December, 1989

TABLE OF CONTENTS

(Continued on next page)

IMPORTANT NOTICE: This Exchange is issued for the sole purpose of timely exchange of information among people studying all aspects of arboviruses. It contains reports, summaries, observations, and comments submitted voluntarily by qualified agencies and investigators. The appearance of any information, data, opinions, or views in this Exchange does not constitute formal publication and should not be either referred to in "Reference" sections of manuscripts or included in lists of publications; it is not a "peer reviewed" publication. Any reference to or quotation of any part of the Exchange must be authorized directly by the agency or person submitting the text.

DENGUE

VECTOR COMPETENCE AND VECTOR BIOLOGY

(continued)

(continued)

EPIDEMICS AND SURVEILLANCE

(continued)

Editor's comments

Dengue, Japanese encephalitis, African horsesickness, yellow fever, Oropouche fever, eastern equine encephalitis, Semliki Forest virus infection, tick-borne encephalitis, bluetongue and other arthropod-borne virus infections continue to rear their ugly heads--arbovirus diseases are with us. No wonder the Arbovirus Information Exchange remains a popular mechanism for trafficking in observations and laboratory data!

It is gratifying to receive so many notes of thanks for editing the Arbovirus Information Exchange from people submitting reports. Such notes encourage me and make my efforts easier, knowing they are worthwhile to arbovirologists; res ipso loquitur. I try to be pragmatic about these things but, as my wife said, "Pragmatism only works in evolution, and then you get knees."

You will note the addition of an INVITED COMMENTARY (pages iv-v). I will try to obtain for each issue a commentary about a timely topic. Commentary volunteers are encouraged.

The next issue is scheduled to be mailed June 1, 1990 (deadline for submissions May 20, 1990). There is nothing that requires you to wait until the last minute. If you have something to communicate in January, February, March, or April, please send it to me.

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

Best regards for a healthy, happy, and peaceful 1989. If your particular calendar does not soon change to a new year, then I send good wishes to you for the remainder of your year and beyond.

Charlie Calisher

Charles H. Calisher, Ph.D.

PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE:

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

We want to keep this mechanism timely and viable. Therefore, send me only recent news and summaries of your work. **PLEASE** limit the submission to 1 or a very few sheets (21.59 cm x 27.94 cm = 8.5 x 11 inches) plus a table or two; condense as much as you can (**single space** the text); **do not** staple pages together; **do not** number pages. This is essentially a one person operation and I am basically a lazy person; the less work I need to do, the better I like it. The American Committee on Arthropod-borne Viruses, Subcommittee on Information Exchange (Chairman, Dr. Nick Karabatsos), which supervises this effort, has agreed that anyone not submitting a report in any two year period will be dropped from the mailing list. Submission of a brief report seems a small price to pay for all this information.

SEMIANNUAL QUOTES (There being no point in wasting space)

Pablo Picasso: "Computers are useless. They can only give you answers."

Samuel Johnson: "I found your essay to be good and original. However, the part that was original was not good and the part that was good was not original."

Damon Runyon: "The race is not always to the swift, nor the battle to the strong, but that is the way to bet."

Oscar Wilde (on his deathbed): "Either that wallpaper goes or I do."

Gustave Flaubert: "May I die like a dog rather than hurry, by a single second, a sentence that isn't ripe."

Greek proverb: "Though boys throw stones at frogs in sport, the frogs do not die in sport but in earnest."

G. B. Shaw: "The man who has never made a mistake will never make anything else."

Theodore Roosevelt: "It is not the critic who counts, not the man who points out how the strong man stumbled, or where the doer of deeds could have done them better. The credit belongs to the man who is actually in the arena; the man whose face is marred by dust and sweat; who strives valiantly, who errs and comes short again and again; who knows the great enthusiasms, the great devotions, and spends himself in a worthy cause; who, at the best, knows in the end the triumph of high achievement; and who, at the worst, if he fails, at least fails while daring greatly, so that his place shall never be with those cold and timid souls who know neither victory nor defeat."

X EDITION OF ASTMH "HEALTH HINTS FOR THE TROPICS" AVAILABLE ON JULY 1, 1989- COMPLIMENTARY COPY TO BE MAILED TO ALL ASTMH INDIVIDUAL MEMBERS

The X (1989) Edition of the ASTMH publication "Health Hints for the Tropics" written by eight members of the American Committee on Clinical Tropical Medicine and Travelers Health (ACTM&TH) is now available as a supplement to the Tropical Medicine and Hygiene News. Individual ASTMH members will receive a complimentary copy by Third Class Bulk (domestic) or surface foreign mail. Additional copies may be purchased through the Editor of the News.

"Health Hints for the Tropics" first appeared in 1948 and has been up-dated in new or slightly revised editions since that time. The past several editions have been edited by Harry Most, M.D. The X Edition is the first produced by a formal editorial committee. At 51 pages, it is 20% larger than the 1986 IX Edition and consists of individually authored chapters on **Preparation for Travel** (Leonard C. Marcus, V.M.D., M.D.); **Immunizations** (Elaine C. Jong, M.D.); **Malaria Prevention** (Jay S. Keystone, M.D.); **Traveler's Diarrhea** (William S. Kammerer, M.D.); **Special Health Problems** (Frank J. Bia, M.D. and Michele Barry, M.D.); and **Advice While Traveling and on Return from Travel** (Martin S. Wolfe, M.D.). Dr. Wolfe served as overall Editor for the X Edition.

The objective of "Health Hints" is to provide a useful and authoritative source of information for the traveler going on limited vacation or business trips, longer term residents in tropical areas and for organizations sending persons abroad. Medical practitioners responsible for advising travelers to the tropics have provided "Health Hints" as a general guide to staying healthy through the knowledge and application of clinical principles, preventive sanitation, and common sense.

In order to encourage and stimulate its wide-spread use by ASTMH members and other health practitioners, the price for orders of 100 copies or more has been kept at the 1986 level. The price for the X Edition of "Health Hints for the Tropics" (post-paid) is:

o Single copies	@ US$	4.00	First Class Mail
o 2 to 9 copies	@	3.75	First Class Mail
o 10 to 49 copies	@	3.50	Third Class Mail
o 50 to 99 copies	@	3.00	Parcel Post
o 100 or more copies	@	2.25	Parcel Post

There will be an additional charge for a higher class of postal service. A check or money order for the appropriate amount in U.S. dollars should accompany orders of less than ten copies. Larger orders may be invoiced for later payment by the recipient. Orders should be sent to:

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AMPLIFICATION OF NUCLEIC ACID ANALYTES OR PROBES FOR ARBOVIRUS DIAGNOSIS

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Arbovirus diagnosis has traditionally depended on standard virus isolation or serologic techniques. Most of these techniques are tedious and time-consuming. Currently, the main emphasis in diagnosis is the development of rapid, reliable tests that can be applied in field and clinical situations. These clinically relevant tests include immunological and molecular techniques that provide diagnostic results in a time frame that permits therapeutic intervention by clinicians and institution of control measures in epidemics by public health officials. These techniques, such as enzyme immunoassays and nucleic acid hybridization, are capable of direct detection of arboviruses in clinical specimens. Hybridization techniques permit diagnosis in conditions where virus isolation and antigen detection techniques are ineffective. Conventional blot hybridization techniques for detection or RNA viruses are relatively insensitive. However, powerful new approaches are now available to increase sensitivity of nucleic acid hybridization techniques.

The polymerase chain reaction (PCR) amplification technique may become a potent tool for arbovirus diagnostic research. PCR is essentially a simple method for amplifying nucleic acids. In the PCR techinique, the anylate sequences are denatured and two oligonucleotide primers are annealed to complementary strands on either side of the analyte sequences. These primers are then extended by the Taq DNA polymerase, a thermostable DNA polymerase derived from the Thermus aquaticus. The new double-stranded analytes are denatured, the primers annealed and then extended by the polymerase. The amount of anylate is increased geometrically with each cycle and a single copy of DNA can be increased up to 1,000,000 copies. After amplification, analyte sequences can be detected using a labeled probe specific for a portion of the amplified sequence.

PCR is being applied in molecular biology laboratories around the world for use in diagnostics, sequencing, cloning, and population genetics. However, for the PCR technique to be applied for arbovirus diagnosis, several considerations need to be addressed. Nearly all of the arboviruses have RNA genomes, therefore, reverse transcriptase is required to make the initial cDNA copy of the viral gene or genome. Sequence information must be available to synthesize the oligonucleotide primers. The technique has been used to amplify La Crosse virus RNA (Grady and Campbell, 1989). They were able to successfully amplify large sequences of the La Crosse virus genome corresponding to the middle-size RNA segment (M segment), starting with first-strand

cDNA. Thus, it is likely that PCR can be widely used in arborvirology.

A new molecular technique that overcomes some of these considerations involves amplifying the reporter system. The Q-beta replicase system can be exploited to amplify quickly, the signal in hybridization tests. The Q-beta replicase can amplify midivariant RNA (MDV-1) 10^9-fold in a single 30 minute incubation (Lizardi, et al., 1988). The probe sequences are inserted into the MDV-1, and after hybridization to the target analyte, the MDV-1 can be rapidly and specifically amplified by the addition of Q-beta replicase. Reverse transcriptase is not needed. This technique could theoretically probe for the concensus sequences on the ends of segmented genome arboviruses, thereby amplifying the target sequences to be detected in a sample.

The technique can also be adapted to be a homogenous assay by the use of a molecular switch strategy described by Kramer and Lizardi (1989). A molecular switch, which can be genetically engineered into a probe, changes its conformation upon specific hybridization. A probe could be inserted into the double-stranded RNA recognition site of ribonuclease III; hybridization to analyte destroys the recognition site. When ribonuclease III is added to the suspension, all unbound probe is destroyed and the bound probe can subsequently be amplified.

The arbovirus diagnostician needs to select the appropriate test to reach the correct diagnosis. The test of choice may not always be the newest, most exciting, or most technologically advanced. For example, PCR and other very sensitive reporter systems may be required for efficient detection of arbovirus analyte in vertebrate specimens. However, these techniques may not be required for the detection of arboviruses in vectors. The vector is a little PCR machine that synthesizes large amounts of analyte. Therefore, more effective hybridization techniques may be all that is necessary for detection of virus in vectors.

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Lizardi PM, Guerra CE, Lomeli H, Tussie-Luna I, and Kramer FR (1988). Exponential amplification of recombinant-RNA hybridization probes. Bio/technology 6:1197-1202.

Report from Instituto Evandro Chagas-FSESP (Av. Almirante Barroso 492, 66050, C.P. 1128), Belém, Para, Brazil

Isolation of dengue-2 virus from a human traveling from Luanda, Angola and first isolation of this virus in Brazil.

We describe the first isolation of dengue-2 virus in Brazil. The virus was isolated from the blood of a 28-year old female from Para State, Brazil on the third day of her illness. At the time of her illness the patient was a government worker residing in Luanda, Angola and had just returned to Brazil for personal holiday. From Luanda her trip took her to Rio de Janeiro (only long enough to make airline flight connections), Brasilia (two days), and Belém, where she arrived ill. Her symptoms were: fever, headache, chills, nausea, general myalgias (particularly severe in the lumbar region of the back and the lower extremities), anorexia, and constipation. The clinical course lasted 8 days; there were no sequelae. The hemogram, taken on the fourth day of illness, showed leukopenia (3,000 leukocytes) and relative lymphocytosis (49%). Examination for Plasmodium, done for differential diagnosis, did not reveal parasites. Virus isolation attempts from patient serum was made using suckling albino Swiss mice 2- to 4-days old, and C6/36 (Aedes albopictus) cells. Mice became sick irregularly 9-14 days after intracranial inoculation and C6/36 cells showed cytopathic effects (syncytia and vacuolization) beginning on the fifth day after inoculation. Identification of the isolate was by complement-fixation of brains from infected suckling mice and by indirect immunofluorescence (monoclonal antibodies) using infected cell cultures. Reisolation was successful.

This finding may be of great importance in terms of hemorrhagic dengue in Brazil. We already have recognized the presence of millions of cases of dengue-1 virus infections in this country. Therefore the presence of this particular dengue serotype (type 2), superimposed on sensitization of humans with dengue-1 virus, and the presence of Aedes aegypti mosquitoes (the classical vector of urban dengue in this hemisphere), are risk factors of considerable interest and potential importance.

A.P.A. Travassos da Rosa, P.F.C. Vasconcelos, J.F.S. Travassos da Rosa, and S.C. Guerreiro (Virus Section)

VERTICAL TRANSMISSION OF DENGUE VIRUSES BY STRAINS OF *AEDES ALBOPICTUS* RECENTLY INTRODUCED INTO BRAZIL

Strains of *Aedes albopictus* from Anchieta and Santa Teresa, Espirito Santo State, and Sao Paulo, Brazil, were examined for their ability to vertically transmit dengue 1 (DEN-1) and dengue 4 (DEN-4) viruses. Parental females were uniformly infected by parenteral inoculation of virus and 2,512 F_1 progeny from DEN-1 infected mothers and 5,609 F_1 progeny from DEN-4-infected females were pooled in lots of approximately 50 and tested for virus. Seven of 60 pools were positive for DEN-1 virus and 1 of 121 pools was positive for DEN-4 virus. In DEN-1 assays, the minimum infection rate for larvae (only 2 pools tested) was 1:84. Among positive cohorts of adults, pooled by sex and by geographic strain of mosquito, the MIR ranged from 1:193 to 1:626 for males and from 1:187 to 1:311 for females. Only a single pool of adult females was positive for DEN-4 virus (MIR 1:1022 for adult female cohort from Santa Teresa). The known susceptibility of *Ae. albopictus* from Brazil and elsewhere to oral infection with dengue viruses, coupled with a high degree of vertical tramsmission, indicate that this species could play a role in the maintenance of dengue viruses in nature.

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CUMMULATIVE DENGUE VIRUS ISOLATIONS 1962-88, DEPT OF VIROLOGY, ARMED FORCES RESEARCH INSTITUTE OF MEDICAL SCIENCES, BANGKOK 10400, THAILAND

A major activity of the Department of Virology has been to provide laboratory support to the hemorrhagic fever ward of the Bangkok Children's Hospital. This hospital is one of several major treatment centers for dengue hemorrhagic fever (DHF) in Bangkok (estimated population 6,000,000). The case series of dengue infections studied in this hospital is representative of severe dengue infections in Bangkok, a city with one of the world's most significant dengue public health problems.

Periodically, investigators have requested information on the prevalence of dengue serotypes in Bangkok. The unpublished data below are the best available estimates of the dengue serotypes causing disease sufficiently severe to require hospitalization, in the metropolitan area during the period 1962-1988. From 1978 onwards, virtually every child admitted to the Children's Hospital hemorrhagic fever unit with clinically suspected dengue was evaluated with culture of at least serum or plasma. More than 90% of cases were confirmed dengue infections (by serology or culture). More than 80% had plasma leakage and fulfilled WHO criteria for diagnosis of DHF.

Dengue Virus Isolations Made at AFRIMS From Patients Admitted to the Hemorrhagic Fever Ward, Children's Hospital, Bangkok, Thailand

Year	Cases	Confirmed Isolations	Number of Isolates (% of Total) Den-1	Den-2	Den-3	Den-4	Major Types
1962	148	50	17 (34)	23 (46)	9 (18)	1 (2)	2/1/3
1963	156	35	8 (23)	10 (29)	17 (49)	0	3/2/1
1964	333	105	29 (28)	53 (50)	20 (19)	3 (3)	2/1/3
1965	88	12	0	8 (67)	3 (25)	1 (8)	2/3
1966	55	10	7 (70)	1 (10)	0	2 (20)	1
			1967-1972, No data				
1973	135	22	5 (23)	13 (59)	4 (18)	0	2/1/3
1974	151	21	8 (38)	7 (33)	6 (29)	0	1/2/3
1975	399	14	1 (7)	8 (57)	5 (36)	0	2/3
1976	176	9	0	6 (67)	1 (11)	2 (22)	2/4
1977	495	66	0	37 (56)	10 (15)	19 (29)	2/4
1978	185	33	0	28 (85)	1 (3)	4 (12)	2
1979	301	61	2 (3)	58 (95)	0	1 (2)	2
1980	788	240	50 (21)	174 (72)	14 (6)	2 (1)	2/1
1981	196	36	11 (31)	21 (58)	3 (8)	1 (3)	2/1
1982	206	25	4 (16)	17 (68)	1 (4)	3 (12)	2/1
1983	400	53	3 (6)	25 (47)	15 (28)	10 (19)	2/3
1984	745	181	1 (1)	69 (38)	46 (25)	65 (36)	2/4/3
1985	506	113	3 (3)	45 (40)	25 (22)	40 (35)	2/4/3
1986	559	86	20 (23)	35 (41)	30 (35)	1 (1)	2/3/1
1987	1370	249	28 (11)	90 (36)	131 (53)	0	3/2
1988	420	108	10 (9)	58 (54)	40 (37)	0	3/2
Totals	7812	1529	207 (14)	786 (51)	381 (25)	155 (10)	2/3/1

Most isolations 1962-1965 were made in suckling mice. Isolations 1966-1978 were made in cell culture, principally LLC-MK_2 (monkey kidney). Isolations made since 1978 were by intrathoracic inoculation of Toxorhynchites splendans. Specimens for isolation were serum, plasma, leukocytes or tissue obtained at autopsy.

Serotyping was by neutralization 1962-81 and by EIA using WRAIR Mabs 1982 to present.

Dengue 2 has been the most frequent isolate in 17 of 20 years. In the past 10 years, the period when the most representative sampling of patients hospitalized with suspected dengue was performed, this serotype was dominant until 1987. In 1987 and 1988, dengue 3 replaced dengue 2 as the leading isolate. This switch was preceeded by a 4 year period during which dengue 3 was noted to be responsible for an increasing proportion of cases to which a specific etiology could be assigned.

Dengue 1 and dengue 4 have been responsible for a major proportion of cases over 2 to 3 year intervals, but these serotypes apparently have been of less significance when the 20 year experience is totaled.

The circulation of dengue viruses in Bangkok is not representative of all of Thailand. Limited studies have been conducted by the Department in cities other than Bangkok. While the proportions of serotypes isolated in a year has varied geographically, we can state that in every recent survey within Thailand, multiple dengue serotypes have been identified.

Contributed by: Ananda Nisalak, M.D., Bruce Innis, M.D., AFRIMS and Suchitra Nimmannitya, M.D., Chidren's Hospital, Bangkok, Thailand

Molecular Characterization of Dengue Viruses

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The nucleotide and encoded amino acid sequences of genes determining the structural proteins of the prototype dengue type 2 (Den-2) virus, NGC strain, and three Malaysian Den-2 viruses, M1, M2 and M3, isolated from patients with dengue haemorrhagic fever, dengue shock syndrome and dengue fever, respectively, have been completed. We have also determined the nucleotide and encoded amino acid sequences of the non-structural protein NS1 gene of M1, M2 and M3.

We determined the nucleotide sequences by direct RNA sequencing, achieved by primer extension with synthetic oligonucleotide primers and the Sanger dideoxynucleotide chain termination method.

Nucleotide and encoded amino acid sequences of the structural protein genes of M1, M2, M3 and NGC

Analyses of the nucleotide and encoded amino acid sequences of the capsid genes of M1, M2, M3 and NGC indicated that M1 and M2 are more related to each other (98% similarity) than to M3 (94-95% similarities) at the nucleotide level. At the amino acid level, M1 and M2 are identical to each other, and each shows 97%

similarity to M3. At the nucleotide level, NGC is slightly more related to M3 (96% similarity) than to M1 and M2 (94% similarity). However, at the amino acid level, NGC shows almost identical similarities (97%) to M1, M2 and M3.

Analyses of the nucleotide and encoded amino acid sequences of the prM genes of M1, M2, M3 and NGC indicated that M1 and M2 are more related to each other (99% similarities) than to M3 (92-94% similarities) at both the nucleotide and amino acid levels. NGC is more related to M3 (93-95% similarities) than to M1 and M2 (90-93% similarities) at both the nucleotide and amino acid levels.

Similarly, analyses of the nucleotide and encoded amino acid sequences of the membrane genes of M1, M2, M3 and NGC indicated that M1 and M2 are more related to each other (93-96% similarities) than to M3 (89-92% similarities) at both the nucleotide and amino acid levels. NGC is more related to M3 (95-96% similarities) than to M1 and M2 (89-92% similarities) at both the nucleotide and amino acid levels.

Comparative analyses of the nucleotide and encoded amino acid sequences of the envelope genes of M1, M2, M3 and NGC indicated that M1 and M2 are more related to each other (98% similarities) than to M3 (91-92% similarities) at both the nucleotide and amino acid levels. NGC is more related to M1 and M2 (94-96% similarities) than to M3 (88-91% similarities) at both the nucleotide and amino acid levels.

From the data generated, we observed that most of the genomic variations occur in the third position of a codon coding for an amino acid. These variations usually give rise to silent mutations and therefore do not result in pronounced amino acid variations in the structural proteins of these viruses. Comparative nucleotide and amino acid sequence analyses showed that M1 and M2 are more closely related to each other than to M3, with NGC showing similar relatedness to all three Malaysian virus isolates.

Nucleotide and encoded amino acid sequences of the nonstructural protein NS1 gene of M1, M2 and M3

The NS1 gene contains 1056 nucleotides, and it encodes a polypeptide of 352 amino acids. The three Den-2 isolates show similarities greater than 95% at the nucleotide level. We noticed that nucleotide changes are scattered throughout the gene, but occur mostly in the third position of the codons, and the majority of these changes do not give rise to amino acid substitutions. Therefore, the degree of similarity is higher at the amino acid level. We found that the NS1 proteins of M1 and M2 are almost identical (99.4% similarity). M3 has 98.7 and 98.3% similarities with M1 and M2, respectively. These data show that the NS1 proteins of M1, M2 and M3 have a high degree of relatedness.

Comparisons of M1, M2 and M3 with other Den-2 strains showed similarities of 90-94% at the nucleotide level and 95-98% at the amino acid level. Like other DEN-2 strains, the Malaysian isolates are less similar to the other dengue serotypes,

with amino acid similarities ranging from 71.6 to 77.6%. It was observed that the NS1 proteins of M1, M2 and M3 have several features which are common in all dengue viruses. The proteins contain (i) two potential N-linked glycosylation sites, at amino acid residues 130 and 207; (ii) twelve conserved cysteine residues, six of which are located within a 50 amino acid stretch from residues 280-330; and (iii) one possible cleavage site with consensus sequence "Val-( )-Ala", at residues 350-352.

The information available thus indicates that the NS1 protein is highly conserved and it may have an important role in viral replication and/or morphogenesis.

ANTIGENIC ANALYSIS OF ENVELOPE GLYCOPROTEIN OF DENGUE VIRUS TYPE 1 (Preliminary Report).

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Dengue type 1 virus (D1V, Mochizuki strain) propagated in Aedes albopictus C6/36 cells or in Vero cells was used.

Anti-D1V monoclonal antibodies were prepared from fusion cultures of D1V-infected Balb/c mice spleen cells and myeloma SP2/0. ELISA and HI titers were measured. So far in our present study, three groups of cell clones were obtained: (I) clones showing common ELISA reactivity to DEN and JE; (II) those showing high reactivity to DEN but not to JE; and (III) those possessing the intermediate reactivity. The group II also showed higher HI titers to DEN than to JE.

