Lam will be involved in are:

1. To develop, in close collaboration with the Regional Offices, a comprehensive programme on simple and rapid techniques for diagnosis of viral diseases, with special emphasis on the training component and the preparation of reagents at the Regional level, while securing quality control of reagents and of tests at the global level.

2. To develop, coordinate and implement programmes on: viral hepatitis, sexual, congenital and perinatal infections caused by viruses, chlamydiae and mycoplasmas, including surveillance, prevention, treatment and research.

3. To be responsible for surveillance of, and to advise on prevention of and research on arboviruses, including yellow fever, and rodent-borne viruses.

4. To develop and coordinate the programme on the preparation, testing and distribution of viral reagents.

5. To be responsible for the programme on enteric viral infections and to cooperate with the Diarrhoeal Diseases Control Programme, with regard to gastroenteritis viruses, with special emphasis on rotaviruses.

International Symposium

An <u>INTERNATIONAL SYMPOSIUM ON CALIFORNIA SEROGROUP VIRUSES</u> will be held at the Bond Court Hotel, Cleveland, Ohio, November 12-13, 1982. This symposium will bring together experts to discuss the current knowledge of the ecology, epidemiology, virus variation, genetics, pathogenesis, clinical aspects, prevalence, economic impact, diagnosis and control of LaCrosse encephalitis and other California serogroup bunyaviruses. Emphasis will be placed on the disease, the viruses, and the arthropod vectors of these viruses.

A copy of the Program is attached. We would appreciate it if you would post this Announcement and Program so that your co-workers can be made aware of this meeting. For additional details, please write or call: Charles H. Calisher, Ph.D., Chief, Arbovirus Reference Branch, VBVDD, CDC, P.O. Box 2087, Fort Collins, Colorado 80522---(303) 221-6459

MONITORING OF AN EPIZOOTIC WAVE OF YELLOW FEVER IN PANAMA

Abdiel J. Adames, Gustavo Justines, Pauline H. Peralta,
William C. Reeves and Pedro Galindo

Gorgas Memorial Laboratory
Republic of Panama

Since 1948, yellow fever (YF) epizootics have occurred at approximately 8-9 year intervals in Panama's Darien rainforest. The last YF epizootic was detected in the area in 1971 and by 1974 had reached the populated lower Bayano River valley, on its way to the Panama Canal. Several human cases and deaths occurred in the wake of that epizootic wave.

YF is monitored by estimating the howler monkey population density and measuring YF antibody prevalence in the populations of howlers and spiders. In 1979 field teams were sent to some areas along the route of previous YF epizootics to determine the status of YF in the area. In May 1979, monkeys from Altos de Nique, in southern Darien adjacent to the Colombian border, were sampled; in June, sera were gathered in Cerro Tacarcuna and in September, Cerro Sasardí, in eastern and northern Darien, respectively. At Nique, forty-five monkeys were collected, 35 black spiders and 10 howlers. All 10 howler monkeys had neutralizing antibody titers ranging between 1:128-1:256. Twenty-six of the spider monkeys had demonstrable YF antibody. At Tacarcuna, a large proportion of monkeys collected was also immune to YF. Nineteen of 31 spider monkeys had high level YF neutralizing antibody. In the trip to Sasardí, blood was collected from 22 howler monkeys of which only 6, all old animals and presumably survivors of the previous epizootic, had low levels of YF antibody.

In 1980-82 attempts were made to determine if the epizootic have had continued on its way to the Panama Canal. In 1980, monkeys were captured in Rio Armila and Cerro Porcona, and in January and October 1981 as well as May 1982, in an area around Cerro Anachanuna. Results indicated no recent YF activity in all collections of 1980 and 1981, but in the 1982 collection five of ten spider monkeys and 1 of 10 howler monkeys (all born since the last epizootic in the 70's) had YF antibody titers of 1:64-1:128.

Results thus indicate that YF is now active in the Darien forest and the wave is moving northward and westward toward the Panama Canal, somewhere between Cerro Anachacuna and the Serrania de San Blas.

Plans have been drawn to attempt to locate the head of the epizootic wave and to try to elucidate the dynamics of transmission of YF virus in the forest.

ARBOVIRAL DISEASE IN AN ENVIRONMENTAL ASSESSMENT FOR A HYDROELECTRIC PROJECT IN WESTERN PANAMA

Abdiel J. Adames, Pauline H. Peralta, Curtis G. Hayes,
Carl M. Johnson and William C. Reeves

Gorgas Memorial Laboratory
Republic of Panama

Arboviral activity was assessed as part of a biomedical impact evaluation for the proposed Tabasara River Hydroelectric Project in Western Panama. The study area ranges from sea level to an elevation of 500 m in the upper watershed. It covers 3 life zones and includes several ecological associations. The human population consists of 3 major ethnic groups: Amerindian (Guaymí), Caucasian and Mixed Race.

Between April and August 1981, a clinical-epidemiological survey was conducted in representative communities, and over 3,000 persons (about one-third of the total population of the area) were examined and bled. Sample collections of blood-sucking mosquitoes, phlebotomine sandflies and *Culicoides* midges were made. Sera were examined for specific arboviral antibodies by plaque neutralization in Vero cell cultures by a constant virus-serum dilution method. Sixteen viruses, all previously isolated in Panama and most recognized as capable of causing human disease, were used. All human sera were tested against VEE (enzootic I-D), SLE and ILH viruses. A systematic 1-in-4 subsample was assayed for antibodies to EEE, MAY, BSQ, OSSA, MAD, GMA, WYO, GRO, CAL, PT, CHG, VSV-I and VSV-N.

None of the human sera tested had antibodies to EEE, BSQ, OSSA, MAD or GMA group virus. Antibodies to WYO and CHG were negligible. Overall prevalence of VEE, SLE, ILH, MAY, GRO, CAL and PT antibodies was quite low. The age distribution indicated continuing, low level endemic activity of VEE, SLE, ILH, CAL and PT viruses; GRO virus had not been active for many years but previously may have occurred epidemically. By contrast, both VSV-I and VSV-N are highly endemic throughout the area. While most of the viruses showed focal distribution, SLE, VSV-I and VSV-N have been ubiquitous. High male: female prevalence ratios among persons 30 years and older indicated that infection with VEE, MAY and CAL virus was frequently acquired away from home. Such a pattern for ILH, PT and SLE viruses was less pronounced. No sex difference was found for either VSV-I or VSV-N at any age, however.

Several positive correlations of virus activity with ecological factors were found. The effect of a future hydroelectric project in the area on arbovirus activity may be predicted.

Regional Variation In *Aedes aegypti* Infectability For Yellow Fever Virus

By

Aitken, T.H.G., Beaty, B.J., Tabachnick, W.J., Wallis, G., Miller, B.M. and Scott, T.

Twenty-four populations of *Aedes aegypti*, encompassing the broad geographic range of the species, were compared in their ability to become orally infected with yellow fever virus. Infection rates ranged from 8% (5/59) in one sylvatic population from East Africa to 57% (104/184) for a domestic population from East Africa. There was overlap in infection rates from the respective geographic regions. However, when the rates from each region were analyzed statistically, there were no statistical differences between mosquito populations from a given geographic region (Table 1). The only exception was East Africa where sylvatic and domestic forms of *Aedes aegypti* coexist and are markedly different in vector competence.

Populations from the West Indies were the most susceptible to oral infection (46%-148/322), and the West African populations were least susceptible (12%-47/401). Asian populations were intermediate in susceptibility (29%-208/715.

Table 1

Vector Competence of Geographic Regions of *Aedes aegypti*

Geographic Region	Number examined/Number positive	Percent
West Indies	148/322	46
East Africa Domestic	169/406	42
South-Central America	112/356	31
Asia	208/715	29
Mexico-U.S.	97/439	22
East Africa Sylvan	13/90	14
West Africa	47/401	12

A MODEL FOR THE ENCEPHALITIC FORM OF RIFT VALLEY FEVER

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The pathogenesis of Rift Valley fever in the gerbil, Meriones unguiculatus, was investigated as a possible model for studying the encephalitic form of the disease in man. The ZH 501 strain of Rift Valley fever virus (RVFV) used was isolated from a fatal human hemorrhagic fever case during a recent Egyptian epidemic. Resistance to subcutaneous (s.c.) inoculation of RVFV was found to be an age-dependent phenomenon with 100% mortality at 3 weeks, decreasing to approximately 25% by ten weeks of age in the outbred gerbils. The mean time of death increased from 6 to 12 days, respectively. The fatal lesion in both groups was necrotizing encephalitis. Encephalitis occurred in about one-fourth of the 10-week-old adults independent of the virus dose (10^1 to 10^7 pfu, s.c.) suggesting that the outbred population consisted of exquisitely resistant and susceptible individuals. Viral replication and histological lesions were followed serially in six tissues throughout the course of the infection in young (4 week) and adult (10 week) gerbils. The liver, spleen, adrenal, kidney, and lung were histologically normal with little or no viral replication, except for the spleen which averaged about 10^3 pfu/g during the infection for both groups. Virus growth was evident in the brain tissue of young gerbils from day 4 (10^3 pfu/g) through day 7 (10^6 pfu/g), the last day the young gerbils survived. Virus replication was first and only detected in the brain tissue of one adult gerbil on day 7 (10^4 pfu/g) in the timed sequential survey. Additional adults selected when moribund, had approximately 10^7 pfu/g of virus in the brain tissue on days 8 and 11. Necrotizing encephalitis was only detected on days 6 and 7 in young gerbils with brain virus titers of approximately 10^6 pfu/g, while all adult with brain virus titers had evidence of necrotizing encephalitis. When young and adult gerbils were inoculated with a low dose (50 pfu) of virus intracranially, there were no detectable differences in the course of infection and all animals died at approximately day 7 from necrotizing encephalitis. Thus, adult gerbils either block RVFV entry into the central nervous system (CNS) or block viral replication there. This model seems to mimic what is known about RVF encephalitis in man. Further, this gerbil model should prove useful in establishing prophylactic means of protection and treatment of Rift Valley fever encephalitis.

Interference between California group viruses in *Aedes triseriatus* mosquitoes

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Interference between California group viruses was demonstrated in *Aedes triseriatus*. Mosquitoes were intrathoracically infected with a ts mutant of La Crosse (LAC) virus. One week later these mosquiotes as well as noninfected control mosquitoes were inoculated with certain wild type California group or other arboviruses. After one week extrensic incubation, mosquitoes were triturated and the suspensions were assayed for virus at a nonpermissive temperature (39.8°C) for the LAC ts mutant. Geometric mean titers are shown in the Table. Prior infection with LAC virus had no effect on subsequent replication of the flavivirus, West Nile, and the bunyavirus, Guaroa. In contrast, there were significant decreases in titer in mosquitoes subsequently infected with wt LAC and Snowshoe hare (SSH) viruses. Replication of the more distantly related tahyna (TAH) and trivittatus (TVT) virus was completely inhibited.

The mechanism of the interference is not known. However, since the homologous LAC and closely related SSH viruses did replicate in previously LAC infected mosquitoes but the more distantly related California group viruses (TAH and TVT) did not, the interference is apparently mediated by a different mechanism than conventionally attributed to defective interfering particles.

Table

Geometric mean titer (PFU) per mosquito

Wild type virus	Preinfected with LAC-II-5	Controls
La Crosse	1.29×10^2	1.51×10^4
Snowshoe hare	8.3	4.9×10^2
Tahyna	0	2.88×10^3
Trivittatus	0	4.79×10^3
Guaroa	1.29×10^4	1.58×10^4
West Nile	2.0×10^4	4.17×10^4

EVIDENCE FOR THE PERSISTENCE OF RIFT VALLEY FEVER VIRUS IN EGYPT*

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Abstract

A seroepidemiological study of cattle, camels, sheep and goats is in progress to detect evidence of RVF virus infections in Egypt. Sentinel herds have been selected, and the animals are being bled at 6 month intervals. Additional sera are being collected from herds with disease problems and from herds resident in other areas of Egypt on an unscheduled basis. The sera will be assayed by indirect fluorescent antibody, complement fixation, and enzyme linked immunosorbent assays and positive sera will be confirmed by plaque reduction assays. To date 168 sera collected from native animals near Aswan have been assayed by the CF test and 10 (6%) were positive. When the positive sera were run in a plaque reduction assay 8 were confirmed. These data indicate that RVF virus is still present in Egypt although no clinical disease attributable to the virus is being reported in man or animals.

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KINETICS OF JAPANESE ENCEPHALITIS IgM AND IgG IN HUMAN SERUM AND CSF

BY: Donald S. Burke*, Thanom Laorakpongse, Suchard Chantavibul, Ananda Nisalak, Michael A. Ussery.

A prospective study of the kinetics of Japanese encephalitis (JE)antibodies in serum and cerebrospinal fluid (CSF) of encephalitis paitnets was conducted at the Kampangphet Thailand Provincial Hospital during the 1981 epidemic season (1 July-1 September). Antibodies were measured by hemagglutination inhibition (HAI) and by newly developed "antibody capture" solid phase enzyme-linked immunoassays for detection of isotype specific, JE specific IgM or IgG (JE MACELISA or JE GACELISA). All patients who met criteria for a clinical diagnosis of acute viral encephalitis had specimens of serum and CSF obtained within 12 hours of hospital admission and again 7, 30, and 180 days later. Also, finger stick blood samples were obtained from all patient family members within seven days of the patients hospital admission.

32 patients with a clinical diagnosis of viral encephalitis were studied: 27 were serologically confirmed to be JE infected, two were definitely not flavivirus infected, and in three results were incomplete or equivocal. All confirmed cases but one were children less than 15 years old. Seven patients died. Of 267 family contacts screened, five (all children) were found to have had recent asymptomatic JE infections as detected by the presence of high levels of serum JE MACELISA antibodies (4% of all patient siblings). The percent of specimens positive from definite JE cases follows:

		Day 0	Day 7	Day 30	Day 180
Serum	JE HAI	79%	100%	100%	96%
"	JE MACELISA	53	100	100	39
"	JE GACELISA	21	79	100	50
CSF	JE HAI	0	11	33	0
"	JE MACELISA	68	100	96	72
"	JE GACELISA	47	89	100	100

Specific anti-JE activity of both IgM and IgG antibodies in CSF was almost always greater than that in serum, probably reflecting local synthesis of antibodies into the CSF. As controls, 25 CSF samples were obtained from patients with other diseases with possible nervous system involvement (but none with a clinical diagnosis of viral encephalitis); all were negative for JE MACELISA antibodies, but 48% had JE GACELISA antibodies. For additional controls, serum and CSF samples were obtained from five asymptomatic but JEV infected siblings of encephalitis cases; all had JE MACELISA antibodies in serum (100%) but none had JE MACELISA antibodies in CSF (0%).

Detection of IgM anti-viral activity in CSF by antibody capture ELISA may be an imporant, new approach to the rapid diagnosis of flavivirus encephalitis.

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Presenting author identified by underline.

A PROSPECTIVE STUDY OF DENGUE INFECTIONS IN A BANGKOK SCHOOL

BY: Donald S. Burke, Ananda Nisalak, David E. Johnson, Robert McNair Scott.*

1748 children (ages 4-15 years) at a lower socioeconomic group school in central Bangkok were prospectively studied in 1980 to determine if pre-existing immunity to flaviviruses conferred added risk of severe dengue illness. Five milliliter clot blood samples were obtained from all children in June 1980 and again in January 1981. Overall, 1043 students (58%) had detectable ($\geq$1:10) HAI antibodies to one or more of the antigens tested (D1, 2, 3, 4, and JE) in their 1980 serum specimens. Classrooms were visited daily to determine absentees; any child absent for two or more days because of a febrile illness had a blood specimen obtained on return to school and again two weeks later. Acute dengue infections were diagnosed by a rise in HAI antibody titer and/or detection of dengue specific IgM antibodies capture radioimmunoassay. If the patient had visited a physician or had been hospitalized, clinical records of the illness were reviewed.

Seven study children were hospitalized with a clinical diagnosis of dengue hemorrhagic fever (DHF) during the six month study period. All seven (100%) had pre-existing HAI antibodies in their June 1980 serum samples and had high, levels ($\geq$1:2560) of broadly reactive HAI antibodies in their convalescent illness blood samples. The rate of hospital admission for DHF of antibody positive children (7/1043) was significantly (p=.027, Fisher's exact test) greater than that of antibody negative children (0/705). An additional nine children were clinically diagnosed as possible DHF by private physicians but were not admitted to hospitals; six were incorrectly diagnosed and lacked serologic confirmation, one was a child with no pre-existing antibody, and two had pre-existing antibody.

Of a total of 37 children showing a seroconversion from HAI negative to positive during the study period (as evidence of a first dengue infection) none was admitted to a hospital with DHF. Of 51 children showing a four-fold HAI titer rise to three or more antigens during the study (as evidence of a second or subsequent dengue infection) seven were admitted (0/37 versus 7/51 p=.018, Fishers exact test). Thus, antibody positive children were at higher risk of development of DHF during the 1980 Bangkok dengue epidemic season than were antibody negative children.

A surprise finding was the polarity of the clinical presentations of the 51 dengue infections in sero-positive children: in 42, the infection was minimally symptomatic with no school absence, while among another nine with symptomatic infection causing school absence, an average of 10.2 $\pm$ 4.3 days of school were missed, and seven of these nine children were hospitalized for DHF.

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CROSS-REACTIVITY OF FLAVIVIRUSES BY NEUTRALIZATION TESTING

Charles H. Calisher, Nick Karabatsos, Joel N. Dalrymple, Robert E. Shope

In 1974 de Madrid and Porterfield reported a cross-neutralization study of 41 registered flaviviruses. Since there are now 62 flaviviruses registered in the Arbovirus Catalogue, we felt that it would be useful to review the previous study and to extend them to include the newly recognized members of the serogroup.

Sixty registered flaviviruses and four unregistered viruses were tested with 65 antisera. The 60 registered viruses were first titrated in serially propagated Vero, $LLCMK_2$ and PS cells and in primary Pekin duck embryo cells. Optimal systems were chosen, based upon titer and size and clarity of plaques, and the homologous serum dilution-plaque reduction neutralization titers determined for each immune reagent. All viruses were then tested against all antisera at 1:20 dilutions and end-points subsequently determined for those which were positive in screening tests.

The results of our tests in general supported and extended the findings of de Madrid and Porterfield. We were able to provisionally divide the flaviviruses into eight subgroups based upon antigenic relatedness (cf de Madrid and Porterfield). Final subdivisions will depend upon further mathematical analyses not available at this time.

1. (12 viruses) Negishi, Langat, Kyasanur Forest disease, Omsk hemorrhagic fever, Louping ill, Central European encephalitis, Powassan, Russian Spring-Summer encephalitis, Apoi, Kadam, Royal Farm and Saumarez Reef.

2. (9 viruses) Dakar bat, Entebbe bat, Bukalasa bat, Rio Bravo, Sokuluk, Saboya, Phnom Penh bat, Carey Island and Yokose.

3. (5 viruses) Modoc, Cowbone Ridge, Jutiapa, Sal Vieja and San Perlita.

4. (13 viruses) Japanese encephalitis, Murray Valley encephalitis, St. Louis encephalitis, West Nile, Kunjin, Usutu, Kokobera, Stratford, Alfuy, Ilheus, Rocio, Koutango and Karshi.

5. (4 viruses) Israel turkey meningoencephalitis, Ntaya, Tembusu, and Bagaza.

6. (4 viruses) Banzi, Edge Hill, Uganda S and Bouboui.

7. (4 viruses) dengue-1, dengue-2, dengue-3 and dengue-4.

8. (1 virus) Sepik.

In addition, 13 viruses showed either no relationship to any other flavivirus, reacted only at marginal levels and could not be placed in a subgroup with confidence, or only the antiserum (Wesselsbron) was available to us: Caciapacore, (CSIRO-122), yellow fever, Bussuquara, Wesselsbron, Montana Myotis Leukoencephalitis, Spondweni, Naranjal, Aroa, Tyuleniy, Jugra, Tamana bat and Zika.

BRAIN RNA POLYMERASES ACTIVITY AFTER VEE VIRUS INFECTION IN MICE

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RNA polymerases activity was measured in brain of young and adult mice 16 hrs after i.p. inoculation of a dilution 10^{-2} of a VEE virus suspension.

No modification in the activity of RNA polymerase I was detected in the animals at any age, however a significant decrease in the activity of RNA polymerase II was observed in both groups of animals.

In the young animals this decrease reached almost 50% while in the adult ones, it was only 25%.

The results showed that young mice are more sensitive than adults to the effect of VEE virus infection on RNA polymerase II activity.

