December, 1990

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PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE:

To exchange information on a timely basis. The recipients are those who study various aspects of arboviruses. The Exchange contains preliminary reports, summaries, observations, and comments submitted voluntarily by qualified agencies and individual investigators. The appearance in the Exchange of any information, data, opinions, or views does not constitute formal publication and should not be referred to in "Reference" sections of papers or included in lists of publications. The Exchange is not a "peer reviewed" publication; in fact, it is not a publication at all. Any reference to or quotation of any part of the Exchange must be authorized directly by the agency or person submitting the text.

HANTAVIRUSES

Editor's comments

Recent submissions to the Arbovirus Information Exchange have been terrific and numerous. Thank you all very much for your support and for your scientific responses and generous letters. I am certain that with such nutriment this vehicle for communications will continue for some time to come. As some of us suspected, there is more interest in arboviruses throughout the world than we have been lead to believe.

As noted in "PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE", which is found in each issue, we encourage you to submit a brief summary of your work. The summary need not be in manuscript style, the results do not have to be definitive, you need not include tables (unless you want to). This is not a peer-reviewed publication. The intent is to communicate among ourselves and to let each other know what we are doing. If you do not send in a report, the inevitable conclusion is that you do not care or you are not doing anything.

Please note the article "Saliva-activated transmission of tick-borne viruses" by PA Nuttall and LD Jones. This is an interesting follow-up to "A suggested experiment" by PA Nuttall, LD Jones, and CR Davies, printed in the June, 1989 issue (page 1) of the Arbovirus Information Exchange.

The next issue is scheduled to be mailed June 1, 1991 (deadline for submissions May 15, 1990). There is nothing that requires you to wait until the last minute. If you have something to communicate in January, February, March or April, please send it to me. Also, there is nothing that prevents you from submitting a report to every issue. There are no pages charges either, but then again, this is not a publication.

PLEASE !!!

Follow the directions for submitting reports. Double-spaced pages take twice as much space as single-spaced pages. Do not double-space or number pages. Single-space them and leave them unnumbered. Do not staple pages together.

Charles H. Calisher, Ph.D.
DVBID/CID/CDC
P.O. Box 2087
Ft. Collins, Colorado 80522 (U.S.A.)

GUIDELINES FOR SUBMITTING REPORTS

We want to keep this mechanism timely and viable. Therefore, send me only recent news and summaries of your work. PLEASE limit the submission to 1 or a very few sheets (21.59 cm x 27.94 cm = 8.5 x 11 inches) plus a table or two: condense as much as you can (single space the text); do not staple pages together; do not number pages. This is essentially a one person operation and I am basically a lazy person; the less work I need to do, the better I like it.

Comments on the 50th anniversary of the isolation of Russian Spring-Summer Encephalitis virus (1987) and the 80th birthday of Dr. Mikhail P. Chumakov (1989)

Mikhail Petrovich Chumakov was born to the family of a veterinary assistant November 14, 1909, in the town of Yepifan. He entered the Medical Faculty of Moscow State University in 1927. In 1931, he graduated from the University and began working as a medical bacteriologist in the laboratory of Professor I.M. Velikanov, where he studied laboratory diagnosis of wound (street trauma) infections for two years, publishing the first of his nearly 1,000 scientific publications and monographs. From 1932-1935, he was a post-graduate student in the Moscow Research Institute of Microbiology under Professor I.L. Krichevsky and defended his thesis, "Role of the reticuloendothelial system in certain infections and in immunity."

In 1936, Professor Chumakov began his career in virology as a senior scientific worker in the U.S.S.R. Institute of Microbiology, Department of Medical Virology, under the guidance of Professor L.A. Zilber. In the spring and summer of 1937, Professor Chumakov was a virologist on the first--and now classic--research expedition studying the etiology and epidemiology of tick-borne encephalitis (TBE) in the Far East of the U.S.S.R. (Khabarovski District). During those studies he was accidentally infected in the laboratory with a strain of what is now known as Russian Spring-Summer Encephalitis virus, and suffered the paralytic form of TBE. Although severely affected, Professor Chumakov continues his long and remarkable career.

1938-1942: Worked as senior scientist in the All-Union Institute of Experimental Medicine.

1942-1944: Organized a research encephalitis laboratory attached to the All-Union Institute of Experimental Medicine clinic, and studied TBE in the European U.S.S.R. and in neighboring countries of Europe. In 1944, his doctoral thesis, "Tick-borne encephalitis of man," was accepted, and he became a Doctor of Medical Microbiology and attained the rank of Professor of Microbiology.

1944-1946: Studied the etiology, immunology, and transmission of Crimean hemorrhagic fever virus by ticks.

1947-1949: Continued studies of TBE and Crimean hemorrhagic fever and began studies of Omsk hemorrhagic fever.

1950-1956: Devoted himself to studies of the etiology and epidemiology of hemorrhagic fever with renal syndrome in endemic foci of the European U.S.S.R. (Volga region and Urals) and conducted investigations on trachoma, developing methods for trachoma (Chlamydia trachomatis) therapy. In 1953-1955, Professor Chumakov began intensive studies of immunology, diagnosis, and epidemiology of poliomyelitis, and organized large-scale production of live poliovirus vaccine strains from Dr. Albert Sabin. From

1950 to 1954, he headed the D.I. Ivanovsky Institute of Virology of the U.S.S.R. Academy of Medical Sciences, and, in 1955, founded the Institute on the Study of Poliomyelitis, which subsequently became the Institute of Poliomyelitis and Viral Encephalitides.

1972-1989: Became Head of the Department of Hemorrhagic Fevers and of Search Investigations, and advised on the management of the Institute. At present, he continues his efforts to elaborate the technologies necessary for production of three new vaccines: one for TBE, a subunit vaccine for influenza, and a live virus measles vaccine.

In 1941, Professor Chumakov was decorated with the State Prize First Class for his scientific studies. He was elected corresponding member of the U.S.S.R. Academy of Medical Sciences (1948), and, later (1960), Academician. Over the years he has been awarded numerous prizes and awards: Member of the German Academy of Naturalists "Leopoldina," Honorary Member of the Hungarian Academy of Sciences, Czecho-Slovak Purkinje Scientific Society, Bulgarian Scientific Society of Microbiologists. In 1984, he was awarded the title "Hero of Socialist Labor" and, later, given the D.I. Ivanovsky Prize, the German Gold Hufeland Medal, and the U.S.S.R. Council of Ministers' Prize.

In addition to his scientific publications, he has written more than 30 technological regulations and instruction manuals on problems of production of anti-viral (poliomyelitis, other enteroviral diseases, TBE, hemorrhagic fevers, influenza, and measles) vaccines.

In addition to these scientific contributions, Professor Chumakov has made many and significant contributions to his fellow humans. His personal integrity alone demands recognition. I am certain that I speak for many when I wish Professor Chumakov all the best. He is a remarkable human being and scientist. May his career continue for many years.

NOTE: I am obliged to Dr. Alexander M. Butenko, D.I. Ivanovsky Institute of Virology, Moscow, U.S.S.R., for sending me the following exceptional photographs and to Dr. Alexis Shelokov, The Salk Institute, Columbia, Maryland, for providing some information about the photographs. For additional information, see above and two fascinating papers: J Casals, H Hoogstraal, KM Johnson, A Shelokov, NH Wiebenga, TH Work (1966), A current appraisal of hemorrhagic fevers in the U.S.S.R., *Am J Trop Med Hyg*, 15:751-764, and J Casals, BE Henderson, H Hoogstraal, KM Johnson, A Shelokov (1970), A review of Soviet viral hemorrhagic fevers, 1969, *J Infect Dis*, 122:437-453.

Figure 1. Far eastern Siberia, Primorskii Kray (Maritime Region), Obor settlement, RSSE Study Team, 1937 (Mikhail P. Chumakov on right).

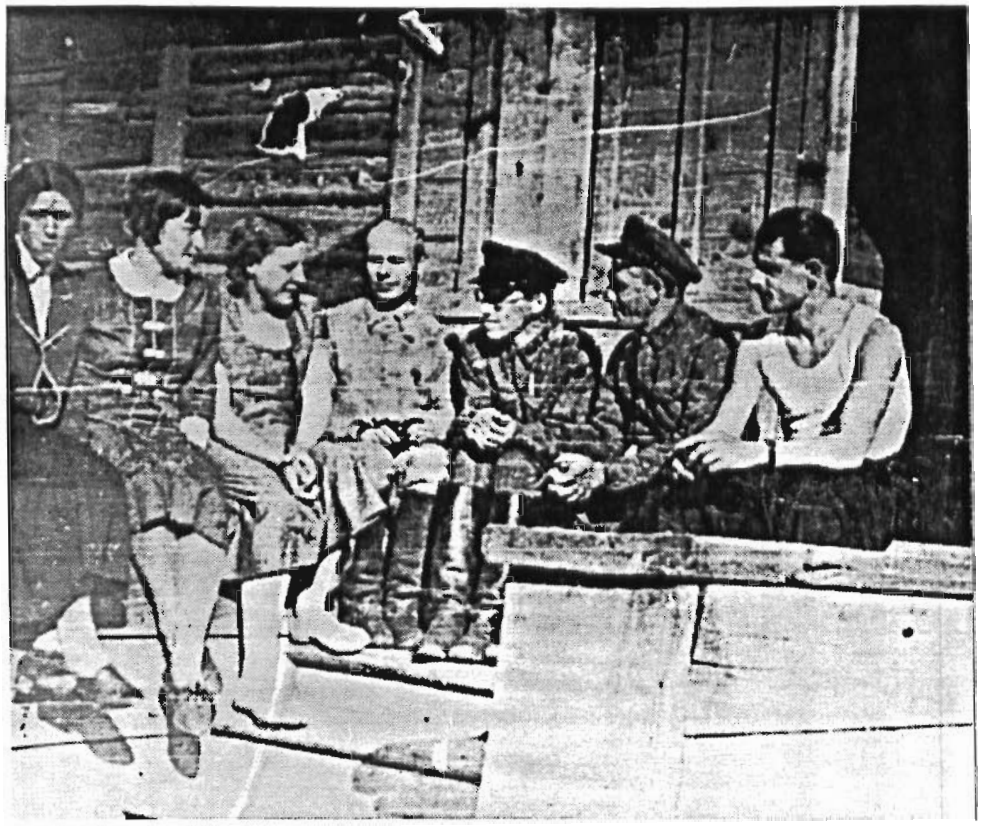


Figure 2. Far eastern Siberia, Primorskii Kray, Obor settlement, laboratory studies, 1937 (Mikhail P. Chumakov on right).

Figure 3. Moscow, 1941 (left to right: Anatolyi A. Smorodintsev, Elena N. Levkovich, Mikhail P. Chumakov).

Figure 4. Moscow, May 1965; members [M] of first U.S. hemorrhagic fever delegation to U.S.S.R. and three Soviet hosts [H] (left to right: Alexis Shelokov [M], Mikhail P. Chumakov [H], Jordi Casals [M], Bela Kaplan [H], Ned H. Wiebenga [M], Karl M. Johnson [M], G. I. Netsky [H]). Harry Hoogstraal and Telford H. Work, also members of the delegation do not appear in this photograph.

ACAV OPEN MEETING HELD IN CONJUNCTION WITH

THE INTERNATIONAL CONGRESS OF VIROLOGY, BERLIN, 26 AUG 1990

Several members of the international arbovirology community gathered on Sunday, 26 August 1990 in Berlin to participate in the ACAV open meeting which was held in conjunction with the International Congress of Virology. The meeting represented a continuation of a longstanding tradition for the ACAV to meet in open scientific session with their International Advisors as part of the International Congress. Brief presentations were made by Dr. Jim Meegan, representing the WHO, and by Dr. Francisco Pinheiro of the Pan American Health Organization. Both Dr. Meegan and Dr. Pinheiro are also members of the ACAV Executive Council.

Several ACAV International Advisors presented summaries of current arbovirus activities in their countries. Dr. Lynn Dalgarno commented on arbovirus activities in Australia, while Dr. Digoutte of the Institute Pasteur in Dakar, Senegal, summarized current investigations in West Africa. Dr. Tom Yuill, ACAV Treasurer, gave an overview on recent VEE activity in Central America, and Academician Lvov spoke about arbovirology in the USSR. The meeting was chaired by Dr. Joel Dalrymple.

USA/USSR JOINT PROGRAM ON ARBOVIRUSES

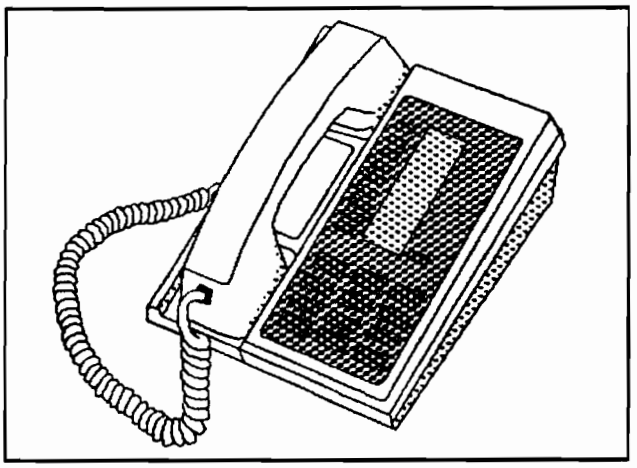
Scientists from the United States of America and the Soviet Union recently participated in the first joint USA/USSR expedition to Western Siberia to collect mosquitoes for arbovirus investigations and taxonomic studies. This was done as part of the USA/USSR Joint Program on Arobviruses under the auspices of the USA/USSR Joint Committee for Health Cooperation. This program is a cooperative effort between the D.I. Ivanovsky Institute of Virology, Moscow, and the Centers for Disease Control (CDC), Atlanta and Fort Collins. The program coordinator for the Ivanovsky Institute is Dr. Dimitry Lvov, and the coordinators for CDC are Drs. Duane Gubler, Brian Mahy, and Frederick Murphy.

Dr. Sergei Lvov, Gamaleya Institute for Epidemiology and Microbiology, Moscow, and Dr. Yevgeni Samockvalob of the Ivanovsky Institute, were accompanied on the expedition by Dr. Carl Mitchell and Dr. Harry Savage, Division of Vector-Borne Infectious Diseases (DVBID), Center for Infectious Diseases, Centers for Disease Control (CDC), Fort Collins, Colorado. Two technicians from the Ivanovsky Institute, Ala Lazarenko, and Valentina Lipatova, also accompanied the group and assisted with the collections.

The expedition arrived in Tyumen City, the capitol of Tyumen Oblast, on 1 July. The group boarded a 30-meter boat and travelled down the Tura, Tobol, Irtysh, and Ob rivers stopping periodically to make collections and freeze them in liquid nitrogen. The journey of more than 1,200 miles from Tyumen to Salekhard, on the Arctic Circle, passed through five distinct ecological zones, i.e., the South, Middle, and North Taiga, Forest Tundra, and Tundra. Dr. Dimitry Lvov joined the expedition in Salekhard and assisted with collections on the southern part of the Yamal Peninsula at the mouth of the Ob and along the Shchooche River, a tributary of the Ob. Collections were made at 19 sites and more than 100,000 specimens were preserved for virus tests.

The results are expected to help clarify questions concerning the ecology and vector relationships of California serogroup viruses in the USSR. In the Soviet Union, Tahyna, Inkoo, and snowshoe hare viruses, and certain antigenic variants, are known to occur. In certain areas of the USSR human infection with CAL serogroup viruses is common and there is evidence of CNS involvement in some cases. Also, there is speculation that reassortment of CAL serogroup viruses may take place in mosquitoes in the arctic and subarctic areas where viruses have been isolated. Clearly, there are important scientific questions to be answered that have significant public health implications.

Report submitted by Carl J. Mitchell, Medical Entomology-Ecology Branch, Division of Vector-Borne Infectious Diseases, Center for Infectious Diseases, Centers for Disease Control, Fort Collins.



American Committee on Arthropod-borne Viruses (ACAV) neckties still are available (in blue, burgundy, brown, or green). Profits from the sale of these ties are used to support various ACAV activities. If you want one or more ties, the prices are:

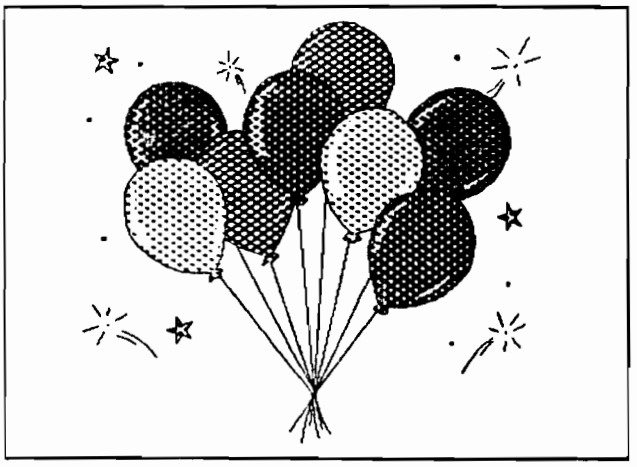
1= $15
2= $25
3= $35
≥4= $10 each

To order, send a check (made out to "ACAV" in U.S. dollars) to either

Dr. James W. LeDuc
Chief, Department of Epidemiology
DAD/Department of the Army
USAMRIID, Ft. Detrick
Frederick, MD 21701-5011

or

Dr. Charles H. Calisher
DVBID/CID/CDC
P.O. Box 2087
Ft. Collins, CO 80522



THE FIRST EPIDEMIC OF DENGUE HEMORRHAGIC FEVER IN THE PEOPLE'S REPUBLIC OF CHINA

FU-XI QIU (FU-HSI CH'IU)[1], DUANE J. GUBLER[2], QIU-QIAN CHEN[3], QI-YA HO[4], WEN-ZHOU CHEN[4], ZHI-GUO ZHAO[4], and BI-WEI ZHAO[1]

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[4] Hainan Provincial Hygiene and Epidemic Prevention Station, Haikou 570003, Hainan Province, People's Republic of China

The first epidemic of dengue in China associated with significant severe and fatal hemorrhagic disease occurred on Hainan Island in 1985-1986. The epidemic began in Zhan County in September 1985, spread throughout the coastal areas and ultimately involved 13 counties and cities of the Island in 1986. The mosquito vector was Aedes aegypti. The morbidity associated with dengue infection on Hainan Island was 1912,75/100,000, with a case fatality of 24.97/10,000. Severe disease was more prevalent in the 10-29 age group. Principal clinical features in laboratory confirmed cases were fever, osteoarthralgia, hemorrhages and/or shock, and thrombocytopenia. Complications such as acute intravascular hemolysis, diffuse intravascular coagulation, hemoconcentration or pleural effusion, altered mentality, and pneumonia were observed in a few cases. One-hundred and twenty-five dengue 2 viruses were recovered from acute-phase serum samples from 278 patients, and 5 viruses of the same serotype were isolated from 5 pools of adult Aedes aegypti.

FURTHER EVALUATION OF THE DENGUE IgM ELISA

S.K. Lam and S. Devi, WHO Collaborating Centre for DF/DHF,
Department of Medical Microbiology, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia.

Introduction

The gold standard serological test in dengue diagnosis has been the haemagglutination inhibition (HI) test. This test is not only labour intensive, taking 2-3 days to perform, but also requires exquisite standardization of reagents and a source of goose red blood cells. Very often, a definitive interpretation is dependent on the availability of paired sera and in our experience, about 75% of sera submitted to our laboratory are single specimens.

In 1987, we recommended that the HI test be replaced by the dengue IgM ELISA developed in our WHO Centre (1). We present here a summary of the further evaluation of this test based on 1,422 clinical specimens sumbitted to the Centre for dengue diagnosis in 1989.

Results

(1) Of the 1,422 serum specimens, only 345 or 24.3% were paired specimens, confirming our earlier finding from previous years that nearly 75% of samples were single specimens.

(2) Of the 345 paired specimens, 171 or 49.6% were positive by both HI and IgM and 151 or 43.8% were negative by both methods. There were therefore 93.3% (322 specimens) agreement between the two test methods.

(3) Of the 1,077 single specimens, 228 were positive by both HI and IgM and 704 were negative by both methods. There were therefore 86.5% (932 specimens) agreement between the two test methods.

Combining (2) and (3), out of a total of 1,422 specimens, 1,254 or 88.2% were in total agreement by HI and IgM ELISA.

(4) Of the 1,077 single specimens, 270 were positive by HI and 331 were positive by IgM ELISA. The IgM ELISA was able to detect an additional 61 positive specimens, proving again that it is a more sensitive test than HI.

(5) We analysed in detail 122 paired positive specimens, 22 of which were the result of a primary dengue infection and 100 from secondary infection.

As expected, none of the 22 acute specimens in the primary dengue infection was positive based on HI titres but 13 or 59% of these specimens were IgM positive. Whilst HI cannot yield a conclusive result based on single specimens in a primary dengue infection, the IgM ELISA can be useful in some instances.

Of the 100 paired positive specimens resulting from a secondary dengue infection, 62 were positive by HI based on tests done on acute specimens (HI titre >640) and 67 were positive by IgM. The IgM ELISA is therefore slightly more sensitive than HI based on tests done on acute specimens in a secondary dengue infection.

Overall, of the 122 paired positive specimens, only 62 or 50.8% of the acute specimens were positive by HI compared to 80 or 65.8% by IgM ELISA.

Conclusion

1. The IgM ELISA that we have developed is easy to carry out even in small laboratories where routine screening of blood donors by ELISA is being carried out. Only one antigen and its homologous monoclonal antibody are required.

2. The IgM ELISA test can be completed within a few hours unlike the 2-3 days required for the HI test.

3. Results can be obtained in most instances by testing acute samples in paired specimens or in single specimens.

4. The IgM ELISA is more sensitive than HI in diagnosing dengue infections irrespective of whether the disease is the result of a primary or secondary dengue infection.