^{35}S-labeling of viral proteins was carried out as follows: Cell cultures were fed with medium supplemented with ^{35}S-methionine. Some of the cultures were added with actinomycin-D. At appropriate time of incubation, culture fluid was taken and centrifuged, first, at 10,000 x g for 30 min, and next, at 105,000 x g for 2 hr. The resulting pellet was used as labeled virions. The infected cells were lysed in Tris buffer (pH 8.0) containing 140 mM NaCl plus 2% NP40 and centrifuged at 10,000 xg for 30 min. The supernatant was used as cellular extracts.

Radioimmunoprecipitation and SDS polyacrylamide gel electrophoresis (SDS/PAGE) were performed by the method of Laemmli (Nature, 227, 680,1970).

The results obtained indicated: Actinomycin-D enhanced production of viral proteins, especially protein E; no significant difference in electrophoretic patterns was noted between the materials derived from C6/36 cells and those from Vero cells. Extended studies are being done based on these preliminary data.

(Reported by SUSUMU HOTTA, M.D.)

AN EASY AND EFFICIENT METHOD FOR ISOLATION OF DENGUE VIRUS (DV) FROM PATIENTS AND ANALYSES OF DATA OBTAIND DURING AN EPIDEMIC OF DHF AT JAKARTA IN 1988.

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Encountering an outbreak of DHF at Jakarta, Indonesia in 1988, we attempted to isolate etiologic viruses from patients. Procedures of virus isolation and typing were as follows: Aedes albopictus C6/36 cell cultures were used. The fluid phase of well-grown cultures was replaced with medium supplemented with heparin (30 μ/ml) and the culture plates were covered with a plastic sealer. Immediately after the peripheral blood was taken from patients by the venipuncture, two drops thereof were inoculated onto the cultures by inserting the needle through the sealer which had been wiped with ethanol. After being put at 28C overnight, the cultures were washed with PBS to remove heparin and blood, added with new medium and incubated at 28C for further several days. The inoculation could be done at bedside without any difficulty.

By applying the above techniques, 17 strains of DV were isolated from 100 blood specimens. The isolates were identified and typed by immunofluorescent antibody methods using anti-dengue hyperimmune mouse ascitic fluids and type-specific monoclonal antibodies. Fourteen strains were of type 3 and the remaining three of type 1.

All the viruses were recovered from patients of the age 3 to 12, with one exceptional case of unknown age. No significant difference was noted in virus isolation rates between male and female patients.

The possibility of virus isolation was apparently related to the time of blood sampling; specimens taken in the earlier stage (within 3 days adter admission to hospital) appeared to be better than those taken later.

The viruses were isolated from patients of various severity, i. e., from each of WHO Grades I - IV, although there was a superficial tendency that the positive rates were higher with specimens of Grades I - II than with those of Grades III - IV.

(The authors are greatly indebted to Dr. Usman and the staff of the Department of Microbiology, University of Indonesia, and medical and nursing staffs of the Department of Child Health, Cipto Mangunkusmo Hospital, all of whom gave us unlimited expert help. Thanks are also due to Prof. Takeo Matsumura of the Department of Medical Zoology, Kobe University, for his kind advice. The study was financially supported by Grants-in-Aid from Ministry of Education, Science and Culture: No. 62041100 and No. 63043070.)

Reported by: SUSUMU HOTTA, M.D.

Report from the
Sir Albert Sakzewski Virus Research Laboratory
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Cross-reaction Patterns of a Panel of Mouse Monoclonal Antibodies Raised Against the Dengue 2 Virus Nonstructural (NS1) Glycoprotein.

Although previous workers have recorded dengue group cross-reactive epitopes on the non-structural glycoprotein, NS1, of dengue viruses using polyclonal sera (1-4), we have found a predominantly type-specific response using mouse polyclonal antisera (see figure). The figure shows the ELISA titre (t_{50}) of two mabs (NS1 and E specific) in addition to polyclonal antisera specific for the four dengue virus serotypes against a substrate of whole virus (left panel) or affinity purified NS1 (right panel). This finding in part, reflects the observation that purified dengue 2 virus NS1 offers only partial protection against homologous but not heterologous virus challenge in the mouse model (3).

Recently, the native configuration of the NS1 glycoprotein has been shown to be a dimer (5), both as an intracellular, membrane associated form (mMS1) and an extracellular, secreted form (sNS1) (own observations, see Brandt,1988). At present, no monoclonal antibodies (mAbs) have been described which react with dengue group cross-reactive epitopes or epitopes present only on the native, dimeric structure of this glycoprotein. In this study, we attempted to generate mouse monoclonal antibodies specific for NS1 epitopes that described both of these characteristics.

Spleen cells of Balb/c mice hyperimmunized with affinity purified, dimeric sNS1 of dengue 2 virus (PR159) were fused with the mouse plasmacytoma cell line SP2/0 Ag14. 173 anti-NS1 clones were selected and supernatants were further screened for dimer specificity and their ability to cross-react within the dengue group. A basic panel of 35 mouse monoclonal antibodies were selected and further assessed for their ability to cross-react on immunoblots with other antigenic groups (6) within the Flaviviridae (see Table). As previously described, many of the mAbs in this panel were found to be dengue 2 specific, but although most of these reacted with conformational epitopes, 3 clones were found to describe a linear, dengue 2 specific epitope thought to be different from that previously recorded (7,8). A number of other clones were specific for conformational epitopes which are cross-reactive with the other dengue virus serotypes (including a sub-group specificity between dengue type 2 and 4 viruses, as seen in the mouse polyclonal sera - see figure). At least two conformational and 1 linear epitopes were found to be present on all 4 serotypes of dengue virus NS1. In addition, a high level of cross-reaction was observed between this panel of mAbs and members of antigenic group III flaviviruses. Interestingly, sera obtained from rabbits hyperimmunized with purified dimeric dengue 2 sNS1 were also found to show a similar pattern of cross-reaction with the exception of one member assigned to the "unknown" antigenic group (MML). Of particular interest are the four mabs which cross-react with members of antigenic group III but only with dengue serotypes 2 and 4.

Three dimer specific mAbs were also obtained which describe at least 2 different epitopes, shown by their variable cross-reaction pattern with other dengue 2 isolates. No clones were found to differentiate between sNS1 and mNS1 and with the exception of the dimer specific clone, 5H10.3, all mAbs in this panel reacted with each of a group of 23 dengue 2 virus isolates representing different geographical topotypes.

Further studies using this panel of mAbs are now being carried out in order to locate potential protective, cross-reactive epitopes on this protein, to synthesize these epitopes and to study their ability to protect animals against homologous and heterologous virus challenge.

(A.K.I. Falconar and P.R. Young)

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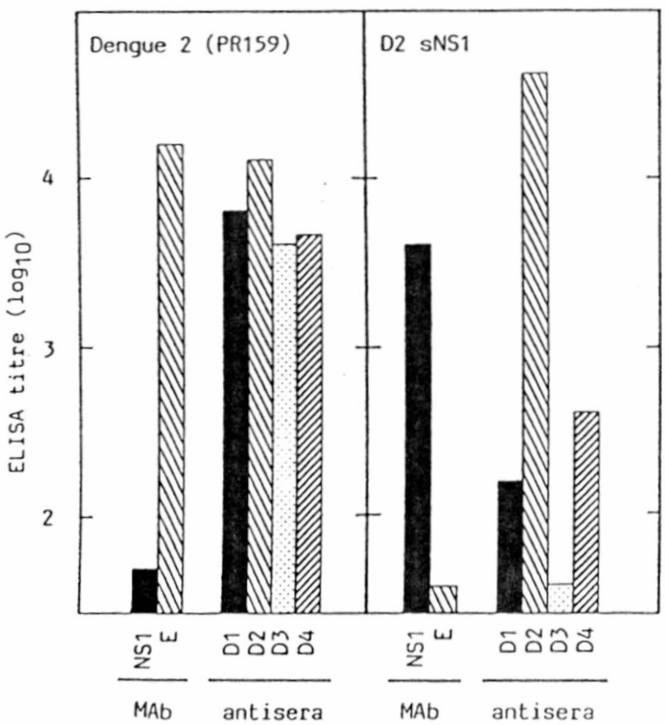

IMMUNOBLOT CROSS-REACTIVITY OF NS1 SPECIFIC MONOCLONAL ANTIBODIES WITH MEMBERS OF THE DIFFERENT FLAVIVIRUS ANTIGENIC GROUPS

CLONE	ELISA t50 DEN-2 PR159	Reduced sNS1 DEN-2 PR159	I		II		III		V	VI		VII (prototypes)				VIII			U		
			TBE	CI	EB	DB	JE	KUN	BAG	BAN	BOU	D1	D2	D3	D4	MOD	JUT	ILH	SEP	YF	MML
5H10.3d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1G5.4d	2.1	-	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-
1B6.4d	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
5H9.3	0.8	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
1F7.3	2.1	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
5C7.3	2.7	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
5D7.3	3.4	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
2H10.4	2.8	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
1H9.3	4.0	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
5B10.3	3.1	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
4D4.4	3.8	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
1B5.3	3.4	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
5E4.3	2.6	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
5A7.3	2.9	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
5C7.4	5.4	-	-	-	-	-	-	+	-	-	-	-	++	-	-	-	-	-	-	-	-
2C9.4	5.3	+++	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-
5H4.4	5.4	++++	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-
1H7.4	6.0	++++	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	-	-
5H6.3	4.6	-	-	-	-	-	-	-	-	-	-	-	+++	-	+	-	-	-	-	-	-
2A5.1	4.8	-	-	-	-	-	-	-	-	-	-	-	+++	-	+	-	-	-	-	-	-
5B6.3	4.5	-	-	-	-	-	-	-	-	-	-	-	+++	-	+	-	-	-	-	-	-
1E2.3	5.5	+	-	-	-	-	-	-	-	-	-	-	+++	-	+	-	-	-	-	-	-
1A4.4	2.8	-	-	-	-	-	-	-	-	-	-	-	+++	-	++	-	-	-	-	-	-
5H5.4	5.7	-	-	-	-	-	-	+	-	-	-	-	+++	-	++	-	-	-	-	-	-
5H4.3	4.7	-	-	-	-	-	+	++	-	-	-	-	+++	-	++	-	-	-	-	-	-
5F10.3	4.9	-	-	-	-	-	+	++	-	-	-	-	+++	-	++	-	-	-	-	-	-
5B9.3	4.8	-	-	-	+	-	+	++	-	-	-	-	+++	-	+++	-	-	-	-	-	-
5B5.3	4.0	-	-	-	-	-	++	++	-	-	-	-	++	+++	+	-	-	-	-	-	-
1C6.3	3.3	++++	-	-	-	-	+	+	-	-	-	++	+++	+++	++	-	-	-	+	-	-
1A12.3	2.7	++++	-	-	-	-	+	+	-	-	-	+++	+++	+++	+++	-	-	-	-	-	-
4H3.4	4.2	++++	-	-	-	-	+	++	-	-	-	+++	++	++	+++	-	-	-	-	-	-
3D1.4	4.2	++++	-	-	-	-	+	+	-	-	-	++++	+++	+++	++++	-	-	-	-	-	-
3A5.4	5.1	++++	-	-	-	-	++	++	-	-	-	++++	+++	+++	++++	-	-	-	+	-	-
1G8.3	5.4	-	-	-	-	+	+	++	-	-	-	++	+++	+	++	+	-	-	++	++	-
1G5.3	5.0	+	-	-	-	-	+	++	+	-	-	+	+++	+	+++	-	-	-	+	++	-
Rabbit anti-NS1	4.1	+++	-	-	-	+	++	+++	+	-	-	+++	++++	+++	+++	-	-	-	++	++	++
Human anti-D2‡	3.2	-	+++	+++	+++	+++	+++	+++	++	+++	+	+++	+++	+++	+++	++	++	+++	++	++	+++

d - dimer NS1 specific
‡ - human anti-dengue2 (predominantly anti-E)

DENGUE AND DENGUE HEMORRHAGIC FEVER IN THE AMERICAS, DIVISION OF VECTOR-BORNE VIRAL DISEASES, CENTERS FOR DISEASE CONTROL, FORT COLLINS, CO 80522

Dengue was first described clinically in the Americas in 1780, when a large epidemic occurred in Philadelphia, Pennsylvania, U.S.A. Little information is available on the occurrence of dengue-like illnesses in the region between 1780 and the late 1820s. From 1826-1828, major epidemics of dengue-like illness occurred in the southern United States and in Caribbean Basin countries. It was during these epidemics that the name dengue came into general use to describe the disease. From that time on, epidemics have occurred in the region at irregular intervals. Of importance is that the intervals between epidemics in recent years have become progressively shorter, with major epidemics occurring every 1 to 5 years.

The virus serotypes involved in early epidemics are not known. Serologic studies in Panama suggested that dengue 2 (DEN-2) was responsible for the 1941-1942 outbreak, but that dengue 3 (DEN-3) was also present. DEN-2 was the first dengue virus isolated in the American region (in Trinidad, 1953). DEN-3 was responsible for major epidemics in the early 1960s and DEN-2 in the late 1960s and early 1970s. In 1977, dengue 1 (DEN-1) was introduced into the Americas for the first time, although retrospective serologic evidence suggests that it may have been present many years earlier. Finally, dengue 4 (DEN-4) was introduced into the region in 1981, an since that time, three serotypes, DEN-1, DEN-2, and DEN-4, have been transmitted simultaneously in many of the countries of the region where Aedes aegypti occurs. DEN-3 transmission has not been documented in the Americas since 1977, but has been introduced into the region by travelers from Asia on numerous occasions in the 1980s.

In the 1950s and 1960s, a major effort was made to eradicate the principal urban vector mosquito (Ae. aegypti) of dengue and yellow fever viruses in the Americas. Success was variable: eradication was achieved in Mexico, Guatemala, Belize, Honduras, El Salvador, Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Peru, Bolivia, Paraguay, Uruguay, Argentina, Chile, Brazil, the Cayman Islands, and Bermuda, but not in other countries of the region. The failure to eradicate Ae. aegypti from the whole region resulted in repeated reinvasions by this mosquito into those countries that had achieved eradication. In the 1970s, support for surveillance and control programs was reduced, and by the end of the decade, many countries of the region had been reinfested with Ae. aegypti. In 1989, only Costa Rica, Chile, Uruguay, the Cayman Islands and Bermuda remain free of this species.

The expanding distribution of Ae. aegypti in the late 1970s coincided with increased movement of dengue viruses both within and into the region by airplane travelers. The result has been progressively increasing incidence associated with increased frequency of epidemic activity caused by multiple virus serotypes. In the past, most epidemics were caused by one, or occasionally two dengue serotypes, but multiple serotypes are now endemic in most countries of the region. This has resulted in the emergence of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) as a major public health problem in the Americas.

The evolving disease pattern in the Americas since the late 1970s is nearly identical to that which occurred in southeast Asia 30 years ago. Expanded distribution and increased densities of Ae. aegypti occurred during and after World War II in Asia and after failure of the Ae. aegypti eradication program in the Americas. In both regions this was followed by hyperendemicity in many countries. In every country in Southeast Asia where epidemic DHF/DSS became a major public health problem, the disease first appeared sporadically for several years, ultimately culminating in a major epidemic. Most of those countries subsequently developed a continuing cycle of epidemic DHF at 3- to 4-year intervals.

In the American region, a large epidemic of DHF occurred during 1981 in Cuba. Sporadic cases of confirmed severe and fatal hemorrhagic disease associated with dengue infection have subsequently been confirmed in Mexico, Nicaragua, El Salvador, Jamaica, Dominican Republic, Puerto Rico, St. Lucia, Aruba, Suriname, Brazil and Colombia while other countries such as Curacao and Haiti have reported cases that were clincially compatible with DHF, but were not laboratory confirmed. Most of the hemorrhagic disease has occurred in recent years. The evidence suggests that epidemic DHF/DSS will become a major public health problem in the Americas.

Several factors are responsible for the increased dengue transmission and occurrence of DHF/DSS in the American region, including 1) a near complete breakdown in mosquito control in most countries, 2) increased movement of viruses within the region as well as frequent introduction of viruses from Asia and Africa by air travelers, and 3) increased urbanization and creation of ecologic conditions in tropical American cities that have allowed coexistence of multiple serotypes of dengue viruses. Prospects for changing these conditions in the near future are not good. Therefore, the American region can expect more and larger epidemics of dengue and DHF/DSS in the future unless effective mosquito control programs are implemented immediately.

To summarize, there has been a constant increase in the incidence of dengue in the Americas over the past 15 years. This has been caused by increased frequency of epidemic activity in most countries which in turn has been caused by increased numbers of virus serotypes circulating in the region. The change in disease ecology has resulted in the emergence of DHF/DSS in the region, first with a major epidemic in Cuba, followed by increased occurrence of sporadic cases of DHF/DSS in many other countries. The sequence of events in the Americas in the 1980s has been nearly identical to the pattern observed in Southeast Asia in the 1960s. Prospects for prevention of epidemic DHF/DSS in the American region, therefore, are not good. In the absence of Ae. aegypti eradication, the only hope for effective prevention and control is to develop more effective, proactive surveillance and emergency vector control programs. Ultimately, effective long-term prevention and control will depend upon developing and implementing integrated, community-based mosquito control programs.

(Duane J. Gubler)

PROGRAM FOR PREVENTION AND CONTROL OF EPIDEMIC DENGUE AND DENGUE HEMORRHAGIC FEVER IN PUERTO RICO, DIVISION OF VECTOR-BORNE VIRAL DISEASES, CENTERS FOR DISEASE CONTROL, FORT COLLINS, CO 80522

As part of the U. S. Public Health Service "1990 Objectives," the Dengue Branch, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, in collaboration with the Puerto Rico Department of Health, in 1984 developed a new program for the prevention and control of epidemic dengue and dengue hemorrhagic fever (DHF) in Puerto Rico. Because epidemic dengue activity had become increasingly frequent, and because there was no effective Aedes aegypti control program in place, the approach was to place emphasis on disease control strategies that would prevent major epidemics rather than deal only with mosquito control or eradication. The program has five major components.

Improved proactive surveillance
Rapid-response emergency vector control
Emergency hospitalization plan
Education of the medical community
Long-term, integrated, community-based mosquito control.

The rationale was to develop a proactive surveillance system that had an "early warning" capability to predict dengue epidemics, and combine that with a rapid-response vector control program that could prevent the spread of an incipient epidemic. A contingency plan for emergency hospitalization was developed, and if implemented properly, could reduce mortality associated with dengue hemorrhagic fever during an epidemic. The last two components of the program, education of the medical community on clinical diagnosis and treatment of DHF, and community-based mosquito control, are designed to provide long-term disease control. Once that has been achieved, eradication can be considered.

The most important part of community-based mosquito control is to involve all segments of the community, including government, private companies, community service and religious organizations and residents from all levels of the community. As part of this goal, a plan was implemented in 1985 to involve Rotary International, a private service organization comprised of business and community leaders, in DHF prevention and control. Initially started as a community service project of the San Juan Rotary Club, it has now evolved into an international program.

Primarily educational, the program was implemented in three phases. The first phase was short term, providing publicity and creating high visibility for the campaign while the second phase emphasized the development of new educational approaches. The goal of the third phase is to introduce the campaign to Rotary Clubs in all parts of the island and to countries outside of Puerto Rico.

(Duane J. Gubler and Mr. A. Casta-Vélez)

SENSITIVITY OF IgM ELISA AND HI IN PATIENTS WITH DENGUE INFECTION CONFIRMED BY VIRUS ISOLATION, DENGUE BRANCH, SAN JUAN LABORATORIES, DIVISION OF VECTOR-BORNE VIRAL DISEASES, CENTERS FOR DISEASE CONTROL, SAN JUAN, PR 00936

The IgM ELISA test is increasingly used as a diagnostic test for dengue despite limited published data on sensitivity and specificity of this procedure. We retrospectively compared the sensitivity of an IgM capture ELISA with the hemagglutination inhibition (HI) test in detecting dengue infection in 131 patients with dengue confirmed by virus isolation. Sensitivity of IgM ELISA of sera from 68 patients using a mixture of all 4 dengue serotype antigens, and 63 patients using the 4 antigens separately was similar. The infecting serotype was indicated correctly by separate antigen testing in only 30 (59%) of 51 samples positive by IgM ELISA to at least one antigen. The HI test showed a 4-fold or greater titer rise to at least one dengue antigen in 130 (99%) of 131 serum pairs. When convalescent samples were obtained 7-20 days from onset (n = 76), IgM ELISA sensitivity was 96% (95%, C.I. = 89-100%), not statistically different from that of HI (100%, p = 0.25). By 60 days IgM ELISA sensitivity had decreased to 30% (95%, C.I. 7-76%). Sensitivity of IgM ELISA was similar in primary and secondary infections as determined by WHO criteria. IgM ELISA using mixed dengue antigens is efficient and highly sensitive in detecting dengue infection if samples are obtained 7-20 days after onset of illness.

(E. B. Hayes, D. J. Gubler, I. Gómez, G. E. Sather)

A STUDY OF ANTIBODY RESPONSE TO DENGUE NS1 BY WESTERN BLOT, DENGUE BRANCH, SAN JUAN LABORATORIES, DIVISION OF VECTOR-BORNE VIRAL DISEASES, CENTERS FOR DISEASE CONTROL, SAN JUAN, PR 00936

As an alternative to attenuated vaccines for dengue, subunit vaccines composed of a nonstructural protein (NS1) are being developed. Before human trials are initiated, it is important to examine the immune response to natural infections. We first studied the dynamics of the immune response in 2 cases of primary and 3 cases of secondary infections from which multiple specimens were available. Anti-NS1 antibody was not detectable in the primary infections but was detectable in 2 of the 3 secondary infections in the specimens collected on day 15 after onset or vaccination. Detection of this antibody was often associated with high HI antibody titer of specimen. In these 2 cases, titers to NS1 were lower than those to E-protein. Three hundred and one serum specimens from confirmed primary and secondary dengue infections collected in Puerto Rico and in Indonesia were examined. It was found that in primary infections anti-NS1 antibody was detectable only in a small proportion of convalescent specimens (8% from Puerto Rico and none from Indonesia). In the acute phase of the secondary infections, the difference in proportions of positive specimens between Puerto Rico (14.7%) and Indonesia (47.7% for classic fever; 62.5% for DHF) was statistically significant. Apparently, in acute phase of secondary infections Indonesians responded immunologically more intensely to NS1 than Puerto Ricans. In the convalescent phase, the difference between Puerto Rico (58%) and Indonesia (84.6%) was only marginally significant, while that between classic fever (82.6%) and DHF (84.6%) in Indonesia was insignificant.

(Goro Kuno, Vance Vorndam, Duane J. Gubler, and Ivette Gómez)

Aedes (Howardina) bahamensis as a host and potential vector of St. Louis encephalitis virus.

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Known previously only from the Bahamas, the peridomestic mosquito Aedes (Howardina) bahamensis Berlin is an invading species with expanding distribution in south Florida[1-2]. Aedes bahamensis utilizes tires and other artificial containers as larval habitats in Florida, and although it is autogenous it is also known to feed on man. An earlier study of Ae. bahamensis from the Bahamas[3] concluded that chikungunya and dengue-2 viruses did not persist or replicate to high titer after parenteral inoculation. Since Ae. bahamensis is now an inhabitant of a part of Florida that is probably endemic for St. Louis encephalitis (SLE) virus, a pilot study was undertaken to assess the vector competence of Ae. bahamensis for this virus. This study was also motivated by a desire to establish whether or not the reported insusceptibility of Ae. bahamensis to parenteral infection with dengue-2 virus was indicative of a general resistance to infection with flaviviruses.

