ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

January, 1993

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NOTE: As noted in "PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE", which is found on the front page of each issue, you are encouraged to submit a brief summary of your work. The summary need not be in manuscript style, the results do not have to be definitive, you need not include tables (unless you want to). This is not a peer-reviewed publication. The intent is to communicate among ourselves and to let each other know what we are doing. This issue consists of 31 reports and three announcements or committee or subcommittee updates. The subcommittee reports will cover 59 pages. The next issue will likely be mailed June 1, 1993, (probable deadline for submissions: May 15, 1993). 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. Also, there is nothing that prevents you from submitting a report to every issue. There are no page charges either but, then again, this is not a publication.

PLEASE!!!

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

Subcommittee on Information Exchange
DVBID/NCID/CDC
P.O. Box 2087
Fort Collins, CO 80522

GUIDELINES FOR SUBMITTING REPORTS

We want to keep this mechanism timely and viable. Therefore, submit only recent news and summaries of your work. **PLEASE** limit the submission to 1 or a very few sheets (21.59 cm x 27.94 cm = 8.5 x 11 inches) plus a table or two; condense as much as you can (single space the text); **do not** staple pages together; **do not** number pages.

UPDATE ON ACTIVITIES OF THE INTERNATIONAL COMMITTEE ON TAXONOMY OF VIRUSES: THE ICTV DATABASE ICTVdB^R AND OTHER INITIATIVES

The International Committee on Taxonomy of Viruses (ICTV) was founded at the International Congress of Microbiology in Moscow in 1966.   When, in 1968, the Virology Division of the International Union of Microbiological Societies (IUMS) was formally organized at the First International Congress of Virology in Helsinki, the ICTV became permanently associated with the Division.   In the years since then, the ICTV has succeeded in bringing virus taxonomy from an amateur, confusing, and ever-changing situation to one of stability and world-wide agreement and use.   Today, the ICTV operates seven Subcommittees and 45 Study Groups -- more than 376 virologists participate, representing virologists working in all fields and in all countries.   The current officers and members of the Executive Committee are:

President: F. A. Murphy, USA
Vice President: K. W. Buck, UK
Secretaries: C. Fauquet, FRANCE/USA;
             C. R. Pringle, UK
Chairman, Vertebrate Virus Subcommittee: D. H. L. Bishop, UK
Chairman, Fungal Virus Subcommittee: S. A. Ghabrial, USA
Chairman, Virus Data Subcommittee: A. J. Gibbs, AUSTRALIA
Chairman, Bacterial Virus Subcommittee: A. W. Jarvis, NEW ZEALAND
Chairman, Plant Virus Subcommittee: G. P. Martelli, ITALY
Co-Chairmen, Coordinating Subcommittee: J. Strauss, USA, and D. McGeoch, UK
Chairman, Invertebrate Virus Subcommittee: M. D. Summers, USA
Member: H. W. Ackermann, CANADA
Member: P. Ahlquist, USA
Member: L. Berthiaume, CANADA
Member: C. H. Calisher, USA
Member: R. Goldbach, THE NETHERLANDS
Member: J. Maniloff, USA
Member: M. A. Mayo, UK
Member: G. Rohrmann, USA

The Executive Committee met from April 6-8, 1992 at the NERC Institute of Virology and Environmental Microbiology in Oxford, UK.   The following provides a brief report of the proceedings of this meeting:

TAXONOMIC DECISIONS: Some 49 taxonomic proposals were considered, 44 of which will be advanced for final consideration at the meeting of the ICTV at the Ninth International Congress of Virology to be held in Glasgow in August, 1993.   Cumulatively, these proposals will

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bring the universal system for virus taxonomy to two Orders, 50 Families, 9 Subfamilies, 126 Genera, 23 floating Genera, and 4 Subgenera (system still contains 2,644 unassigned viruses, largely because of a lack of data):

- **PLANT VIRUSES:** Decisions were reached, based upon proposals from the Plant Virus Subcommittee and its Study Groups, to form six new families (there were already three families containing plant viruses, the families *Bunyaviridae*, *Reoviridae*, and *Rhabdoviridae*): these are the families *Cryptoviridae*, *Geminiviridae*, *Tombusviridae*, *Comoviridae*, *Bromoviridae*, and *Potyviridae*. Agreement on these proposals represents a major advance that brings plant virus taxonomy/nomenclature in line with that of other viruses. This agreement follows upon recent findings of remarkable sequence similarities between some plant and vertebrate viruses.

- **BACTERIAL VIRUSES:** The second order in the universal virus taxonomy system was approved (the first was the order *Mononegavirales*, comprising the families *Paramyxoviridae*, *Rhabdoviridae* and *Filoviridae*): this is the order *Caudovirales*, comprising the three families of tailed bacteriophages, the families *Myoviridae*, *Siphoviridae* and *Podoviridae*.

- **VERTEBRATE VIRUSES:** A decision was reached to form a new family, the *Circoviridae*, comprising those newly characterized very small viruses with circular dsDNA genomes. Three viruses of current notoriety, rabbit hemorrhagic disease virus, Norwalk virus (and related viruses), and hepatitis E virus, were proposed to become members of the family *Caliciviridae*. The genus *Arterivirus* was removed from the family *Togaviridae*, and left as a free-floating genus pending the assessment of ongoing research; it is presumed that viruses such as lactic dehydrogenase elevating virus of mice, simian hemorrhagic fever virus, and possibly blue ear virus of swine, Borna virus of horses and carrot mottle virus may become members. The genus *Torovirus*, which had been a free-floating genus, was proposed to become a genus of the family *Coronaviridae*.

- **AGENTS OF SPONGIFORM ENCEPHALOPATHIES:** A decision was reached to form a Study Group which is to be charged with developing a system of taxonomy/nomenclature for these agents. Thus, these agents will be managed by ICTV as viroids and satellites are presently.

PLANS FOR FUTURE REPORTS OF THE ICTV (THE CLASSIFICATION AND NOMENCLATURE OF VIRUSES): Future reports of the ICTV will be produced as database-driven, software-based venues for easy taxonomic data access. From the database, each Report will be made available as a book, and if resources allow, as a CD-ROM. The Sixth ICTV Report will be published in 1994; with this Report the

process of switching to the database will start. This Report will have greater depth, and greater uniformity than earlier Reports, and for the first time it will begin to be developed in the form of a searchable database.

THE CONCEPT OF VIRUS SPECIES: The Sixth ICTV Report will be the first to incorporate decisions regarding the adoption of the concept of virus species. The species taxon has always been regarded as the most important taxonomic level in classification, but with the viruses it has proved to be the most difficult to deal with. After years of controversy, in 1991, the ICTV Executive Committee, accepted the definition of a virus species proposed by M. H. V. van Regenmortel, as follows: "*A virus species is a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche.*" The major advantage in this definition is that it can accommodate the inherent variability of viruses and it does not depend on the existence of a single unique virion characteristic. Members of a polythetic class are define by more than one property and no one property is essential or necessary. Thus, in each virus family it will be possible to determine the set of properties of the type species and then it will be possible to compare family members with this type species -- this, in turn, will allow precise definitions of genera and other sub-taxa within each family. The members of the ICTV Study Groups will now determine the precise definition of virus species in the taxon for which they are responsible.