Based on our findings, the IgM ELISA should be recommended to replace the HI test where rapid results are required to help in patient management and disease control. For laboratories where ELISA test is not done because of cost, HI is still useful. In research where it is necessary to differentiate between a primary from a secondary dengue infection, the HI or other quantitative ELISA tests can be helpful.

Reference

1. S.K. Lam, S. Devi & T. Pang. Detection of specific IgM in dengue infection. Southeast Asian J. Trop. Med. Pub. Hlth. 18: 532-538 (1987).

Detecting and typing dengue viruses by genomic amplification in a reverse transcriptase-polymerase chain reaction: application to rapid diagnosis.

The flaviviruses, originally defined as the group B arboviruses, cross-react in standard serological tests. They have been arranged in 7 serogroups on the basis of their antigenic interrelationships. Genetic and other characteristics have been used to place them in the family Flaviviridae, of which yellow fever is the type species. At present, 68 antigenically related but distinct viruses are recognized as members of the family. Included are 4 that cause "dengue fever". Dengue (DEN), originally considered to cause only a mild three-day fever, has increasingly become recognized as a disease with severe, even life-threatening, complications, such as hemorrhagic fever (DHF) and shock syndrome. Millions of infections with these viruses occur each year in areas where two billion are at risk. DHF is a leading cause of hospitalization and death among children in southeast Asia, where more than a million cases were recorded between 1987 and 1989. A DHF epidemic occurred in Cuba in 1981 and has been reported in 12 other countries in this hemisphere since then. Thus DHF now may be emerging in the Western Hemisphere as it did in southeast Asia in the 1960's.

The pathogenesis of severe disease caused by these viruses is still not well understood. Whether the pathology is caused by immune enhancement or by other viral attributes, there is a critical need for rapid and specific virus typing so that we can quickly understand and respond to the problem in epidemic areas.

There are excellent serological tests to identify antibody to DEN viruses and to identify the viruses themselves. HI, CF, neutralization, and, more recently and preferable, IgM antibody capture ELISA have been used singly or in combination to provide evidence for recent infections with these viruses. The principal problems with these tests are that they are not absolutely specific and sensitive, multiple sera are needed (and this creates the need for precious time to elapse between specimen collections), and results are rarely unequivocal. Virus isolation, from human sera or from arthropod vectors, can be done using cell cultures, mosquito inoculation, or even laboratory mice; however, virus isolation may take from days to weeks and is not always successful because of low virus titer, virus-antibody complexes, mishandling of specimens and other complications.

What is needed is an assay that is done rapid and the results of which are sufficiently specific to allow for bedside-relevant treatment and application of suitable and appropriate vector control measures. Direct detection and identification of DEN viruses in sera from febrile humans or in individual or pooled mosquitoes would be an assay of choice and polymerase chain reaction (PCR) potentially is such an assay.

Flaviviruses have a linear, positive-stranded infectious RNA molecule about 10,000 nucleotides long. The genomes of several have been sequenced and shown to be similarly organized. The viral proteins are encoded in one open reading frame, which represents about 90% of the genome. Seven non-structural virus-coded proteins are recognized and the entire gene order of both the structural and non-structural proteins is now known.

To isolate viral RNA we found that a published procedure, using guanidine isothiocyanate, was most reproducible. Rather than design type-specific primers that might be too specific, two oligonucleotide "consensus" primers were designed. These primers possess greater than 90% homology to the published sequences of the four dengue types and amplify a 510 bp product in a RT/PCR reaction. The amplification strategy was to purify the viral RNA, then add deoxynucleotide triphosphates, one of the primers, and reverse transcriptase (RT); this secures the production of a cDNA copy of a portion of the viral genome. The cDNA is then amplified by the addition of the second primer, followed by 35 cycles of heat denaturation, primer annealing, and primer extension. Using purified DEN viruses, the RT/PCR was optimized by testing the effect of Mg^{2+}, primer concentration, and various commercially-available RT preparations. Also, we tried a two-step and single incubation RT/PCR. The combined reaction yielded results identical to that obtained with a two-step reaction. To develop a type-specific assay we first used restriction enzymes Bgl II and Sau 3A to produce predicted fragments of the 510 bp amplified product: restriction digestion of the amplimers generated a unique digest pattern for each of the DEN types.

However serviceable the technique, the cost of these enzymes makes use of this method prohibitive for routine application. Therefore, we synthesized type-specific probes by copying conserved sequences of each of the 4 DEN types, as published. Virus typing was then accomplished by hybridization of the amplimer to all type-specific probes; each type hybridizes to only one of the probes. All DEN types were detected, but we did not detect other flaviviruses or the alphavirus WEE. In addition, as few as 50 pfu of each DEN type were detected in "spiked" human sera.

We then tested 30 serum samples from a larger group collected during 1976-1978 in Indonesia. These sera were from humans with clinically-, virologically- and serologically-confirmed DEN but the sera had undergone multiple freeze-thaw cycles since then. Viruses were originally isolated in *Aedes aegypti* by intrathoracic inoculation and subsequent direct fluorescent antibody of head squashes. Identification was by CF using reference immune mouse ascitic fluid and antigen to the isolate prepared in mosquitoes. Sera were treated with guanidine isothiocyanate and tested by PCR with consensus and type-specific primers. Two were identified as DEN-1, 7 as DEN-2, 5 as DEN-3, and 1 as DEN-4. The identity of 2 of these 15 differed from the original identification; both are in the process of being reidentified by PCR and by virus isolation and serologic identification.

Mosquitoes were experimentally-infected with DEN-2 virus by intrathoracic inoculation and frozen at essentially daily intervals after inoculation, beginning on day 2; 5 mosquitoes from each group were frozen at -70°C. Uninoculated mosquitoes were also collected. We pooled the 5 mosquitoes from each group, macerated them, denatured with guanidine isothiocyanate, and tested them by PCR. None were positive, although by parallel IFA these mosquitoes were known to contain virus. We then tried a technique using primer-coated magnetic beads to capture viral RNA and this was successful. All inoculated mosquitoes, including those collected only two days after inoculation, were positive.

Certainly these are preliminary, yet encouraging, results. We will next assay sera that have been titrated recently, test a large number of these, test sera containing viruses from different geographic areas, and assay mosquitoes that have been inoculated with small doses of viruses.

Cost is certainly a factor in performing PCR. In addition to expensive equipment and reagents needed, it might be best to have on hand an oligonucleotide synthesizer to prepare large lots of primers or smaller lots more often; commercial products are very costly and the wait for the product either is a nuisance or an obstacle. Another problem that we have encountered is cross-contamination when amplifying cDNAs: we now amplify in a room separate from the room in which we carry out other steps. We have yet to determine the role of RNAse, temperature, freezing-thawing, and other factors that might lead to degradation of viral RNA; obviously, decrease in RNA available to be amplified would reduce the applicability of this PCR assay. Finally, considerable expertise is required to trouble-shoot. These problems and potential problems in application of DEN-PCR are counterbalanced by the relative rapidity with which DEN viruses are specifically identified.

We believe that the procedure described appears to be excellent for detecting and typing DEN viruses. Obviously, further work is required. However, this assay appears applicable for clinical situations, molecular epidemiology, and for experimental analyses. We know of no reasons why this general approach could not be used to identify other flaviviruses in viremic sera, in arthropods, or in other tissues and hosts. In fact, given sequence information, it might be useful for detecting many more RNA viruses than DEN viruses.

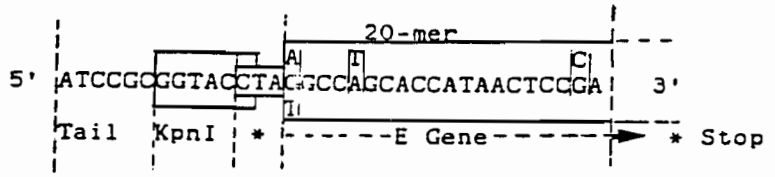
R.S. Lanciotti, C.H. Calisher, D. J. Gubler, and A.V. Vorndam
(Division of Vector-borne Infectious Diseases, Center for Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado 80522 and San Juan, Puerto Rico

Report from the Health Research Council of New Zealand's Virus Research Unit, University of Otago, P.O.Box 56, Dunedin, N.Z.

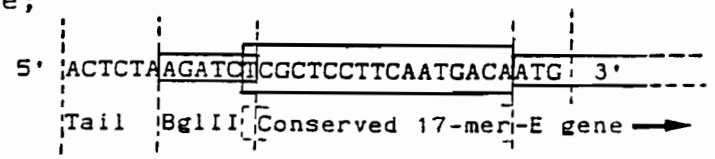
PRODUCTION OF DENGUE VIRUS GENE SEQUENCES FOR EXPRESSION STUDIES.

We have used the polymerase chain reaction (PCR) to produce defined gene sequences from dengue viruses for cloning, sequencing and expression studies. Since the PCR reaction requires primers specific for sequences at, or just outside, the termini of the sequences to be amplified, pairs of oligonucleotides have been designed and synthesised for each of several gene constructs from dengue type 2 (Tonga 1974 strain) and dengue 3 (H-87 strain). Basically, these primers consist of three parts, a 6-mer tail, a restriction enzyme cutting site and a sequence homologous to a portion of the gene. As needed, ATG initiation codons and stop signals are also included. As an example of these paired primers, the design of the two primers for the intact dengue 2 envelope glycoprotein (E) gene is shown below:

(a) The first primer which attaches to the 3' end of the virion RNA for first strand cDNA synthesis and is also used in the PCR, contains a sequence complementary to the last 20 nucleotides at the 3' end of the E gene, followed by a stop codon, a Kpn I site and a 6-nucleotide tail which ensures that the Kpn I will cut. It has the sequence;



(b) The second primer contains a 20-mer sequence identical to the conserved 17-nucleotide sequence immediately outside the 5' end of the E gene as well as the ATG initiation codon. Added to these are a Bgl II restriction site and a 6-mer tail. This oligo primes the first Taq polymerase copy of the first strand cDNA. It has the sequence;



The choice of restriction sites will depend upon which vector the genes are to be cloned into. Using this strategy we have been able to clone intact genes (E, NS12a, NS3 for dengue 2, and E and NS12a for dengue 3) as well as variants of these with shortened anchor sequences or lengthened leader sequences. We are now studying these recombinants in baculovirus and E.coli (pGEX) expression systems.

(T.Maguire, Wenbin Chen, Qu Xinyong).

AN IMMUNOPEROXIDASE TECHNIQUE FOR LOCALIZATION OF DENGUE VIRUS ANTIGENS IN FORMALIN FIXED TISSUES

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Immunohistochemical localization of antigen is a powerful investigative technique for pathophysiological studies. In response to the dearth of data on the distribution of dengue antigens in human tissues, we systematically optimized a dengue antigen staining protocol using commercially available avidin-biotin horseradish peroxidase immunocytochemical reagents (Lipshaw Corporation, Detroit, MI). 2 um thick sections of mouse brain infected with dengue virus types 1-4, Japanese encephalitis virus or chikungunya virus, or uninfected brain served as control tissues. These were fixed for 24 hours in Mellonig's buffered formalin and paraffin embedded by standard methods. Anti-dengue type 2, anti-dengue type 3, anti-chikungunya and normal mouse ascitic fluids (MAF) as well as monoclonal antibody (Mab) 4G2 (from WRAIR) reactive with a flavivirus group determinant were tested as primary antibodies. Trypsin digestion for 20 minutes was employed to unmask antigens denatured by formalin. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in methanol. Anti-dengue type 2 MAF pre-absorbed with mouse acetone liver powder (Sigma, St. Louis, MO), Sarcoma 180 cells and normal human peripheral blood mononuclear cells was selected as the optimum primary antibody. To eliminate background staining, the primary antibody was diluted in 40% anti-flavivirus antibody-negative human plasma. Using this MAF, intense staining of neurons was seen in sections of mouse brain infected with all types of dengue virus and Japanese encephalitis virus. Anti-dengue MAF did not stain chikungunya-infected mouse brain or normal brain. Conversely, normal or anti-chikungunya MAF did not stain any flavivirus-infected mouse brain section. Mab 4G2 also stained flavivirus-infected mouse brain sections.

To evaluate the specificity of the staining protocol with human tissues, liver blocks from 28 patients who died of causes other than dengue were examined under code. No dengue antigen was detected in these blocks, however abundant dengue antigen was detected in coded liver and lung tissue from a patient from whom dengue type 2 was isolated from serum, liver and lung. Mab 4G2 failed to stain the human positive control tissue (which contained very much less antigen than infected mouse brain tissues). We concluded that when care was taken to block non-specific reactions and endogenous peroxidase activity, dengue antigen may be specifically detected in formalin fixed tissues with appropriately prepared polyclonal anti-dengue antisera.

The staining procedure was then used to evaluate liver tissues from 19 cases of fatal dengue hemorrhagic fever (1 case not confirmed by serology or virus isolation). Dengue antigen was visualized in 15/18 confirmed cases and in the single case that was unconfirmed by usual laboratory methods. Antigen was sparse and best visualized at 400x or 1000x. It was detected principally in Kupffer cells, occasionally in the rare inflammatory cells present and in some hepatocytes peripheral to areas of coagulative

necrosis. In seven cases, liver tissue was both cultured for virus and stained for virus antigen. All 7 tissues were antigen positive, but only a single tissue yielded a virus isolate (dengue type 2).

To better understand how field conditions affect the detection of dengue antigens by the staining protocol, we investigated the effects of delayed autopsy, delayed fixation after autopsy, and prolonged fixation, using infected mouse brains. Delay of autopsy for 24 hours slightly reduced the intensity of staining; a 48 hour delay had a marked deleterious effect. Delay of fixation for one to several hours had little effect. Prolonged fixation reduced staining but could be partly compensated for by more prolonged trypsin digestion.

Our laboratories now regularly use this protocol to examine tissues obtained at autopsy from patients dying of hemorrhagic fever or liver failure. We offer to examine tissues from outside institutions and to promptly report our findings and return submitted tissue blocks. Laboratories which wish to submit tissues or to collaborate in clinical investigations may contact Bruce Innis, M.D. to obtain more information. Tissue blocks may be mailed to Bangkok along with a brief case description and 0.1 ml of patient serum for anti-dengue IgM detection, if available (collected as late in the illness as possible, azide added and sent at ambient temperature), using the address below:

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ORAL SUSCEPTIBILITY OF AEDES ALBOPICTUS TO DENGUE VIRUS TYPE 2: A STUDY OF MIDGUT INFECTION KINETICS USING THE POLYMERASE CHAIN REACTION FOR VIRAL DETECTION

In research on arthropod borne virus, studies dealing with virus vector interactions contribute to better a understanding of the mechanisms involved in viral transmission to the vertebrate hosts and increase our knowledge about the viral life cycle and epidemiology. Such a preocupation requires both a sensitive method of viral detection and/or localization and also to examine large samples for epidemiological surveys.

Towards our goal, i.e. the detection of few ARN viral sequences within mosquito tissue, one could predict that the contributions of the polymerase chain reaction (P.C.R.) were going to be crucial. In our study, we focuss on *Aedes albopictus* midgut infections by dengue virus type 2 (DEN 2). Using both reverse transcription and P.C.R. procedures, we attempted to study the kinetics of micgut infection from 24 hours to 24 days after an infectious blood feeding experiment, in order to check i) wether P.C.R. could be useful in dengue detection within a single mosquito, ii) wether some females were refractory to oral infection, iii) how did viral particles evolve in the midgut during the time of the experiment: did they completely leave the midgut epithelium when they invaded hemocoel? Were events the same for all individuals? Was there replication within the midgut?...

The females were taken out, six by six, at each of the following times of incubation at 28°C after the infectious meal (virus titer :10^{10} MID 50/ml) 24 hrs, 48 hrs, 72 hrs, 96 hrs, 5 days, 6 days, 8 days, 11 days, 14 days, 18 days and 24 days. The entire midgut portion, deprived of the ventral and dorsal diverticula, of each female was removed, washed and then treated for RNA extraction and viral cDNA transcription. Amplification was carried out as previously described (Tardieux & Poupel, 1990). For each point of the kinetics, we analyzed at least 16 females.

All the results are illustrated in figure 1. They show that i) we could not detect the target sequence of dengue virus from some RNA extracts, ii) a rather low percentage of samples were detected positive one day after the infectious meal, but this ratio increased in the two subsequent days, reached a maximum in day 3 and then decreased until day 8. A second peak was observed around day 14 followed by progressive decrease, iii) At day 24 some samples were still positive.

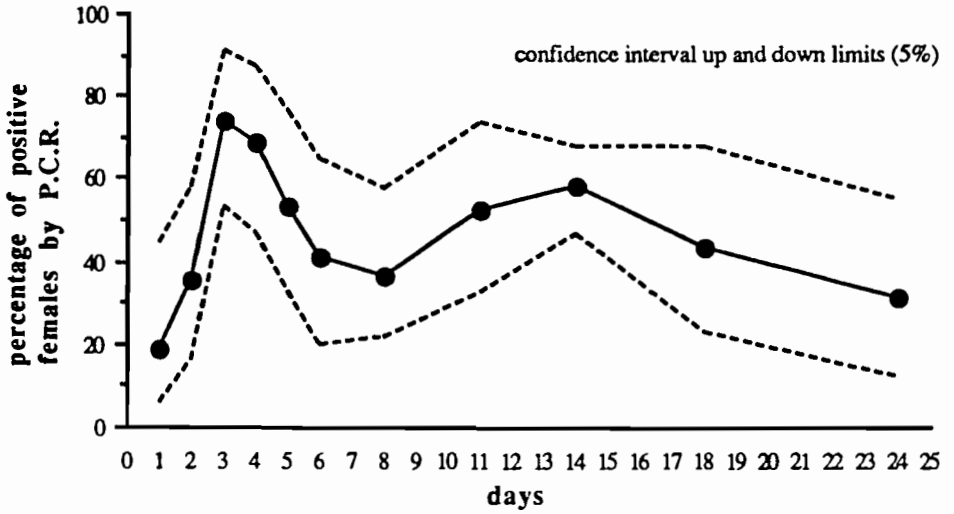

Figure 1: Kinetics of *Aedes albopictus* midgut infection with DEN2 using the P.C.R. technique for viral detection.

The combination of reverse transcription and P.C.R. allows to detect viral particles in the infected midgut epithelium of a mosquito. Analysis of controls showed that we did not detect false positives, even for females tested one day after the infectious meal when the blood meal is still visible in the midgut lumen. This result was confirmed by our kinetics observations since the ratio of positive samples is lower during the two first days compared to that of the following days.

Another point to note is that some females seemed to be "midgut resistant" to DEN 2 infection, since after two days, some samples were still found negative. The low percentage of infected females during the first two days also pointed to the limitations of our detection procedure and we detected particles when genome replication had already occurred. Whatever the level, variation of midgut behavior towards the virus exists.

A third point concerns the fate of the particles within midgut epithelium. It showed that replication took place during the first three days since ratio of positive samples increased until the third day. Since we could consider that the sensitivity threshold remained stable during the entire study, the decrease in the positive female percentage (from day 3 to 8) indicated that for some females, viral release from the midgut epithelium occurred. The second maximum revealed that there was an additional replication phase around day 10-15, since more samples became positive: it might represented viral contamination of neighbouring columnar cells.

There are limitations to this type of application of the P.C.R., but the technique was helpful in improving sensitivity and specificity of the detection. Such an improvement allows us to define phenotype in order to analyze the genetic basis of the mosquito midgut susceptibility to DEN 2 in selected mosquito lines.

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Reference: I. Tardieux and O. Poupel, 1990. Use of D.N.A. amplification for rapid detection of dengue viruses in midgut cells of individual mosquitoes. Res. Virol., 141: 455-457.

CULICOIDES - ARBOVIRUS RESEARCH AT VETERINARY RESEARCH INSTITUTE, ONDERSTEPOORT, SOUTH AFRICA

In 1983, a research group was formed to study Culicoides midges and the diseases they transmit. Researchers in this group are Errol Nevill, Gert Venter, Rudy Meiswinkel, Mark Edwardes and Hilda Nevill. The group works closely with the Virology and Biochemistry Sections at Onderstepoort on the virological, serological and biochemical aspects of these studies. Current studies cover the following aspects:

(a) Adult taxonomy

The last taxonomic revision of this genus in South Africa was done by O.G.H. Fiedler in 1951. He recorded 22 species. Since then the number of species has risen to more than 100. It is important, therefore, that a fresh revision of this genus be published. However, before this is possible, many new species have to be described, others have to be redescribed, new records must be noted, subgenera have to be established, and, most difficult of all, sibling species, especially in the subgenus Avaritia, must be differentiated. Culicoides imicola, our major virus vector, falls in this subgenus and, as there are a number of species almost identical with it, their recognition is the most important taxonomic problem facing us at the moment.

(b) Pupal taxonomy and larval habitats

In South Africa, in the past, the significance of the morphology of the pupal stage of Culicoides has been largely overlooked. Since this stage has to be able to survive in the larval medium and since every Culicoides species presumably has its own ecological niche, morphological differences between the pupae of these species can be expected. Detailed studies during the past 2 years, on the pupae of about 20 Culicoides species, revealed common subgeneric characters as well as habitat-related morphological differences. The differences between pupae of closely related species were sometimes far more striking than between adult females of these species and, in these cases, helped clarify the taxonomy and biology of their adults. The specific larval habitat requirements of many species are also a valuable aid in clarifying their taxonomy. This is especially the case in the subgenus Avaritia where sibling species use the dung of different large herbivores for their development. For example 4 Culicoides spp. which breed in the dung of cattle and elephants have been shown to feed on those animals. One may eventually be correct in deducing that a species that has such a close association with its host could also be the vector of certain virus infections of that host.