The SLE virus strain used (77V-12908) was an epidemic strain isolated from Florida Culex nigripalpus in 1977. All assays and titrations were accomplished by parenteral inoculation of Culex quinquefasciatus mosquitoes, which were examined by indirect immunofluorescent staining of head squashes (IFAT) after 10 days incubation at 28° C. The Ae. bahamensis used were colonized from mosquitoes collected in Broward and Dade counties, Florida and were used in lab generations 2 and 3.

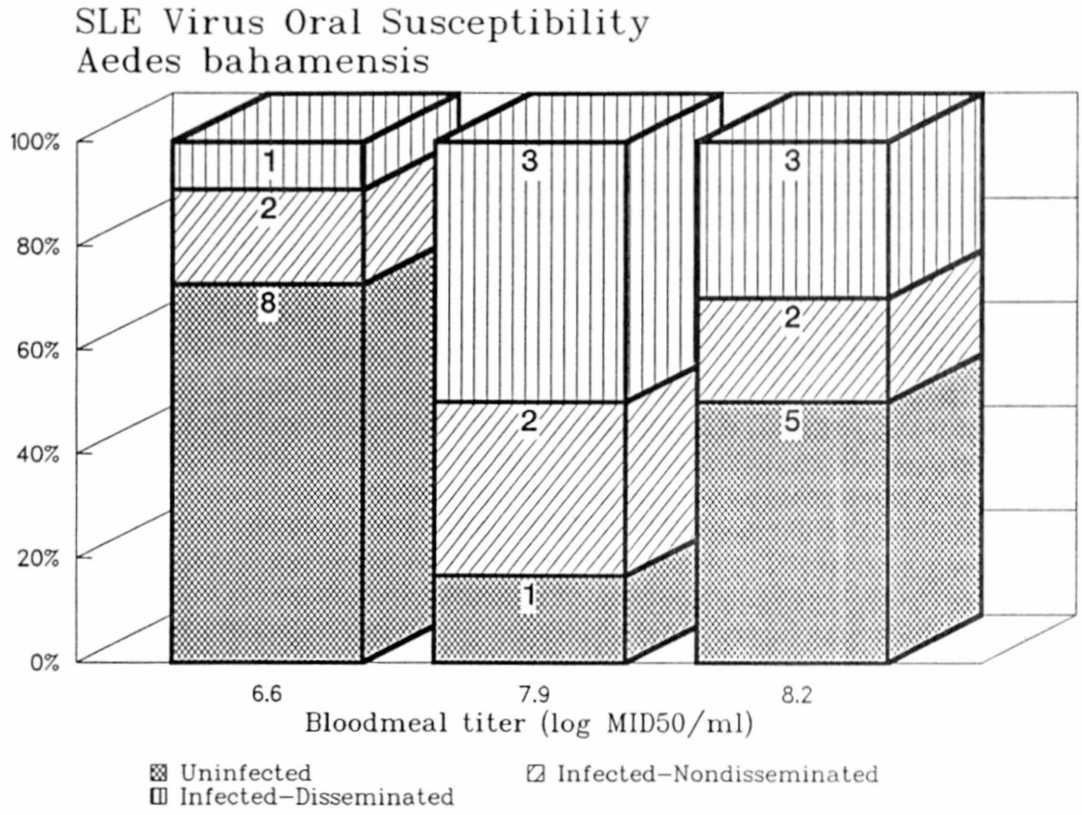
Ae. bahamensis females inoculated with $10^{3.8}$ mosquito infectious doses$_{50}$ (MID_{50}) all possessed SLE-positive head squashes by day 14, and had mean virus content of $10^{6.8}$ MID_{50}/mosquito. An attempt was then made to orally infect Ae. bahamensis females by allowing them to engorge on viremic baby chickens; titers of chick blood at time of feeding were estimated by assaying sample blood meals immediately after engorgement. Engorged females were held 14 days before their heads were examined by IFAT. Body remnants of individuals with SLE-negative heads were then triturated in 0.5 ml diluent and assayed for presence of virus. Mosquitoes with SLE-negative heads, but with detectable virus in body remnants were scored as "Infected-Nondisseminated;" mosquitoes with SLE-positive heads were scored as "Infected-Disseminated." The figure below shows that some individuals developed disseminated infections at each of the oral input titers used.

To determine whether mosquitoes with disseminated infections could transmit virus by bite, females were inoculated with SLE virus and tested 16 days later for ability to transmit by an in vitro "capillary tube" method[4]. Of 20 females so tested, 60% were capable of transmission.

One additional experiment sheds further light on the suitability of Ae. bahamensis as an SLE virus host. After parenteral infection of 148 females 3-8 days post-emergence, autogenous eggs were collected from an unknown number of these females 10-14 days later. One pool of larvae reared from these eggs contained at least one vertically infected mosquito (estimated infection rate 0.17%, method of Le[5]).

These results indicate that (a) the tested SLE virus strain grows to high titer in Ae. bahamensis following parenteral infection; (b) Ae. bahamensis females can be infected by the oral route; (c) most females with disseminated infections can transmit virus by bite; (d) SLE virus can be vertically transmitted by Ae. bahamensis females. The oral

susceptibility of Ae. bahamensis is markedly less than normally seen in Florida Culex nigripalpus or Culex quinquefasciatus ingesting comparable virus doses (data not shown).



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ENDOPHILIC BEHAVIOR OF AEDES AEGYPTI IN PUERTO RICO, DENGUE BRANCH, SAN JUAN LABORATORIES, DIVISION OF VECTOR-BORNE VIRAL DISEASES, CENTERS FOR DISEASE CONTROL, SAN JUAN, PR 00936

A study of the resting behavior of Aedes aegypti in San Juan, Puerto Rico, clearly demonstrated the endophilic nature of this species. It was undertaken after a series of ultralow volume (ULV) insecticide applications in urban areas of San Juan resulted in no impact on the wild mosquito population. An average of 17 to 33 females was collected with a backpack aspirator during weekly visits to houses in a lower middle class area of San Juan over a 52-week period. During three weeks in August and September, 1988, a period when increasing dengue transmission was detected in Puerto Rico, collections were made on 5 consecutive days and an average of 14 to 39 female Ae. aegypti was collected per house per day. Approximately 90% of these had recently taken a blood meal. If female blood feeding was evenly distributed between the 2 or 3 elderly occupants of these houses, from 7 to 13 bites per person per day would have occurred. Following the introduction of a dengue virus into an area with such high Ae. aegypti densities, rapid dissemination of the virus would likely ensue, unless a high level of immunity existed prior to its appearance. The endophilic behavior of this species clearly hampers the potential efficacy of ULV insecticide applications.

(Gary G. Clark, H. Seda, and D. J. Gubler)

NUMBER OF EGGS PER SITE LAID BY AEDES AEGYPTI AND IMPLICATIONS FOR DISPERSAL AND VIRUS TRANSMISSION, DENGUE BRANCH, SAN JUAN LABORATORIES, DIVISION OF VECTOR-BORNE VIRAL DISEASES, CENTERS FOR DISEASE CONTROL, SAN JUAN, PR 00936

It is generally accepted that an Aedes aegypti female does not deposit all her eggs at one oviposition site. We examined the frequency of the numbers of eggs laid in 1,000 ovitraps in San Juan, Puerto Rico, and found clear peaks at a mean interval of about 23 eggs. We believe that these peaks represent multiples of the average number of eggs laid by individual females per site-visit. From the number of eggs in fully gravid wild caught mosquitoes, we deduce that a female Ae. aegypti must find up to eight suitable sites to deposit her entire batch of eggs. If we assume that mosquitoes oviposit only once at any particular site, we can predict that during every oviposition cycle an individual female must search at least 15 premises in our study area for a suitable oviposition site. The time required for searching and selecting these sites is not known, but is likely to span more than one circadian cycle, especially in these insects. During daily periods of inactivity, many mosquitoes rest indoors, in close proximity to man. In collections from indoor resting sites, we found Ae. aegypti with fully developed eggs and a recent blood meal. Insects in this condition have been described on several occasions in the literature, and sometimes comprise more than 50% of landing collections. We suggest that in a container-breeding vector, such as Ae. aegypti, feeding by gravid females that have laid some of their eggs is influenced by the availability of oviposition sites, and could be a significant factor in the dynamics of arbovirus transmission.

(Paul Reiter, M. A. Amador, and D. J. Gubler)

Isofemale Line Analysis of the Genetic Basis of Oral Susceptibility of Aedes aegypti aegypti and Aedes aegypti formosus for Yellow Fever Virus. B.R. Miller and C.J. Mitchell, Division of Vector-Borne Infectious Diseases, CID, CDC, Fort Collins, Colorado, USA.

We have constructed isofemale lines of Aedes aegypti that are resistant and susceptible to oral infection with yellow fever virus. We are currently testing the F_1, F_2 and backcross progeny of resistant and susceptible lines for segregation analysis of the yellow fever virus susceptibility phenotype.

Two sub-genomic libraries of Aedes aegypti (West African and Caribbean) have been constructed in pUC19-derived vectors (Bluescribe, Stratagene). Three size-classes (500 bp, 1,000 bp and 2,000 bp) of genomic DNA have been cloned. The cloned fragments are currently being screened and characterized. The cloned fragments will be used as probes in Southern blots to discover restriction fragment length polymorphisms (RFLPs) that are linked to the quantitative trait of yellow fever virus susceptibility. We eventually hope to discover the molecular basis of virus susceptibility by finding chromosomal markers that are tightly linked to this important phenotype.

Susceptibility of the dog tick Haemaphysalis leachi to West Nile virus.

WN virus was isolated from Haemaphysalis leachi removed from a dog. Serological studies previously reported indicated that a high proportion of dogs at the locality of the tick isolate had significant WN virus specific antibodies. A study was therefore carried out to determine if the tick H. leachi is susceptible to WN virus and to assess whether it could be involved in a second transmission cycle of this virus. The ticks were infected at the larval and nymphal stages by allowing them to feed on WN virus infected hamsters. WN virus was recovered from very few of the larvae (3/100) and subsequent instars (4/80 nymphae, 0/60 adult ticks). Virus was isolated from 57.5 per cent (46/80) nymphae after they had taken an infected blood meal with a virus titre of at least 5.0 $\log_{10}$/ml and 35 per cent (21/60) of the subsequent adult ticks were infected. Unfortunately H. leachi will only feed on carnivores, mainly dogs, which were unavailable for transmission experiments. It was still possible to come to a conclusion from the study, namely that H. leachi is likely to represent a dead end in the transmission chain and is unlikely to be important in the epidemiology of WN virus. This was based on the observations that although nymphae of H. leachi may feed on dogs the low level of canine viraemia, determined in previous studies, is unlikely to be sufficient to infect a significant proportion of this instar as the 50 per cent infection threshold for the nymphal stage is probably 5.0 $\log_{10}$/ml according to the degree of susceptibility shown in our study. This would suggest that the recovery of WN virus from the tick removed from a dog was because this tick still contained undigested infected canine blood, the dog having been infected by bites of the main vector of WN virus, the mosquito Culex univittatus.

Unidentified virus isolates.

Several viruses isolated at this laboratory over the years had not been fully identified. A start has been made to clear this backlog. Three of these isolates have been provisionally identified as viruses which have not been previously recognized in southern Africa.

a) AR11194 - GOMOKA - Isolated from Ae. lineatopennis (= Ae. mcintoshi) collected on a farm just outside Harare on 24.05.69.

b) AR12431 and AR12537 - THIMIRI - Isolated at Lake Chrissie, R.S.A. from Culicoides spp and from Bethulie, R.S.A. from Culicoides nivosus respectively.

Reverse capture ELISA for detection of Sindbis and West Nile IgM antibodies.

Sera which are positive for SIN or WN antibodies by the HI test are routinely tested for specific IgM antibodies using the ELISA method. A comparison of the ELISA results with those obtained by IIF antibody tests using both fluorescein and biotinylated anti-human IgM conjugates, showed that the former technique was both more sensitive and specific than the IIF methods. There were no false positives by ELISA whereas with the IgM-IIF assays a high percentage of false positives were obtained. These were due to the rheumatoid factor (RF) and also to an interfering factor which was not detected by the RF latex agglutination test. Absorption of the sera with anti-IgG was necessary to eliminate this interference in the IgM-IIF tests. Because of its higher specificity, as well as no necessity for serum pre-treatment, the capture ELISA is clearly preferable to the IIF antibody methods for Sindbis and West Nile virus specific IgM detection.

N K BLACKBURN P G JUPP T G BESSELAAR B A PATTERSON

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

Vector competence of Aedes provocans for Jamestown Canyon virus in northeastern New York.

During the past twenty years, annual surveys of human and mosquito populations for arboviral infections have revealed that Jamestown Canyon (JC) virus, a member of the California serogroup, is the most widespread arboviral neuro-pathogen in New York State. This virus, which is most prevalent in the northeastern part of the state, was isolated most frequently from the Aedes communis complex, a group of closely related, morphologically similar mosquito species often difficult to distinguish from each other. During the spring of 1988, a site in Saratoga County was selected for an in-depth study of the vector potential of this mosquito complex; JC virus had been isolated on numerous occasions from this area in the past. High field infection rates were demonstrated in adult female Aedes provocans, a member of the Ae. communis group, collected in 1988 by aspiration with human bait.

Transovarial transmission by naturally-infected males: Between May 1 and May 9, 1989, approximately 5000 immature mosquitoes were collected at this same site. These specimens were reared in the arbovirus insectary to the adult stage, identified and pooled according to species, date of collection and sex. A total of 4,985 reared adult specimens were tested for arbovirus in 143 pools. Of these, 4,220 (85%) were identified as Ae. provocans, consisting of 506 females and 3,714 males. The remainder of the collection (15%) was comprised of Aedes canadensis, Aedes stimulans group and other members of the Ae. communis complex. Jamestown Canyon virus was isolated from two pools of reared mosquitoes, each containing 50 male Ae. provocans collected on May 4 and May 9, 1989, respectively.

Experimental transmission by intrathoracicly-infected females: Following the demonstration of high field infection rates in 1988 and transovarial transmission of JC virus by laboratory-reared male Ae. provocans in 1989, experiments were initiated to test the vector competence of this species for JC virus. Two groups of adult female Ae. provocans, one reared from pupae collected 5/3-4/89 and one collected as adults on 6/1/89 were injected intrathoracicly with approximately 340 TCID50 of JC virus. After incubation for 12 days at 20°C and 80% RH, the mosquitoes were allowed to feed individually from a capillary tube containing a 50% mixture of 10% sucrose and fetal bovine serum (FBS). After the feeding period, the mosquito was removed from the capillary tube and a head-squash preparation was fixed on a microscope slide which was examined by indirect fluorescent antibody (IFA) technique for evidence of JC virus infection. The body of the mosquito and the contents of the capillary tube were assayed for virus in Vero cell cultures. In the first group of 32 reared females, 100% of the mosquito bodies and heads were found infected with JC virus and 75% transmitted the virus to capillary tubes. In the second group of 40 field-collected adult females, all of the specimens developed JC virus-infected heads and bodies and 95% of these transmitted virus; the amount of virus transmitted ranged from 2.5 - 6.6 log10 TCID50 per ml with a mean of 4.4 log10 (n=13).

Experimental transmission by orally-infected females: Reared adult female Ae. provocans collected as pupae on 5/9/89 were allowed to feed on defibrinated rabbit blood containing 5.4 log10 TCID50 per ml of JC virus. Fully engorged bloodfed mosquitoes were incubated for 12 days at 20°C/80% RH, fed on sucrose/FBS filled capillary tubes and processed as previously described. Eighty-five percent (17/20) of the mosquito bodies became infected and 47% (8/17) of these developed JC virus-infected heads; none of the latter mosquitoes (0/8) transmitted JC virus to the capillary tubes. A second group of Ae. provocans collected as adults on 5/25/89 were fed an infectious bloodmeal containing 5.6 log10 per ml of JC virus. However, these mosquitoes were incubated for 14 days, resulting in 100% (36/36) infection of the mosquito bodies and 50% (18/36) infection of the heads; 50% (9/18) of those specimens with infected heads transmitted virus. The quantity of JC virus in this group of capillary tubes ranged from 0.5 - 6.5 log10 TCID50 per ml with a mean of 3.2 log10 (n=9).

The high field infection rates exhibited by this species in man-baited collections from Saratoga County in 1988 coupled with the evidence of transovarial transmission in reared males and transmission of the virus by experimentally infected females in 1989 clearly incriminate Ae. provocans as a competent vector of JC virus in northeastern New York.

(Margaret A. Grayson, Ph.D., Robert D. Boromisa, Ph.D. & Leo J. Grady, Ph.D.)

Report from the Arbovirus Research Program, University of California, Berkeley
(J Hardy, E Houk, L Kramer, M Milby, S Presser, W Reeves & W Reisen)

Modulation of alphaviral replication in Culex tarsalis

Earlier studies done in our laboratory indicated that some *Culex tarsalis* females could genetically control or modulate the replication of western equine encephalomyelitis (WEE, strain BFS 1703) virus after either peroral or parenteral infection. This concept was substantiated when we were able to select 2 genetic lines of *Cx. tarsalis*, one that allowed WEE virus to multiply to high titers (i.e., high viral producers or HVP) and the other that modulated viral replication to low titers (i.e., low viral producers or LVP). Optimal expression of modulation was obtained by inoculating males or females with 100 PFU of virus and then incubating them for 3-5 days at 32°C. The HVP trait was dominant and appeared to be controlled by a single autosomal gene. More recent data suggest that LVP mosquitoes produce a protein(s) that inhibit(s) WEE viral replication.

Other investigators have reported that *Aedes albopictus* cells produce a virus-specific inhibitory substance when persistently infected with Sindbis (SIN) or Semliki Forest (SF) virus. Therefore, it was of interest to determine if the ability of LVP *Cx. tarsalis* to modulate WEE viral replication was specific for WEE virus. LVP and HVP females were infected parenterally with 2 strains of WEE virus, 6 other alphaviruses that were evolutionally distant from and closely related to WEE virus [eastern equine encephalomyelitis (EEE), Fort Morgan (FM), Highland J (HJ), SF, SIN and Venezuelan equine encephalomyelitis (VEE)], a flavivirus [St. Louis encephalitis (SLE)], a bunyavirus [Turlock (TUR)] and a rhabdovirus [vesicular stomatitis (VS-NJ)]. The results shown in the table below clearly indicate that viral modulation in *Cx. tarsalis* is not WEE viral specific, but it is alphaviral specific.

		Mean (range) $\log_{10}$ PFU titer/female*	
Group	Virus	HVP	LVP
Alphavirus			
	WEE (BFS 1703)	6.3 (6.0-6.5)	<1.0 (<1.0-4.5)
	WEE (A7712)	5.7 (5.0-6.2)	<1.0 (<1.0-3.8)
	EEE	5.8 (4.4-6.5)	<1.0 (<1.0)
	FM	6.2 (6.0-6.4)	<2.8 (<1.0-4.5)
	HJ	4.4 (1.0-6.4)	<1.3 (<1.0-4.9)
	SF	5.6 (5.0-6.5)	<1.0 (<1.0-4.0)
	SIN	5.5 (4.6-6.4)	<2.7 (<1.0-4.5)
	VEE	5.5 (4.3-6.6)	<1.0 (<1.0-3.6)
Flavivirus			
	SLE	6.5 (6.1-6.8)	6.7 (6.2-7.0)
Bunyavirus			
	TUR	3.5 (3.0-4.2)	3.7 (3.0-4.1)
Rhabdovirus			
	VS-NJ	5.0 (4.6-6.1)	5.4 (4.5-6.4)

* Inoculated intrathoracically with approximately $10^{2.0}$ PFU/female and incubated at 32°C for 5 days before plaque assay in vero cells.

Feeding and habits of *Aedes scapularis* and *Culex* (*Melanoconion*) *sacchettae* mosquitoes in southern Brazil.

Research on the appentential flight of *Aedes scapularis* and the frequency of this mosquito in peridomestic environments indicated that after dispersal some mosquitoes were always found in domiciliary resting places. Observations were made at Ribeira Valley, São Paulo State, Brazil, in an area surrounded by primitive Atlanta plain forest. *Aedes scapularis* showed a clear preference for bovine hosts and, in general, large domestic mammals. *Culex* (*Melanoconion*) *sacchettae* also frequented the peridomestic environment; its potential for entering houses is also emphasized.

Contributed by:

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Iná Kakitani

Maria Anice Mureb Sallum

CONTEMPORARY TECHNIQUES FOR DIAGNOSIS OF AUSTRALIAN FLAVIVIRUSES:

A REPORT FROM THE VECTOR BIOLOGY AND CONTROL UNIT, QUEENSLAND INSTITUTE OF MEDICAL RESEARCH, BRISBANE AND THE GRADUATE SCHOOL OF TROPICAL VETERINARY SCIENCE, JAMES COOK UNIVERSITY, TOWNSVILLE, AUSTRALIA

The antigenic characteristics of Murray Valley Encephalitis (MVE), Kunjin (KUN) and Kokobera (KOK) viruses were examined at the epitope level using a panel of monoclonal antibodies produced to the envelope (E) and non-structural (NS1) proteins of these viruses. These antibodies were assayed for HI and neutralising activity and tested in ELISA to 15 different flaviviruses (Figure 1). Monoclonal antibodies produced to MVE virus revealed a strong antigenic similarity between this virus and Alfuy (ALF), Japanese encephalitis (JE) and KOK viruses. Fifteen monoclonal antibodies produced to KUN virus reacted with at least 4 different epitopes on the E protein. These antibodies also reacted to West Nile (WN) virus. Sixteen of 36 antibodies produced to KOK virus were type-specific. The remainder revealed a strong similarity between KOK and Stratford (STR), dengue type-2 (DEN2), and Saint Louis encephalitis (SLE) viruses. The overall reactions of the monoclonal antibodies used in this study suggest that an uncharacterised flavivirus, CS946, has a high degree of antigenic homology with STR virus.

It was also observed that epitopes present on the NS1 proteins of MVE and KOK, tended to be type-specific and were not involved in HI or neutralisation. In comparison, antibodies to epitopes on the E protein of MVE and KUN viruses showed strong HI and/or neutralising activity and were more likely to react with other flaviviruses. These monoclonal antibodies will be useful reference reagents and are being utilised as research and diagnostic probes.

Nineteen monoclonal antibodies produced to MVE virus were assayed by Western Blotting to determine the viral proteins they recognised. Nine antibodies reacted with the E protein as a 53kDa band in the unreduced state. When the antigen was reduced by 2-mercaptoethanol, a single band of 55kDa was recognised by five of these antibodies. Seven mabs reacted with the NS1 protein which appeared as a diffuse 45/53kDa dimer in the unreduced state and as five more discrete bands forming a doublet of 47-49kDa and a triplet of 54-58kDa after reduction. When anti-NS1 antibodies were reacted with unreduced, unboiled antigen, a diffuse band of about 80-85kDa was observed. Unidentified proteins of 90kDa and 32kDa (M_r) were recognised by two monoclonal antibodies. Anti-E and anti-NS1 antibodies were shown to react with two high molecular weight proteins (98kDa and 107kDa M_{ur}) that may represent E-NS1 complexes.

