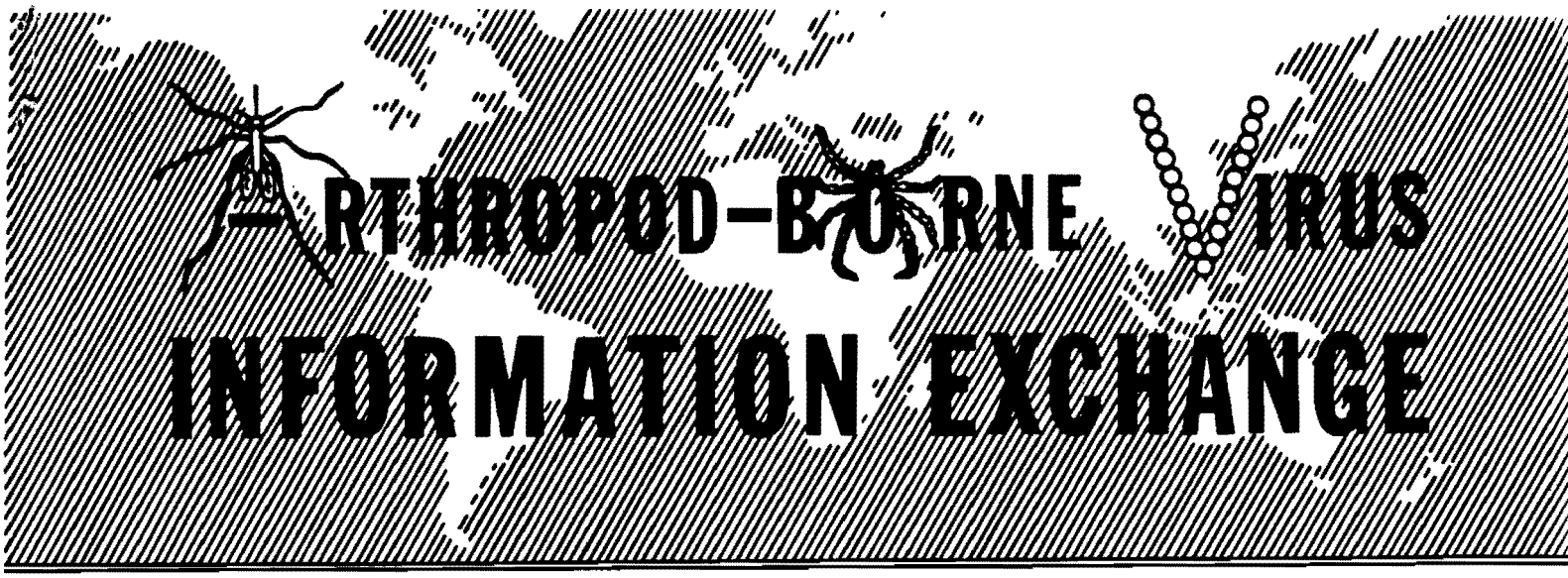


June, 1994

TABLE OF CONTENTS

The Arbovirus Information Exchange is a newsletter prepared under the auspices of the Subcommittee on Information Exchange (Nick Karabatsos, Chairman), American Committee on Arthropod-borne Viruses. Printing and mailing costs of the Arbovirus Information Exchange are paid by the **Division of Vector-Borne Infectious Diseases, Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado, USA.**

The purpose of the Arbovirus Information Exchange is the timely trade of information. Recipients are those who study various aspects of arbovirology. The Arbovirus Information Exchange contains preliminary reports, summaries, observations, and comments submitted voluntarily by qualified agencies and individual investigators. The appearance in the Arbovirus Information 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 Arbovirus Information 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 Arbovirus Information Exchange must be authorized directly by the agency or person submitting the text. Reports need not be in manuscript style, the results do not have to be definitive, and you need not include tables (unless you want to). The intent is to communicate among ourselves and to let others know what we are doing.

PLEASE READ CAREFULLY

INSTRUCTIONS FOR SUBMITTING REPORTS

PLEASE follow these instructions for submitting reports. We want to keep this mechanism timely and viable. Therefore, submit 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; double-spaced pages take twice as much space as single-spaced pages); **do not** staple pages together; **do not** number pages.

Send reports to the Editor, Charles H. Calisher, Ph.D.

c/o DVBID, NCID, CDC
P.O. Box 2087
Ft. Collins, CO 80522 (U.S.A.) or

Arthropod-borne and Infectious Diseases Laboratory
Colorado State University Foothills Campus
Ft. Collins, CO 80523 (U.S.A.)
TEL: (303) 491-8604 or (303) 223-8586
FAX: 303-491-1815 or 303-225-1903

You may send reports to me by e-mail at: ccalisher@vines.colostate.edu

If you have an e-mail address, please let me know what it is. Thanks.

C.H. Calisher

NEXT ISSUE

The next issue likely will be mailed December 1, 1994 (probable deadline for submissions: November 15, 1994). There is nothing that requires you to wait until the last minute. If you have something to communicate in July, August, September, or October, please send it. 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.

Page

DENGUE

VECTOR COMPETENCE AND VECTOR BIOLOGY

DEVELOPMENT AND APPLICATION OF TECHNIQUES

Editor's comments

Given the accelerated speed with which scientific advances are made these days, time seems to be increasingly compressed, such that discoveries made a relatively short time ago seem, in retrospect, to have been made eons ago. Not long ago, while visiting a laboratory in Europe, a young investigator told me he was somewhat embarrassed because he thought I was dead. Seems he had read some papers I had written many years ago and simply made an incorrect (and unscientific) assumption. I told him that whereas some may have thought I was dead a couple of years ago, a component of the US Government sends me a check each month and ostensibly will continue to do so until it receives notice from my wife to start sending it to her. I also told him I had recently bought a truck and they do not sell trucks to people who are dead (unless they pay cash). It is tough enough to be aging but to have to make personal appearances and to breath on a mirror for young virologists is humiliating. As Robert Orben said, "Every morning I get up and look through the Forbes list of the richest people in America. If I'm not there, I go to work." I do much the same but when I arrive at Colorado State University each morning no one appears to be surprised I am alive. For this, I am grateful.

Nevertheless, the arbovirus community has lost many of its Founding Fathers. Because they are no longer with us we will never be the same, but there are other "gray beards" who still make contributions or who have left the field but are quite sharp. For example, a year ago I was asked to write a review of medically important arboviruses of the United States and Canada. I put together what I thought was a pretty good draft and sent it to Roy Chamberlain, one of the best scientific writers and editors I know, and asked him to edit it, if he did not consider it too much of an imposition on his retirement. As he revised it, the manuscript not only was better, it was readable. You can see for yourself (Clin. Microbiol. Rev. 7:89-116, 1994). Roy seems to have lost none of his editorial abilities.

I try to stay in touch with some of my other heroes and old friends, people such as Tommy Aitken, Trygve Berge, Jordi Casals, Adrian Chappell, Glynn Davies, Sophia Gaidamovich, Milota Gresikova, Jack Hayes, Herald Johnson, Karl Johnson (soon to be portrayed by the better looking Robert Redford in a movie), Ian Marshall, Don McLean, Nils Oker-Blom, Akira Oya, James Porterfield, Marta Sabattini, Toby St. George, Carlos Sanmartin, Alex Shelokov, and Telford Work. Most of them continue to be directly or peripherally involved in arbovirology, albeit on a less regular basis than when they were younger and the world was thought to be flat. A second group of people (no need to mention their names) have left the field for personal reasons or to obtain funding and meet new challenges. All retain their love of arbovirus ecology and their fascination with diseases caused by arboviruses. None has ever indicated to me that they "do not care anymore".

And lest you think that just because you have not seen a recent publication by these people they are sitting on a beach or breathing only with assistance, check out Elizan TS and Casals J (1991) Astrogliosis in von Economo's and postencephalitic Parkinson's diseases supports probable viral etiology. J. Neurol. Sci. 105:131-134.

I have addresses and telephone numbers of all these good folks, in case you are interested.

Diseases in Russia Caused by California Serogroup Viruses (CSV)

Lvov D.K., Kolobukhina L.V., Butenko A.M., Gromashevsky V.L.,
Skvortsova T.M., Galkina, I. V., Nedyalkova, M.S.

The D.I. Ivanovsky Institute of Virology,
Department of Virus Ecology, Moscow, Russia

As a result of virological examination of 2,239,000 mosquitoes collected in 1986-1993 in all landscape-climatic zones of Russia, 241 strains of CSV were isolated. Neutralizing antibodies to CSV in healthy human and domestic animals were determined at a rate of 50-90% in the taiga belt of Euroasia, 30-40% in the Central region of the European part, and 10-20% in southern regions (in forest-steppen and steppen belts). The highest activity of Inkoo virus was found in the western and central regions of the European part and in western Siberia, Tahyna virus in the southern regions of the European parts, and snowshoe hare virus (SSH) in the northern and north-eastern regions of Eurasia. In order to study the role of these viruses in human pathology, sera from 3,214 patients with acute febrile illnesses of unknown etiology were examined by neutralization tests, MAC-ELISA (some collected in 1989-1993) and HI. Two hundred and sixty-three cases of viral disease were diagnosed. This amounted to 8.2% of all those examined. Those patients with disease originated in the taiga zone, the zones of mixed and deciduous forests, in the forest-steppe, steppe and semi-desert zones. The highest indices of specific diagnosis of disease due to viruses of the California encephalitis group took place in the taiga zone.

Seventy-six of the 263 diagnosed cases were classified as Tahyna fever, 96 as Inkoo fever, 14 as disease caused by snowshoe hare viruses, and 77 as due to one or more viruses of the California encephalitis group.

The data shown in the accompanying table concern the analysis of the clinical picture of 176 of the most completely studied cases of disease. Of those studied, there were 19 children (10.8%), age 3-14 years, 91 persons (51.7%), age 15-30 years, 48 persons (27.3%), age 31-50 years, and 18 persons (10.2%) over 50 years of age. In 139 (82.0%) patients, the clinical disease involved an acute neuroinfection syndrome (serious meningitis, meningoencephalitis). Based on etiology, the 176 analyzed cases were categorized as 61 patients with Inkoo fever, of which 22 (36.1%) had CNS symptoms and 39 (63.9%) manifested an influenza-like disease. Fifty-eight other patients had Tahyna fever, of which 3 (5.2%) had CNS injury and 55 patients manifested an influenza-like form of disease. Fourteen patients diagnosed with snowshoe hare viral infections all manifested an influenza-like form of the disease. Forty-five patients were diagnosed with CVS infection, of which 12 (26.7%) had CNS injury and 33 (73.3%) manifested an influenza-like form of disease.

The "influenza-like" form of the disease was characterized by high and short-term fever (4.5 ± 0.3 day), headache (100%), which in some cases was accompanied by sickness and vomiting (31.3% and 21.7%, correspondingly). In one-third of the patients (26.5%), auscultation brutal breathing and sometimes short-term dry rales were determined; upon x-ray examination, an aggravation of the bronchovascular picture was noted. Clinico-roentgenological changes in the bronchopulmonary system were seen in 13 patients (7.4%); in 4 of them, bronchitis was diagnosed; 9 manifested focal pneumonia.

In serous meningitis, a hypertensive syndrome was the prominent clinical picture. In 37.5% of the patients, symptoms from the part of the vegetative nerve system (face and neck hyperemia, bradycardia, tachycardia, instability in Romberg pose) took place. Inflammatory changes in cerebrospinal fluid (CSF) were temperate, lymphocytic pleocytosis, normal or a slightly reduced protein. In 34.5% of the patients with serious meningitis, clinico-roentgenological changes in lungs were noted; brutal breathing, dry rales, an aggravation of the bronchovascular picture and inflammatory changes according to the type of focal pneumonic infiltration. In electroencephalographic studies, diffuse changes in the state of alpha-rhythm irregularity were observed. Positive dynamics corresponded to clinical recovery. The meningoencephalitis caused by Inkoo virus, the acute onset, generally infectious syndrome, generally cerebral and focal symptomatology and inflammatory changes in CSF were peculiar. However, the changes in EEG persisted for two more years after recovery (the absence of alpha-rhythm, prevalence of low-amplitude activity, the features of intracranial hypertension).

The Distribution of Confirmed Cases of California Group Viral Infections in the Russian Federation, 1986-1993

Natural zone, administrative unit	Quality of observed patients*	Number of diagnosed cases				Total
		Tahyna	Inkoo	SSH	CSV	
European part						
Coniferous forests						
Vologda	55	-	2	-	4	6
Perm	143	3	4	6	6	19
Udmurtia	55	3	3	-	2	8
Kirov	35	2	-	-	4	6
Mixed forests						
Bashkiria	55	1	2	2	1	6
Rep. Mariel	69	1	-	-	3	4
Moscow	974	9	35	-	19	63
Tatarstan	32	-	2	2	-	4
Tver	1	-	-	-	1	1
Yaroslavl	56	-	-	-	7	7
Deciduous forests						
Orel	9	-	-	-	1	1
Ryazan	675	16	38	-	5	59
Forest-steppe						
Belgorod	8	-	-	-	1	1
Chuvashia	28	1	1	-	1	3
Mordovia	44	1	1	-	1	3
Penza	1	-	-	-	1	1
Samara	54	1	-	-	1	2
Tula	307	-	-	-	6	6
Steppe						
Stavropol	29	1	-	-	-	1
Semideserts						
Astrahan	376	33	4	-	-	37
Asiatic part						
Taiga						
Irkutsk	9	-	-	-	1	1
Kamchatka	15	-	-	-	1	1
Krasnoyarsk	15	-	-	-	2	2
Tumen	89	-	4	4	5	13
Steppe						
Altai	80	4	-	-	4	8
Total	3214	76	96	14	77	263

* Patients with acute febrile illnesses of unknown etiology were observed.

Dengue - 1 outbreak in Grande Comore Island (Federal Islamic Republic of Comoros. Indian Ocean) Virological and serological results.

J. M. Morvan (*), N. Charrier (**), P. Boisier (*) and J. F. Roux (*).

(*) Unité de Virologie. Institut Pasteur de Madagascar. BP 1274. Tananarive. Madagascar. (**) Laboratoire de Biologie. Hopital El Maarouf. Moroni. République Fédérale Islamique des Comores.

An extensive epidemic of dengue-like illness occured in Grande Comore island during March to May 1993. The clinical picture was that of classical dengue. No fatality or case of dengue haemorragic fever was reported. The infection was found out from all group age persons (with a preponderance of exile population).

1. Material and methods

a. Population: two groups were studied.

- Group 1 clinical specimens: blood samples were collected from 146 outpatients at El Maarouf Hospital in Moroni, the capital of Grande Comore island.

- Group 2 surveillance of the epidemic.
  * Grande Comore island: a random serological survey was conducted in 30 villages (527 sera collected).
  * Anjouan island: 151 sera were collected (61 sera collected in 2 villages where children deaths were reported in february 1993).
  * Moheli island: 151 sere were collected.

Sera were stored in liquid nitrogene for virus assays (58 patients tested), at -20°C for serological tests.

Virus isolation

Virus assays were attempted by inoculation into clone C6/36 of *Aedes albopictus* cells. Cells were screened by an indirect fluorescent antibody test (IFAT) using DEN-1,-2,-3, and -4 (dengue 1, 2, 3, and 4) hyper immune ascitic fluid (kindly provided by V. Deubel, WHO Regional Center of Reference and Research on Arboviruses, Institut Pasteur, Paris, F) and fluorescein isothiocyanate conjugated sheep anti-mouse IgG (Biosys). Identification was achieved by IFAT using serotype specific monoclonal antibody (kindly

provided by E. Chungue. Malarde Institute. Tahiti. French Polynesia). Virus assays were also conducted by inoculation intracranialy into suckling mice.

b. Serological methods

IgM specific antibodies were detected by IgM capture enzyme-linked immunosorbent assay (ELISA) as previously described by Chungue *et al.*, using 4 serotype antigens. IgG were detected using IgG ELISA.

2. Results

Group 1.

Of 146 patients subjected to laboratory testing, 95 (65%) were confirmed as positive by virus isolation and/or IgM detection. Dengue-1 virus was isolated from 19 of 58 (32.7%) patients tested for virus assay (from which 14 sera were collected in exile population). 16 virus strains were isolated in clone C6/36 of *Aedes albopictus* cells, and 3 virus strains in suckling mice. All virus strains were isolated from patients with <5 days of illness.