(c) Arbovirus distribution

Questionnaires have been sent out to veterinarians throughout the country and the information has been used to compile distribution maps for bluetongue, African horsesickness and 3-day-stiffsickness. Donkeys are being used throughout the country to monitor the distribution of African horsesickness. An ELISA test is used for the detection of antibody to horsesickness virus.

(d) Culicoides distribution and prevalence

Using light traps, truck traps and emergence traps, especially near stock, the distribution and prevalence of the various species of Culicoides in different farming areas of South Africa, are systematically being determined. This is also being related to possible causative factors such as temperature, rainfall, irrigation, host animals, vegetation, etc. Clear patterns are starting to emerge. For example warm areas with good rainfall or irrigated pastures favour C. imicola. Similar areas with lower temperatures favour C. magnus, C. milnei, C. zuluensis, C. gulbenkiani and C. bolitinos. However, semi-arid areas favour members of the subgenus Remmia. Through these studies the Culicoides species likely to be involved in arbovirus transmission in various regions can be anticipated, and will form the basis for vector competence studies and investigations into the epidemiology of Culicoides-transmitted viral diseases.

(e) Culicoides host preference

Although more than 100 Culicoides species are known from South Africa most of these are not associated with stock and will play no role in the transmission of virus diseases in livestock. To determine which Culicoides species are potential vectors their blood-meals are being identified by means of a cross-over electrophoresis test using anti-sera to horse, cattle, sheep, chicken and pig. To date 14 Culicoides species have been shown to have fed on livestock (cattle 12, horse 7, sheep 5, chicken 5). C. imicola, C. zuluensis, C. magnus, C. bolitinos, C. gulbenkiani, C. schultzei group and C. enqubandei fed almost solely on mammals while C. leucostictus and C. pycnostictus fed mostly on birds.

(f) Culicoides vector competence

We are concentrating on bluetongue and African horsesickness transmission at present, as so far only C. imicola has been shown to transmit this virus in South Africa. Other species must be involved, as bluetongue occurs commonly in regions where C. imicola is rare. The same study must be repeated for bovine ephemeral fever, Akabane virus and some encephalosis viruses of horses. Results to date indicate the infection rate of C. imicola from the north-eastern Transvaal to be approximately 25% for bluetongue serotypes 3 and 6. It has also been shown that African horsesickness can survive for at least 10 days in C. imicola.

SALIVA-ACTIVATED TRANSMISSION OF TICK-BORNE VIRUSES

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A factor produced in the salivary glands of feeding ticks has been shown to cause a 10-fold increase in the number of ticks infected by Thogoto (THO) virus (Jones *et al.*, 1989, J.Gen. Virol. **70**:1895). This SAT (saliva-activated transmission) factor probably accounts for non-viraemic transmission, i.e. virus transmission from infected to uninfected ticks co-feeding on a non-viraemic host (Jones *et al.*, 1987, Science **237**:775).

To determine whether SAT could be demonstrated with other tick-borne viruses, guinea pigs (Dunkin Hartley strain) infested with uninfected ticks were inoculated with a virus mixed with a salivary gland extract (SGE) derived from uninfected, partially engorged ticks (see Table). Animals inoculated with Central European encephalitis (CEE) virus or Kemerovo (KEM) virus were infested with uninfected *Rhipicephalus appendiculatus* nymphs, those receiving Dugbe virus (DUG) were infested with *Amblyomma variegatum* nymphs, and with Dhori (DHO) virus, *Hyalomma dromedarii* nymphs. The ticks were harvested on the day of drop-off (*R. appendiculatus*) or 10 days after repletion (*A. variegatum* and *H. dromedarii*) and then screened for virus by plaque assay. Blood samples were collected from the guinea pigs on day 5 p.i.: virus was not detected by plaque assay.

Infected ticks were obtained from animals inoculated with DHO or CEE virus but not with KEM or DUG virus. The numbers of infected ticks were low compared with those reported previously for THO virus (up to 80%). DHO virus is genetically related to THO virus but is transmitted by different tick species. The difference in SAT activity between THO and DHO viruses may reflect different feeding characterisitics of the tick vectors and/or different infection strategies in the vertebrate host. For CEE virus, the results may have been influenced by the fact that *R. appendiculatus* is not a natural vector of CEE virus. The results warrent further investigation using *Ixodes ricinus*, a natural vector of CEE virus. This is of particular interest in view of a report of non-viraemic transmission of CEE virus (Alekseev, A. & Chunikhin, S.P., 1990, Med. Parasitol **2**: 48-50).

Inoculum		No. ticks infected/ no.ticks tested	Percentage infected
Virus	SGE		
4.0 $\log_{10}$PFU DHO virus	40µg *H. dromedarii*	13/51	25%
7.0 $\log_{10}$PFU CEE virus	40µg *I. ricinus*	8/60	13%
6.7 $\log_{10}$PFU KEM virus	40µg *I. ricinus*	0/60	0%
6.0 $\log_{10}$PFU DUG virus	40µg *A. variegatum*	0/43	0%

MOSQUITO LANDING BEHAVIOR, WITH REFERENCE TO PREFERRED SITES ON THE HUMAN BODY IN THE CANOPY OF AN AMAZONIAN RAIN FOREST (DIPTERA: CULICIDAE)[1, 2]

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ABSTRACT - Collections and observations were done in primary forest near Belém, Pará, Brazil, from June, 29 to October, 29, 1987 and from May, 6 to July, 12, 1988. Mosquito preferences for various parts of the human body were noted during daily three-hours runs (0:30 - 3:30 p.m. in 1987, and 2:00 - 5:00 p.m. in 1988) of human bait collecting. Two tree platforms were used, at 20 m and 15 m high in 1987 and 1988, respectively.

The twelve collected species (791 specimens) were Haemagogus leucocelaenus, Hg. janthinomys, Limatus flavisetosus, Psorophora albipes, Runchomyia magna, Sabethes amazonicus, Sa. belisarioi, Sa. chloropterus, Sa. cyaneus, Sa. glaucodaemon, Sa. quasicyaneus, and Sa. tarsopus.

Of all mosquitoes, 47.7%, 7.1%, 17.9%, 27.1% landed on the head and neck, trunk and abdomen, upper limbs, and lower limbs, respectively. The only species which showed some variation between dry and rainy seasons was Sa. tarsopus.

Six species were abundant enough to allow more detailed considerations. Hg. janthinomys was the most abundant on lower limbs (88.4%), with 79.5% collected on the feet, whereas the sabethine species were more attracted to the head. Sabethes spp., with the exception of Sa. chloropterus, were more common on the nose than on the ears; Ru. magna and Sa. cyaneus were also collected in large numbers on the legs and the arms, respectively.

These results raise questions about the bioecological implications of such differences in mosquito landing behavior. Without any doubt, they have to be considerated when evaluating the efficacy of repellent products.

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MODIFICATIONS OF ARBOVIRUSES' ECO-EPIDEMIOLOGY IN TUCURUI, PARA, BRAZILIAN AMAZONIA, RELATED TO THE CONSTRUCTION OF AN HYDROELECTRIC DAM[1, 2].

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Physical, ecological, and climatic data about the Amazonian Basin may be summarized as follows: the Amazon River and its tributaries hydrographic basin covers 3,984,467 km^2; approximately 3,373,000 km^2 (84 %) of Brazilian Amazonia are covered by dense equatorial forest; this region is yet sparsely inhabited, with less than 10 % of the total brazilian population and, excluding the state capital cities, it has one of the world's lowest population densities, less than to 2 inhabitants /km^2; with its equatorial or humid tropical climate and potential of natural resources and the resultant employment, Amazonia is very attractive to people from regions which suffer from severe droughts like the Northeast.

The Amazonian region of Brazil seems to be the world's richest reservoir of arboviruses. At this time, 173 different types of arboviruses have been detected in the Amazonian region, accounting for one third of the 532 world arboviruses. Of these, 146 (84 %) are endemics.

This report concerns the preliminary results of a 6-years-duration project, which was initiated in 1982.

The main objective of the project was to evaluate what would be the effects of the construction and flooding of a dam on the transmission and epidemiology of sylvatic arboviruses, in order to define the potential health hazards to which the human population would be subjected.

Comparisons were made between a number of surveys, done: 1) inside the dam region, before, during and after the flooding period; 2) outside the dam region, but in an area with a similar climate, landscape and vegetation, during the total time of the studies.

The two chosen areas, namely Altamira and Tucurui, Para, were studied since 1974, and since September, 1982, respectively. The former, and the latter before the outset of flooding (september, 6, 1984) were considered as a control or reference for comparisons with data obtained in Tucurui during flooding and thereafter.

The surveys consisted of sampling as many wild vertebrate and hematophagous Diptera as possible. Human sera were collected from febril cases and random serological surveys. Field collecting and conservation, inoculation, serological testing and identification procedures were as routinely done in arbovirus laboratories.

The geology, climate and vegetation of the two areas are very similar.

As regard to the evolution of human serology, very few variations seem to have occurred between the three phases of the study, with a maximum of positive serologies during the flooding period, but the differences were not statistically significant. Similarly, in the reference region, the prevalence of antibodies against the four more important arboviruses' groups have not varied significantly.

Some eco-epidemiological topics have been selected, allowing the grouping of the different arboviruses under somewhat arbitrary but convenient categories, for a more detailed analysis: 1) new or already known arboviruses which caused epizootics, 2) transmission of arboviruses from possible exogenous origin, 3) epizootics caused by endemic arboviruses, 4) arboviruses whose transmission has shown a slight enhancement, whether or not caused by the new ecological conditions, and 5) those which showed no apparent modification in their transmission patterns (referring to the control area).

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[1] Presentation made at the Fifth Australian Arbovirus Symposium, 28th August - 1st September, 1989, Brisbane, Queensland, Australia, and at the Symposium "Forest 90", Manaus, Brazil, 7th - 13th October, 1990.

[2] The present work has beneficiated of financial and/or logistic support from Eletronorte (Eletrobras), SUDAM (Polos Agropecuarios da Amazonia), CNPq, ORSTOM and Foundation SESP.

The main results are the following:

1) Three new and three already known types in the Anopheles A group of Bunyavirus were favoured by the great proliferation of Anopheles nuneztovari and An. triannulatus which accompagned the flooding of the dam.

2) An epizootic of the Gamboa Bunyavirus, newly registered for Brazil, occurred one year after the outset of flooding, when the mosquito Aedeomyia squamipennis showed very large populations.

3) Guaroa (Bunyavirus, group California), an endemic arbovirus showed an epizootic probably as a consequence of the proliferation of anopheline mosquitoes.

4) A temporary proliferation of Culex spp. mosquitoes and the presence of a rich avifauna in the dam area during flooding are probable causes of the enhanced circulation of Turlock (Bunyavirus, group Turlock) and Kwatta-like (Rhabdoviridae, group Kwatta) viruses. Oropouche (Bunyavirus: group Simbu), Saint Louis Encephalitis (Flavivirus, group B), Maguari/Xingu (two jointly studied Bunyavirus: group Bunyamwera), and Triniti (ungrouped Togaviridae) arboviruses showed at least patterns of enhanced circulation but probably for diverse reasons, not all due to the dam flooding. The last two types showed a possible shift of mosquito vectors, from diurnal ones to the nocturnal above cited anopheline.

5) Eight arboviruses showed no clear-cut difference between their transmission patterns in the reference region and the ecologically modified dam area. They are: Yellow Fever (Flavivirus: group B), Mayaro (Alphavirus: group A), Ilheus (Flavivirus: group B), Tucunduba (Bunyavirus: group Bunyamwera), Eastern (and Western) Equine Encephalitis (Alphavirus: group A), Icoaraci (Phlebovirus: group Phlebotomus), and Itaporanga (Phlebovirus : group Phlebotomus) viruses. The first six are pathogenic for man.

Some new data concerning the hosts and cycles, were occasionally collected for Bush Bush (Bunyavirus: group Capim), Aruac (unclassified Rhabdoviridae), Munguba (Phlebovirus, Phlebotomus group), Santarem (ungrouped Bunyaviridae), and Acado-like (Orbivirus: group Corriparta) arboviruses.

52 probably new types of arboviruses were classified in the Changuinola group of the genus Orbivirus (Reoviridae), the Phlebotomus, Anopheles A, and Simbu groups of Bunyaviridae (genera Phlebovirus and Bunyavirus). Seven remained neither classified nor grouped.

The usefulness of classifying ecologically the arboviruses is highlighted, when studying or forecasting possible impacts of dam impoundment on the human health hazards due to these agents. The scarcity of such studies does not allow comparisons but for future studies, it would be most important a) to define the ecological characteristics of the new equilibrium which becomes established in modified regions, and b) to adopt a more previsional attitude (hypothesis testing) during project planning.

REPORT FROM THE VECTOR BIOLOGY LABORATORIES, UNIVERSITY OF NOTRE DAME, NOTRE DAME, IN 46556

The introduction of *Aedes albopictus* into the La Crosse virus (LACV) endemic region of the midwestern United States over the last five years presents an opportunity for this mosquito to become exposed to and perhaps vector LACV. However, that species probably would not be a potential vector of other California group viruses in the same region (Grimstad *et al.* 1989). We have conducted laboratory studies to determine rates of transmission of LACV by numerous field populations of *Ae. albopictus*, horizontally to vertebrate hosts (this report) and vertically to progeny (later reports).

An extensive study of numerous Asian and American *Ae. albopictus* strains has revealed the ready susceptibility of this mosquito to oral infection with LACV. Only 4 of 1,554 mosquitoes failed to show midgut infection with LACV after imbibing viremic blood meals. And 1,511 of 1,554 *Ae. albopictus* tested (97.2%) from 27 separate populations showed disseminated LACV infection beyond the midgut. When mosquitoes were fed viremic blood meals with titers sufficient to ameliorate dose effects, it became evident that midgut barriers to LACV were insignificant in *Ae. albopictus* (the blood meals used ranged in titer from 3.7 to 4.6 $\log_{10}$ $TCID_{50}$/0.025ml in Vero cells).

These experiments also focused upon oral transmission of LACV to suckling mice by *Ae. albopictus*. Following a 14-day period for extrinsic incubation of virus in the mosquito, 43% of the 1,554 *Ae. albopictus* tested transmitted LACV to mice. These transmission data were then analyzed by geographic origin or region for each *Ae. albopictus* strain tested. Among the 14 strains of *Ae. albopictus* tested that originated from locales within the mainland United States, a wide range of transmission rates was found (i.e., 18-64%). This range of transmission rates is similar to that found for the natural LACV vector, *Aedes triseriatus* (Grimstad *et al.* 1977). In total, 40.5% of the *Ae. triseriatus* freshly colonized from mainland U.S. sites orally transmitted virus to suckling mice in that earlier study.

Figure 1 compares barriers to infection and transmission rates by region of origin for the 27 strains of *Ae. albopictus* we tested. It is interesting to note that the overall transmission rate for U. S. mainland strains is similar to that found for the temperate Asian strains tested, and similar to that found for a long-colonized laboratory strain (OAHU) used in our studies as a control. Significant differences were measured however, between transmission rates for these temperate Asian and U. S. strains on the one hand, and the overall transmission rates for 5 Brazilian strains and 3 tropical Asian strains on the other. Also included in this figure are barrier and transmission data for *Ae. triseriatus*; these latter data are taken from an earlier work (Grimstad *et al.* 1977) and depicted here for comparison with *Ae. albopictus*.

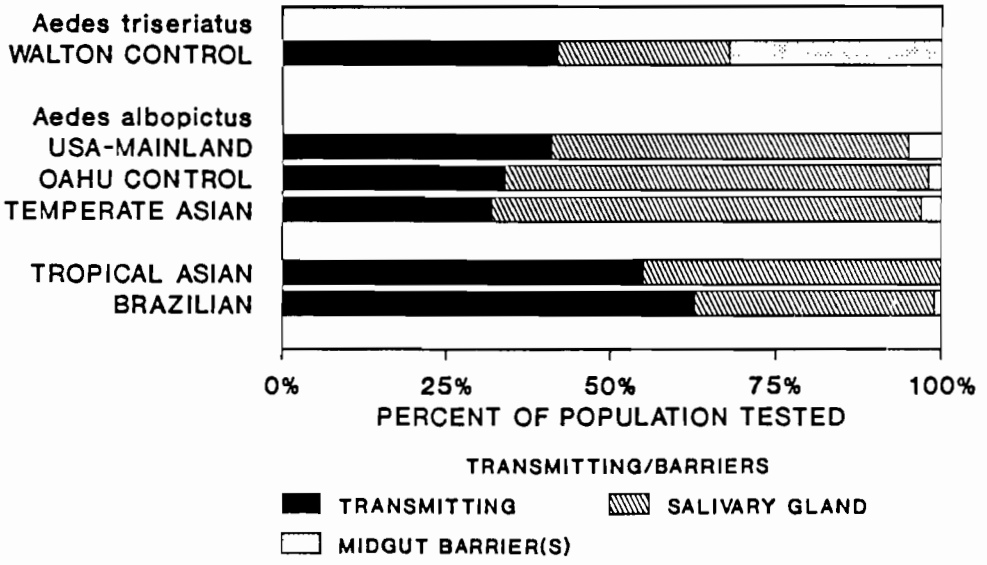
Our data suggest that genetic differences exist among populations in regard to vectorial capacity for oral transmission of LACV. Moreover, the correlation between introduced United States' populations of *Ae. albopictus* and those of temperate Asia are supported by findings on photoperiodism, cold tolerance, and allozymes (Hawley & Craig 1989). These findings then, lend additional credence

to the hypothesis that United States' populations of *Ae. albopictus* trace their origin to temperate Asia (Hawley *et al.* 1987).

Thomas G. Streit, George B. Craig and Paul R. Grimstad

FIGURE 1.

TRANSMISSION OF LA CROSSE VIRUS TO SUCKLING MICE



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A simple device for sterile collection of baby mouse brains.

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Baby mouse intracerebral inoculation is widely used for isolation and propagation of arboviruses. The brain tissue of the dying mice has to be collected for passages or preparation of antigens. In the latter case usually a large number of litters are used and to collect the brains by dissecting the skull can become rather difficult and labor-intensive. We have obtained very good results with a self-made device built from materials available in any laboratory.

One 50 ml centrifuge tube (glass or plastic) is fitted with a rubber stopper with two holes. Through one of the holes a thin (3-4 mm OD) plastic tube is inserted to the bottom of the centrifuge tube leaving outside 10-15 cm, fitted, at its end, with a syringe needle (gauge 14, 1/4" length) either by inserting the tube in the syringe receptacle of the needle or by cutting and using a tube with a needle from the bags used for i-v perfusion or blood collection. We found this very effective because the continuous needle-tube minimize any lost of tissue at the junction.

Through the second hole is inserted a glass tube (6 mm OD). To avoid sucking brain fragments into the vacuum system, the part of the glass tube which is in the centrifuge tube is bend to 180 degrees, taking care not to obstruct it (Fig 1). The ready mounted device can be sterilized by autoclaving at 121^0 and is ready for use. Before starting the brain harvest, the centrifuge tube is placed in a rack or a clamp on a stand. The mouse is held with two fingers of the left hand and the needle is introduced through the occipital area, the brain is instantly aspirated into the tube and the upper part of the skull collapses. The time necessary for each mouse is no longer than 3-4 seconds. When all the material is harvested, the solution to be used for the homogenization of the collected material is aspirated through the needle flushing it and its tube of any fragments. After removing the rubber stopper it is easy to transfer the material or to process or store it.

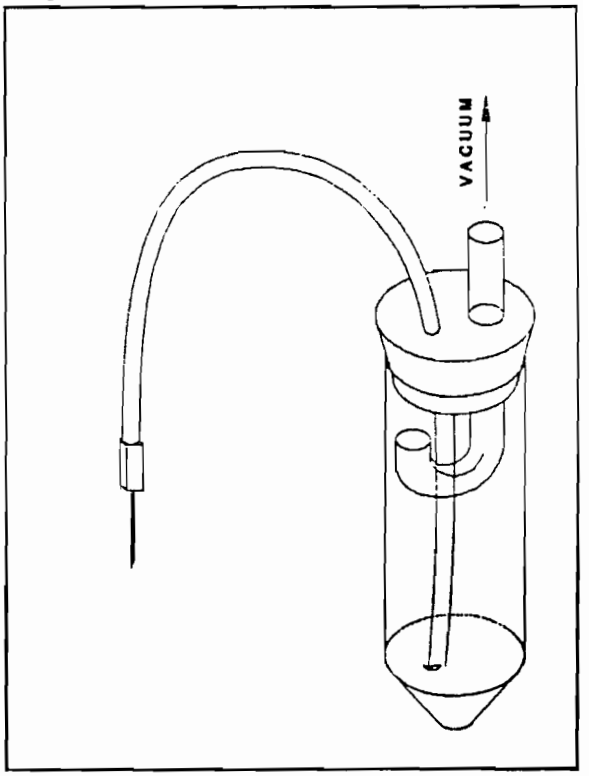

Figure 1

If the material needs to be kept sterile, it is recommended to immerse the mice in a disinfectant solution for a few minutes. When working with hazardous material it is recommended to connect between the vacuum line and the tube a receptacle holding a strong disinfectant solution which will trap any infectious aerosols.