Competitive antibody binding studies were used to determine the relative positions of epitopes on the major glycoproteins of the flavivirus Murray Valley encephalitis virus. Seven monoclonal antibodies reacting to the structural glycoproteins (E and PrM) were labelled with biotin and allowed to compete for epitopes with a panel of unlabelled antibodies. Six epitopes in four non-overlapping domains were observed on the E protein of MVE virus. They included two sub-group reactive, one partially group reactive and three group reactive epitopes. This represented two distinct HI sites and three separate sites of neutralisation. One epitope was identified on the prM protein.

Further studies were carried out using rabbit polyclonal sera to MVE and KUN. Viral specific and group specific competitive binding was demonstrated. The MVE specific anti-NS1 MAb 10C6 and the anti-prM MAb MB11 are strongly inhibited by rabbit serum collected at 7 days and 28 days after infection. This implies that they are inhibited both by IgM and IgG. It indicates that these MAbs are useful for indicating the early and late immune responses to MVE. They were not inhibited by rabbit antiserum to KUN or either acute or convalescent sera to dengue infections. The binding of the anti-E monoclonal antibody M2-3H6 was inhibited by rabbit serum to both MVE and KUN but not by control serum. This indicates a group specific reaction. These studies form the basis of the development of diagnostic tests which can be designed to detect either specific or group reactive immune responses as the need arises (Figure 2).

Five MAbs to the nonstructural protein (NS1) were similarly labelled and allowed to compete for binding sites on NS1 with unlabelled mabs. Six spatially unique epitopes were identified on this protein.

Eleven isolates of MVE virus collected from different regions of Australia and New Guinea over a 35 year time period were examined for antigenic variation using 8 monoclonal antibodies. In ELISA, one antibody (M2-10G4), produced to the F3/51 strain, detected an epitope present on only eight of these strains. The three strains apparently lacking this epitope included two isolates from New Guinea (NG and MK4686) and one isolate from the Ord River (OR 156). From our Western blotting studies, this mab appears to react with an epitope present only on the high molecular weight form (56kDa) of the NS1 protein of MVE. The remaining seven monoclonal antibodies did not differentiate between the eleven strains. The findings of this study essentially correlate with the results of partial nucleotide sequence analysis previously reported for MVE and indicate that at least two phenotypes of MVE virus exist.

An antigen-capture ELISA has been developed to detect antigens of Australian flaviviruses in mosquito pools, suckling mouse brain and infected cell culture supernatant fluid. A monoclonal antibody reactive to an epitope on the envelope glycoprotein common to all flaviviruses was used as the capture antibody. Purified rabbit IgG, produced against Murray Valley encephalitis (MVE) virus, which reacted with eight Australian flaviviruses in haemagglutination inhibition and in an indirect fluorescent antibody test, was used as the indicator antibody in direct and indirect antigen-capture ELISA. A monoclonal antibody specific for a subgroup of encephalitic flaviviruses was conjugated to horseradish peroxidase and used as the indicator antibody to distinguish MVE, Kunjin and Alfuy viruses from the remainder tested. This ELISA could detect viral antigen in mosquito cell culture fluids and suckling mouse brain preparations at titres as low as 1000 TCID50/100 µl. Viral antigen in a single mosquito infected with MVE could be detected in a pool of 500.

A second generation antigen detection ELISA was developed utilising a polyclonal rabbit capture antibody with MAbs as indicators. The sensitivity for MVE was probably better (ca 200 TCID50/mosquito in preliminary assays) than that obtained with the first generation assay. For KUN, antigen was detected in one orally fed *Culex annulirostris* which contained <20 $TCID_{50}$ /mosquito. Using these MAbs separately or in combination, we now have the ability to specifically detect MVE, JE, KUN, ALF, KOK, Stratford, CS946, dengue, and YF either in mosquito pools, infected cell cultures or in body fluids. The sensitivity of this assay may be higher than any other assay for the detection of flavivirus antigens described to date. However, there is potential for increased sensitivity when hyperimmune polyvalent serum is used as the source of globulin for the capture step.

R.A. Hall, B.H. Kay, G.W. Burgess, P. Clancy and I.D. Fanning.

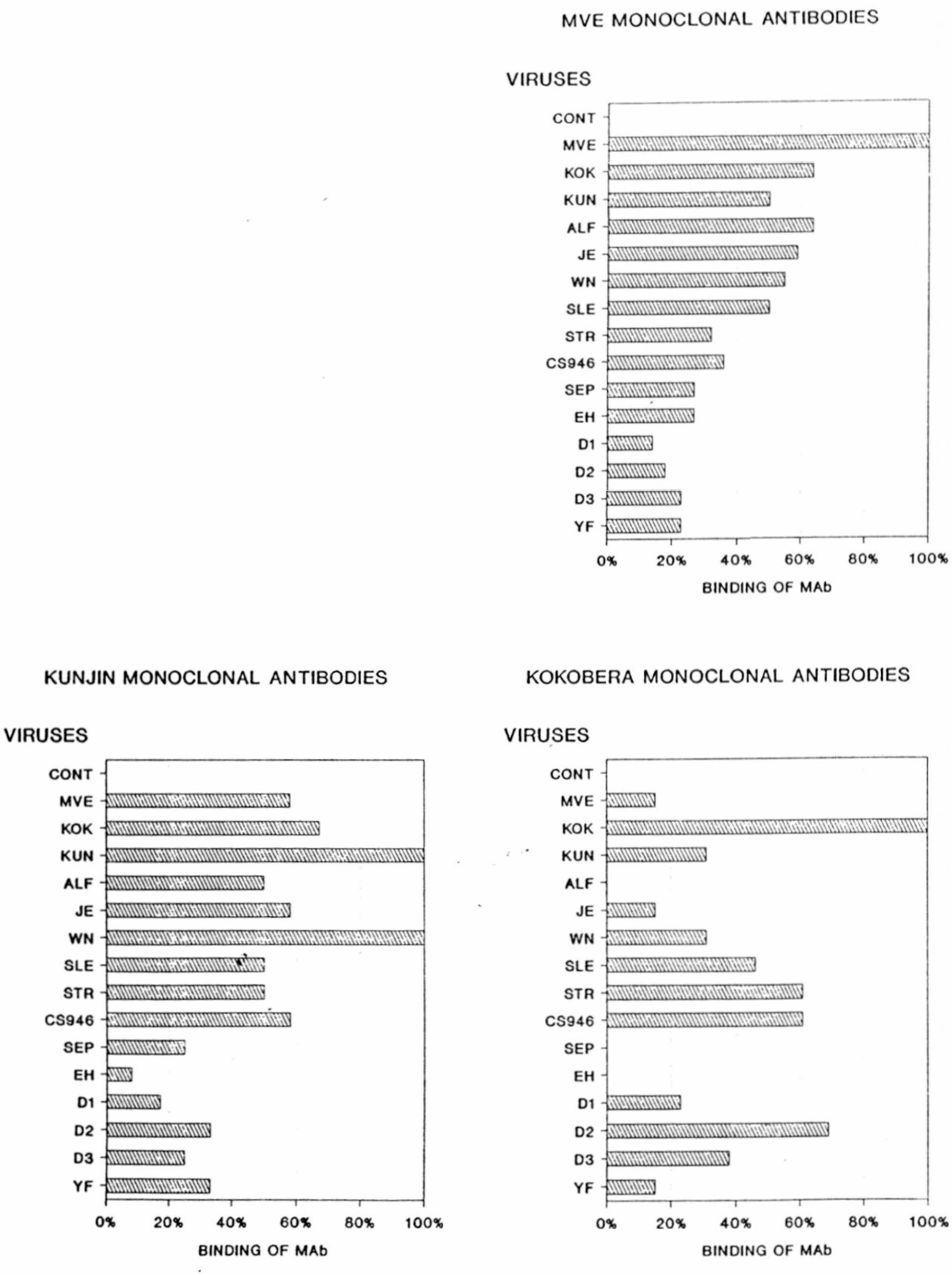


Figure 1 Binding patterns of three panels of monoclonal antibodies to 15 different flaviviruses in ELISA. Results are expressed as the percentage of monoclonal antibodies in each of the panels reacting with each of the viruses

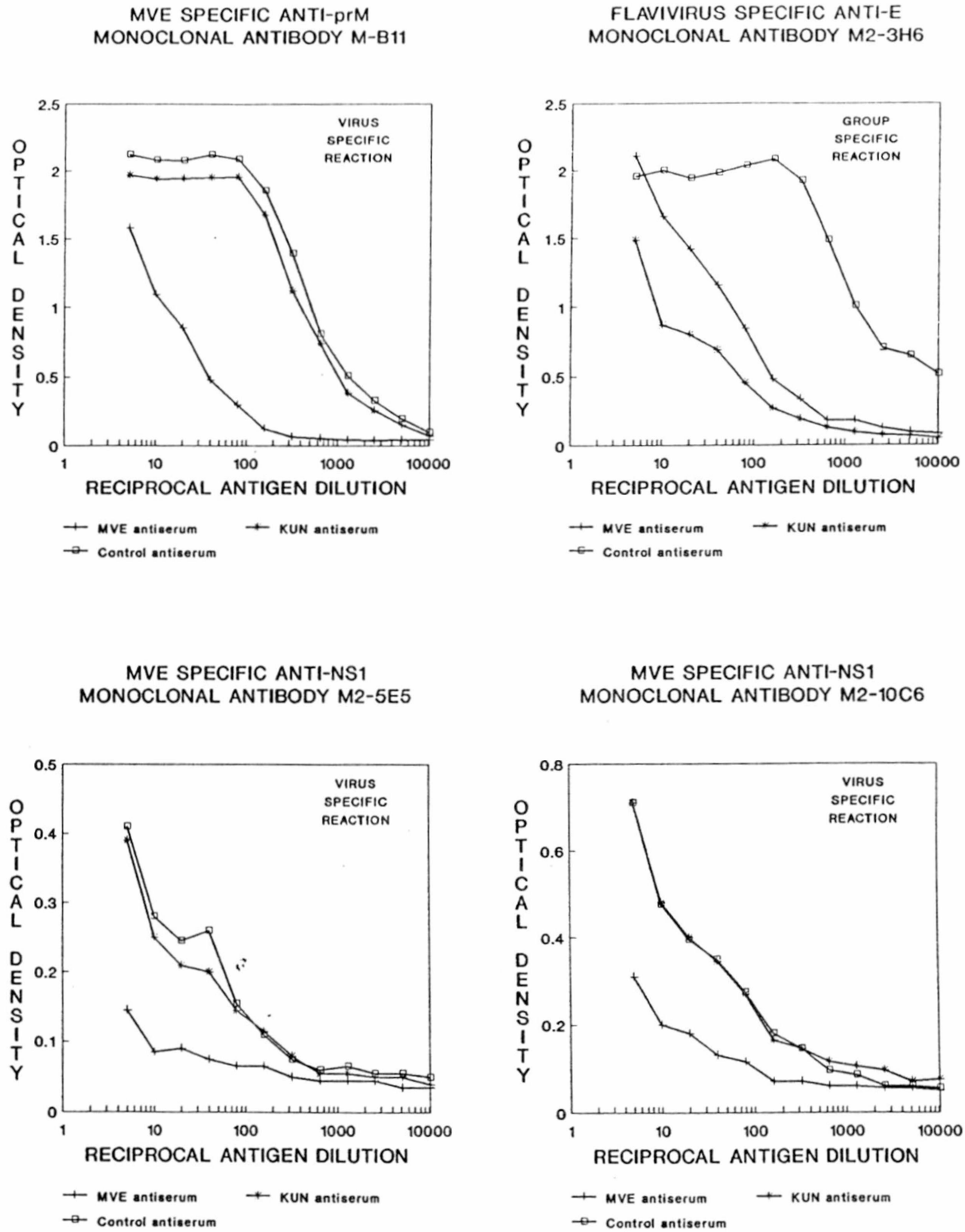


Figure 2 Competitive ELISA for measuring immune responses to flaviviruses. Control rabbit serum or antiserum to MVE or KUN competed with labelled monoclonal antibodies for viral specific or group specific epitopes on a range of dilutions of MVE antigen.

Enzyme Immunoassay for the Detection of Antibodies to WEE, EEE, and SLE in Chickens

During the 1988 arbovirus season, we compared our standard hemagglutination inhibition assay with an enzyme immunoassay (EIA) developed in our laboratory for the detection of antibodies to WEE, EEE, and SLE in chicken sera.

This assay is a solid phase assay in which the antigen is bound to the microtiter plate. The chicken sera is diluted 1:200 and added to the plate. After a one hour incubation, the unbound serum is washed off the plate and a goat anti-chicken horseradish peroxidase conjugate is added. After another one hour incubation, the unbound conjugate is washed off the plate and a peroxide-tetramethylbenzidine substrate solution is added. This reaction is stopped after 30 minutes and the optical density is read on a spectophotometer.

In the comparison, all chicken sera received in 1988 were tested by both HI and EIA. Any specimens that were positive by the EIA and negative by HI were tested by a blocking assay to confirm the EIA results.

The EIA result is considered positive if the optical density is greater than or equal to 0.220 above normal mouse brain background. The EIA result is negative if the optical density is less than or equal to 0.180 above the normal mouse brain background. Specimens with results between 0.180 and 0.220 were repeated.

The results of the comparison are shown on the below.

SUMMARY OF ALL SERA TESTED IN 1988

	WEE		SLE		EEE		
COUNTY	HI	EIA	HI	EIA	HI	EIA	#TESTED
Lubbock	252	240	13	35	0	8	379
Dallas	2	2	4	5	0	1	560
Dallas(City)	0	0	0	0	1	1	409
Galveston	0	0	0	0	0	2	395
Harlingen	0	0	0	0	0	0	129
Montgomery	0	0	0	0	0	0	28

SUMMARY OF CHICKENS SEROCONVERTING IN 1988

	WEE		SLE		EEE		
COUNTY	HI	EIA	HI	EIA	HI	EIA	#TESTED
Lubbock	62	59	6	15	0	5	103
Dallas	1	1	3	3	0	1	79
Dallas(City)	0	0	0	0	1	1	87
Galveston	0	0	0	0	0	2	76
Harlingen	0	0	0	0	0	0	50
Montgomery	0	0	0	0	0	0	9

A total of 1911 specimens were tested. Of these, 254 were positive for WEE antibodies by HI and 242 were positive for WEE antibodies by EIA. The sensitivity of HI for WEE antibodies was 97% and of EIA was 93%. There were no locations from which sera were collected which had specimens positive by HI that did not also have some specimens positive by EIA.

There 17 specimens positive for SLE antibodies by HI and 40 specimens by EIA. These additional specimens positive by EIA were all confirmed by blocking and came from locations which had specimens positive by HI. Thus the sensitivity of HI for SLE antibodies was 42% and of EIA was 100%.

There was one specimen positive for EEE by both HI and EIA. There were an additional 11 specimens positive by EIA only. These specimens could be blocked with only EEE antigen. However, the first of these specimens were from Lubbock. Since EEE is not expected from Lubbock, several specimens were sent to CDC to test for neutralizing antibody. There was no neutralizing antibody detected in these specimens. Those specimens which were EIA positive, HI negative for EEE antibodies were thus considered false positives for EEE. Since there was only one specimen positive for EEE by HI and EIA, the sensitivity for both methods was 100%.

Since HI was considered the reference test, the specificity of HI was 100%. Due to the 11 false positive EEE specimens, the specificity of EIA was 99.4%.

These data show that this EIA is a sensitive alternative to HI for testing chickens for seroconversion to WEE, EEE, and SLE. The EIA is slightly less sensitive than HI for WEE, but significantly more sensitive than HI for SLE. Chickens that seroconverted to WEE or SLE were identified at the same locations with the same bleeding with either test. These data also indicate that we must be careful with EEE positive results, such that any EEE positive must be confirmed by another test, either HI or virus neutralization.

The EIA is a much quicker and simpler test to perform than the HI test. The EIA can be completed in three to four hours, while the HI test requires three days. This allows a much quicker turn around time for specimens. The regions can be notified earlier of positive results and thus institute their mosquito control efforts in a more timely manner.

Due to this, we are changing to the EIA for surveillance of chickens for seroconversion to WEE, SLE, and EEE. Positive results for WEE and SLE will be based on the EIA alone. Positive results for EEE will be confirmed by HI or virus neutralization. No titers will be reported since HI titers and EIA titers do not always correspond.

This change in procedure will allow more timely reporting of results and more efficient testing in the laboratory.

Susan U. Neill, Ph.D.
Texas Department of Health
Medical Virology Laboratory

REPORT FROM THE DEPARTAMENT OF VIROLOGY - INSTITUTE OF MICROBIOLOGY - RIO DE JANEIRO FEDERAL UNIVERSITY - BRASIL

ADAPTATION OF THE SINGLE RADIAL IMMUNODIFFUSION (SRID) SEROLOGICAL TEST TO EASTERN EQUINE ENCEPHALOMYELITS VIRUS (EEE).

We standardized the Single Radial Immunodiffusion (SRID) methodology to perform serological tests with EEE virus.

The reagent kit consists of one plastic slide support covered with 3 ml of an antigen-containing gel prepared with the following materials: 10^{10} $TCID_{50}$ of purified viable virus (Figure 1), agarose 0,8%, sodium azide 0,1% and buffer pH 7,2 with phenol red.

Eighteen wells (4 mm diameter) were cut in the gel and each one of them was filled with 15 µl of the total serum to be tested.

Each slide test unit were covered with another plastic slide and incubated during 5 to 7 hours al 37ºC, in humid chamber, them we could notice the formation of opalescent lines around the wells, that had been filled with serum from immunized chickens or rabbits.

The diameter of the opalescents halos is proportional to the antibody concentration present in the serum (Figure 2). The antigenic kit is usuable, during, at least, 15 days when kept at 37ºC, but its reactivity is better conserved when it is maintained in refrigerator.

The SRID results were compared with Neutralization test and are shown in Table 1. The results suggest that SRID test could be used for EEE serological surveillance because the SRID test reveals, at least, 90% of the negative sera.

Improvements in the SRID-EEE test are being made aiming to present it as a commercial kit.

(CABRAL, M.C.; VILLELA, M.T.; RABELO NETTO, E.J.; COUCEIRO, J.N.S.S.; CABRAL, T.M. & MACHADO, R.D.)

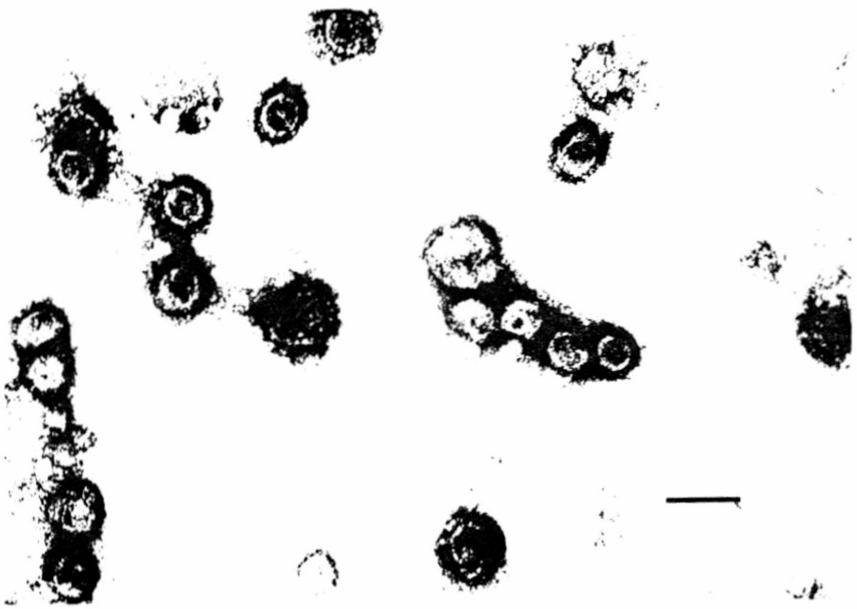
SRID-EEE serological test

FIGURE 1

EEE virions, from chicken embryos extracts, purified by ultracentrifugation in tartrate/glycerol linear density gradient. Electromicroscopy after negative staining.

Infectivity titre: 10^{10} $TCID_{50/ml}$

Scale: 1 cm = 71 nm



SRID-EEE serological test

FIGURE 2

Direct observation of the test after 7 hours of incubation (scale = 1:1).

Legend:

1 to 6 = Serial dilution of chicken anti - EEE immune serum (1/1, 1/2, 1/4, 1/8, 1/16 and 1/32).

7 - 11 = Serial dilution of guinea pig anti - WEE immune serum (1/1, 1/2, 1/4, 1/8 and 1/16).

12 = Rabbit normal serum

13 - 18 = Serial dilution of Rabbit anti EEE immune serum (1/1, 1/2, 1/4, 1/8, 1/16 and 1/32).

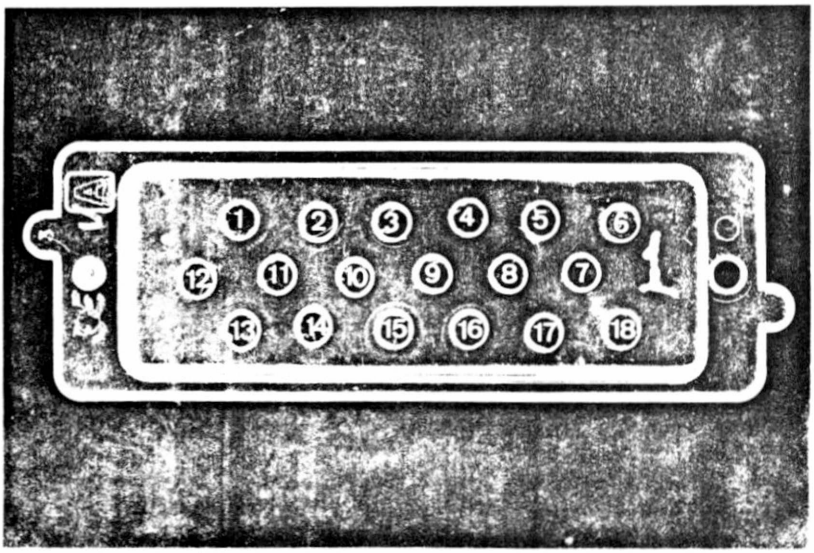


TABLE 1:

Relation between the results of SRID and Neutralization tests, against EEE virus, with 67 serums - 48 from human (H) and 19 from equine (E).

TN \ SRID	+	−
+	1 (1 H)	0
−	6 (4 H) (2 E)	60 (43 H) (17 E)

Expression of the Lassa Virus Nucleocapsid Protein in Insect Cells Infected with a Recombinant Baculovirus: Application to Diagnostic Assays for Lassa Virus Infection

Report from the Pathology Division, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury SP4 0JG, U.K.

The coding region of the gene for the nucleocapsid protein of Lassa virus has been inserted into the genome of *Autographica californica* nuclear polyhedrosis virus (AcNPV) using the transfer vector pAcYM1, so that expression of the foreign DNA is under the control of the promoter of the AcNPV polyhedrin gene. Infection of cultured *Spodoptera frugiperda* cells with recombinant virus resulted in synthesis of high levels of a protein which was indistinguishable from the authentic Lassa virus protein by SDS gel electrophoresis and immunoblotting with a variety of specific immune sera and monoclonal antibodies. The kinetics of appearance of the protein were comparable to those of polyhedrin production in wild-type AcNPV-infected cells. The recombinant material was antigenic when used in ELISA for Lassa virus-specific antibodies, reacting well with monoclonal antibodies specific for the nucleocapsid protein and with sera from experimentally infected guinea pigs.