IgM antibodies were detected from 86/169 (50.8%) sera (corresponding to 76 patients).

Group 2.

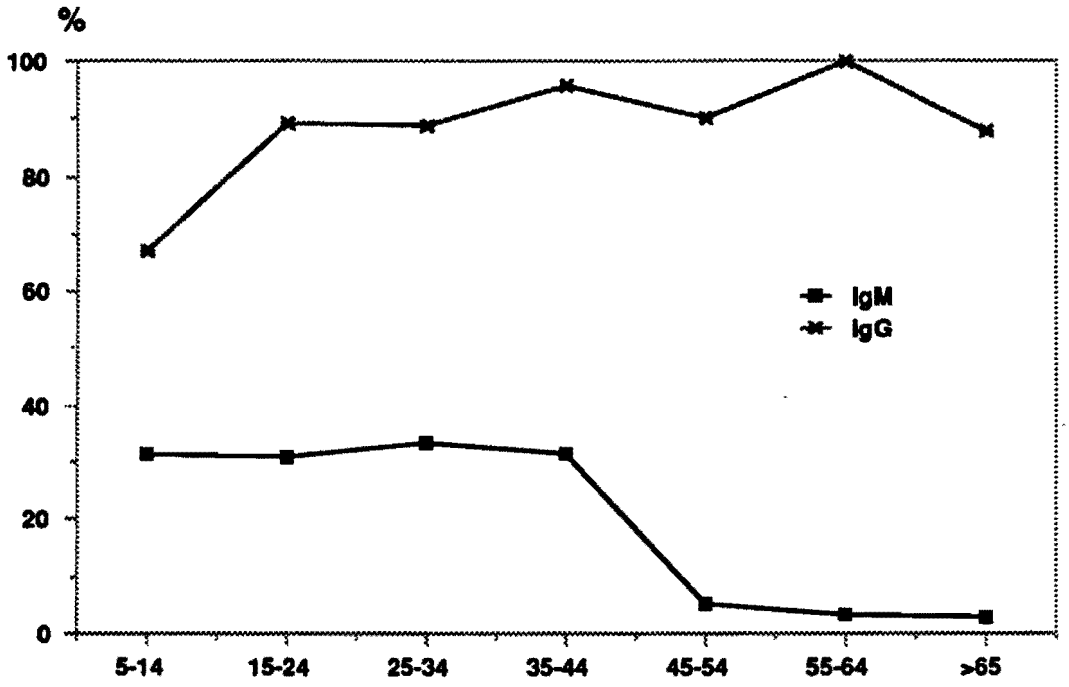
* In Grande Comore island, the IgM antibody prevalence rate obtained in the random sample of >5 years old population was 26% (22.3% to 29.7%). The estimated number of recent dengue virus contact (estimated population 251,000) was between 56,000 and 75,000. 83.2% had IgG antibodies. IgM and IgG antibody prevalence rate according to age groups are presented in figure 1.

* In Anjouan island 4/151 (2.6%) sera were found IgM positive (from which 3 sera were collected in patients recently coming from Grande Comore island), and 34/151 (22.5%) were IgG positive.

* In Moheli island 3/131 (2.3%) were found IgM positive, and 53/131 (40%) were IgG positive.

The results of virus isolation and IgM antibody detection indicate that an extensive outbreak of dengue-1 occured recently only in Grande Comore island. Dengue fever was reported in Comoros islands in 1943, and dengue-2 outbreaks also occured in Seychelles, Reunion island and Kenya during the past 15 years, but it is the first documented dengue-1 epidemic in this part of the Indian Ocean. Serological results suggest that probably dengue-1 has circulated in Grande Comore island before 1950.

Figure 1. Random serological survey in Grande Comore island. IgM and IgG antibody prevalence rate according to age groups.



REPORT FROM CELL CULTURE LABORATORY TROPICAL MEDICINE INSTITUTE "PEDRO KOURI".
P.O.Box. apdo 601, Marianao 13, ZP 11 300, Havana City, Cuba.

OBTENTION OF A C6/36 (Aedes albopictus) SUBLINE ADAPTED TO GROW IN A SERUM-FREE MEDIUM.

A subline adapted to grow in a serum-free medium (C6/36-SF) was obtained from C6/36 cloned cell line. Several media were tested to support the cell growth.Eagle MEM supplemented with glutamine, BME vitamine, non essential amino acids and tryptose-phosphate-broth was the one that showed the best results.

There are no morphological differences between the adapted subline C6/36-SF and the original C6/36, but the first one grows slower than the second. C6/36-SF needs between 4 and 5 days to form a monolayer with a subcultivation ratio of 1:3 at 28° C.

Both cell systems were inoculated with Dengue-1 and Dengue-2 viruses non adapted to grow in mosquito cells, decreasing the multiplicity of infection (m.o.i) from 1 to 0.1 in order to compare the sensitivity to these agents.

The inoculated cells were observed during nine days in order to detect cytopathic effect (CPE) but it was not oberved in any case. Nevertheless, Dengue-1 virus was detected by Indirect Immunofluorescence (IF) in both cell systems from m.o.i. of 1 to 0.5 three days post infection. In those cells inoculated with Dengue-2 virus, IF began at 48 hours, m.o.i. of 0.5 but it was clearer in C6/36 than in C6/36-SF.

C6/36-SF is an alternative choice for the study of these viruses without undesirable effects of different bachts of sera and it is as sensitive as the orifinal culture.

(A. Castillo, L. Morier)

A preliminary report on variation of prM (M) gene of dengue-2 virus strains isolated in China.

Yang Peiying Qin Ede

Department of Virology, Institute of Microbiology and Epidemiology, Beijing, China

We have analyzed and compared two strains of dengue-2 virus, D2-04 and D2-43, isolated in China, with reference to dengue-2 virus strains: JAM, NGC and S1, relating to their homology of genomic nucleotide sequences, similarity of amino acid sequences, site of glycosylation, cysteine residue, cleavage site of proteins and hydropathy profile, of structural proteins C, prM(M), E and nonstructural protein NS1.

Strain D2-04, non-pathogenic for suckling mice, was isolated from patient serum of Hainan Province in 1985 while strain D2-43, pathogenic for suckling mice, was isolated from patient serum of Guangxi Province in 1987.

The results showed that the open reading frames (ORF) from C to NS1 genes of D2-04 and D2-43 strains were identical and both were composed of 3381 nucleotides, which encoded a total number of 1127 amino acids. The number and position of glycosylation sites and conservative cysteine residues, hydropathy profiles and cleavage sites of encoded proteins were also identical.

The homology of the nucleotide sequences was 93.8% and the similarity of amino acid sequences was 91.3% between D2-04 and D2-43. However, on further investigation, we found that the homology of C, prM(M), E and NS1 genes of two isolated strains was 95%, 90.1% (86.7%), 95.8% and 92.2% respectively.

These results indicated that C and E were the most conservative genes, while prM(M) gene was the most variable one. The difference between D2-04 and D2-43 strains was laid on the variation at prM(M) gene.

When the nucleotide sequence of D2-04 and D2-43 strains were compared with that of the reference strains of dengue 2 virus, we found that variation of prM (M) gene of D2-04 isolate was occurred. This suggests that variation in prM(M) gene may lead to the change of encoded amino acid. In other words, the different pathogenicity of two isolates for suckling mice may be directly related to the variation of their prM(M) genes.

NEUTRALIZATION ESCAPE MUTANTS OF DENGUE VIRUS TYPE 2

B. Lin, J. M. Murray and P. J. Wright, Department of Microbiology, Monash University, Clayton, Victoria, 3168, Australia.

Two neutralization resistant variants of dengue virus type 2 were selected using a neutralizing monoclonal antibody G8D11. This antibody was known to bind to a bacterial trp E-E fusion protein containing amino acids 298-397 of E (Megret *et al.*, [1992] Virology 187, 480-491). DEN-2 virus was initially incubated with G8D11 ascitic fluid (diluted1:6) and plaqued on Vero cells. Plaques were picked, and virus was passaged three times in C6/36 cells in the presence of G8D11 (diluted 1:6 in the inoculum, 1:10 in the maintenance medium). Virus N-GV4 was derived from the New Guinea C strain and virus P-GV3 from the PUO-218 strain (Gruenberg *et al.*, [1988] J. Gen. Virol. 69, 1391-1398). cDNA corresponding to nucleotides 757 to 2478 of the DEN-2 genome was amplified using RT-PCR and cloned into pUC18. The complete E gene of four clones for each variant was sequenced. Although the two variants were from different parental strains, both had an identical change of A to G at nucleotide 919 in the E gene. The corresponding amino acid change was from lysine to glutamic acid at E residue 307. By chance, the nucleotide change destroyed a unique *Dra* I site in the E gene, thus facilitating the screening of additional amplifications for the presence of the variants.

The substitution abolished the ability of antibody G8D11 to bind to the E glycoprotein in immunoprecipitation experiments. The G8D11 epitope was sensitive to treatment with SDS and was dependent on the formation of the disulphide bridge between cysteine residues eleven and twelve in the E glycoprotein. To demonstrate this dependency the codons for these two residues were changed, individually and together, to encode alanine in the E gene expressed via the vector pcEXV (Miller & Germain [1986] J. Exp. Med. 164, 1478-1489).

The position of the G8D11 epitope of DEN-2 corresponds to that of a neutralizing epitope of louping ill virus (Jiang *et al.*, [1993] J. Gen. Virol. 74, 931-935).

Partial nucleotide and encoded amino acid sequences of dengue 2 viruses isolated from mosquitoes and their genetic relatedness to isolates from humans.

G. Subramaniam, I.M. Kautner, C.L. Koh* and S.K. Lam

Department of Medical Microbiology, Faculty of Medicine, University of Malaya,

*Department of Genetics and Cellular Biology, Faculty of Science, University of Malaya.

The nucleotide and encoded amino acid sequences of the structural and nonstructural NS1 protein genes of three dengue 2 (DEN 2) viruses, P8-377, P7-863 and P7-843, isolated from mosquitoes, were determined. The nucleotide sequences were obtained by either direct RNA sequencing using total viral RNA isolated from infected *Aedes albopictus* C6/36 cells or sequencing a cDNA fragment of approximately 2 kb obtained after reverse transcriptase-polymerase chain reaction (RT-PCR). The sequence data were then analysed using the IBI Pustell DNA sequencing program and the Phylogenetic Analysis Using Parsimony (PAUP) program.

Overall, NS1 was the most conserved among the genes analysed at both the nucleotide and amino acid levels in all the three mosquito DEN 2 isolates. There was more similarity between P7-863 and P7-843, the strains from *Aedes aegypti*, than between P8-377, the strain from *Ae. albopictus* and the two isolates from *Ae. aegypti*. The differences between P8-377 and the isolates from *Ae. aegypti* were more pronounced at the amino acid level. The results indicated that it was possible to distinguish between P8-377, the strain from *Ae. albopictus*, and P7-863 and P7-843, the strains from *Ae. aegypti*, by comparing the amino acid sequences of the prM proteins.

Comparison of the nucleotide and encoded amino acid sequences of P8-377, P7-863

and P7-843 with other DEN 2 viruses from humans showed that the three mosquito DEN 2 isolates had greater similarity with JAM and NGC than with the three Malaysian human DEN 2 isolates (M1, M2 and M3), the Chinese DEN 2 isolate (D2-04), and the candidate vaccine strain PR-159S1 (S1). However, P8-377 showed considerable sequence divergence at both the nucleotide and encoded amino acid levels in the structural protein genes when compared to the other DEN 2 viruses. Base changes were found to be generally transitions rather than transversions and codon usage was nonrandom. However, cysteine residues and glycosylation sites were not strictly conserved in the mosquito DEN 2 isolates.

A phylogram was generated using the PAUP program in order to ascertain the genetic relationships among various flaviviruses including the three mosquito DEN 2 isolates. DEN 2 viruses were clearly clustered into two groups - the mosquito isolates and the human isolates. P8-377, from *Ae. albopictus*, showed high sequence divergence at both the nucleotide and encoded amino acid levels, and was clustered independently from the other DEN 2 strains. Among the human DEN 2 isolates, the Malaysian human DEN 2 viruses (M1, M2 and M3) were grouped separately from the other human DEN 2 isolates (JAM, NGC, PDK53, S1 and D2-04).

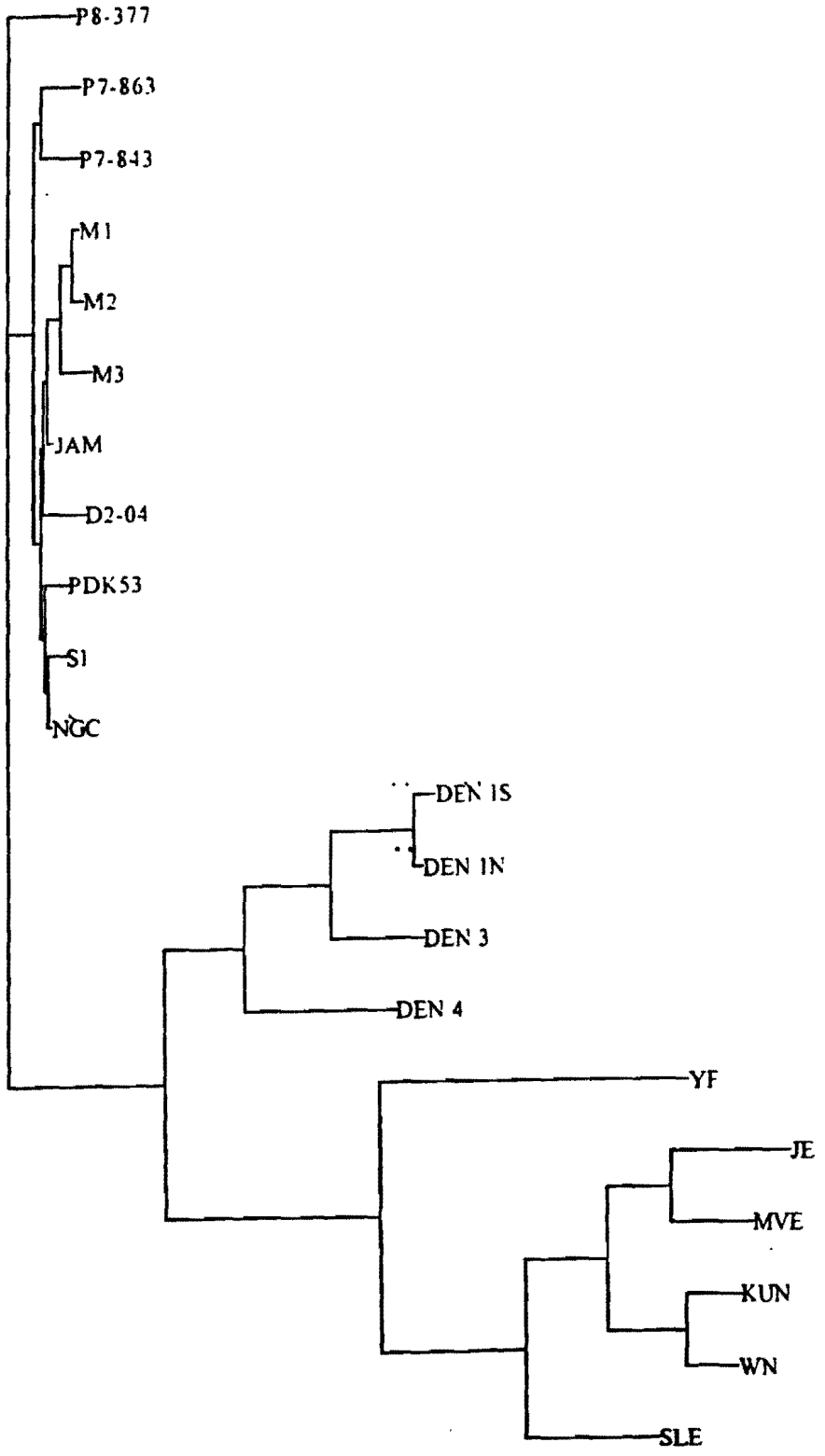


Figure 1. **Phylogram depicting genetic relationships among flaviviruses.**

REPORT FROM CELL CULTURE LABORATORY AND ARBOVIRUS LABORATORY
TROPICAL MEDICINE INSTITUTE "PEDRO KOURI".
P.O. Box. Apdo 601, Marianao 13, ZP 11 300, Havana City, Cuba.