ANTIGENIC RELATIONSHIPS BETWEEN SINDBIS, OCKELBO, KARELIAN FEVER AND BABANKI VIRUSES AS DETERMINED BY CROSS NEUTRALIZATION TESTS

Virus strains identified as Sindbis virus or as closely related to Sindbis virus have been isolated in Europe, Africa, Asia and Australia.[1] Studies using highly discriminating techniques, such as RNA-RNA hybridization[2], T_1 oligonucleotide fingerprinting and tryptic peptide mapping,[3] indicated that ancestral Sindbis virus may have evolved into 4 groups, with geographical borders similar to the Palaearctic, the Ethiopian, the Oriental, and the Australian zoogeographical regions. Traditionally, the classification of alphaviruses is based on serological cross-reactivity by hemagglutination inhibition (HI), complement fixation (CF), and neutralization tests.[4]

We evaluated the antigenic relatedness between Sindbis virus strains from the Palaearctic, the Ethiopian, and the Australian zoogeographic regions, by complete cross neutralization test.

The virus strains tested are listed in table 1. Polyclonal antisera to each virus strain, except the AR 18132, were produced in mice.[5] Antiserum to AR 18132 was produced in hamster. Neutralizing activity in antisera was tested in plaque reduction neutralization test.[5]

The 11 virus strains tested for antigenic relationship were all very closely related (Table 2). With few exceptions, the virus strains tested were indistinguishable (Ho/Ht ratio$\leq$4) in a one-way cross-test, but differed (Ho/Ht ratio$\geq$8) from several other strains in the other direction. The most pronounced one-way differences were observed between two strains (EgAr 339 and ACROCEPHALUS) from the southern Palaearctic region, and strains from the Australian, Ethiopian and northern Palaearctic regions. Antisera to strains Egar 339 and ACROCEPHALUS neutralized the homologous viruses $\geq$16 times more efficiently than the majority of other viruses tested.

In conclusion, all the virus strains tested in the complete cross-neutralization test were shown to be varieties of Sindbis

virus. The observed one-way differences were not related to the zoogeographical regions from which the virus isolate originated.

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Table 1.
Virus strains used for cross comparisons, listed by geographic area, virus, virus strain and source of isolation.

Zoogeographic region	Country	Virus	Strain	Source
Palaearctic	Egypt	Sindbis	EgAr 339	Cx. univittatus
	Czechoslovakia	Sindbis	Acroceph.	Acrocephalus scirpaceus
	Sweden	Ockelbo	Eds 82/5	Culiseta spp.
	Sweden	Ockelbo*	86-520	Cx pipiens/torrentium
	Sweden	Ockelbo*	86-752	Cx pipiens/torrentium
	Karelia,USSR	Karelian*	LEIV-9298	Aedes spp
Etiopian	South Africa	Sindbis	AR 18132	Culex univittatus
	South Africa	Sindbis	Girdwood	Human
	Cameroon	Babanki*	Y-251	Mansonia africana
Australian	Quuensland	Sindbis	MRM 18520	Mosquitoes
	Queensland	Sindbis	C-377	Mansonia septempunctata

* name used in the literature but the taxonomic status is not defined.

Table 2.

Relationships of Sindbis and Sindbis related viruses by plaque reduction neutralization test of polyclonal antisera.

A) Homologous and heterologous neutralization titre of the antisera tested.

	Antisera										
Strain	ACRO	EGAR	C377	MRM	GIRD	18132	752	82/5	520	LEIV	Y251
ACROCEPH.	5120*	1280	640	320	640	2560	640	640	320	640	640
EGAR-339	1280	1280	80	160	320	2560	640	640	320	320	640
C-377	1280	160	640	320	320	2560	320	640	320	320	320
MRM18520	160	20	80	320	80	320	20	160	160	160	160
GIRDWOOD	160	20	80	320	320	160	40	320	320	320	320
AR 18132	40	80	40	80	40	1280	160	160	80	80	640
86-752	80	40	80	80	320	1280	640	640	1280	320	80
82/5	80	80	80	160	160	2560	1280	1280	1280	640	640
86-520	80	80	80	40	320	1280	1280	1280	640	160	160
LEIV-9298	160	160	40	40	320	1280	640	640	640	160	320
Y-251	160	40	40	40	80	1280	320	160	80	160	320

B) Ratio of homologous/heterologous neutralization titre for each virus and antisera combination tested.

	Antisera										
Strain	ACRO	EGAR	C377	MRM	GIRD	18132	752	82/5	520	LEIV	Y251
ACROCEPH.	1#	1	1	1	0.5	0.5	1	2	2	0.2	0.5
EGAR-339	4	1	8	2	1	0.5	1	2	2	0.5	0.5
C-377	4	8	1	1	1	0.5	2	2	2	0.5	1
MRM18520	32	64	8	1	4	4	32	8	4	1	2
GIRDWOOD	32	64	8	1	1	8	16	4	2	0.5	1
AR 18132	128	16	16	4	8	1	4	8	8	2	0.5
86-752	64	32	8	4	1	1	1	2	0.5	0.5	4
82/5	64	16	8	2	2	0.5	0.5	1	0.5	0.2	0.5
86-520	64	16	8	8	1	1	0.5	1	1	1	2
LEIV-9298	32	8	16	8	1	1	1	2	1	1	1
Y-251	32	32	16	8	4	1	2	8	8	1	1

* underlined indicates homologous neutralization titre for each antisera.

underlined indicates that the homologous/heterologous titre ratio equals one by definition.

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Comparative Nucleotide and Amino Acid Sequence of an Attenuated Japanese Encephalitis (JE) Virus ML-17 Strain for Swine Immunization and its Virulent Parental JaOH0566 Strain

Swine immunization has been used to interrupt JE virus circulation in nature by immunization of the major amplifier vertebrate of JE virus. One of the attenuated vaccine strains, ML-17, was developed from a virulent JaOH0566 strain isolated from fatal human brain by repeated passages in monkey kidney cell cultures at step-wise reduced temperatures (Yoshida et al., Biken J., 24: 47-67, 1981). The ML-17 strain was reported to possess several different biological markers from its parental JaOH0566 strain, such as reduced mouse neurovirulence, absence of swine viremia and limited growth in vector mosquitoes. In order to analyze pathogenicity of JE virus at a molecular level, comparative nucleotide and deduced amino acid sequencing was performed for this parent-progeny paired strain.

There were no deletion or insertion mutations, but a total 29 nucleotide (nt) substitutions were observed between JaOH0566 and ML-17 strains, which resulted in 14 amino acid (AA) replacements (sense mutation rate: 47 %). The AA substitutions were not random and concentrated in following 3 loci: PrM-M, ns4b, and N-terminal of NS5, while C, E, NS1, ns2b, NS3, and ns4a proteins were conserved. Although 5'noncoding region (ncr) was identical for both strains, 3 nt substitutions were found in the 3'ncr outside its conserved sequences. When the sequence of JaOH0566 strain was compared with that of JaOArS982 strain, there were 288 nt and 18 AA differences (sense mutation rate: 6.3 %).

Table 1. Nt and AA difference between JaOH0566, ML-17, and JaOArS982 strains of JE virus

Genome segment	Total nt/AA	JaOH0566 vs ML-17 Change nt/AA	JaOH0566 vs ML-17 % Change nt/AA	JaOH0566 vs JaOArS982 Change nt/AA	JaOH0566 vs JaOArS982 % Change nt/AA
5'ncr	95/	0/	0 /	0/	0 /
C	381/ 127	0/ 0	0 / 0	8/ 1	2.10/0.78
PrM	276/ 92	1/ 1	0.36/1.09	7/ 1	2.53/1.09
M	225/ 75	2/ 2	0.88/2.67	5/ 1	2.22/1.33
E	1500/ 500	2/ 0	0.13/ 0	31/ 3	2.07/0.60
NS1	1236/ 412	1/ 0	0.08/ 0	28/ 0	2.26/ 0
ns2a	501/ 167	3/ 1	0.60/0.60	17/ 1	3.39/0.60
ns2b	393/ 131	0/ 0	0 / 0	12/ 0	3.05/ 0
NS3	1857/ 619	2/ 0	0.11/ 0	50/ 2	2.69/0.32
ns4a	867/ 289	0/ 0	0 / 0	29/ 2	3.34/0.69
ns4b	345/ 115	3/ 3	0.87/2.60	12/ 0	3.47/ 0
NS5	2715/ 905	12/ 7	0.44/0.77	77/ 7	2.83/0.77
3'-ncr	585/	3/	0.51/	12/	2.25/
Total	10976/3432	29/14	0.26/0.41	288/18	2.26/0.52

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FURTHER STUDIES ON THE HEMAGGLUTINATING AND HEMOLITIC ACTIVITIES OF PURIFIED WESTERN EQUINE ENCEPHALOMYELITIS VIRUS.

This work was proposed to study some biological aspects associated to the surface structure of the Western Equine Encephalomyelitis (WEE) virus.

The development of this work depend upon purified viral preparations. Therefore, previously standardized method for virus purification was modified in order to be able to carry out functional activities studies, specially concerned with the fusion process evaluated by hemolysis test.

Viral concentration was performed with 8% polyethylene glycol 6000. This procedure allowed better results than previously described. The resuspended viral preparations were ultracentrifuged in a potassium citrate glycerol continuous gradient(45% - 0% potassium citrate and 0% - 30% glycerol). A 90% decrease in the infectivity titre was detected when the viral band was collected, diluted to 10% and ultracentrifuged.

Polyacrylamide - sodium dodecyl sulphate (SDS) gel electrophoresis demonstrated that the viral particles are constituted by three structural proteins, C, E_2, E_1, with molecular weights of 31 - 32 x 10^3, 47 - 50 x 10^3 and 53 - 57 x 10^3, respectively.

Egg grown purified WEE virus preparation exhibited hemagglutinating and fusogenic activities at pH 6.0 and pH 6.0 - 6.2, respectively. Both activities were lost upon previous treatment of the viral preparations at pH 6.0.

The treatment of purified WEE virions with mild acidic conditions resulted in rapid and irreversible alteration in the viral particles specific density. Electron microscopy preparation also demonstrated that the viral particles were agregated and more stained with sodium phosphotungstate.

The treatment of purified WEE virus with the nonionic detergent (Nonidet P - 40) followed by ultracentrifugation allowed the purification of nucleocapsid protein but the envelope proteins still contained capsid components as shown by SDS - PAGE analysis.

It also demonstrated that the purified viral preparation can be used as antigen for antibody titration by hemagglutination inhibition, hemolysis inhibition and neutralization tests.

Studies on Molecular-Biological Characterization of Flaviviruses, Especially of Japanese Encephalitis and Dengue

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Viruses: Mainly used were JEV (JaGAr-01 strain) and DlV (Mochizuki strain and A88 strain). The Mochizuki strain has been passed through mice and tissue cultures for a number of generations and has become completely nonvirulent to humans. The A88 strain, isolated from the blood of a DHF patient in Indonesia, has been passed through tissue cultures only for a few generations and is perhaps highly virulent to humans.

Fractionation of virus-infected cells: Virus-infected C6/36 cells were homogenized in Tris-buffer (pH 7.5) and fractionated by centrifugation into nuclear, membrane and supernatant fractions. Nonstructural proteins NS5 and NS3 were prepared by SDS-PAGE analyses and electro-elution. Structural protein E was also obtained.

Antiserum: Antisera against the above components were obtained by immunizing rabbits with each of the components.

Western blot (WB) analyses, immunofluorescence (IF) tests, and nucleotides and aminoacids sequences determination were carried out by techniques which are commonly applied.

RESULTS:

(1) Anti-NS5, anti-NS3 and anti-E sera recognized specifically the respective components of JEV and DlV.

(2) NS5 and NS3 were located mainly in the nuclear and membrane fractions of cells. The IF reactions were revealed distinctly in the perinuclear sites and also in the cytoplasm.

(3) The NS's of JEV detected by WB and IF techniques were revealed 6 to 12 hr after infection and became distinct at 24 hr, while the same reactions to DlV-NS were positive 1 day after infection and were distinct at 2-3 days. These time sequences were compatible with growth curves in tissue culture of the two viruses; the growth of DlV being much slower than that of JEV. It was suggested that one of the factors controlling the growth of viruses may be the speed of NS formation.

(4) In in vitro RNA synthesis experiments, anti-NS antibodies inhibited the RNA synthesis. The data suggest that NS proteins have essential role(s) in viral RNA synthesis.

(5) In nucleotides and amino acids sequences determination experiments concerning E protein, (i) the old (Mochizuki) and new (A88) strains of DlV were shown to have the similar pattern of sequence, with few points of difference, and (ii) such DlV sequences were different from those of D2, D3 and D4. However, Mochizuki and A88 showed different images of NS 5 bands revealed by WB immunoanalyses. Particular significance(s) of NS proteins were suggested.

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(Reported by S. HOTTA)

EXPRESSION OF THE BUNYAMWERA VIRUS L PROTEIN USING RECOMBINANT VACCINIA VIRUSES

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The complete nucleotide sequence of the Bunyamwera virus L RNA segment was recently determined in this laboratory from cloned cDNAs (Elliott, 1989; Virology **173**, 426-436). The L segment is 6875 bases in length, and in the complementary sense RNA encodes the L protein (2238 amino acids, MWt 259,000) which is thought to be the virion associated transcriptase or RNA polymerase. We have made monospecific rabbit antisera to the amino- and carboxy-termini of the L protein by fusing appropriate cDNA sequences of the L segment to the bacterial ß-galactosidase gene using the pUEX series of plasmids (Bressan & Stanley, 1987; Nucl. Acids Res. **15**, 10056). These antisera recognise the L protein in Bunyamwera virus infected cells by both immunoprecipitation and Western blotting.

With the long term aim of defining the functional domains within the L protein we have expressed a full length cDNA using vaccinia virus vectors. Two systems have been used: firstly the "standard" system where the L segment cDNA has been cloned into a transfer vector (pSC11) under control of the vaccinia virus 7.5K promoter and recombined into the thymidine kinase (TK) locus in the vaccinia virus genome (Chakrabati *et al.*, 1985; Mol. Cell. Biol. **5**, 3403-3409); and secondly the "T7" system where the cDNA has been inserted into the TK locus but under the control of a bacteriophage T7 promoter - expression of the target gene in this case only occurs when T7 RNA polymerase, supplied by a second recombinant vaccinia virus (vT7-3), is synthesised in the same cell (Fuerst *et al.*, 1987; Mole. Cell. Biol. **7**, 2538-2544). Antigenically authentic L protein of apparently the same size as that of Bunyamwera virus was detected in cells infected with either type of vaccinia virus vector. More L protein was synthesised using the standard system than the T7 system.

We have demonstrated that the expressed protein is functional by a nucleocapsid rescue assay: Bunyamwera virus nucleocapsids were purified from infected cells by CsCl centrifugation, and then transfected into CV-1 cells which had previously been infected with the recombinant vaccinia viruses. Total cell RNA was extracted at 20hr post transfection and analysed by Northern blot hybridization. A dramatic amplification of the nucleocapsid RNA, as monitored by studying the level of the S RNA genome segment with an S segment specific probe, was observed in cells which expressed the L protein from the vaccinia viruses, whereas in control cells the S RNA was barely detectable. The level of amplification was higher using the standard rather than the T7 vaccinia virus system. We hope to exploit this methodology to investigate the functions of the bunyavirus L protein in fine detail.

Detection of St. Louis Encephalitis Virus and Other Flaviviruses by Reverse-transcriptase Polymerase Chain Reaction

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RNA sequences of St. Louis encephalitis (SLE) virus and 4 other flaviviruses were detected by a modified polymerase chain reaction (PCR) incorporating a reverse transcriptase [1, 2, 3, 4]. RNA of 7 flaviviruses (Table 1) was extracted from virus-infected mouse brains or C6/36 mosquito cells [5]) in guanidine isothiocyanate [3, 6, 7]. RNA was transcribed into cDNA by avian myeloblastosis virus reverse transcriptase (RT) incorporated into the PCR mixture [8]. Regions of the genomes of 5 flaviviruses that appeared to be highly conserved were selected using the SAS Bestfit and Gap programs of the GenBank database, Genetics Computer Group, University of Wisconsin [9]. Genomic sequences for the other 2 flaviviruses were not available to us. The genomic regions were also chosen to contain suitable restriction-endonuclease cleavage sites. Oligonucleotide primers (20-mers) complimentary to the ends of the selected cDNA regions were synthesized (Table 2). For SLE virus a second pair of "nested" primers internal to the first pair was also synthesized; aliquots of the SLE cDNA products of the initial reverse-transcriptase PCR (RT-PCR) served as templates for a conventional PCR [10] with nested primers (N-PCR). All PCR was performed using commercial reagent kits and thermal controllers (Perkin Elmer Cetus, Norwalk, CT) as previously described [3, 10]. The amplified PCR products, ranging from 302 to 465 base pairs long, were visualized after electrophoresis in agarose gels containing ethidium bromide, and the identity of the products was confirmed by treatment with restriction endonuclease to generate fragments of predicted lengths.

Each of the 5 primer pairs amplified cDNA transcribed from homologous RNA but did not amplify cDNA from any of the 6 heterologous viruses. The SLE primers amplified cDNA transcribed from RNA of 2 different strains of SLE virus extracted from both mouse brain tissues and C6/36 cells. The sensitivity of RT-PCR and N-PCR for detecting SLE virus compared favorably with that of 2 conventional assays of infectivity (Table 3). RT-PCR also amplified cDNA transcribed from an RNA extract of formalin-fixed paraffin-embedded brain tissue infected with SLE virus.

PCR appears to offer a specific, sensitive and rapid method for detecting flaviviruses, even in materials not suitable for conventional assays of infectivity.

Table 1. Strains and passage histories of flaviviruses studied by PCR

Flavivirus	ACAV Abbreviation	Strain	Passage[a]
Dengue 2	DEN-2	New Guinea	24
Dengue 4	DEN-4	H-241	42
Japanese Encephalitis	JBE	M543	2
Langat	LGT	TP21	?
Powassan	POW	MacLean	7
St. Louis Encephalitis	SLE	Parton	4
		Hubbard	5
Yellow Fever	YF	17D vaccine	1

a Number of mouse-brain passages in Laboratory of CNS Studies.

Table 2. Flaviviral oligonucleotide primer sequences and PCR products

Viral Primers	Primer Sequence (5' to 3')	Size of PCR product (base pairs)	Sizes of Alu I digest fragments (base pairs)	Position of target sequence in viral RNA (gene)	Strain of reference viral sequence	Reference
DEN-2	1 CGC GCT GCC CAA CAC AAG GG	400	217 & 183[a]	envelope	S1 vaccine	[11]
	2 GCT TTG TCT TTC ATT TGC AG					
DEN-4	1 TTG TGG TTG ACA ACG TGC AC	440	276 & 164	nucleocapsid	Caribbean	[12]
	2 GTT GGT CGT GAA CAT GCC AA					
JBE	1 ATG ACT AAA AAA CCA GGA GG	350	243 & 107	envelope	JaOArS982	[13]
	2 CTT GCG AGC CAC ATG ATT GA					
SLE (outer)	1 GGG AAT TAC CCA ATG TCT AA	382	198 & 184	nucleocapsid	MSI.7	[14]
	2 ACT GCA ATG AAC TCG CCA GT					
SLE (nested)	1 GAA ACC GGG TTG TCA ATA TG	302	158 & 144	nucleocapsid	MSI.7	[14]
	2 GAG CAA CGA TCT GGT CCC TC					
YF	1 TAC CCT GGA GCA AGA CAA GT	465	287 & 178	envelope	17D vaccine	[15]
	2 GCT TTT CCA TAC CCA ATG AA					

a Predicted sizes are for Eco N1 digest fragments; there was no Alu I site in the predicted product.

Table 3. Comparison of PCR and infectivity assays for detection of SLE virus

Assay	Highest dilution of infected brain tissue in which virus was detected		
	Tissue sample number		
	1	2	3
SMB[a]	7.0[b]	4.3	5.3
C6/36-SMB[c]	nd[d]	5[e]	nd
RT-PCR[f]	5	5	7
N-PCR[g]	nd	8	8

a Suckling mouse intracerebral (ic) inoculation, 0.03 ml per inoculum.
b Reciprocal $\log_{10}$ LD50.
c Cultured 7 days in C6/36 cells, then cells and supernatant fluids inoculated ic into suckling mice.
d Not done.
e Reciprocal of $\log_{10}$ highest positive dilution.
f Reverse-transcriptase PCR.
g Nested-primer PCR.

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BANK VOLE-MOUSE HETEROHYBRIDOMAS PRODUCING MONOCLONAL ANTIBODIES AGAINST PUUMALA VIRUS

Nephropathia epidemica is a milder form of hemorrhagic fever with renal syndrome caused by Puumala (PUU) virus. Several viruses serologically related or identical to PUU virus cause significant human morbidity in Europe. The lack of specific monoclonal antibodies (MAbs) have hampered more detailed studies of these viruses. Attempts to produce mouse MAbs against PUU virus have been unsuccessful, probably due to absent or low immunological responses in laboratory mice. To overcome this problem we infected the natural host, bank vole (Clethrionomys glareolus), with PUU virus and obtained activated specific B-lymphocytes for the hybridoma production. Bank vole spleen cells were fused with the mouse myeloma cell line SP2/0 and fourteen PUU-specific monoclonal heterohybridomas were selected for further characterisation. Eleven of the bank vole MAbs were specific for the nucleocapside protein (NP) of PUU virus. One MAb was specific to glycoprotein 1 (G1) while two MAbs reacted with glycoprotein 2 (G2), as shown by immunoprecipitation. The anti G1-MAb and one of the anti-G2 MAbs showed significant neutralizing activity when tested in a new neutralisation test developed for PUU virus.
The MAbs have been successfully used in several assays including immunoprecipitation, immunofluorescence and immunoblotting with methods and reagents developed for mouse MAbs. They all have characteristics very similar to mouse IgG MAbs, which make them easy to handle and powerful tools for the investigation of viruses associated with hemorrhagic fever with renal syndrome.