To test the ability of the recombinant baculovirus-derived Lassa N protein to differentiate clearly between sera negative and positive for anti-Lassa virus antibodies, several sera from patients recovering from Lassa fever and 50 human sera obtained from Uganda and known to be negative for anti-Lassa antibody (as determined by conventional immunofluorescence assay) were screened at a dilution of 1/100 by ELISA using the recombinant antigen. All the negative sera gave low responses (mean A_{492} 0.07±0.05). However, even a convalescent serum which had an immunofluorescence titre as low as 1/32 gave an absorbance reading of 0.62, and was thus clearly distinguishable from the negative sera.

Current methods of diagnosis of cases of Lassa fever generally rely on the detection of virus-specific antibodies using virus-infected cells as targets in an immunofluorescence assay. The performance of insect cells infected with the recombinant baculovirus in such an assay was therefore examined. Such cells fluoresce brightly with monoclonal antibodies specific for the Lassa virus N protein, and also with human sera known to be positive in the conventional immunofluorescence test using Lassa virus-infected cells. Uninfected *S. frugiperda* cells included as controls show only weak background fluorescence, easily distinguishable from that of recombinant-infected cells. These results suggest that the recombinant baculovirus-infected insect cells can function as a direct replacement for Lassa virus-infected cells in this type of assay.

The insect-cell produced recombinant antigen has several advantages over alternative materials which may be equally effective in terms of ability to detect Lassa virus-specific antibodies. It is inherently safe to produce and use, whereas growth of Lassa virus itself requires specialised containment facilities, and the inactivation process required so that the antigen can be used elsewhere must be stringently monitored. The use of antigen produced in mammalian cells infected with recombinant vaccinia viruses has the disadvantage that vaccinia virus-specific antibodies may give potentially confusing side reactions.

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Specific seroepidemiological monitoring of flavivirus infections using monoclonal antibodies

We have recently employed a competitive ELISA to monitor sentinel chickens for serum antibodies to specific flavivirus epitopes. A monoclonal antibody (M2-3H6), reactive to an immunodominant epitope on the E protein of all 15 flaviviruses tested was used to detect anti-flavivirus antibodies. A second antibody (M2-10C6), specific for Murray Valley encephalitis (MVE) virus and reacting to an immunodominant epitope on the NS1 protein was used to detect specific anti-MVE antibodies. We have tested sera from a variety of species (man, rabbit, chicken, pig and horse) by allowing them to compete with the monoclonal antibodies for binding sites on crude MVE antigen adsorbed to the solid phase. We can reliably detect anti-flavivirus antibody in sera from animals that have been infected with any of the flaviviruses common to Australia (MVE, Kunjin, Alfuy, Kokobera and dengue) by the inhibition of the group-specific monoclonal antibody (M2-3H6). However, only sera from MVE infections inhibit the binding of the MVE-specific monoclonal antibody (M2-10C6), allowing us to differentiate these infections from the remainder.

The method is rapid and easy to perform and the results are highly reproducible. The sensitivity of both the flavivirus-group and the MVE-specific assays appears to be at least equivalent to that of haemagglutination inhibition (HI) and both epitopes appear to be recognised by sera from recent (4-7 days post infection) and past infections (at least 6 months post infection). There are several advantages in using a competitive ELISA of the configuration described here. Monoclonal antibodies can be provided as standardised, highly consistent reagents while species-specific reagents are unnecessary even when a range of animal species are to be tested. There is also no need to purify antigen (we use infected Vero culture supernatant that has no added serum factors) nor for fresh reagents such as gander or chicken erythrocytes. In addition, antibody levels can be quantified by standard titration or by measuring the optical density at two serum dilutions (1/10 and 1/100) and calculating arbitrary ELISA units from a standard curve of reference).

In South-eastern and Western Australia, the established methods of screening sentinel chicken sera (by HI or indirect ELISA) are unable to confidently distinguish between flavivirus infections. While some of the flaviviruses that circulate in these areas appear to be nonpathogenic, Kokobera and Kunjin have been implicated as the causative agents of a poly-articular disease and mild encephalitis respectively. MVE on the other hand is the proven aetiological agent of a severe and sometimes fatal encephalitis. As these agents may be active in the same region concurrently or independently it is imperative to specifically identify human and sentinel seroconversions to Kunjin, Kokobera and particularly MVE to evaluate the extent of the public health risk. We are currently evaluating monoclonal antibodies to Kunjin and Kokobera viruses as reagents to detect serum antibodies specific for these viruses.

(R.A. Hall, N. King, A.K. Broom and J.S. Mackenzie).

REPORT FROM ARBOVIRUS UNIT
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DETECTION OF IgG AND IgM ANTIBODIES TO TOSCANA VIRUS BY ELISA ASSAY.

Toscana (TOS) virus (family Bunyaviridae genus Phlebovirus), has been repeatedly isolated from both Phlebotomus perniciosus and P. perfiliewi sand flies collected in some rural areas of Central Italy and antibodies to it have been detected in human sera. Moreover, more than 150 cases with clinical diagnosis of meningitis or meningo-encephalitis, occurring during last 10 years in the same regions where natural foci have been identified, were serologically diagnosed as cases of Toscana virus infection. Antibodies in sera of patients can be detected by conventional techniques such as NT, HI, CF and IFA. However, none of these approaches has been proven entirely satisfactory for routine diagnostic work. Therefore, ELISA IgG and IgM assays have been developed and assayed for their possible use for both serosurvey and diagnosis.

Briefly, for the ELISA IgG test, a sucrose-acetone-extracted suckling mouse brain TOS antigen was captured by mouse TOS affinity-purified antibodies adsorbed to polystyrene plates. After adding test sample, the binding of specific antibodies was indicated by alcaline phosphatase-conjugated anti-human IgG. For the ELISA IgM, polystyrene plates coated with anti-human IgM were incubated successively with serum sample, TOS antigen and indicator antibodies.

The IgG ELISA proved to be more sensitive than HI and PRNT in detecting antibodies to TOS virus in human sera from healthy population. In fact, in a sample of 237 sera from healthy population of an area of the Tuscany region we obtained 48 (20.2%) monospecific positives for TOS virus by PRNT and 63 (26.6%) by IgG ELISA. Of 7 sera found monospecific reactive to Sandfly fever Naples (SFN) virus by PRNT, 5 resulted positive by TOS IgG ELISA. No cross-reactions were demonstrated among Sicilian Sandfly fever (SFS) virus monospecific reactors. High levels of cross-reaction were also detected by TOS IgG ELISA between TOS and SFN MIAFs, but not between TOS and SFS MIAFs.

These data indicate that the TOS IgG-ELISA, while rapid and sensitive, cannot be used alone for serological surveys in areas where both TOS and SFN are endemic or for old population in countries like Italy where SFN circulation has been discontinued in recent years.

The development of an ELISA assay for the detection of virus specific IgM was, on the contrary, a significant advance for the rapid diagnosis of TOS viral infections in clinical cases of meningitis. In fact, as by ELISA all TOS patients were found positive for IgG both in acute and convalescent sera we are

basing the serological diagnosis of TOS virus meningitis upon detection of specific IgM.

In the Table the titers of antibodies to TOS virus detected by ELISA in acute and convalescent sera from 14 selected meningitis patients are shown. The values are compared with the titers found in healthy population.

Antibody titers to TOS virus

	IgM		IgG	
	Mean	Range	Mean	Range
Patients				
Acute serum	3822	1200-51,200	1520	200-12,800
Convalescent serum	4973	<100-25,600	4973	1600-12,800
Healthy population			66	50-100

IgM antibodies were detected in 88% (46/52) of patients tested 15 days after onset of symptoms, in 71% (5/7) of those tested after 6 months and only in 1 out of 11 tested after one year. By ELISA, IgG were still detected up to 4 years after onset of meningitis.

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Epitope Mapping of the Lassa virus Nucleoprotein using Monoclonal Anti-Nucleocapsid Antibodies

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Monoclonal antibodies with differing specificity were prepared against the Josiah strain of the Lassa virus. All monoclonal antibodies were characterized by subclass determination and the immunofluorescence test against Lassa, LCM (WE & Arm strain), Junin, Machupo and other arenavirus antigens (tab 1). In radioimmune precipitation tests using purified Lassa virus antigen all monoclonal antibodies precipitated a single band of 60 kd, specific for the viral nucleoprotein (p 60). To determine the different epitopes of the Lassa virus nucleoprotein we used an ELISA-inhibition test. The Sandwich-ELISA test was carried out using all possible combinations of antigen detection with the monoclonal antibodies prepared. Three domains (A,B,C) were identified on the surface of the Lassa virus nucleoprotein because we could demonstrate three different groups of inhibiting clones (tab 2). All domains carried different Lassa virus specific epitopes. In addition, the A-domain carried a group specific epitope present within the arenavirus family as a whole as shown by cross-reaction in immunofluorescence tests. The B-domain only carries Lassa virus specific epitopes, whereas the C-domain has a type specific and a subgroup specific (Lassa, LCM) epitope.

Frank Hufert, M.D.
Wilfried Lüdke
Herbert Schmitz, M.D.
Department of Virology

Table 1:

Cross-reactivity of the anti-Lassa virus monoclonal antibodies in immunofluorescence test using different Arenaviruses

Hybridoma line	Tested viral antigen								
	Amapari	Junin	Lassa	LCM	Machupo	Parana	Pinheiro	Pichinde	Tamiam
L4F4*	neg.	neg.	++	neg.	neg.	neg.	neg.	neg.	neg.
L2B5*	++	+	+++	++	+	+	+	+	+
L1A12*	neg.	neg.	++	neg.	neg.	neg.	neg.	neg.	neg.
L4C3*	neg.	neg.	++	neg.	neg.	neg.	neg.	neg.	neg.
L2F1*	neg.	neg.	+++	neg.	neg.	neg.	neg.	neg.	neg.
L4F5*	neg.	neg.	+	neg.	neg.	neg.	neg.	neg.	neg.
L2G8*	neg.	neg.	+++	+++	neg.	neg.	neg.	neg.	neg.
L2D9*	neg.	neg.	++	neg.	neg.	neg.	neg.	neg.	neg.
L2A9*	neg.	neg.	++	neg.	neg.	neg.	neg.	neg.	neg.

Table 1:

* TCS = tissue culture supernatant, +, ++, +++ = intensity of fluorescence, neg. = negative

Table 2:

The mapping of antigenic domains on the surface of Lassa virus nucleoprotein with the ELISA-inhibition test.

Groups of inhibiting clones

A	B	C
L4F4	L1A12	L2G8†
L2B5*	L4C3	L2D9
	L2F1	L2A9
	L4F5	

Table 2:

Table 2 shows the 3 different groups of anti-Lassa virus monoclonal antibodies we conclude from the ELISA-inhibition data.
† = cross-reactive with LCM (WE & Arm) in IFT,
* = cross-reactive with all tested arenaviruses in IFT

MOLECULAR STUDIES

FLAVIVIRUS PROTEINS DEFINITION, NOMENCLATURE AND CLEAVAGE SITES OF ALL GENE PRODUCTS

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The *Flaviviridae* was accepted by the International Committee for Taxonomy of Viruses as a new RNA virus family (see Westaway *et al.*, 1985) prior to the introduction of a revised nomenclature for flavivirus proteins, based on the gene order obtained from sequencing data for YF virus, viz., 5'-C.prM.E.NS1 to NS5-3'. Thus Rice *et al.*, (1985) showed that the RNA genome contains one long open reading frame coding for about 3400 amino acids, and positively identified six gene products (C, prM, E, NS1, NS3 and NS5). Subsequently, we confirmed the gene order from sequencing data for Kunjin (KUN) virus, and identified the four hypothetical nonstructural proteins NS2A, NS2B, NS4A and NS4B, thus establishing all the gene boundaries and the cleavage sites within the polyprotein (Coia *et al.*, 1988; Speight *et al.*, 1988; Speight and Westaway, 1989a).

Table 1 is provided as an aid to identifying all flavivirus-specified proteins, and to relate the earlier nomenclature to the current (and hopefully final) nomenclature. The theoretical sizes are for KUN virus proteins, calculated from the deduced amino acid sequences between defined termini. Cleavage sites and approximate sizes appear to be conserved among the ten species for which complete or incomplete sequence data are available. However, when apparent sizes are measured by reference to molecular weight markers in polyacrylamide gels, these vary in different gel systems and several products appear to often migrate too rapidly e.g. NS2A, NS2B, NS4A, NS4B and NS5.

Recent carboxypeptidase analyses of WN virus C (from virions) and of KUN virus C (from infected cytoplasm) show that the carboxy terminus is truncated by a Type 1 cleavage (see Table) at a conserved site 18 residues upstream from the amino terminus of prM (Nowak *et al.*, 1989; Speight and Westaway, 1989b).

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(E.G. Westaway, G. Speight, G. Coia).

TABLE 1

DEFINITION OF FLAVIVIRUS-SPECIFIED PROTEINS

Nomenclature[a]			Theoretical Size	Cleavage site preceding
1	2	3	(Kilodaltons)[b]	the N-terminus[c]
NV5	P98	NS5	103.6	Type 1 (LYS/ARG.ARG↓GLY/SER/ALA)
NV4	P71	NS3	68.9	Type 1
V3	E	E	53.7	Signal peptidase site
NV3	GP44	NS1	39.8	Type 2 (VAL-X-ALA↓)
NV2[1]/2	P21	NS4B	27.5	Type 2
NV2	GP20	prM	18.4	Signal peptidase site
(NV2)[d]	P19	NS2A	25.4	Type 2
-	-	NS4A[e]	16.1	Type 1
V2/NV1[1]/2	C	C	13.4	Met
NV1	P10	NS2B	14.4	Type 1
V1	M	M	8.2	Golgi protease site?

[a] For origins of nomenclature systems, see Westaway, E.G. (1987) Advances in Virus Research *33*, 45-90.

[b] Based on size of Kunjin virus polypeptides. Attached glycans may increase the apparent molecular weights of prM, E, and NS1 by several thousand. The polypeptides of other flavivirus species are similar in size although some heterogeneity exists.

[c] The Type 1 and Type 2 cleavage sites are present at corresponding positions in the deduced translation sequences published for KUN, WN, MVE, SLE, JE, YF, TBE, DEN-1, DEN-2 and DEN-4 viruses with the following exceptions. The Type 1 cleavage site of DEN-2 and DEN-4 is modified by insertion of GLN or THR-GLN, respectively, between LYS.ARG for NS3, and the pair of basic residues for NS4A is ARG.LYS. The Type 2 cleavage site for DEN-4 NS4B is apparently modified by a conservative substitution of VAL to ILE-X-ALA↓. Amino acid residue X is relatively nonspecific, its distribution in 28 sites of 10 species examined being HIS(8), GLN(1), GLY(1) in NS1, THR(4), ASN(2), ASP(1), GLN(1), VAL(1), SER(1) in NS2A, and ALA(8) in NS4B.

[d] The distinction between the glycoprotein GP20 (NV2 or prM) and the unrelated but similarly migrating P19 (NS2A) has only recently become apparent.

[e] NS4A was only recently identified.

ANALYSIS OF NUCLEOTIDE SEQUENCE AT THE 3'END OF FLAVIVIRUS GENOMIC RNA.

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Viruses used were JE (strains JaGAr-01, Nakayama-Yoken, Mie 44-1, Sagayama and Beijin), WN and DEN-1. All the viruses were cultivated in Aedes albopictus c6/36 cells and precipitated by polyethyleneglycol and NaCl. Viral RNA was extracted from virions by SDS/phenol/chloroform.

Nucleotide sequences were determined by the chain termination method using reverse-transcriptase. Oligonucleotides corresponding to the 3'end of vRNA were used as primer(Takegami et al, Virology, 152, 483, 1986). The reaction was carried out at 42C for 1 hr, followed by a 30 min chase, and the samples were subjected to the polyacrylamide gel electrophoresis. Of three synthetic nucleotides (3a, 3b and 3c), 3a was useful for JE, while this did not work as primer for DEN-1.

Of the five strains of JEV, sequence of 130 nucleotides at the 3'end was determined. One hundred and twenty-five nucleotides were completely the same with each other. At the position 126 there was a difference. Nakayama-Yoken, Mie 44-1 and Beijing had an A residue while JaGAr-01 and Sagayama had a C. Among the JE viruses examined, three common sequences were noted: AACACA---CU at the end, GCACAG at position 45-50, and CAUAUUGACACCUGGGAAUAGAC at position 99-111.

The putative secondary structure at the 3'end of JE appeared stable and similar to that of YF (Rice et al, Science, 229, 726, 1985). Free energy for the secondary structure composed of ca. 100 nucleotides were estimated and the values were: -28 Kcal for JE, and -26 Kcal for WN.

Similarities and/or differences of nucleotides of flaviviruses may be related to specific biological properties of each virus and deserve further studies.

Related report: T. Takegami and S. Hotta, Virus Research, 13, 337, 1989.

(Reported by SUSUMU HOTTA, M.D.) Figures attached.

[Fig.1.]

```
                                        130
(J)                                      CCGUCUUUGC AUCAAACAGC AUAUUGACAC
(S)                                          C
(M)                                          A
(N)                                          A
(B)                                          A
   100                                   70
(J) CUGGGAAUAG ACUGGGAGAU CUUCUGCUCU AUCUCAACAU CAGCUACUAG GCACAGAGCG
(S)
(M)
(N)
(B)
    40
(J) CCGAAGUAUG UAGCUUGUGG UGAGGAAGAA CACAGGAUCU  3' (JEV)
(S)
(M)
(N)
(B)
```

(J) JaGAr-01, (S) Sagayama, (M) Nie44-1
(N) Nakayama-Yoken, (B) Beijing

Fig.1. Comparison of nucleotide sequences at the 3'end of genomic RNAs of several JE virus strains.
(J),(S),(M),(N) and (B) mean JaGAr-01, Sagayama Nie44-1, Nakayama-Yoken and Beijing strains, respectively.

[Fig.2.]

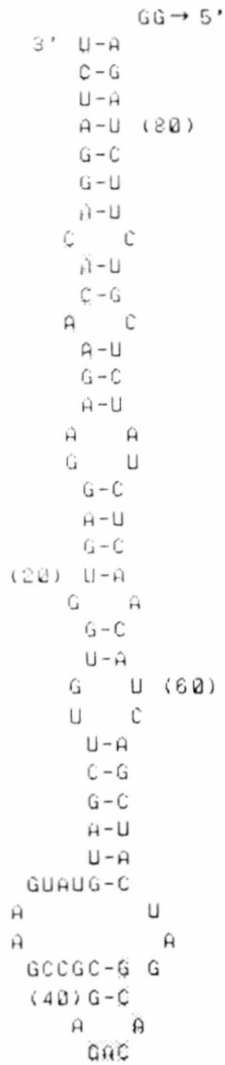

Fig.2. A putative secondary structure at the 3'end of JE virus genomic RNA.
Representative common sequences among genomic RNA of JW,WN,YF and dengue viruses are shown as the bracketed nucleotides.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, COLORADO STATE UNIVERSITY, FORT COLLINS, CO, 80523. REASSORTMENT OF LA CROSSE AND TAHYNA BUNYAVIRUSES IN AEDES TRISERIATUS MOSQUITOES.

Experiments were conducted to determine if La Crosse (LAC) and tahyna (TAH) viruses reassort in Ae. triseriatus mosquitoes and to determine the genotypic frequencies of viruses selected by in vivo vector interactions. Since it is difficult to determine the parental origin of RNA segments of California group reassortant viruses using serologic techniques, analysis of RNA of progeny viruses was used to determine if reassortment occurred in the mosquitoes. A molecular hybridization technique, utilizing specific probes for the L, M, and S segments, was used for genotyping these viruses.

Plasmids containing LAC-virus specific sequences were obtained from Dr. N. Nathanson at the University of Pennsylvania. The names of the plasmids and their segment specificity are: pLAC4.16 (LAC L RNA), pLAC4.27 (LAC M RNA) and pLAC4C-26 (LAC S RNA). These plasmid DNAs, when used as probes in hybridization to LAC or TAH viral RNA, hybridize specifically to LAC viral RNA, but not to TAH viral RNA.

Non-mutagenized parental (wild-type, wt) viruses were used in this study. Ae. triseriatus adult (3-day-old) female mosquitoes were inoculated with approximately 30 $TCID_{50}$ of a mixture of LAC and TAH viruses. Control mosquitoes were inoculated with the same amount of LAC or TAH virus alone. Following inoculation, mosquitoes were held in 1-pint cartons for 2 days, then transferred to large cages containing male mosquitoes for mating. Mosquitoes were offered an adult mouse as a source of blood on days 2, 5, 12, 15 and 18 post-inoculation (PI). Oviposition liners containing eggs were collected on days 13, 18 and 20 PI. Eggs were air dried for 24 hrs, then held at 27C 80% RH in humidified boxes. All female mosquitoes were harvested for assay on 23 days PI. Mosquitoes were triturated in 1 ml of tissue culture medium, stored at -80C until the time of assay.

Mosquito suspensions were inoculated onto VERO cells. Twenty four individual virus plaques were picked. Each plaque isolate was propagated in BHK-21 cells in a well of a 24-well tissue culture plate. Cytoplasmic RNA was extracted from the cells in each well, and blotted in triplicate to Nytran using a Schleicher and Schuell slot blot apparatus. Each blot was then hybridized with ^{32}P labeled pLAC4.16, pLAC4.27 or pLAC4C-26 to determine the parental origin of each RNA segment.

Forty-eight plaques from 2 mosquitoes of each of the control groups were genotyped. No hybridization occurred to the RNA extracted from plaque isolates of mosquitoes inoculated with TAH virus alone (Figure 1B). Significant hybridization occurred to the RNA extracted from the plaque isolates of mosquitoes inoculated with LAC virus alone (Figure 1A). Ninety mosquitoes were inoculated with the mixture of LAC and TAH viruses. 612 plaques isolated from 26 mosquitoes were genotyped. High frequency reassortment occurred in these mosquitoes (Figure 1D, E, F). All of the expected genotypes resulting from a cross of LAC and TAH were obtained from these mosquitoes. Genotypic frequencies of the 612 plaques analyzed are presented in Figure 2.

Studies are currently in progress to determine if resultant progeny were infected with reassortant viruses by transovarial transmission, and if so, whether progeny could transmit virus orally to a susceptible host.

(Laura J. Chandler, Barry J. Beaty)

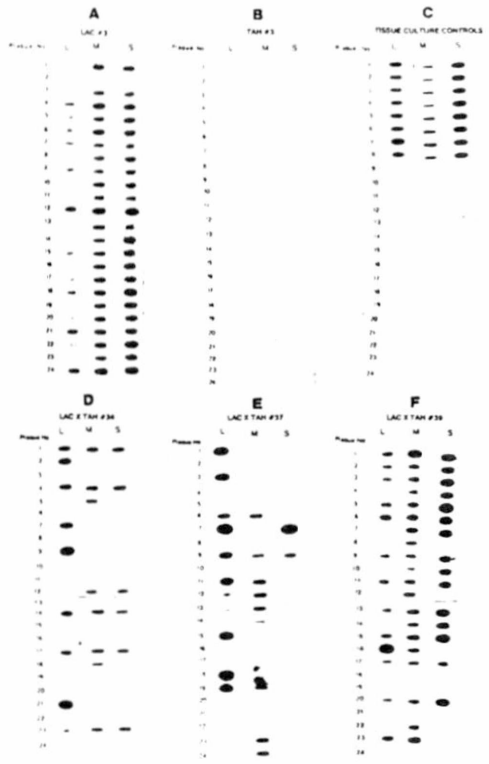

Figure 1. Hybridization of pLAC 4.16, pLAC4.27 and pLAC4C-26 to RNA extracts for genotyping reassortant viruses. BHK-21 cells in 24 well plates were inoculated with one plaque per well of virus isolated from A) LAC mosquito #3; B) TAH mosquito #3; C) Stock virus (Plaques #1-8, LAC; #9-16 mock-infected; #17-24, TAH); D), E), F), LAC x TAH dually infected mosquitoes #34, 37, and 39. RNAs were extracted and blotted as described in the text. Hybridizations were at 45C (pLAC 4.16); 48C (pLAC4.27) or 55C (pLAC 4C-26). Washes were at 56C (pLAC 4.16), 60C (pLAC 4.27), or 65C (pLAC4C-26). Genotypes are determined by the presence or absence of a hybridization band. L = Large RNA; M = Middle RNA; S = Small RNA.