Use of Virus-Specified Protein Profiles From Infected Cells for the Identification and Classification of Orbiviruses

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Classification of orbiviruses has been done by the use of group-reactive serological tests, (agar gel diffusion precipitin, complement-fixation and fluorescent antibody), to group isolates and type-specific tests, (virus neutralization and cross-protection), to type viruses. Many isolates do not always fit neatly into serogroups, and the problem of intergroup relationships and intragroup variation is becoming more important as detailed serological comparisons are being made and some new isolates cannot be definitely classified. The serological tests probably only involve protein products from 2 or 3 of the 10 double-stranded ribonucleic acid gene segments. It was proposed to analyse all of the proteins translated from the 10 gene segments. Virus-specified proteins were labeled with ^{35}S-methionine in infected cells and a whole cell extract made in sodium dodecyl sulfate containing buffer which was then analysed by electrophoresis in 10% Laemmli polyacrylamide slab gels. The gel profiles (10 to 12 possible virus-specified protein bands) were detected by autoradiography. The analyses showed that each orbivirus serogroup had a distinctive protein profile for its members when compared to different serogroup members. Specific serotypes, within a serogroup, usually varied in the migration pattern of 2 to 5 proteins. Analyses of a number of different isolates of serotypes in the bluetongue and Palyam serogroups showed that isolates of the same serotype had indistinguishable protein band patterns, except for CSIRO 156 (Bluetongue virus type 1) isolates where differences were observed in the migration of one protein band. Analyses have been performed for virus-specified protein profiles of Australian bluetongue, epizootic hemorrhagic disease of deer, Eubenangee, Palyam, Wallal, Warrego and Corriparta serogroup isolates. The technique is easy to perform; results are rapidly obtained (much faster than having to produce reference antisera for serological comparisons); and it not only gives similar findings to serological tests but also additional information which may prove useful for orbivirus classification.

Vector Competence of *Haemagogus equinus*, a Sylvatic Vector of Yellow Fever in Tropical America

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The capacity of *Haemagogus equinus* to transmit yellow fever virus by bite was demonstrated by Waddell and Taylor (1945). *Hg. equinus* is the most widely distributed member of the genus in Middle America. Collection records indicate that morphological variations exist and that the species breeds in several different habitats suggesting a good deal of genetic variability within the species.

To study variation of *Hg. equinus* vectorial capacity for yellow fever virus, we tested *per os* susceptibility in females of a colony initiated in 1975 from specimens collected at Bayano, Panama and in the F_1 female progeny from 1981 field collected specimens from Bayano. The 1981 collections were grouped as early, mid and late season mosquitoes. Mosquitoes were infected by feeding on cotton-pledgets saturated with different YF virus concentrations. Females that fed were incubated for 10 days at 28°C and assayed for virus by plaquing in Vero cells. Colony females were more susceptible than females from the field collected in the same area six years later. The results showed that 13/13 colony females were infected with a virus dose of $10^{5.9}$ PFU; while a dose $10^{7.0}$ PFU was necessary to infect 62/86 field females. Among field mosquitoes the late season females were slightly more susceptible than those from early and mid season. The ID_{50}'s were $10^{4.5}$ PFU for the colony mosquitoes, $10^{6.6}$ PFU for the field early season, and $10^{5.5}$ PFU for the field mid and late season. The eggs from the more resistant and more susceptible females from the colony and field strains have been collected individually, and susceptibility testing is in progress to select lines for genetic studies.

The other parameter of vector competence quantitated was transmission. In experiments infecting the colony mosquitoes by pledget feeding, results showed the following pattern of transmission: After 10 days of incubation, 1/11 infected females successfully transmited; on day 14, 0/17; on day 21, 2/10 and on day 28, 5/7. Colony females

were also infected by feeding on a viremic _Aotus_ monkey circulating $10^{10.3}$ PFU/ml of YF virus. Ten females were assayed for virus each day until day 12. The virus decreased and disappeared by day 3. Virus was measurable on day 4, and titers increased thereafter. On day 6, 5/5 females were able to transmit YF virus to suckling mice.

Pledget feeding in wild females or newly established colonies of _Hg. equinus_ is not efficient. A technique employing male mice injected in the tail vein with virus is proving useful. With field collected specimens, between 90-95% feeding is observed. These and other results will be presented and discussed.

Transmission of Dengue Virus by Orally Infected Aedes triseriatus

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Three species of mosquitoes belonging to the Aedes triseriatus group were tested for their potential to serve as vectors of dengue (DEN) 1 virus. Mosquitoes were infected orally by allowing 5-day-old females to feed through a membrane on an artificial feeding mixture with a virus titer of 10^7 MID_{50} per ml. After engorgement, mosquitoes were held for 14 or more days at 28°C before immunofluorescent examination of head squashes. Transmission trials were conducted by allowing mosquitoes to feed in groups of 10 on a small volume (300 μl) of defibrinated blood after 14 or more days of incubation. After the mosquitoes had fed, the remaining blood was centrifuged and the supernatant fluid assayed for the presence of dengue virus by inoculation into Toxorhynchites brevipalpis mosquitoes. These were held for 14 days at 28°C and examined by the head squash technique. Aedes aegypti VILLALBA, a strain previously shown to be highly susceptible to oral infection with DEN virus, served as a control.

Variation in susceptibility to infection was noted among the 3 strains of Ae. triseriatus tested. Infection rates were highest in the KERRVILLE strain (78.1%) followed by the WALTON (61.5%) and VERO BEACH (45.1%) strains. Aedes aegypti VILLALBA used in these same experiments had an infection rate of 59.2%. In vitro transmission was demonstrated for all three strains of Ae. triseriatus and for the Ae. aegypti control.

Two other species of the Ae. triseriatus group, Ae. brelandi CHISOS and Ae. hendersoni SOCOL, were also tested for susceptibility to infection with the same virus dose indicated above. Infection rates were 46.6% and 25.0% respectively.

Aedes triseriatus WALTON was tested for ability to transmit DEN 1 virus transovarially. Twelve-day-old females were infected by inoculation and given normal blood meals 5 days later. The resulting progeny were tested as fourth instar larvae. Supernatant suspensions from homogenized pools containing 100 larvae each were assayed for DEN virus by inoculation into Tx. brevipalpis. No infected progeny were detected among the 1,105 larvae tested.

These results will be discussed in terms of the relative abundance of Ae. triseriatus group mosquitoes in the southern United States and the urbanization of this group through the exploitation of discarded tires as larval habitats. The role that may be played by this potential new vector in an outbreak of dengue will also be discussed.

Evidence for a Minimum of Two Distinct Antigenic Domains on the G_1 Protein of LaCrosse Virus

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A number of monoclonal antibodies to CAL serogroup viruses have recently become available. In our laboratory we have seven monoclonals specific for the G_1 protein of LaCrosse virus and with different reaction patterns with other members of the CAL serogroup. In biological tests, some are positive for HI and neutralization, some for HI alone, and others are negative in both assays. To begin to determine the number of distinct antigenic regions that are involved in these responses, four of the antibodies which have similar affinities for the G_1 protein have been used in cross-competition studies. Each antibody was conjugated with enzyme and used in ELISA with homologous and heterologous competiting antibodies. The data obtained indicate that at least two separate antigenic domains exist in the G_1 protein. One of these is involved with the HI and neutralization responses. An epitope found only on the G_1 of the original LaCrosse isolate occurs in this region. It also contains a minimum of two other epitopes. One of these is common to all LaCrosse isolates so far examined but is not found on other CAL viruses. The second is common to LaCrosse, Snowshoe hare, Jamestown Canyon, California encephalitis, and Keystone viruses. The second antigenic domain detected is not involved in either HI or neutralization and the only epitope identified at present is common to LaCrosse, Snowshoe hare, and Keystone viruses.

Jamestown Canyon virus infections in the Indiana human and deer populations.

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Sera from humans and white-tailed deer were used to determine the extent of infection in the statewide Indiana human population and select deer populations with Jamestown Canyon virus (JCV). Specifically we wanted to identify the foci of infection in the human population by geographic location and determine the antibody prevalence in that population. An area with the highest prevalence of antibody to JCV in humans was selected for a determination of the age-specific antibody prevalence in its white-taled deer population.

More than 10,200 human blood samples were collected statewide by the Indiana State Board of Health with informed consent through the cooperation of local physicians, clinics and hospitals, medical laboratories, and blood banks. Sera were screened for antibody to California group arboviruses, JCV, La Crosse, and trivittatus, using the standard serum dilution neutralization test in microtiter in African green monkey kidney (Vero) cell cultures. Antibody prevalence data for the human population were visually measured by use of a computer-drawn "map" as a means of identifying foci of infection. Deer sera were collected in northern Indiana through the cooperation of the Indiana Department of Natural Resources and with the aid of individual hunters; we provided hunters with vials and requested they collect fresh blood at the kill site. Deer sera were screened for antibody to California group viruses using the same method as for human samples.

Antibody prevalence to JCV in the overall human sample was low; only .63% of the sample population was seropositive. However, the 64 seropositive samples came from residents distributed throughout the state. Approximately three-fourths of the seropositives came from rural residents, the remainder from residents of counties predominantly urban-suburban. Specific foci of human infection were seen throughout the state through the use of the computer-drawn "map" and were most pronounced in the northern third of the state. Antibody prevalence in 116 deer harvested within the major focus was above 95% for California group viruses in deer 1½ years old or more; 82% of deer of that age were specifically seropositive for JCV. Antibody prevalence in yearling deer to JCV was only 4% from the same population, however.

We conclude that JCV infections in humans, while apparently not prevalent, is widespread and occurs more frequently in rural residents. Computer-drawn mapping of antibody prevalence was useful in locating a focus of human infection in southwestern Michigan residents and locating a deer population with very high rates of antibody to JCV. Age-specific antibody prevalence in deer suggests yearlings may be protected by maternal antibody their first year, thus providing numerous susceptible primary hosts for JCV the following spring. Further studies now in progress will be discussed.

Use of C6/36 Cell Cultures and Specific Monoclonal Antibody for Dengue Virus Surveillance in Puerto Rico

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During the fall of 1981, a new method utilizing C6/36 cell cultures and type specific antidengue monoclonal antibodies was implemented for the routine isolation and identification of dengue viruses in Puerto Rico. A blind comparison of the monoclonal antibody indirect fluorescent antibody test and the complement fixation test for identification of 53 newly isolated dengue viruses showed a 100% agreement. Isolation rates were slightly higher by mosquito inoculation than by the C6/36 cell cultures, but use of the latter system was much less time consuming and allowed the processing of larger numbers of sera. Beginning in November, acute sera from suspected dengue cases were selected for virus isolation attempt on the basis of geographic area on the island, day after onset the blood was taken and clinical signs and symptoms. These were inoculated into C6/36 cell cultures, incubated at 28°C for 10 days, the cells harvested, and the cells from each culture spotted on two slides. These were air-dried and fixed for 10 minutes in cold acetone. The cells on slide one were tested for dengue virus infection by direct FA using a conjugate prepared from high titrated human serum. The remaining slides from the positive cultures were then used for virus identification by the indirect FA using the monoclonal antibodies.

Using this system, 1587 sera were tested from November 1981 to April 1982. Dengue virus was isolated from 368 for an isolation rate of 23%. Dengue 1 was the predominant virus until December 1981 when dengue 4 became dominant. The changing patterns dengue 1 and 4 distribution by time and geographic location on Puerto Rico will be discussed. This system allows the dengue viruses being transmitted in an area to be monitored with a minimal amount of effort.

Use of Monoclonal Enhancing Antibodies to Map Dengue Virion Surface Antigens

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Antibody dependent enhancement of dengue 2 infection can be demonstrated in human peripheral blood monocytes as well as several mammalian continuous macrophage cell lines. Based on preliminary studies with polyclonal or monoclonal antibodies it is thought that interactions between antibodies with virion group or sub-group determinants result in infectious immune complexes which enhance infections in mononuclear phagocytes. When antibodies raised in man to natural dengue virus infections are tested for enhancement activity, wide variations are noted in enhancementt titers and in fold-enhancement. One explanation of this heterogeneity of biological activity is that there is a comparable heterogeneity in enhancing antigenic sites. When an epitope on a virion provides an appropriate match to an antibody idiotype, enhancement occurs.

One method of testing for the heterogeneity of enhancing determinants is to determine the enhancement profile of a battery of monoclonal antibodies agaist a large number of dengue 2 virus strains. In this study, five dengue 2 monoclones were obtained from the WRAIR (courtesy of W. Brandt). Three;of these exhibit gropp specificity (1B8) (1B10) (4G2) and 2 are type specific by HI and neutralization tests (3H1, 3H5). Using P388D$_1$ continuous mouse macrophages, these sera were tested against 7 dengue2 strains; TR 1751, NGC, PR 159 ;and four Thailand strains, (AHF-191-80, AHF-110-80, AHF-183-80 and 16881). In these experiments, both group and type specific monoclones enhanced dengue 2 virus infections. A low-titered group specific monoclone, 4G2, gave negative or weak enhancement aginst all 7 dengue 2 viruses. The monospecific antisera.3H1 enhanced four dengue 2 viruses, but TR 1751, AHF-183-80 and AHF-110-80 were not enhanced This is the first described inhomogeneity in the enhancement system related to dengue virus strains .

It is planned to enlarge and refine the collection of both monoclones and dengue virus strains. Further tests should permit the identification of discrete enhancing determinants and permit their topographic distribution on virions and their geographic distribution in nature.

Modulation of WEE viral titers in Culex tarsalis following oral and parenteral infection.

J. L. Hardy, L. D. Kramer, S. B. Presser, E. J. Houk, W. C. Reeves

During the "acute" infection of susceptible mosquito tissues or cell cultures with a high dose of an arbovirus, the virus initially multiplies to a high titer and within a few days the viral titer decreases markedly to a level significantly below the peak titer. The tissue or cell culture is then persistently infected and can continue to release low levels of infectious virus for the life of the mosquito or culture. This quantitative limitation of the amount of infectious virus produced by an infected mosquito cell has been called "modulation of viral titer" by Murphy et al. and has been advanced as a mechanism to explain the cellular resistance of susceptible invertebrate cells to the potential cytopathic effects of arboviruses. The precise mechanism(s) which brings about the modulation of viral titers in mosquitoes is unknown but could involve viral factors.

At present, the concept of modulation of viral titers is the best explanation for the dissemination barriers (i.e., midgut escape [ME] and salivary gland infection [SGI]) found in some Culex tarsalis females that become infected with western equine encephalomyelitis (WEE) virus but can not transmit virus by bite. If they are not initially overwhelmed with the virus, it would appear that these females have the ability to modulate viral titer at various stages of the infection and prevent successful infection of the salivary glands. Although modulation of viral titers does occur even when mosquitoes are infected on high viral doses, modulation occurs too late to prevent the rapidly spreading virus from infecting the salivary glands. Further, it appears to be the mosquito and not the virus that controls the production of "modulating factors." Indirect evidence that will be presented to support this hypothesis is as follows:

1. Field populations of mosquitoes are polymorphic in their ability to modulate viral titers when allowed to ingest intermediate doses of virus by the pledget feeding technique.

2. Female Cx. tarsalis can modulate WEE viral titers even when the virus is introduced by intrathoracic inoculation and this trait varies from one colonized strain to another. There are obviously "high producer" and "low producer" females in most unselected colonized strains. Studies are in progress in genetically selected low and high producer lines.

3. The few WR female Cx. tarsalis that become infected after ingestion of WEE virus allow the production of only low titers of virus (i.e., $10^{3.0}$ PFU/infected female) whereas most WS females infected on similar concentrations of virus allow the production of high titers of virus (i.e., $10^{6.0}$ PFU/infected female). Mean viral titers in F_1 progeny derived from reciprocal matings between WS and WR parents are intermediate to those found in the two parents. This is dose-dependent and modulation of viral titers are more pronounced with the WR ♂ X WS ♀ cross than the WS ♂ X WR ♀ cross.

4. A high proportion of ME and SGI barriers to WEE virus are found in F_1 progeny of matings between WR and WS parents.

5. Incubation of F_1 (WS ♂ X WR ♀) females at 32°C after they ingest WEE virus enhances their ability to modulate WEE viral titers, possibly "curing" them of the infection. This might occur if a viral modulating factor is synthesized more rapidly or functions more effectively at higher temperatures.

Experimental Infection of Cormorants and Sloths with St. Louis Encephalitis Virus

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Field studies have shown that the olivaceous cormorant (*Phalacrocorax olivaceous*) and the two-toed sloth (*Choloepus hoffmanni*) form part of the SLE transmission cycle in Panama. To further evaluate their host potential, both species were experimentally infected with SLE virus.

Cormorants readily became infected after receiving as little as 40 PFU of virus. The mean duration of viremia was 4 days. The average peak titer varied depending on the infectious dose and the strain of virus used. An average peak titer of $10^{4.2}$ PFU/ML of blood was obtained with a strain of SLE originally isolated from cormorants; whereas, the average peak titer obtained with a mosquito isolate was $10^{2.6}$ PFU/ML. Cormorants also could be infected with a North American epizootic strain of SLE virus.

Sloths also readily became infected after inoculation with a low titered dose of SLE virus. This species developed a prolonged viremia lasting from 8-14 days. The average peak viremia titer was 10^7 PFU/ML of blood, and the titer remained elevated ($\geq 10^5$ PFU/ML) for an average duration of 6.8 days. The viremia levels developed by sloths were sufficient to infect mosquitoes. *Culex quinquefasciatus*, *Haemagogus equinus* and *Sabethes cyaneus* all became infected after feeding on sloths circulating $\geq 10^{5.0}$ PFU/ML of blood; although the *Cx. quinquefasciatus* appeared to be more susceptible than the other two species.

The present ecological and virological data incriminate both the cormorant and 2-toed sloth as likely amplifying hosts in the tropical SLE transmission cycle. In addition the cormorant probably plays an important role in the dissemination of SLE virus.

Detection of Eastern Equine Encephalitis virus and Highland J virus antigens within mosquito pools by enzyme immunoassays (EIA)

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Enzyme-linked immunoassays were developed to replace common virological isolation and identification assays used in surveillance of arboviruses. These techniques were demonstrated capable of detection and identification of EEE and HJ viruses within 24 hours after specimen procurement.

An indirect sandwich ELISA method was used. Micro-titer plates wre coated with anti-virus mouse IgG, followed by the sequential addition of mosquito pool sample, anti-virus rabbit IgG, goat anti-rabbit IgG conjugated with alkaline phosphatase, and substrate (either chromogenic= p-nitrophenyl phosphate or fluorogenic= 4-methyl umbelliferyl phosphate).

In laboratory studies, the minimum number of EEE or HJ infected _Ae. triseriatus_ mosquitoes was determined using pools of known composition. One hundred percent concordance was found between virus isolation with BHK-21 cells and the EIA with mosquito pools containing various numbers of non-infected mosquitoes (7 to 99) and 1 or 3 infected mosquitoes (all at 15 days post inoculation). Growth curve studies of EEE in parenterally infected _Ae. triseriatus_ revealed that virus isolation was more sensitive than EIA (95.5% and 36.4%, respectively) during the first 3 extrinsic incubation days. After 3 days, however, there was complete concordance between the two assays; both techniques detected 100% of the infected pools. Specificity was 100% by both assays; serological cross-reactivity between EEE and HJ was minimal in the EIA.

In a retrospective study, the EIA was used to re-test 495 mosquito pools collected by the Massachusetts Department Public Health (MDPH) between 1978 through 1981. These pools were tested for virus with a chick embryo tissue culture plaque assay. Virus isolates were identified by the plaque-reduction method. Of the 495 pools, 215 (43.4%) were diagnosed by MDPH to contain either EEE, HJ, or both viruses. Of these, 165/215 (76.7%) were found positive when tested by EIA. When the 50 "false negatives" were retested, virus was isolated from only 8 pools. Thus, an adjusted estimate of the sensitivity of EIA was approximately 95%. Of the mosquito pools classified as positive by both assays, there was 98.7% agreement (kappa=0.987) in the identification of the two viruses. In one pool previously classified only as a EEE positive by MDPH, the EIA also detected HJ antigen. Of the 280 pools classified by MDPH as negative, 278 (99.3%) were also negative by EIA, but 2 (0.7%) were positive. HJ virus was subsequently isolated from these two "false positive" pools.

For the purpose of surveillance of a known virus within a mosquito population, the EIA method for the detection of viral antigens appears to be as sensitive and specific as a standard surveillance method.

PRELIMINARY OBSERVATIONS OF THE VECTOR POTENTIAL OF SANDFLIES WITH RIFT VALLEY FEVER VIRUS

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Laboratory studies at this Institute are presently being conducted to evaluate the vector potential of various species of sandflies with Rift Valley fever virus (RVFV), strain Zagazig 501.

RVFV replicated in Lutzomyia longipalpis, a new world species of Phlebotominae, following intrathoracic (i.t.) inoculation. Adult female L. longipalpis were inoculated with approximately $10^{2.1}$ plaque-forming units (PFU) of RVFV, and placed in an incubator maintained at 24°C. Samples of postinoculated sandflies were removed periodically and frozen at -70°C until assay for virus. Sandflies were assayed individually by infectious virus assay on Vero (R-6) cell monolayers to determine titers. During initial incubation, virus titers increased with time postinoculation and peaked between 10^4 and 10^5 PFU on day 3. Once the virus titer reached this level, it remained fairly constant throughout the observation period of 3-10 days, regardless of extrinsic incubation.

L. longipalpis infected with RVFV by i.t. inoculation transmitted virus by bite to susceptible golden Syrian hamsters. Infected flies were maintained at 24°C during the experiment, except during feeding. Approximately 5 female sandflies were exposed to each of 28 test hamsters on days 6-9 post-sandfly inoculation. Six of these hamsters died between days 2 and 5 after they were fed on by the inoculated sandflies. Virus was recovered from the livers of each of these hamsters, and confirmed to be RVFV by a virus dilution neutralization test.