COMPARATIVE STUDY OF XL-2 (Xenopus laevis) AND VERO CELL LINES FOR THE DETECTION OF EASTERN ENCEPHALOMYELITIS VIRUS (EEE) USING RAPID DIAGNOSTIC TECHNIQUES.

Five samples previously detected from natural foci, and stored at -70° C since then, were used in this study, they were inoculated in XL-2 and Vero cell lines. Indirect Immunofluorescence technique (IF) and a double antibody sandwich enzyme immunoassay (EIA) were applied from 2 to 12 hours and from 2 to 24 hours respectively in order to detect the viral presence.

In XL-2 cells, specific IF was observed in 4 samples in 5 hours postinoculation and all were positive in 6 hours. Meanwhile, Vero cells showed specific fluorescence in 1 sample in 6 hours and the rest of the samples were positive in more than 7 hours after inoculation the fluorescence in XL-2 cells was always clearer than in Vero cells.

In both cell systems the EEE virus was detected in four samples by EIA between 8 and 10 hours post infection. Only one sample was positive in less than 8 hours, however the optical density values were higher in XL-2 cells than in Vero cells.

This in the first time that EIA has been applied to XL-2 cells for the detection of virus from samples.

(L. Morier, S. Vazquez, A. Castillo, JL. Pelegrino, MG. Guzman)

Arbovirus Surveillance in New York State.

1992: During the surveillance season of 1992, arbovirus testing was conducted on 3,295 pools of 232,878 mosquitoes comprising at least 22 species from 8 counties in New York State. The upstate Syracuse area, where epizootics of Eastern Equine Encephalitis (EEE) periodically occur, was represented by 4 counties (Madison, Oneida, Onondaga, Oswego); the western region by 3 counties (Chautauqua, Erie, Niagara) and the southeastern area by 1 county (Suffolk). Arbovirus isolates were obtained from 140 pools of 12,298 specimens comprising 3 species of mosquitoes captured in 5 counties. Of these, 138 Alphavirus isolates were identified as Highlands J (HJ) virus, a non-pathogenic member of the Western Equine Encephalitis (WEE) complex, while 2 Bunyaviruses were identified as Jamestown Canyon (JC), in the California (CAL) serogroup of arboviruses. The minimal field infection rate (MFIR) for each of these viruses is shown by county and mosquito species in Table 1.

No virus isolates were obtained from 94 non-human vertebrate tissues tested in 1992; however, 9 of 21 sentinel pheasants exposed in Onondaga County exhibited serologic conversions to HJ virus between 7/08/92 and 10/14/92, with hemagglutination-inhibition (HI) titers ranging from 1:160 to 1:2560.

1993: A total of 3,349 pools of 212,073 mosquitoes in at least 26 species from 7 counties was tested for arbovirus infection in 1993. Collections were provided by the same counties, except Niagara, which submitted mosquitoes the previous year. Eighty-five pools consisting of 6,925 mosquitoes (in 7 species) from 4 counties yielded isolates: EEE (6), HJ (77) and JC (2). The MFIR is shown for each of these viruses by county and mosquito species in Table 2.

Virus isolation attempts from 92 avian tissue samples collected in 1993 yielded negative results; however, 9 of 26 sentinel pheasants exposed in Onondaga County developed HI antibody to HJ virus between 7/28/93 and 8/14/93.

Experimental Transmission of Powassan Virus by Ixodes dammini Ticks.

Powassan virus (POW) is a tick-borne virus which causes human encephalitis in the northeastern U.S. and Canada. Because the geographic and host distribution of Ixodes dammini and POW overlap, it was relevant to study the potential of this tick species to vector this arbovirus. **Oral infection rates** (OIR) in larvae and nymphs fed on POW-infected hamsters were 10% and 40%, respectively; in females fed on infected rabbits, 57%. There was a significant difference between OIR of larvae and nymphs ($p=0.045$) and larvae and females ($p=0.002$); no difference was found between nymphs and females ($p=0.23$). **Transstadial transmission rates** (TSTR) for nymphs exposed to POW as larvae, adults exposed as larvae, and adults exposed as nymphs, were 9.5%, 10% and 54%, respectively. A significant difference was found between the TSTR of females originally exposed to virus as larvae and females exposed as nymphs ($p=0.004$). Evidence of transovarial transmission occurred when 2 clean hamsters feeding F_2 larvae and nymphs (originally exposed to virus in the F_1 nymphal stage) seroconverted to POW with HI titers of 80 and 5120, respectively, on week 4 post tick drop off. **Transovarial transmission rates** were 16.6%.

Our results indicate that larval, nymphal and female I. dammini can become infected with POW when feeding on experimentally infected hosts; OIR increase with each developmental stage; there is transstadial transmission of virus from larvae to nymphs to adults, including males, regardless of the stage in which the ticks were originally exposed to virus; TSTR in adults are higher when original infection occurs at the nymphal rather than at the larval stage; all stages can transmit POW orally to clean hosts regardless of when the ticks were originally exposed to virus; transovarial transmission of POW by I. dammini occurs.

Margaret A. Grayson, Adriana Costero and Leo J. Grady

Table 1: Arbovirus isolations from mosquitoes collected in New York State: 1992.

County	Species	No. tested Pools	No. tested Specs.	No. of Isolates HJ	No. of Isolates JC	MFIR 1:
Erie	Aedes stimulans	99	6,849		1	6,849
Madison	Culiseta melanura	7	225	2		113
Onondaga	Aedes canadensis	317	28,391	5		5,678
	Culiseta melanura	672	55,831	128		436
Oswego	Aedes canadensis	164	13,012		1	13,012
	Culiseta melanura	215	8,018	2		4,009
Suffolk	Culiseta melanura	31	2,137	1		2,137
	Totals	1,505	114,463	138	2	

Table 2: Arbovirus isolations from mosquitoes collected in New York State: 1993

County	Species	No. tested Pools	No. tested Specs.	No. of Isolates EEE	No. of Isolates HJ	No. of Isolates JC	MFIR 1:
Madison	Aedes trichurus	7	165			1	165
	Culiseta melanura	61	3,784		6		631
	Culiseta morsitans	21	844		1		844
Onondaga	Culiseta melanura	629	54,576		64		853
Oswego	Coquilletidia perturbans	230	20,062		1		20,062
	Culiseta melanura	194	4,927		2		2,464
Suffolk	Aedes canadensis	62	5,775		1		5,775
	Anopheles punctipennis	11	443			1	443
	Coquilletidia perturbans	103	9,957		1		9,957
	Culiseta melanura	34	2,521	6			420
	Culex species	83	7,665		1		7,665
	Totals	1,435	110,719	6	77	2	

Arbovirus Information Exchange

Aedes albopictus in Nigerian Yellow Fever Foci

An epidemic of yellow fever in eastern Nigeria, adjacent to the areas where *Aedes albopictus* was discovered in 1991, prompted an investigation to confirm the presence of *Aedes albopictus* in villages with yellow fever activity and to attempt virus isolation from biting mosquitoes collected in the villages.

Epidemiological data was used to identify villages with yellow fever activity. The data indicated that transmission started in August, 1993, peaked during November and December, and ended in January of 1994, as dry season conditions reduced mosquito population density. Human bait collections, larval surveys, and ovitrapping were conducted from February 23 to March 6, 1994 in 12 villages with a history of presumptive yellow fever cases.

Mosquito population density was very low. The biting density for all villages combined was 0.14/hour for *Ae. aegypti*, 0.05/hr for *Ae. albopictus*, 0.06/hour for *Ae. africanus*, and 0.15/hour for *Ae. leuteocephalus*. Despite the low densities, *Ae. albopictus* was found biting in 7 of 12 villages sampled. No immature *Ae. albopictus* were found. For comparison, the other domestic container mosquito in the area, *Ae. aegypti*, was found biting in 10 of 12 villages and as immatures in water pots in 6 of 12 villages.

Eggs from the oviposition traps have been hatched. *Ae. albopictus* eggs were collected from 7/12 villages and from one of two forests sampled. *Ae. aegypti* eggs were collected in 10/12 villages, but none were collected in the forests.

Virus isolation attempts on the mosquitoes collected coming to human bait are still in progress.

Submitted by: R.S. Nasci, Research Entomologist with the CDC Division of Vector-Borne Infectious Diseases, and Mr. V.I. Ezike of the Nigerian Federal Ministry of Health and Social Services, National Arbovirus and Vector Research Division. J. Weisfeld, Nigeria Combatting Childhood Communicable Diseases Project, USAID.

Detection of California Serogroup Viruses in Infected Mosquitoes by Time-Resolved Fluoroimmunoassay

V. Pomelova et al. (Acta Virol., 1992, v.36, p 260-268) have used time-resolved fluoroimmunoassay (TR-FIA) for detection of tick-borne encephalitis virus in *Ixodes persulcatus* ticks. Currently, this method has not been used for detection of other arboviruses in arthropod vectors.

This communication presents the results of comparative examination of TR-FIA, ELISA and inoculation of continuous pig embryo kidney cell culture (SPEV) for detection of California serogroup viruses (CSV) in mosquito suspensions, as well as for simultaneous identification of these viruses. The TR-FIA method is similar to ELISA. It is a solid phase immunochemical method based on the use of lanthanide ion labelled antibodies and a newly developed method for detecting the fluorescence of these ions with a time-resolved fluorometer.

Females of *Aedes aegypti* were infected by feeding through a biological membrane with Tahyna, Lumbo, Inkoo, California encephalitis, snowshoe hare (SSH) viruses and two original strains, LEIV-8545 and LEIV-12812, from Karelia and Tverskaya regions of Russia. In every experiment, fifty mosquitoes were included.

In infectious brain suspensions, virus titers varied from 6 to 8 $\log_{10}$CPD 50/ml; in suspensions of infected mosquitoes, 3 to 6 $\log_{10}$CPD 50/ml when they were examined in CPEV cells.

Titers of virus antigens in mosquitoes estimated by TR-FIA (usually 1:1600 - 1:12800) were 8 - 16 times higher as compared with ELISA (1:400 - 1:800).

It was possible to detect SSH virus antigen in mosquito suspensions by TR-FIA when the concentration of infectious virus was 1.0 $\log_{10}$CPD 50/ml. Detection of specific antigen by ELISA was successful when the titer of the SSH virus was >2.8 $\log_{10}$CPD 50/ml. On the other hand, a direct correlation between infectious titers of virus in mosquitoes and titers of antigens detected in the same suspension by TR-FIA and ELISA tests was not found.

The specificity of TR-FIA and ELISA for differentiation of CSV and two original strains, LEIV-8545 and LEIV-12812 in infected mosquito suspension was compared. Test systems employed were prepared using polyclonal antibodies to Tahyna, Inkoo and SSH viruses.

Tahyna, Inkoo and SSH viruses were clearly different one from another when they were tested by TR-FIA and ELISA. LEIV-12812 and LEIV-8545 were identified as SSH virus strains; Lumbo and California encephalitis viruses were found to be CSV members by TR-FIA but could not be detected by the less sensitive ELISA test.

When two different viruses of CSV were simultaneously presented in mosquito suspensions, identification was impossible both by TR-FIA and ELISA.

These findings have demonstrated the efficiency of TR-FIA for detection and identification of CSV in infected mosquitoes.

Ivanovsky Institute of Virology RAMS, Department of Arboviruses, WHO Collaborating Center, etc.

V.G. Pomelova, S.D. Lebedeva, T.A. Bichenkova, N.V. Khutoretskaya, A.M. Butenko.

Preliminary Studies on the use of Solid-Phase Immunosorbent Techniques for the rapid detection of Wesselsbron Virus (WSLV) IgM by Haemagglutination-Inhibition.

By

S.S. Baba, A.H. Fagbami, C.K. Ojeh
Department of Virology, College of Medicine, University of Ibadan, Nigeria

The adoption of solid-phase techniques including the enzyme immunoassays and the radioimmunoassay, for rapid detection of viral IgM in human and animal sera, is becoming increasingly difficult for developing countries. These procedures require the availability and maintenance of specialised equipment and suitably trained personnel, a luxury in non industralized countries.

The flavivirus haemagglutination-inhibition (HI) test has been modified for the detection of WSLV-specific IgM. The WSLV HI test is performed in microplate wells in which IgM from human sera has been selectively retained by antihuman IgM bound to the polystyrene surface[2]. In addition to the standard flavivirus HI reagents, the test requires only antihuman IgM coated microtitre plates.

Using this inexpensive HI test adapted to solid-phase immunosorbent technique (SPIT), we detected IgM to WSLV in 33(12%) of the 274 human sera collected from different ecological zones in Nigeria. Few (6%) of the WSLV IgM positive sera cross-reacted with one or more other flaviviruses (Yellow fever, Westnile, Potiskum and Uganda S) used in the test. In addition, majority of the positive sera [15(45%) of the 33 positive sera] reacted to high titres (>1:10240). With the conventional HI test, 78% of the sera tested were positive for presence of antibodies to 3 or more flavivirus antigens[1].

Although SPIT may be less sensitive than the conventional HI test, it has been found to be more specific and could be used for rapid detection of early flavivirus infections. In addition, the procedure has the potentials to resolve the problem of flavivirus cross-reactivity, a continuing diagnostic problem in flavivirus hyperendemic communities in tropical countries. We hope to compare the sensitivity, specificity and practicality of this procedure with the existing solid-support techniques.

References:

1. Baba, S.S. (1991). Wesselsbron virus: biological characteristics and seroepidemiological survey in Nigeria. Ph.D Thesis, University of Ibadan, Ibadan, Nigeria. 1-296.

2. Krech, U. and Wilhelm, J.A. (1979). A solid-phase immunosorbent technique for the rapid detection of rubella Igm by haemagglutination inhibition. J. gen. Virol. 44: 281-286.

Reverse Transcription-PCR Detection of LaCrosse Virus in Mosquitoes: Comparison with Enzyme Immune Assay and Virus Isolation

Leonard P. Wasieloski Jr., Alfredo Rayms-Keller, Laura A. Curtis, Carol D. Blair, and Barry J. Beaty*

*Arthropod Borne and Infectious Diseases Laboratory (AIDL), Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523. Telephone 303-491-8604, FAX 303-491-8304, Electronic mail address: bbeaty@vines.colostate.edu

Monitoring vector populations for arbovirus prevalence is an important component of assessing disease risk to humans and animals. Virus isolation in bioassays, such as suckling mice and cell cultures, and subsequent serologic identification of isolates, has classically been used to determine field infection rates in vectors. However in the last 20 years, modern biotechnological assays such as enzyme immune assays (EIA) have become the method of choice for arbovirus surveillance. Detection of viral specific nucleic acids is an alternative to virus isolation and/or antigen detection. The two principal approaches have been detection of analyte by hybridization and more recently by PCR. We have developed a reverse transcription PCR (RT-PCR) assay for the detection of LAC viral analyte in mosquitoes, and have compared RT-PCR, VI and EIA in their ability to detect LAC virus and/or analyte in pools of *Aedes triseriatus* mosquitoes.

Virus isolation and EIA. The titers of individual and pooled mosquitoes were determined by microtitration in BHK-21 cells. LAC antigen was detected in a capture enzyme immune assay for LAC nucleocapsid protein.

RNA extraction. Total mosquito RNA was extracted by the acid guanidinium thiocyanate—phenol—chloroform (AGPC) method described by Chomczynski and Sacchi.

RT-PCR primers. Primer-1 (5'-TCAAGAGTGTGATGTCGGATTTGG) is identical to nucleotides 71-94 of the LAC S segment virus complementary sequence, and used to prime cDNA synthesis from S segment genomic RNA. Primer-2 (5'-GGAAGCCTGATGCCAAATTTCTG) is complementary to nucleotides 763-785 of the LAC S segment virus complementary sequence, and is used with Primer-1 for PCR amplification.

Reverse transcription PCR. One tenth of the RNA from each sample was reverse transcribed with Primer-1 using SuperScript (BRL, Gaithersburg, MD). One tenth of the cDNA was PCR amplified with Primers 1 and 2, and the products were analyzed by electrophoresis in 1.5% agarose gels.

Detection of LAC in parenterally infected mosquitoes. The ability of RT-PCR, EIA, and VI to detect a single LAC infected mosquito in pools of 1, 10, 50 and 100 total mosquitoes was examined. RT-PCR of mosquito pools resulted in a specific 715 bp product in the LAC positive pools and no specific product in the negative controls. All three techniques detected LAC at every pool size with no false positive results.