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FLANDERS VIRUS RNA

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Flanders virus (FV) is a member of the Hart Park Serogroup of the Rhabdovirus Family. Previous studies have identified eight virus-specific proteins in FV-infected Vero cells as well as in purified virions [Boyd and Whitaker-Dowling (1988), Virology 163, 349-358]. In this study, FV was propagated in BHK cells, RNA was labelled with ^{3}H-uridine in the presence of Actinomycin D, and the RNA analyzed by electrophoresis in 1.5% agarose formaldehyde gels.

While no bands were detected in RNA from mock-infected cells, at least 7 bands were present in RNA extracted from virus-infected cells. Of these, one was located slightly behind vesicular stomatitis virus genome RNA and comigrated with radiolabeled RNA extracted from purified FV virions. The other 6 RNA bands bound efficiently to oligo [dT] cellulose and their sizes were estimated: RNA 2, 6.3kb; RNA 3, 2.2 kb; RNA 4, 1.3 kb; RNA 5, 1.0 kb, RNA 6, 0.7 kb; and RNA 7, 0.5 kb. RNA 4 consistently migrated as a broad heavy band which occassionally separated into two distinct bands.

In order to demonstrate that FV virion RNA is of negative polarity, we assessed the ability of poly A^{+} RNA from FV-infected cells to hybridize to and protect radiolabeled virion RNA from cleavage by RNases A and T-1. RNase resistance increased with increasing amounts of FV poly A^{+} RNA and at the highest concentration over 90% of virus genome was protected. Equivalent concentrations of poly A^{+} RNA from mock-infected cells afforded no protection.

The capacity of poly A^{+} RNA from FV-infected cells to direct virus-specific protein synthesis was assessed in rabbit reticulocyte lysate preparations and the products analyzed by SDS-PAGE. Seven FV-specific proteins were detected in reactions primed with FV RNA. Of these seven, six comigrated with FV proteins synthesized in infected Vero cells. The seventh protein (mw = 64,000) produced *in vitro* probably represents the nonglycosylated form of the 81,000 mw glycoprotein detected in infected cells but absent from the *in vitro* reaction products.

At the molecular level, FV shares several fundamental characteristics with other rhabdoviruses including a single species of virion RNA with negative polarity. The largest FV poly A^{+} RNA comigrated with VSV L mRNA, and probably encodes the analogous FV "L" protein which was produced *in vivo* and *in vitro*. In contrast to most rhabdoviruses, FV poly A^{+} RNA appears to encode seven viral proteins.

We are currently undertaking similar molecular studies of other Hart Park Serogroup viruses as well as cloning of cDNA derived from FV RNA.

FIELD STUDIES ON POTOSI VIRUS, A NEW BUNYAVIRUS ISOLATED FROM AEDES ALBOPICTUS COLLECTED IN POTOSI, MISSOURI.

Sixteen isolates of a newly recognized Bunyavirus, provisionally named Potosi virus, were made from pools of Aedes albopictus collected at a scrap-tire dump in Potosi, Washington County, Missouri, during August and September 1989 (Francy et al., in press; C.J. Mitchell, G.C. Smith, T.F. Tsai, and C. Frazier, unpublished data). Experimental studies have shown that Ae. albopictus is a competent vector of this virus, but vertical transmission of virus was not detected in 6,635 progeny of infected females (Mitchell et. al., 1990, J. Am. Mosq. Control Assoc. 6:523-527). Therefore, it is unlikely that the virus was introduced into Missouri via infected Ae. albopictus eggs in imported used tires. Instead, the existence of a transmission cycle in nature involving another vector seems more likely. To investigate this possibility, we conducted field studies in Washington County, Missouri, during the spring and summer of 1990.

Mosquitoes were collected by sweep-net, mechanical aspirator, and CO_2-baited CDC light traps and cone traps during June 11-16, July 27 - August 1, and September 7-13, 1990. The results are summarized in Table 1. Three virus isolations were made in Vero cell culture. One isolate from Culex restuans, collected on July 28, 1990, has been identified as Flanders virus. Identification of the other two isolates, from Culex erraticus collected on September 10 and 11, 1990, is pending; however, neither isolate appears to be Potosi virus.

During September 1990, 50 captures of 39 different vertebrates were also made in and around the tire yards at Potosi, Missouri. Blood specimens were obtained and tested for virus isolation and neutralizing antibody against Potosi virus for the 39 animals (Table 2). No virus was isolated from the sera and only 1 animal (eastern chipmunk) had neutralizing antibody specific for Potosi virus.

The apparent absence, or extremely low level of activity, of Potosi virus during 1990 in the same area that yielded 16 virus isolations during the same time period in 1989 remains unexplained.

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Table 1. Total number of mosquitoes and other biting flies collected in Washington County, Missouri, and tested for virus during 1990.

Arthropod	No. Specimens	No. Pools	No. Pools Positive
Aedes albopictus	9301	248	
Ae. canadensis	66	9	
Ae. epactius	1463	64	
Ae. vexans	2418	107	
Ae. triseriatus-hendersoni	5343	177	
Ae. spp.	353	32	
Anopheles barberi	2	2	
An. crucians	80	19	
An. punctipennis	512	70	
An. quadrimaculatus	689	73	
An. spp.	15	3	
Coquillittidia perturbans	476	57	
Culex erraticus	4335	104	2[a]
Cx. p. quinquefasciatus	277	35	
Cx. restuans	250	25	1[b]
Cx. salinarius	422	51	
Cx. territans	21	13	
Cx. (Mel.) spp.	527	45	
Cx. (Cux.) spp.	1064	94	
Cx. spp.	10	3	
Culiseta inornata	3	1	
Cs. melanura	36	14	
Orthopodomyia signifera	2	1	
Or. spp.	3	1	
Psorophora ciliata	5	3	
Ps. colombiae	15	3	
Ps. cyanescens	23	8	
Ps. ferox	208	29	
Ps. howardii	15	5	
Ps. spp.	1	1	
Uranotaenia sapphirina	13	4	
Culicoides spp.	124	15	
Simuliidae	12	4	
Total	28084	1320	

[a]Identification pending
[b]Flanders virus

Table 2. Laboratory results on animals captured at Potosi, Missouri.

Species	Virus Isolation	Neutralizing Antibody		
		Potosi Virus	Cache Valley virus	Tensaw virus
Opossum	0/11	0/11	0/11	0/11
Raccoon	0/6	0/6	0/6	0/6
Cattle	0/6	0/6	0/6	0/6
White-footed mouse	0/2	0/2	0/2	0/2
Eastern chipmunk	0/2	1/2	0/2	0/2
Eastern cottontail	0/2	0/2	0/2	0/2
Eastern woodrat	0/1	0/1	0/1	0/1
Fox squirrel	0/1	0/1	0/1	0/1
Domestic cat	0/1	0/1	0/1	0/1
Eastern box turtle	0/7	0/7	0/7	0/7
Total	0/39	1/39	0/39	0/39

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Two Cases of Chikungunya Fever

Chikungunya virus belongs to the genus Alphavirus, family Togaviridae. The virus was first isolated from the blood of patients by Ross in 1956 during an epidemic in Tanzania. Chikungunya virus is widely distributed in Africa, the Western Pacific and South East Asia and causes sporadic cases and epidemic outbreaks. Often, outbreaks of Chikungunya fevers occur simultaneously with dengue fever epidemics. The disease is characterized by abrupt onset, high fever, headache, joint pains and rash. The outcome of the disease is, as a rule, benign.

This report describes two cases of Chikungunya fever in Soviet citizens who had visited Angola. Both became ill on June 19, 1989 and were incapacitated by sudden high fever, headache, and severe myalgia. Rash appeared on the trunk and extremities on days 4 and 5. Lymphatic nodes were enlarged. The fever lasted about one week, but the joint pains persisted more than 3 months. The illnesses were diagnosed clinically as dengue fever.

Blood samples were taken on August 21, 1989. The sera were examined for antibodies to dengue 1, dengue 2, West Nile, Chikungunya, Sindbis, Semliki Forest, yellow fever and Rift Valley fever in HI, CF, indirect IF and plaque reduction neutralization tests. The reactions were positive for Chikungunya virus only. From the results shown below, it was concluded that both patients had Chikungunya fever.

Titer of antibodies to Chikungunya virus in sera from two patients who became ill in Angola

Patient	CF	HI	Indirect IF	PRNT in Vero Cells
1	32	80	80	1280
2	64	160	100	2560

Submitted by S. Ya. Gaidamovich, N. A. Sveshnikova, E. E. Melnikova and N. V. Khutoretskaya

Serological detection of California Encephalitis group viruses infection in mammals in The People's Republic of China.

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Serological evidence of infection with California serogroup viruses in human residing in a suburb of Shanghai (China) was reported in 1984 (Gu H.X. *et al.* Transactions of The Royal Society of Tropical Medicine and Hygiene 78, 780-781, 1984). In order to investigate more on the prevalence of these viruses in China, sera of resident animals were collected in Shanghai area during February 1988. 149 rabbit and 274 horse sera were tested for the presence of antibodies to California serogroup viruses (snowshoe hare, La Crosse and Tahyna) antibodies. The results obtained demonstrate the exposure of resident mammals in China to a California serogroup virus closely related or identical to snowshoe hare virus. Antibody to Tahyna virus appears to be due to heterologous cross-reactivity to this virus, which is closely related to snowshoe hare virus. Neutralizing antibodies to Snowshoe hare and/or Tahyna viruses were detected in 7.30% of the horse sera. Only one rabbit was found to be seropositive to snowshoe hare virus. Attempts to isolate these viruses from their mammalian reservoir(s) and from the mosquito vector(s) in China as well studies on the ecology of these viruses in South East Asia are warranted and will be the next research step.

Table 1: Neutralization serology of resident horse sera

Serum	TITER			TITER	
number	SSH[1]	TAH[2]	number	SSH	TAH
33	40*	-	148	40	-
37	≥160	-	158	80	10
38	160	-	159	20	20
39	40	-	160	40	-
49	20	-	169	-	10
52	120	-	170	40	-
96	≥160	-	173	20	-
97	20	10	177	-	20
98	40	-	178	80	-
139	-	10	182	40	-

[1]SSH = snowshoe hare virus; [2]TAH = Tahyna virus; *Reciprocal titer

ARBOVIRUS/VECTOR MONITORING IN NSW, AUSTRALIA; 1988/89, 1989/90

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Arbovirus activity appears to occur in southeastern Australia each year. There has been no evidence for activity of the encephalitogenic flavivirus Murray Valley Encephalitis (MVE) in this region of Australia since 1974, and the flavivirus Kunjin (KUN) which also is known to cause the disease Australian Encephalitis has only been detected on a few occasions during the past decade. However, the alphavirus Ross River (RR) which is responsible for a debilitating epidemic polyarthritis appears to be active every year and evidence for activity of other arboviruses, alphaviruses (e.g. Barmah Forest and Sindbis), flaviviruses (e.g. Kokobera and Stratford) and bunyaviruses (e.g. Gan Gan and Trubanaman), is detected occasionally. Of the less well-known viruses, Barmah Forest, Sindbis, Kokobera and Gan Gan viruses have all been reported to cause illness.

The summer of 1988/89 in southeastern Australia was one of extensive and considerable activity of Ross River virus, with more than 500 clinical cases being serologically confirmed for the State of New South Wales (NSW) alone. However, during the recent summer of 1989/90, although Ross River virus was active in most areas of NSW, less than 200 suspected clinical cases were confirmed serologically and this appears to represent a real decrease in incidence of RR infection although the possibility that other arbovirus/es were responsible has not been excluded.

A programme to monitor mosquito vector populations in NSW has been underway since 1984 with the support of the State Department of Health. The programme has a number of collaborating centres in country areas and reference laboratories in Sydney, and is coordinated through the Medical Entomology Unit of the University of Sydney at Westmead Hospital in Sydney. During the past two years we have incorporated arbovirus isolation and identification procedures for alphaviruses, flaviviruses and bunyaviruses, and also an antigen detection procedure for flaviviruses.

The mosquito monitoring programme is conducted typically from November through May (i.e. late-spring through autumn) each year and over the past two seasons has included up to 30 sampling locations representing coastal and inland regions of the state. Each of these locations has at least two sampling sites, and weekly samples are collected by dry-ice baited EVS traps and submitted live to the Medical Entomology laboratory for sorting, identification and processing for arbovirus detection/isolation.

Ground mosquitoes are put through cell culture using C6/36, BHK-21 and Vero cells, with repassage, and subsequent virus isolates are identified by microneutralisation and ELISA tests. During the 1988/89 season, the alphaviruses Ross River and Sindbis, and a few as yet uncharacterised viruses, were isolated from the mosquito samples. During 1989/90, Ross River and Sindbis viruses, and the bunyavirus Trubanaman, were isolated.

No flavivirus activity was detected during the two seasons, either from the mosquito processing or from a programme of weekly bleeding of sentinel chicken flocks maintained at 10 of the inland localities where mosquito populations were sampled (the sentinel chickens are currently tested only for seroconversion to flaviviruses).

During the past two seasons, more than 50 species of mosquito have been recorded from the coastal sampling sites and more than 25 from the inland locations. The species most often yielding virus from inland locations has been the freshwater riverine species Culex annulirostris, and the most 'productive' species in coastal areas has been the saline-marsh species Aedes vigilax.

Although our data are limited as yet, they are revealing a definite seasonal relationship between mosquito population abundance, virus activity in the vector mosquitoes, and infections in humans. We hope that a continuation of the programme will result in a valuable data base on the interactions between vectors and viruses in the different regions of the State, in order to better understand the epidemiology of the diseases and provide for more informed approaches to vector control in the various situations. In general terms there are no routine mosquito control programmes undertaken in NSW as a precaution against arboviral disease outbreaks, although a few local authorities in both inland and coastal regions do undertake some control of pest mosquitoes on an ad hoc basis.

[Submitted jointly by Richard Russell, Peter Wells and John Clancy of the Medical Entomology Unit, Westmead Hospital, Westmead (Sydney, Australia); Linda Hueston and Tony Cunningham, Virology Unit, Westmead Hospital (Sydney); Michael Cloonan, Greg James and Michael Fennell, Virology Section, Prince Henry Hospital (Sydney); Royle Hawkes and Helen Naim, Department of Microbiology, University of New South Wales (Sydney); and Clement Boughton, Department of Infectious Diseases, Prince Henry Hospital (Sydney)].

REPORT FROM THE VIRUS LABORATORY
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Wild rabbit (Oryctolagus cuniculus L.) and arbovirus in South-east France : results of two serological inquiries.

During field studies conducted by "Office National de la chasse" on the biology of wild rabbit (Oryctolagus cuniculus) in south-east France, blood was collected on paper strips from 269 specimens trapped in 1985 and 1986 in Camargue and Vaucluse.

Wild rabbit is a popular game species in France and one of the objectives of the Office was to appreciate the epidemiological role of this lagomorph in arbovirus infections and the eventual effects of these infections on population dynamics of this species.

In south-east France, O. cuniculus is the host of many tick species including Ixodes ventalloi, Haemaphysalis hispanica and Rhipicephalus pusillus. During certain periods, each individual rabbit may harbour up to one hundred adult ticks (Gilot et al, 1985). At the beginning of rabbit shotting season the density of ectoparasitism averages 20-30 adult ticks/day (Legrand, 1986). This period also corresponds to the return in Camargue of migrating birds from western and south Africa. All these data and results of previous serosurveys in Europe point out that wild rabbit may be an excellent host for arboviruses in south-east France.

Rabbit blood specimens were examined using hemagglutination inhibition (HI) test for antibody to Sindbis, West Nile, Tahyna, Uukuniemi, Arumowot and Sicilian Sandly fever viruses and complement fixation test for antibody to Tribeč, Eyach and Erve viruses.

From 269 blood specimens surveyed, 4 (or 1,5 %) were found positive : one for West Nile virus (titer 1:20), two for Eyach virus (titer 1:10 each) and one for Tribec virus (titer 1:10).

It is not too surprising to detect some low activity of West Nile virus in wild rabbit sera since this mosquito-borne flavivirus was isolated from Camargue many years ago from both patients and Culex modestus mosquito (Hannoun et al, 1964).

Tribeč virus was first isolated in Slovakia from Ixodes ricinus ticks (Libikova et al, 1965) and small wild mammals (Grasikova et al, 1965). This tick-borne orbivirus is largely distributed in the paleartic region where it circulates among birds, small wild mammals and cattle. However, it was not yet isolated from France.

Eyach virus, another tick-borne orbivirus of the Colorado tick fever group, was isolated in france from ticks parasiting O. cuniculus in Mayenne (Chastel et al, 1984) after its original description from West-Germany (Rehse-Kupper et al, 1976). Thus, O. cuniculus appears as a new mammalian host for this virus in France after Cervus elaphus, Rupricapra rupricapra and Ovinus (Le Lay-Roguès et al, 1987). Antibody to Eyach virus was also found in Tchecoslovaquia in patients with various neuropathis (Frankova et al, 1981 ; Malkova et al, 1980 ; 1982).

It is hypothetized that Eyach virus may have originated from Colorado tick fever virus through the recent introduction into

Europe of infected Sylvilagus floridanus rabbits from U.S.A. S. floridanus was imported in France for game purposes first in 1953 and subsequently in 1972 (Arthur and Chapuis, 1982).

The potential effects of the circulation of West Nile, Eyach and Tribeč viruses on the population dynamics of wild rabbit in south-east France remain to be clarified.

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ENCEPHALITIS SURVEILLANCE
NEW ORLEANS

MAY through AUGUST 1990

Encephalitis Surveillance efforts produced 1022 wild bird blood samples from New Orleans during the past year. Fifty percent of these birds were common sparrows (*Passer domesticus*), the remaining bird samples included Red-winged Blackbirds (*Angelaius phocniceus*), Cardinals (*Richmondena cardinalis*), Cowbirds (*Molothrus ater*), Blue Jays (*Cyanocitta cristata*), and others.

Sixty-eight samples were found to be positive for SLE by *MacElisa* test performed by the State Lab. Most of the positives occurred in the eastern portion of New Orleans.

Although there were no subsequent human cases, and the positives could not be confirmed, the areas where positives occurred and their grouping at certain bird traps convinces us that this was not lab error; rather, the *MacElisa* test appears to have been less specific than expected.

Mosquito pools collected from positive areas yielded several avian viruses other than SLE.

Because of the problems we have experienced with the *Elisa* test results during the past season, we are hoping to return to the H.I. test next year.

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Mosquito Control Specialist

CJL/lhk

STUDY OF ARBOVIRUSES AND ARBOVIRUS INFECTIONS IN THE USSR

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of the USSR AMS, Moscow

D.K. Lvov, A.M. Butenko and V.L. Gromashevsky

The circulation of the following arboviruses has been demonstrated in the USSR.

Family Togaviridae : Getah, Karelian fever, Kyzylagach, Semliki Forest, Sindbis.
Family Flaviviridae : Alma-Arasan, Japanese encephalitis, Karshi, Negishi, Omsk hemorrhagic fever, Powassan, Sokuluk, tick-borne encephalitis, Tyuleniy, West Nile.
Family Bunyaviridae :
Bunyavirus : Batai, Tahyña, Inkoo, snowshoe hare.
Phlebovirus : SF-Naples, SF-Sicilian, Karimabad.
Nairovirus : CHF-Congo, Clo Mor, Paramushir, Rukutama, Sakhalin.
Uukuvirus : 'Jukuniemi, Zaliv Terpeniya.
Hantavirus : Hantaan, antigenic subtypes of Hantaan, Prospect Hill, Puumala and Seoul.
Other antigenic groups: Bhanja.
Antigenically ungrouped viruses: Artashat, Caspiy, Hasan, Issyk-Kul, Razdan, Tamdy, Uzun-Agach, Zevashen.
Family Reoviridae : Aniva, Baku, Chenuda, Kemerovo, Okhotskiy, Tribec, Wad Medani.
Family Rhabdoviridae : Isfahan, bovine ephemeral fever.
Family Orthomyxoviridae : Batken, Dhori.
Family Picornaviridae : Sikhote-Alin, Syr-Dariya Valley.

According to the available evidence, the following viruses are pathogenic for man: Bhanja, SF-Naples, SF-Sicilian, Dhori, Hantaan, Inkoo, Issyk-Kul, Japanese encephalitis, Karelian fever, Kemerovo, Omsk hemorrhagic fever, Negishi, Powassan, Tahyña, Tamdy, tick-borne encephalitis, Sindbis, West Nile. Epidemiologically most well known are tick-borne encephalitis, hemorrhagic fever with renal syndrome, and Crimean hemorrhagic fever.

In 1985 - 1989, the Department of Virus Ecology surveyed the territories of the European and Asian USSR, a total area of 12.3 million km^2 (55% of the entire country). Inoculations of newborn white mice were used to examine 1,700,000 blood-sucking mosquitoes, about 92,000 ixodid ticks and more than 3,500 specimens of wild vertebrates. As a result of this work, 459 strains of viruses were isolated (Table 1).

For the study of antibody to California serogroup and Batai viruses in humans, sera of 7,000 normal residents and 1,500 domestic animals (cows, deer, horses, sheep, swine, camels) were examined by neutralization tests. The highest prevalences (50% - 90%) to these viruses were found in the population of northern Eurasia. In central regions of the European USSR, the prevalence was 30% - 40%, and in the southern regions (the zones of steppes and forest-steppes), 10% - 20%. The results indicate simultaneous circulation in the same territory of Tahyña, Inkoo, and snowshoe hare viruses as well as of antigenic variants differing from them. Inkoo virus circulation is most active in western and central regions of the USSR, Tahyña virus in southern, snowshoe hare virus in northern and eastern regions.

Antibodies to Batai virus were found very rarely (1% - 2%) in humans, but were found in > 50% of domestic animals, especially in central and southern regions.