Following ingestion of 10^3-10^4 PFU of FVFV from viremic hamsters (titer range = 10^7-10^8), 10^1-10^2 PFU were recovered from individual sandflies for up to 7 days post-feeding. However, no transmission of RVFV has been successfully demonstrated for L. longipalpis after ingesting viremic blood and subsequently re-fed on susceptible hamsters after 6-7 days of extrinsic incubation.

The ability of RVFV to replicate in sandflies and the recent classification of this virus as a member of the sandfly fever virus serogroup of the Bunyaviridae indicate the need for further studies to evaluate the vector potential of African species of Phlebotomine flies.

TICKS ASSOCIATED WITH FERAL PIGS IN FLORIDA, AND THEIR RELEVANCE TO THE EPIDEMIOLOGY OF AFRICAN SWINE FEVE.

K.H. Holscher, P.P. Humphrey, E.C. Greiner, J.F. Butler and E.P.J. Gibbs

African swine fever (ASF) is a highly contagious, usually fatal viral disease which threatens the swine industry throughout the world. Since 1978, it has been recognized in the Caribbean region and, therefore, poses an immediate threat to the United States. To date, the only known biological vectors of ASF involved in the natural transmission of the virus are certain species of soft ticks of the genus Ornithodoros found in Africa and the Iberian peninsula. Although species of these ticks are known to exist in Florida, their geographical distribution and association with feral pigs is unknown.

From 1979-1981, a total of 691 feral pigs were trapped at Fisheating Creek, near Palmdale, Florida, mainly during August and September. Each pig was closely examined for ticks, resulting in the collection of 37,040 adults of the following species: Dermacentor variabilis (American dog tick), Amblyomma maculatum (Gulf Coast tick), Amblyomma americanum (lone star tick), and Ixodes sp. (probably the black-legged tick). Average seasonal tick burden was 12 ticks/pig in the spring, 76 summer, 38 fall, and 4 winter. Dermacentor variabilis was the dominant tick parasitizing pigs in spring (99.7% of the collection), summer (87.9%), and fall (73.7%). Ixodes sp. dominated the winter collection, comprising 82.3% of the sample. No soft ticks were collected. Since soft ticks are burrow dwelling, nocturnal feeders that do not usually remain on the host, the failure to find soft ticks by examination of trapped pigs was not unexpected.

In Florida, Ornithodoros turicata has previously been reported from gopher tortoise (Gopherus polyphemus) burrows in seven counties. To facilitate and expedite further sampling of this particular tick, sophisticated vacuum equipment was developed. Using such equipment, 222 gopher tortoise burrows were sampled in 27 counties throughout Florida in the past year. Fifty-one (23%) burrows from 21 different counties were found to contain populations of O. turicata, ranging from 1 to 1000+ ticks/burrow. Collections were made from several different vegetative habitats, and extended along both the Atlantic and Gulf Coasts in southern Florida, throughout the central portion of the state, and into areas of the panhandle and northeastern region. Many of these collections were also in areas which have a large feral pig population (10+ pigs/square mile).

Collaborative research with USDA, Plum Island Animal Disease Laboratory indicates that O. turicata is capable of transmitting ASF. With an estimated population of 500,000 feral pigs in Florida, the possible establishment of an endemic focus of ASF exists, which in turn threatens the entire swine industry of the United States.

EXOTIC ALPHAVIRUSES IMPLICATED IN DISEASES OF DOMESTICATED ANIMALS

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Ten registered alphaviruses have been associated with disease in domesticated animals. Seven of these viruses (Aura, Getah, Middelburg, Ndumu, Ross River, Semliki Forest, and Una) are exotic to the U.S. Little is known about the disease induced by these agents both in laboratory rodent models or in domesticated animals; reports of disease in livestock are anecdotal or poorly documented. Each virus was inoculated peripherally and intracerebrally into mice and hamsters of various ages to determine mortality and infectivity ratios. All 7 viruses were highly lethal for suckling mice by all routes of inoculation. By the IP route all viruses except Semliki Forest had low mortality ratios (0-21%) in weanling mice, adult mice, and adult hamsters. By the IC route of inoculation mortality ratios also were generally low but showed more variation (0-100%). In contrast, Semliki Forest virus by either route of inoculation was highly lethal for all age groups of rodents (morality 75-100%). All 7 viruses produced infection regardless of inoculation route, as determined by neutralizing or HI antibody tests with infectivity ratios approaching 100%. Comparative pathogenesis studies were done in peripherally-inoculated suckling mice. Fourteen different tissues were serially collected and evaluated using virus titration, histopathology, immunofluorescence, and electron microscopy. Results of these studies will be presented.

Horses were inoculated with approximately 10^6 suckling mouse IC LD_{50} ($SMICLD_{50}$) of Ross River, Semliki Forest, and Una viruses, with no resulting clinical disease. Low level viremias were detected on post-inoculation days 2 and 4 (maximum titer 1.3 $\log_{10}$/ml) in one horse inoculated with Ross River virus; on PI days 2 and 6 (maximum titer 1.6 $\log_{10}$/ml) in one horse inoculated with Semliki Forest virus; and on PI day 6 (titer 1.0 $\log_{10}$/ml) in another horse inoculated with Semliki Forest virus. HI antibody developed to high titer in all 3 horses inoculated with Ross River virus, 2 of 3 horses inoculated with Semliki Forest virus, but 0 of 3 horses inoculated with Una virus.

Neonatal lambs were inoculated with approximately 10^6 $SMICLD_{50}$ of Middelburg, Ndumu, and Ross River virus. Middelburg virus induced clinical disease (weakness, anorexia, fever) and viremias in 3 of 3 lambs on post-inoculation days 1 and 2 with peak viremas reachin 4 $\log_{10}$/ml. _Culex tarsalis_ mosquitos fed on lambs inoculated with Middelburg virus became infected (maximum titer 3.3 $\log_{10}$). Ndumu and RR virus failed to produce clinical disease or detectable viremia in lambs, but did cause infection as shown by development of neutralizing antibodies.

Vector competence for arboviruses of a strain of *Culex tarsalis* infected with the microsporidian *Amblyospora californica*

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Field studies in 1979 in Kern County, California, revealed the presence of an infection in *Culex tarsalis* with the microsporidian *Amblyospora californica*. Infected females show no demonstrable symptoms, but transmit the parasite transovarially to male and female progeny. However, the infection is fatal to males usually in the 3rd or 4th instar, thereby allowing the emergence of female adult progeny only.

An *Amblyospora californica* infected strain (Ac+) of *Culex tarsalis* and a microsporidian-free strain (Ac-) were colonized from adult females collected during 1980 at the Breckenridge study area in Kern County. Cured strains were developed by allowing infected females to pass through 2-3 oviposition cycles, after which time some female progeny appeared to have lost the infection.

Vector competence studies for various arboviruses have been completed which compare the susceptibility of the Ac+ strain with an Ac- and cured strains of *Cx. tarsalis*. The Ac+ strain was significantly less susceptible than Ac- or cured strains to WEE virus following pledget feeding. When females were inoculated intrathoracically, the titers in the remnants were equal regardless of *A. californica* infection status; however, only 5% of Ac+ females showed evidence of mesenteronal infections with WEE virus as compared with 50% of Ac- females. Therefore, Ac+ females had greater mesenteronal infection barriers to WEE virus on both the lumenal and ablumenal sides of the mesenteron.

Following feeding on viremic chicks, there was essentially no difference in vector competence for SLE virus between Ac+ and Ac- females. Yet, Ac+ females were more susceptible to Turlock virus following pledget feeding than were Ac- females.

Histologic sections of infected and clean *Cx. tarsalis* are currently being examined to determine the extent of pathologic changes in the mesenteron or body due to the parasite. However, because of the different vector competence results with different arboviruses, one would not expect pathologic changes to account for the susceptibility changes associated with the microsporidian infection. Electron microscopic sections of WEE and Turlock-fed Ac+ and Ac- females are also currently being examined.

Cultivation of Mosquito Cells in Serum-Free Media and Its Effects on Dengue Virus Replication

by

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Bovine sera are an important component of growth media for many animal cell cultures. However, they must be of good quality (free of Mycoplasma and viruses) and are often very expensive. It is, therefore, sometimes desirable to grow cells in inexpensive serum-free media (SFM) so long as the beneficial traits of cells are not lost. For those engaged in arbovirus research, one of the most important cellular traints concerned is the sensitivity to viral infection.

Four mosquito cell lines of known sensitivity to dengue virus infection were adapted to 3 SFMs, and studies were done to determine whether there were changes in sensitivity to dengue virus infection or alterations of other cellular characteristics. The first SFM(SFM (L-15)) was composed of equal volumes of L-15 medium and tryptose phosphate broth (TPB). The second and the third SFMs contained either Eagle's MEM with Earle's salts or Medium-199 with Hanks' salts in place of L-15 medium. All cell lines tested were eventually adapted to the SFM (L-15), with the minimum length of period and subcultures necessary for complete adaptation ranging from as short as several weeks for the TRA-171 cells to as long as several months for other cells. Morphological changes were observed in some cell lines adapted in SFM. The differences in chromosome number between the cells in SFM and those of the original cultures was recognized in the AP-61, RML-12, and TRA-171 cells. The results of growth kinetic study showed that all cell cultures in SFM grew more slowly than in the original cultures in conventional media and that the cells grown either in the SFM(Eagle) or in the SFM(Hanks) replicated faster than in the SFM(L-15). With respect to dengue virus replication, a significant drop in virus sensitivity to all serotypes was found in the C6/36 cells grown in SFM. On the other hand, virus titers in the RML-12, AP-61, and TRA-171 cells were generally only slightly (by less than 0.5 dex PFU/ml) less than the corresponding values in the original cultures. No significant difference in viral replication was observed between the cell cultures in the SFM(L-15) and in the SFM(Eagle).

Since a previous study(unpublished) showed that a subline of the TRA-284 cells grown in specimen (1-dram) vials with the SFM(L-15) was equally effective for dengue virus isolation from human sera, when it was compared with the same cells grown in tubes or flasks, the combination of the uses of SFM and of inexpensive culture vessels, such as specimen vials, may assist in economizing the cost of mosquito cell culture for routine dengue diagnostic work.

INFECTION WITH HEPATITIS B VIRUS IN FRENCH VOLUNTEERS WORKING IN TROPICAL AFRICA

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Hepatitis is hyperendemic in Tropical Africa so it is assumed that volunteers working there are at high risk of becoming infected.

The incidence of hepatitis B virus (HBV) infection was measured in 227 French "Volontaires du Progrès" who had lived for 18 to 30 months in the bush in West and Central Africa (mean age: 23.6 years, range 20-34 years; sex ratio: 1.91). 25.6% were medical workers (mostly nurses), 54.1% technical advisers, 20.3% community workers. During their stay in Africa, 50 (22%) of those field workers developed jaundice, 27 of whom were tested when jaundiced; the SGPT level showed a marked increase in all the tested cases.

Sera obtained from each volunteer before and after their stay in Africa were tested by radioimmunoassay for HBsAg, anti HBs and anti HBc.

One of the HBV markers was detected in 14 (6.1%) of those 227 volunteers before leaving for Africa (3.5% were anti HBs+, 2.6% were positive for anti HBc alone, none were HBsAg+).

Among the 213 individuals presenting no marker before leaving, 22 (10.3%) developed HBV infection during their stay in Africa. On their return, 0.9% were HBsAg+, 6.6% were anti HBs+, 2.8% were positive for anti HBc alone. The multifactorial analysis of correspondence showed that the overall prevalence of markers was higher in the group which had developed jaundice (among the 49 jaundice cases, 9 (18.4%) showed an HBV related seroconversion; 13 (59%) of the 22 subjects infected with HBV had not developed jaundice). No relationship was found between hepatitis B markers/jaundice and age, sex, occupation, country of residence or duration of stay.

HBV infection is often asymptomatic. However, its evolution is unpredictable in the short and long term and its incidence is high in studied population. Therefore, in such populations, vaccination against HBV would be advisable in association with the prevention of hepatitis A.

Mode of Entry of a Neurotropic Arbovirus into the Central Nervous System: Reinvestigation of an old Controversy

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The mechanism by which neurotropic arboviruses gain access to the central nervous system remains uncertain, although it is generally assumed that viremic infection results in growth across or passive diffusion through brain capillaries. In contrast to the natural reservoir hosts of these arboviruses, however, clinical hosts (e.g. equines, humans) have viremias of very brief duration and low magnitude. We investigated the question of neuroinvasion in 6 week-old Syrian hamsters infected with St. Louis encephalitis virus. This model shares with the human disease low or undetectable viremia and many clinical and pathoanatomical features. Mortality ratios after intraperitoneal inoculation of a moderate viral dose was 88%. No viremia was detectable by sensitive assay in 31% of the animals. In the remaining hamsters, the mean peak viremia was 1.0 $\log_{10}$ PFU/0.05 ml and the mean duration 1-2 days. There was no correlation between viremia and outcome of infection, length of incubation period, or brain virus titer. Tissue infectivity studies showed a pronounced rise in titer in the olfactory neuroepithelium on day 4 postinoculation (PI), then in the olfactory bulbs (day 5 PI), and finally in the remainder of the brain (day 6 PI). Specific immunofluorescence was demonstrated in the bipolar neurons of the olfactory epithelium and in axon bundles of the olfactory nerves in the submucosa. By electron microscopy, virus particles and associated tubular structures were demonstrated within dendrites, perikarya, and axons of olfactory neurons, and to a lesser extent in macrophages and Bowman's gland cells in the lamina propria. In cells of Bowman's glands large numbers of virions were sequestered within secretory granules. Virus was recovered in relatively large amounts from nasal washings on days 3-5 PI. These data taken together indicate that in this model, the olfactory pathway is the principal route of viral entry into the CNS. After peripheral inoculation a low-level viremia results in infection of highly susceptible cells in the olfactory neuroepithelium, allowing centripetal axonal transport of virus to the olfactory bulb, whence spread is unimpeded throughout the neuropil of the CNS. Infection of Bowman's gland cells in the olfactory mucosa and shedding of virus in nasal mucus suggest possible new diagnostic methods. The significance of infection of the olfactory apparatus is discussed in terms of the evolution and ecologic relationships of the flavivirus genus.

Aedes aegypti Habitat Analysis and Targeted Source Reduction in Puerto Rico

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An analysis of the artifical container habitats of Aedes aegypti in 3 cities in Puerto Rico will be presented. The study areas in each city were chosen to insure that variations in socio-economic groups, environmental characteristics and dengue virus activity were represented. Within each study area initial sampling was completed in order to establish Breteau and Container Indexes as well as to establish the number of containers needed to be sampled in detail, i.e. total contents, for significant absolute density calculations. Six species of mosquitoes, Aedes mediovittatus, Culex pipiens quinquifasciatus, Cx. secutor, Anopheles grabhami, Toxorhynchites portoricensis and Wyeomyia sp. have been found associated with Aedes aegypti. The frequency of overlap between these species and Ae. aegypti by container type and its effects on productivity will be discussed. Absolute population densities by container type of Ae. aegypti from Bayamon show that tires, buckets and small miscellaneous containers (less than 1 gallon) contributes 60% of the total vector population. This is contrasted to Fajardo where flower pots, tin cans and tires represent a similar population percent. Also variation in habitat types of Ae. aegypti by socio-economic groups have been found within these 3 study cities.

The results will be presented by (1) mosquito species diversity and numbers and (2) significant containers in terms of Aedes aegypti productivity for each study site and city. Correlations between the 3 methods of sampling as well as the effects of container target source reduction for vector population control will be included.

Detection of dengue viral antigen in infected cell culture fluids and in suckling mouse brain suspensions by the modified double antibody sandwich enzyme linked immunosorbent assay

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An antigen detection enzyme linked immunosorbent assay (ELISA) for dengue virus (DEN) was developed for virus stocks prepared in *Aedes albopictus* clone C6-36 cells and in suckling mouse brains. A pool of human convalescent sera from laboratory confirmed dengue fever patients was coated on 96-well flat bottomed microtiter plates. The virus stocks, previously titrated by either hemagglutination (HA) or by cell culture, and uninfected control antigens were added after the coating step. Mouse DEN immune ascitic fluids were added next followed by goat anti-mouse immunoglobulin conjugated with alkaline phosphatase. The enzyme substrate, p-nitrophenyl phosphate added in the final step, is colorimetrically altered in the presence of the bound enzyme. The subsequent reactions were read spectrophotometrically on a Titer-Tek Multiscan plate reader at 405 nm. Absorbance values exceeding the mean of multiple replicates of the uninfected control antigens plus three times their standard deviation were recorded as positive.

Using a combination of hyperimmune mouse ascitic fluids and mouse ascitic fluids which contained monoclonal DEN antibodies, we were able to detect and identify all 4 serotypes of DEN. ELISA was a slightly less sensitive technique than cell culture and more sensitive than HA for detecting DEN antigen. The ELISA was capable of detecting antigen at levels below those normally found in human patients with DEN infections. Attempts to detect DEN antigen in human serum are underway.

IMPACT OF TOXICANTS AND DISEASE ON GROWTH AND REPRODUCTION USING DEER MICE (PEROMYSCUS MANICULATUS)

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In order to survive, an endotherm must expend a specified amount of energy on basic metabolic processes, dependent on the environmental variables of ambient temperature, radiation, and wind velocity plus the biotic coefficients of fur properties and allometry. Any additional energy consumed may be used for locomotion, growth, or reproduction. Disease may affect this equation in several ways: 1) fever increases the body temperature, elevating the basal metabolic rate, and effectively decreasing the amount of surplus energy; 2) behavioral changes may occur which decrease food consumption; or 3) the ability to properly digest food may be affected, decreasing the total amount of energy available.

Studying the energy balance system has been difficult because many variables act simultaneously. Our experiment employs full and fractional factorial designs described by Box, Hunter and Hunter (1978) which are able to manipulate multiple variables in a series of iterative steps. With this method, the effects of several variables and their interactions can be measured simultaneously, using a minimum of experimental animals. Insignificant variables can be eliminated and the dose-levels of the remaining variables can be manipulated until a complete resolution of the significant variables is performed. This experimental design has worked well in industry and the physical sciences but this is one of its first applications to biological studies.

The present series of experiments examines the effects of limited availability of food and water, the presence of a sublethal viral disease (Venezuelan equine encephalitis, Strain TC-83 -- VEEV), and an immune suppressant (cyclophosphamide -- CP) on survivability and growth to weaning (21 days) of young deer mice (Peromyscus maniculatus) and Swiss-Webster white mice. Pregnant females were given either CP (20mg/kg mouse/day) or saline-control food beginning approximately 5 days prior to parturition. Upon giving birth, females were given either ad lib or 80% normal consumption of food and/or water, and inoculated with an ID_{100} of VEEV (0.4ml of 8 X 10^4 pfu/ml) or a diluent control. Weight at weaning, survivability, and food and water consumption were monitored.

Results from the white mice and deer mice experiments were similar, although deer mice had a much lower survivability. Weight at weaning was reduced by 50% when food was limited. Limited water decreased weaning weights by 30%. Limitation of both food and water decreased weaning weight to half the weight of control animals. Administration of VEEV decreased weaning weights by 15%, except when food was limited and weights decreased by 75%. CP alone had no effect on weaning weight, regardless of food or water availability. When food and water were available ad lib, virus and CP, either alone or in combination, had little to no effect on weaning weight. The stress of viral infection and CP-induced immune suppression during food and water limitation caused the greatest decrease in weaning weight, by 80%. Limited water decreased food consumption to 80% of normal consumption. Water consumption increased by 50% when food was in short supply. CP alone increased both food and water consumption, VEEV increased water but not food consumption, and the combination of CP + VEEV increased food and water consumption

in white mice but not in deer mice.

In summary, for both white mice and deer mice the presence of an immune suppressant and disease in combination with limited food and water represents a substantial stress on the animals, leading to very little or no survival of young to weaning. In contrast, when both the immune suppressant and disease are present in addition to unlimited food and water, the animals compensate successfully and bring young to weaning, although at values significantly below control values.

Characteristics of Junin Virus Nucleic Acids

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Junin virus (JV), the etiologic agent of Argentine hemorrhagic fever, is a highly virulent member of the Tacaribe complex of arenaviridae. Because of difficulties associated with laboratory containment, little molecular characterization of strains has been attempted. Unique containment facilities at USAMRIID have allowed analysis of several laboratory attenuated and field JV strains to determine nucleic acid characteristics.

^{32}P-labeled JV propagated in Vero cells was concentrated by pelleting and purified by sequential centrifugation on 20%/60% discontinuous sucrose and 15% to 40% Renografin® gradients. Infectivity titers in excess of 10^8 PFU/ml coincident with maximum radioisotope label were observed in a single optically defined virus band. As with other arenaviruses, electrophoretic analysis of heat-denatured JV RNAs on 2.2% polyacrylamide gels revealed four distinct RNA species. Large (L) and small (S) viral RNAs (vRNAs) were observed together with cellular 28S and 18S ribosomal RNAs. Apparent molecular weights of L and S vRNAs were determined to be 2.7×10^6 and 1.5×10^6 daltons, respectively. The L vRNA appeared to possess secondary structure based upon differences in electrophoretic migration observed under denaturing and non-denaturing conditions.

Oligonucleotide fingerprint analysis of the two vRNAs confirmed their uniqueness as distinct vRNA species. Few, if any, similarities were noted between fingerprint patterns of JV vRNAs and those reported for other arenaviruses. Ongoing fingerprint analyses of multiple JV strains have demonstrated the utility of this technique for distinguishing JV strains of varying biologic behavior on a biochemical basis.