Sensitivity of RT-PCR, VI, and EIA. To determine the sensitivity of RT-PCR, EIA, and VI, mosquitoes were parenterally infected with LAC and assayed at hours 0 and 10, and days 1, 2, 3 and 7 post infection. VI was the most sensitive technique; virus was isolated from 4 of 4 LAC infected mosquitoes immediately following inoculation. RT-PCR was second in sensitivity and detected LAC RNA in 3 out of 3 LAC infected mosquitoes 1 day post inoculation. EIA was the least sensitive and detected 4 of 4 LAC infected mosquitoes 2 days post inoculation.

Specificity of RT-PCR. The specificity of the LAC RT-PCR was investigated by analyzing individual mosquitoes infected with serologically related and unrelated viruses. RNAs extracted from three LAC, SSH, and TAH inoculated *Aedes triseriatus*, and 3 DEN 2 inoculated *Aedes aegypti* were reverse transcribed and PCR amplified as before. All the LAC, SSH and TAH infected mosquitoes yielded a diagnostic 715 bp PCR product as expected from LAC; while the 715 bp product was absent from the *Aedes aegypti*/ DEN 2 samples.

Effect of freeze-thawing on LAC detection. Since mosquito pools frequently undergo multiple freeze-thaw (F-T) cycles during the process of collection, mosquito identification, and virus isolation, the effect of multiple F-T cycles upon RT-PCR, VI, and EIA detection of LAC virus was investigated. Individual positive mosquitoes, which had been demonstrated to be infected by IF, were subjected to 7 cycles of freezing to -70°C followed by incubation at room temperature for 1 h. Samples were from F-T cycles 3, 5 and 7. Virus was isolated in one of 3 mosquitoes after 5 F-T cycles, but could not be isolated after 7. Both RT-PCR and EIA detected LAC analyte after 7 F-T cycles.

Stability of RT-PCR samples. To determine the stability of AGPC extracted mosquito RNA, individual LAC mosquitoes were ground in 500 μl of solution D and left at room temperature. Three mosquito triturates were transferred to -20°C after 1, 3 and 8 days post trituration. RT-PCR amplification resulted in LAC specific product in all of the samples.

DISCUSSION. The results show that RT-PCR compares favorably to VI and EIA for detection of LAC analyte in *Aedes triseriatus* mosquitoes. RT-PCR shares many of the attributes of EIA; the test is sensitive, specific, and capable of providing diagnostic results within hours rather than days. Additionally, freeze-thaw and RNA stability studies demonstrate that RT-PCR is practical from field collected samples even in the absence of a cold chain. However, while EIA can be completed in the field for immediate results, specialized equipment is required for RNA isolation, reverse transcription and PCR amplification. Finally, the practicality and cost effectiveness of EIA for routine surveillance is well established, but unknown for the RT-PCR. The diagnostic efficacy of RT-PCR in clinical and field applications remains to be determined.

Methodological problems of tick environmental studies in long-term field experiments

Milan Daniel

Postgraduate Medical School, 100 05 Prague 10, Czech Republic

Long-term field experiments dealing with the development and behaviour of ticks under different ecological conditions often need to keep the ticks in very limited space, in suitable microcages. This gives rise to three main questions about the microcages: 1/ their shape, 2/ their size and 3/ the material used for their construction. Our many years' experience with Ixodes ricinus and other tick species shows that the most suitable, in many ways are cylindrical cages (50 mm long and 25 mm in diameter) made of wire specially protected against rust and covered by very fine silon (nylon) fabric. (This method was described by Daniel et al., 1972.) These cages can be used in all tick habitats and microhabitats and for all developmental stages (starving, engorged, egg-laying etc.).

When studying the specific features of the life cycle of I. ricinus influenced by the microclimates of different microhabitats, we tried simultaneously to recognize the possible role of the silon-fabric cages described above on the character and changes of the microclimate inside. For comparison we also used cages (of the same shape and size, i.e. 50 mm tubes, 25 mm caliber) made from other materials (referred to in the literature - wood, glass, metal (aluminium) and plastic (Novodur) - all closed by silon fabric. For the whole vegetation period, under the conditions of the South Moravian thermophilic oak forest, we evaluated specific changes in the microenvironment (temperature, relative air humidity) of free forest litter and of five types of tick microcages made from different materials. These changes were measured continuously by electroresistance thermometers and hygrometers containing bead thermistors; recordings were made at 20-second intervals during the whole vegetation period. The features of the microclimate were compared using descriptive statistics, specifically: analysis of trend of variables, covariance and spectral analysis, analysis of relations between two bioclimatological variables based in cross-variance and cross-spectral functions. All these indices show the best coincidence of microenvironmental character and its changes in free litter layer and in the wire cages covered by silon fabric. The temperature and relative air humidity in other types of cages varied considerably. To demonstrate these differences the accompanying three-dimensional histograms are used to illustrate the temperature and relative air humidity (RH) values measured in individual cages (temperature and RH = abscissa; % time relative to the entire observation period = ordinate). On Figure 1 the curve depicting the temperature measurements in a silon-fabric cage (site 5) is very similar to the conditions in free litter (site 6) in respect of the shape and maximal values reached (26, resp. 25° C). The curve for site 4 demonstrates the conditions in a cage protected by glass. It shows a higher peak, a longer period spent at higher temperatures and a maximal temperature (30° C). Curves for sites 1-3 clearly show how the protective characters of the mate-

rials used (wood, plastic, metal) influence the courses of the temperatures measured and also the maximal values reached (29, 31, and 30° C). The histogram of RH values shows not only the differences between values measured in free litter and those in the various cages but also the low values reached in all cages, with the exception of the silon-fabric microcage. In cages made from plastic, glass and metal (and partially this is valid for wooden cages too), the relative air humidity falls for long periods below the critical equilibrium humidity (86-96% RH) for tick I. ricinus (Knülle and Rudolph, 1983). Moreover, the wooden microcage has been attacked by fungi.

References

Daniel M., Honzáková E., Černý V., 1972. Overwintering of the tick Ixodes ricinus (L.) in the conditions of field experiments. Folia parasit. (Prague), 19: 305-314.
Knülle W., Rudolph D., 1983: Humidity ralationships and water balance of ticks. In: Obenchain F. D., Gaalun R., "Physiology of ticks". Pergamon Press, Oxford: 34-70.

Figures 1 and 2

Histograms of values of microclimate measured in the growing season (April-October)

Axis x: temperature (° C) / relative air humidity (% RH)
Axis y: relative frequency of values measured (in %) during the whole vegetation period
Axis z: sites of measurements in microcages made from:
1 - wood; 2 - plastic; 3 - metal; 4 - glass;
5 - silon-fabric; 6 - microenvironment of free forest litter;

Figure 1

TEMPERATURE
Relative Frequency of Measured Values(%)

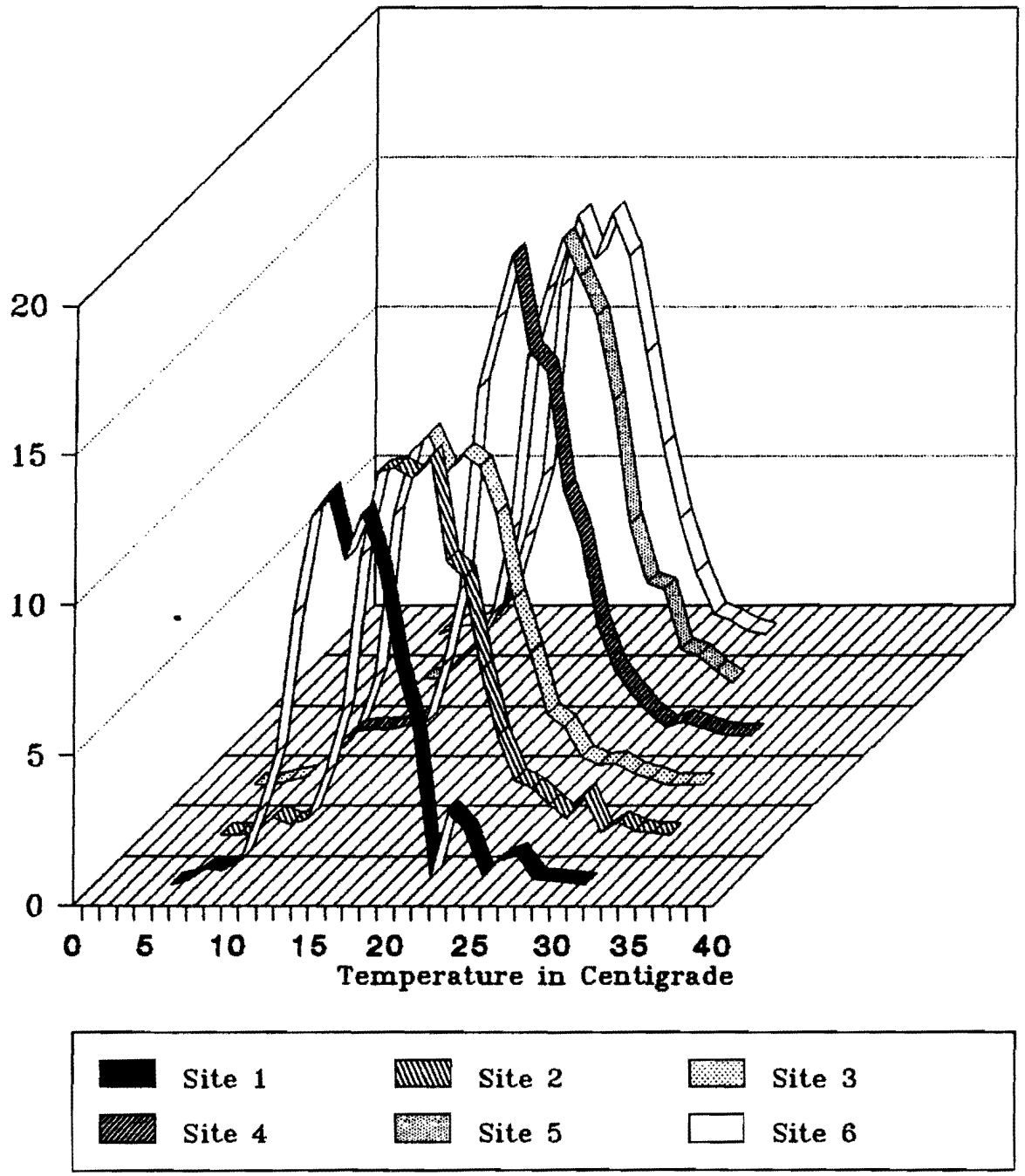
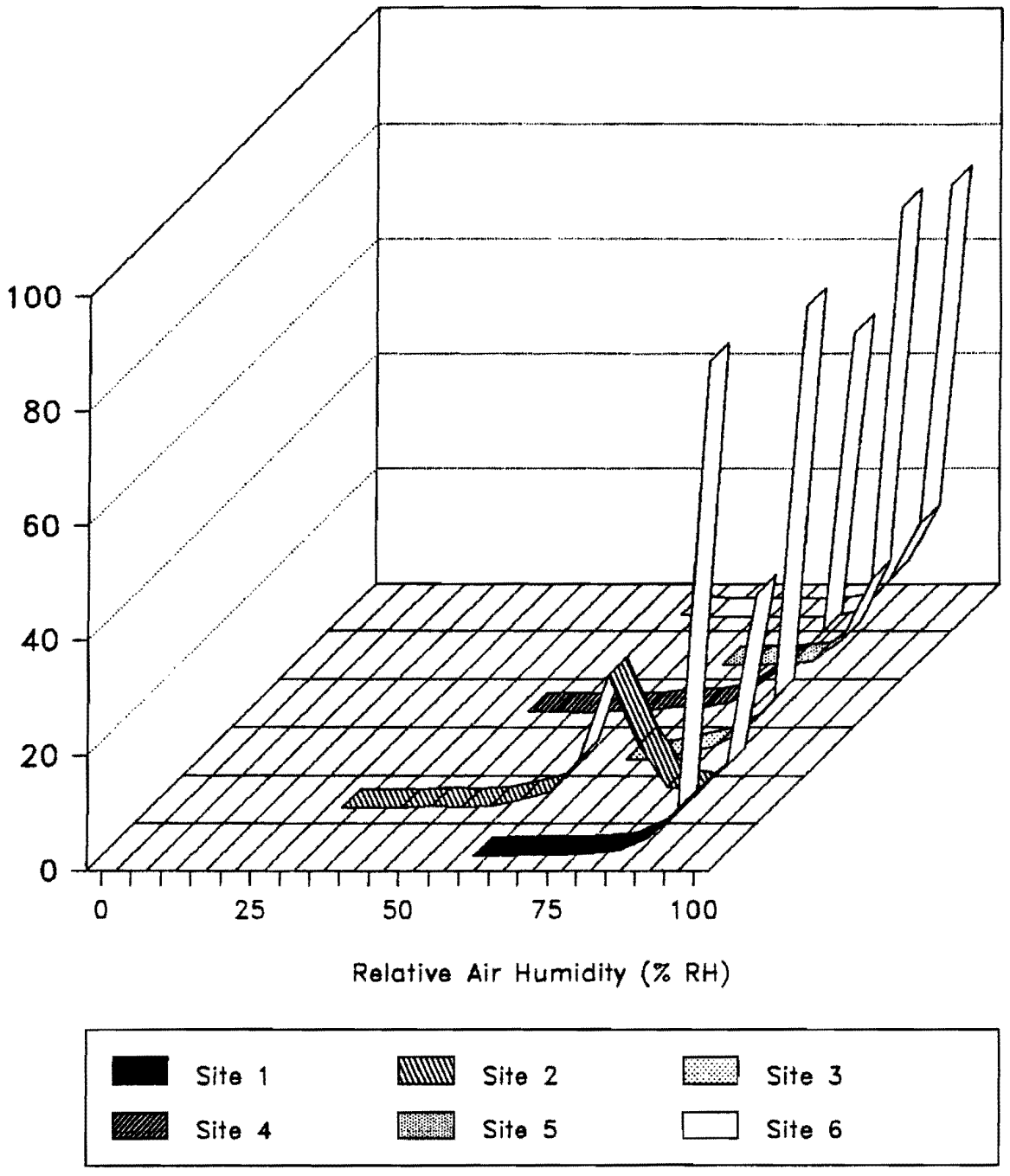


Figure 2

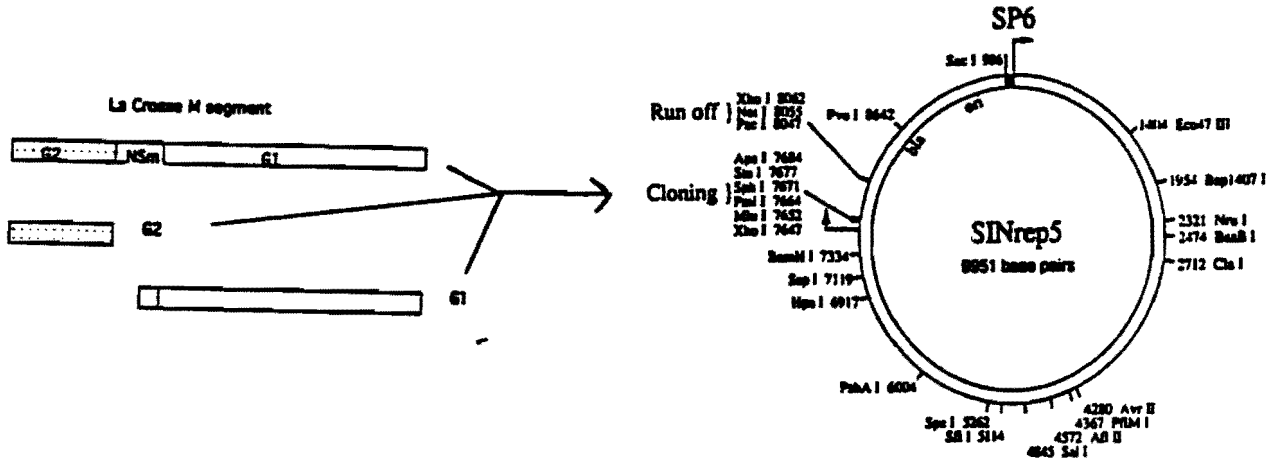

AIR HUMIDITY
Relative Frequency of Measured Values(%)
100
80
60
40
20
0
0
25
50
75
100
Relative Air Humidity (% RH)
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6

Expression of La Crosse virus glycoproteins in *Aedes albopictus* (C6/36) mosquito cells using the Sindbis virus replicon system.