PLANS FOR THE ICTV DATABASE (ICTVdB^R): Viral taxonomy has reached a critical juncture, requiring new ideas for keeping the universal system from breaking down because of data overload and data chaos. To relieve this problem and to provide a base for delivering many kinds of practical products from core information, the ICTV, at its 1991 midterm meeting committed itself to the development of a universal virus database, the ICTVdB^R. Agreement was reached on the nature (hardware, software), the principal participants, and the international locations of various facets of this database and the necessary ICTV activities to support it. In Oxford in 1992, agreements were reconfirmed that the DELTA software system, developed in the CSIRO and the Australian National University in Canberra, will become the base for the ICTVdB^R. The DELTA system was specifically designed for handling taxonomic information. This work will be led by A. J. Gibbs, M. Dallwitz and colleagues. Efforts are underway to obtain necessary financial support for development and adaptation of the DELTA system to the requirements of the ICTVdB^R. Financial support is being sought on an international basis from several agencies. An immediate task, being coordinated by A. J. Gibbs, is the compilation of an appropriate set of virus

descriptors.   This set,   which   will   include   500-700
characters for each virus.

The members of the ICTV are in a dynamic and enthusiastic
frame of mind -- the members know that virologists need
the ICTVdB^R, and they know that the key to this
development lies in completing the universal taxonomic
system at the levels of family, genus and species (and in
some cases at the level of order).   The members of the
ICTV look forward to presenting these matters to the
world's   community   of   virologists   at   the   Ninth
International Congress of Virology in Glasgow in August,
1993.

For the ICTV
Craig R. Pringle
June 1992

SEAS Update

The Subcommittee on Evaluation of Arthropod-Borne Status
(SEAS) was formed in 1970 as a working subcommittee of the
American Committee on Arthropod-Borne Viruses (ACAV). Its
primary function is to evaluate newly registered viruses (and to
review existing ones) with an aim toward determining the role
played by arthropods in their transmission. The original
subcommittee, under the direction of Dr. William McD. Hammon,
adopted the 1967 WHO definition of an arthropod-borne virus:
"Arboviruses are viruses which are maintained in nature
principally, or to an important extent, through biological
transmission between susceptible vertebrate hosts by
haematophagous arthropods; they multiply and produce viraemia in
the vertebrates, multiply in the tissues of arthropods, and are
passed on to new vertebrates by the bites of arthropods after a
period of extrinsic incubation." Based on this definition,
members of SEAS established criteria by which viruses were
classified as **arthropod-borne viruses (arboviruses), possible
arboviruses**, or **non-arboviruses**. This was later expanded to five
categories, including **probable arboviruses** and **probable non-
arboviruses** in addition to the three listed above. Since 1971,
SEAS has evaluated more than 500 viruses.

At the International Congress of Virology held in Edmonton in
August 1987, it was suggested that, because many of the viruses
that are now thought not to be arboviruses (e.g. hantaviruses,
arenaviruses, etc.) are associated with rodents but not
arthropods, we should develop a new category, **rodent-borne
viruses**. The members of SEAS have finally agreed on a new
classification scheme. Category assignments depend on how the
virus is maintained in nature. Virus registrations are first
examined to determine if the viruses meet traditional definitions
of an **arbovirus** or **probable arbovirus**. If not, and insufficient
information is available to make a decision, they are still
classified as a **possible arbovirus**. However, if there is
information that indicates association with rodents but not
arthropods (i.e., persistent infection in rodents, direct rodent
to rodent transmission without the presence of arthropods,
demonstrated non-susceptibility of arthropods to infection with
the virus, etc.), then the virus is classified as either a
rodent-associated or a **probable rodent-associated virus**,
depending on the strength of the evidence. Similarly, a virus
recovered only from bats is classified as a **bat-associated** or a
probable bat-associated virus. It should be emphasized that an
arbovirus associated with rodents (e.g. La Crosse virus) is still
considered to be an **arbovirus** and not a **rodent-associated virus**
(i.e., rodent (bat)-associated implies non-arbovirus). Analogous
to the definition for an arbovirus, our definition for a **rodent
(or bat)-associated virus** is one chiefly maintained in nature by
direct intra- or interspecific transmission from rodent (or bat)
to rodent (or bat). Virus infection is usually chronic in at
least a portion of the small mammal population and viremia may be
prolonged. Transmission may occur through one or more of the

following means: direct contact, salivary or venereal
secretions, milk, urine, or intrauterine infection. Virus is
passed indirectly to humans, usually by means of the urine or
saliva (possibly by aerosols) of the chronically infected small
mammal. Naturally infected arthropods are rarely encountered and
arthropod transmission is not considered to be the usual route of
transmission."

The recent advances in molecular techniques provide evidence
for a classification system for viruses analogous to that used
for plants and animals. The recommendations of the SEAS are not
meant to supplant these, but rather to provide additional
information about the natural history of these viruses. Because
of the rapid growth in the number of registered viruses, we are
no longer able to be familiar with all of the registered viruses.
Thus, the purpose of the SEAS classification is to give the
reader a feel for the epidemiology and ecology of a given virus,
rather than to serve as a taxonomic classification.

Based on our evaluation of viruses currently registered, the
SEAS recommends that the following viruses be classified as
either rodent-associated or bat-associated viruses. We are
currently reviewing viruses that might go into the "probable"
rodent- or bat-associated virus categories.

Rodent-associated Viruses

Arenaviridae
Junin	JUN
Lassa	LAS
Latino	LAS
Machupo	MAC
Pichinde	PIC
Tamiami	TAM

Bunyaviridae
Hantaan	HTN
Prospect Hill	PH
Puumala	PUU
Seoul	SEO

Flaviviridae
Modoc	MOD

Bat-associated Viruses

Flaviviridae
Dakar bat	DB
Rio Bravo	RB

Reoviridae
Ife	IFE

Collection of Insect Cell Lines

As part of our reference center activities, we have recently begun to collect and store cell lines of medically important insects. The table below gives those that we have acquired so far. Most are not available from the American Type Culture Collection. All are stored in liquid nitrogen and are available at no cost to qualified investigators. We would also appreciate receiving cultures of other insect cell lines not listed below, as these represent a valuable resource and should be preserved in several different localities. Persons wishing to use or to donate cell lines should contact Dr. Robert Tesh, or Dr. Robert Shope, Yale Arbovirus Research Unit, Department Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, P.O.Box 3333, New Haven, CT 06510.