Effect of temperature on quantity of saliva and virus excreted by mosquitoes

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Dengue infected mosquitoes incubated at a high temperature induced infection in monkeys, but not so at a lower temperature. This led us to ask whether there was a temperature related salivary barrier or a difference in salivary volume and virus particles in those mosquitoes. We measured salivary output of virus using a capillary tube device that allowed a mosquito's mouthparts to be inserted in a stream of oil. Oil acts as a salivary stimulant. It was thus possible to measure the volume of output and virus titer of individual mosquitoes. We compared *Aedes aegypti* mosquitoes infected with Dengue and Chikungunya incubated at 26°C or 32°C for 14 days to similarly treated non-infected mosquitoes. Our results indicate that both temperature and virus infection influence saliva output but paradoxically that this allows more virus to be inoculated into the host. Information on such quanta of inoculation have important consequences in vaccine development and epidemiology.

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Nucleotide Sequence and Molecular Analysis of Bluetongue Virus

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The 3' termini of the plus and minus strands of the individual genes of bluetongue virus (BTV, a member of Reoviridae family) serotypes 10 and 11 were sequenced by chemical and enzymatic digestion. As expected, it was found that the 3' terminus of each gene is conserved among the different BTV serotypes. However, this sequence is quite different from that of Reovirus, a different genus of the same family. Proteins coded by each gene of different BTV serotypes were also analyzed.

Epidemic Dengue 1 in Puerto Rico, 1981

G. Sather, D.J. Gubler, S.B. Waterman, G. Kuno

Puerto Rico experienced rather large dengue epidemics in 1977 (Dengue 2 and 3) and 1978 (Dengue 1). After nearly 3 years of only sporadic transmission, another epidemic of dengue 1 occurred in 1981. Increased numbers of cases began to be reported in late July and the number of reported dengue-like illnesses peaked in October. Serological studies, however, showed that the epidemic peaked in September 1981. Age and sex distributions indicated little or no difference from the 1978 dengue 1 epidemic suggesting that the magnitude of the 1978 outbreak may have been exagerated. An analysis of the geographic distribution in 1978 and 1981, however, showed that the two epidemics were localized in different areas of the island. In 1978, most of the cases were reported from the San Juan metropolitan area whereas in 1981, the majority of cases were reported from the South west of the island. Dengue 4 was introduced into Puerto Rico in the summer of 1981, but transmission of this type remained sporadic until November, when the number of dengue 4 isolates began to increase. Clinically, the epidemic was similar to previous outbreaks in Puerto Rico. The majority of reported cases were adults and the illness was generally compatible with classical dengue fever. The epidemic will be discussed from a historical, epidemiologic and virologic point of view.

The development and distribution of eastern equine encephalitis virus antigen in orally infected *Culiseta melanura* as determined by the fluorescent antibody technique

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Although *Culiseta melanura* is considered the principal enzootic vector of eastern equine encephalitis virus (EEE), only limited information is available on the replication and tissue tropism of EEE in *C. melanura*. Furthermore, efforts to detect alphavirus antigen in infected mosquito organs or mosquito smear preparations with fluorescent antibodies have not succeeded or have not been reproducible. Therefore, this study was conducted in order to: 1) demonstrate that the direct fluorescent antibody technique can be used to reliably detect an alphavirus antigen (EEE) in mosquito organs; 2) test the hypothesis that EEE is transovarially transmitted (TOT) by *C. melanura*; and 3) describe the development and distribution of EEE in *C. melanura*.

Two-hundred sixty *C. melanura* fed on chicks circulating 4.8 - 7.8 log_{10} BHK $TCID_{50}$ of EEE (ME-77132). Eight mosquitoes were collected and dissected approximately every other day from day 3 until day 21 as well as on days 26 and 33 after the blood meal.

Results show that when compared to uninfected controls, EEE infected *C. melanura* can be reliably detected by direct immunofluorescent staining of dissected organs. Essential components of detection are an epifluorescent microscope equipped with a mercury vapor light source. The technique is improved by purification of immunoglobulins prior to conjugation.

No evidence for TOT by *C. melanura* was observed. Although other reproductive tissues contained specific fluorescence, ovarioles never contained detectable antigen.

Viral antigen was detected from the third through the thirty-third day of extrinsic incubation. Organs containing the most fluorescence were the foregut, midgut, hindgut, rectum, dorsal aorta, lateral and common oviducts, and thoracic muscle. Organs mildly or occasionally infected included sub- and/or supra-esophageal ganglia, thoracic ganglia, abdominal ganglia, abdominal fat bodies, spermatheca ducts, dorsal and ventral diverticula, and pericardial cells. Salivary glands and malpighian tubules were not included in the analysis because of non-specific staining and autofluorescence, respectively.

Work in progress includes determination of a growth curve for EEE in *C. melanura* and an additional experiment with orally infected *C. melanura* dissected daily from day 0 until day 7 after blood feeding. The latter study will determine when and where viral antigen can be first detected.

SEROLOGIC EVALUATION OF EQUINE ENCEPHALITIS VACCINATION PROGRAMS IN COSTA RICA

Charles Seymour and Donna M. Davison

The hemagglutination-inhibition (HI) test was used to evaluate rural vaccination programs against equine encephalitis. Apart from governmental administration of TC-83 vaccine during the 1971 epizootic of Venezuelan equine encephalomyelitis (VEE) virus, vaccination in Costa Rica is left to the private individual. The "Jen-Sal" trivalent killed vaccine* is the most widely used; in addition to a VEE component it also contains killed eastern (EEE) and western (WEE) equine encephalomyelitis viruses.

Horses of four Pacific coastal haciendas using this vaccine were studied for HI antibody response to these three viruses. The owners of these haciendas were educated, well-informed individuals who were aware of and claimed to adhere to the manufacturers' recommendations on refrigeration and expiration dates, though all attested to periodic power losses affecting refrigeration.

In baseline tests of 88 unvaccinated horses, 14 were positive to VEE virus suggesting enzootic circulation of this agent.

In tests of vaccination schedules following the manufacturers' recommendations, 21 horses aged 4-12 months without preexisting HI antibody were bled 5-8 weeks after their primary dose. None was positive against VEE and EEE, and only one was positive against WEE antigen. Fourteen horses received the recommended booster 5-6 weeks after the primary dose; 3 weeks later, no more than half were positive against WEE and VEE, and none was positive against EEE antigen. Titers were no higher than 1:40 (median 1:20 for both VEE and WEE).

On a different schedule, 21 adult (1.5 - 8 years) horses were vaccinated once per year. A year after the first dose, no more than 3 of 7 horses were positive against any antigen. Two months after the second annual dose, 7 of 14 animals reacted against EEE, and 10 and 11 reacted against VEE and WEE antigens respectively (median titers 1:10 - 1:20).

Of 50 adult horses vaccinated 2-4 times in the past 12 years, 36 were positive against VEE, 7 were positive against WEE, and none was positive against EEE antigen (median titers 1:20 against both VEE and WEE).

Neutralization (N) tests in Vero cells using very low antigen doses indicate that no more than 14% of the HI reactions are false positives. N tests are in progress to identify false HI negatives and to assess N antibody stimulation.

We conclude that the "Jen-Sal" trivalent vaccine, as widely used in Costa Rica, is ineffective, particularly against EEE virus. This finding is significant because protection against EEE virus, the documented cause of sporadic epizootics on the Pacific coast of neighboring Panamá, may be more important than protection against VEE and WEE viruses. WEE virus has not been isolated between Mexico and South America, and horse-virulent VEE virus is known in this area from only a single epizootic.

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* Reg. Trademark: Cephalovac. Jensen-Salisbury Laboratories, Division of Burroughs-Wellcome Co.

Transovarial transmission of dengue virus in *Aedes albopictus* mosquitoes: influence of female reproductive history on filial infection rates

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Aedes albopictus, an important vector of dengue viruses, is capable of transovarial transmission of all four dengue serotypes. The infection rates among progeny of infected females are influenced by poorly understood variables, including some factors related to female reproductive history. Mosquitoes having taken a single non-infective blood meal prior to inoculation with dengue type 1 virus produced a significantly larger proportion of infected progeny (2.1%) than did females not given a pre-infection blood meal (0.7%). Physiological or morphological changes associated with the pre-infection ovarian cycle are presumed to underlie this phenomenon.

When females were orally infected with dengue type 1 virus, progeny resulting from a subsequent non-infective blood meal had much lower infection rates when the blood meal occurred on day 3 after the first (<0.1%) than when it occurred on day 17 (1.7%). Other recent studies suggest that, within a single gonotrophic cycle, FIR may be influenced by timing of oviposition.

In mosquitoes orally infected with dengue type 1 virus, FIR's did not differ with timing of the second gonotrophic cycle when at least 7 days were allowed after the infective blood meal (FIR's ranged from 0.6–0.8%). But when a parallel group of infected females experienced multiple gonotrophic cycles timed to occur at the same intervals, FIR's declined markedly. FIR's of progeny from gonotrophic cycles 2-5 were 0.6%, 0.2%, <0.1% and 0.1% respectively. Thus, filial infection rates declined with increasing physiological age, but not with chronological age.

The experiments described all employed a single strain of *Ae. albopictus* from Hawaii and a single strain of dengue type 1 virus from Fiji. Other mosquito-virus combinations offering substantially higher background FIR's are needed to fully assess the significance of these observations.

Prevalence of Cytomegalovirus Antibodies in Nigerian School Children

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Serum samples from 69 school children (8-16 years of age) from Epe, Nigeria, were examined for cytomegalovirus (CMV) antibodies employing an enzyme immune assay. The mean age of the students was 10.6 $\pm$ 1.9 with a male to female ratio of 1.1. Serum samples from 97% of this school age population were positive for CMV antibodies. Six percent of this population presented antibody titers ($\geq$ 1.00 corrected absorbance reading at 1/50 serum dilutions). Sixty five percent fell into the medium titer range (0.46 - 0.99) and 26% were in the low titer category (0.31 - 0.45). No individuals fell in the questionable range (0.21 - 0.30) while 3% were negative ($\leq$0.20). The distribution of results revealed that there was no significant difference in the percentage positive for antibody or the antibody titers according to age or sex. These results differ from earlier papers which report that antibody levels tend to rise with age. However, the age range of our study is rather small.

Seroepidemiologic studies have been conducted in developed and less developed countries with antibody levels generally reported higher in the less developed areas of the world. Our results were consistent with these findings. It has been theorized that socioeconomic factors significantly affect CMV antibody positivity and antibody levels. This may be at least a partial explanation of the similarities of our data with an investigation by Krech, 1973 at Ibadan, Nigeria and, conversely, the variation between our results and a recent study by Okafor and Marshall, 1978, in student nurses at Enugu, Nigeria. Different serologic techniques may have contributed to the differences detected, but it is more likely that they are a consequence of Nigerian geographic considerations (of which the socioeconomic variations are a part). For example, Enugu is in Eastern Nigeria while Epe and Ibadan are located in Western Nigeria. The high level of CMV antibody positivity and the relatively high titer levels (70% of this population had moderate to high antibody titers) are indicative of a hyperendemic focus of CMV infections. Further, the percent positive and the percent with high titers suggest that there is a moderate level of herd immunity in this local. It could be argued that these antibody levels may not correlate with immuno-protection. However, the data suggests that exposure to CMV occurs early in life for Epe dwellers.

CMV is of major concern primarily because of the deleterious sequelae for the infant and child following intrauterine infection. Since there are no significant differences amongst the sexes and a high percent of this population is positive for CMV antibody, including females of child-bearing age, (assuming that these detectable antibody levels are protective) the risk of intrauterine infection may in fact be reduced in this hyperendemic area. A small percent of the female population, however, as seronegative. These individuals, particularly those of child-bearing age, are at risk and, in addition, the children they may bear are at risk. Because of the hyperendemicity of CMV in Epe, it may be advisable to periodically screen females of child-bearing age by the rapid, simple, and yet sensitive and specific enzyme immune assay technique. Those who are seronegative could be advised concerning preventive measures especially if pregnancy is planned.

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ABSTRACT - ORAL PRESENTATION

The American Society of Tropical Medicine and Hygiene

Cleveland, Ohio

"Genetic studies on the trait susceptibility to oral infection with yellow fever virus in *Aedes aegypti*"

The ability of *Aedes aegypti* to become infected with yellow fever virus is a complex trait which probably involves many different interactions between mosquito and virus. Our studies to understand the genetic control of this trait in *A. aegypti* have been based on the hypothesis that different genetic loci are involved. In order to understand the role of genetic variation and environmental modification in controlling infectivity in *A. aegypti* we have artificially selected mosquito strains which are highly resistant to infection and strains which are highly susceptible. Two isofemale lines from a population from Austin, Texas were chosen to initiate selection, one was selected for resistance, the other for susceptibility to oral infection with yellow fever virus. The general *A. aegypti* population in Austin, Texas showed 84/119 or 0.71 resistance to infection. In the resistant line three generations of individual selection resulted in 224/275 or 0.82 resistance. The susceptible line showed 6/21 or 0.29 resistance. The results of this selection scheme will enable several inferences to be made concerning genetic control. First by comparing the greater similarity in infectivity among related individuals (sibs and half-sibs) with unrelated individuals we have been able to estimate the proportion of variation in infectivity among individuals in the original population due to genetic differences. This is the broad heritability $h^2 = 0.71$. Secondly by observing the infectivity differences between generations of the same family, we have demonstrated the nongenetic or environmental component to variability in this trait. These results and the implications for future studies will be discussed.

Genetic Control of Susceptibility of *Culex pipiens* to Rift Valley Fever Virus

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Evidence obtained during vector competence studies of Egyptian strains of *Culex pipiens* for Rift Valley fever (RVF) virus suggested that susceptibility to infection was under the genetic control of this mosquito. Three distinct populations of *Cx. pipiens* were observed at 7-12 days of extrinsic incubation following ingestion of 10^6 plaque-forming units (pfu) of RVF virus from a viremic hamster: 1) uninfected females, 2) females which contained a moderate amount of virus, median titer $10^{3.1}$ pfu, and 3) females which contained a high viral titer, median titer $10^{5.4}$ pfu. Assay of legs from individual infected mosquitoes indicated that mosquitoes with a moderate titer (group 2) had a non-disseminated infection limited to the gut, while those with a high titer (group 3) had a disseminated infection. Analysis of viral titers of mosquitoes with non-disseminated infections suggested that this group consisted of 2 subgroups. Thus, 4 populations were identified: 1) refractory (R), 2) non-disseminated-low (ND-L) ($10^{0.7}$-$10^{3.4}$ pfu), 3) non-disseminated-high (ND-H) ($10^{3.5}$-$10^{4.4}$ pfu), and 4) disseminated (D) ($\geq 10^{5.2}$ pfu).

Female *Cx. pipiens* in the F_{11} generation since colonization of specimens collected in the Sharqiya Governorate in Egypt were used to select for the 4 populations above. After ingestion of 10^6 pfu of RVF virus from a viremic hamster, egg rafts were collected from individual females and each female was examined for virus and classified into 1 of the 4 groups on the basis of viral titer. Progeny from 2 females in each of the 4 groups were reared in separate containers at 26°C. After being allowed to mate with their siblings, females were permitted to feed on a viremic hamster, egg rafts were collected, and viral levels were determined for individual females. In each line, progeny from the female whose progeny had the highest percentage of appropriately classified progeny were used to produce the next generation. This process was repeated each generation in each of the lines.

The distribution of females in the F_{11} parental population was 4 (14%), 5 (17%), 11 (38%), and 9 (31%) in the 4 populations R, ND-L, ND-H, and D, respectively. After 4 consecutive generations of within-population selections, the percentage of females appropriately classified in each of the 4 selected lines increased from that in the parental population. For instance, the percentage of R females in the line selected to be refractory increased from 14% to 48% (10/21). Likewise, the percentage of ND-L females in the ND-L line increased from 17% to 67% (28/42); of ND-H females in the ND-H line, from 38% to 72% (20/28); and of D females in the D line, from 31% to 37% (7/19). The existence of these 4 populations in unselected mosquitoes, and the ability to selectively increase the percentage of mosquitoes in each of these populations strongly implies that susceptibility of *Cx. pipiens* to RVF virus infection is under the genetic control of the mosquito.

The history of *Aedes aegypti* populations in the New World as reflected by their genetic structure.

Graham P. Wallis, Walter J. Tabachnick and J.R. Powell

Department of Biology, Yale University

Over 100 collections of *Aedes aegypti* representing some 70 localities world-wide have now been analysed electrophoretically. For the 10 loci scored in all collections, average overall expected heterozygosity per locus is 0.117 ± 0.005. The gene frequency data have been used to calculate Nei's overall genetic distance value (D) for every pairwise population comparison, and have been submitted to stepwise discriminant analysis. Both analyses reveal that the New World populations analysed to date form 3 distinct genetic geographic groupings. The south-eastern US group (SEUS) comprises 8 populations; all the other 20 mainland populations from Texas through Central America to Suriname (including Trinidad) form another entity (TEXMEX). Five collections from Puerto Rico and Jamaica form the third group (CAR). Further extensive sampling of the Caribbean Islands will enable us to comment more thoroughly on genetic differentiation in this region.

Mean D values from intragroup and intergroup New World population comparisons are given in Table 1. It is striking that several Texan populations have a greater genetic affinity to Mexican and South American populations than they do to ones from neighbouring states to the east. Populations in the CAR group are more similar to those in the TEXMEX group than they are to SEUS populations.

Possible reasons for these patterns will be discussed in light of the historical literature on the presence of *A. aegypti*. The contact zone of the two groups may not be stationary; one gene pool may be spreading to the exclusion of the other. If group commonalities in enzyme genotype are indicative of other similarities such as parameters of vector competence, this is a matter of great importance.

Table 1. Average D values within and between the three New World groups

	SEUS	TEXMEX	CAR
SEUS	0.016 ± 0.002		
TEXMEX	0.028 ± 0.001	0.013 ± 0.001	
CAR	0.045 ± 0.002	0.033 ± 0.002	0.037 ± 0.007

Epidemic Acute Hemorrhagic Conjunctivitis, Puerto Rico

S. H. Waterman, R. Casas Benabe, R. Muñoz Jimenez

In late 1981 the Western Hemisphere's pandemic of acute hemorrhagic conjunctivitis (AHC) spread to Puerto Rico. Over 6,000 cases were reported to the Puerto Rico Department of Health from November 1981 to March 1982. The peak of the epidemic was in January and cases occurred island wide. Female to male sex ratio was 1.56:1 for cases for whom sex data were available. Available age data for the San Juan metropolitan area show that all age groups were affected but that attack rates were significantely higher for those <30 years ($p < .001$). A lower middle socioeconomic section of Cidra municipio with relatively intense transmission was studied in depth. In a house-to-house survey 151/649 (23.3%) persons reported illness consistent with AHC. Forty-nine out of 130 households (38%) reported at least 1 case of AHC. The secondary attack rate in affected households was 50%. The female/male ratio in this sector was 1.5/1. Highest attack rates were in the 5-14 year (43%) and 25-40 year (36%) age groups. Household index cases were also more likely to be of school age (5-14 years) than of any other age group. Household index cases not of school age often reported contact with infected school age children prior to the onset of illness. Households from Cidra and other areas with high attack rates and low attack rates were compared to look at household transmission factors. Crowding and sharing of beds were important variables ($p < .02$ and $p < .01$, respectively). Serologic and virologic data from this study will be discussed.

Neurologists and opthalmologists on the island were contacted retrospectively in search of patients with neurologic complications of AHC. One presumptive case was identified, a 49 year old woman with a Bell's palsy 10 days following the onset of hemorrhagic conjunctivitis.

The results of this study suggest that the epidemiology of AHC in Puerto Rico is similar to that reported in Florida. School age children appear to play an important role in the transmission of AHC. Stringent school exclusion would appear justified as a control measure. Heightened personal hygiene measures should help prevent secondary infection within households but crowding may limit the effectiveness of such efforts.

Barriers to dissemination of Middle American Venezuelan encephalitis viruses in the enzootic vector mosquito Culex (Melanoconion) taeniopus from Guatemala.

S.C. Weaver, W.F. Scherer, E.W. Cupp and C. Taylor

Our previous studies have shown that Culex (Melanoconion) taeniopus exhibits a mesenteronal infection barrier to Middle American epizootic, HI subtype 1AB VE viruses, but not to enzootic, subtype 1E strains. Mosquitoes which do become infected with epizootic VE strains fail to transmit virus to hamsters after 21 days of extrinsic incubation at 27°C, while flies infected with enzootic strains transmit virus in most cases. Mean titers of virus per infected mosquito after extrinsic incubation were $10^{4.1-5.4}$ CECpfu of enzootic strains, and $10^{2.6-3.5}$ of epizootic strains.

In order to explain the failure of Cu. taeniopus to transmit epizootic VE strains, F_7–F_9 generation colonized Guatemalan mosquitoes were given bloodmeals on hamsters circulating various titers of enzootic or epizootic VE strains. Samples of individual mosquitoes were frozen immediately after engorgement and assayed for mean bloodmeal titers. Remaining engorged mosquitoes were held at 27°C. 80% relative humidity, with crepuscular lighting and a 15 hour light period daily. Individual mosquitoes were randomly taken at selected intervals following engorgement and mesenterons dissected, washed and assayed separately from remaining tissues (remnants).