Serological examinations of patients with unidentified seasonal febrile diseases resulted in diagnosis of 104 cases of illnesses etiologically associated with California encephalitis serogroup viruses. The illnesses ran an influenza-like course or appeared as central nervous system illnesses (aseptic meningitis and meningoencephalitis).

Table 1. Results of arbovirus isolation, 1985 - 1989.

Viruses	Mosquitoes	Ixodid Ticks	Wild Mammals	Total
California serogroup viruses [a]	137 [b]		4	141
Batai-like	129			129
TBE		56	2	58
Tyuleniy		27	2	29
Getah	17			17
Zaliv Terpeniya	2	15		17
Sakhalin		14		14
Paramushir		10		10
Rukutama		10		10
Okotskiy		5		5
Aniva		2		2
Kemerovo		2		2
Uukuniemi		2		2
Unidentified	15	7	1	23
Total	300	150	9	459

[a] The data on identification will be presented in the next report.

[b]. Number of strains isolated

PROBABLE LABORATORY-ACQUIRED INFECTION WITH VEE (SUBTYPE IF) VIRUS

(Amélia Paes de Andrade Travassos da Rosa, Pedro Fernando da Costa Vasconcelos, Jorge Fernando Soares Travassos da Rosa, and Sueli Guerreiro Rodrigues: Evandro Chagas Institute, SESP Foundation, Av. Almirante Barroso, 492, Caixa Postal 1128, CEP 66050, Belém, Pará, Brazil)

A probable laboratory infection with Venezuelan Equine Encephalitis (VEE) virus occurred in a staff member of the Virus Section of this institute. The causative agent was VEE subtype IF (prototype strain SP An 50783), which had been isolated from whole blood of a bat, ***Carrollia perspicillata***, captured 20 April, 1976 at Ribeira Valley, Iguape County, São Paulo, Brazil. Clinical manifestations consisted of fever (39°-40°C maximum), headache (generalized and very severe), nausea without vomiting, and dizziness. Illness lasted for three days.

Leukopenia (5,200 white blood cells) was observed on the first day of illness. On the third day white cells were at 4,000. Serum SGOT, SGPT, and bilirubin levels were within normal limits. A virus strain (BE H 427922) was isolated from a blood sample obtained on the first day of illness.

The mode of infection remains unknown, but it is suspected that it was through aerosol transmission. The patient denied contact with the infecting agent for the six days prior to onset of illness and he reported no cuts or abrasions while working with it prior to that time. He had not been outside the city of Belém for several months before the onset of this illness. This is the first documentation of human disease caused by this subtype of VEE virus in Brazil.

Editor's note: At the American Society for Tropical Medicine and Hygiene annual meeting in New Orleans, Louisiana (November 5-8, 1990) a paper was presented (see abstract below) describing additional human infections with this VEE subtype in Brazil.

HUMAN DISEASE CAUSED BY VENEZUELAN EQUINE ENCEPHALITIS SUBTYPE I. IN RIBEIRA VALLEY, SAO PAULO, BRAZIL. L.B. Iversson+, A.P.A. Travassos Da Rosa, S.G. Rodrigues, and M.D.B. Rosa. Public Health School, of the University of Sao Paulo, Evandro Chagas Institute, Ministry of Health, Belem, Para, Brazil.

In 1976, a new subtype (IF) of VEE virus was isolated from a bat, Carollia perspicillata, and from a pool of Culex (Melanoconion) mosquitoed in Ribeira Valley, a densely forested area in the state of Sao Paulo, Brazil. Human sera collected in the region between 1977 and 1987 had a high prevalence of HI and neutralizing antibodies to this virus. Development of disease in a laboratory researcher provided evidence that this enzootic VEE virus was pathogenic. We investigated a group of 25 soldiers aged 21 to 35, who underwent 15 days of training in the forested area of Ribeira Valley. Twenty (20) of these soldiers had a febrile disease around 7 days after their return. HI tests to 19 arboviruses and ELISA for Hantaan virus were performed. HI-positives were tested by neutralization, complement fixation test and MAC-ELISA to subtype IF of VEE. Neutralizing antibodies to this virus were present in 6 soldiers; in 2 of them with fever, severe headache, general malaise, diarrhea and sleepiness, MAC-ELISA and CF tests were positive to VEE subtype IF. Evidence of past infections caused by three other arboviruses was observed in 3 men. This is the first report of naturally-acquired disease caused by VEE subtype IF.

REPORT FROM THE INSTITUTO DE INVESTIGACIONES VETERINARIAS, LABORATORIO DE ARBOVIRUS, APARTADO 70, MARACAY 2102. VENEZUELA.

FIRST REPORT OF BLUETONGUE ANTIBODY IN VENEZUELA

Julieta de Siger; Elvira Pulgar G., Gladys Medina.

On February 29, 1988, eight serum samples from convalescent bovines collected at "Las Delicias", (a farm in a hot dry forest with permanent surface water by irrigation, less than 100 m altitude, located in Carmelo Municipality, Urdaneta District, Zulia State) were sent to this laboratory by J.A. Quintero, a private veterinarian in charge of the farm. He reported clinical cases of Bluetongue (BT). On requirement from this laboratory, three ovine serum samples, were received without history of BT from the same farm.

The sera were tested for group-specific antibody using the agar gel precipitation test (AGPT), and antigen supplied by National Veterinary Services Laboratory, Ames, Iowa, USA and a Comercial Bluetongue antibody test Kit, supplied by Michael M. Jochim, Veterinary Diagnostic Technology, Inc. The protocols sent by both institutions were used in these studies.

Attempts to isolate virus were made as described in the protocol for Bluetongue virus isolation (National Veterinary Services Laboratory, Ames, Iowa. November 1982) but using bovine serum samples instead of whole blood.

Positive precipitin lines were observed in three of the eight bovine and two of the three ovine serum samples. Two isolations of Vesicular Stomatitis virus, (New Jersey and Indiana type) were made from bovine samples.

Since then a total of 2653 serum samples from domestic ruminants have been analized by AGPT. Samples were divided in three groups.

1. Samples from a serological survey which comprised 1643 healthy bovines and ovines, collected in 32 farms located in Carmelo Municipality. Results are shown in Table 1.

2. Serum samples from 781 animals collected during 1988 - 1990 in 11 Venezuelan States with different ecological habitats and altitudes, most of them from healthy bovines (Table 2)

3. Samples of 229 bovine, ovine and caprine collected in five Venezuelan States between 1973 - 1983. In this group only 18 samples were collected from a farm that reported congenital abnormalities and abortion in bovines and ovines (Table 3)

Results showed:

- Positivity in goats, sheep and cattle, although in the latter a certain percentage of reactors may have been due to Epizootic Hemorrahgic Disease.
- Disease expression of the virus similar to that observed in Colombia, Guyana and the Caribbean islands.
- Viral activity in our country since at least 1973.
- Virus circulating at different altitudes and ecological environments
- Serological conversion in both imported and native cattle in the states of Apure, Tachira and Zulia
- In two of the three States mentioned above, virus was probably being transmitted simultaneously with other pathogens according to an epidemiological study carried out in farms with problems.

TABLE 1

AGPT REACTORS USING BTV ANTIGEN AND SERUM SAMPLES(1) COLLECTED AT ZULIA STATE, URDANETA DISTRICT, CARMELO MUNICIPALITY. 1988

Age group (Years)	Number Positive/Total Tested	Percentage Positive
< 5 *	48/137	35.0
6 - 11 *	97/198	49.0
1 - 2	323/412	78.4
3 - 4	177/273	64.8
5 - 6	171/281	60.9
> 7	193/336	57.4
u	5/6	83.3
Total	1014/1643	61.7

(1): 1579 bovines, 64 ovines; *: months; u: unknown

TABLE 2

AGPT REACTORS USING BTV ANTIGEN IN SAMPLES COLLECTED IN 44 FARMS IN 11 VENEZUELA STATES. 1988 - 1990

States	Number Positive/Total Tested	Percentage Positive
Apure	89/257	34.6
Aragua	2/4	50.0
Bolivar	19/26	73.1
Carabobo	14/21	66.7
Cojedes	63/159	39.6
Guarico	6/18	33.3
Lara	33/61	54.1
Merida	6/6	100.0
Tachira	55/99	55.5
Yaracuy	11/14	78.6
Zulia	64/116	55.2
Total	362/781	46.3

TABLE 3

RETROSPECTIVE STUDY. DISTRIBUTION OF BTV REACTORS USING THE AGPT IN DOMESTIC RUMINANTS IN VENEZUELA DURING THE PERIOD 1973 - 1983.

States	Year	Number Positive/Total Tested	Percentage Positive
Delta Amacuro	1973	9/17	52.9
Zulia	1973-1974	32/113	28.3
Aragua	1977	10/18	55.5
Monagas	1977	6/6	100.0
Apure	1978	12/20	60.0
Aragua	1981	3/5	60.0
Zulia	1982-1983	27/50	54.0
Total	Seven Years	99/229	43.2

DISTRIBUTION BY SPECIES

Bovines	62/110	56.4
Ovines	29/74	39.2
Caprines	8/45	17.8
Total	99/229	43.2

CONCOMITANT INFECTIONS BY MALARIA AND ARBOVIRUSES IN THE BRAZILIAN AMAZON REGION (1).

Pedro Fernando da Costa Vasconcelos (*), Amélia Paes de Andrade Travassos da Rosa (*), Jorge Fernando Soares Travassos da Rosa (*) and Nicolas Dégallier (**). (*) Arbovirus laboratory, Virus Section, Evandro Chagas Institute, SESP Foundation, Av. Almirante Barroso 492, CP 1128, 66050, Belém, Pará, Brazil. (**) ORSTOM, CP 75, 66050, Belém, Pará, Brazil, & Instituto Evandro Chagas.

We describe six cases, in which infections of arboviruses and malaria were observed in the same patient and at the same time, in the Amazon Region of Brazil. The arboviruses isolated are included in family Bunyaviridae, genus Bunyavirus. Plasmodium falciparum (diagnosed by thick and thin smears) was associated with the following arboviruses: Guaroa (California serogroup) 3 times; Tacaiuma (Anopheles A serogroup) twice; Catu (Guama serogroup) once. The latter was also infected by P. vivax.

Five patients were male and one female. All were seventeen years old or more. None were born in Pará State, although all were living there. The female was a domestic help, while four men were agricultural workers and one was a commercial traveller.

The main clinical history of disease was fever with headache, chills, myalgia and arthralgia. Sometimes we noted abdominal pain, nausea, vomiting and dizziness. Jaundice was recorded in two cases of P. falciparum in association with Tacaiuma. The typical periodic fever associated with malaria was not observed. It was continuous. Patients were treated by SUCAM with chloroquine, primaquine, quinine, or other drugs when necessary. Five recovered quickly, but one died.

These cases are important because in Amazonia thousands of people are diagnosed and treated for malaria. About 10% of the strains of P. falciparum are considered to be drug resistant. The possibility (probably much underestimated) of concomitant infections with arboviruses may obscure the effectiveness of the treatment, or may lead to an erroneus diagnosis.

It is proposed that the joint infections with malaria and Guaroa or Tacaiuma viruses were due to the fact that in Amazonia, both agents share the same mosquito vectors in forested areas, either Anopheles nuneztovari or An. triannulatus.

(1) Work done with joint funding by SESP Foundation/CNPq/ORSTOM.

WHO COLLABORATING CENTER OF REFERENCE AND RESEARCH FOR ARBOVIRUSES
Institut Pasteur - P.O.B.220 - DAKAR (Senegal)

During 1989, the Center had identified 258 strains from the Pasteur Institute un Abidjan (41), Bangui (13), Madagascar (8), Dakar (178) and from USAMRIID(1) Fort-Detrick.
Since its opening, the Center has identified 4016 viral strains.
The viruses of this year came from various areas in Africa and were derived from mosquitoes, ticks, human and animal samples. They include the following: Chikungunya (24), Babanki (7), Middelburg (2), Bagaza (5) Wesselsbron (3), Yaoundé (1), West-Nile (19), Bouboui (1), Yellow fever (1), Zika (31), Dengue 2 (27), Dengue 2/Zika association (16), Bunyamwera (1), Ngari (14), Simbu (1) Bwamba (1), Congo (3), Dugbe (2), Bandia (2), ArD 46672a Wad-Medani variant (76), ArD 56184 another Wad-Medani variant (1), Gomoka (1), Orungo (1). Two viruses are recognized as probably new: AnB 4151 isolated by the Bangui Pasteur Institute from a **Rattus rattus** sample, and ArMg 966 isolated from a mosquito pool (**Mansonia uniformis**).
The reference virus collection actually includes 189 arboviruses or vertebrate viruses. Some changes have been made in their classification.
Some noteworth accomplishments has been observed :
The exploitation of mosquito pools, caught during 1989 in the Kedougou area, shows an important Dengue 2 epizootic in the usual wild vectors : **Aedes furcifer, A. taylori** and **A.luteocephalus**. The Zika epizootic, in the same area,is now 6 YEARS OLD. We found Dengue 2-Zika viruses associated in the same pools at a significatly higher level than expected, and this is now being studied.
In the same area, the isolation and identification of a Yellow Fever viral strain proves the low level circulation of this virus in the Kedougou semi-moist savannah, outside of an epizootic context.
For the first time, West-Nile virus has been isolated from Senegal, as part of a study of the culicidian vectors from the Senegal River.
Ngari virus has been isolated from mosquito pools caught by USAMRIID entomologists from Fort-Detrick.Several mosquito species are involved.During 1988 this virus has been isolated from samples of a sick sheep in the Eastern part of Mauritania.
The laboratory of arboviral epidemiology has, in particular, studied the CCHF virus cycle in nature and the risk, for humans, of contracting this disease. Concerning the same virus, the Experimental Virology laboratory has developed some interesting hypothesis, based on infection in the laboratory of several domestic and wild animals, and transmission to some ticks (**Hyalomma, Rhipicephalus** and **Amblyomma**) at different stages.
The studies from Pasteur Institute of Dakar are done in very close collaboration with a team of entomologists from ORSTOM (2).

1)USAMRIID : United States Army Medical Research Institute on Infectious Diseases

2)ORSTOM : French Institute for the Development of research in Cooperation.

Jean-Pierre DIGOUTTE

ARBOVIRUS STUDIES IN MADAGASCAR

REPORT FROM ARBOVIRUS RESEARCH UNIT
INSTITUT PASTEUR MADAGASCAR
BP 1274 TANANARIVE. MADAGASCAR

1. West Nile (WN) virus activity in Madagascar. 1990

We have recently evaluated the prevalence of WN virus antibodies in human population of different regions of Madagascar. During 1990, 2844 sera were sampled among scholastic population (6 to 21 years old apparently healthy subjects). WN virus antibodies were tested by an immunoglobulin-G capture enzyme-linked assay. The results are presented in Table 1. These data show the presence of WN virus throughout island, with a lower prevalence in Highlands areas.

Table 1

WN virus antibody prevalence (ELISA) in different regions of Madagascar. Scholastic population (6 to 21 years old).

Place	Region	N*	Pos	%
Sainte-Marie	East	504	91	18,0
Mananara	East	395	70	17,7
Marovitsika	East (forest)	239	99	41,4
Andriba	West	340	191	56,1
Mandoto	West	386	116	30,0
Ampijoroa	West (forest)	92	62	67,3
Ankazobe	Highlands	258	43	16,6
Manarintsoa	Highlands	319	27	8,4
Amboasitra	Highlands	118	1	0,8
Ampanihy	South	99	18	18,1
Betioky	South	94	30	31,9

N* number of samples

2. Rift Valley fever virus (RVFV) activity in Madagascar. 1990.

A RVFV epizootic outbreak in cattle zebu has been observed in the East coast of Madagascar during May 1990 (Fenerive district. 140 km north from Tamatave). A serological survey in human population has been conducted in 5 villages where cattle abortions have been notified. Sera were sampled in apparently healthy population and in 17 patients hospitalized in Fenerive Hospital with fever and headache. Sera were tested for RVFV antibody by an immunofluorescent assay (IFA), by an IgM capture ELISA for IgM specific antibodies,

and by Plaque reduction neutralization test (PRNT). Virus isolation (Vero cells, AP 61 cells, and suckling mice) and antigen detection (ELISA immunocaptation) were also performed.

Among human sera, 37/471 (7,8 %) were positive for RVFV antibodies, 25/438 (5,7 %) had IgM antibodies to RVFV. All positive sera were confirmed by PRNT. Among patients hospitalized 5/17 (29,4 %) were positive by IFA, and 4/16 (25 %) were positive for RVFV IgM antibodies. No virus was isolated, and antigen detection was negative.
The epizootic outbreak was associated to an important virus circulation in human population.

(Jacques Morvan, Pierre Coulanges. Institut Pasteur. BP 1274. Tananarive. Madagascar.)

REPORT FROM ARBOVIRUS UNIT
DEPARTMENT OF VIROLOGY
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MONITORING OF PHLEBOTOMINAE SAND FLY TRANSMITTED VIRUSES ACTIVITY IN MARCHE REGION (ITALY).

Results of field and laboratory studies suggest that Toscana and Arbia viruses (Bunyaviridae, Phlebovirus) are transovarially transmitted in their insect vector in nature. However, it is uncertain whether these agents can be maintained exclusively by vertical transmission in their vector or if periodic amplification in a vertebrate-insect cycle is necessary for virus survival. In order to verify whether amplification cycles occur under natural conditions, we have planned to monitor the infection rates of sand flies during the months of their activity in a "focus" of Toscana virus.

The data related to the first year of surveillance are here reported. From June to September 1989, collection of Phlebotominae sand flies was performed using CDC ligth traps. The area of collection was the farm near Fermo (Marche Region), were in the previous years a Toscana virus focus has been identified. Fourty-one pools, containing 50 Phlebotomus perfiliewi each, were tested for virus. Virus isolation was performed in parallel in both Vero cells and suckling mouse brain; identification of isolates was performed by complement fixation test. From 1990 sand flies we isolated 13 strains of Toscana (TOS), 2 strains of Arbia (ARB), and 5 strains of a new virus, recently isolated in our laboratory from P.perfiliewi: Radi (Rhabdoviridae: Vesiculovirus).

In table 1 the month of collection and the infection rate of sand flies for the three viruses are shown. As typical of the italian natural foci the three types of viruses are active simultaneously in the same sand fly population. From two pools we isolated both TOS virus in Vero cells and Radi virus in suckling mouse. The difference in the number of sand flies tested in each month depends on variation of climatic conditions which affect sand fly activity. However, at least for TOS virus, which is more represented among the isolates, a comparison of virus isolation rates in different months can be attempted. For this virus, the isolation rate found in June (reflecting the infection rate of overwintering sand flies) and that found in subsequent months is similar. With the limitation of the small number of sand flies tested, it seems from these results that amplification mechanisms are not occurring. Data on viral activity in the same area and in different years could confirm this observation. Isolation attempts from sand flies captured during 1990 summer are in progress.

TOS and Radi viruses were isolated both from female and male sand flies (Table 2). For Radi virus, this is the first isolation from male sand flies implying that also for this agent, as demonstrated for TOS and ARB viruses previously, transovarial transmission occurs under natural conditions.

TABLE 1. Monthly distribution of virus isolations in Fermo province during 1989

Month of capture	N°sand flies N° pools	N° of virus isolation TOS	ARB	Radi	Total
June	661/13	5(0.7)*	1(0.1)	3(0.4)	9(1.4)
July	133/4	1(0.7)	0	0	1(0.7)
August	1160/22	6(0.5)	1(0.1)	2(0.2)	9(0.7)
September	36/2	1(2.8)	0	0	1(2.8)
Total	1990/41	13(0.6)	2(0.1)	5(0.2)	20(1.0)

* Minimum field infection rate expressed as number of infected per 100 Phlebotomus perfiliewi tested.

TABLE 2. Sand fly virus isolation by sex

Sex	N° sand flies N° pools	N° of virus isolation TOS	ARB	Radi	Total
Males	307/7	1(0.3)	0	1(0.3)	2(0.6)
Females	1683/34	12(0.7)	2(0.1)*	4(0.2)	18(1.0)
Total	1990/41	13(0.6)	2(0.1)	5(0.2)	20(1.0)

* Infection rate per 100 sand flies tested.

M.G. Ciufolini, M. Maroli, N. Miceli, L. Nicoletti, S. Cecchetti, R. Amore, S. Percopo, P. Verani.

A SEROEPIDEMIOLOGICAL STUDY OF CALIFORNIA SEROGROUP VIRUSES IN SRI-LANKA.

Viruses belonging to the California serogroup have been documented in North and South America, Europe and Africa, and some of these viruses cause human infection and disease. However, there is limited information on California serogroup (CAL) viruses in Asia (Bardos et al 1983; Gu et al 1984; Peiris et al. 1986; Gu and Artsob 1987). We have carried out a seroepidemiological investigation of human (n=952) and animal (n=1834) sera collected from different ecological zones of Sri Lanka (latitudes 5^{o} - 10^{o} N; longitudes 79^{o}-82^{o} E).

The sera were screened for neutralising antibody to Lumbo (LUM), snowshoe hare (SSH) and trivittatus (TVT) viruses by a microtitre adaptation of a plaque neutralisation test on Vero cells, and 262 (9%) of the 2786 sera screened had antibody to one or more virus. Nineteen sera - selected to represent different species of origin and reaction profiles - were titrated against nine CAL viruses: LUM; SSH; TVT; Tahyna (TAH); California encephalitis (CE); La Crosse (LAC); Inkoo (INK); Melao (MEL) and Guaroa (GRO). Results from representative sera are shown in Table 1.

a) the majority of sera reacted with LUM / CE / SSH / LAC viruses. These sera gave highest titres to LUM or SSH viruses, with lower titred cross reactivity to CE and LAC. Surprisingly, these sera reacted poorly if at all, to Tahyna virus, usually regarded as antigenically closely related to LUM. It has been previously reported that whereas TAH and LUM viruses are cross neutralised by antisera produced in laboratory animals, sera from humans naturally infected with one virus does not reciprocally neutralise the other (Calisher 1983). The LUM/SSH reaction profile was seen in sera from humans, cattle, pigs, dogs, rabbits, chicken and ducks.

b) a few sera gave highest titres to MEL (eg N47; N1771). Some of these sera cross neutralised LUM, SSH and TAH viruses at low serum dilutions. In these instances the titres against LUM and TAH were comparable, in contrast to profile a) above. Antibody with the Melao profile was found in cattle, goat, sheep, chicken and occasional human sera.