Insect species	Cell designation	Origin
Aedes aegypti	Singh's	larvae
Aedes aegypti	RML-12	?
Aedes albopictus	Singh's	larvae
Aedes albopictus	C6/36	clone of Singh's
Aedes psuedoscutellaris	AP-61	larvae
Aedes triseriatus	MAT	larvae
Toxorhynchites amboinensis	TRA-171	larvae
Toxorhynchites amboinensis	TRA-284	larvae
Toxorhynchites amboinensis	TA-14	?
Culex tarsalis	Chao/Ball	embryonated eggs
Culex quinquefasciatus	Hsu	adult ovary
Culex theileri	lines 1 and 3	embryonated eggs
Anopheles stephensi	MOS.43	larvae
Anopheles gambiae	Mos.55	larvae
Culicoides variipennis	Cu Va	embryonated eggs
Lutzomyia longipalpis	LL-5	embryonated eggs
Phlebotomus papatasi	PP-9	embryonated eggs

REPORT FROM VIRUS LABORATORY, DEPARTMENT OF TROPICAL
MEDICINE, MEDICAL RESEARCH INSTITUTE, KANAZAWA MEDICAL
UNIVERSITY, UCHINADA, ISHIKAWA-KEN, JAPAN 920-02

Molecular Comparison of DEN-1 Mochizuki Strain Virus
and Other Selected DEN Viruses Regarding Nucleotide
and Amino Acid Sequences.

The virus cultivated in vero cells was purified by
sucrose density gradient centrifugation. Viral RNA was
extracted from purified virions and amplified by PCR
method. The products were cloned and confirmed to be
DEN-specific. Nucleotide sequences were determined and
amino acid sequences were deduced. Regions encoding C,
preM, M and parts of E proteins were major subjects of
investigation.

The glycosylation sites in preM and hydrophobic
regions at caboxyl termini of both M and E of DEN-1 were
well conserved.

It was noted that the Mochizuki strain virus was
closer to Philippine 836-1 strain and Nauru Island strain
than to Thai AHF 82-86 strain and Caribbean CV1636/77
strain. The Mochizuki strain was isolated in 1943 in Japan
while the Philippine strain was isolated in 1984 and the
Nauru strain in 1974 in the respective places. It may be
pointed out in this connection that the first onset of
Japanese dengue epidemics was among the crew of a cargo
boat which was connecting Japan proper and South western
Pacific islands at that time (rf: J. Trop. Med. Hyg. 56:
83, 1953).

On the other hand, difference between Mochizuki strain
and Philippine and Nauru strains was shown at the cleavage
site of preM/M; Mochizuki possesses RRGKR/S while the latter
two possess RRDKR/S. The DEN-1 viruses were distinguished
from other types of virus (DEN-2 to 4) regarding the sequence
patterns.

(Conducted by: EDWARD ZULKARNAIN and TSUTOMU TAKEGAMI)

(Reported by: SUSUMU HOTTA)

Analysis of Variation in Dengue Virus Type 2 Using the Chemical Cleavage at Mismatch Method

P. J. Wright [1], B. Lin [1], D. W. Trent [2] and R. G. H. Cotton [3].

1. Monash University, Clayton, Vic. Australia.   2. Centers for Disease Control, Fort Collins, Co. U.S.A..   3. Murdoch Institute, Parkville, Vic. Australia.

The genetic variation in twelve strains of DEN-2 was studied by the chemical cleavage at mismatched cytosine (CCM method) in DNA:RNA heteroduplexes. The DEN-2 isolates used are listed below.

Strain	Location	Year	Topotype
NGC	New Guinea	1944	New Guinea
PUO-218	Thailand	1980	Thailand
S44552	Seychelles	1977	Seychelles
S44554	Seychelles	1977	Seychelles
UV0199	Burkina Faso	1982	Africa/epidemic
HA242-82	Ivory Coast	1982	Africa/epidemic
1408	Jamaica	1983	Caribbean
1409	Jamaica	1983	Caribbean
S16803	Thailand	1974	Thailand
D79-069	Thailand	1979	Thailand
S35179	Philippines	1975	Philippines
TC16677	Thailand	1964	Thailand
S10099	Philippines	1966	Philippines

The viruses were passed twice in C6/36 cells, then total RNA from infected cells was extracted.   End-labelled DNA probes were prepared from cDNA of the New Guinea C strain in the E (nts 26 to 428) or NS2A (nts 141-369) genes and hybridized to RNA.   Mismatched cytosine in the heteroduplexes was modified by hydroxylamine, the nucleic acid strands cleaved by piperidine, and the radiolabelled fragments were analysed by electrophoresis and autoradiography (Cotton & Wright, [1989] J. Virol. Methods 26, 67-76) (Lin et al.[1992] J. Virol. Methods, in press).   The patterns of bands generated by both probes corresponded to the geographical groupings of the isolates.   Our results demonstrated that the CCM method is suitable in epidemiological studies for surveying a large number of isolates.   It is possible to choose the gene to be targeted, and the pattern of mismatches allows the typing or grouping of isolates.   It is a rapid and relatively simple approach to the study of virus variation.

Monoclonal antibodies against the New Guinea strain of dengue 2 virus.

A panel of 24 monoclonal antibodies was prepared against the New Guinea strain of dengue 2 virus. Some of their biological characteristics are tabulated below.

MAb	Virus specificity	Neut. titre	H.I. titre	C.F. titre
E protein specific				
20/7	Type	<10	20	<10
128/5	Group	16	3,200	<10
136/9	Subgroup	<10	160	<10
152/10	Type	100	12,800	<10
174/6	Type	250	320	<10
183/11	Flavivirus	1,250	25,600	<10
184/12	Type	>10,000	12,800	<10
234/15	Strain	1,250	<10	<10
294/10	Flavivirus	25	6,400	<10
316/17	Type	125	40	<10
NS1 protein-specific				
123/4	Subgroup	<10	<10	<10
237/14	Type	<10	<10	1,000
298/7	Type	<10	20	3,200
Pre-M protein-specific				
9/15	Subgroup	<10	<10	<10
167/3	Type	<10	10	<10
Undetermined molecular specificity				
145/12	Subgroup	<10	10	<10
157/11	Type	<10	10	<10
158/4	Subgroup	<10	<10	<10
161/5	Type	<10	20	<10
194/3	Flavivirus	<10	<10	<10
209/4	Flavivirus	<10	20	<10
218/10	Type	<10	20	<10
235/5	Subgroup	<10	<10	<10
308/12	Type	<10	<10	<10

MAb 174/6, an E protein-specific neutralizing antibody which recognizes a conformational epitope, has been used to select two neutralization escape mutants. One of the mutants does not bind either MAb 174/6 or 316/7 and is not neutralized by them. The other is still being characterized. The E genes of both mutants are being sequenced and the sequences compared with that of the parent virus in order to locate the amino acid substitution(s) responsible for inactivation of the epitope.

F J Austin, Health Research Council of New Zealand Virus Research Unit, P O Box 56, Dunedin, New Zealand.

A PCR-Restriction Enzyme Technique for Determining the Topotype of Dengue Viruses.