In two experiments, mosquitoes were infected with 10^{4-5} CECpfu of epizootic strains 69Z1 and 69U332, respectively. Bloodmeal titers declined in the gut about 7-fold by day 2 after engorgement, and remaining undigested blood was excreted by day 4. Most mosquitoes dissected contained no infectious virus by day 4, in either mesenteron or other tissues. However, 17-20% of mosquitoes did become infected, and virus was limited to the mesenteron in all specimens, including those dissected 25 days past infection. This represents a mesenteron escape barrier to epizootic VE strains. Virus replicates in the mesenteron, but fails to reach other tissues.

Other mosquitoes were allowed to engorge on hamsters circulating $10^{5.1}$ and $<10^{0.7}$ CECpfu per bloodmeal of enzootic strain 63U2. Bloodmeal virus decay was similar to that seen in epizootic strains, dropping about 7-fold during the first 2 days. With the large dose bloodmeal, virus was detected in both mesenteron and remnant tissues within 4 days of infection. Titers in both tissues peaked between days 4 and 9, before dropping about 10-fold by day 15. In mosquitoes infected with the small virus dose, remnant titers peaked later, and at a slightly lower titer than with a large oral dose.

With both large and small oral enzootic doses, several flies dissected contained virus in the mesenteron but not in other tissues, exhibiting a mesenteron escape barrier. This barrier was expressed in 11% of mosquitoes ingesting $10^{5.2}$ CECpfu, and 51% of those ingesting $<10^{0.7}$ pfu of enzootic virus. Thus, this mesenteron escape barrier to enzootic viruses appears to be dose dependent.

REPORT FROM THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH
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Investigation of dengue outbreak, 1981-82

Dengue fever has had a long association with Queensland probably since 1879 at Townsville but certainly since 1885 at Charters Towers when the first death certificate, ascribed to dengue, was issued in this State. Subsequent outbreaks were common and sometimes extensive e.g. 1897, 1904-05, 1910-11, 1916, 1925-26, 1941-43, 1953-55 and most recently from April 1981. In 1906, of course, Dr T.L. Bancroft published his pioneering work to elucidate the vector of dengue *Stegomyia fasciata*, now known as *Aedes aegypti*. Epidemics sometimes extended southward to just north of Sydney, New South Wales.

By 1965, control by health surveyors and the advent of reticulated water saw *Aedes aegypti* restricted in distribution from parts of south-east Queensland (but absent from Brisbane) with greater numbers in provincial towns in tropical northern Queensland and the Torres Strait islands. A progressive downgrading of routine house to house inspectors by local government authorities has probably allowed *Aedes aegypti* not only to widen its distribution but also, in some towns, to build up sizeable populations.

In April 1981, a 46 year old female from Cairns in northern Queensland presented with a typical dengue-like illness which was subsequently diagnosed as infection with dengue type 1. The woman had no recent history of travel outside Australia. Subsequent sporadic cases due to dengue type 1 were recorded in Townsville and Cairns until in late October, an outbreak of a dengue-like illness was reported on Thursday Island in the Torres Strait. Subsequent clinical infection has been reported at Badu, Yorke, Coconut and Yam Is., Mareeba, Innisfail, Mossman, Cooktown, Atherton, Gordonvale and Charters Towers.

Typical symptoms include high (40-41°C) biphasic fever, headache, malaise, bodyaches especially back and legs, a rash which developed first on the upper trunk and subsequently, at the end of convalescence, on the limbs. Diarrhoea and haematuria were recorded once each. No deaths could be positively attributed to dengue. Adults were most severely affected although some had mild symptoms; children had few or mild sequelae.

This outbreak was investigated (i) from an epidemiological standpoint (ii) to provide dengue isolates for comparison with known world strains and (iii) as a public health exercise. Interestingly enough, Dr E.N. Marks *et al* warned that such an outbreak could occur at Townsville in December 1980 issue of the Medical Journal of Australia.

To date, several things are clear: (1) the indices of larval abundance adopted by the World Health Organization are not ideal but nevertheless demonstrate the prevalence of *Ae aegypti* in three centres of northern Queensland (Table 1), (2) local authorities responsible for mosquito control have failed, for various reasons, to deal effectively with *Ae aegypti*, (3) only a small proportion of cases are being notified and (4) confirming diagnostic procedures have been too slow to be of clinical use.

Thirteen and possibly 14 strains of dengue have been isolated from patients using a C6/36 *Aedes albopictus* cell line, inoculated with acute lymphocytes separated by zonal centrifugation with Ficoll - Paque. Some of these have been identified as type 1. No strains were isolated from mosquitoes collected either biting or resting. However, during March 1982, the medical superintendant of Thursday Island reported that persons with clinical dengue during late 1981 were representing. This may suggest that another type is also circulating.

From clinical and serological survey data, an estimated 3000 cases have occurred mainly in Thursday Island, Cairns and Townsville. Although dengue is notifiable, unfortunately many medical practitioners have not done so. This in turn, makes it more difficult to convince people that Queenslanders ever had a dengue problem in 1981-82 and that an *Ae aegypti* eradication program sould be instituted. We now join Texas as a First World locality with a recent dengue problem. [B.H. Kay, B.M. Gorman, P.Barker-Hudson]

Third Arbovirus Symposium

The Institute, in collaboration with CSIRO, hosted the Third Symposium "Arbovirus Research in Australia" at the Bardon Professional Centre from February 15-17, 1982. The meeting was highly successful and attracted approximately 115 participants including 25 from overseas: Drs D.S. Burke, Kanai Chatiyanonda (Thailand); J. Sulianti Saroso, Suharyone Wuryardi, Rondhardyo Purnomo, P.L. Young, (Indonesia); B.M. McIntosh (South Africa); J.U. Mataika (Fiji); P. Fauran, P. Lucet (New Caledonia); F. Austin (New Zealand); F.R. Brown, J.S. Porterfield (U.K.)D.J. Gubler (Puerto Rico); W.C. Reeves, L. Rosen, K.M. Johnson, F.A. Murphy, A. Dian, P. Roy, W.K. Joklik, C.H. Campbell (U.S.A.), L.S. Self (Philippines) Abdullah bin Ghana (Malaysia) and P. Halonen (Finland). Copies of the Proceedings will be available soon.

[B.H. Kay]

TABLE 1

Prevalence of *Aedes aegypti* larvae in Thursday Island, Cairns and Townsville

Locality	Date inspected	Index*		
		Breteau	House	Container
Thursday Is.	Oct.28-Nov.4	20	16	12
	Jan. 23-28	112	48	27
	April 19-23	141	51	22
Townsville (7 suburbs)	Feb. - April		ca 50	
Cairns (4 suburbs)	June 1-4	12.2-37.5	12.0-27.1	7.7-23.1

* Breteau index (number of positive containers/100 houses; House index (% of positive houses); Container index (% of positive containers)

REPORT FROM THE DEPARTMENT OF PARASITOLOGY, TEIKYO UNIVERSITY SCHOOL OF MEDICINE, 11-1, KAGA 2 CHOME, ITABASHI-KU, TOKYO 173, JAPAN

1. Susceptibility of Japanese mosquitoes to dengue-2 virus

Females of 15 species of Japanese mosquitoes were studied to determined if they were susceptible to mouse-adapted dengue-2 virus (New Guinea C strain) by oral administration of the virus mixed with rabbit blood and sucrose. The following species were tested: *Aedes (Stegomyia) albopictus, Ae. (Stg.) flavopictus, Ae. (Stg.) riversi, Ae. (Ochlerotatus) dorsalis, Ae. (Aedimorphus) vexans nipponii, Ae. (Finlaya) japonicus, Ae. (Fin.) togoi, Armigeres (Armigeres) subalbatus, Anopheles (Anopheles) sinensis, Culex (Culex) orientalis, Cx. (Cux.) pipiens molestus, Cx. (Cux.) pipiens pallens, Cx. (Cux.) pipiens quinquefasciatus, Cx. (Cux.) tritaeniorhynchus* and *Tripteroides (Tripteroides) bambusa*. Several hundred mosquitoes were used for each species. They were kept at 30°C after the infection, and were stored at -80°C after the incubation of various time length from 0 to 30 days. The detection and titration of the virus were made by the intracranial injection of a suspension pool of 10 mosquitoes in suckling mice (ddY strain). The virus was detected at varying titers from all the mosquito species immediately after the infection, but was detected after 20 days of infection only from the four species, i.e., *Ae. (Stg.) albopictus, Ae. (Stg.) flavopictus, Ae. (Stg.) riversi* and *Ae. (Och.) dorsalis*, ranging from "trace" to $\geq 10^{4.6}$ LD_{50} per pool. In more detailed studies with the positive mossquito species, the virus was found to multiply after an eclipse period of 7 to 10 days and to reach a peak titer after 20 to 30 days.

2. Improved methods for the laboratory rearing of Toxorhynchites amboinensis (Doleschall), a laboratory host of dengue viruses

The purpose of this study was to examine methods of improving the mass rearing techniques of a non-biting mosquito *Toxorhynchites amboinensis* (Doleschall), which is an efficient laboratory host for dengue virus when the virus is injected intrathoracically. Experiments were directed at (1) the establishment of individual rearing techniques thus preventing larvae cannibalism; (2) the development of a suitable food for the larvae; and (3) the definition of the optimum physical conditions for larvae growth.

(1). The larvae were reared individually in 3 ml disposable trays containing 2 ml of water or in 200 ml plastic cups containing 50 ml of water with *Aedes albopictus* (Skuse) larvae provided as food. The larval period at 27°C was 18.1 days with a pupation rate of 89.1% in the disposable trays and 18.6 days and 94.1% in the plastic cups. In group rearing of 50 larvae in plastic trays (27 x 35 x 8 cm) containing 3 liters of water, the larval period was 18.1 days and the pupation rate was 29.9%. It was shown that the individual method gives higher pupation rates and that the use of larger containers give slightly better results.

(2). The larvae were reared using the following feed: Tetramine®, Vita-shrimp®, liver powder, dried water fleas, dried blood worms, and drone powder. The average larval periods were 47.7, 45.5, 56.0, 53.7 and 52.0 days, respectively. The pupation rates were 70, 70, 50, 80, 40 and 10%, respectively. Larvae reared with a combination of a living diet of newly hatched brine shrimp *Artemia salina* (Linnaeus) for the young larval stage and the blood worm *Limnodrilus hoffmeisteri* Claparède

for the third and fourth larval stages pupated after an average of 23.2 days at 25°C with a pupation rate of 100%. These results were almost the same or slightly better than those obtained with group rearing by the conventional method providing *Aedes albopictus* larvae in which the larval period was 24.1 days and the pupation rate 90%.

(3). The larvae were reared by providing a combined living diet at the temperature levels of 20, 25, 27 and 30°C. No larval growth was seen at 20°C, and the average preadult periods and pupation rates at the higher temperatures were 32.2, 29.4 and 27.4 days, and 100, 90 and 80%, respectively. At 30°C the death rate of the blood worms was high enough to cause fouling of the water.

(Yuuki Eshita)

REPORT FROM THE DEPARTMENT OF MEDICAL ZOOLOGY,

KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

Evaluation of Enzyme-Linked Immunosorbent Assay for Quantitation of Antibodies to Japanese Encephalitis Virus in Swine Sera

The enzyme-linked immunosorbent assay (ELISA) was evaluated for the quantitation of antibodies to Japanese encephalitis (JE) virus in swine sera, in view of its application to seroepidemiological studies. In this study, we adopted the microadapted ELISA system in which polycarbonate-coated iron beads were used as the solid phase with magnetic transfer devices.

The preliminary experiments in which parameters and other conditions were examined indicated that the optimal assay condition was brought about by (i) the sensitization with formalin-inactivated, purified JE virus vaccine (5 μg/ml, pH 7.4), (ii) the first reaction with 100-fold diluted test sera, (iii) the second reaction with 100-fold diluted alkaline phosphatase-conjugated anti-porcine IgG, and (iv) the third reaction with *p*-nitrophenyl phosphate: Each reaction was performed at 37 C for 1 hr.

The assay was highly reproducible (coefficient of variation of the data was less than 5%) and significantly correlated with HI test (correlation coefficient was 0.921). As a borderline differentiating positive from negative sera, 0.204 was determined by the statistical analysis based on the frequency distribution of absorbance values for 366 swine serum samples. Under this diagnostic criterion, all of the sera positive for HI antibody turned out to be positive for ELISA antibody and also all negative for ELISA turned out to be negative for HI. Inconsistency was observed in only six cases where the antibody was detected in ELJSA but not in HI test.

On the basis of these results, the present ELISA system was considered to be a good diagnostic tool for seroepidemiological studies of JE virus in which examinations of many samples are required within a limited time.

(Investigations conducted by E. Konishi and M. Yamaoka)

(Reported by T. Matsumura)

REPORT FROM THE W.H.O. COLLABORATING CENTRE FOR ARBOVIRUS REFERENCE AND RESEARCH (DENGUE AND DENGUE HAEMORRHAGIC FEVERS) AT THE DEPT. OF MEDICAL MICROBIOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF MALAYA, KUALA LUMPUR 22-11, MALAYSIA

The Arbovirus laboratory at the Dept. of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur was designated as a W.H.O. Collaborating Centre for Arbovirus Reference and Research (Dengue and Dengue Haemorrhagic Fevers) in June, 1982.

The last report submitted by the Department was in the September 1981 issue of Arbovirus Information Exchange. The present report covers the year 1981 and the period up to August 1982.

1. Overall disease activity in the country

The year 1981 saw 558 cases of dengue fever/dengue haemorrhagic fever (DF/DHF). Of these 558 cases, 269 (48.2%) were DHF cases and 9 deaths were recorded (3.3% fatality rate). However, the first half of 1982 saw a significant increase in disease activity and the country is presently in the midst of quite a severe outbreak of DF/DHF. As of August 9, 1982 a total of 866 DF/DHF cases have been reported; of these 529 were DF and 337 were DHF cases with 22 deaths (6.5% fatality rate) (Table 1). This compares with 368 DF/DHF cases (183 DF, 185 DHF) with 9 deaths for the same period in 1981, representing a 135% increase in the number of cases and a 133% increase in deaths. The outbreak has affected all states of Peninsular and East Malaysia except Perlis and Sabah (Table 1). The most number of deaths was recorded in the Federal Territory (Table 1). The majority of DHF cases were noted in the 5-14 years age group (34% of cases).

2. Diagnostic Service

a. Serology

Dengue serology is routinely carried out using the complement fixation test (CFT). Table 2 shows the monthly distribution of positive dengue cases for 1981. June, 1981 was again a peak month but, unusually, September-December, 1981 also showed significant activity. This observation may be of significance in light of the outbreak mentioned above which began in April, 1982. Out of 464 patients in 1981, 125 (26.9%) were diagnosed as positive or presumptive positive flavivirus infection. The positive cases up to July, 1982 are presented on Table 3; the slightly lower percentage positive rate (17.2%) may be a reflection of the increased number of specimens being sent to the laboratory during the present outbreak.

Table 4 shows the age distribution of confirmed dengue cases. Approximately 20% of the cases were in children under 10 years of age with 50% of the cases in the 10-24 age group. There was fairly even distribution of cases in males and females in the various age groups.

Table 5 presents the data based on sex and ethnic distributions. Again, there was no apparent difference in dengue incidence between the two sexes. As in previous years, more Chinese are affected by dengue and reflected the fact that there is a larger Chinese population in the larger urban areas.

b. Virus Isolation

Viruses are routinely isolated from clinical specimens using two methods : *Aedes pseudoscutellaris* (AP-61) cells and *Toxorhynchites splendens* mosquitoes. Cells and mosquito head squashes are routinely examined 10 days after inoculation by direct immunofluorescence using fluorescein-conjugated pooled convalescent human sera. Preliminary data indicate that AP-61 cells are more sensitive than mosquitoes for dengue virus isolation. Typing of virus isolates is being carried out using monoclonal antibodies to all four dengue serotypes by indirect immunofluorescence on infected AP-61 cells. These antibodies have been kindly provided by Dr. T.P. Monath, W.H.O. Collaborating Centre for Arbovirus Reference and Research, Centers for Disease Control, Fort Collins, Colorado. Table 6 shows that dengue-1 and dengue-2 were the main types isolated in 1981. Although many isolates are still being typed, it appears likely that dengue-1 and dengue-2 are also associated with the 1982 outbreak (Table 6).

4. Research activities

Several projects are being carried out in the Department at the present moment. Firstly, a study is in progress to document the clinical presentation of DF/DHF cases at the University Hospital and correlate these data with serological and/or virus isolation results. Secondly, a project has just commenced to study the biochemistry of dengue virus replication in infected mammalian and mosquito cells. The aim of this work is to characterize virus-induced proteins in infected cells and to see if any correlation exists with virus virulence. Thirdly, extensive studies are being carried out to characterize the cell-mediated immune response (CMI) in experimental dengue infection of mice. Studies to date indicate that a definite CMI response, as measured by delayed-type hypersensitivity(DTH) in the footpad, was elicited in infected mice. Further studies are being carried out to further characterize this response and to investigate the role played by CMI in dengue virus infection. Lastly a collaborative project is being initiated with the Queensland Institute of Medical Research (Dr. B. Gorman) to

perform oligonucleotide fingerprinting of dengue viruses isolated from DF/DHF cases at the University Hospital.

5. Publications

1. T. Pang, S.K. Lam. Immunopathogenesis of dengue haemorrhagic fever. Immunology Today (in press).

2. T. Pang, P.Y. Wong, R. Pathmanathan. Induction and characterization of delayed-type hypersensitivity (DTH) to dengue virus in mice. J. Inf. Diseases, in press (August 1982).

(T. Pang, S.K. Lam, P.Y. Wong, S. Devi)

Table 1

Dengue Activity in Malaysia - 1982*

State	DF	DHF	Total	Deaths
Perak	108	72	180	2
Penang	71	88	159	1
Selangor	100	47	147	5
Johore	78	38	116	1
Federal Territory	44	43	87	6
Kelantan	67	9	76	3
Malacca	23	23	46	2
Negeri Sembilan	12	13	25	1
Pahang	13	0	13	0
Kedah	6	3	9	0
Sarawak	5	1	6	1
Trengganu	2	0	2	0
Perlis	0	0	0	0
Sabah	0	0	0	0
Total	529	337	866	22

* Situation on August 9, 1982
Source of data : Ministry of Health, Malaysia.

Table 2

Monthly Distribution of Dengue Cases - 1981*

Month		No. of patients	No. of dengue positive	Percentage positive
Jan	1981	27	5	18.5
Feb	1981	26	7	26.9
Mar	1981	32	10	31.2
Apr	1981	46	9	19.6
May	1981	53	11	20.7
Jun	1981	53	18	34.0
Jul	1981	37	5	13.5
Aug	1981	31	3	9.7
Sep	1981	27	9	33.3
Oct	1981	45	19	42.2
Nov	1981	39	14	35.9
Dec	1981	48	15	31.2
Total		464	125	26.9

* Cases admitted to the University Hospital, Kuala Lumpur.

Table 3

Monthly Distribution of Dengue Cases - 1982*

Month		No. of patients	No. of dengue positive	Percentage positive
Jan	1982	52	15	28.8
Feb	1982	62	10	16.1
Mar	1982	37	1	2.7
Apr	1982	40	12	30.0
May	1982	67	6	8.9
Jun	1982	87	8	9.2
Jul	1982	72	19	26.4
Total		417	71	17.2

* Cases admitted to the University Hospital, Kuala Lumpur.

Table 4

Age Distribution of Laboratory Confirmed Dengue Cases, January 1981 - July 1982

Age group (yrs)	No. of male positive	No. of female positive	Total positive	Percentage positive
< 1	2	0	2	1.6
1 - 4	6	2	8	6.3
5 - 9	5	9	14	11.1
10 - 14	10	7	17	13.5
15 - 19	12	11	23	18.2
20 - 24	11	13	24	19.0
25 - 29	8	3	11	8.7
30 - 34	6	5	11	8.7
35 - 39	3	6	9	7.1
40 - 44	0	1	1	0.8
45 - 49	4	0	4	3.2
≥ 50	0	2	2	1.6
Total	67 (53.2%)	59 (46.8%)	126	

Table 5

Sex and Ethnic Distribution of 126 Laboratory-confirmed Cases of Dengue, January 1981 - July 1982

		Number positive	Percentage positive
SEX	Males	67	53.2
	Females	59	46.8
ETHNIC	Malay	37	29.4
	Chinese	66	52.4
	Indian	17	13.5
	Others	6	4.7

Table 6

Dengue Virus Isolation

Year	No. specimens tested	No. of isolates	Virus type				
			D1	D2	D3	D4	Untyped
1981	302	29 (9.6%)	11	6	1	1	1
1982*	240	18 (7.5%)	3	3	-	-	12

* Until July, 1982

REPORT FROM NATIONAL INSTITUTE OF VIROLOGY
20-A, DR.AMBEDKAR ROAD, PUNE-411 001, INDIA.