K.I. Kamrud and B.J. Beaty

Arthropod Borne and Infectious Diseases Laboratory, Colorado State University, Fort Collins, CO 80523.

La Crosse (LAC) virus, a member of the Bunyaviridae family, has a three segmented single-stranded negative-sense RNA genome. The medium (M) genome segment encodes the two viral glycoproteins (G1 and G2) and a non-structural (NSm) protein in a single continuous open reading frame with a gene order of 5' G2-NSm-G1 3'. We are interested in expression of the LAC virus M segment in mosquito cells. The Sindbis replicon system was chosen as our expression system because Sindbis is an arbovirus which can infect *Aedes albopictus* (C6/36) mosquito cells and the replicon system can accommodate the large M segment fragment. The LAC virus M segment was cloned into the Sindbis replicon vector pSinRep5. In addition, truncated versions of the M segment which encode the G2 and G1 genes individually have been cloned into the pSinRep5 vector. Run-off transcription of pSinRep5 constructs, with SP6 RNA polymerase, produces a transcript which contains the Sindbis virus non-structural genes and the LAC virus M genome fragments in place of the Sindbis structural genes. Electroporation of the pSinRep5 M segment construct RNA's into C6/36 cells allows for transient expression of the LAC virus glycoproteins in 10-20% of the electroporated cells, as determined by IFA. RNA produced from helper Sindbis virus plasmids co-electroporated with the pSinRep5 M segment RNA's into BHK-21 cells produced infectious particles capable of at least one round of replication. When C6/36 cells were infected with the pSinRep5 M replicon particles both the G1 and G2 envelope glycoproteins were expressed. Replicon virus were produced with pSinRep5 G2 and pSinRep5 G1 and their respective proteins were expressed individually in C6/36 cells.



References:

Bredenbeek, P.J., Frolov, I., Rice, C.M., Schlensinger, S. (1993). Sindbis virus expression vectors: Packaging of RNA replicons by using defective helper RNAs. J. Virol. 67(11), 6439-6446.

Evaluation of molecular strategies for intracellular immunization against bunyaviruses.

A.M. Powers, K.I. Kamrud, K.E. Olson, S. Higgs, J.O. Carlson, and B.J. Beaty

Arthropod Borne and Infectious Diseases Laboratory (AIDL), Colorado State University, Ft. Collins, CO 80523

A double subgenomic Sindbis virus (TE/3'2J/αS) was used to express the negative sense small RNA segment of LaCrosse (LAC) virus in C6/36 *Aedes albopictus* cells. Cells infected with TE/3'2J/αS at an moi of 50 and subsequently challenged with LAC virus were resistant to LAC infection; LAC titers were consistently $\geq$ 4.0 $\log_{10}TCID_{50}$/ml less than titers in cells infected with AR339 SIN or a control dsSIN virus (Figure 1). This dsSIN virus system was used to express other portions of the small or medium RNA segments of LAC virus in C6/36 cells. Recombinant viruses were generated which contained the full length small segment in sense orientation, the small (NS_s) segment nonstructural coding region in sense or antisense orientation, the consensus sequence of the LAC S segment, or portions of the glycoprotein coding regions in sense or antisense orientation. C6/36 cells were infected with these recombinant viruses or a control dsSIN virus and challenged 48 hours later with LAC. The relative abilities of these recombinant viruses to induce interference to LAC virus were compared using TE/3'2J/αS as a standard. Recombinant viruses that inhibited LAC infection were then used to infect C6/36 cells before challenging with heterologous bunyaviruses to determine the potential of these constructs to be cross-protective. The recombinant dsSIN viruses are a useful tool for evaluating molecular strategies for intracellular immunization of mosquito cells against arthropod-borne viruses.

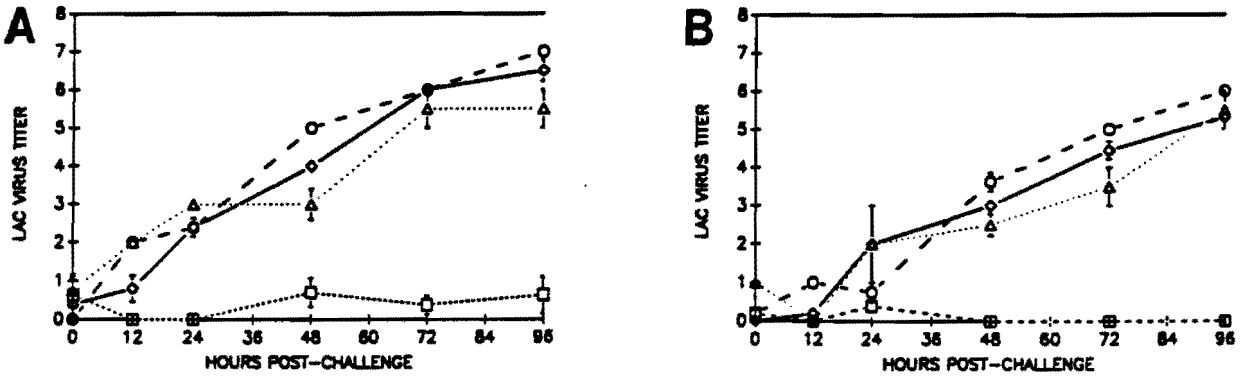


Fig. 1. Titers of LAC virus in dsSIN infected/LAC challenged C6/36 cells. Cells were infected with wild type SIN (○), TE/3'2J (△), TE/3'2J/ANTI-S (□), or no SIN virus (◇), before challenge with LAC virus at an moi of 0.1 (A) or 0.01 (B).

Recombinant Sindbis viruses expressing the C and prM genes of dengue-2 in sense and antisense render C6/36 mosquito cells resistant to dengue-2 challenge in culture.

P.J. Gaines, K.E. Olson, S. Higgs, and C.D. Blair

Arthropod Borne and Infectious Diseases Laboratory (AIDL), Colorado State University, Fort Collins, CO 80523

The capsid (C) and premembrane (prM) genes of dengue-2 were cloned into the Sindbis virus double promoter plasmid pTE/3'2J. In vitro transcription was performed and the RNA electroporated into BHK-21 cells. Recombinant Sindbis viruses were harvested 30 hours following electroporation. Northern blot analysis demonstrated that these viruses expressed the C and prM RNA transcripts in both sense and antisense in C6/36 cells. C and prM protein expression was confirmed by in vitro labelling and fluorescence antibody labelling.

C6/36 cells infected with the recombinant Sindbis viruses were resistant to challenge with dengue-2 virus, whereas uninfected cells or cells infected with the parent TE/3'2J Sindbis virus readily supported dengue-2 replication. Expression of the dengue C and prM genes in sense or antisense may be useful in the development of dengue resistant transgenic mosquitos.

Geographic distribution and evolution of yellow fever viruses based on direct sequencing of genomic cDNA fragments

Loïc Lepiniec, Lynn Dalgarno, Vu Thi Que Huong, Thomas P. Monath, Jean-Pierre Digoutte and Vincent Deubel

Report from: *Unité des Arbovirus et Virus des Fièvres Hémorragiques, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris cedex 15, France*

We have compared the nucleotide sequence of an envelope protein gene fragment encoding amino acids 291 to 406 of a large panel of yellow fever (YF) virus strains of diverse geographic and host origins isolated over a 63 year time span. The nucleotide fragment of viral RNA was examined by direct sequencing of a polymerase chain reaction product derived from complementary DNA (Deubel et al., 1993; Huong et al., 1993). Alignment with the prototype Asibi strain sequence showed divergence of 0-21.5%.corresponding to a maximum of 5.2% divergence in the amino acid sequence Phylogenetic analysis of a 348 nucleotide-long sequence revealed broader genetic relationships between different topotypes established by T1 oligonucleotide mapping (Deubel et al., 1985; 1986) and provided quantitative data which were easier to interpret (Fig.1). Taking 10% nucleotide divergence as a cut-off point, the 22 YF virus strains fell into three topotypes which corresponded to different geographical areas: the African strains can be grouped into two main clusters, a western African cluster and a central-eastern cluster including strains from Ethiopia, Zaire and Central African Republic. The South American strain previously described by Ballinger-Crabtree and Miller (1990) corresponded to the third topotype. Two subgroups were defined in West Africa : the first subgroup corresponds to the historical strains Asibi and FV, which were responsible for the outbreaks along the West African coast in 1927, and to the strains circulating in the sylvatic zone of the western part of Africa, from western Ivory Coast-Mali to Senegal. The second subgroup in West Africa includes the epidemic strains isolated during explosive outbreaks in Burkina Faso (1983), in Nigeria (1986) and in Cameroon (1990). The strain similarity indicates that the recent epidemics in Cameroon represent an extension of YF activity in Nigeria.

Despite the temporal separation of 14 years and the different sources of virus isolates in two separate regions of YF endemicity, Senegal and Central African Republic, five strains from Senegal (1976-1990) showed the same nucleotide sequence in the gene fragment analyzed and four strain from Central African Republic (1977-1991) showed less than 1% nucleotide change, thus confirming the genetic stability of the YF virus in each ecological zone previously noted in T1 oligonucleotide map (Deubel et al., 1985, 1986)

Intense sylvatic circulation in Central and East Africa has been recorded but human cases are very rare and occur only at low level or in infrequent epidemics. It is not clear if the lack of epidemics in Central Africa is due to a less virulent genotype of virus.or to ecological factors limiting its transmission (Aedes aegypti may not be susceptible to this virus strain). A large epidemic developed in southern Sudan and in Ethiopia in 1959-1962. No evidence of previous circulation of YF virus could be found in Ethiopia and the outbreak was probably the result of introduction of the virus from

afar rather than an emergence from a local, enzootic reservoir. The genetic characterization indicates that the source of the epidemic strain was elsewhere in East Africa, as might be expected on the ground of geographic proximity of Uganda, Sudan and Kenya where YF sylvatic circulation has been observed. The virus isolated in 1961 during the Ethiopian epidemic shared 98.6% similarity with a virus isolated from a human case in Zaire in 1959 where YF is mainly endemic with sporadic cases. Therefore, these strains are closer to each other than to the endemic strains in the more geographically distant Central African Republic (94.5 to 95.4% similarity).

The South American strain constitutes the third topotype, and shows 14 to 16% divergence in nucleotide sequence from West African strains and about 21.5% divergence from Central-East African strains. Our results support the concept that the virus originated in Africa was introduced into the New World in slave ships bearing active cases of YF or infected Ae. aegypti.

Our results offer new insights on genetic relationships between YF isolates and provide useful tool for the understanding of YF distribution and evolution.

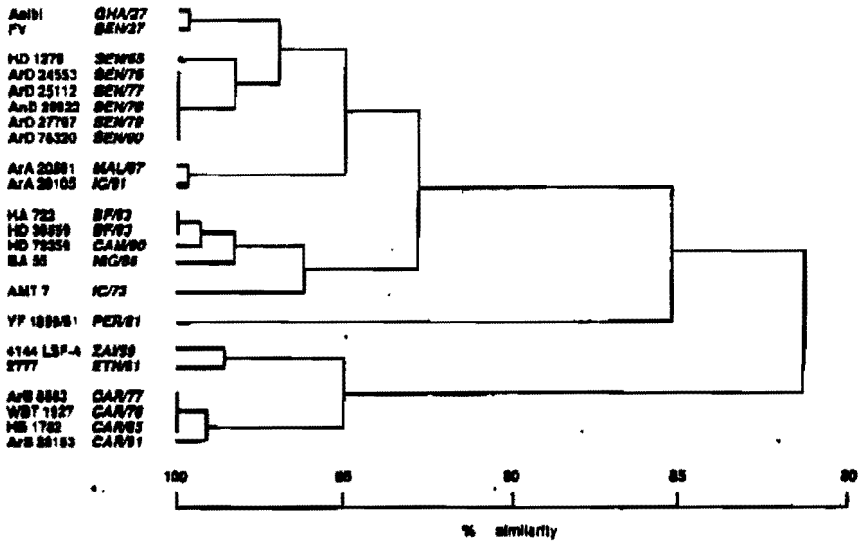


Figure 1. Dendrogram showing the extent of base sequence similarity between yellow fever viruses. The origins of the strains were Ghana (GHA), Senegal (SEN), Mali (MAL), Ivory Coast (IC), Burkina Faso (BF), Cameroun (CAM), Nigeria (NIG), Peru (PER), Zaire (ZAI), Ethiopia (ETH) and Central African Republic (CAR). The following two numbers indicate the year of isolation (i.e./27=1927).

BALLINGER-CRABTREE, M. E. & MILLER, B. R. (1990). *J. Gen. Virol.* **71**, 2115-2121.

DEUBEL, V., PAILLIEZ, J. P., CORNET, M., SCHLESINGER, J. J., DIOP, M., DIGOUTTE, J. P. & GIRARD, M. (1985). *Am. J. Trop. Med.* **34**, 976-983.

DEUBEL, V., DIGOUTTE, J. P., MONATH, T. P. & GIRARD, M. (1986). *J. Gen. Virol.* **67**, 209-213.

DEUBEL, V., NOGUEIRA, R. M., DROUET, M. T, ZELLER, H., REYNES, J. M. & HA, D. Q. (1993). *Arch. Virol.* **129**, 197-210.

HUONG, V.T.Q., HA, D.Q. & DEUBEL, V. (1993). *Am.J.Trop.Med.* **49**, in press.

EASTERN EQUINE ENCEPHALITIS IN A HORSE FROM SOUTHWESTERN ONTARIO

P.S. Carman[1], H. Artsob[2], S. Emery[1], M.G. Maxie[1], D. Pooley[3], I.K. Barker[4], G.A. Surgeoner[5], M.S. Mahdy[6]

[1]Ontario Ministry of Agriculture, Veterinary Laboratory Services Branch, Guelph, Ontario, N1H 6R8
[2]Zoonotic Diseases, Laboratory Centre for Disease Control, Tunney's Pasture, Ottawa, Ontario, K1A 0L2
[3]Pooley Equine Veterinary Services, R.R. #1, Embro, Ontario, N0J 1J0
[4]Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, N1G 2W1
[5]Department of Environmental Biology, University of Guelph, Guelph, Ontario, N1G 2W1
[6]Vector-Borne and Special Pathogens Unit, Laboratory Services Branch, Ontario Ministry of Health, Etobicoke, Ontario, M9P 3T1

Two outbreaks of eastern equine encephalitis (EEE) have been documented in Canadian horses. In 1938, 12 Ontario horses were afflicted with encephalomyelitis and EEE virus was isolated from the blood of one animal (1). A subsequent outbreak due to EEE virus was observed in 1972 in the Eastern Townships of Quebec in which approximately 30 horses died (1). We report herein a third instance in which EEE virus has been shown to infect Canadian horses.

In mid-August, 1992, a five-year-old Belgian mare near Woodstock in southwestern Ontario was examined because of neurologic signs that were of acute onset. The mare appeared to be blind, pressed into fence posts, gates, and into the manger of the stall, lost coordination, and had a flaccid lower lip. Rectal temperature was 40°C. The mare became recumbent after 8-10 hours, convulsed, and died within 18 hours of examination. It had been vaccinated against rabies in the spring. The clinician suspected viral encephalitis, particularly EEE virus.

Histologic examination of brain sections revealed that numerous vessels in white and gray matter of the cerebrum and cerebellum had narrow cuffs of cells, predominantly lymphocytes plus a few neutrophils. Within 48 hours of inoculation of brain tissue supernatants onto BHK-21 cell cultures, an aggressive cytopathic agent was recovered (isolated G92-03127).

Supernatant from isolate G92-03127 reacted by complement fixation to EEE virus hyperimmune ascitic fluid, with a titer equivalent to the reaction of EEE virus with its homologous ascitic fluid (Table 1). Thus, G92-03127 was presumptively identified as EEE virus. This presumptive identification was confirmed by immunofluorescent antibody staining. Isolate G92-03127 reacted with monoclones 1B5C-3 and 1B1C-4 kindly supplied by Dr. Nick Karabatsos, CDC, Fort Collins, showing that it was the North American variant of EEE virus.