A. V. Vorndam and Noemi Rosado.   Dengue Branch, DVBID, NCID, CDC, San Juan, Puerto Rico.

Genetic analysis of dengue virus isolates from different regions has shown that geographic variations occur within the serotypes which are referred to as topotypes. Previously, these analyses have been conducted using radioisotopic techniques and relatively large amounts of viral RNA (Trent et al., 1990) The polymerase chain reaction (PCR) can provide useable amounts of viral genetic material which can be digested by a large selection of restriction enzymes (RE) and the resulting fragments can be detected by nonisotopic methods. We have found that RE digestion of an amplified part of the dengue viral genome can differentiate among the various topotypes of the dengue viruses.

The viruses used in this study were obtained from the reference collection of the Centers for Disease Control in San Juan, Puerto Rico or Ft. Collins, Colorado, and were propagated in Aedes albopictus tissue culture cells. Total cellular RNA from approximately 10^6 virus-infected cells was extracted by a guanidinium thiocyanate, phenol procedure (STAT-60, Tel-Test B, Inc.) and kept frozen until use. The PCR procedure described by Lewis, et al., 1991 was used. The primers used (D1, D2) amplified a 2400 base sequence encompassing virtually all of the structural protein region of the viral genome. The fragments produced by enzyme digestion were separated by electrophoresis and visualized by ethidium bromide staining.

In selecting restriction enzymes to use in this study we surveyed by computer the predicted cutting sites in the amplified region based on published sequences. We selected Alu I and Dde I as the most useful since they produced 9 and 8 fragments, respectively, after digestion of the amplification product. Table 1 summarizes the results obtained using these enzymes to compare dengue 2 isolates representing the Jamaica (A-F), Puerto Rico (G-K) and various foreign topotypes (L-Q). There is a clear distinction between the these groups, with the Puerto Rico topotype sharing only 6% of the Jamaica topotype fragments. Various Asian and Pacific isolates showed higher degrees of similarity, but were readily distinguishable when compared to the high degree of similarity within the Jamaica group.

It is important for surveillance laboratories to be able to rapidly screen multiple isolates to detect introductions or major genetic changes within regions. Using a PCR-based technique, it is possible to rapidly test new isolates using a small number of infected cells and without the use of radioisotopes or computer analysis.   Our results obtained using the amplified structural protein region of the dengue virus genome show the same topotype relationships as those previously obtained by other techniques (Trent et al. 1990). Among the American region dengue 2 viruses there is a geographical separation, with the Puerto Rico topotype present in Mexico, Central America and western South America.

The Jamaica topotype has spread from its original Caribbean focus to the northern and eastern part of South America.

Virus Strain

		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Alu	1	+	+	+	+	+	+	o	o	o	o	o	o	o	o	o	o	o
	2	+	+	+	+	+	+	o	o	o	o	o	o	o	o	o	+	+
	3	+	+	+	+	+	+	o	o	o	o	o	o	o	o	o	+	o
	4	+	+	+	+	+	+	o	o	o	o	o	+	+	+	+	+	+
	5	+	+	+	+	+	+	o	o	o	o	o	+	+	+	o	+	+
	6	+	+	+	+	+	+	o	o	o	o	o	+	o	o	o	+	o
	7	+	+	+	+	+	+	+	+	+	+	+	o	o	o	o	o	o
	8	+	+	+	+	+	o	o	o	o	o	o	o	o	o	o	o	o
	9	+	+	o	+	+	+	+	+	+	+	+	o	o	o	+	o	+
Dde	1	+	+	+	+	+	+	o	o	o	o	o	o	o	+	o	o	o
	2	+	+	+	+	+	+	o	o	o	o	o	+	+	+	+	+	+
	3	+	+	+	+	o	+	o	o	o	o	o	+	+	+	o	+	+
	4	+	+	+	+	+	+	o	o	o	o	o	+	+	o	+	+	+
	5	+	+	+	+	+	o	o	o	o	o	o	o	o	+	+	o	o
	6	+	+	+	+	o	+	o	o	o	o	o	+	+	+	+	+	+
	7	+	+	+	+	+	+	o	o	o	o	o	+	+	+	o	+	o
	8	+	+	o	+	+	+	o	o	o	o	o	+	+	+	+	+	+

A. Brazil, 1990
B. Puerto Rico, 1991
C. Puerto Rico, 1984
D. Jamaica, 1988
E. Venezuela, 1991
F. French Guyana, 1991
G. Ecuador, 1990
H. Honduras, 1991
I. El Salvador, 1987

J. Mexico, 1984
K. Mexico, 1990
L. India, 1988
M. Sri Lanka, 1991
N. Thailand, 1989
O. Palau, 1988
P. Indonesia, 1989
Q. Philippines, 1983

Table 1. Comparison of dengue 2 Jamaica topotype fragments (A-F) with the Puerto Rico topotype (G-K) and other foreign isolates (L-Q).

1. Trent, D.W., Manske, C.L., Fox, G.E., Chu, M.C., Klicks, S.C., and Monath, T.P. 1990. Appl. Virol. Res. 2: 293- 315.

2. Lewis, J.G., Chang, G-J., Lanciotti, R.S. and Trent, D.W. 1992. J. Virol. Methods 38: 11-24.

COLLECTION OF DENGUE VIRUSES ISOLATED IN MEXICO

Celso Ramos, Herlinda García, Jorge Villaseca & Verónica Cruz

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Dengue is a viral disease widespread in tropical and subtropical regions of the world. The disease is caused by one of 4 serotypes of dengue virus. Dengue in Mexico appeared in the southern border in 1978. Since then, the virus spread through the country. Up to date serotypes 1, 2 and 4 circulate in different regions.

We report here the collection of dengue viruses isolated in Mexico from serum of acute cases of dengue fever. The viruses were isolated in mosquito cell line cultures (TRA-284) and the serotype was identified by indirect immunofluorescence using monoclonal antibodies. These viruses will be used in future research projects and they are available for people who request them for research purposes.

COLLECTION OF DENGUE VIRUSES ISOLATED IN MEXICO

CODE NUMBER	SEROTYPE	LOCATION/STATE	YEAR
0001	DEN-1	NAYARIT	84
0002	DEN-1	NAYARIT	84
0003	DEN-1	SONORA	84
0004	DEN-1	SONORA	84
0005	DEN-1	TAXCO, GUERRERO	88
0006	DEN-1	TAXCO, GUERRERO	88
0007	DEN-1	TAXCO, GUERRERO	88
0008	DEN-1	TAXCO, GUERRERO	88
0009	DEN-1	TAXCO, GUERRERO	88
0010	DEN-1	SONORA	84
0011	DEN-1	SONORA	85
0012	DEN-1	JALISCO	88
0013	DEN-1	PUERTO VALLARTA, JALISCO	88
0014	DEN-1	PUERTO VALLARTA, JALISCO	84
0015	DEN-1	PUERTO VALLARTA, JALISCO	84
0016	DEN-1	PUERTO VALLARTA, JALISCO	84
0017	DEN-1	NAYARIT	84
0018	DEN-1	PUERTO VALLARTA, JALISCO	84
0019	DEN-1	PUERTO VALLARTA, JALISCO	84
0020	DEN-1	OAXACA	84
0021	DEN-4	GUERRERO	84
0022	DEN-1	PUERTO VALLARTA, JALISCO	84
0023	DEN-1	PUERTO VALLARTA, JALISCO	84
0024	DEN-1	PUERTO VALLARTA, JALISCO	84
0025	DEN-1	PUERTO VALLARTA, JALISCO	89
0026	DEN-4	PUERTO VALLARTA, JALISCO	84
0027	DEN-4	–[b]	84
0028	DEN-4	MERIDA, YUCATAN	84
0029	DEN-4	NAYARIT	86
0030	–[a]	JALISCO	88