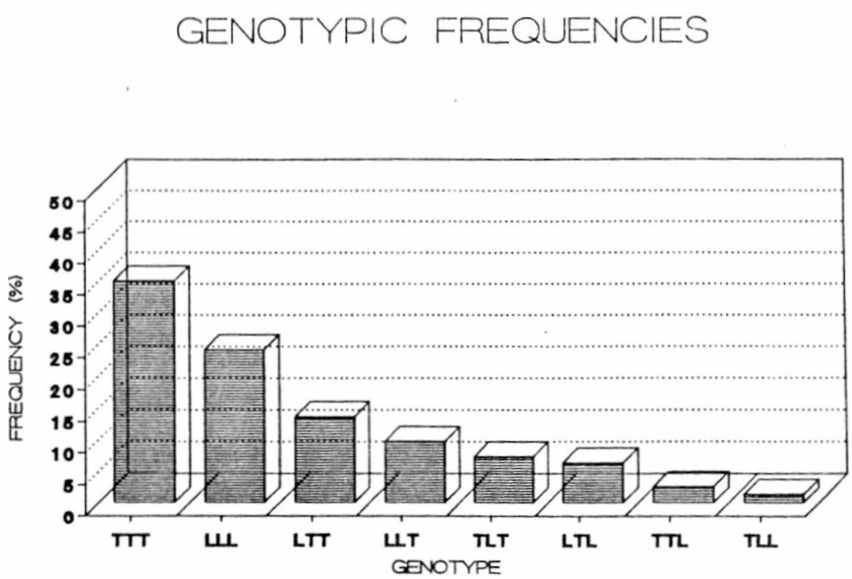

Figure 2. Genotypic frequencies of 612 viruses isolated from dually infected female mosquitoes.

NUCLEOTIDE SEQUENCES OF THE 26S RNAs OF VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUSES P676, 3880, AND EVERGLADES

We have reported a comparative sequence analysis of the complete 42S RNA genomes of VEE Trinidad donkey (TRD) virus and its attenuated vaccine derivative, TC-83 virus (Kinney et al. 1989. Virology 170:19-30). Nine of the 11 nucleotide differences detected between the sequences of these two viruses occurred in the 26S RNA region, which encodes the structural proteins of the virus (Kinney et al. 1986. Virology 152:400-413); (Johnson et al. 1986 J. Gen. Virol. 67:1951-1960).

We have recently sequenced the 26S RNA regions of the genomes of epizootic P676 (VEE subtype 1C) virus and two enzootic VEE viruses, strains 3880 (1D) and Everglades (2). These two enzootic strains are closely related to the epizootic VEE viruses both antigenically (France et al. 1979. J. Gen. Virol. 44:725-740) and structurally (Kinney and Trent. 1982. Virology 121:345-362).

Relative to TRD virus, we have identified 138, 220, and 357 nucleotide differences which translate to 20, 22, and 53 amino acid differences in the 26S RNAs of P676, 3880, and Everglades viruses, respectively. Twelve, 15, and 30 amino acid differences occurred in the E2 glycoproteins of P676, 3880, and Everglades viruses. The E1 glycoproteins of P676, 3880, and Everglades viruses were more similar to that of TRD virus. Only 1, 2, and 7 E1 amino acid differences were detected, respectively.

(Communicated by Richard M. Kinney and Dennis W. Trent, Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado)

RECOMBINANT VACCINIA-VENEZUELAN EQUINE ENCEPHALITIS VIRUS (VEE) PROTECTS HORSES FROM PERIPHERAL VEE VIRUS CHALLENGE

We have recently evaluated the efficacy of a recombinant VACC/VEE vaccine to protect horses against peripheral challenge with equine-virulent VEE virus (strain 71-180). The genes encoding the structural proteins of VEE TC-83 virus have been inserted into the thymidine kinase gene of the Wyeth strain of vaccinia virus and are under control of the vaccinia 7.5 K promoter. The recombinant vaccinia/TC-83 virus, which is designated VACC/TC-5A virus, expresses the capsid protein and the E1 and E2 envelope glycoproteins of VEE TC-83 virus in recombinant virus-infected cells (Kinney et al. 1989. J. Gen. Virol. 69:3005-3013).

Four horses were immunized intradermally at four sites (neck and shoulders) with a total of 2.0×10^8 PFU of partially-purified recombinant VACC/TC-5A virus. Two horses were immunized similarly with wild-type vaccinia (VACC) virus, while two horses received the standard live, attenuated TC-83 virus vaccine (3200 PFU) subcutaneously. Primary immunization resulted in high-titer VEE virus-specific ELISA and neutralization (Nt) antibodies in TC-83 virus-immunized animals and low-level antibody titers in VACC/TC-5A virus-immunized horses. The recombinant horse group received a secondary immunization with VACC/TC-5A virus 91 days following the primary immunization. This boosted Nt and ELISA antibody titers in all four recombinant virus-immunized horses.

All horses were challenged subcutaneously with 10,000 PFU of VEE 71-180 virus 21 days after the secondary VACC/TC-5A immunizations. Following challenge, the horses immunized with recombinant VACC/TC-5A virus or TC-83 virus showed no clinical symptoms or histopathology characteristic of VEE disease. The horses immunized with wild-type VACC virus, however, developed no VEE virus-specific antibodies and, following challenge with VEE 71-180 virus, exhibited classical signs of VEE disease, including biphasic fever, depression, anorexia, oligourea, ataxia, and severe, non-suppurative meningoencephalitis characterized by widespread lymphocytic perivascular cuffing, hyperemia and focal hemorrhage, endothelial swelling, focal glial nodules and diffuse gliosis.

Immunity afforded by the recombinant vaccine was sufficient to fully protect horses from peripheral challenge with equine-virulent VEE virus. The clinical and histopathologic profile of VEE virus disease, so clearly evident in the VACC virus-immunized horses, was essentially absent in the horses immunized with recombinant VACC/TC-5A or TC-83 virus. The results obtained in this equine study are similar to our results obtained from mice immunized with the VACC/TC-5A vaccine (Kinney et al. 1989. J. Virol. 62:4697-4702). A recombinant vaccinia virus that expresses the structural genes of Sindbis virus elicits anti-Sindbis Nt antibodies in cattle (Rice et al. 1985. J. Virol. 56:227-239); (Franke et al. 1985. Res. Vet. Sci. 39:113-115).

(Communicated by Richard A. Bowen (Colorado State University) and Dennis W. Trent (Centers for Disease Control), Fort Collins, Colorado.)

ARBOVIRUSES STUDIES IN LUANDA. POPULAR REPUBLIC OF ANGOLA

In co-operation with the Health Services in Angola recent work involving the study of the present situation of the Arboviruses in Luanda has come to light.

The period extending from 1970 to 1988 the city of Luanda underwent a tremendous increase in rural population that could at the present moment be close to 300%. The population increased from 600 000 inhabitants in 1970 to 1 800 000, this total constituting the population of the city and its boundaries.

The movement of the population from the interior of the country to the capital also resulted in the fact that certain individuals who had contracted diseases in other regions of Angola came to be hospitalized in Luanda. Concerning the Flavivirus the studies that took place in 1970 showed that only 11,5% of the population had antibodies against this group of virus, of which 5% reacted with Yellow Fever. After the epidemic that took place in 1971, the percentage of the population with antibodies increased to 22,2%. In 1988, the studies showed that 59,8% of the same population had contact with Flavivirus - infected mosquitoes.

The samples that were observed were taken from a very young population, the serological answer with multiple infection shows that this population was frequently bitten by Flavivirus - infected mosquitoes during the last years. We think that this resulted in not having an explosiv epidemic in 1988 similiar to the one that occurred in 1971. This study in the concerned area, did not permit us to determine any serological evidence as far as the activity of the Dengue virus is concerned. This we can conclude that this virus has never been present in Luanda.

A pathogenic virus for laboratory mice was isolated from mosquitoes that were captured in Luanda. The virus is presently being identified.

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REPORT OF ARBOVIRUS RESEARCH UNIT
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Serological prevalence of West Nile virus antibodies in Antananarivo suburbs. Madagascar.

A serological survey on West Nile antibodies frequency has been run among the human population in a Central Highlands village of Antananarivo suburbs.

In december 1988, 461 serum samples were collected among 1 to 78 years old subjects of both sex, in apparent good health. A second series (493 serum samples) was collected in july 1989. On the whole, 363 pair serum samples were collected.

West Nile virus antibodies were tested by immunoglobulin G capture enzyme-linked assay: two contiguous wells were sensitized with West Nile immune ascite and with specific antigen (brain of sucking mice infected with Mg Ar 945 virus strain) and with control antigen (brain of not infected sucking mice). After serum incubation, fixation was revealed by horseradish peroxydase conjugated with antihuman IgG antiglobulin, and then with o. phenylene diamine.

Results: Prevalence rate in december 1988 and july 1989 are respectively 13,4 % and 21,7 %; and incidence rate for six month 14,7 % Antibody rate is increasing with age (Table 1), but seroconversions are observed in age grades. Sex has not effect on frequency. Most of the seroconversions are observed between december and april, during the rainy season when culicidae are numerous and more aggressive, essentialy *Culex antennatus*.

In the village, five areas have been defined with ecological differences (paddy field or forest proximity). No variation in seropositive rate has been noticed according to the repartition into areas. In each area we have recorded animals (hens, zebus, pigs) outside and inside the houses and the index has been calculated animal number per inhabitants. In area 3, it seems that hens have protective effect but statistically we haven't noticed any difference according to the presence or the absence of animals in houses (Table 2).

Table 1

Serum samples repartition according to age and sex.
Series of decemmber 1988 (n = 461) and july 1989 (n = 493).

	December 1988			July 1989		
	POS	NEG	% pos	POS	NEG	% pos
sex men	27	185	(5,8)	43	183	(8,6)
sex women	35	214	(7,6)	65	205	(14)
AGE						
< 4	5	26	(16,1)	6	27	(18,1)
5 - 9	8	94	(7,9)	15	92	(14)
10 - 14	10	68	(12,8)	16	70	(18,6)
15 - 19	8	48	(14,2)	15	59	(25,4)
20 - 24	4	38	(9,5)	10	37	(21,2)
25 - 29	6	27	(18,1)	13	23	(36,1)
30 - 34	-	23	(-)	3	19	(13,6)
35 - 39	6	25	(19,3)	9	26	(25,7)
40 - 44	3	9	(25)	4	10	(28,5)
45 - 50	1	8	(11,1)	-	12	(-)
50 - 54	1	12	(7,7)	3	13	(18,7)
> 55	10	33	(23,2)	14	16	(36,6)

Table 2

Record of animals outside and inside of houses. Comparison with prevalence and incidence rates. (animal index = animal number per inhabitants)

	hens index		zébus index		pigs index		PREVALENCE		INCID
	EXT	INT	EXT	INT	EXT	INT	DEC	JUL	
area 1	-	1,97	0,09	0,14	0,12	0,05	15,5	28,6	23,8
area 2	-	2,06	0,20	0,09	0,05	0,19	12,1	17,3	12,5
area 3	-	2,33	0,59	0,02	0,04	0,11	9,3	15,7	3,7
area 4	-	0,97	0,20	0,01	0,12	0,01	13,4	20,4	15,8
area 9	-	1,26	0,09	-	0,05	-	16,6	24,3	11,1

POWASSAN ENCEPHALITIS IN CANADA, 1988.

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Powassan (POW) virus was isolated in September, 1958 from a fatal case of encephalitis in a 5-year-old boy from Powassan, Ontario. From 1958 to 1981 there were nine diagnosed cases of POW encephalitis in Canada including five from Ontario and four from Quebec. Three of these infections resulted in fatalities - one during the acute phase of the disease (the index case) and two additional patients expired, 1 and 3 years respectively after onset, from sequelae directly related to their disease (1).

No diagnostic seroconversions to POW virus were reported in Canada from 1981 to 1987 although a possible case was described of an Ontario patient who, in 1987, developed right leg weakness which was associated with high antibody titers to POW virus (2). Two diagnostic seroconversions to POW virus were recognized in Canada in 1988. Brief histories of these two cases follow.

CASE 1

In August, 1988 an 8-year-old-boy died of a fulminating encephalitis one week after initial symptoms. Sera taken 5 days apart showed an 8 fold increase in hemagglutination inhibiting antibodies to POW virus (acute 1:20, convalescent 1:160). No POW complement fixing antibodies were detected in his sera. This boy resided on a farm near Sherbrooke, Quebec in the vicinity of a previously recognized POW case.

CASE 2

In September, 1988 a 76-year-old-man was diagnosed as having viral encephalitis. Serological tests on sera drawn September 30 and October 10 and 27, 1988 revealed a diagnostic seroconversion to POW virus both by hemagglutination inhibition and complement fixation tests. The patient had been staying at a cottage near Moncton and had not travelled outside New Brunswick for over a year. No history of tick bite was elicited. The patient recovered and by April, 1989 was suffering only minor memory impairment.

These are the tenth and eleventh diagnosed cases of POW encephalitis in Canada. The potential severity of this disease is once again shown by the fatal Quebec case. The New Brunswick case marks the first diagnosed case of POW virus to be contracted in Atlantic Canada. Ixodes cookei, a tick commonly associated with POW virus, has been documented in both Quebec and New Brunswick.

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REPORT FROM THE ARBOVIRUSES AND SPECIAL PATHOGENS LABORATORY

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ARBOVIRUSES AND RICKETTSIA:

Surveillance of arboviral and rickettsial infections, excluding Q fever, revealed 9 cases in 1989 from which five, at least, were associated with histories of travel from areas with known agent(s) activity.

Six cases of arboviral disease included: a 29 year old male, 46 and 37 year old females with seroconversion to flavivirus antigens, clinical diagnosis of dengue and recent history of travel from Jamaica and the Virgin Islands in two patients, indeterminate area of travel in the third.

A two year old boy with meningo-encephalitis had seroconversion to Powassan virus antigen without cross-reactivity to St. Louis encephalitis or dengue antigens. The family lives about 100 kilometres East of Toronto, and there was no history of tick bite.

Two cases of California group virus were also identified. The first involved a one year old boy who had asceptic meningitis and was confirmed to have snowshoe hare virus infection; the second was a 13 year old boy returning from Guyana; the California sub-type in this patient is indeterminate.

South African tick bite fever was identified in two travellers, one from the Zululand and the other from South Africa. A third patient was seroreactive to Murine typhus, with titres ranging between 64 to 128 over a period of six months. Information on the patient's travels could not be obtained. Surveillance of Q fever will be separately reported.

SPECIAL PATHOGENS:

1. Ontario Maximum Containment Laboratory -

Dangerous exotic diseases (DED) have been exported through travels and animals brought from endemic areas. The potential for such exportation is illustrated by reports describing the outbreak of Marburg hemorrhagic fever in Germany and Yugoslavia, cases of Lassa fever in at least 12 instances which occurred in the United Kingdom, the U.S.A., the Netherlands, Japan and Canada, and of Hantavirus in the U.K.

Episodes have occurred in Ontario where large numbers of contacts with suspect cases of DED were involved. Examples include a hospital which was closed for admissions, a large hotel, a major teaching hospital, and a research institute which imports primates. Four of these episodes had to be resolved at the Special Pathogens Branch of the Centers for Disease Control (CDC), Atlanta. We have recently reported the first confirmed case of Lassa fever imported into Canada earlier this year.

The Government of Ontario has built two facilities, each the first of its kind in Canada, to provide immediate response in such episodes of DED. One is a strict patient isolation facility, located in Toronto General Hospital and operated by the Tropical Disease Clinic. The other is a maximum containment laboratory (MCL) which is almost completed and presently being tested, soon to be in operation. The program of this MCL will include studies and diagnosis of DED pathogens as well as other high hazard agents with notorious records of laboratory associated infections. The MCL is located on the third floor of the Central Laboratory of this Service, has a maximum containment area of 102^2; it

meets all national and international requirements for such facilities. The MCL will operate under cabinet mode containment with suit backup in cases of spills, accidents or unusual emergencies.

Containment is provided through negative pressure, assuring air flow from outside to inside the MCL; sealed floors, walls and ceilings; independent air supply and exhaust systems, the latter being decontaminated twice before discharge by high efficiency particulate air (HEPA) filters with computerized scanning; super-heating all liquid waste in pressurized, glass-lined tanks; and 17 laboratory units of special containment equipment. These units include a continuous line of 14 Class 3 absolute barrier cabinets with built-in refrigerator, freezer, incubator, centrifuges, microscope and in-line autoclave. Backup systems and operations guard against failures.

Control panels inside and outside the laboratory allow monitoring negative pressure throughout the containment zones of the MCL, exhaust and standby fans, integrity of exhaust filters, sterilization tanks, liquid spills on the floors, operation of pumps and interlocking of pneumatic (airtight) doors on all rooms of the MCL.

Features which provide further safety for laboratory personnel and containment of operation, include: a) a halon fire-control system with automatic sensors and manual stations which can be activated to release gas in laboratory zone(s), b) alarm stations, c) a short ciruit TV system with recording capabilities for emergencies, and with monitors outside the MCL, d) a communication setup for local and long distance relay and receipt of verbal and

written information, and e) "buddy" type operation at all times.

2. Lassa Fever: An Imported Case with No Transmission to Family Contacts

A 38 year old Canadian and his family had been living since 1986, south of Jos, Nigeria. He fell ill on December 17, 1988 with headache, fever, malaise, sore throat, and was admitted to hospital where he developed dyspnea, rigors and rash. He had albuminuria, leukopenia, negative malaria tests and did not respond to anti-malarial or typhoid treatments. He gradually improved and was discharged, but re-admitted after two days with bilateral orchitis which resolved in a week. The family returned to Canada three weeks later and all have been well. Through this episode, his wife and one child (10 yrs.) were asymptomatic, but another child (7 yrs.) became ill with jaundice; her brother (5 yrs.) felt tired and weak two days later. The father had IgG (512) and IgM (64) antibodies by immunofluorescence to LF 24 days post-illness; the IgM titre declined to 16 after one month and became undetectable 67 days after onset. Mumps was excluded as a possible cause of the patient's development of orchitis, a rather rare complication of Lassa fever.

There was no evidence to suggest that the patients's wife and children had prior infection with Lassa fever or became infected through their close contact with him. This finding is consistent with other episodes of exported cases where there has been no evidence of either secondary infection or disease in close contact, and health care workers of cases exported to the U.K., the U.S.A., the Netherlands and Japan.

The three children, however, had positive IgM for hepatitis A virus, which explains the earlier disease of two of them. Both parents were receiving regularly human immune globulin and did not have any evidence of infection with hapatitis A virus.

This is the first diagnosed importation of LF into Canada. Meanwhile, the New York Times Medical Sciences reported the death from LF of a 43 year old engineer in Chicago after his return from Nigeria, thus making two imported cases of this disease to North America in one year.

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15/11/89

Mosquitoborne Encephalitis Virus Activity in California, 1989 - Preliminary Report

As usual, extensive surveillance for human and equine cases of encephalitis and for evidence of St. Louis encephalitis (SLE), western equine encephalomyelitis (WEE), and California group (CE) viruses was carried out in a large cooperative program by local mosquito abatement agencies; local health departments; physicians and veterinarians; the Arbovirus Laboratory of the School of Public Health, University of California, Berkeley (AL, UCB); the California Department of Food and Agriculture; and the Environmental Management Branch, the Infectious Disease Branch and the Viral and Rickettsial Disease Laboratory (VRDL) of the California State Department of Health Services.

The 1989 season in California was highlighted by extensive St. Louis encephalitis (SLE) virus activity, including a resurgence in the San Joaquin Valley, particularly Kern County, after a period of quiescence for many years.

As of November 13, 1989, 10 serologically-confirmed human cases of SLE were recognized. Further search of hospital records and retrospective serologic surveys may reveal additional cases. The 10 cases identified so far are:

Age	Sex	County	Date of Onset
65	Male	Los Angeles	8/10/89
16	Female	Kern	9/12/89
61	Female	Kern	9/17/89
28	Female	Kern	9/19/89
62	Female	Kern	9/20/89
60	Female	Kern	9/20/89
27	Male	Kern	9/28/89
42	Male	Kern	10/5/89
37	Male	Kern	10/8/89
27	Male	Kern	10/8/89

The increasing use of private clinical laboratories for diagnostic tests of suspected encephalitis cases has resulted in some delays in notification to local and state public health laboratories of suspect or confirmed cases.

Serum samples from only 8 horses have been submitted thus far, and no cases of WEE were found. However, an 8 year old mare from Kern County had onset 9/16/89 of an encephalitis-like illness, and paired sera showed high antibody titers to SLE virus, suggesting but not proving an etiologic association. SLE antibody is occasionally found in equines (35 of 633 tested in the VRDL from 1971-1988), but rising titers in association with illness are quite rare. One such case was reported from Madera County in October, 1961.

There were 3,860 mosquito pools tested by the VRDL during the May-November season, yielding 118 SLE, 13 WEE, and 2 CEV isolates. The CEV were from Aedes melanimon; 1 WEE and 28 SLE were from Culex quinquefasciatus; 3 SLE were from Cx stigmatosoma; and the rest were from Cx tarsalis. Of 7,717 sentinel chickens tested so far there were SLE seroconversions in 220, and WEE seroconversions in only 34. Virus activity was most prominent in Imperial, Riverside, and Kern counties.

A final report for the 1989 season will be published in the Proceedings of the 58th Annual Conference of the California Mosquito and Vector Control Association, Inc., and will be abstracted for the next Arbovirus Information Exchange.

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**REPORT FROM THE VICTORIAN ARBOVIRUS DISEASE CONTROL PROGRAM
VETERINARY RESEARCH INSTITUTE ATTWOOD
MELBOURNE AUSTRALIA**

SURVEILLANCE FOR MURRAY VALLEY ENCEPHALITIS VIRUS

Weather patterns for 1988 and 1989 suggest an increased probability of the reappearance of Murray Valley Encephalitis (MVE) virus in the state of Victoria.

A preliminary survey of nestling ibis (32) in the Kerang area gave a negative result for antibody to MVE, Kunjin, Ross River Bannah Forest and Sindbis. Mosquito collection at the site showed Aedes camptorhynclus to be the dominant species.

Further surveys are planned to coincide with increased water bird activity and rise in temperature. Sentinel chickens are being established in mid November at 10 sites along the Murray River and its tributaries.

ROSS RIVER VIRUS IN SOUTH-EAST GIPPSLAND

Aedes camptorhynchus was shown to be the main species in 5 regions of S.E. Gippsland, Victoria. Four isolates of Ross River virus (RRV) were made during January to March 1989 from this species.