Sera from nine horses that had been in contact with the deceased horse were tested by hemagglutination-inhibition for antibodies to EEE, western equine encephalitis (WEE), Venezuelan equine encephalitis (VEE) and Highlands J (HJ) viruses. One reactor was obtained. This reactor had a hemagglutination-inhibition titer of 1:320 to EEE virus with no cross-reaction to the other alphavirus antigens. Complement fixation testing of this serum revealed a 1:32 titer both to EEE virus and to an antigen produced from isolate G92-03127, but no antibodies (titer <1:8) to WEE, VEE and HJ antigens.

Sera from 81 birds housed on the farm in open-windowed facilities were tested by hemagglutination-inhibition including 48 chickens, 30 ducks and three geese. One duck serum was positive at 1:20 dilution to WEE and at 1:10 to HJ antigens; sera from all other birds housed on the farm were negative for HI antibodies to EEE and WEE viruses.

This is the first reported case of confirmed EEE in a horse in Ontario since 1938 although EEE virus was isolated from the blood of a migratory bird captured at Long Point, Ontario in 1961 (3). The deceased and in-contact horses were infected in August, 1992. They were pastured with access to a permanent swamp, filled with water as a result of the summer rains. The farm chickens, geese and ducks housed a few hundred meters from the swamp, that could be regarded as sentinels and were found to be HI antibody-negative to EEE, illustrate the localized habitat of the vector in this situation.

The occurrence of the two equine cases warrants surveillance against potential resurgence of EEE activity. This view is supported by three factors: the recognition of these two cases after 55 years without records of EEE disease; the activity of this virus in Michigan, Ohio, and New York, three states contiguous to Ontario; and the nature and similarity of habitat to that of a recent Ohio outbreak in which 12 confirmed and seven presumed equine cases were observed but with limited avian involvement (4).

References

1. Schofield FW, Labzoffsky N. Rep Ont Dept Agric OVC 1938;25-29.
2. Bellavance R, Rossier E, Le Maitre M, Willis NG, Belanger P. Can J Pub Hlth 1973;64:189-190.
3. Karstad L. Ontario Bird Banding 1965;1:1-9.
4. Nasci RS, Berry RL, Restifo RA, Parsons MA, Smith GC, Martin DA. J Med Entomol 1993; 30:217-222.

Table 1. Complement fixation test for the identification of isolate G92-03127.

	Hyperimmune mouse ascitic fluid to:			
Antigen*	**EEE**	**HJ**	**WEE**	**VEE**
G92-03127	1:128	<1:8	1:8	<1:8
EEE	**1:128**	<1:8	1:16	<1:8
HJ	1:16	**1:16**	1:512	<1:8
WEE	1:16	1:8	**1:2048**	<1:8
VEE	<1:8	<1:8	<1:8	**1:32**

*Appropriate antigen controls from uninfected material were included for all antigens.

EEE = eastern equine encephalitis virus; HJ = Highlands J virus; WEE = western equine encephalitis virus; VEE = Venezuelan equine encephalitis virus.

Arbovirus encephalitis surveillance, California, 1993

A review of the 1993 season will be published in the Proceedings and Papers of the 62nd annual CMVCA conference, and is also briefly summarized here. Following the recognition of early WEE activity in Sacramento County in mid to late June, a major effort was made to alert the medical community to watch for suspect cases of aseptic meningitis and encephalitis. Over 200 suspect cases were tested at the State's Viral and Rickettsial Disease Laboratory, and an unknown number by other laboratories, revealing three confirmed SLE cases: (1) a 64 year old male resident of San Bernardino City, San Bernardino County, who became ill 8/12/93, was hospitalized from 8/15-8/23, and recovered completely. His only known recent travel had been within 20-30 miles of his home; (2) a 19 year old male resident of San Diego County, who became ill 9/11/93 with fever, headache, nausea, stiff neck and transient diplopia, but recovered completely. On 8/31, he had travelled to Imperial County and fished in the West Main Canal, the most likely source of mosquito exposure; (3) a third case was a 30 year old male resident of Orange County at the time of illness onset 9/24/94. He was hospitalized 3 days but recovered completely. Probable place of exposure was San Bernardino County along the Colorado River south of Needles. The case was reported late in the season since the serologic test results were atypical and special extra testing was required.

In addition to the SLE cases, extensive WEE activity was detected in sentinel chicken flocks and mosquito pools, especially in the Sacramento Valley northward. There was one fatal case of WEE in a four month old quarter horse filly from Red Bluff, Tehama County, 8/21/93. Also, there was extensive involvement of a relatively new and exotic species in California, the emu, which is being raised commercially and is quite susceptible to the WEE virus. Veterinarians began vaccinating emus with equine WEE vaccines, although the efficacy for emus is unknown.

The routine surveillance program utilizing sentinel chickens and mosquito collections for virus isolation has again proven to be a useful and successful means of providing early alerts to virus activity in various regions of the State and has helped focus mosquito control efforts in the most critical areas. The 157 flocks of sentinel chickens were bled every two weeks, using a new filter paper blood collection technique, yielding over 17,000 blood samples for testing. Of these, 281 were positive for WEE antibodies and 91 for SLE antibodies. The dried blood samples were mailed at ambient temperature, saving significantly on postage. Of 4,395 mosquito pools tested, 180 were positive for WEE virus, and 35 were positive for SLE virus, including isolates by the Arbovirus Research Program, U.C. Berkeley. The filter paper collection method for serum testing and streamlined laboratory methods for virus isolation and identification have helped to reduce the costs and extend the surveillance network for this program.

Prepared by the Viral and Rickettsial Disease Laboratory, Vector Biology and Control Branch and Communicable Disease Control Division of the California Department of Health Services, 2151 Berkeley Way, Berkeley, California 94704.

Richard W. Emmons, M.D., Ph.D.

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

Arbovirus Surveillance in New Jersey, 1993

During the 1993 surveillance period from June into October, 821 mosquito pools containing up to 100 mosquitoes each were tested for viruses in day old chicks. There were 21 mosquito pools positive for Eastern enciphalitis (EE) virus and Highlands J (HJ) virus was isolated from 1.

Table 1 summarizes the collection area totals, species of mosquito and time of collection for the EE isolates. Activity began with late July collections and continued into October. All of the 21 isolates were from pools containing Culiseta melanura mosquitoes at 7 sites.

A mid September (9/17/93) collection from the Centerton site gave the only isolate of HJ isolates from a Culiseta melanura pool.

(Shahiedy I. Shahied, Bernard F. Taylor, Wayne Pizzuti)

New Jersey, Department of Health
Trenton, N.J. 08625

Table 1
1993
EE MOSQUITO POOL ISOLATES
FOR WEEK ENDING

AREA COLLECTED	MOSQUITO SPECIES	7/30	8/6	8/13	8/20	8/27	9/3	9/10	9/17	9/24	10/1	AREA TOTALS
Bass River	Cs. melanura					1					1	2
Centerton	Cs. melanura	1			1	1		1		1	1	6
Dennisville	Cs. melanura	1			3		2			3		9
Hammonton	Cs. melanura	1										1
Ocean City	Cs. melanura							1				1
Turkey Swamp	Cs. melanura								1			1
Waretown	Cs. melanura										1	1
WEEKLY TOTALS		3	0	0	4	2	2	2	1	4	3	21

DECREASE IN NATURAL IMMUNITY OF RIFT VALLEY FEVER NEUTRALIZING ANTIBODIES FROM SENTINEL DOMESTIC RUMINANTS IN THE SENEGAL RIVER BASIN AFTER THE 1987 EPIZOOTIC. REPORT FROM THE LABORATORY OF VIROLOGY AND THE RIFT VALLEY FEVER SURVEY CENTER, INSTITUT SÉNÉGALAIS DE RECHERCHES AGRICOLES

A domestic ruminant serosurvey of Rift Valley fever (RVF) neutralizing antibodies has been carried out annually after the 1987 Senegal River basin epidemic. A follow-up survey of cattle, sheep and goats herds has been maintained during the past five years looking for clinical symptoms and taking blood samples to test for neutralizing antibodies.

An annual decrease in RVF neutralizing antibodies is observed(Table 2).And now has returned to a low antibody prevalence as that which was observed before the 1987 epidemic. This fact can be explained by the natural decrease in antibody prevalence without active reinfection and the high turn over in the local herds. Globally there is a negative gradient of RVF virus antibody prevalence from the Delta to the Upper Sénégal River Basin(Table 1) and it is observed every year. In accord with the suggested origin of the 1987 epidemic, the Delta Sénégal River Basin appears to be an hyperenzootic area.

Seroprevalence in cattle appears higher than that observed in goats and sheep. Cattle are known to be more resistent to the RVF and survivors are more likely found in such population of ruminants. In these animals, females present an unexplained higher rate of antibody than males (data not shown).

Continued surveillance is planned which will allow us to evaluate the risk of RVF re-emerging in this area and provide information for the understanding of RVF virus circulation in West Africa.

ANTIBODY PREVALENCE OF DOMESTIC RUMINANTS , 1990

Sénégal River	Cattle	Sheep & Goats
Delta	36.9 (249)*	8.6 (105)
Middle	27.0 (222)	10.0 (259))
Upper	12.6 (159)	1.7 (231)
Total	27.4 (630)	06.6 (595)

*% (total tested)

Temporal evolution in RVF virus seroprevalence from domestic ruminants of the Dagana district (Sénégal River delta region).

Year	Total tested	% pos.	Test
1982	23	.0	ELISA[1]
1985	63	.3	ELISA[1]
1987	**87**	**85.0**	**ELISA[1]**
1988	**39**	**71.7**	**NUT[2]**
1989	159	23.8	NUT[2]
1990	105	8.5	NUT
1991	190	9.4	NUT
1992	111	6.3	NUT

[1] KSIAZECK, T., JOUAN, A., MEEGAN, ET COLL.,1989,*Research in Virology*, **140**, 67-77
[2] THIONGANE Y., GONZALEZ JP., FATI N. A. & AKAKPO JA.,1991,*Research in Virology*,**142**, 67-70

REPORT BY: YAYA THIONGANE, H. ZELLER, M. M. LO, N. A. FATI, J. A. AKAKPO & J.-P. GONZALEZ. Institut Sénégalais de Recherches Agricoles, B.P 2057 Dakar, Sénégal; Institut Pasteur, Dakar, Sénégal; Ecole Inter-états des Sciences et Médecine Vétérinaires, Dakar; Institut Français de Recherche Scientifique pour le Développement en Coopération

Isolation of New A Flavivirus from *Ixodes Lividus* Ticks

Lvov, D.K., Gromashevsky, V.L., Aristova, V.A., Skvortsova, T.M.,
Melnikova, E.E., and Gushchina, E.A

The D.I. Ivanovsky Institute of Virology,
Department of Virus Ecology, Moscow, Russia

Three identical strains of a new virus (LEIV 24075Tat, LEIV 24077Tat, LEIV 24078Tat) were isolated from 220 *I. lividus* ticks collected in the nests of *Riparia riparia* in June 1990 on the islands of the northern part of the Kuibyshev storage lake (Volga-Kama pool). The collected feeding ticks were immediately separated into 5 pools and were stored in liquid nitrogen.

Isolation was carried out by intracerebral inoculation of newborn mice. The incubation period of fixed strains was 3-4 days. Reisolation attempts were positive and were carried out three months after storage of tick suspensions at -65°C.

The isolated strains agglutinated goose erythrocytes at an optimal pH range of 6.4-6.6.

Complement-fixation (CF) and immunofluorescent antibody (MFA) assays with standard mouse immune ascitic fluid (IAF) and electron microscopy were used for the identification of the isolates. These isolates were determined to be members of the *Flaviviridae* family. They were found to be more closely related to Tyuleniy virus than to other flaviviruses (Langat, Negishi, Powassan, Kyasanur Forest disease, Japanese encephalitis, St. Louis encephalitis, Kokobera, Ntaya, Uganda S, Louping ill, Royal Farm, Cowbone Ridge, Entebbe bat, Koutango, Sokuluk and Banzi).

With the exception of their relationship to Tyuleniy virus, the isolates were not found to be related to most other flaviviruses by CF or minimally to a few other flaviviruses (RSSE, Apoi, West Nile and Dengue 1). Results of the identifications are presented in Table 1.

On the basis of our data, isolates LEIV 24077Tat, LEIV 24075Tat and LEIV 24078Tat may be considered to be strains of a single virus. These isolates appear to be antigenically different from all known flaviviruses except for their close one-way CF relationship to Tyuleniy virus. Thus, we would consider these isolates to be strains of a new flavivirus, provisionally named Kama virus.

Table 1

Identification of Kama virus according to the data of CF and MFA

	CF*			MFA*		
Antigen	Tyuleniy	LEIV 24077	LEIV 24075	LEIV 24078	Tyuleniy	LEIV 24077	DEN-1
Tyuleniy	160**/128***	40/16	20/8	40/16	+ + + +	+ + +	n/t
LEIV 24077Tat	40/64	160/128	80/128	80/128	+ + +	+ + + + + +	neg
Dengue 1	10/8	n/t	20/8	20/16	n/t	+ + +	+ + + +

* Initial dilution of IAF in CF = 1:8, in MFA = 1:20
** Titre of antigen
*** Titre of antibodies

SHORT COMMUNICATION

VIRUSES ASSOCIATED WITH THE EPIDEMIC NEUROPATHY OUTBREAK IN CUBA.

By the end of 1991 on the Western part of Cuba an unusual numbers of cases of optic neuritis were detected among middle aged male who were tobacco growers and that smoked heavily. they used to modestely consume alcoholic breverages. By 1992 the central part and the eastern part of the country detected cases too. During the first semester of 1993, the epidemic affected all provinces in variable degree and about 58 000 patients have been reported. According to the predominance of the symptoms two clinical form were present during the epidemic: The optic and the peripheric forms. Also mixt form were detected.

Toxic-nutritional and biological factors are argued to be involved in the pathogenesis of the epidemic. Only the biological factor has been demonstrated although it may not be the exclusive pathogenic factor in this case.

During the first semester of 1992, the isolation of a Coxsackie virus was obtained in Vero cells from two feces samples and one cerebro spinal fluids (CSF) sample, all from different patients. A weak cytopathogenic effect (CPE) was observed in more than 90% of the feces samples inoculated in the same cell line. No significant difference in the percentage or the geometric mean titer of neutralizing antibodies were detected in the sera from patients and the control group. 94.7% of 19 sera from patients neutralized the weak CPE when tested at 1:10 dilution.

In 1993 an exponential increase in the number of reported cases

took place. Ten CSF and sera were obtained from same number of patients with optic neuritis. The CSF were inoculated in 12 cell lines (namely: Vero, Hep 2, C_6-36, MRC-5, BGMK, RD, MDCK, RK-13, XL-2, BHK-21, HeLa and FL). Except XL-2 and C_6-36 which were incubated below 30 ^{o}C all the others cell lines were incubated at 37^{o}C. Two blind passages were done and the cultures were daily observed under the microscope until day 6 after inoculation. Two types of CPE were observed: in two cases a tipical enterovirus CPE (strains 35/93 IPK and 47/93 IPK) was observed after the second passage in Vero and RD cells. In the others 8 CSF a weak, slowly progresing and delayed CPE was observed in Vero cells. The weak CPE was more evident at the edges of the cell culture monolayer after three days of incubation. In the others cell lines CPE was not observed.When a passage of the weak CPE found in Vero cells was inoculated in the others cell lines a complete CPE was observed in MDCK after 24 hours, but desappeared after two more passages.

The typical enterovirus CPE was identified as Coxsackie A9 by neutralization test with LBM pool of sera.

All the patient's sera were positive by microneutralization test against 100 TCD_{50} of the 47/93 IPK strain. The titer ranged from 20 to >320 (SMT-1:69.6) The IgM were positive in 8 of the sera diluted 1:10 and tested by indirect immunofluorescence with the above mentioned strain.

In conclusion, two types of CPE were observed in Vero cells inoculated with CSF of patients with optical neuritis. One was a typical enterovirus CPE obtained in a low number of patients and it was identified as Coxsackie A9. The others was a weak and

delayed CPE observed in the Vero cell culture of all the others samples. The clinical picture and the epidemiological history of this disease has not been related to viruses infection so far. A direct transmission has not been demonstrated either. Several investigations continues in order to clarify the role these agents play in the etiology of the epidemic neuropathy in Cuba.