CODE NUMBER	SEROTYPE	LOCATION/STATE	YEAR
0031	–	JALISCO	88
0032	–	PUEBLA	88
0033	–	JALISCO	88
0034	–	NUEVO LEON	88
0035	–	–	85
0036	–	CAYEHUACAN, MORELOS	89
0037	DEN-4	–	84
0038	–	–	89
0039	–	JALISCO	88
0040	–	–	–c
0041	DEN-1	–	–
0042	–	–	–
0043	–	–	–
0044	DEN-4	AJALPAN, PUEBLA	91
0045	DEN-4	AJALPAN, PUEBLA	91
0046	DEN-4	AJALPAN, PUEBLA	91
0047	DEN-4	AJALPAN, PUEBLA	91
0048	DEN-4	AJALPAN, PUEBLA	91
0049	DEN-4	AJALPAN, PUEBLA	91
0050	DEN-4	AJALPAN, PUEBLA	91
0051	DEN-4	AJALPAN, PUEBLA	91
0052	DEN-4	AJALPAN, PUEBLA	91
0053	DEN-4	AJALPAN, PUEBLA	91
0054	DEN-4	AJALPAN, PUEBLA	91
0055	DEN-4	AJALPAN, PUEBLA	91
0056	DEN-4	AJALPAN, PUEBLA	91
0057	DEN-4	AJALPAN, PUEBLA	91
0058	DEN-4	AJALPAN, PUEBLA	91
0059	DEN-4	AJALPAN, PUEBLA	91
0060	DEN-4	AJALPAN, PUEBLA	91
0061	–	AJALPAN, PUEBLA	91
0062	–	AJALPAN, PUEBLA	91
0063	–	AJALPAN, PUEBLA	91
0064	DEN-4	AJALPAN, PUEBLA	91
0065	DEN-4	AJALPAN, PUEBLA	91
0066	DEN-4	AJALPAN, PUEBLA	91
0067	DEN-4	AJALPAN, PUEBLA	91
0068	DEN-4	AJALPAN, PUEBLA	91
0069	–	AJALPAN, PUEBLA	91
0070	DEN-4	AJALPAN, PUEBLA	91
0071	DEN-4	AJALPAN, PUEBLA	91
0072	DEN-4	AJALPAN, PUEBLA	91
0073	DEN-4	AJALPAN, PUEBLA	91
0074	DEN-4	AJALPAN, PUEBLA	91
0075	DEN-4	AJALPAN, PUEBLA	91
0076	DEN-4	AJALPAN, PUEBLA	91
0077	DEN-4	AJALPAN, PUEBLA	91
0078	DEN-4	AJALPAN, PUEBLA	91
0079	DEN-4	AJALPAN, PUEBLA	91
0080	DEN-4	AJALPAN, PUEBLA	91
0081	DEN-4	AJALPAN, PUEBLA	91
0082	DEN-4	AJALPAN, PUEBLA	91
0083	DEN-4	AJALPAN, PUEBLA	91

COLLECTION OF DENGUE VIRUSES ISOLATED IN MEXICO

CODE NUMBER	SEROTYPE	LOCATION/STATE	YEAR
0084	DEN-4	AJALPAN, PUEBLA	91
0085	DEN-4	AJALPAN, PUEBLA	91
0086	DEN-4	AJALPAN, PUEBLA	91
0087	DEN-4	AJALPAN, PUEBLA	91
0088	DEN-4	AJALPAN, PUEBLA	91
0089	DEN-4	AJALPAN, PUEBLA	91
0090	DEN-4	AJALPAN, PUEBLA	91
0091	DEN-4	AJALPAN, PUEBLA	91
0092	DEN-4	AJALPAN, PUEBLA	91
0093	DEN-4	AJALPAN, PUEBLA	91
0094	DEN-4	AJALPAN, PUEBLA	91
0095	DEN-4	AJALPAN, PUEBLA	91
0096	DEN-4	AJALPAN, PUEBLA	91
0097	DEN-4	AJALPAN, PUEBLA	91
0098	DEN-4	AJALPAN, PUEBLA	91
0099	DEN-4	AJALPAN, PUEBLA	91
0100	DEN-4	AJALPAN, PUEBLA	91
0101	DEN-4	AJALPAN, PUEBLA	91
0102	–	AJALPAN, PUEBLA	91
0103	–	AJALPAN, PUEBLA	91
0104	DEN-1	–	–
0105	DEN-1	CHIETLA, PUEBLA	91
0106	DEN-1	CUAYUCA, PUEBLA	91
0107	DEN-1	CUAYUCA, PUEBLA	91
0108	DEN-1	CUAYUCA, PUEBLA	91
0109	DEN-1	CUAYUCA, PUEBLA	91
0110	DEN-1	CUAYUCA, PUEBLA	91
0111	DEN-1	CUAYUCA, PUEBLA	91
0112	DEN-1	CUAYUCA, PUEBLA	91
0113	DEN-1	CUAYUCA, PUEBLA	91
0114	DEN-1	CUAYUCA, PUEBLA	91
0115	DEN-1	CUAYUCA, PUEBLA	91
0116	DEN-1	CUAYUCA, PUEBLA	91
0117	DEN-1	CUAYUCA, PUEBLA	91
0118	DEN-1	CUAYUCA, PUEBLA	91
0119	DEN-1	CUAYUCA, PUEBLA	91
0120	DEN-2	NAVOJOA, SONORA	92
0121	DEN-2	NAVOJOA, SONORA	92
0122	DEN-2	NAVOJOA, SONORA	92

a) NOT YET SEROTYPED

b) UNKNOWN PLACE OF COLLECTION

c) UNKNOWN YEAR OF COLLECTION

DENGUE 3 OUTBREAK IN MALAYSIA

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Malaysia is experiencing an outbreak of dengue 3 virus since the beginning of 1992. From January to October 1992, 4,225 cases of dengue infection have been reported, of which 525 were dengue haemorrhagic fever, with 19 deaths. In 1991, dengue 2 was the predominant strain which resulted in 5,880 reported cases of which 741 were DHF, with 39 deaths.

Using mosquito larvae inoculation and AP/61 cells, we have isolated 249 dengue viruses to date, of which 198 strains were identified as Dengue 3 and 24 strains as dengue 2. Dengue 1 and 4 have also been isolated but to a lesser degree.