Sera from 507 residents from 2 areas in S.E. Gippsland had a prevalence of antibody (IGg, ELISA) to RRV of 25% in October - December 1988.

Seronegative donors (207) were retested in May 1989 and were shown to have an infection rate of 10% by the RRV ELISA antibody test.

Wildlife sera collected between March and June 1989 showed RRV antibody prevalences of eastern grey kangaroo 74% (n = 28), deer 18% (n = 22), feral pig 33% (n = 15) and fox 60% (u = 5). Domestic animal sera test for RRV antibody during the summer period showed prevalences of - horses 50% (u = 102) dogs 6% (n = 63) and goats 21% (n = 24).

ISOLATION OF RRV FROM A HORSE WITH NERVOUS DISORDERS

Blood was collected from a 10 year old thoroughbred mare at Yarragon in Gippsland, Victoria on 26/389. This animal was very ataxic with muscle rigidity and twitching. She was hypersensitive to sound. Heart rate was rapid with occasional arythmia. The mare gradually improved, with ataxia decreasing over the next few days.

Using $C_{6/36}$, BHK, Vero cell lines, and sucking mice, RRV was isolated from the blood sample.

It was identified as RRV by Plaque Inhibition. This virus caused paralysis of short duration but not death in 3 day old sucking mice.

A further sample was obtained and tested in August 1989. The serum contained HI (1.160) and ELISA antibody.

Ref: Campbell J, Aldred J. and Davis G. Ross River Virus From Aedes Camptorhynchus. MJA (1989) May 15, 150:603 - 604.

(J CAMPBELL, J ALDRED, G DAVIS)

REPORT FROM THE PASTEUR INSTITUTE
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HUMAN PATHOGENICITY OF SEMLIKI FOREST VIRUS

Since its first isolation in 1942, Semliki Forest virus (SFv) has become a very familiar laboratory model, especially used in studies on the mechanisms of neurovirulence and virus-induced demyelination of the central nervous system.
However, a fatal laboratory infection apart, SFv was up to now considered as non pathogenic for human, and no SFv isolation has ever been obtained from human in nature.

SFv was responsible for an outbreak of febrile illnesses in Bangui, the capital of the Central African Republic (CAR), from October to December 1987.
Physicians in Bangui collaborating to a surveillance program of arboviruses and haemorrhagic fever viruses, collected during this period 89 sera samples from patients presenting with a clinical picture compatible with an infection by arbovirus or haemorrhagic virus, i.e. acute febrile syndrome without malaria, associated with at least one more sign such as algia, haemorrhage, encephalitis, hepatitis, renal failure, shock syndrome. Twenty-two SFv isolations were made from the sera collected.

Europeans, particularely soldiers recently arrived from France, were mostly affected, suggesting a protective effect of antibodies to alphaviruses present in a high proportion of the resident population in CAR.
The clinical picture was mild, including fever, persistant headache, myalgia, arthralgia, and convalescence with asthenia.

During the outbreak 8 SFv isolates were obtained from 148 pools of mosquitoes in Bangui. Five strains were isolated from Aedes aegypti which is suggested to be responsible for the human-to-human transmission of SFv.
Previously 17 SFv strains had been isolated from 282 mosquito pools, mainly from Aedes africanus, collected from August to December 1987 in a gallery forest close to a village 100 km north of Bangui.
We suggest that SFv may have been transported from the gallery forest to Bangui by a viremic host.

As the epidemic SFv strains show antigenic differences with prototype SFv (Charles H. Calisher, personal communication), studies are in progress to determine whether the genetic variation reflected in the antigenic variation is the condition for the virulence pattern of our SFv isolates.

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Epidemiologic Investigations on the New Jersey Serotype of Vesicular Stomatitis Virus on Ossabaw Island, Georgia

During the summer of 1988, six isolates of the New Jersey serotype of vesicular stomatitis virus (VSNJ) were made from 610 pools of unengorged female phlebotomine sand flies (Lutzomyia shannoni) on Ossabaw Island, Chatham County, Georgia. Annual VSNJ activity has been documented in juvenile free-ranging feral swine on this island since 1980; thus VSNJ is enzootic at this focus.

To learn more about the involvement of Lu. shannoni in the epizootiology of VSNJ, a closed laboratory colony of this species was established from specimens collected on Ossabaw Island. The colony was maintained at the Yale Arbovirus Research Unit, New Haven, CT, where vector competence studies were undertaken using the Ossabaw Island strain of VSNJ.

Sand flies were susceptible to oral infection with VSNJ when offered blood meals containing 6.1-9.1 logs of VSNJ per ml in membrane feeders. Infection rates ranged from about 7% with the low dose to 88% with the high dose. Virus was recovered from flies as long as 13 days postinfection, indicating a persistent VSNJ infection. More than 4 logs of VSNJ were recovered from the heads of orally infected flies on day 12 postinfection, indicating a disseminated infection.

Infected Lu. shannoni transmitted VSNJ by bite to laboratory rodents. Virus was recovered from the brains of exposed suckling mice, and VSNJ antibodies were detected in the serum of an adult hamster which had been exposed to a group of flies that had been inoculated with VSNJ.

Transovarial transmission of VSNJ occurred with females infected with higher doses of VSNJ. Filial infection rates were between about 1 and 2%. Similar rates of transovarial transmission were obtained in the first and second ovarian cycles.

Laboratory studies, together with the association between Lu. shannoni seasonal abundance and activity and seroconversion among feral sentinel swine on Ossabaw Island, indicate that Lu. shannoni is a capable vector of VSNJ. Although rates of transovarial transmission obtained were low, a possible mechanism for the overwintering of VSNJ on Ossabaw Island has been demonstrated.

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OUTBREAK OF OROPOUCHE FEVER IN PANAMA SINCE AUGUST, 1989.

GORGAS MEMORIAL LABORATORY, P.O.BOX 6991, PANAMA 5, REPUBLIC OF PANAMA

Dengue virus has been absent from Panama since 1942, but Aedes aegypti was reintroduced in 1985 and reached critical, epidemic-compatible levels in and around Panama City in 1988. By 1989, Ae. aegypti larvae were being found in many towns at some distance from the capital. In one such town, Bejuco, a coastal community 35 miles west of the capital, larvae were reported in early August. The occurrence of an outbreak of dengue-like illness was reported to us on August 24, and sera from three patients were submitted. We isolated from one of them Oropouche virus, reported previously only in Trinidad and Brazil.

Patients from the outbreak area were interviewed and additional sera obtained in order to determine whether dengue virus was also involved. Blood specimens and questionnaires from our ongoing dengue surveillance program were being received at the same time from the greater Metropolitan Area of Panama City. Some of these cases mentioned residence, work or recent travel outside the City west of the Canal, especially in the coastal area. Therefore, all acute sera received after September 15 were inoculated into Vero for Oropouche, as well as the usual TRA-284 cultures for dengue.

Isolation attempts on 191 sera have yielded Oropouche virus from 14 patients in whom infection was confirmed. Twelve isolates await convalescent sera. Seven of 68 paired sera from patients without isolates showed an antibody rise, while 5 had a very high titer in both sera.

Records of the Bejuco Health Center from July 1 to September 30 were examined for cases of acute viral syndrome. No such cases were recorded until August 21, but thereafter 70 cases visited the clinic. Review of 64 charts showed 34 male and 30 female patients, of whom only 7 (11%) were children under 14, an age-group which makes up about 50% of the population. Complaints were recorded with the following frequency (%): fever 89, headache 84, arthralgia 61, lumbar pain 42, chills 25, myalgia 20, dizziness 17, sore throat 9, and other complaints 59. One or 2 relapses were not uncommon.

Concomitantly, we have not isolated dengue virus from any patients anywhere in Panama. Nor have any dengue infections been demonstrated serologically in 393 patients enrolled during one year of active dengue surveillance.

On September 12, hematophagous insects were collected using CDC light traps at 4 sites near Bejuco where patients had been recently ill, but the few arthropods obtained (23 pools, 12 species, mostly Aedes taeniorhynchus) were virus-negative.

The question arose as to whether Oropouche cases have occurred previously in Panama or whether this virus was recently introduced into Panama. Serologic testing of a small set of 1978 sera from Bejuco and 1968 sera from rural areas from the hills behind Bejuco revealed 25% positive and confirmed that Oropouche virus is not new here.

(Evelia Quiroz, Pauline H. Peralta and Bedsy E. Dutary)
November 20, 1989.

An Epidemic of Polyarthritis Due to Ross River Virus in Western Australia

The largest outbreak of epidemic polyarthritis to be reported in Western Australia occurred in the south-west of the State in the summer of 1988-89. Epidemiological studies on Ross River virus, the aetiological agent of epidemic polyarthritis, have been in progress in our laboratory since 1987, thus allowing a comparison of mosquito vector populations and of environmental factors affecting virus activity between epidemic and non-epidemic years.

A total of 607 cases of epidemic polyarthritis were diagnosed by the State Health Laboratory Service between October, 1988 and May, 1989, with peak monthly totals of 178 and 164 in January and February respectively. This compares with annual average of 46 cases over the previous 4 years in the south-west of the State. The ratio of male to female cases during the epidemic was 1.1:1.0, and the most commonly infected age group was 31-40.

The initial focus of virus activity occurred in and between the coastal towns of Mandurah and Bunbury, 80 to 180 kms south of the State capital, Perth. This area encompasses one of the largest areas of saltmarsh and tidal wetlands in Australia. In October and November, 1988, almost all cases were from this narrow coastal belt, but by the end of the epidemic, cases had been reported from throughout the south-west of the State, including 90 cases from Perth and its suburbs. This suggests that a gradual dissemination of virus might have occurred from the original focus.

The saltmarsh breeding *Aedes vigilax* mosquito was shown to be the most likely epidemic vector of the virus, whilst its winter-spring ecological counterpart, *Ae. camptorhynchus*, may have been responsible for initiating a build-up of virus. Indeed virus was isolated from both species, and both species were present in plague proportions. In addition, *Anopheles annulipes*, a mosquito found in very low numbers throughout the year in the south-west, yielded two isolates of Ross River virus from a total of 21 mosquitoes trapped early in the epidemic, tempting speculation that this species may have a role as a maintenance vector. Several other species of mosquito, especially *Culex annulirostris*, may have been involved in minor cycles of Ross River transmission in Perth and some inland areas.

During the second half of 1988, the Southern Oscillation Index, a predictor of weather conditions based on barometric pressures in Tahiti and Northern Australia, moved into a positive phase. As a consequence, above average rainfall occurred in winter and spring in the south-west, and the mean sea level off the Western Australian coast rose to 5.5 cm above the long term mean, resulting in higher tides and higher sea surface temperatures than normal. Thus conditions became more favourable for saltmarsh mosquito breeding. This was reflected in the increased numbers of *Ae. camptorhynchus* and *Ae. vigilax* trapped during the periods, which were 3 fold and 40 fold respectively above the numbers trapped during the same periods in the previous year, 1987/88. In addition, there was a considerable overlap in the population of the two species, which was not observed in 1987. The high spring rainfall may also have enhanced the breeding of certain vertebrate hosts of Ross River virus, particularly the macropod population, resulting in increased numbers of non-immune recruits entering the virus maintenance cycle. Indeed, high numbers of seropositive western grey kangaroos were found during the epidemic. Seroprevalence studies also indicated a possible amplifying role for horses during the epidemic.

(M.D. Lindsay, J. Latchford, J.S. Mackenzie, A.E. Wright and M.R. Bucens).

ARBOVIRUS - LABORATORY TESTING, 1989

Since 1982 the Virology Section of the Division of Public Health Laboratories has collaborated with the Mosquito Control Section of the Department of Natural Resources and Environmental Control for the Surveillance of Arbovirus activity in Delaware.

During the period from July to October, 1989, the surveillance involved in testing 40 mosquito pools, 334 bloods of sentinel flocks, 4 horse brains and one group of birds.

Animal brains and mosquitoes trapped were processed for virus isolation which was undertaken by intra-cerebral inoculation of new born mice and/or inoculation of vero cell cultures. Bloods of sentinel birds and antigens of the above mentioned virus isolates were tested for identification of EEE, WEE or SLE viruses by either Hemagglutination Test or Complement Fixation Test.

The earliest evidence of arbovirus activity of this year was obtained from mosquito pool collected on July 18, 1989 and continued into October.

Up to the date, November 15, 1989, the total of seven EEE virus isolates were made from three horse brains, three mosquito pools and a group of birds. EEE seroconversion of 3 sentinel flocks indicative of current infection were also found during late August to mid September of this year.

The results of laboratory testing of arbovirus in 1989 clearly indicated that the threat of EEE epizootic activity in the southern part of Delaware. Thus far, however, no transmission to humans has been documented.

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Studies on Lyme Disease Vectors

A landscape ecology study has been initiated in Westchester Co., NY in collaboration with Dr. Durland Fish, New York Medical College. A total of 67 properties have been examined, and the habitats containing the 3 life stages of Ixodes dammini determined. Preliminary results indicate that the majority of infected nymphs are in wooded habitats, while smaller populations are present in edge, ornamental plantings, and maintained lawns. The goal of this project is to make landscaping recommendations to homeowners to enable them to reduce their risk of contacting infected ticks.

An ear punch biopsy method has been developed to detect spirochetes in reservoir hosts. This method has already been used to detect spirochetes in rodents from Massachusetts, New York, and Connecticut.

A scutal index of engorgement has been developed to allow investigators to estimate the duration of attachment of nymphal I. dammini. The scutal index is being used to estimate the average duration of attachment of nymphal ticks submitted by physicians or individuals to the Westchester Co. Health Dept. This project is being performed in collaboration with Dr. Richard Falco.

Colonies of I. dammini have been established, and will serve as a base for acaricidal bioassay tests and vector competence trials.

J. Piesman, G. Maupin, E. Campos, and C. Happ. Lyme Disease Vector Section, Medical Entomology & Ecology Branch, Division of Vector-borne Infectious Diseases, CDC, PO Box 2087, Ft. Collins, CO 80522

LYME DISEASE STUDIES IN CHINA

Lu Bao Lin*
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In China, Lyme disease was firstly discovered in Hailin County of Heilongjiang Province in 1985 (Ai Chengxu et al., 1987). The causative agent, Borrelia burdorferi, had been isolated from the tick, Ixodes persulcatus and from rodents and blood of patients (Wen Yaxin et al., 1988; Xu Xaihai et al., 1989c). Antibodies of the disease were found in the patients suspected to be Russian tick-borne encephalitis (Zhang Zhide et al., 1989). The disease was also observed in regions of Yanbien of Jilin Province (Zhang Zhefu et al., 1988).

The ultrastructural and immuno-chemical studies of the American strain amd Chinese isolates showed that they were similar but not identical and a new type of B. burdorferi was suggested (Xu Zaihai et al., 1989a,b; Zhang Zhefu, 1989a). The spirochaetes were found in various organs of the infected tick, including ovaries, oviducts, Malpigian tubules, salivary glands, etc. (Dan Guilan and Yang Zhen, 1989). It could be cultured and maintained in the tick cell culture (Peng Chunji, 1989) and a modified BSK medium (Zhang Zhefu, 1989b).

In 1988, a natural foci of the disease was found in Nalati Xinyuan County of Uygar Autonomous Region of Northwest China (Zhang Qien et al., 1989a). I. persulcatus was showed to be the major vector in the foci too and some rodents, including Clethrionomys frater, Microtus arvalis, Apodemes sylvaticus and Sicita tianshanica acted as important reservior animals (Zhang Qien et al., 1989b). B. burdorferi was also isolated from Neomys fodieus and Sorex asper (Zhang Qien et al., 1989c).

Studies had been also made on the earlier clinical manifestations of the disease (Feng Wenxiang et al., 1989).

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(All the papers were written in Chinese and with English summary except the one indicated "in Chinese")

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REPORT FROM THE CENTER FOR ZOONOSES RESEARCH
NATIONAL INSTITUTE OF HEALTH
ÁGUAS DE MOURA-PORTUGAL

The laboratory of arbovirus in the National Institute of Health, Lisbon, Portugal, recently moved to Águas de Moura, a small village, 60 Km south of Lisbon, where a new laboratory for arbovirus, rodent-borne diseases and parasitology has been organized.

The librarian will be very happy to receive reprints or books about any area of microbiology, in order to help to organize a modern library in this new Center of Research.

HANTAVIRAL ANTIGENS AND ANTIBODIES IN WILD RODENTS IN PORTUGAL

For some years we have tried to identify human cases of HFRS disease in Portugal. Several patients living in rural areas of Southern Portugal have been found with some kind of nephropatia. Hemodialysis patients have also been studied in order to determine the eventual responsability of hantavirus causing the disease.

Some of the studied situations had a clinical disease and epidemiologic features compatible with a HFRS case, mainly workers involved in rural work, in farming areas where wild rodent populations were very high.

Slides with Hantaan virus and observed using the indirect immunofluorescence antibody technique showed several human cases with a low titer of antibodies, usually 1:32. In some cases we noted that these "positive" IFA sera reacted better with the NE antigen than with other hantavirus antigens. However, when the western blot confirmation test was used with the IFA positive sera, the obtained results were not conclusive.

Due to these difficulties it was decided to carry on the research in order to localize some foci of hantavirus in small wild rodents.

One area of Southern Portugal where our research effort was concentrated is located around a village called Águas de Moura, Palmela county. In this region no Apodemus species exist and the wild rodent population is mainly composed of Rattus norvegicus, Rattus rattus and Mus species.

Our results have shown that some captured Rattus norvegicus had antigens for hantavirus in the lungs with high titers of antibodies in the sera. Curiously, the obtained results have shown that the sera reacted intensively with the antigen of Tchoupitoulas virus, a R. norvegicus virus isolated from North America. Probably the Águas de Moura strain of hantavirus is more antigenically closely related to the Tchoupitoulas virus than to any other of the virus strains used as antigens.

Work leading to the isolation of the Portuguese strain of hantavirus is now on the way. Studies to characterize the clinical features of any human HFRS related infections in Portugal are expected to be better elucidated once we have isolated the hantavirus surviving in the farms around the north of Águas de Moura.

Report from WHO Collaborating Centre for Arbovirus Reference and Research, Institute of Virology, Bratislava, Czechoslovakia

Detection of antibodies to western and eastern types of hemorrhagic fever with renal syndrome (HFRS) virus in sera from patients in Slovakia.

In 1984 we detected antigen of HFRS virus in lung tissues from small rodents in Eastern Slovakia. By complement-fixation, these antigens were found to be closely related to the western type of HFRS virus (Puumala virus). Antibodies to this virus were detected in sera of Clethrionomys glareolus, Apodemus agrarius, and Pitymys subterraneus from Eastern Slovakia and in sera of Clethrionomys glareolus, Apodemus sylvaticus, Microtus arvalis, and Microtus economus from Western Slovakia. Antigen of the western type of HFRS virus was demonstrated in Clethrionomys glareolus and Microtus arvalis; antigen of the eastern type of HFRS virus (Hantaan virus) was detected in Apodemus agrarius. Therefore we have focused our attention on the presence of antibodies to the western and eastern types of HFRS virus in serum samples from patients.

Sucrose-acetone extracted antigens for hemagglutination and hemagglutination-inhibition (HI) tests were prepared from the brains of suckling mice infected with Fojnica strain of Hantaan virus (kindly supplied by Dr. A. Gligic, Institute of Immunology and Virology, "Torlak", Beograd, Yugoslavia). HI tests were done with 4- to 8-units of antigen. Serum samples were treated with 2-mercaptoethanol and then extracted with acetone and adsorbed with concentrated goose cells. Antibodies in patient sera were detected by immunofluorescence (IF).

During 1987 and 1988, sera were obtained from patients with clinically diagnosed HFRS, nephritis, or influenza. The results of IF tests with serum samples from six patients are shown in Table 1. Five of six patients (three with clinically-diagnosed HFRS) had antibody; three with highest antibody titers to prototype Hantaan virus (eastern type HFRS virus). In two of the five highest antibody titers were to western type HFRS virus (strain CG-1820). In addition, sera from three individuals clinically diagnosed as having nephritis and one with "influenza" were positive by IF, with highest titers to Hantaan virus.

HI tests (Fojnica antigen) for IgM antibody were also done with human sera (Table 2). Two of nine samples from humans had IgM HI antibody to the eastern type (Hantaan) of HFRS virus.

It has already been shown that the eastern type of HFRS occurs in Far Eastern Asia (Korea, China, Far East of USSR) and the western type of HFRS occurs in Europe. The European form of HFRS (Nephropathia epidemica) is considered less hemorrhagic than Korean hemorrhagic fever. However, in Slovakia, the first HFRS cases had fatal outcomes. Both the eastern type (Hantaan) and the western type (Puumala) of HFRS virus have been detected in small rodents in Slovakia. Clethrionomys glareolus and Microtus arvalis have been the common hosts for the western type of HFRS and correlate with the milder form of the disease. Apodemus species seem to be the host for the eastern type of HFRS virus and correlate with the more severe form of the disease.

It is of interest that we have been able to demonstrate IgM antibodies

to hantaviruses by HI tests. By IF tests antibodies to both eastern and western types of HFRS viruses have been detected.

Table 1. Immunofluorescent antibody (IFA) titres to two hantaviruses in serum samples from six patients in Czechoslovakia.

Patient	Serum No.	Date	Clinical diagnosis	IFA titre with antigen	
				Hantaan	CG-1820
1	1a	12.X.87	HFRS	16	64
	1b	23.X.87		16	512
	1c	30.X.87		16	512
	1d	1.XII.87		16	512
2	2a	7.XII.87	Nephritis	512	16
	2b	18.XII.87		512	16
3		1.VI.88	Nephritis	128	<16
4		18.VIII.88	Influenza	128	2048
5		29.IX.88	HFRS	128	16
6		3.VIII.88	HFRS	<16	<16

Table 2. Hemagglutination-inhibition (HI) titres to Fojnica strain of Hantaan virus in serum samples from nine patients in Czechoslovakia.

Patient	Clinical diagnosis	HI titre	HI titre after treatment of serum with 2-mercaptoethanol
1	HFRS	10	<10
2	Nephritis	160	40
3	Nephritis	80	10
4	Influenza	<10	<10
5	HFRS	10	10
6	HFRS	<10	<10
7	HFRS	<10	<10
8	HFRS	<10	<10
9	HFRS	<10	<10
"Bulgaria"	HFRS	80	10
"Yugoslavia"	HFRS	80	<10
"USA"	HFRS	40	20
"Healthy"	None	<10	<10

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FLAVIRUS STUDIES IN NATIONAL INSTITUTE OF HEALTH, JAPAN

(April,1987 - March,1988)

Takashi Kitamura and Tadahiko Kitano
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1. Field Trial of the Efficacy of Japanese Encephalitis (JE) Vaccine Produced in India.