Más, P., Guzmán, G., Palomera, R., Castillo, A.

Institute of Tropical Medicine "Pedro Kouri"
Box 601. Havana. Cuba.

REPORT FROM VETERINARY RESEARCH INSTITUTE, IPOH, MALAYSIA

In 1987 there were severe cases of bluetongue disease amongst recently imported Australian sheep in several States of Peninsular Malaysia. BLU 1 was isolated from one of the affected sheep.

Since February 1991, bluetongue in Malaysia has been investigated in a collaborative research project by this institute and the Bureau of Resource Sciences, Canberra, Australia. This research has been partly funded by the Australian Centre for International Agricultural Research (ACIAR).

Four herds of sentinel cattle, and one sentinel sheep flock, were sited on government farms in four States and the animals were bled regularly between March 1991 and February 1992. Most of the cattle, and 12 % of the sheep, seroconverted during this period. The heparinised bloods of some of the animals were tested for virus by inoculation to eggs and cell cultures and 18 cytopathic agents were isolated.

The 18 isolates were tested by an immunospotting technique and 16 were identified as bluetongue. When the viruses grouped as bluetongue were tested by plaque reduction, the isolates were serotyped as BLU 1, 2, 3, 9, 16 and 23. The 2 isolates which did not react in the bluetongue immunospot test have been provisionally identified as EHD 6.

Current research involves investigating the role of bluetongue in a sheep pneumonia complex which occurs in native, crossbred and imported sheep and the role of bluetongue and EHD in sudden death of deer.

BLUETONGUE LABORATORY METHODS TRAINING COURSE

In August 1993, an ACIAR-sponsored training course on bluetongue laboratory methods was held at VRI, Ipoh. Laboratory scientists from China, India, Indonesia, Malaysia, Phillippines and Thailand attended. The participants from China, India and Indonesia are from laboratories with current bluetongue research projects. The others expect to be working on bluetongue soon. Most activities of the 2 week course concerned hands-on practical sessions and participants were involved in all routine methods used for antibody, antigen and virus detection.

Sharifah Syed Hassan and Geoff Gard

Outbreak of probable milk-borne tick-borne encephalitis in Slovakia

Report from WHO Collaborating Centre for Arbovirus Reference and Research, Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovak Republic

In September, 1993, 7 family members and a close friend and neighbor were hospitalized in Povazska Bystrica Hospital with suspect tick-borne encephalitis. Onset of disease occurred between 1-9 September, with high fever, headache, and influenza-like symptoms. All seven patients were later hospotalized between 21-27 September during the second phase of illness with meningoencephalitis. All cases were serologically confirmed as tick-borne encephalitis (TBE), with antibody titres rising 2-4 fold between acute and convalescent (10 days later) serum samples when examined by haemagglutination inhibition (HI) tests (titres 1:80 to 1:1280) and virus neutralization assays (titres 1:32 to 1:256).

All affected family members and associates regularly drank unboiled milk from two goats owned by the family. These goats routinely grazed in the area of high tick populations. Blood and milk specimens were obtained from the goats, and virus isolations attempted. Although no virus was isolated from the milk or blood, one goat had HI and neutralizing antibodies to TBE virus.

In Slovakia, TBE is most frequently found in the western and southern regions, with 15-20 human cases diagnosed annually. A natural foci of TBE also exist in Povazska Bystrica region in central Slovakia. From 1971 to 1991, 49 human TBE was recorded in this region. Previously, alimentary TBE was recorded in 1984 (4 cases), and in 1989 (2 cases). Both outbreaks were associated with drinking unpasteurized goat milk. The numbers of goats maintained in rural areas of Slovakia are increasing, and the reported outbreak of TBE serves as a warning of the possible re-emerging threat to human health of consumption of unpasteurized milk from domestic animals, especially goats.

(**I. Kohl**, Regional Hygiene Station Povazska Bystrica; **O. Kozuch, E. Eleckova, M. Labuda**, Institute of Virology, Bratislava, Slovakia)

Outbreaks of Barmah Forest virus disease in Western Australia.

by M.D. Lindsay[1], D.W. Smith[2], C.A. Johansen[1] and J.S. Mackenzie[1].

[1]Department of Microbiology, The University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands, WA, 6009

[2]Virology Section, State Health Laboratory Service, Queen Elizabeth II Medical Centre, Nedlands, WA, 6009.

BF virus is a mosquito-borne alphavirus, found only in Australia, that causes a similar disease to Ross River virus in humans. Most studies in eastern and northern Australia suggest that the principle mosquito vectors of BF virus are probably the same as for Ross River virus, but little is known about its vertebrate hosts. BF virus activity was first detected in western Australia (WA) when it was isolated from mosquitoes trapped in the south-east Kimberley in 1989 (Broom, *et al.*, 1989). Small clusters of human cases in WA were diagnosed by the State Health Laboratory Service in 1992 (D.W. Smith, unpublished results) and the first substantial outbreak occurred in the south-west during spring and summer of 1993-94.

The first Western Australian cases of BF virus disease were reported individually or in small clusters from towns in the Pilbara, Gascoyne, Murchison and Goldfields regions between April and September 1992. Most activity was reported from the towns of Exmouth (6 cases) and Carnarvon (4 cases). All of these early cases were during or just after much larger outbreaks of Ross River virus disease in those regions. This suggested that the two viruses may have similar mosquito vectors and require similar environmental conditions. The main environmental factor contributing to the 1992 outbreaks was extremely heavy rain in these normally arid regions during autumn and winter (Lindsay, *et al.*, 1992). Six cases of BF infection were also reported following record wet season rains in the Kimberley region in 1993. As BF virus infection is not yet notifiable in WA, the incidence of disease may be under-reported.

Research in our laboratory has shown that BF and Ross River viruses can co-circulate in the Kimberley and Pilbara regions. Both viruses have been isolated from different individuals of the same mosquito species caught in the same trap. In coastal regions the main vector of BF virus appears to be *Aedes vigilax*, a saltmarsh breeding species. Large numbers of this species develop after very high tides or heavy rains on saltmarshes. This species is implicated as a vector on the basis of numerous virus isolations just prior to and during the 1992 outbreaks, however nothing is known of its vector competence for BF virus. We have also isolated BF virus from several other species of freshwater-breeding mosquitoes in inland areas of the north-west of WA. These include *Aedes normanensis*, *Aedes eidsvoldensis*, *Aedes* E.N. Mark's species No. 85, *Culex annulirostris*, *Culex quinquefasciatus*, and *Anopheles amictus*.

In early January 1993, we isolated BF virus from *Culex annulirostris* and *Coquillettidia* species near *linealis* trapped at Karnup, 40 kms south of the capital, Perth. Two weeks later a single case was reported from an address less than 1 km from our trap site. Then, between August 1993 and March 1994, a larger outbreak occurred in the south-west of WA. Of twenty reported cases, over half were in the Peel region, some 80 to 120 kms south of Perth on the coast. Cases were also reported from Perth, and the south-west towns of Collie, Busselton, Mullalyup, Manjimup and Esperance. BF virus was isolated on 12 occasions from *Aedes camptorhynchus* mosquitoes trapped just prior to and during the outbreak in the Peel and Busselton regions, thereby implicating that species, along with *Culex annulirostris* and *Coquillettidia* species near *linealis* as vectors in the south-west region. *Aedes camptorhynchus* is also the main vector of Ross River virus in the south-west (Lindsay, *et al.*, 1989; 1992). BF virus was also isolated from *Culex annulirostris* mosquitoes trapped in the southern suburbs of Perth suggesting that under certain circumstances the virus may be transmitted to humans

in the metropolitan area. This has occurred with Ross River virus in two previous outbreaks in the south-west.

The 1993/94 outbreak of BF virus is also the first occassion anywhere in Australia in which a substantial number of cases have occurred in the absence of Ross River virus activity. Only one case of Ross River virus disease was reported from the Peel region during spring-summer 1993/94 which is the lowest recorded number of cases in spring and summer in that region. Environmental conditions and vector mosquito populations in the south-west were found to be unfavourable for Ross River virus transmission during the BF outbreak (M.D. Lindsay, C.A. Johansen, J.S. Mackenzie, unpublished results). It is not known whether the outbreak occurred because BF virus can circulate under conditions that are not suitable for Ross River virus activity, or whether extremely low levels of immunity in 'virgin' vertebrate host and human populations in the south-west may have enhanced transmission cycles.

The only evidence of BF virus activity anywhere in WA prior to 1989 and in the south-west prior to late 1992 was from a serosurvey of Ross River virus-like illnesses in the south-west in 1988-89 (L. O'Connor and M. Bucens, unpublished results). In this study, two patients were found to have evidence of infection with BF virus by HI. However, the samples were not tested for IgM antibody and no travel histories were available so it was not possible to determine whether the infections were recent and acquired in WA. No BF virus isolates were obtained from more than 400,000 mosquitoes trapped throughout the south-west between 1987 and 1992 (Lindsay, *et al.*, 1989; 1992; M.D. Lindsay, C.A. Johansen, A.E. Wright and J.S. Mackenzie, unpublished results). This suggests that the virus responsible for the recent outbreaks may have been recently introduced or re-introduced to WA after a long period of absence. The means of introduction, initially to the north-west and more recently to the south-west is not known. It is hoped that a proposed study to compare the nucleotide sequence of our recent isolates with interstate isolates, and a sero-survey of vertebrates sampled before and after the south-west outbreak, will shed some light on this matter.

We do not know whether BF virus will persist in WA, like RR virus has, either in low level maintenance cycles or by vertical transmission in certain *Aedes* mosquitoes. These questions along with others about the vector competence of different mosquito species for BF virus and environmental conditions which predispose to outbreaks must be addressed before future outbreaks can be predicted. The Health Department of WA and our laboratory recently commenced more intensive monitoring of BF and Ross River viruses in the Peel region where mosquito breeding may be enhanced by more frequent tidal inundation caused by the opening of a second channel into the Peel Inlet and Harvey Estuary. In view of the recent BF activity in WA, BF virus should be added to the differential diagnosis of patients with arthritis, arthralgia, myalgia or rash, who may have been exposed to these viruses.

References:

1. Broom, A.K., Mackenzie, J.S., Lindsay, M.D. and Wright, A.E. (Year) Epidemiology of Murray Valley encephalitis and Kunjin viruses in Western Australia, 1980-89 *Arbovirus Research in Australia,* **5**: 14-18. CSIRO and Queensland Institute of Medical Research.
2. Lindsay, M.D., Johansen, C., Wright, A.E., Condon, R., D'Ercole, M., Smith, D. and Mackenzie, J.S. (1992) The epidemiology of outbreaks of Ross River virus infection in Western Australia in 1991-1992 *Arbovirus Research in Australia,* **6**: 72-76. CSIRO and Queensland Institute of Medical Research.
3. Lindsay, M.D., Latchford, J.A., Wright, A.E. and Mackenzie, J.S. (1989) Studies on the ecology of Ross River virus in the south west of Western Australia *Arbovirus Research in Australia,* **5**: 28-32. CSIRO and Queensland Institute of Medical Research.

Nairobi Sheep Disease virus isolated from *Haemaphysalis intermedia* ticks collected in Sri Lanka

L.P. Perera[1], J.S.M. Peiris[1], C.H. Calisher[2], R.E. Shope[3], and D.J. Weilgama[4].

Departments of Microbiology[1] and Parasitology[4], Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka.
Division of Vector-Borne Infectious Diseases[2], National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado 80522,
United States of America.
Yale Arbovirus Research Unit[3], Department of Epidemiology and Public Health, School of Medicine, 60 College Street, P.O.Box 208034, New Haven, Connecticut,
United States of America.

Adult (engorged or unengorged) and nymphal ticks were collected from healthy goats at the Goat Breeding Station, Kotukachchiya, situated climatologically in the in the Intermediate zone of Sri Lanka. They were identified as *Haemaphysalis intermedia* (156 adults; 73 nymphs) or *Rhipicephalus* spp. (2 adults and 5 nymphs) held at ambient temperature (26° to 28°) for 3-4 days to allow digestion of any blood meal, and virus isolation was attempted by inoculation into 2-4 day old Swiss albino mice by the intracranial route (0.02ml/mouse). Four pools (TK260, TK257, TK258 and T4) of *H. intermedia* ticks yielded a virus. An antiserum raised in rabbits to isolate T4 neutralised all four isolates to similar titre suggesting that all four isolates were similar if not identical. Limited seroepidemiological studies using a plaque neutralizing assay showed that 6/14 of the workers at the farm, 8/9 sheep and 6/13 goats had antibodies to T4 virus.

Isolate T4 lost viability on storage, and subsequent studies to identify the tick viruses was carried out on isolate TK-260. It reacted in immunofluorescence tests with a polyvalent immune serum containing antibody to Crimean-Congo haemorrhagic fever (C-CHF) and other tick borne nairoviruses (family Bunyaviridae).

An inactivated antigen prepared from the isolate TK 260 was compared with the antigens and mouse immune ascitic fluids to nairoviruses C-CHF, Hazara, Nairobi sheep disease virus, Bhanja and Dugbe (the components of the polyvalent nairovirus immune serum) by the complement fixing test (Table). The results suggest that the isolate TK-260 is identical or closely related to Nairobi sheep disease virus.

This is the first report of the isolation of NSD virus in Sri Lanka. NSD has long been known to be a major veterinary pathogen in East Africa (reviewed by Glyn Davies 1989). Ganjam virus, now recognized as indistinguishable from Nairobi sheep disease virus, has been isolated in India from the vector *Haemaphysalis intermedia* (Dandawate & Shah 1969), and less

commonly from other species of ticks and mosquitoes (reviewed in Banerjee 1984). Veterinary and human disease has been documented (Dandawate et al 1969) and the virus has caused infections and disease in laboratory workers. Hence the isolation of NSD virus in Sri Lanka has both veterinary and medical significance.

REACTIONS OF ISOLATE TK-260 WITH MOUSE IMMUNE ASCITIC FLUIDS TO NAIROVIRUSES IN THE COMPLEMENT FIXATION TEST.

	Mouse immune ascitic fluids to				
Antigens	*CCHF*	*Hazara*	*NSD*	*Bhanja*	*Dugbe*
TK-260	0/0*	0/0	8/16	0/0	0/0
CCHF	8/8	0/0	0/0	0/0	0/0
Hazara	0/0	8/256	0/0	0/0	0/0
NSD	0/0	0/0	8/16	0/0	0/0
Bhanja	0/0	0/0	0/0	16/16	0/0
Dugbe	0/0	0/0	0/0	0/0	64/256

* reciprocal of antibody/antigen titres.

REFERENCES

Banerjee K (1984) Emerging arboviruses of zoonotic and human importance in India. In *Virus ecology*. eds. Misra A & Polasa H. South Asian Publishers, New Delhi, pp109-121.

Dandawate CN & Shah KV. (1969) Ganjam virus: A new arbovirus isolated from ticks *Haemaphysalis intermedia*, Warbuton and Nuttal 1909, in Orissa-India. *Indian Journal of Medical Research* 57: 799-804.

Dandawate CN, Work TS, Webb JKG, Shah KV (1969) Isolation of Ganjam virus from a human case of febrile illness: a report of laboratory infection and serological survey of human sera from three different states of India. *Indian Journal of Medical Research*. 57: 975-82.

Glyn Davies F (1989) Nairobi sheep disease In: *The Arboviruses: Epidemiology and Ecology*. Monath TP (ed). CRC Press, Boca Raton, Florida, Vol III, pp: 191-203.

Canines are not a Good Source for Vector Transmission of African Horse Sickness

Braverman, Y. and A. Chizov-Ginzburg
Kimron Veterinary Institute, P.O.B. 12, Bet Dagan 50250, Israel

At Bet Dagan Culicoides imicola Kieffer, C. schultzei gp. (a mammal feeder), Culex pipiens L. (a laboratory vector) and Culex univittatus Theobald were caught in significantly ($P<0.05$) smaller number near dogs than near other hosts, while Phlebotomus spp. were caught in significantly ($P<0.05$) higher numbers in dog kennels than near other farm animals. Blood meals of Culicoides spp. from Israel and Zimbabwe were negative to dogs. Only one blood meal out of sixteen of Culex pipiens caught in Israel was positive to dogs. The results indicate that the feasibility that dogs can act as a field reservoir for African horse sickness virus is remote.