The emergence of dengue 3 as a predominant strain comes after three years of dengue 2, from 1989 to 1991. The last outbreak of dengue 3 was in 1985 and 1986. In the present outbreak, severe dengue cases are seen in all age groups and among the three major ethnic groups of Malays, Chinese and Indians. In DHF, bleeding manifestations may appear late and may be minimal, with predominant features of plasma leakage. The clinical diagnosis may not be obvious and other diseases suspected. Severe liver dysfunction and encephalopathy have been reported in several cases.

Of the 35 DHF/DSS laboratory confirmed cases, 6 were shown to be due to dengue 3 by virus isolation. Dengue 3 was isolated from the liver and serum of one fatal case. Using polymerase chain reaction, we were able to demonstrate dengue 3 in ten cases, including in three fatal cases. Dengue 2 was not incriminated in any of these cases.

RETRIEVING INFORMATION ON DENGUE

Goro Kuno*

Full-time investigators of dengue, like other scientists, need to spend a considerable amount of time searching for pertinent, scientific information. To minimize the time spent for the search, many of them depend on computerized information databases. In this study, the characteristics and problems of some of the databases popularly used for retrieval of information on dengue were analyzed.

The databases studied were five on-line databases (Medline,Biosis Previews,CAB Abstract,Excerpta Medica,and Scisearch) and 3 off-line sources (Tropical Diseases Bulletin,Annotated Bibliography on Mosquito-Borne Diseases in Southeast Asia, and listed publications in the Literature References for Mosquito and Mosquito-Borne Diseases, which is published periodically as a service to the recipients of the Journal of American Mosquito Control Association). Using a variety of methods and sources, including the above 8 sources, I obtained original copies from 931 out of 962 source items that met the definition of dengue article between 1983 and 1988. The following analyses were based on those 931 articles.

The body of dengue literature consisted of 777 source items published in periodicals and other numbered publications, 136 items in books, and 18 items in all other publications. The number of items retrieved and its percentage for each type of publication are shown in Table 1. When the 5 on-line databases were combined, 67.9% of source items in periodicals were retrieved. Although a combination of all 8 databases further increased the percentages of retrievable source items, about 17%, 44%, and 61% of the items in periodicals, books, and other publications, respectively, could not be retrieved. Although missing 17% of source items in periodicals and other numbered publications may appear negligible to some people, this percentage included many important articles. The retrieval rate was also affected by other factors, such as subject (Table 2) and location of publisher (Table 3).

The findings are briefly summarized as follows:
(1) The recognition of publication in book is regrettably low. The existing sources specializing on book contents are similarly unsatisfactory.
(2) As most people expect, publications in English in the periodicals from the countries in western Europe, north America, and a small number of other countries are more retrievable than those from the tropics.

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For example, it is noted that not a single periodical is covered in the Index Medicus for more than 17 dengue-endemic countries including the Philippines where DHF was first documented and continues to be a major health problem.

(3) When broken down on the basis of subject, the retrieval of publications in clinical medicine and epidemiology was inadequate, in contrast to the retrieval on molecular biology for which reliance on only one or two databases was sufficient. Since most investigators rarely check as many as 8 databases, retrieval rates using on-line services would be expected to be much lower for the above subject.

(4) Selection of periodicals and other sources for databases varies considerably among indexing companies and institutions because of difference in objectives, fields of emphasis, and many other factors. Thus, the above study should not be interpreted as a comparison of the qualities among databases. Nevertheless, unless current policies on source selection are modified, retrieval, and hence recognition, of the publications from most tropical countries would continue to suffer. Alternatively, emergence of new types of databases with different criteria for source selection is highly desirable.

Table 1. Information Retrieval among Primary Databases

Kind of Publication	Total No. Source items	No. Relevant Source Items Retrieved (% total) by the Primary Databases[1]							
		A	B	C	D	E	F	G	H
Periodicals and All Numbered Publications	777	475 (61.1)	380 (48.9)	296 (38.1)	192 (24.7)	311 (40.0)	184 (23.7)	211 (27.2)	168 (21.6)
Books	136	2 (1.5)	5 (3.6)	2 (1.5)	9 (6.6)	1 (0.7)	2 (1.5)	7 (5.1)	48 (47.0)
Others	18	0	1 (5.6)	0	4 (22.2)	0	2 (11.1)	2 (11.1)	2 (11.1)
Total No. Relevant Source Items	931	477	386	298	205	312	188	220	218
Total No. Items Retrieved by On-Line Database (% relevancy)		2208 (21.6)	2350 (16.4)	1142 (26.1)	1877 (10.9)	1976 (15.8)			

[1] Abbreviation of Databases
A: Medline; B: Biosis Previews; C: Excerpta Medica; D: CAB Abstract; E. Scisearch; F: Tropical Disease Bulletin (printed version); G: J. Am. Mosquito Control Assoc.; H. SEAMEO Bibliography

Table 2. Retrieval According to the Subject of Source Item

Subject	No. Source Items	No. Source Items Retrieved (%) by Databases						
		A	B	C	D	E	F	G
Clinical Medicine and Epidemiology	313	150 (47.9)	112 (35.8)	70 (22.4)	64 (20.4)	61 (19.5)	69 (22.0)	72 (23.0)
Laboratory Diagnosis	142	71 (50.0)	69 (48.6)	48 (33.8)	20 (14.1)	43 (30.3)	36 (25.5)	42 (29.6)
Basic Virology & Immunology	187	123 (65.4)	110 (58.8)	102 (54.3)	13 (6.9)	118 (62.8)	55 (29.3)	43 (22.9)
Molecular Biology	48	39 (81.3)	36 (75.0)	33 (68.8)	6 (12.5)	35 (72.9)	13 (27.1)	16 (33.3)
Vector Biology & Control	130	57 (43.8)	47 (36.2)	34 (26.2)	75 (57.7)	43 (33.1)	5 (3.8)	32 (24.6)
General Subjects	111	37 (33.3)	12 (10.8)	11 (9.9)	27 (24.3)	12 (10.8)	10 (9.0)	15 (13.5)

Table 3 Retrieval According to the Geographic Location of Publisher

Region	No. Source Items	No. Source Items Retrievable (% of total no. per region) by the Following Databases						
		A	B	C	D	E	F	G
Europe	231	149 (64.5)	90 (39.0)	115 (49.8)	61 (26.4)	130 (56.0)	58 (25.1)	72 (31.2)
Africa	4	3 (75)	3 (75)	2 (50)	4 (100)	2 (50)	1 (25)	1 (25)
India	32	20 (62.5)	21 (65.6)	4 (15.6)	7 (21.9)	19 (59.4)	2 (6.3)	4 (12.5)
Southeast Asia	207	65 (31.4)	57 (27.5)	11 (5.3)	32 (15.4)	5 (2.4)	14 (6.8)	49 (23.7)
East Asia	129	35 (27.1)	63 (48.8)	45 (34.9)	13 (10.1)	23 (17.8)	23 (17.8)	11 (8.5)
Australia & New Zealand	8	6 (75.0)	5 (62.5)	3 (37.5)	3 (37.5)	5 (62.5)	3 (37.5)	2 (25.0)
USA & Canada	238	163 (68.8)	127 (53.6)	112 (47.3)	72 (30.3)	121 (51.6)	73 (30.8)	71 (30.0)
Mexico & Central America	14	8 (57.1)	2 (14.3)	0	4 (28.6)	2 (14.3)	4 (28.6)	3 (21.4)
Caribbean Basin	37	22 (59.5)	12 (32.4)	2 (5.4)	2 (5.4)	3 (8.1)	6 (16.2)	2 (5.4)
South America	31	6 (20.0)	6 (19.4)	4 (12.9)	7 (22.6)	2 (6.5)	4 (12.9)	4 (12.9)

Transmission of Tick-borne Encephalitis Virus Between Ticks Co-Feeding on Natural Hosts.