From the above results, it was concluded that there were multiple CAL viruses active in Sri Lanka - a LUMBO / SSH related virus/es and a MELAO related virus. Definitive identification of the CAL virus/es active in Sri Lanka can only be made following virus isolation. Although over 100,000 mosquitoes collected from these study areas have been processed for virus isolation, and a number of virus isolations have been made, none of these belong to the CAL virus serogroup.

Table 1. Neutralisation profile of representative California virus-reactive sera.

	Reciprocal plaque neutralising antibody titer to virus[a]:								
Serum	TAH	LUM	SSH	CE	LAC	INK	MEL	TVT	GRO
NT22N/83	<10	640	160	160	40	<10	20	20	<10
NK/C5	<10	160	10	40	10	<10	10	20	<10
H22	80	>2560	>2560	640	160	160	320	320	<10
N47	40	20	80	20	10	<10	320	40	<10
N1771	20	20	<10	<10	<10	<10	80	10	<10
N1778	10	10	10	<10	<10	<10	80	<10	<10

[a] TAH= Tahyña, LUM= Lumbo, SSH= snowshoe hare, CE= California encephalitis, LAC= LaCrosse, INK= Inkoo, MEL= Melao, TVT= trivitattus, GRO= Guaroa

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REPORT SUBMITTED BY:

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REPORT FROM THE DEPARTMENT OF HYGIENE, TEIKYO UNIVERSITY SCHOOL OF MEDICINE, ITABASHI TOKYO 173, JAPAN, AND NATIONAL INSTITUTE OF HEALTH, SHINAGAWA TOKYO 141, JAPAN

An analysis of Japanese encephalitis (JE) epizootic in pigs during 1965 - 1989 in Japan

A nationwide surveillance of JE virus spread based on positive sero-conversion of HI antibody of domestic pig was started in 1965. The accumulated data from 1965 to date demonstrated clearly some changes in intensity of JE virus spread in Japan.

Domestic swine sera were collected for detection of JE antibody at approximately 50 fixed observation points located from the southern part (Kyushu) to the northern part (Hokkaido) of Japan. Blood specimens from six- to eight-month-old pigs (20 pigs per observation point), which have no experience of JE enzootic in the former year, were collected three to four times per month from May or July through September. The intensity of JE virus spread in 1965 - 1989 has been observed by the month of appearance of positive antibody in 50% of the sera ("JE positive month").

The observation years were categorized into three different status by difference in "JE positive month".

(1):1965 - 1969: At all the observation points except Hokkaido "JE positive month" were detected during July and August through 1965 - 1968. In the positive months, the observation points became "JE positive month" from the south to the north in geographical order. In 1969, a few observation points in the middle and the northern part of Honshu island became "JE positive month" in September.

(2):1970 - 1979: At many of the observation points, "JE positive month" moved to August. Some of the observation points located in the northern part of Honshu island could not be "JE positive month" during entire JE epidemic season of the each year. Moreover, in 1972, 1974 and 1977, the observation points even in the southern part (Kyushu, Shikoku and Chugoku), could not be "JE positive month" in each of those years.

(3):1980 - 1989: In general, similar tendency observed during 1965 -1969 was obtained in this period. That is, many observation points located in the southern and the northern parts turned to be "JE positive month" in July and August.

JE is an epidemic diseases in Japan reporting human cases every year. However, number of human cases decreased remarkably since 1969. In the JE epidemic before 1968, more than 1,000 human cases per year were commonly observed in Japan. In the 70's and 80's, human JE cases per year are less than 50. Although it is still not clear that why JE epidemic among Japanese people disappeared, it is required to pay attention that spread of JE virus still keep similar intensity like as observed in the 1965–1968, when severe JE epidemic had occurred.

(Ogata, T., Sugamata, M., Oya, A.)

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

Studies on recurrent outbreaks of Japanese encephalitis(JE) in Nagaland, India.

An outbreak of high fever associated with headache, convulsion, drowsiness and unconsciousness in a few, simulating viral encephalitis was observed in Nagaland - a north eastern state of India, in the middle of 1985, involving several hundreds of people. Recurrent illness of similar nature was also noted in the years 1986 to 1989. These outbreaks were investigated both virologically & epidemiologically from time to time. A few sporadic cases of malarial infection were also detected during the investigation.

A total number of 83 clinically diagnosed encephalitis cases could be investigated virologically during the period from 1985 to 1989. Although no viral agent could be isolated from clinical specimens, evidence of JE infection could be established by detecting JE specific IgM antibodies by MAC ELISA test as well as by HI & CF tests in 34 (40.9%) and flavivirus (including JE) in 17 (21.5%) cases (Table - I). A limited serosurvey amongst the human contacts revealed presence of JE antibody in 26.6% (Table - II). Moderately high prevalence of JE antibody (36%) was also detected in pig sera.

The ecological, epidemiological & other bioenvironmental factors favouring JE transmission were conspicuous by their presence in Nagaland. Various species of Culicine mosquitoes, particularly C.vishnui, a predominant vector of JEV and which have been incriminated with most of the JE epidemics in India and elsewhere, have also been found in adundance.

Pigs, which are considered as the 'amplifier' host for JE in most JE endemic countries, are steadily increasing in Nagaland. More so, the presence of JE antibody in a considerable number of pig sera, indicate their active involvement in JE transmission at Nagaland.

The fact, that JE establishes itself in countries with pre-existing favourable ecological, epidemiological and bioenvironmental factors, holds quite true in case of Nagaland also. Therefore, early institution of containment programmes such as antimosquito measures, controlled pig rearing & immunization of susceptible human population in this hilly state are expected to prove beneficial in combating the extensiveness of future outbreaks.

Table - I

Result of serological testing

Year	Number of Cases	Clinical materials	Antibody testing for Exclusively JE: MAC ELISA	JE: HI	JE: CF	Flavivirus: HI	Flavivirus: CF	Evidence of: JE	Evidence of: Flavivirus
1985	11	Acute blood (11)*	6	5	2	2	1		
		Convalescent blood(1) (paired)	nil	1	1	-	-	6	2
		C S F (5)	3	-	-	-	-		
1986	12	Acute blood (12)	3	3	nil	2	nil	3	2
1987	10	Acute blood (10)	5	5	3	nil	nil		
		Convalescent blood(6) (paired)	-	3	3	2	2	5	2
1988	9	Acute blood (9)	3	3	2	2	1	3	2
		C S F (4)	2	-	-	nil	nil		
1989	41	Acute blood (37)	10	10	3	8	1		
		Convalescent blood(3)	nil	2	2	1	1	17	9
		C S F (1)	1	-	-	-	-		

* Number tested.

Table - II

Age and sex wise distribution of JE antibody in healthy individuals (contacts) of Dimapur, 1985.

Age group (in yrs.)	Male	Female	Total
1 - 10	2/21	2/10	4/31
11 - 20	2/9	5/12	7/21
21 - 30	3/6	1/6	4/12
31 - 40	1/6	2/7	3/13
41 and above	2/5	4/8	6/13
	10/47 (21.5%)	14/43 (32.5%)	24/90 (26.6%)

(M.S. Chakraborty, S.K. Chakraborti, K.K. Mukherjee, M.K. Mukherjee, P.N. De, S. Chatterjee & P. Chatterjee)

REPORT FROM THE NEW YORK STATE DEPARTMENT OF HEALTH
WADSWORTH CENTER FOR LABORATORIES & RESEARCH

EASTERN EQUINE ENCEPHALITIS IN NEW YORK STATE: 1990

Upstate New York. During the summer and fall of 1990 an epizootic of eastern equine encephalitis (EEE), initiated in mid-July in Oswego County, amplified and spread to 3 other counties (Onondaga, Oneida, and Madison) in the Syracuse region. Tests for alphavirus isolation were completed on 1215 vertebrate tissues and 2030 pools of 131,388 mosquitoes from the 4-county area. One hundred and twenty-four isolations of the virus were recovered from equines, sentinel pheasants, 12 species of wild avians and 6 species of mosquitoes.

Mosquitoes. Eastern equine encephalomyelitis virus (EEEV) was detected in 86 pools of 4561 mosquitoes collected in all 4 counties between 7/23/90 and 10/16/90. Seventy-three (85%) of the isolates were from Culiseta melanura, the primary vector of EEEV among avians in this area. The remainder came from Culiseta morsitans (5), Aedes canadensis (3), Aedes vexans (2), Coquilletidia perturbans (2) and Anopheles quadrimaculatus (1). The seasonal minimal field infection rate (MFIR) for EEEV in the region was highest in Cs. melanura (1:418), followed by An. quadrimaculatus (1:837), Cs. morsitans (1:1171), Ae. vexans (1:3163), Ae. canadensis (1:13,284) and Coq. perturbans (1:20,274). Both species of Culiseta prefer to feed on birds whereas the other 4 species have a broader host range which includes mammals.

Avians. A total of 34 isolates was obtained from domestic and wild birds. EEEV infections were diagnosed in 25 viremic wild birds sampled between 7/16/90 and 9/03/90 in Oswego County. Brain tissue collected 9/16/90 from a dead Black-capped Chickadee also yielded virus. The following species exhibited the highest viremia rates: House Wren (25%), Least Flycatcher (25%), Yellow-bellied Flycatcher (20%), White-throated Sparrow (12.5%) and Common Yellow-throat (10%). Two weeks after the first viremic birds were recognized in this area, 28 of 72 (39%) wild avians bled between 7/29/90 and 8/01/90 were seropositive for EEEV and 18 of these (64%) exhibited high hemagglutination-inhibiting (HI) antibody titers (1:160 - 1:2560), suggesting recent infection. In Onondaga County, EEEV was isolated from blood clots of 4 sentinel pheasants sampled on 8/22/90 and from the brains of 3 dead pheasants and 1 House Finch collected between 9/4/90 and 9/6/90. In addition, serologic confirmation of EEEV infection was demonstrated in 7 pheasants which converted from negative to positive during the period of 8/6/90 to 9/6/90; 3 of these were found to be viremic on 8/22/90.

Equines. Fourteen suspect equine cases were investigated in 7 upstate counties; 4 fatal cases of EEE were confirmed in 2 horses from Oswego, 1 from Onondaga and 1 from Oneida by isolation of virus from brain tissue collected between 8/16/90 and 10/03/90. In addition, 2 equines from Oswego County with clinical symptoms of EEE and no history of recent vaccinations had HI antibody titers to EEEV (1:160 and 1:1280) compatible with recent or current infection.

Downstate New York. Although surveillance for arboviruses was minimal in southeastern New York this year, EEEV activity was recognized in 2 downstate counties, namely, Orange and Suffolk. A yearling colt from Orange County with no history of travel or vaccination became acutely ill on 9/13/90 with central nervous system (CNS) symptoms suggestive of EEE; the horse was euthanized the next day and EEEV was subsequently isolated from post-mortem brain tissue. This animal was pastured near a large swamp north of Orange Lake (about 12 miles northwest of Newburgh) where EEEV was active in 1952. In Suffolk County, 188 pools of 14,640 adult female mosquitoes collected from 6/05/90 to 9/13/90 were tested for arbovirus; 1 isolation of EEEV and 4 of Highlands J (HJ) virus were obtained from 5 pools of 497 Cs. melanura mosquitoes. The EEEV isolation came from 117 females captured in Riverhead on 8/28/90, whereas all of the HJ isolates were from specimens taken 8/17/90 in Connetquot Park. The MFIR for EEEV and HJ virus in Cs. melanura was 1:2986 and 1:747, respectively.

Comments: The 1990 EEE outbreak in upstate New York had all the components of a full-blown epizootic; I.e., infected horses, pheasants, wild birds and mosquitoes. However, in contrast to the outbreaks of 1983 and 1988, the 1990 epizootic was notable in that it was not preceded by the appearance of HJ virus, a non-pathogenic member of the western equine encephalitis complex, which shares the same habitats, mosquito vectors and avian hosts and was considered to be predictive for EEEV activity in this area. Furthermore, with respect to EEEV infections in mosquitoes and wild avians, the intensity and duration of this outbreak was unprecedented in the Syracuse region, particularly in view of extensive efforts to control mosquito vector populations. Despite these efforts to intervene in the epizootic transmission cycle, EEEV was continuously isolated from viremic wild birds sampled weekly in one treated site in Oswego County and from Cs. melanura collected throughout the 4-county area; no previous outbreak has yielded as many EEEV isolations. As of 11/14/90, however, no evidence of EEEV infection has been uncovered in tests of 47 patients with symptoms of CNS disease from New York State.

Margaret A. Grayson, Ph.D., Robert D. Boromisa, Ph.D., and Leo J. Grady, Ph.D.

Centre for Arbovirus Reference and Research.

Laboratory for Microbiology and Pathology, Queensland Department of Health, and The School of Life Science, Queensland University of Technology, Brisbane, Australia.

1. **Dengue outbreak in North Queensland.**

The first clinical dengue infections due to transmission of indigenous dengue since the outbreak in 1981 were recorded in North Queensland. From May to September a total of 31 patients, from an area centered on Cairns, were diagnosed as having a flavivirus infection. Dengue 1 was isolated from 10 of these patients and a further 16 possessed IgM to Dengue 1. Serum from two patients contained IgM antibody which reacted only with Dengue 2 while serum from three patients contained IgM antibody reacting with more than one dengue serotype.

2. **Barmah forest Virus Disease.**

Clinical cases of human infection with Barmah Forest virus has been recognized in Australia. Forty-six patients over a two year period have been diagnosed by detection of IgM or virus isolation. Symptoms associated with infection include arthritis, arthralgia, myalgia, headache and rash.

3. **Other viral isolates.**

An as yet unidentified flavivirus isolate has been made from a patient in Queensland. Serological analysis indicated that the isolate is related to, but distinct from, Edge Hill and Kokobera viruses.

4. **Functional epitopes on dengue virus.**

Following our earlier mapping of serological epitopes in the E protein of Dengue 2 (Arch. Virol. 105, 209 - 221;1989), we have attempted to identify any role these epitopes might have.

Peptides corresponding to three of the serologically defined epitopes interfered with agglutination of gander erythrocytes by dengue viruses suggesting the peptides could correspond to part of the viral haemagglutinin. ("In Arbovirus Research - Australia", 59 - 62;1989).

Three strains of mice (Balb C, CBA and Quakenbush) were immunized with Dengue E protein octapeptides, either alone in Freunds adjuvant, or coupled to known T cell epitopes from influenza virus. None of the mice produced neutralizing antibody with a titre of greater than 1 in 20.

This project is continuing but by employing immunization protocols likely to elicit stronger anti-peptide responses.

Studies on the biological characteristics of monoclonal antibodies A35 against hemorrhagic fever with renal syndrome virus.

Monoclonal antibody A35, prepared against a strain of hemorrhagic fever with renal syndrome (HFRS) virus reacts in indirect immunofluorescence tests with all HFRS viruses from China, U.S.A., Japan, U.S.S.R., North Europe, Brazil, Thailand, and Korea available to us. This includes type I (76-118), II (Seoul), III (Hällnäs), IV (Prospect Hill), V (Marji), and VI (Leaky). Obviously this monoclonal antibody is broadly cross-reactive.

Immunoprecipitation showed that monoclonal antibody A35 is anti-nucleocapsid antibody, possessing hemagglutination-inhibiting and neutralizing antibodies.

Experimental treatment with A35 of HFRS virus-infected suckling mice gave 100% protection when one dose of monoclonal antibody was given by the intraperitoneal route 18-48 hours after HFRS virus infection.

(Chen Bo Quan, Liu Qin Zhi, We Mei Ying, Lian Mi Fan, Hang Chang Shou, Song Gan: Institute of Virology, Chinese Academy of Preventive Medicine, Beijing 100052, China)

HANTAVIRUS INFECTION OF RATS IN HAWAII.

Dept. of Tropical Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI.

Antibody to Hantaan virus in Hawaiian Rattus rattus, R. norvegicus and R. exulans was first demonstrated by our laboratory in 1983. The highest prevalence was in R. norvegicus (17.7%) followed by R. rattus (7.2%) and R. exulans (5.9%). Vector control personnel working as rat-catchers also had antibody (11/36 positive). Attempts to isolate the virus at that time were not successful. Recently we have confirmed the serological evidence for presence of a Hantaan-like bunyavirus in feral and laboratory rats in Hawaii; 364/1272 (29%) feral rats, 163/420 (39%) laboratory rats, and 33/337 (14%) feral cats had antibody to Hantaan virus (76-118 prototype strain) by indirect immunofluorescent antibody test (IFAT) at a titer of 1:32 or greater. However, the antibody is non-reactive to the major Hantaan virus proteins and only weakly reactive to some minor proteins by Western blot analysis.

Several strains of virus were isolated from sero-positive feral and laboratory rats by co-cultivation in Vero E6 cell cultures of lung and spleen cells disaggregated in a Stomacher blender. Adapted virus causes mild cytopathic effect in Vero E6 cells. Fine fluorescent granules detectable by IFAT early after infection, eventually fill the entire cytoplasm of infected cells. The isolates, only partially characterized at this time, are enveloped (deoxycholate sensitive), approximately 100 nm in diameter, and are morphologically similar to Hantaan virus in transmission electron micrographs. Large electron-dense granular inclusion bodies characteristic of hantaviruses are prominent features in thin sections of infected cells.

The Hawaii isolate appears to be a bunyavirus and possibly a Hantavirus, but it differs antigenically form other rat-associated hantaviruses, all of which belong to the Seoul subgroup. In view of its unique characteristics, the Hawaii isolates have been tentatively named Manoa virus. Further characterization of the virus is underway.

(Michael J. Langford, Arwind Diwan, Nyven J. Marchette)

Preparation of a candidate recombinant vaccine for HFRS

Hantaan virus is the etiologic agent of Korean hemorrhagic fever, the most serious form of hemorrhagic fever with renal syndrome (HFRS). Like other viruses in the Bunyaviridae family, Hantaan virus has a three-segmented, single-stranded RNA genome. The small (S) segment encodes the viral nucleocapsid protein, the medium (M) segment encodes the two envelope glycoproteins, G1 and G2, and the large (L) segment presumably encodes the virion-associated polymerase. We previously reported construction of recombinant vaccinia and baculoviruses expressing the S segment, the M segment, or portions of the M encoding either G1 or G2 (Schmaljohn, et al., 1990). Our data demonstrated that both vaccinia virus and baculovirus recombinants expressing the complete M segment, and baculovirus recombinants expressing the S segment could protect animals from infection when challenged with Hantaan virus. Based upon this information, we have constructed two recombinant vaccinia viruses as candidate human vaccines for HFRS.

Our original vaccinia virus recombinants were prepared by homologous recombination between vaccinia virus DNA and a plasmid transfer vector (pSC-11) that contained either the Hantaan virus M or S segment and also the Lac Z gene of E. coli. Recombinants thus expressed two foreign genes: the Hantaan gene under control of the vaccinia virus 7.5 K promoter and the Lac Z gene under control of the vaccinia virus 11 K promoter. Both foreign genes were inserted into the thymidine kinase gene of vaccinia virus. The inclusion of the Lac Z gene allowed easy selection of recombinants in that the Lac Z gene product, betagalactosidase, forms a blue color in the presence of appropriate substrates added to the cell culture medium. Recombinant viruses, therefore, formed blue plaques on monolayers and non-recombinants formed clear plaques.

For use as a human vaccine, we felt that recombinant viruses should not contain superfluous foreign genes such as Lac Z. Consequently, we constructed two new plasmid vectors for homologous recombination. The first plasmid was formed by simply using the restriction enzymes Pst 1 and Xho 1 to delete the Lac Z gene from pSC-11 already containing the M segment. The second transfer vector was constructed by similarly deleting the Lac Z gene, and then replacing it with the S segment of Hantaan virus. Recombinants formed by homologous recombination with the first vector contained Hantaan M under control of the vaccinia virus 7.5 K promoter. Recombinants formed with the second vector contained Hantaan M under control of the 7.5 K promoter and Hantaan S under control of the vaccinia 11 K promoter.

Because recombinants could no longer be selected from wild-type vaccinia viruses by color, an immuno-blot selection procedure was devised. For this method, dilutions of vaccinia recombination mixtures were plaqued under liquid on monolayers of Vero E6 cells. When plaques became visible (3 to 5 days post infection), the liquid was removed and dry nitrocellulose filters were placed on top of the monolayers. The cell monolayers, which adhered to the nitrocellulose filters, were then treated as if they were Western blots. That is, filters were blocked with a buffer containing 10% fetal calf serum and then reacted with hyperimmune mouse ascitic fluid to Hantaan virus or with monoclonal antibodies to the viral nucleocapsid or envelope glycoproteins. Normal mouse ascitic fluid was used for control immuno-blots. Alkaline phosphatase-conjugated goat anti-mouse serum was then reacted with the filters followed by addition of substrate (BCIP/NBT). Plaques assayed from recombinants expressing the M segment were detectable with the polyclonal antibodies and the monoclonals to G1 and G2, and plaques obtained from recombinants expressing both the M and S segments were detectable with monoclonal antibodies to the nucleocapsid protein, the envelope glycoproteins or with polyclonal ascitic fluid. Recombinants were recovered by cutting the colored plaques out of the dry nitrocellulose filters and sonicating in cell culture

growth medium. We found that virus could be recovered from processed filters stored at room temperature for several weeks.