<u>Replication of Japanese encephalitis (JE) virus in human mononuclear leukocytes *in vitro*</u>

JE virus (803835 strain, isolated from brain necropsy of a case of encephalitis in 1979 epidemic at Kolar, Karnataka State, India) was studied for its ability to replicate in the mitogen stimulated and unstimulated human mononuclear leukocytes (MNL) and their subpopulations. MNL cultures of all the seven individuals stimulated by Phytohemagglutinin-P (PHA-P) and four individuals with Pokeweed mitogen (PWM) supported the replication. No replication was observed in Conconavalin A (0/6)* or Formolinized *Staphylococcus aureus* (Cowan I)(1/6) stimulated MNL. Unstimulated human MNL from only three out of nine individuals studied supported the replication of JE virus, although to a lesser extent.

Macrophage cultures (2/2) derived from peripheral blood leukocytes, also supported replication of JE virus, while unstimulated non-adherent MNL (lymphocytes) did not support the replication. Purified T and B lymphocytes obtained after complement dependent lysis of non-adherent lymphocytes from two individuals, using Hybritech monoclonal antibodies against T and Ia antigens were examined for their ability to support replication of JE virus. Studies have revealed that PHA and PWM stimulated T cells supported replication, while mitogen stimulated B lymphocytes did not.

Replication of JE virus was also studied in MNL from acute and convalescent encephalitis cases which were

* Number of donor cultures showing replication/Total number studied.

transported at room temperature from field to laboratory using Park and Terasaki's modification of McCoy 5A medium. It was observed that PHA stimulated MNL obtained from acute (2/4) and convalescent (2/2) cases supported the replication. Also unstimulated MNL from the above two patients and convalescent cases supported the replication of JE virus.

Isolation of JE and West Nile (WN) viruses from peripheral blood of encephalitis cases

Since PHA stimulated human MNL seemed to be a good host cell for JE virus replication, we attempted isolation of virus from peripheral blood obtained from encephalitis epidemic cases at Kolar in Nov-Dec. 1981. The plasma and MNL from patients were obtained by centrifugation of heparinized blood of lymphocyte separation medium (Density 1.077) in siliconized glass vials. MNL ($1x10^5$) or plasma (0.1 ml) from encephalitis cases were inoculated into PHA stimulated MNL ($1x10^6$/ml) cultures from normal individuals. After 72 h incubation at 37°C, the cultures were stored frozen at -70°C and later passaged 3 times in PS (Porcine kidney) cell line and in suckling swiss albino mice by simultaneous intracerebral and subcutaneous inoculation. Three strains of JE and one strain of WN virus were isolated from plasma of a total of 11 acute patients studied. Preliminary studies indicated that these strains differed in their biological and serological properties as compared to other strains in our laboratory. No virus was isolated from MNL of acute cases.

(N.Kedarnath, M.M.Gore, A.C.Banerjea, C.Dayaraj, S.V.Ranbhor, C.N.Dandawate, S.R.Prasad, S.George, A.A.Koshy and S.N.Ghosh)

COMPARATIVE EFFICIENCY OF MOSQUITO INOCULATION AND INFANT MOUSE INOCULATION TECHNIQUES FOR ISOLATION AND DETECTION OF DENGUE VIRUSES.

In 1979, an epidemic of dengue had occurred in Rahuri, Ahmednagar District in Maharashtra State. Twenty four serum specimens from patients in the acute phase of illness collected during this epidemic from febrile cases were earlier processed by infant mouse inoculation, and a total of 8 strains of Dengue viruses were isolated. Besides, in 5 cases dengue aetiology was determined by sero-conversion or by the cross challenge test in mice.

Recently, indirect fluorescent antibody technique on the head squashes of *Aedes aegypti* mosquitoes inoculated with field collected specimens has been standardised in this laboratory. The sera specimens collected during the Rahuri epidemic, which were stored at -70°C for over two years were processed by this technique in an effort to isolate dengue virus and to compare the results with the earlier diagnosis done by virus isolation in infant mice. The comparative results are given in the following table. The identification was done by complement fixation test on crude mouse brain extract or inoculated mosquitoes suspension.

Dengue virus was isolated in 13 of the sera specimens inoculated in mosquitoes against eight isolated in the infant mice. However, the 5 sera specimens from which virus was not isolated in infant mice protected the surviving mice when challenged with dengue virus.

(V. Dhanda, J.J. Rodrigues and M.A. Ilkal)

Mosquito inoculation			Infant Mouse inoculation		
No.of speci-mens proces-sed	Positive by FA	Identification by CF test	Positive by mouse inocu-lation	Identification by CF test	Positive by cross challenge test
24	13	3 Dn type 1 1 Dn type 2 1 Dn type 1 or 2 8 not done	8	5 Dn type 1 2 Dn type 1 or 2 1 Dn type not identified	5

EXPERIMENTAL TRANSMISSION OF JAPANESE ENCEPHALITIS VIRUS BY A COLONIZED STRAIN OF *CULEX VISHNUI* THEOBALD.

Culex vishnui Theobald has been considered an important potential vector of Japanese encephalitis in Taiwan and in Southern India, mainly based on isolations of the virus from wild caught mosquitoes and on ecological considerations. However, no experimental transmission studies were reported because earlier attempts to colonize this species in laboratory were unsuccessful. A strain from Kolar, Karnataka State of this species has been successfully colonized under normal laboratory condition. Experimental studies revealed that Japanese encephalitis virus was successfully transmitted to susceptible chicks by mosquito bite on 10th and 15th days after ingestion of infective blood meal.

(A.C. Mishra, K. Banerjee and H.R. Bhat)

MONITORING OF THE ACTIVITY OF JAPANESE ENCEPHALITIS VIRUS BY USING SENTINEL PIGS IN KOLAR, KARNATAKA STATE

Subsequent to the JE outbreak in 1979 in Kolar District of Karnataka State, studies were initiated to monitor the activity of the virus by using the sentinel pigs. Locally available piglets of 2 to 4 weeks old, were used as sentinels. Eighty piglets were studied in 1981 in five localities. These pigs were marked and bled regularly approximately once in a month. Of 34 pigs kept in three villages, 33 showed seroconversion between October and December apparently coinciding with the occurance of JE outbreak in humans in Kolar district.

All the 9 piglets kept at yet another village in January 1981 had maternal antibodies to JE/WN viruses. The maternal antibodies disappeared by March. These piglets were negative for haemagglutination inhibiting and neutralizing antibodies upto August but were found positive in December. Thus the occurance of infection was indicated in the pigs some times during the epidemic season. However, the period of infection of these pigs could not be determined as the samples were not available for the months of September to November.

(G. Geevarghese, H.R. Bhat, B.H. Shaikh, K.M. Pavri)

REPORT FROM THE DEPARTMENT OF VIROLOGY,
ISRAEL INSTITUTE FOR BIOLOGICAL RESEARCH, P.O.B. 19,
NESS-ZIONA 70450, ISRAEL.

DETERMINATION OF ANTIBODY LEVELS TO RIFT VALLEY FEVER VIRUS (RVFV).

Antibodies to RVFV were titrated in our laboratory by reverse passive hemagglutination inhibition (RPHI) and by ELISA neutralization. The titers obtained by these techniques were compared to the titers obtained by plaque reduction neutralization (PRN) and by hemagglutination inhibition (HI).

Bovine sera were collected by the Veterinary Services, Government of Israel, at various stages of immunization of cattle with the South African RVF-killed-vaccine. Randomly assorted samples from the collection were analysed in the present study.

ELISA-neutralization: Various dilutions of the serum samples were incubated with a constant amount of RVF-HA antigen (1)(E-Ag), equivalent to about 0.1 HAU/0.1 ml. The Ag-Ab mixture was then transferred to wells in PVC microtiter plates. The wells were pre-coated with ascitic fluid from mice immunized with RVF (Entebbe) killed vaccine (2) (E-Vac). The unreacted Ag from the Ag-Ab mixture was immobilized in the coated wells, and free antigenic sites were allowed to react with serum from rabbits immunized with the Smithburn strain of RVF (3) (S-SA). The amount of bound rabbit serum was determined with peroxidase-conjugated goat anti-rabbit IgG (4). Antibody titer to RVFV in the tested bovine sera was scored as the highest dilution that suppressed the color reaction to 50% of its maximal value obtained by using about 0.1 HAU to the coated wells.

RPHI: This procedure was as described by Goldwasser, Elliott and Johnson (5). Globulins for conjugation to glutaraldehyde-treated chicken erythrocytes were prepared from ascitic fluids of mice immunized with E-Vac.

HI: The HI titers of the bovine sera were obtained by microassay using 4-8 HAU/0.025 ml of E-Ag and goose erythrocytes at pH = 6.0.

PRN: Dilutions of the serum samples were mixed with constant amounts of S-SA virus. Titers were determined for 80% plaque reduction in BHK cells. Zero neutralization level was about 60 plaques per plate.

Table 1 presents the anitbody titers of the 19 sera, tested by the various methods. Table 2 compares the methods by the titer-level attained.

(1) HA-Ag, Entebbe, sucrose-acetone extract of suckling mice livers, Salk Institute, U.S.A.

(2) NDBR 103 RVF (Entebbe) inactivated vaccine. Research Laboratories, National Drug Company, U.S.A.

(3) The Smithburn neuro-adapted RVF strain was obtained from Dr. McIntosh, South Africa.

(4) Miles Yeda, Israel.

(5) Goldwasser, R.A., Elliott, C.H. and Johnson, K.M. (1980). J. Clin. Microb. 11, (6): 593-599.

(A. Keisary, D. Ben-Nathan, R.A. Goldwasser, R. Barzilai).

Table 1. RVF-antibody titers in bovine sera assayed by various techniques.

Serum Number		80% PRN	HI	RPHI	50% - ELISA
1.	3012	20	20	<10	<20
2.	3013	<10	10	<10	<20
3.	3014	<10	20	<10	<20
4.	3015	80	40	20	20
5.	3016	<10	20	<10	<20
6.	3027	<10	10	<10	<20
7.	3028	80	40	<10	<20
8.	3030	80	80	<10	20
9.	3038	1280	+320	+60	200
10.	3040	640	320	+80	200
11.	3051	640	320	+40	80
12.	3055	320	160	+40	40
13.	3112	<10	<10	<10	<20
14.	3113	<10	<10	<10	<20
15.	3114	<10	<10	<10	<20
16.	3115	<10	<10	<10	<20
17.	3116	<10	<10	<10	<20
18.	3118	<10	<10	<10	<20
19.	3119	<10	<10	<10	<20

+: End point differs in 1 dilution step between two separate determinations, and only the lower dilution factor is recorded.

Table 2. Number of sera scored as positive (out of 19) by different methods at various titer levels.

LEVEL	80% PRN	HI	RPHI	50% ELISA
≥ 10	8	12	5	n.d.
≥ 20	8	10	5	6
≥ 40	7	7	4	4
≥ 80	7	5	2	3
≥160	4	4	1	2

(A. Kesary, D. Ben-Nathan, R.A. Goldwasser, R. Barzilai).

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

Establishment and characterization of hybridomas secreting monoclonal anti tick-borne encephalitis virus antibodies

Inst. Virol. Slovak Acad. Sci 817 03 Bratislava

Hybrid cell lines that produce monoclonal antibodies against tick-borne encephalitis /TBE/ virus have been prepared by the fusion mouse myelòma cells /NS1/1/ with the lymphocytes of mice that were immunized with the Skalica strain of TBE virus /spontaneous variant with decreased virulence for mice/. Some of these cell lines were cloned and inoculated into syngeneic mice for the production of ascitic fluids. The hybridomas were found to produce monoclonal antibodies of IgM class with type of light chain /gel double immunodiffusion, agarose gel electrophoresis, SDS-PAGE/. Electron microscopy of hybridomas revealed the presence of some cells with several nuclei in sub-clones obtained after long-term incubation and after as many as five recloning. Monoclonal antibodies were tested for their ability to react with II different togaviruses. In HI assay, only TBE virus gave positive reaction. These monoclonal antibodies have potential application to epidemiology of TBE virus infection and may be used for rapid differential diagnosis.

(Novák, M., Grešíková, M., Sekeyová, M., Russ, G., Čiampor, F.)

Report from the Federal Research Institute for Animal Virus Diseases
Tübingen, Federal Republic of Germany

Isolation of P7 core protein of Bluetongue virus via chromatofocusing

The double immunodiffusion test for the detection of Bluetongue (BTV) antibodies is used routinely by the majority of laboratories dealing with BTV antibody survey. The advantage of the above test is that antibodies to all existing serotypes can be detected. Therefore it has been postulated that all serotypes share one common antigen responsible for eliciting group specific antobodies. This has been confirmed recently by other workers using the immunoprecipitation assay. Their results indicated that a core protein designated P7 is the common antigen for all BTV serotypes. In addition it has been shown that P7 is produced in excess during viral replication and released into the culture medium. We have therefore tried to isolate P7 from infectious supernatant in order to obtain a clearly defined precipitating antigen. Purification procedures by Sephacryl S-200 chromatography yielded a partially purified antigen but an untolerably high percentage of nonspecific protein remaining. However the application of chromatofocusing using concentrated infectious supernatant in a gradient from pH 4 to pH 7 enabled us to isolate pure P7. SDS-PAGE clearly demonstrated the presence of a protein band at the same position as protein P7 when complete virus was run on gelelectrophoresis. Immunodiffusion tests with BTV reconvalescent serum showed one distinct band only. Tests performed with crude antigen often yielded two or more precipitation bands of which one usually accounted for the specific reaction whereas the others were nonspecific precipitation bands. Chromatofocusing is therefore recommended for the preparation of the BTV precipitating antigen.

(O.J.B. Hübschle)

REPORT FROM THE ARBOVIRUS LABORATORY, MEDICAL INSTITUTE OF
ENVIRONMENTAL HYGIENE, AUF'M HENNEKAMP 50
D-4000 DUESSELDORF, WEST GERMANY

Tahyna Virus Surveillance in Central Europe during 1980 and 1981

A total of 42,073 mosquitoes belonging to 25 species were caught between January 1980 and September 1981 in West Germany (4 regions), Austria (1 region) and northern Italy (1 region). There were six (6) TAH isolates, one from *Aedes caspius* mosquitoes (1 out of 148 pools), caught at the eastern shore of Lake Neusiedl (Austria) on August 18, 1981. Five (5) TAH isolates came from 62 pools (6066 mosquitoes) caught in the Middle Rhine area (near Germersheim) on September 10, 1981, consisting of two (2) isolates from identified *Aedes vexans* mosquitoes and three (3) from unidentified mosquitoes. This is the first TAH virus isolation in the Rhine area. Further details will be presented in the next issue.

(H. Mackenstein, J. Pilaski)

USE OF SCANNING ELECTRON MICROSCOPY IN THE STUDY OF HAEMAGGLUTINATION INDUCED BY ARBOVIRUSES

The characteristics of the haemagglutinating antigens of arboviruses, obtained by the classical techniques of Clarke and Casals (1958), may show considerable variation between different types of viruses.

While most viruses of the Togaviridae family yield potent haemagglutinins, producing compact and firm agglutinates, other viruses (in particular those of the Bunyaviridae family) only have weak haemagglutinins in low titer, leading to the formation of fragile agglutinates.

Tawara et al (1976) successfully used scanning electron microscopy (SEM) to visualise very precisely the mechanism by which particles of Myxovirus influenza B become adsorbed to the surface of chicken red blood cells (RBC's) producing haemagglutination. We therefore decided to use this technique to study haemagglutination caused by various arboviruses.

By this mean we studied six viruses of the Togaviridae family: Sindbis, Getah, West Nile, Dengue type 2, Tick-borne encephalitis, Wesselsbron, and from the Bunyaviridae family: Calovo, Tahyna, Uukuniemi and Bhanja.

1. HAEMAGGLUTINATION CHAMBER FOR THE PREPARATION OF SAMPLES FOR SEM

We designed a sterilisable and re-usable haemagglutination chamber in the form of a broad and almost flat-bottomed cup, into which an object slide is placed: the agglutinates settle on to this slide, which can then be easily removed from the chamber and treated appropriately.

It is made from a square glass salt-cellar measuring 30 x 40 mm, and 15 mm thick (fig. 1). It is hollowed out into a brod U-shaped cup 30 mm in diameter and 10 mm deep. This is covered by a 40 x 40 mm glass slide 1 mm thick to prevent drying-out of the reaction. A circular object-slide 10 mm in diameter is placed at the base of the cup. The haemagglutination reaction takes place in this chamber, which must not be moved during the process.

Haemagglutination by each virus was tested simultaneously in three chambers as follows:

- a haemagglutination test using 4 haemagglutinating units, at optimal pH and temperature;
- a haemagglutination test using 4 haemagglutinating units, at the same temperature but at an unfavourable pH, i.e. a pH outside the range within which haemagglutination was seen in previous microtitrations;
- a control test comprising only RBC's, at optimal pH and temperature for the corresponding haemagglutinin.

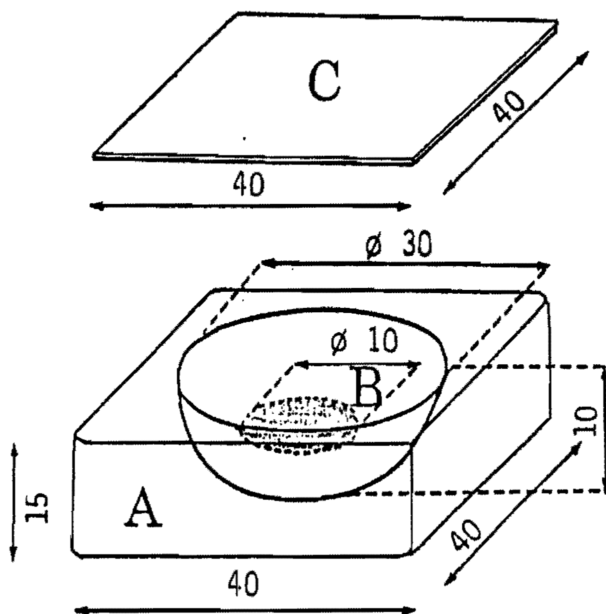

Fig. 1. Haemagglutination chamber (sizes in mm).

A: Salt-cellar with an almost flat-bottomed cup.

B: Circular object-slide 10 mm in diameter placed at the base of the cup.

C: 40 x 40 mm glass slide 1 mm thick assigned to prevent drying-out of the reaction.

2. *TREATMENT OF SPECIMENS FOR SEM*

For each reaction, once the agglutinates or non-agglutinated RBC's had settled, i.e. 2 to 3 hours after setting up the reaction, the buffer was carefully aspirated and replaced by a 2.5% solution of glutaraldehyde in 0.1 M phosphate buffer, pH 7.0. Fixation was carried out for 3 days, after which the fixative was removed by aspiration. The specimen was then washed 3 times in distilled water, and rehydrated by passage through a series of baths of progressively increasing concentrations of ethanol in distilled water, followed by increasing concentrations of amyl acetate in ethanol.

The samples were then treated by critical-point evaporation and metal-coated in vacuum by gold-palladium. Finally, the metal-coated specimens were examined under a Jeol type 15 SEM and photographed at the usuals enlargements of 30x to 10,000x.

3. *RESULTS AND COMMENTS*

a. For any haemagglutinin, the agglutinated red blood cells (RBC's) were arranged in more or less well-structured clumps, separated from one another by 'clefts' containing non RBC's (fig. 2 and 3). The shape and surface of RBC's appeared grossly normal. The control reactions contained no structured clumps of RBC's nor clefts. With the more potent haemagglutinins i.e. those of West Nile and dengue type 2, structured clumps were also observed in the presence of haemagglutinin, but at an unfavourable pH.

b. Haemagglutination caused by Togaviridae and Bunyaviridae seems not due to the presence of complete viral particles.

c. With dengue type 2 haemagglutinin, star-shaped structures were seen adhering to the RBC surface. They may correspond to haemagglutinating subunits of virion; however they are too small in number to produce a lattice capable of aggregating RBC's.

d. The mechanism of haemagglutination by arboviruses may differ fundamentally from that of myxoviruses. It is known that the haemagglutinins of alphaviruses, certain flaviviruses and certain bunyaviruses also have haemolytic properties. The fixation of haemagglutinating sub-units on erythrocyte membrane receptors might produce microlesions capable on their own of causing first agglutination and then sedimentation of damaged RBC's.

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[F. Le Goff, G. Le Lay-Roguès and C. Chastel, Virus Laboratory, Faculty of Medicine, Brest, France].