Assessment of the Mouse Open-Field Activity after Infection with Ťahyňa Virus (California Serogroup, Bunyaviridae)

The laboratory mouse has become the most frequently used host model in the pathogenetic studies of arbovirus infections. However, symptoms of infection in mice usually are described only in terms of clear signs of encephalitis (convulsions, paralysis) or as total mortality, while more subtle signs remain generally unstudied. The object of this paper is to report results of our effort to quantify a specific behavior, open-field (exploratory) activity of mice infected with Ťahyňa (TAH) virus.

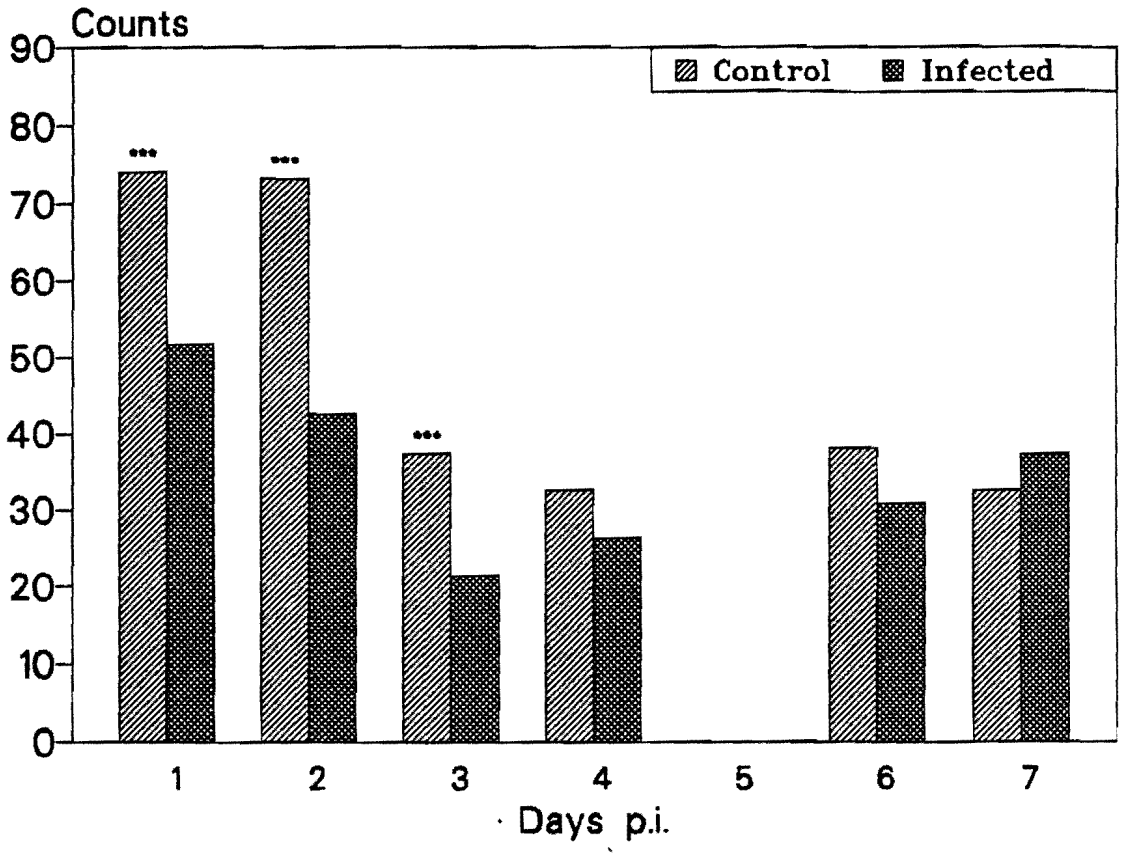
Male C57Bl/6 mice 25 days old, weighing 13 g, were distributed into two groups. Ten mice were inoculated intraperitoneally (i.p.) with 0.2 ml of 0.01% clarified infectious suckling mouse (SM) brain suspension in phosphate buffered saline, containing 2,100 SM intracerebral (i.c.) LD_{50} doses of TAH virus strain T16 at its 4th SM brain passage. Ten control mice were inoculated i.p. with 0.2 ml of 0.01% clarified normal SM brain suspension in PBS. Behavior was tested in an arena 23x30 cm in a plastic cage with a grid of six 10-cm squares painted on the floor. The animals were introduced separately to the arena and observed for 5 minutes on days 1, 2, 3, 4, 6, 7, 13 and 28 post-inoculation (p.i.). Each subject was placed in the centre of the arena, and its behavior was recorded visually as the number (counts per 5 min) of: (a) quadrant crossings (the front half of the mouse body crossing into a new area); (b) vertical erections (standing on hind limbs); (c) head raisings with sniffing. Behavior (a) was regarded as the horizontal activity, while (b) combined with (c) as the vertical activity. Between successive observations, the test field was disinfected with 1% chloramin, washed and dried to minimize olfactory signals.

The open-field activities (horizontal, vertical, and total) of infected mice were significantly ($P<0.01$) lower than those of the uninfected animals during days 1, 2, 3 and 13 p.i. The most pronounced reduction of exploratory activity in infected mice was observed on days 2-3 p.i. In control mice, a significant ($P<0.05$) consecutive decrease of open-field activity was found between days 2 and 3, 3 and 4, and 13 and 28 p.i., while a significant increase was noted between the 7th and 13th day p.i. In infected mice, a significant consecutive decrease of the activity was observed only between days 1 and 2, and 2 and 3 p.i., and no significant increase was registered. The gradual decrease of exploratory activity observed during the first days of the experiment in both control and infected mice can be attributed to habituation.

(The paper will appear in the journal '*Laboratory Animal Science*').

Zdeněk Hubálek (Academy of Sciences, Institute of Landscape Ecology, Květná 8, 60365 Brno, Czech Republic), and
Pavel Rödl (National Institute of Public Health, Šrobárova 48, Prague, Czech Republic)

Fig. Mean counts of total open-field activity of mice during the first 7 days p.i. Asterisks indicate a significant ($P<0.001$) difference between infected and control mice.



XXXIst Congress of the European Dialysis & Transplant Association, European Renal Association, Vienna, Austria. 3-6 July 1994.

1993 HANTAVIRUS (HV) EPIDEMIC IN THE ARDENNES

J. Clement, P. Colson, Ph. Damoiseaux, P. Mc Kenna, J. Coeck, J. Neyts, H. Leirs, R. Verhagen
Queen Astrid Mil. Hospital, Brussels, C.S. des Fagnes, Chimay, C.H.de Dinant, and RUCA, Antwerp, Belgium

During the most important outbreak so far of HV nephropathy (HVN) in Belgium, a total of 57 serologically proven cases were registered from Sept '92 through Jan '94 in the Southern Ardennes. IgG IFA screening established a significantly higher mean geometric titre of 989.1 for the "European" serotype *Puumala* (PUU) vs only 72.5 for *Hantaan* (X^2=37 p<0.001), respectively 48.8 for *Seoul* (X^2=33 p<0.001), the latter being both "Asian" serotypes. μ capture IgM EIA confirmed PUU as the serotype involved in all acute samples. A very dense local population of bank voles (the main rodent vector for PUU) was noted, probably related to the abundance of beechnuts (the staple food of voles) and the mild 92'-93' winter. With a capture EIA assay, we found the presence of a PUU-like antigen in a total of 10/160 (6%) voles trapped in June, Sept. and Nov. '93 around the habitats of HVN patients. All other rodent species captured (total 94) were HV- antigen negative.

Analysis of 34 completed clinical records disclosed: fever in 100%, lumbalgia in 79%, acute myopia in 26%, S.creatinine >1.2 mg% in 88% (range 1.1-9.9), and platelets < 100,000/ml in 76%. Combined dialysis and mechanical ventilation was necessary in 2 patients, 1 presenting with fluid overload, the other with shock (BP 65/40 mm Hg), diffuse intravascular coagulation, internal hemorrhages and adult respiratory distress syndrome (ARDS) (pO2 40 mmHg). Thus, European PUU-induced HVN may present with ARDS, a feature hitherto considered specific for the current HV-epidemic in the USA.

An investigative report of a febrile episode among a floating population of the members of Central Reserve Police Force, stationed at Calcutta

Report from the Department of Virology,
School of Tropical Medicine,
Calcutta, INDIA.

Dr. N. Bhattacharyya, Dr. S. K, Chakravarti, Dr. K. K. Mukherjee,
Dr. P. N. De, Dr, S. Chatterjee,& Dr. M.S. Chakraborty.

A sudden outbreak of febrile illness among members of the Central Reserve Police Force, temporarily stationed at Calcutta from various parts of the country, was reported during the month of May - July, (onset of monsoon), 1993,

The illness started with fever (ranging between 38°C - 40°C) of 3 - 5 days duration accompanied by headache, bodyache and arthralgia. No rash or, lymphadenopathy was observed in any of the victims. Convalescence was slow and prolonged. The average age group was between 20 - 45 years. No fatality was, however, reported from any of the cases.

A total of 28 blood samples were collected. Nineteen samples tested for blood culture were negative. None of the sera was positive for widal test and malarial parasites could not be detected from any of them in peripheral blood smear.

Virological studies :

Three mouse pathogenic agents could be isolated from 28 acute phase blood samples. The isolates were identified as DEN-3 by indirect immunofluorescence test (IFT), A total of 21 single sera (acute) and 7 paired sera (acute & convalescent) were tested by HI & CF test. Sero-conversion to DEN-3 was found in 4 out of 7 (57.14%) paired samples and evidences of dengue infection were found in 17/21 (80.95%) single sera. Flavivirus group reaction was observed in 2/7 paired sera. Twelve out of 21 acute phase samples, tested by MAC ELISA, showed recent infection to dengue,

Thus, the investigative report detailed above, suggests that the febrile illness is of dengue in origin. Evidences of primary infection against dengue is the hallmark of the study, This fits well with the floating nature of the members of Police Force coming from nonendemic zone to an endemic zone like Calcutta.

2º SIMPOSIO INTERNACIONAL SOBRE FLEBOTOMINOS

INTERNATIONAL SYMPOSIUM ON PHLEBOTOMINES SANDFLIES

ISOPS

MERIDA / VENEZUELA

SEPTIEMBRE 1995

PRIMER AVISO / FIRST ANNOUNCEMENT

ORGANIZACION DEL SIMPOSIO

El evento cuenta con organización internacional y local integrada de la manera siguiente:

- Presidente del Simposio
- Secretaría del Simposio
- Comité Honorario
- Comité Científico Internacional
- Comité Organizador Local
- Comité Editor Internacional

LUGAR Y RESERVACION HOTELERA

La ceremonia de apertura del evento se realizará en el Aula Magna del edificio del Rectorado de la Universidad de Los Andes en la ciudad de Mérida y el programa científico será realizado en otras instalaciones de la misma ciudad, donde existen hoteles confortables y de bajo costo.
Se proveen descuentos especiales para estudiantes.
Mérida es una ciudad turística y universitaria, ubicada en una meseta de la Sierra Nevada en los Andes venezolanos.
Más detalles serán dados en el segundo aviso.

ORGANIZATION OF THE SYMPOSIUM

This event will be organized locally and internationally as folows:

- Presidency of te Symposium
- Secretary of the Symposium
- Honorary Committe
- International Scientific Committee
- International Editorial Committee
- Local Organizing Committee

SITES AND HOTELS PROGRAM

Mérida is a touristic and universitary city, located in an upland of the Sierra Nevada of the Venezuelan Andes.
The opening ceremony will take place in the Aula Magna, auditorium of the Rectory of the Universidad de Los Andes, and the scientific program will take place in other buildings in the city. There are confortable hotels with reasonable prices and with probable special discounts for students.
More details will be given in the next comunication.

REQUEST TO PRESENT A PAPER AT THE 1995 SECOND INTERNATIONAL SYMPOSIUM ON PHLEBOTOMINE SANFLIES

IMPORTANT!!!
REQUEST TO BE RECEIVED BY APRIL 94

MAIL TO:

J.V. Scorza / Elina Rojas
Secretaría de II ISOPS
Centro "José Witremundo Torrealba" U.L.A. - NURR
Laboratorio de Quimioterapia y Control de Vectores.
Av. Carmona Sector Los Ilustres
Apdo. Postal 100
Telf.: 58-72-33503
Fax: 58-72-33503
Trujillo 3102 - Venezuela

QUOTES

Robert Orben: "Illegal aliens have always been a problem in the United States. Ask any Indian."

Groucho Marx: "If I had my life to live over, I'd live over a delicatessen."

Robert Byrne: "One reason people get divorced is that they run out of gift ideas."

Larry Brown: "Capital punishment is either an affront to humanity or a potential parking place."

Eugene McCarthy: "The only thing that saves us from the bureaucracy is its inefficiency."

Bertrand Russell: "It is undesirable to believe a proposition when there is no ground whatever for supposing it is true."

Ashok Kumar: "What will it say about the human race if we let the tiger go extinct? What can we save? Can we save ourselves?"

Maurice Provost: "I can think of no better way to be corporeally united with the biosphere than to have one's blood spread over the landscape in the form of eggs from a thousand flies."

A. Bartlett Giamatti: "Of course, there are those who learn after the first few times. They grow out of sports. And there are others who were born with the wisdom to know that nothing lasts. These are the truly tough among us, the ones who can live without illusion. I am not that grown-up or up-to-date. I am a simpler creature, tied to more primitive patterns and cycles. I need to think something lasts forever, and it might as well be that state of being that is a game; it might as well be that, in a green field, in the sun."

Aldous Huxley: "Experience is not what happens to a man; it is what a man does with what happens to him."

Jean de la Bruyère: "Life is a tragedy for those who feel, and a comedy for those who think."

Catherine Drinker Bowen: "I speak the truth, not so much as I would, but as much as I dare; and I dare a little more, as I grow older."

John Heisman (explaining on the first day of practice what a football is): "A prolate sphere-- that is, an elongated sphere-- in which the outer leathern casing is drawn tightly over a somewhat smaller rubber tubing. Better to have died a small boy than to fumble this."

Inscription found on the wall of a cellar in Cologne, where Jews had hidden from the Nazis: "I believe in the sun even when it is not shining. I believe in love even when I do not feel it. I believe in God even when He is silent."

George Bernard Shaw: "A man who has had his dinner is never a revolutionist: his politics are all talk."

Frederick Sanger (Nobel Laureate, Chemistry): "Most experiments go wrong."

Spanish proverb: "God helps those who get up early."

Harry Collins and Trevor Pinch (in The Golem: What everyone should know about Science): "Scientists are neither Gods nor charlatans; they are merely experts, like every other expert on the political stage. They have, of course, their special area of expertise, the physical world, but their knowledge is no more immaculate than that of economists, health policy makers, police officers, legal advocates, weather forecasters, travel agents, car mechanics, or plumbers. The expertise that *we* need to deal with them is the well-developed expertise of everyday life; it is what we use when we deal with plumbers and the rest. Plumbers are not perfect -far from it- but society is not beset with anti-plumbers because being anti-plumbing is not a choice available to us. It is not a choice because the counter-choice, plumbing as immaculately conceived, is likewise not on widespread offer."

George B. Shaw: "If all economists were laid end to end they would not reach a conclusion."

Dorothy Parker: "If all the girls at Vassar were laid end to end I wouldn't be a bit surprised."

Will Durant: "Education is the progressive discovery of one's own ignorance."

C. Beaudelaire: "Being unable to abolish love, the Church has decided at least to disinfect it, and has invented marriage."

Mao Zedong: "Revolution is not a dinner party, nor an essay, nor a painting, nor a piece of embroidery; it cannot be advanced softly, gradually, carefully, considerately, respectfully, politely, plainly, and modestly."

Jordi Casals: "The essence of science is to try to prove oneself wrong."

Gelett Burgess: "To appreciate nonsense requires a serious interest in life."

Kin Hubbard: "Some people are so sensitive that they feel snubbed if an epidemic overlooks them."

Blaise Pascal: "I have made this letter longer than usual, because I lack the time to make it short."

Herb Gold: "Haiti under the Duvalliers was a Kleptocracy."

Branch Rickey: "Luck is the residue of design."

Roy Chamberlain: "No amount of work can change a weak piece of work into a strong one."

Emiliano Zapata: "Tierra y Libertad" ("Land and Liberty")

Oscar Wilde: "It is only shallow people who do not judge by appearances."

Persius: "Confront disease at its onset." ("Venienti occurrite morbo.")