To prepare candidate vaccines of these recombinants, we performed three succesive immuno-selections as described, followed by two normal plaque selections under agarose. The recombinants were then passed three times through certified cells and a small master seed was made. The master seed was transferred to a vaccine production laboratory where it was expanded to a production seed and lot one of a candidate vaccine. The candidate vaccine was subjected to extensive tests for contaminating agents and was aliquoted into vials and lyophylized. Currently, we are repeating animal experiments to evaluate the efficacy of these potential recombinant vaccines.

Connie S. Schmaljohn, Sherman E. Hasty, and Joel M. Dalrymple
Virology Division, USAMRIID, Ft. Detrick, Frederick, MD 21702-5011

Reference:Schmaljohn, C.S., Chu, Y.K., Schmaljohn, A.L. and Dalrymple, J. M. (1990). Antigenic subunits of Hantaan virus expressed by baculovirus and vaccinia virus recombinants. J. Virol., 64: 3162-3170.

REPORT FROM THE DEFENCE MICROBIOLOGY DIVISION,
CHEMICAL DEFENCE ESTABLISHMENT, PORTON DOWN, SALISBURY, WILTSHIRE

A cytopathic indicator to distinguish Nairobi Sheep Disease Virus from Ganjam Virus

Nairobi Sheep Disease Virus (NSD) is a major tick-transmitted pathogen of sheep and goat populations of East Africa. Ganjam virus was originally isolated from goats in Orissa State, India. Ganjam virus is closely related to NSD virus and it is claimed to be the same virus.

In preliminary work leading to studies on the molecular biology of these two viruses we have been attempting to optimise the growth conditions and yields of the viruses in vertebrate and invertebrate continuous cell lines at various multiplicities of infection, varying temperatures and pH conditions.

NSD was found to replicate successfully in several cell lines including BHK21, SW13 and XTC2 cells while Ganjam virus replicated to the highest titres in BHK21 cells. In BHK21 cells both viruses caused a very marked cell fusion. In each case fusion was not detected until 20 hours pi indicating the probability that this effect is due to fusion from within. However the later stages in the cytopathic process with the two viruses were markedly different. The multinucleate cells in the NSD virus-infected monolayers remained attached to the flasks, while in the Ganjam-infected cultures after forming multinucleate cells, the areas of fused cells readily lifted off to form large membrane-bound structures. Various input multiplicities have been used with both viruses and the differences in cytopathic effect are still readily identified.

Current serological techniques have failed to distinguish between these two viruses, and the viruses grow to relatively low titres in culture systems which has slowed the establishment of biochemical criteria for comparison. Further examination of the difference in cytopathic effect during the fusion event with these viruses, which were initially isolated from different locations and different animals (albeit a common host), may give a reliable cell marker for the two viruses.

S M Eley, L G Bruce, R M Henstridge and N F Moore

DMD3/A/002/90/MG

INFECTION AND TRANSMISSION OF VESICULAR STOMATITIS VIRUS BY BLACK FLIES

The possibility that an epizootic vector(s) occurs in the ecology of vesicular stomatitis (VS) virus (New Jersey serotype) has been postulated for many years based on epidemiological findings (virus isolations) and dispersion patterns of the virus during the time of year when insects are numerous. One group of biting flies that might serve in this capacity is the Simuliidae (black flies). To investigate that possibility, we have begun to evaluate the susceptibility of *Simulium vittatum* (a colonized species) to several strains of the New Jersey serotype of VS.

In preliminary studies using the Verde Valley strain (the index strain of the 1982 epizootic), one-day old flies were injected intrathoracically with 1 ul of RPMI-1640 cell culture medium containing approximately 10^3 pfu of virus. The flies were held for 10 days at 27°C (survival was $\geq$ 50%) and then allowed to feed individually into capillary tubes containing RPMI-1640 culture medium (+ 20% calf serum). After an hour, the fluids from each tube were collected and analyzed per individual fly. Salivary glands (SG) were also dissected, homogenated, and examined for the presence of virus. Ten of 12 flies had infected salivary glands as revealed by culture of SG homogenates for virus in a Vero M cell line (+ CPE). Saliva from 8/10 flies that had fed into capillary tubes contained virus as revealed by culture of capillary tube fluids in Vero M cells (+ CPE) and immunofluorescence of infected cells using conjugated anti- VS(NJ) immune serum.

We next will conduct *per os* infections trials of *S. vittatum* with VS (Verde Valley strain) using an *in vitro* bloodfeeding system developed specifically for *Simulium* spp. (Bernardo & Cupp, J. Med. Ent. 23: 666) and then use the capillary pipette method for collection of putatively-infected saliva. If this aspect of the study is successful, we plan to evaluate *Simulium argus* as a vector. This species, which is a pest of cattle and livestock in the southwestern USA and northern Mexico, may be important for the periodic introduction of VS into the US via the Colorado and Rio Grande river valleys.

E. Cupp, Department of Entomology, and J. Maré, Department of Veterinary Sciences; University of Arizona; Tucson, AZ 85721.

CHIKUNGUNYA VIRUS VACCINE AVAILABLE FOR AT-RISK LABORATORY PERSONNEL

Chikungunya (CHIK) is a mosquito-borne alphavirus found throughout Africa, southeast Asia, the western Pacific, and India that produces a dengue-like illness, with severe joint pain and has often caused epidemics with high attack rates. Other alphaviruses such as Mayaro, Ross River and o'nyong nyong are antigenically related to CHIK and cause similar human disease. In addition, numerous laboratory workers have become infected with CHIK through parenteral or aerosol exposure in the laboratory.

A live, attenuated vaccine (CHIK 181-25) has been developed from a southeast Asian human isolate (CHIK 15561) by serial plaque-to-plaque passage in certified human embryonic lung cells (MRC-5). Safety trials conducted at USAMRIID with medical research volunteer subjects indicate that this vaccine is safe and highly immunogenic. In a double-blind, placebo-controlled trial with 36 subjects receiving vaccine there were no differences between the placebo and vaccine groups with respect to the number of volunteers who experienced symptoms, to include headaches, muscle aches, joint aches, and fatigue. There were more total days of joint aches in the vaccine group but there was no evidence of arthritis and symptoms were short-lived, responding to non-steroidal anti-inflammatory agents such as ibuprophen. In addition, 20 at-risk employees at USAMRIID have received the vaccine without adverse reactions; serologic studies in these employees are pending. Low grade fever (99 to 100.5 ^{0}F), accompanied by mild fatigue and arthralgia, occurred in just less than 10% of volunteers, lasted 24 to 48 hours, and resolved spontaneously.

Studies done with mosquitoes feeding on rhesus monkeys inoculated with parent (CHIK 15561) virus indicate that at viremia levels of 200 PFU/ml of serum infection of the most competent feeding mosquito (Aedes albopictus Gentilly strain) is a relatively rare event (3 positive mosquitoes out of 362 feeding mosquitoes). In order to approach that level of viremia with the CHIK vaccine virus it is necessary to infect monkeys with 1000 times the normal human dose of vaccine. Therefore, it is considered highly improbable that CHIK vaccine virus would be transmitted from vaccine recipients and thus it is **not** necessary to limit inoculations to seasons or locations characterized by low mosquito populations.

Although serologic studies indicate good antibody response to CHIK among alphavirus naive individuals, we have no field trial or efficacy data

and have not yet characterized the antibody response in employees that have received other alphavirus vaccines such as Venezuelan, eastern, or western equine encephalitis virus vaccines. Thus we cannot as yet predict that this vaccine would protect humans against laboratory infection with CHIK in persons who have received any of these other alphavirus vaccines prior to receiving the CHIK vaccine.

Persons or laboratories interested in having their employees receive this vaccine should contact Dr. Frank Malinoski, Virology Division, USAMRIID, Ft. Detrick, Frederick, MD 21702-5011 (Ph: (301) 663-7241; Fax (301) 663-2290) for more details or a copy of the at-risk protocol. Once your immunizations clinic physician is approved as an Associate Investigator on the Investigational New Drug protocol for administering the vaccine, your immunizations clinic will receive the protocol, vaccine, and appropriate guidance to administer the vaccine and follow-up volunteers.

Other investigators interested in field testing of this vaccine should also feel free to contact Dr. Malinoski.

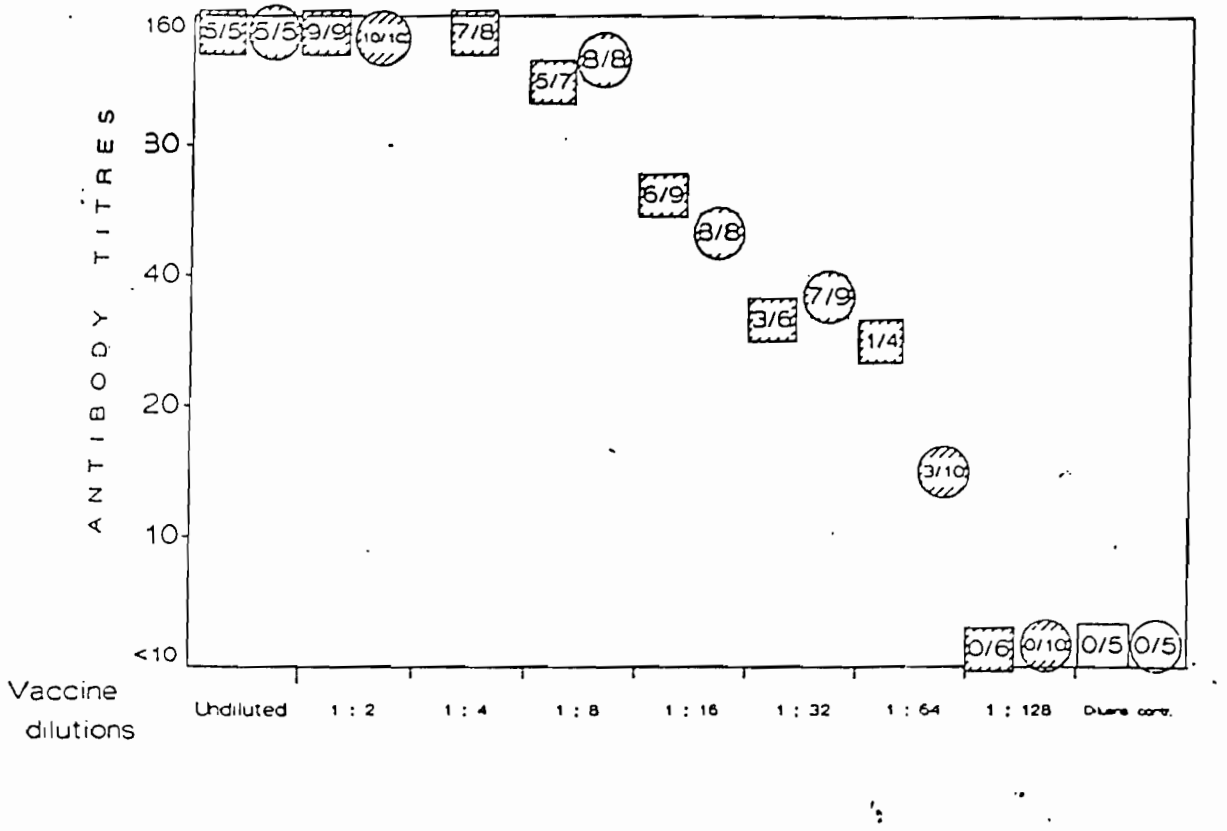
(submitted by F. J. Malinoski, T. P Monath, T. Ksiazek, M. Turell)

HUMORAL ANTIBODY TITRES AND PROTECTION OF MICE AGAINST TICK-BORNE ENCEPHALITIS (Emöke Ferenczi, "Béla Johan" National Institute of Public Health, Budapest, Hungary)

Antigenic differences found in various isolates of tick-borne encephalitis (TBE) virus were thought to influence the efficacy of vaccination with commercial vaccines in different countries. In order to test whether an Austrian vaccine was capable of conferring protection against a Hungarian TBE virus strain, model experiments were carried out using mice.

Weaned mice were immunized intraperitoneally with serial dilutions of a commercially-produced killed TBE vaccine (FSME-IMMUN Inject [R], Immuno, Austria). Serum samples were taken seven weeks after the second dose of the vaccine and the pooled sera were tested for the presence of specific antibodies by hemagglutination-inhibition (HI) and indirect immunofluorescence (IF) tests. No detectable antibody response was obtained in mice vaccinated with dilutions higher than 64-fold. The protective effect of the antibody measured against a Hungarian field isolate (Fornosi F, Molnár E, Acta Microbiol Acad Sci Hung 1:9-21, 1954) was tested by intraperitoneal challenge using 50 mouse LD_{50}. Symptoms of encephalitis were not observed in the group immunized with 0.2 ml of undiluted vaccine. In all others groups, clinical symptoms of encephalitis were observed and the virus was reisolated from the brain of one sick mouse per group, which was sacrificed for that purpose. The results indicate a positive correlation between the amount of antigen and both antibody titres and protection against the Hungarian wild-type virus.

Cumulative results of antibody titres and survival rate of mice in two experiments as a function of antigen dose are shown in the following figure.



n/m Survival rate of mice at antibody titres of pooled sera from experiment 1.

n/m Survival rate of mice at geometric mean antibody titres from experiment 2.

In the past, the lack of a standard case definition for Lyme disease (LD) surveillance as well as the absence of uniform criteria for its laboratory diagnoses have made it difficult to interpret the available data on the occurrence of the disease in this country. It has been even more difficult to compare the occurrence of the disease in different provinces.

To help improve the quality of the data available, the Communicable Disease Subcommittee of the Advisory Committee on Epidemiology is developing a standard Canadian surveillance case definition for LD.* This new case definition will include criteria for laboratory confirmation of the disease, which are being defined by the Technical Advisory Committee. The new case definition should be available later this summer and its implementation will assist in obtaining a much better overall picture of LD in Canada.

In the meantime, LCDC is receiving many requests for information on the occurrence of LD in Canada. The increasing public concern about the disease is based mainly on information coming from the United States where approximately 7400 cases were provisionally reported for 1989[1]. To help allay fears that a similar situation exists in Canada, LCDC feels that it is important to publish the following currently available data, which reveal the existence of approximately 100 cases. Nevertheless, because of the limitations of these data, discussed above, caution must be exercised when interpreting them. Furthermore, the application of the new case definition may well have an impact on the estimate of the prevalence of LD in Canada.

Provisional Data on the Occurrence of Lyme Disease in Canada

In June 1990, the provincial and territorial epidemiologists provided the following information on the cases of LD that were known to them. It should be noted that, at present, the disease is only notifiable in Ontario and Nova Scotia.

Newfoundland: One case, which was not acquired in the province, was reported in 1989.

New Brunswick: Five cases, only 1 of which is believed to have been acquired in New Brunswick, were reported in 1989. Three cases had onset of illness in 1989 and there was one case in 1988 and one in 1987.

Quebec: One case, reported in 1989.

Ontario: Sixty-seven cases, 39 of which were acquired in Ontario. Of the latter cases, 21 (54%) were reported in 1989, 8 (20%) in 1988, and the remaining 10 (26%) were reported between 1987 and 1984.

Manitoba: Seventeen cases that meet the surveillance case definitions in use in Manitoba at the time (please see the last article in this issue entitled "Lyme disease in Manitoba?"); 12 of these 17 cases were from 1989 and 5 from 1988.

*It is important to note that the criteria for a surveillance case definition are not necessarily appropriate for defining a clinical case.

Alberta: One case, reported in 1989.

British Columbia: Eleven cases: 1 reported in 1990, 9 from 1989 and 1 from 1988. Of the 11 cases, 10 had travelled to endemic areas outside of the

province and may have acquired the disease while there. The investigation on the 11th case is continuing.

There are no documented cases from Prince Edward Island, Nova Scotia, Saskatchewan, the Northwest Territories or the Yukon.

Reference
1. Centers for Disease Control. Tickborne diseases - Georgia, 1989. MMWR 1990; 39:397-9.

Source:
SG Mackenzie, PhD, Disease Surveillance Division, Bureau of Communicable Disease Epidemiology, LCDC, Ottawa.
Reprinted from Canada Diseases Weekly Report 1990;16:141-2

NOTE ADDED BY EDITOR: For additional information on Lyme Disease in Canada, see Canada Diseases Weekly Report, 16-30:141-152, 1990.

ANNOUNCEMENTS

To celebrate the Tricentenary of the first description of a phlebotomine sandfly (published in Rome by Philippo Bonanni in 1691), the First International Symposium on Phlebotomine Sandflies has been scheduled for Rome, Italy, 4-6 September, 1991. Organized by the Istituto Superiore di Sanità in collaboration with Accademia Nazionale delle Scienze "detta dei XL, Società Italiana di Parassitologia, and Società Italiana di Medicina Tropicale. For additional information and registration forms contact:

The Symposium Secretariat
Dr. Michele Maroli
Istituto Superiore di Sanità
Department of Parasitology
Viale Regina Elena, 229
00161- Rome, Italy

Telephone: (06) 4990 ext. 302
Telefax: (06) 4957364
Telex: 610071 ISTISAN

XIII INTERNATIONAL CONGRESS FOR TROPICAL MEDICINE AND MALARIA

FIRST ANNOUNCEMENT

The International Federation for Tropical Medicine Organizing Committee for the XIII International Congress for Tropical Medicine and Malaria cordially invites the participation in this congress of all persons interested in the various fields of Tropical Medicine.

VENUE and DATE: The XIII International Congress for Tropical Medicine and Malaria will be held at The Ambassador Jomtien Beach hotel, Cholburi, East Coast Thailand (157 km. from Bangkok), November 29th - December 4th, 1992.

SCIENTIFIC PROGRAM: The Congress will cover the broad field of Tropical Medicine and Hygiene. It will provide a forum for review of current knowlege and for presentation of new findings relating to health and health services in the tropics. The program will include Plenary Addresses, Reviews, Round Table Disscussions, and Poster Sessions. Most free communications will be in the form of posters.

LANGUAGE: The Congress language will be English.

XIII International Congress for Tropical Medicine and Malaria Thailand, November, 29 - December 4, 1992

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TELEFAX: 916-752-2801

October 4, 1990

9

Second & Final Notice

Participants

RE: 2nd International Symposium on Bluetongue, African Horse Sickness, & Related Orbiviruses

This is the second and final call for papers and posters for the Second International Symposium on Bluetongue, African Horse Sickness and Related Orbiviruses to be held at the Office International des Epizooties (OIE) June 17-21, 1991, in Paris, France. The due date for papers and posters has been extended

A registration form is being forwarded to you at this time that is due back to my office no later than February 15, 1991. The registration fee for the Symposium has been set at $150.00 and covers costs for the following:

Attendance
Translators/Recorders
Reception
Abstracts
Published Proceedings.

For your convenience, we are sending you a list of hotels in the general area of the OIE facilities. I urge you to make your reservations early as summer is a very busy tourist season in France.

I look forward to receiving your papers/posters and look forward to your participation at this very important meeting.

Sincerely,

Bennie I. Osburn, Co-Chair

Attachments
Announcement
Registration
Hotel List/Registration Form

cc: Tom Walton, Co-Chair

NAIROBI KENYA

International Congress for Infectious Diseases

June 7-11, 1992

Request for Information

Name _____

Name of Organization _____

Title _____

Address _____

City _____

State _____ Zip _____

Postal Code _____

Country _____

Telephone (____) _____

FAX (____) _____

Please return this form to:

International Society for Infectious Diseases
180 Longwood Avenue
Boston, MA 02115
USA
Tel: (617) 432-2270
Fax: (617) 731-1541
Telex: 4974739

BY POPULAR DEMAND, ANOTHER PAGE OF IRRELEVANT QUOTES

Woody Allen: "I don't want to achieve immortality through my work, I want to achieve it through not dying."

Frank Layden: "The decline of western civilization began when the Dodgers and Giants moved to the West Coast."

Johnny Carson: "The Richard Nixon Library is nearing completion. It will be locked 24 hours each day but anyone can break in any time."

A. Bartlett Giamatti: "It breaks your heart. It is designed to break your heart. The game begins in the spring, when everything else begins again, and it blossoms in the summer, filling the afternoons and evenings, and then as soon as the chill rains come, it stops and leaves you to face the fall alone. You count on it, rely on it to buffer the passage of time, to keep the memory of sunshine and high skies alive, and then just when the days are all twilight, when you need it most, it stops."

Russian proverb: "Mosquitoes sing over the living, priests over the dead."

Alexander Chase: "Psychiatry's chief contribution to philosophy is the discovery that the toilet is the seat of the soul."

Isak Dinesen: "What is man, when you come to think upon him, but a minutely set, ingenious machine for turning, with infinite artfulness, the red wine of Shiraz into urine?"

George Gissing: "It is because nations tend to stupidity and baseness tnat mankind moves so slowly; it is because individuals have a capacity for better things that it moves at all."

Edward Abbey: All men are brothers, we like to say, half-wishing sometimes in secret it were not true. But perhaps it is true. And is the evolutionary line from protozoan to Spinoza any less certain? That also may be true. We are obliged, therefore, to spread the news, painful and bitter though it may be for some to hear, that all living things on earth are kindred."

William Shakespeare: "In the world I fill up a place, which may be better supplied when I have made it empty."

Kingsley Amis: The rewards for being sane may not be very many but knowing what's funny is one of them."

Chinese proverb: "You want no one to know it?... Then don't do it."

Neil Young (sent by Ed Cupp): "Rust never sleeps."

Groucho Marx: "Outside of a dog, a book is a man's best friend. Inside of a dog it is too dark to read."