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Arthropod-borne virus infections result in little damage to the arthropod vector whereas advantages are gained by inducing disease in the vertebrate host. This dogma is based on the rationale that, to maximise transmission, the bite rate of the vector should be unimpaired; in contrast, immobilization of the host and virulence related to high virus titres in the blood (increasing the chances that the blood-sucking vector becomes infected) are likely to increase the transmissibility of the virus[1-4].

Most transmission studies of arboviruses have used syringe inoculation to infect the vertebrate host. We mimicked natural conditions of virus transmission by allowing infected and uninfected arthropod vectors to feed together on uninfected wild vertebrate hosts. Suprisingly, the greatest numbers of infected vectors were obtained from susceptible hosts that had the lowest levels of virus infection (Tables 1 and 2). Thus, when *Ixodes ricinus* adult females infected with tick-borne encephalitis (TBE) virus fed together with uninfected *I. ricinus* nymphs on wild rodent species, 68% nymphs became infected on mice (*Apodemus* spp.) and 28% on bank voles (*Clethrionomys glareolus*) even though bank voles developed much higher levels of virus infection than mice. Pine voles (*Pitymys subterraneus*) had high levels of viraemia; only 14% ticks fed of which 71% ticks became infected. Hedgehogs (*Erinaceus europeus*) and pheasants (*Phasianus colchicus*) yielded negligible numbers of infected ticks although the ticks fed well.

The results suggest an optimum strategy for TBE virus that minimizes the impact of infection on the susceptible vertebrate host. Our previous studies indicate that such a strategy is facilitated by saliva-activated transmission (SAT) in which modification of the skin site of feeding, by tick saliva, enhances virus transmission[5-7].

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TABLE 1   Transmission of TBE Virus Between Infected and Uninfected Ticks Feeding Together on Natural Hosts.

Species	No. of animals	Ticks in cell 1 + /total	Ticks in cell 2 + /total	Total infected %
Apodemus flavicollis	6	76/95 (80%)	46/90 (51%)	66%
Apodemus agrarius	2	18/24 (75%)	7/8 (87%)	78%
Clethrionomys glareolus	8	15/46 (33%)	21/84 (25%)	28%
Pitymys subterraneus	3	11/12 (92%)	1/5 (20%)	71%
Erinaceus europeus	2	2/21 (10%)	0/41 (0%)	3%
Phasianus colchicus	5	0/38 (0%)	0/57 (0%)	0%

Feeding ticks were retained within two neoprene cells. Cell 1 contained two TBE virus-infected female ticks, two uninfected males, and 20 uninfected nymphs; cell 2 contained 20 uninfected nymphs. Nymphs were allowed to feed to repletion (4 days). The animals were then humanely killed and ticks, blood, brain, spleen and lymph nodes collected, and frozen. Ticks and target organs were individually homogenized in microtissue grinders in 1 ml medium and then plaque titrated.

TABLE 2 Detection of TBE Virus in Blood and Organs of Natural Hosts (shown in Table 1)

Species	Viraemia $\geq 2.0 \log_{10}$ $LD_{50}/0.01ml$	Spleen	Lymph nodes
Apodemus spp.	12% (1/8)	13% (1/8)	38% (3/8)
Clethrionomys glareolus	75% (6/8)	75% (6/8)	86% (6/7)
Pitymys subterraneus	100% (3/3)	100% (3/3)	100% (3/3)

SUSCEPTIBILITY PARAMETERS OF AEDES ALBOPICTUS TO PER ORAL INFECTION WITH EASTERN EQUINE ENCEPHALITIS VIRUS.

The recent isolation of 14 strains of eastern equine encephalitis (EEE) virus from Aedes albopictus (Skuse) collected in Polk County, Florida (Mitchell et al., 1992, Science 257:526), has increased concerns that this mosquito may become an epizootic and epidemic vector of EEE virus. Previously, Scott et al. (1990, Am. Mosq. Control Assoc. 6:274) showed that a strain of Ae. albopictus from Houston, Texas, could become infected with EEE virus by feeding on viremic chicks and could transmit the virus by bite in the laboratory. In that study, the EEE virus titers in the infectious bloodmeal were $>10^8$ baby hamster kidney tissue culture 50% infective doses (BHK $TCID_{50}$). Consequently, 100% of the mosquitoes that fed became infected and no information was obtained on the threshold of infection, i.e., the lowest concentration of virus capable of causing an infection in approximately 1 to 5 per cent of specimens ingesting it (Chamberlain et al., 1954, Am. J. Hyg. 60:278), or on the virus dose required to infect 50% of the mosquitoes (ID_{50}).

We fed Aedes albopictus mosquitoes on snowy egrets, Egretta thula, from New Jersey, which had been infected by subcutaneous inoculation of EEE virus (strain NJO/60). Freshly-fed mosquitoes were frozen and tested to determine how much virus they had ingested (Table 1). Other fed mosquitoes from the same lots were incubated for 7 days at 27°C prior to testing. Seven lots of Ae. albopictus fed on viremic birds. Based on average amounts of virus ingested and day-7 virus infection rates in mosquitoes from the same lots, the amount of virus required to infect 50% of the mosquitoes was calculated to be $10^{2.8}$ Vero cell plaque-forming units (PFU). The infection threshold, i.e., the amount of virus required to infect from one to five percent of mosquitoes, was determined to be ≤ 10 PFU per bloodmeal. These parameters indicate that Ae. albopictus is sufficiently susceptible to infection with EEE virus to enable it to acquire infectious doses from a wide variety of viremic birds and possibly from equines.

Report submitted by C.J. Mitchell, R.G. McLean, R.S. Nasci, G.C. Smith, Medical Entomology-Ecology Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, P.O. Box 2087, Fort Collins, Colorado 80522, and W.J. Crans and D.F. Caccamise, Department of Entomology, Rutgers University, New Brunswick, New Jersey 08903.

Table 1. EEE virus infection rates and representative titers in _Ae_. _albopictus_ immediately after feeding and on day-7 postfeeding.