A lot of JE vaccine produced in Central Research Institute, Kasauli,India, with Nakayama strain virus was given to 42 Indian volunteers(age 17-54) twice at 1 week interval and sera were collected 6 weeks after the first inoculation. Sera from individuals with no prior JE vaccination well neutralized the homologous virus strain(Nakayama) but less the isolates from India and Sri Lanka. Sera from those with prior JE vaccination(booster group) showed equally high neutralizing activity against virus strains isolated either in Japan,India or Sri Lanka. A system with vaccination and yearly booster vaccinations may be the practical one which may cover all strains of JEV. (M.Kobayashi, L.N. Rao & A.Oya)

2. Immunogenicity of JE Vaccine Produced with Beijing Strain Virus.

JE virus strain for vaccine production in Japan was shifted from Nakayama to Beijing strain in 1987. Immunogenicity of new vaccine products against various strains of JE virus(JEV) was surveyed by collecting sera of virgin recipients, those receiving the vaccination of standard protocol without prior JE vaccination history, and assaying neutralizing antibody levels against fresh isolates of JEV from Japan, China, India and Sri Lanka, in comparison with serum collection obtained by former vaccinations with Nakayama vaccines. New vaccines produced with Beijing strain gave neutralizing antibody response reacting with all strains equally, while Nakayama vaccine recipients showed selective neutralization of homologous virus strain. The use of Beijing strain was indicated to be better for the purpose of preventing JE in newly emerging endemic areas as India and Sri Lanka. (T.Kitano, S.Yabe, & M.Kobayashi)

3. Structural Proteins of JEV Reacting with Neutralizing Antibody.

Purified virion fraction was digested with chymotrypsin(Cm) or V8 protease, resulting in either 5(Cm-29K,22.5K,39K,37K,& 27K) or 7 bands(39K - 14K), respectively, of structural peptides. By Western blotting, 5 peptides(Cm-29K, 22.5K,V8-39K,37K,27K) were identified to be reacting with neutralizing antibody(NA). No difference in the number and molecular size of NA-reacting structural peptides and reactivities with several monoclonal antibodies was observed among various strains of JEV. (T.Kitano, & S.Yabe).

4. Incidence of Dengue Fever (DF).

There were 14 cases (12 Japanese and 2 foreigners) who developed dengue-like symptomes after entering Japan from endemic areas were subjected to virological diagnostic procedures in our laboratory during fiscal 1987 (April, 1987 - March,1988).Six cases were negative in dengue HI antibody. Among other 8 cases, 6 were diagnosed positive by HI antibody higher than 1:640, 2 cases had low titers(1:80) of both dengue and JE HI antibodies and could not positively diagnosed. (S.Yabe & T.Kitano)

Report from the Instituto Evandro Chagas-FSESP, Brazilian Ministry of Health, (Av. Almirante Barroso 492, 66050, C.P. 1128) Belém, Para, Brazil

Yellow fever in Francisco Dumont, Minas Gerais State, Brazil.

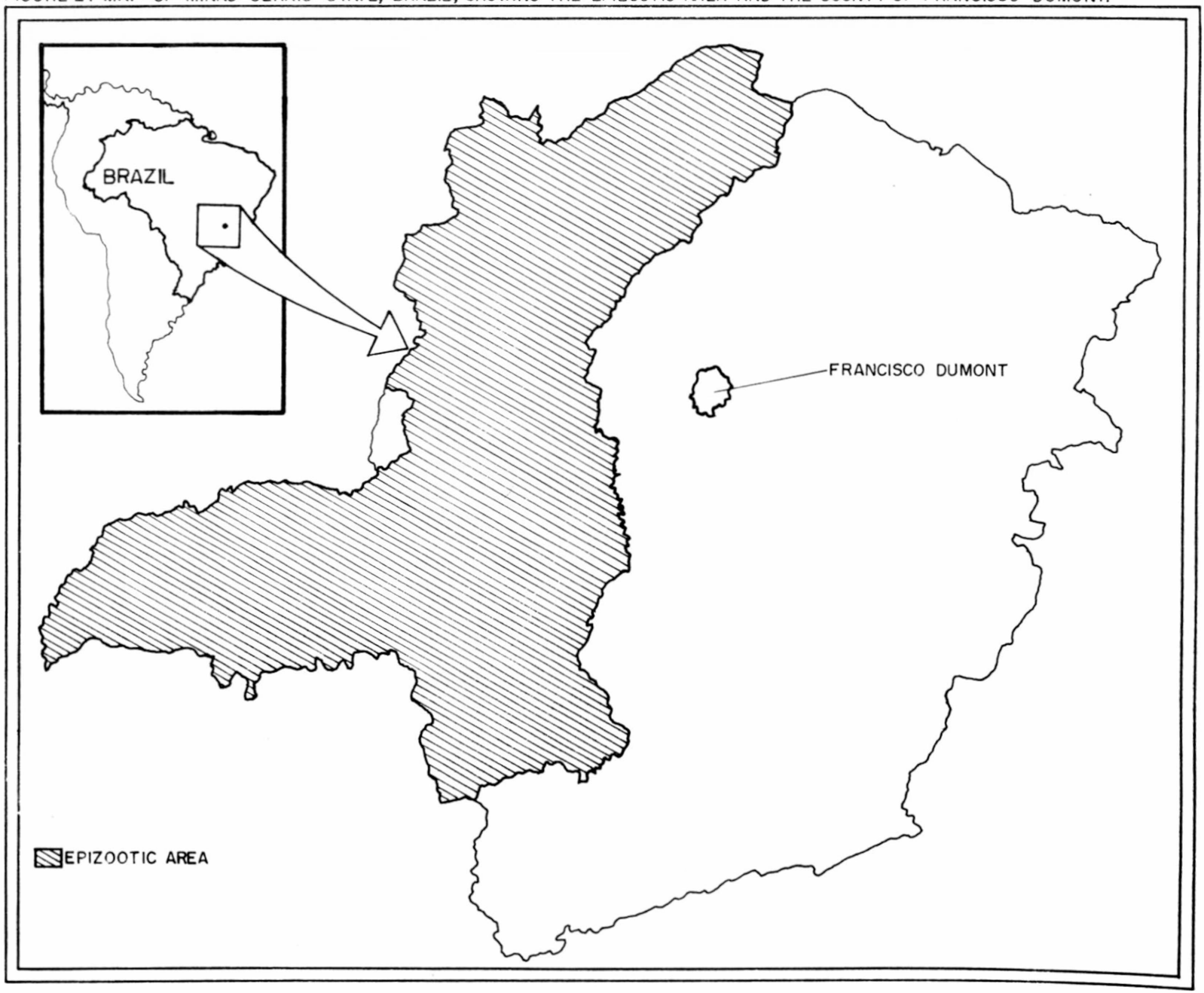
Between March 6 and April 6, 1989 seven cases of yellow fever (YF) were diagnosed using serum samples from 44 clinically-suspect patients or contact residents of Francisco Dumont County, which is located in the rural San Francisco Valley, Minas Gerais State, Brazil (Figure 1). This finding is of considerable interest because YF virus because YF virus has never been isolated in this area, YF vaccination therefore not having been performed routinely. Diagnosis was made on the basis of serology and virus isolation. Hepatitis A and hepatitis B also were present at the time of the outbreak.

Six cases were diagnosed by the presence of IgM antibody (ELISA) to YF virus. The serological pattern of hemagglutination-inhibiting antibodies was compatible with primary YF virus infection. YF virus was recovered from the serum of a seventh patient. The sample was collected on 8 March from a 26-year old male in his seventh day of illness. He had developed high fever, headache, back pain, weakness, and vomiting.

Most of the patients (6 males, 1 female) were between 13 and 29 years old. An intensive vaccination campaign with YF 17D virus was initiated in the area and no further cases were reported after completion of the vaccination efforts.

A.P.A. Travassos da Rosa, S. G. Rodrigues, J.F.S. Travassos da Rosa, and P.F.C. Vasconcelos (Virus Section)

FIGURE 1: MAP OF MINAS GERAIS STATE, BRAZIL, SHOWING THE EPIZOOTIC AREA AND THE COUNTY OF FRANCISCO DUMONT.



REPORT OF DEPARTAMENT OF VIROLOGY - MICROBIOLOGY

INSTITUTE - RIO DE JANEIRO FEDERAL UNIVERSITY - BRAZIL

Ultrastructural aspects of cells treated with Ouabain (OUA) and infected with RNA viruses (WEE and Poliomyelitis viruses).

Chicken fibroblast embryo cells treated with Ouabain (OUA), had been shown to be more active for Sindbis Virus replication. We intended to verifi if the same phenomenon occurs in human cells infected with other viruses belonging the same class IV of Baltimore's classification (Western Equine Encephalitis Virus - WEE and Poliovirus).

HEp2 cells (human laringeal carcinoma) treated or not with OUA (inhibitor of NA+K+ATPase) were infected with viruses and observed at optical and electron microscopy. Virus titration was performed by $TCID_{50}$ dose (Reed & Muench Method).

At 16 hours post-infection, the cells were observed by optical microscopy. Cytophatic effect (CPE) was not detected in only infected cells but instead, the cells treated with OUA showed intense refringence (CPE). Viruses were recovered from these infected cells with titre of 10^5 $TCID_{50}/ml$. At electron microscopy the cells treated with OUA were not so altered, showing slight vacuolization and few microvilosities. Extracellular viruses and inclusion bodies were observed in vacuolated cells infected with WEE. On the other hand, the cells infected with WEE and treated with OUA had vesicles full of viral particles and showed virus budding (Figure 1). High amount of mature viral particles were seen extracellularly. The cells infected with Poliovirus did not show any difference. These results suggest that OUA increase the cell potenciality to biossintetize WEE virus.

(MADEIRA-LIBERTO, M.I.; GALVÃO-CARVALHO, T.C.; RIBEIRO-BISCAYA, D.; VON HUBINGER, M.G. & FÉO-VEIGA, V.)

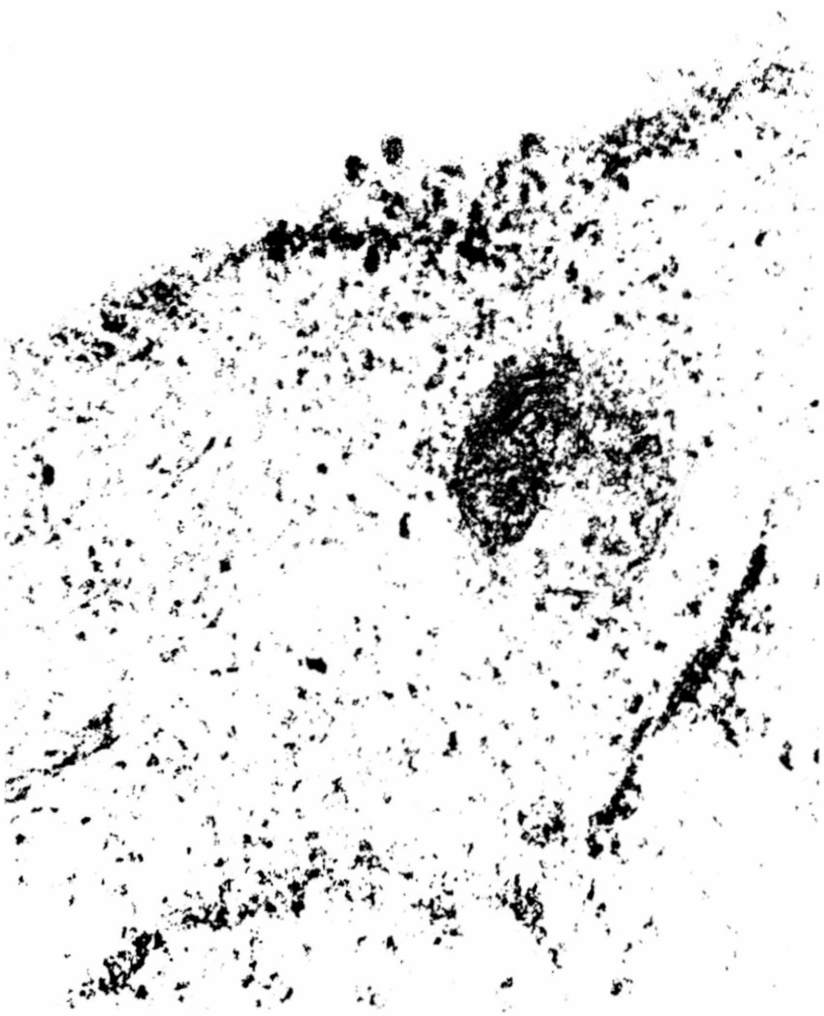
Effect of OUA in viral replication process.

FIGURE 1

Eletromicroscopy of ultra thin section of HEp2 cell culture infected with WEE virus.

Arrows indicating virus budding.

Scale : 1 cm = 100 nm



REPORT FROM NEUROVIROLOGY UNIT, RAYNE INSTITUTE, ST. THOMAS' HOSPITAL, THE UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST. THOMAS' HOSPITAL, LONDON SE1 7EH, ENGLAND.

A killed brain-drived Semliki Forest virus vaccine stimulates anti-brain antibodies and inflammatory lesions in rabbits.

During maturation enveloped viruses bud from external and/or internal cellular membranes and incorporate host-cell antigens in their coat. For the Togaviridae family the lipid composition of several viruses have been analysed and reflects the lipid content of the host cell membranes in which the virus replicates (1). Previous work in the laboratory has shown that following infection with the Togavirus, Semliki Forest virus (SFV) antibodies directed to brain glycolipid, galactocerebroside (GalC) and gangliosides are produced (1 & 2).

This study was designed to investigate whether a killed vaccine of SFV would also produce an immunological response to the lipid component of the viral envelope.

SFV, A7(74) strain was purified from suckling mouse brain using the method of Amor and Webb (3). The purified virus was concentrated using a tangential filtration method and further concentrated using a continuous sucrose gradient (20-50%). The vaccine produced using acetylethineimine (3) did not cause deaths in suckling mice and was considered fully inactivated.

New Zealand white rabbits were inoculated subcutaneously with a total of 2 ml of emulsion containing equal volumes of purified brain derived SFV and complete Freund's adjuvant enriched with 5 mg/ml Mycobacterium tuberculosis. Control animals were given either saline only or saline in place of the vaccine in the emulsion. Inoculation and sampling regimes are shown below.

Table 1 Inoculation and sampling days of vaccinated rabbits.

Rabbit	1	2	3	4	5	6
1st inoc.	vaccine	vaccine	vaccine	vaccine	saline	-
PVD 54	-	-	vaccine	vaccine	-	-
PVD 89	sampled	-	-	-	-	-
PVD 420		vaccine	vaccine	vaccine	saline	-
PVD 434		sampled	sampled	sampled	sampled	sampled

PVD - Post vaccination day

An ELISA was used to measure IgG levels to SFV, GalC and gangliosides. All the SFV vaccinated animals showed high levels of IgG antibodies to the three antigens. Rabbits inoculated with saline in the emulsion also showed a slight reactivity but was not significaly different from the uninoculated rabbit.

The CNS was examined histologically. Perivascular cuffing and inflammatory cells were seen in the grey and to a lesser extent the white matter, in 3/4 vaccinated animals but not controls (rabbits 5 & 6).

This preliminary study suggests that host cell glycolipids in the envelopes of a budding virus, i.e. SFV, stimulates a humoral response and is capable of initiating inflammatory lesions in the rabbit CNS.

Brain glycolipids have been shown to be encephalitogenic when inoculated with adjuvants resulting in both experimental allergic neuritis and experimental allergic encephalitis (4 & 5). These are animal models for the human 'autoimmune diseases', Guillain-Barre syndrome and multiple sclerosis respectively. Thus budding viruses may be capable of initiating autoimmune disease due to incorporation of host cell glycolipids in the viral envelope.

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(S Amor and HE Webb).

REPORT FROM NEUROVIROLOGY UNIT, RAYNE INSTITUTE, ST. THOMAS' HOSPITAL, THE UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST. THOMAS' HOSPITAL, LONDON SE1 7EH, ENGLAND.

Multiple Inoculations of Semliki Forest Virus In Mice

The A7(74) strain of Semliki Forest Virus (SFV) has been used as a model for studying virus induced demyelination within the central nervous system (Webb et al, 1978; Kelly et al, 1982; Illavia et al 1982; Pathak et al, 1983). Some of the lesions seen resemble multiple sclerosis (MS) and because of the possibility that the relapses in MS may be due to recurrent viral infections, the effect on the brains of mice after multiple inoculations of SFV A7(74) was investigated.

The virus was inoculated intraperitoneally into Swiss A2G mice at weekly intervals for 8 weeks. Twice weekly after each booster dose the blood and brains were sampled. The blood was used for virus titres and serum seperated for IgG antibody levels. The brains were used for virus titres and histopathological study. Groups of mice were also given a single inoculation and inoculations at two or more weekly intervals. Normal controls underwent the same sampling.

Results showed that the blood virus titres were measurable after the 1st inoculation only and the brain virus after the 1st and 2nd inoculations up to day 14. The serum IgG antibody levels reached their peaks five weeks after either a single or repeated inoculations.

Grossly enhanced perivascular cuffing and large focal demyelinating lesions were seen particularly after the 2nd inoculation. The demyelinating lesions showed necrotic areas with calcification and intense infiltration of mononuclear cells. It was very uncommon for an animal to die. However, with further inoculations the severity of the lesions did not increase and by the 8th booster dose the brain appeared to have recovered. The maximum and most prolonged damage to the brain was seen after the first two inoculations when given weekly. As the interval between the inoculations was increased the severity of the lesions did not increase. Uninoculated control mice showed no abnormality.

It was concluded that in immunocompetent mice repeated inoculations of SFV A7(74) did not produce a relapsing demyelinating disease.

The enhanced lesions seen after the 2nd inoculation appear to be due to prolonged virus multiplication in the brain. A possible reason for this could be that when the large 2nd dose of SFV is inoculated, it absorbs the low levels of antibodies already present. It seems to act as an immunosuppressant allowing the virus to replicate for a longer time in the brain. This has been shown to be the case in our experiment. Severe damage is still seen 7 days after the 3rd inoculation. After this, due to the higher levels of antibody subsequent virus infections were not able to prolong the infection, and there was no further increase in damage to the brain.

In spite of the gross damage inflicted by the 2nd and 3rd virus inoculations, the brain lesions had apparently recovered by eight weeks.

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(SJ Illavia and HE Webb).

REPORT FROM THE VIRUS LABORATORY,
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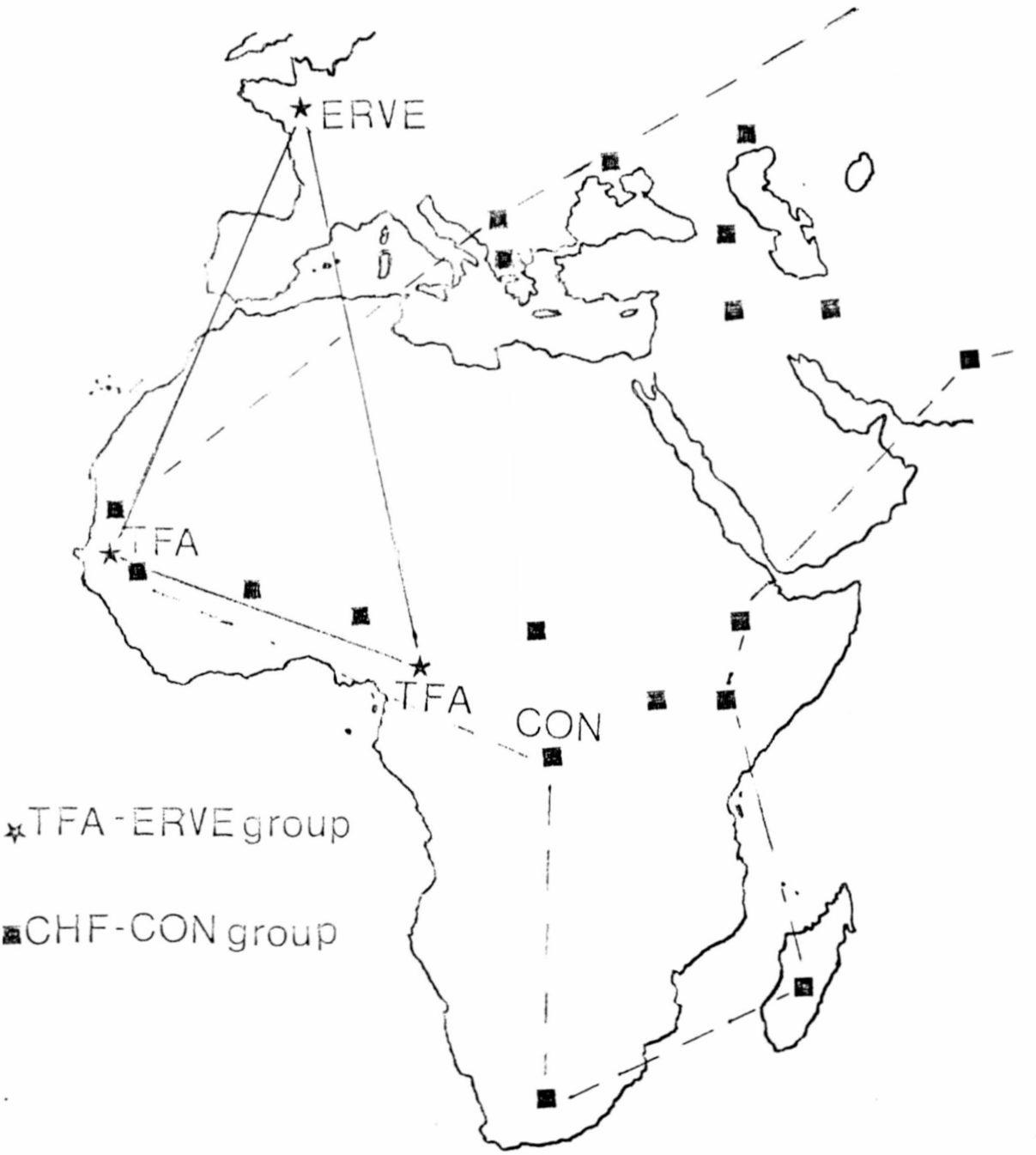
Erve virus, a nairovirus from France, related to CCHF group ?

Erve virus was isolated in 1962 from tissues (spleen + kidney) of three white toothed shrews (Crocidura russula L.) trapped near Saulges village, Erve river valley, Western France, an established focus of Eyach virus infection (Chastel et al. 1964).
Results of virological, physico-chemical and ultrastructural studies suggested that this virus belongs to the Bunyaviridae family (Chastel et al. 1987).
Serosurveys indicated that Erve virus had apparently a large geographical distribution in France and may infect rodents, insectivores, wild boars (Sus scrofa), red deer (Cervus elaphus), sheep, herring gulls (Larus argentatus) and human beings. Healthy blood donors living in the vicinity of the Saulges area exhibited the highest incidence of antibody to Erve virus.
Recently, it was shown by Hervé Zeller, working in Calisher's Laboratory that Erve and Thiafora viruses were antigenically related as demonstrated by IFA. Thiafora virus is an ungrouped virus which was isolated from African shrews (Crocidura sp.) in Senegal and Cameroons.
Furthermore, antigenic relationship was also evidenced between Thiafora and Congo-Crimean haemorrhagic fever virus and protein profiles of Thiafora virus were found mimecking those of nairoviruses by Georges Saluzzo (pers. com. ; 1989).
CF antibody to Erve virus was found to a low titer in four patients in France exhibiting an fatal haemorrhagic syndrome (a child) or suffering from polyradiculoneuritis and arthritis (three adults).
Thus Erve virus appears as a potential human pathogen and probably belongs to the Nairovirus genus in the Bunyaviridae. The geographical distribution of Thiafora-Erve complex and of CCHF (not exhaustive !) is presented in the adjoining map.

Ref. : C. Chastel, A.J. Main, P. Richard, G. Le Lay, MC Legrand-Quillien and J.C. Beaucournu. Acta Virol., 1989, 33, 270-280.


ERVE
TFA
TFA
CON
TFA - ERVE group
CHF - CON group