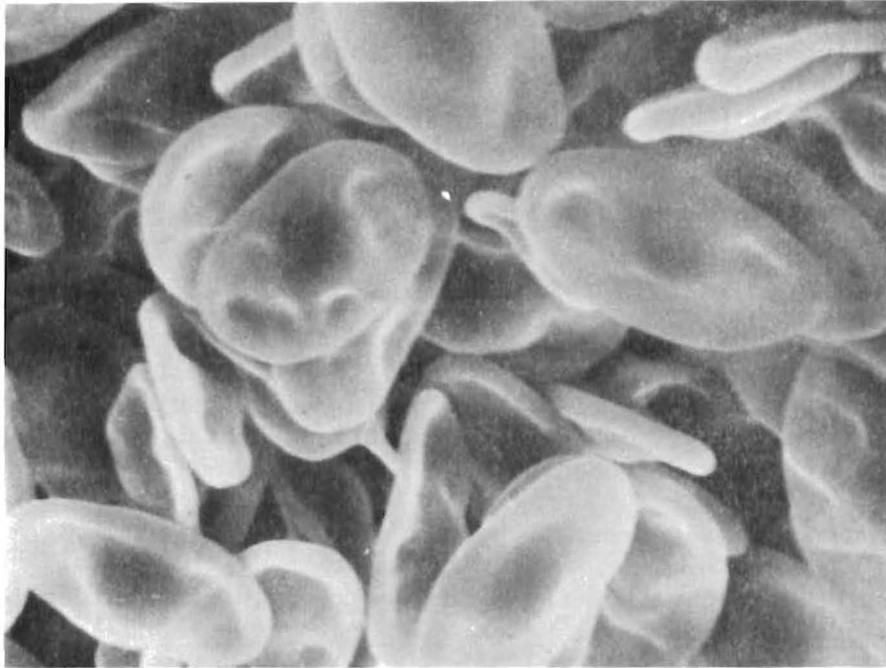

Fig. 2. Uukuniemi: haemagglutination at optimal pH. (x 380)

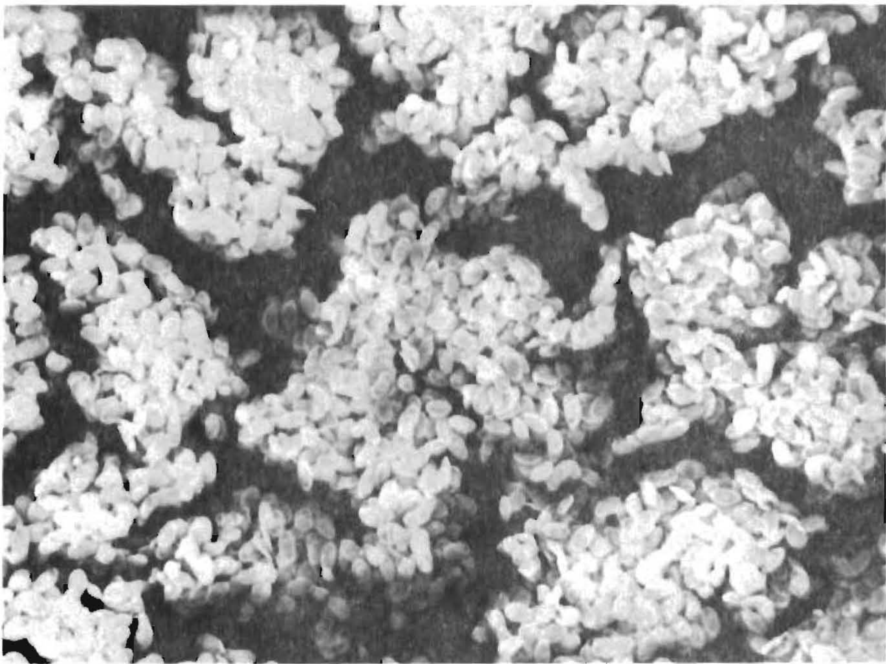

Fig. 3. West Nile: haemagglutination at optimal pH. (x 4,000)

REPORT FROM THE INSTITUT PASTEUR OF MADAGASCAR
B.P. 1274 TANANARIVE MADAGASCAR

PRELIMINARY RESULTS OF ARTHROPOD-BORNE VIRUSES RESEARCH PROGRAM IN MADAGASCAR FROM 1976 TO 1980

Since 1976, an arthropod-borne virus research program has been conducted in Madagascar, in collaboration with Institut Pasteur (Paris and Dakar) and World Health Organization.

Interesting results were obtained in virological and serological studies. Also, an important entomological finding was done. A new species of anthropophilic phlebotomus, Sergentomyia berentiensis, was discovered.

I. Virus isolation

In four years, a total of 36,742 specimens were inoculated into suckling mice by the IC route. This mainly included pools of Culicoides, Ixodid; blood samples from lemurs (the only non-human primate living in Madagascar), psychodidae, ceratopogonidae; and various samples from febrile hospital patients, lemurs, wild birds, and cheiropterans captured from different places.

The main results obtained are:

- Seven strains of Mengovirus isolated from pools of Culicoides (Eretmapodites quinquevittatus, Anopheles gambiae, Culex tritasmorhynchus) and Aedes (Aedes pembaensis).

- One strain of Dengue-2 virus, isolated from a patient who had an history travel (La Réunion) and had dengue-like symptoms.

- One strain of West Nile virus isolated from parrots, Coracopsis vasa, trapped in Morondava area.

- Twelve strains of Zinga virus, originally isolated in Central African Republic in 1960, were also recovered 5 times from pools of Culicoides, alone or in association with another virus Y 251.

- Six strains of Y 251 virus, Sindbis virus subtype, were isolated from pools of Culicoides in the same place.

- Perinet virus, a new virus, was isolated 6 times from Sergentomyia berentiensis and pools of Culicoides in Perinet. It was characterized and shown to be a Rhabdovirus and belong to Vasiculovirus group (TESH, Yale Arbovirus Research Unit).

Mg Ar 807 virus strain, isolated from pools of Culicoides collected in Perinet was also considered as a new virus by the Virus Reference Center, Dakar.

Presently, identification of 7 other strains is in progress:

One strain isolated from pools of Boophilus microplus
Six strains from homogenized insectivore bat captured in Ampijoroa area.

II. Serological studies

The hemagglutination inhibition method has only been performed to investigate wild bird, lemur, and human sera.

A. Human sera

A total of 545 sera essentially collected from young military recruits coming from 7 different places were screened for antibodies against the following antigens:

1 - Alphavirus group

Chikungunya
Semliki forest
Sindbis

2 - Flavivirus group

West Nile
Wesselsbron
Ouganda S
Yellow fever
Spondweni
Zika
Dengue 1-4
Tick-borne encephalitis
St. Louis encephalitis

3 - California group (Tahyna)

4 - Phlebotomus fever group (Siciliam Sandfly fever)

5 - Bunyamwera group

The results of serological examination indicated:

1 - An appreciable activity of Sindbis virus (Alphavirus group). The maximum percentage of antibodies obtained was 22.9% in Ambila Etrotroka area.

2 - A high incidence of flaviviruses in the Southeast, North and Northwest of the Island, with an obvious prevalence of West Nile virus. The maximum percentage of antibodies was 31.3% in Marovoay area.

3 - Low or no incidence of the other groups. The highest percentage of antibodies against SFS was 2.3% in Majunga area.

B. Lemur sera

Lemur sera have been processed in the same manner as human sera.

A total of 237 sera collected from different lemur species trapped from several places were tested for antibodies and gave the following results:

a. Alphavirus group: low activity

b. Flavivirus group: appreciable activity but less important than humans (the highest percentage of antibodies was 12.5%).

c. The 3 other groups: low or no activity

Otherwise, two blood samples from lemurs marked and trapped in Ampijoroa were collected within one year and showed a seroconversion: April 1979 (<1:10); April 1980 (1:160).

C. Wild bird sera

A total of 49 sera collected from parrots (*Coracopsis vasa* and *nigra*) in Morondava area, indicated Sindbis and Wesselsbron activity. The percentage of antibodies were respectively 18.3% and 13%.

III. Conclusions

Although limited means were used to conduct this arbovirus research program which was started in 1976, positive results have been obtained.

It allowed us to isolate a high number of arboviruses with the help of the Institut Pasteur (Dakar and Paris). Some were with widespread activity (Dengue, West-Nile, Sindbis viruses). Others, known to date, occurred in restricted areas, (Zingavirus). Others were new (Perinet virus, Mg Ar 807 strain virus).

Investigations are continuing and should be carried on in order to examine thoroughly this knowledge because Madagascar, by her original feature and her endemic flora and fauna, constitutes an entity very distinct from the African continent with respect to arbovirus species known to circulate there.

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REPORT FROM THE ARBOVIRUSES LABORATORY

INSTITUT PASTEUR

01 B.P. 490
ABIDJAN - IVORY COAST

RURAL EPIDEMIC OF YELLOW FEVER IN IVORY COAST

During the month of May 1982, the Pasteur Institute was advised of an outbreak of febrile icteruses in the region of M'Bahiakro (prefecture of Bouake).

Several multidisciplinary surveys were organised in the epidemic area with the purpose of establishing its virological, entomological and primatological characteristics.

Human study

135 blood samples were collected with special regards to virus isolation. One fraction of each blood was immediately immersed in liquid nitrogen, the remaining part beøing kept for serological studies. During the first investigations eleven cell culture flasks (C6/36 - clone of *A. albopictus* cells) were inoculated at the moment of the blood sampling (dilution : 1:40). Two out of the eleven permitted the isolation of viral strains. In such conditions, immuno-fluorescence diagnosis is now possible as soon as the staff returns to laboratory.

As a whole, Yellow Fever virus was isolated from 27 patients bloods.

In regard to the age-classes, the distribution of isolations is homogeneous, under 25 years :

0/ 4 years	6 strains
5/ 9 "	3 "
10/14 "	4 "
15/19 "	6 "
20/25 "	9 "
25 and more	0 "

It is to be noted that the last systematic Yellow Fever vaccination occured more than 22 years ago in that region.

The serological techniques employed are hemagglutination inhibition test, complement fixation and E.L.I.S.A. The study reveals, beyond the 27 virus isolations :

13 primary sero-conversions
27 secondary sero-conversions which indicate a recent outbreak of a flavivirus.

At least 67 persons out of 120 tested have thus been infected by the Yellow Fever virus. As a whole 35 persons died of Yellow Fever during this epidemic, mortality beøing in the average of 5 %.

Entomological study

It showed the extreme importance of *Aedes aegypti* (40 % of the larvae in the water collecting containers and 73 % for the inside catches). This is linked to unfrequent climatic conditions and water storage habits which differ from those generally encountered in the preforest region.

Seven Yellow Fever virus strains were isolated from female *Aedes aegypti* caught in the vicinity of the icterus cases. No strain was isolated from the males or egg-issued mosquitoes. The catches in the outskirts of the villages have shown the existence of sylvatic and peridomestic Yellow Fever vectors: *Aedes (Stegomyia) africanus*, *A. (Diceromyia) taylori* and *A. (Aedimorphus) vittatus*. No strain was isolated from these vectors. Two ORUNGO strains were isolated from *Anopheles gambiae* at the time of the Yellow Fever outbreak.

Primatological survey

It was conducted on 24 monkeys. Most of them were gun shot in the immediate border of one of the most infected village (Dezidougou). No virus strain was isolated from the blood or organs of the shot animals. Most of them are *Cercopithecus petaurista*. Among them, 64 % have antiamaril antibodies, 30 % of which with a very high titer.

Two other species *Colobus verus* and *Erythrocebus patas* had antibodies too; the only *Cercopithecus (Mona) campbelli* had none.

The prophylactic measures proposed were, together:

- widespread vaccination of the populations in all the region (30000 vaccination doses)

- vector destruction:
  against larvae: ABATE
  against adults: DECIS inside the houses
  ACTELLIC outside

- population move limitation (difficult).

The application of those measures permitted the extinction of the outbreak in less than two weeks.

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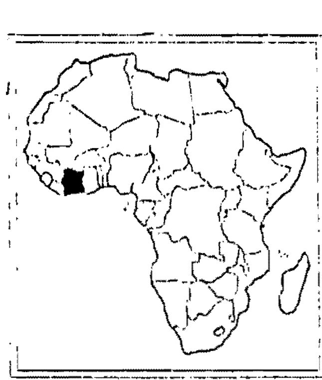


MALI

UPPER VOLTA

Ferkessedougou

Epidemic area

GUINEA

Bouake

Man

M Bahiakro

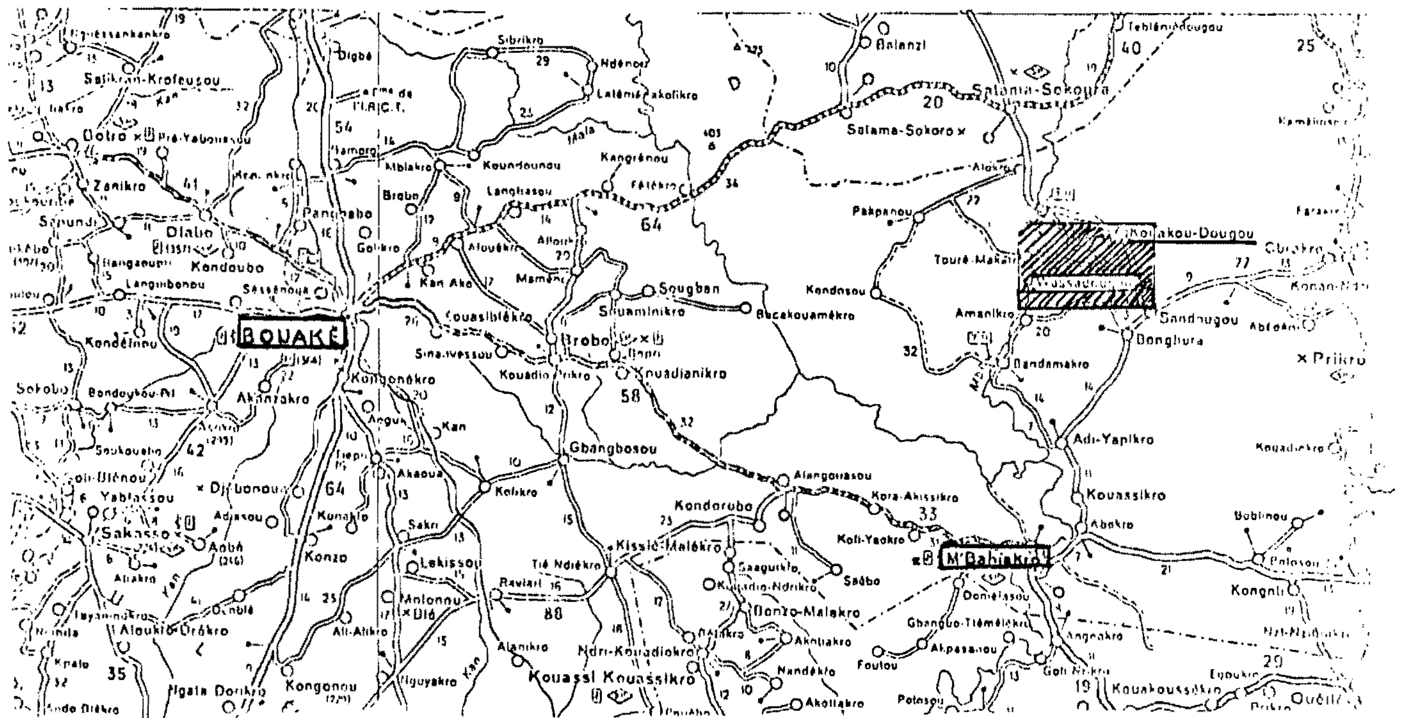
IVORY COAST

GHANA

LIBERIA

Abidjan

South Atlantic Ocean



REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUT PASTEUR AND ORSTOM
INSTITUT PASTEUR BP 220 - DAKAR - SENEGAL

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1. The second semester of 1981 was marked by two particularly interesting facts in Senegal.

1.1 The simultaneous reappearance of yellow-fever in two different places.

1.1.1 The first case, a typical isolated manifestation of sylvatic yellow-fever, concerned a young Lebanese woman, non-immunized and living in Dakar. She very probably caught the disease during a brief stay in a hunting camp in the vicinity of Koungheul, 300 Kms east of Dakar, north of the center of the Gambia, a region belonging to the fluctuant area of the virus emergence. She fell ill in Dakar and died of her yellow-fever during which the virus was isolated. No secondary case was registered.

1.1.2 In the medical district of Mekhe particularly, some 100 Kms in the North-North-East of Dakar, situated in the virus area of epidemicity. The vaccinal protection there being less effective than elsewhere on account of the situation of this region in between the three Great Endemic Diseases Services of Louga, Thies, and Diourbel (where the 1965 epidemic is still remembered) did not exceed 60 per cent and did not reach children under five, because no immunization team had come there since 1978-9.

The epidemic probably made its first appearance in August but was noticed by those in charge at the beginning of September only. The virological enquiry has permitted the isolation of five yellow-fever strains : - 3 human ones, thanks to the simultaneous inoculation to the new born mouse and to continuous cell line _Aedes aegypti_ ; - 2 in _Aedes aegypti_ mosquitoes after intra-thoracic inoculation to the _Toxorhynchites_ bred mosquito and identification by fluorescent foci method and simultaneously by inoculation to _Aedes aegypti_ cells.

The entomological enquiry revealed a high index of _Aedes aegypti_, but equally the activity of potential yellow-fever vectors : _Aedes furcifer_, _Aedes taylori_, and _Aedes metallicus_.

The serological enquiry objectified a wide diffusion of the virus among the infantile population, sparing nevertheless some villages of the area, a lack of vaccinal scars among the children under five, and the circulation two years before of a flavivirus antigenically closed to the yellow-fever virus and probably of an epidemical form.

Its combined action with the recurrent effect due to the yellow-fever infection entailed the appearance of IHA and FC anti-B antibodies in extremely high level in the blood of numerous children, a phenomenon of heterologous secondary reaction absolutely comparable with that obtained by the consecutive infection of two flaviviruses, one of which could be related to the Dengue or Zika viruses. It may be thought that this former epidemic possibly caused, by its immunizing action, a certain benignity of the yellow-fever epidemic among the non-immunized infantile population.

The prophylactic measures taken by the Senegalese authorities entailed a large-scale immunization bearing on more than 700,000 individuals.

1.2 The confirmation of the existence in Senegal of two Dengue serotypes, thanks to new sensitive techniques of isolation on *Toxorhynchites* mosquitoes.

Already, two strains, gathered, the one in 1970 from human blood in Bandia, the other from a lot of *Aedes luteocephalus* captured in Kedougou in November 1974, were identified later on as belonging to Dengue 2.

Then, two other human strains obtained in Bandia in October and November 1979 were identified as belonging to Dengue 1 by the fluorescent monoclonal antibodies technique on continuous cells line *Aedes pseudo-scutellaris*.

In September 1981, an *Erythrocebus patas* monkey, killed in Kedougou, supplied a new Dengue 2 strain.

This phenomenon is not limited to Senegal only, and is found nearly everywhere simultaneously in West-Africa. The recent isolation of numerous Dengue 2 strains from various *Aedes* (4 in Guinea, 23 in Ivory Coast, 67 in Upper Volta) confirmed the general diffusion of this virus.

In fact, there is a risk that the four serotypes of this virus gradually take root in Africa, as it can be foreseen by the isolation in 1981 in Dakar of a Dengue 4 serotype, in a woman coming from Haïti, who fell ill in the plane which brought her to Senegal.

In order to make parallel studies of the isolations on the new-born mouse and on the *Toxorhynchites* mosquito, numerous lots of mosquitoes, captured during the 1981 rainy season, have not been exploited yet on account of the difficulties met in the breeding of *Toxorhynchites*. However, thanks to the present development of the insectarium, isolations have restarted again and the exploitation of these lots will be finished soon.

In other respects, the laboratory has proceeded with its virological and serological investigations on human beings in various Senegalese regions and on Bandia rodents and their parasites.

Finally, the serologic survey of the Eastern Senegal monkeys has been achieved like every year, in order to study the arboviral, and more particularly yellow-fever, circulation in this province.

2. Virological studies

2.1 Human blood samples

309 sera were inoculated and four strains of yellow-fever virus were obtained :

Three from Mekhe, by I.C inoculation of suckling mice and by inoculation of continuous cell line A. aegypti.
The fourth, from Dakar, was obtained by these two methods and by intra-thoracic inoculation of Toxorhynchites.

2.2 Wild vertebrate samples

2.2.1 Rodents from Bandia

212 rodents were examined in 1981 and 4 strains isolated :

2 Bandia viruses
1 Koutango virus
1 Nafada = Bornu = Fika virus (Sud An 754 not yet registered).

2.2.2 Monkeys, principally from Kedougou

193 monkeys were caught and inoculated. One single strain, a dengue 2 virus, was obtained from an Erythrocebus patas.

2.2.3 Other wild vertebrates

6 samples were examined but no viral strain was isolated.

2.3 Arthropods

2.3.1 Mosquitoes

26.399 mosquitoes were collected during the 1981 rainy season and processed in 1101 pools. 6 strains were isolated :
4 strains of Dengue 2 virus from mosquitoes caught in Mali (Republic of Guinea), about fifty kilometers from Kedougou ;
3 from A. africanus and 1 from A. luteocephalus in November.
These strains were isolated only by intra-thoracic inoculation of Toxorhynchites.
2 strains of Yellow-fever virus were isolated from A. furcifer collected in Mekhe during October by two methods :
Toxorhynchites mosquito and continuous cell line A. aegypti
Numerous pools of mosquitoes caught in 1981 are yet in progress, waiting for a sufficient quantity of bred mosquitoes to be inoculated.
8 pools of 171 mosquitoes obtained in the insectarium from scraped holes of trees in order to confirm the natural transovarial transmission, were inoculated but no strain was isolated.

2.3.2 Ticks

4117 ticks, processed in 95 pools, permitted the isolation of 32 Bandia virus strains.

3. Serological studies

3.1. Human sera

3.1.1 From Mekhe

116 sera principally of children, were harvested in Mekhe and in 10 villages in the surroundings. They were investigated by HI an CF tests against YF, WN and Zika antigens. Indubitable serologic signs of a yellow fever outbreak were found among yound children. But very soon as they grew in age, numerous and strong heterologous reactions appeared and interpretation became more difficult.

4 villages seemed to be less affected by the disease. At present, these sera are examined with the Dengue antigens to determine, the circulation of these viruses in the country.

3.1.2 From Eastern Senegal and Casamance

434 sera, collected in April, were investigated by the HI test but the CF test is not finished yet. With this single method, 40 per cent of the sera contain anti B antibodies and the fourth part anti YF antibodies but at a low level. Two sera showed a possible Zika infection and a possible YF infection which could be confirmed only with the CF test. Some hetorologous reactions were also noted, but they were insignificant.

3.1.3 From Bandia

These 157 sera, harvested in the 1980 rainy season, were examined only in 1981. Among them, 33 were paired sera, collected at an interval of 2 or 3 months, before and after the rainy season. They were examined only by HI test and CF test is now in progress. 48 per cent contain antibodies against flaviviruses, 37 per cent anti YF antibodies and 44 per cent anti Dengue D1 antibodies. About twenty sera contain isolated anti-D1 antibodies at a level of 1/80 and some sero-conversions against this virus were noted but they showed a light increase of antibodies.

3.1.4 From Kolda

During an expanded programm of immunization made in Casamance in 1980, a serologic evaluation study was carried out among 472 children. 190 of them were vaccinated against YF during a first passage and 100 at a second passage. The antibodies were detected by three methods = HI, SN and ELISA tests. For the ELISA test, a mixing of 2 specific proteins of YF virus was used as antigens and the sera were diluted at the 50th and 200th. The results are being computer-processed at present and will be known in a few days.

3.2 Vertebrate sera

178 sera were collected in 1981, and only from monkeys. They were tested against the following antigens by the HI test = YF - WN - Zika - Dengue 1 and 2. The CF test is in progress and this does not allow formal conclusions at present.

But the antiflavivirus antibody percentage fell from 100 % in January to 60 % in March, then went gradually up again to 90 % in September.

In June, some isolated antibodies against YF and Zika viruses were observed at a middle level (1/640 - 1/1280). In August, some low heterologous reactions were noted and they became stronger in the following months. It is impossible to conclude without the results of CF method, but Zika virus and perhaps YF virus seemed to persist in the country but at a very low density level.

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Dr M. GERMAIN, Dr M. CORNET and Dr J.L. CAMICAS (ORSTOM)

Report from San Juan Laboratories, VBVDD, CID, CDC

FATAL ENCEPHALOPATHY ASSOCIATED WITH DENGUE 4 INFECTION IN PUERTO RICO

Dengue virus has been isolated for the first time in Puerto Rico from a patient with an illness compatible with viral encephalitis. The patient, a 15 year old male Puerto Rican from San Juan, with seizure disorder on anticonvulcent therapy, was admitted to San Juan Municipal Hospital on March 7, 1982 with a history of 2 days fever, headache, conjunctivitis and vomiting. On admission he had an erythematous rash and a few petechiae, but there were no other hemorrhagic manifestations. Over the next 2 days, the patient's condition deteriorated rapidly and he died on March 9 after developing a clinical picture of encephalopathy characterized by altered mental state, seizures, progressive spastic paresis and coma.

A lumbar puncture done on March 7, showed a clear CSF with 21 red blood cells and 5 white blood cells, 43mg% protein (N=15-30) and glucose 70mg% (N=40-70). Dengue 4 virus was isolated from a blood sample taken on March 8. Virus isolation from the CSF was negative. Serology on blood samples taken on February 16 and March 8 showed low level HI antibody to all dengue antigens. There was no detectable HI antibody in the CSF taken on March 7. Pathology and virus isolation from tissues are pending.

This patient had a 10 year old brother who died in 1974 from an illness compatible with progressive spastic paraparesis and adrenal insufficiency. Since that time the patient had been watched carefully for any signs of a similar illness. Since October 1981 his gait became progressively abnormal (he walked on the tip of his toes). He was hospitalized on February 16, 1982 for an endocrine and neurologic evaluation. The neurologic evaluation did not reveal any objective evidence of CNS deterioration and the endocrine studies were all normal. The patient was discharged on February 26, 1982.

Another brother, aged 22, who lived in the same house, had a febrile illness at the same time as the patient. Dengue 4 virus was also isolated from him.

Although concurrent infections with herpes or enteroviruses cannot be ruled out at this time, there was no clinical, laboratory or epidemiological evidence that would specifically suggest these agents.

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*San Juan City Hospital

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, COLORADO STATE UNIVERSITY, FORT COLLINS, CO. 80523 and THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF ALABAMA, BIRMINGHAM, AL 35294.

We previously demonstrated that dual-infection of Aedes triseriatus with two California group viruses could result in RNA segment reassortment and thus formation of new virus genotypes. We have subsequently embarked upon studies to determine the effective phylogenetic range of segment reassortment and to determine if interference between bunyaviruses occurs in mosquitoes.

Aedes triseriatus mosquitoes were intrathoracically inoculated with a ts mutant of La Crosse (LAC) virus and after 7 days incubation with a selected wt California group virus, Guaroa (bunyavirus), West Nile (flavivirus), or vesicular stomatitis virus (rhabdovirus). Mosquitoes were held for an additional 7 days and then harvested. Virus titers were determined at both 33° C and at 40° C (permissive and nonpermissive temperatures, respectively).

In the first experiment (Table 1), mosquitoes were inoculated with the LAC-I-16 mutant of La Crosse virus. This mutant contains a ts lesion in the M RNA segment which codes for the glycoproteins. In the second experiment (Table 2), mosquitoes were inoculated with the LAC-II-5 mutant virus. This mutant contains the ts lesion in the L RNA segment which presumably codes for the polymerase.

When mosquitoes were infected with the LAC-I-16 mutant, there was evidently complete interference to superinfection with California group viruses (Table 1). No wt virus was detected at 40° C. In contrast, previous infection with LAC-I-16 had no effect on the replication of West Nile or VSV.

When mosquitoes were infected with the LAC-II-5 mutant, the pattern of interference was different. Instead of complete inhibition of replication of California group viruses, both wt LAC and snowshoe hare virus infected the mosquitoes (Table 2). However, the geometric mean titers of both viruses were substantially lower than those of the control wt viruses. No wt virus was detectable in the mosquitoes inoculated with Tahyna or Trivittatus virus. Guaroa, West Nile, and VSV infected and replicated to high titer in both the previously infected and control mosquitoes.

The molecular basis of the interference remains to be ascertained. When the LAC-I-16 mutant was the initial infecting virus, subsequent infection and replication of California group viruses was completely suppressed. This pattern of interference to superinfection is what would be expected with homologous or defective particle interference. However, when the LAC-II-5 mutant was the original infecting virus, both wt La Crosse and snowshoe hare viruses infected and replicated in the vectors, but the more distantly related Tahyna and Trivittus viruses were completely inhibited. This pattern of interference could not be explained by a DI particle hypothesis. Since the ts lesion in the LAC-II-5 mutant is in the L RNA segment, a polymerase phenomenom may be involved. Regardless of the molecular basis of the interference, the implications for bunyavirus evolution via RNA segment reassortment are major; reassortment would seem to be limited to closely related viruses.

(B.J. Beaty, F. Fuller, and D.H.L. Bishop)

Table 1

Virus titers in mosquitoes originally infected with LAC-I-16 virus and subsequently with a selected wild type virus

Virus		Mean titer (pfu/ml)*		
Day 0	Day 7	33°C	40°C	% inhibition
Control	LAC**	2.3×10^4	1.0×10^3	
LAC-I-16	LAC	2.5×10^4	< 10	> 99
Control	SSH	1.3×10^4	2.1×10^3	
LAC-I-16	SSH	1.2×10^4	< 10	> 99
Control	TAH	1.2×10^3	2.6×10^2	
LAC-I-16	TAH	1.2×10^4	< 10	> 99
Control	TVT	2.2×10^4	4.2×10^2	
LAC-I-16	TVT	6.2×10^3	< 10	> 99
Control	WN	1.9×10^5	1.5×10^4	
LAC-I-16	WN	1.7×10^5	2.5×10^4	0
Control	VSV	2.0×10^4	2.1×10^2	
LAC-I-16	VSV	3.4×10^4	2.3×10^3	0

* Geometric mean titer of a minimum of 4 mosquitoes.

** LAC - La Crosse virus; SSH - snowshoe hare virus; TAH - Tahyna virus; TVT - trivittatus virus; GRO - Guaroa virus; WN - West Nile virus; VSV - vesicular stomatitis virus.

Table 2

Virus titers in mosquitoes originally infected with LAC-II-5 virus and subsequently with a selected wild type virus

Virus		Mean titer (pfu/ml)*		
Day 0	Day 7	33°C	40°C	% inhibition
Control	LAC**	$3.9x10^4$	$1.6x10^4$	
LAC-II-5	LAC	$1.2x10^4$	$4.0x10^2$	97.5
Control	SSH	$3.4x10^3$	$1.8x10^3$	
LAC-II-5	SSH	$2.3x10^4$	$3.8x10^1$	97.9
Control	TAH	$1.8x10^4$	$3.1x10^3$	
LAC-II-5	TAH	$5.6x10^3$	< 10	> 99
Control	TVT	$4.2x10^4$	$1.8x10^4$	
LAC-II-5	TVT	$7.9x10^3$	< 10	> 99
Control	GRO	$2.6x10^4$	$1.6x10^4$	
LAC-II-5	GRO	$2.2x10^4$	$1.3x10^4$	0
Control	WN	$9.7x10^4$	$2.0x10^4$	
LAC-II-5	WN	$1.4x10^5$	$2.0x10^4$	0
Control	VSV	$1.0x10^5$	$1.2x10^4$	
LAC-II-5	VSV	$7.8x10^4$	$9.0x10^3$	0

* Geometric mean titer of a minimum of 4 mosquitoes

**LAC - La Crosse virus; SSH - snowshoe hare virus; TAH - Tahyna virus; TVT - trivittatus virus; GRO - Guaroa virus; WN - West Nile virus: VSV - vesicular stomatitis virus.

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

Arbovirus Surveillance, 1982

Two confirmed and eight presumptive cases of California encephalitis (CE) complex were detected by serologic testing of sera from 169 CNS patients who were residents of New York State. The infectious agents in two confirmed cases in patients aged 3 and 24 years old were subtyped as La Crosse and Jamestown Canyon (JC), respectively. Three of the presumptive cases in patients aged 79, 56 and 45 years were fatal; all three were serologically demonstrated to be JC virus infections. High neutralizing antibody to JC was detected in IgM fraction of the serum from the 45-year-old woman. However, she also had a diagnostic serologic finding of herpes simplex virus infection. Five other presumptive cases of CE in patients aged 70, 33, 22, 14 and 4 years were probably due to JC infections.

Another confirmed arbovirus infection, diagnosed as dengue at the San Juan CDC Laboratory was detected in an ill female physician, resident of Erie County, who had just returned from a refugee camp in Thailand.

During June through August a total of 571 pools of wild-caught mosquitoes from nine counties in New York State were inoculated in cell cultures. Seven isolates of California encephalitis complex viruses were obtained, five from Warren County north of Lake George and two from the western part of the state.

Serologic Subtyping of California Serogroup Viruses

Complement-fixation tests of 135 California serogroup viruses from mosquitoes captured in New York State between 1965 and 1981 indicated JC as the most prevalent subtype, comprising 65% of all isolates identified thus far. During the period 1971 to 1981, JC was obtained from at least 14 species in 2 genera of mosquitoes and was widely distributed throughout the state, occurring in 18 counties from Niagara in the northwestern region to Suffolk in Long Island. This virus was most frequently encountered in mosquitoes of the *Aedes communis* group in northeastern New York.

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

Report from the Department of Veterinary Science
University of Wisconsin, Madison, Wisconsin

From January 1981 to July 1982 serological studies of bluetongue prevalence in cattle were carried out under the auspices of our cooperative programs with the Faculty of Veterinary Medicine of the University of Antioquia, Colombia, and with the School of Veterinary Medicine of the National University, Costa Rica.

Blood samples were collected from cattle on farms in the Departments of Antioquia and Cordoba, Colombia with the cooperation of practicing veterinarians and a milk cooperative. In addition 107 blood samples were collected at slaughter in Medellin municipal abattoir. Blood samples were collected throughout Costa Rica with the aid of Ministry of Agriculture field veterinarians, private practitioners and clinicians of the School of Veterinary Medicine. Questionaires were completed detailing the topography of each farm, and the composition and clinical history of each herd.

Sera were tested in our laboratories at the University of Antioquia, and at the School of Veterinary Medicine in Costa Rica. The micro agar gel precipitin test was performed using bluetongue group specific antigen and enhancer serum prepared by the National Veterinary Diagnostic Services Laboratories, Ames.

Results in Colombia (figures 1a and 1b) and Costa Rica (figures 2a and 2b) show a marked association of prevalence with altitude. Over 2000 meters prevalence are zero or near zero, rising to over 50% below 1000 meters. Analysis of data by region indicates that cattle throughout both study areas were exposed to infection. In Costa Rica, no significant difference in prevalence could be demonstrated between farms using a bull (49% prevalence), artificial insemination (43%), or both (44%). Apparent differences in prevalence according to breed of cattle were found not to be distinguishable from differences attributable to altitude.

In Colombia, limited collecting of Culicoides has shown C. insignis to be prevalent in the Valle de Aburra, feeding on seropositive cattle. Light trapping is in progress in both study areas.

In neither country have clinical cases of bluetongue been reported. This may be due to a lack of alertness to the clinical presentation of the disease, a failure to differentiate in adult cattle from vesicular diseases, or possible circulation of milder strains of the virus. No isolations have yet been reported from these two countries. Our isolation attempts continue.

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Results of Agar Gel Precipitin Testing for Bluetongue in the Departments of Antioquia and Cordoba, Colombia

Figure 1a: Distribution by Region.

Region	Number of Farms	Total Animals		Prevalence (%)
		Tested	Positive	
Caucasia	2	75	47	63
Monteria	2	48	31	65
Montelibano	3	40	16	40
Uraba	4	12	6	50
Fredonia	1	24	24	100
Valle De Aburra	3	117	68	58
Oriente de Antioquia	5	67	27	40
Frontino	5	76	33	43
Yarumal	5	82	2	2
Total for Study Area	30	541	254	47

Figure 1b: Distribution by Altitude

Altitude (meters)	Total Animals		Prevalence (%)
	Tested	Positive	
0-1000	199	124	62
1001-2000	260	128	49
over 2000	82	2	2

Results of Agar Gel Precipitin Testing for Bluetongue in Costa Rica.

Figure 2a: Distribution by Region.

Region	Number of Farms	Total Animals		Prevalence (%)
		Tested	Positive	
Guanacaste	9	107	74	69
San Carlos	4	43	31	72
Limon	4	41	16	39
Cordillera Central	14	129	28	22
Cordillera de Talamanca	10	57	10	18
Meseta Central	21	259	114	44
Puntarenas - coastal strip	10	143	78	55
Puntarenas - Panama border	36	391	180	46
Totals for Country	108	1170	531	45

Figure 2b: Distribiton by Altitude

Altitude (meters)	Number of Farms	Total Animals		Prevalence (%)
		Tested	Positive	
0-500	32	377	207	55
501-1000	27	242	133	55
1001-1500	24	306	130	43
1501-2000	21	195	61	31
over 2000	4	50	0	0

REPORT FROM THE HORMEL INSTITUTE, UNIVERSITY OF MINNESOTA, AUSTIN, MINNESOTA

STUDY OF POLYENE MACROLIDE ANTIBIOTICS AGAINST DENGUE VIRUS

Our previous studies (1) have shown that some polyene macrolide antibiotics inhibit *in vitro* propagation of dengue virus type 2 (New Guinea strain). Using a dengue plaque assay in BHK-21 cells (2), we have determined the concentration of several polyene macrolide antibiotics reducing plaque development by 50%. These concentrations are 8-100 times lower than the toxic concentrations for BHK-21 cells. The highest anti-dengue activity was observed with aureofacin and its methyl ester derivative (1).

Aureofacin, as well as its methyl ester, induce in the membrane of BHK-21 cells specific permeability changes for uncontrolled diffusion of potassium ions which leads to cell death. However, these permeability changes could be repaired by the cells and the polyene toxic effect could be reversed.

Recently we have studied the mechanism of the antiviral effects expressed by the polyene antibiotics. Dengue type 2 virus was propagated in BHK-21 cells grown in serum-free medium (3) in order to eliminate a negative feedback of serum on polyene antibiotic action. BHK-21 cells were treated with aureofacin methyl ester for 60 min at various times after dengue virus infection under standard conditions (3). After antibiotic treatment, cells were washed and incubated in the antibiotic-free media to the completion of the dengue virus replication cycle which lasted about 24 h. During the replication cycle, we measured cell ability to repair polyene-induced damage in the cell plasma membrane (4). After the replication cycle was completed, the dengue virus titer produced by BHK-21 cells was measured as well. We observed that dengue virus infected cells treated with polyene antibiotic after the adsorption period were able to recover fully, similar to the uninfected BHK-21 cells treated with the same dose of aureofacin methyl ester. However, such treatment reduced the virus titer about 10-fold of that obtained after the replication cycle was completed (4).

When the polyene antibiotic was used in the later stages of dengue virus replication, an inhibitory effect was observed on the cells' ability to repair polyene-induced membrane damage. However, it did not influence, to a large degree, the virus production by the cells.

In further research we intend to use polyene antibiotics to study the cell-virus relationship during the dengue replication cycle.

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(B. Malewicz, H. M. Jenkin and M. Momsen)

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

Arbovirus surveillance during 1981 and preliminary report on 1982

The twelfth special annual report on arbovirus surveillance since 1969, when testing for viruses in mosquitoes was resumed as a surveillance activity by the VRDL, has been prepared and will be published in the Proceedings and Papers of the Fiftieth Annual Conference of the California Mosquito and Vector Control Association, Inc. A brief summary follows:

During 1981, 306 patients suspected of having viral encephalitis were treated serologically by the VRDL or the 6 county public health virus laboratories which assist in surveillance, and ten human brain samples were tested in suckling mice. No human cases of WEE or SLE were detected. Of 26 suspected equine cases tested serologically, only 1 was confirmed as WEE: an unvaccinated mare from Sacramento county, with onset October 5. There were only 13 equine brain samples submitted, and none yielded WEE virus. However, from one brain sample Main Drain Virus (Bunyawera group) was repeatedly isolated. Main Drain Virus is a _Culicoides_-transmitted virus involving rabbit and rodent hosts; equine infection has been demonstrated previously (serologic surveys) but there has been no previous evidence of pathogenicity, and this is the first isolation of the virus from an equine.

There were 2,205 mosquito pools (104,439) mosquitoes) tested from sampling sites in 23 California counties and one Arizona county, yielding 224 viral isolates: 48 WEE, 2 SLE, 100 Hart Park, 65 Turlock, 8 CEV group, and 1 unidentified agent. Sentinel chicken flocks at 22 sites showed seroconversion for WEE antibody (indirect immunofluorescence method) in 11 and for SLE antibody in 3 by the end of the season.

The near-record heavy rainfall and deep Sierra snowpack during the 1981/82 winter season has again threatened the State with the potential for extensive mosquito breeding. Unusually late, cool temperatures and extensive control efforts by local mosquito abatement districts may be successful in averting a major problem. However, as of August 16, surveillance indicators showed considerable WEE virus activity. Of 1,337 mosquito pools tested for virus, 58 contained WEE virus, and 168 contained other viruses (largely Hart Park and Turlock). A high percentage of sentinel chickens had seroconverted for WEE in Imperial county, and a few chickens had done so in southern San Joaquin Valley areas. One equine case of WEE had been detected in Fresno county. No isolates of SLE virus had been made as yet, and only 2 sentinel chickens in Imperial county had seroconverted for SLE. Direct immunofluorescent staining to identify virus isolates in suckling mice, and indirect immunofluorescence to determine antibody in sentinel chickens are continuing to be rapid and helpful surveillance tools.

REPORT FROM THE NATIONAL ARBOVIRUS REFERENCE SERVICE

DEPARTMENT OF MEDICAL MICROBIOLOGY,

UNIVERSITY OF TORONTO,

TORONTO, ONTARIO, CANADA.

Studies on Powassan Virus Activity in Ontario

Five cases of Powassan (POW) encephalitis have been diagnosed in Ontario including four since 1976. A study was undertaken between 1979 and 1981 of POW activity in parts of Ontario. Ticks were collected, divided into pools, processed and virus isolation was attempted by intracerebral inoculation of suckling mice. A total of 974 *Ixodes* and *Dermacentor* species ticks were examined. One isolate of POW virus was obtained from a pool of *I. cookei* ticks collected on July 25, 1981, in the Guelph area.

Serological studies were performed on groundhog and dog sera. Twenty-one of 91 groundhogs and 12 of 891 dogs tested showed hemagglutination inhibiting antibodies to POW virus for positive antibody rates of 23.1 and 1.3% respectively. In addition POW virus was isolated from the blood of a yearling groundhog.

This study describes the first isolation of POW virus in Ontario since 1966, documents a newly recognized area of POW activity (Guelph), confirms the importance of groundhogs in the POW virus cycle and implicates dogs as having a potentially important role in bringing infected ticks into contact with humans.

(H. Artsob, L. Spence, C. Th'ng and V. Lampotang, National Arbovirus Reference Service, Toronto,in collaboration with G.A. Surgeoner, J. McCreadie and J. Thorsen, University of Guelph.)