Egret Host	Freshly Fed			Day-7 Postfeeding		
	No. Pos./ No. Tested	Avg. Amt. of Virus Ingested*	No. Pos./ No. Tested	% Pos.	Representative Virus Titers**
2042	1/5	≤ 1.0	1/34	2.9	4.1
127	5/5	2.7	19/40	47.5	4.2, 5.0, 5.3
149	5/5	3.1	26/43	60.5	5.0, 5.3, 6.2
2050***	5/5	1.9	11/47	23.4	5.0, 5.8, 6.2
126	2/5	≤ 1.0	1/41	2.4	6.7
128	5/5	1.4	0/19	0	--
2050***	1/5	≤ 1.0	0/29	0	--

*Log_{10} Vero cell PFU/female; only positive females included.

**Log_{10} Vero cell PFU/female.

***Mosquitoes were fed on this host on two successive days.

BIOECOLOGICAL STUDIES ON MOSQUITO VECTORS OF ARBOVIRUSES IN BRAZILIAN AMAZONIA: THE GONOTROPHIC CYCLE AND SURVIVAL*.

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The potential of a mosquito population for arboviruses' transmission is mostly related to its mean daily survival rate. This may be estimated by the mean duration of the gonotrophic cycle, used as the root exponent of the parity rate of the mosquito population. Although the duration of the gonotrophic cycle may be relatively easely determined under natural conditions by mark/release/recapture experiments, almost no data is available concerning the south american species of vectors of arboviruses. In a forested area near Belém (PA, Brazil), eight different species of mosquitoes (*Haemagogus janthinomys*, *Hg. leucocelaenus*, *Sabethes chloropterus*, *Sa. cyaneus*, *Sa. belisarioi*, *Sa. amazonicus*, *Sa. quasicyaneus* and *Sa. glaucodaemon*) were collected and engorged on human bait, powdered with fluorescent dyes (5 different colours on 5 successive days), and released each day on May, 8, 9, 12, 15 and 16, 1989. Catching sessions have been conducted daily to July, 6, 1989.

8 *Hg. janthinomys* out of the 74 marked (10,8 %) have been recaptured. The mean duration of the gonotrophic cycle, estimated by the median value, was 15 days The "older" individual have been recaptured 19 days after its release. The second species in abundance was *Sa. chloropterus*, another vector of Yellow fever in Brazil. 12 out of 59 marked (20,3 %) have been recaptured. Although the first mosquitoes began to go back to bite on the 6th, 8th and 11th days, a peak may be distinct from 15th to 18th day. 1 mosquito have been recaptured after 44 days, showing a very long survival. 7 out of 32 (21,9%) and 7 out of 62 (11,3 %) specimens have been recaptured for *Sa. amazonicus* and *Sa. cyaneus*, respectively. The former species have furnished marked specimens between the 14th and the 39th days, suggesting both a long gonotrophic cycle and high survival rate. Individuals of the latter species have been recaptured almost evenly from the 7th day to the 24th. Only 2 out of 34 (5,8 %) *Sa. belisarioi* have been recaptured, one of them twice. *Hg. leucocelaenus* and *Sa. quasicyaneus* have furnished each only one recaptured mosquito, 21 days "old".

From these preliminary results, its is deduced that: (I) *Hg. janthinomys* has a longer gonotrophic cycle than previously inferred from laboratory studies and (ii) the *Sabethes* species may be very long-lived and probably with a more complex pattern of blood-feeding cycle

Table 1 Minimum infection rates (%) of each positive species for YF virus, according to the place of collection in the region of Campo Grande, MS; the dates of the most recent human cases in each locality give an indication of the end of the epizootics.

Species \ Place	Jaraguari	Campo Grande	Sidrolandia	Total
Most recent human case	Dec., 20, '91	Dec., 31, '91	Feb., 3, '92	
Haemagogus (Hag.) janthinomys		1.27	4.41	2.17
Sabethes (Sbo.) chloropterus			1.67	0.48
Sabethes (Sbn.) soperi	5.26			5.00

Table 2: Summary of eco- epidemiological parameters for the *Haemagogus janthinomys* mosquito population, collected in the region of Campo Grande, MS, by county, January, 24 to February, 14, 1992.

	Jaraguari	Campo Grande	Sidrolandia	Estimated[a]
Nr of days since the last human case	52	41	17	0
MIR (%)	0	1.27	4.41	6.54 (r2=0.99)
Relative density	0.20	0.37	0.62	0.83 (r2=0.99)
Parity rate (%)	57.5	53.62	61.81	63.45 (r2=0.48)
Daily survival rate (%)[b]	96.37	95.93	96.84	
Surviving infected mosquitoes (%)[c]	14.62	18.20	57.93	
MIR at time of epizootics	?	6.97	7,61	

[a] Values at origin estimated by linear regression of the known values, the values of the coefficients of regression are indicated between parenthesis
[b] assuming a 15 days long gonotrophic cycle.
[c] this value represents the proportion of infected mosquitoes surviving at the end of the period = (daily survival rate)$^{(Nr\ of\ days\ since\ the\ last\ human\ cases)}$

NEW ENTOMOLOGICAL AND VIROLOGICAL DATA ON THE VECTORS OF SYLVATIC YELLOW FEVER IN BRAZIL[1].

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The present report focuses on recent eco-epidemiological data on Yellow fever, obtained in two very distinct eco-epidemiological contexts. the Barcarena (PA) area (1° 31' S; 48° 40' W; alt. <100 m), situated in the dense amazonian rainforest, and the Campo Grande (MS) region, situated in the cerrado with gallery forest in central Brazil (20° 27' S; 54° 37' W; alt.: 566 m). In the first region, one strain have been isolated from a pool of 6 *Hg. janthinomys* (collecting period: June 12 to 26, 1991). In the other region, 2480 anthropophilous mosquitoes have been collected, of which near 40 % were potential YF vectors (collecting periods: January, 24 to February, 10; January, 26 to February, 5; and February 6 to 14, 1992 in three Fazendas Cabeceira do Jaraguari, Rincão and Agua Encanada, respectively). Classified by decreasing relative abundance, the latter were *Ae. scapularis*, *Sa. chloropterus*, *Hg. janthinomys*, *Hg. leucocelaenus*, *Hg. spegazinii*, and *Sa. soperi*. Four strains of YF virus were isolated from *Hg. janthinomys*, one from *Sa. chloropterus* (first report as natural host in South America) and one from *Sa. soperi* (first report as YF natural host). The minimal infection rates varied among the three localities but were all high (table 1). The mean daily survival rate was 0.9635 for the populations of *Hg. janthinomys*, allowing to extrapolate the value of the infection rate when people was infected most recently.

Some eco-epidemiological parameters of the *Hg. janthinomys* populations (infected or not) from each of the 3 places of study in the Campo Grande region have been compared. It seems that the time lags between the last human contaminations and our collecting of infected mosquitoes would be 41 and 17 days for the Faz. Rincão and Sidrolandia, respectively. With survival rates equal to 0.9593 and 0.9684, the infected mosquitoes would represent 18.20 % and 57.93 % of those infected during the epizootics in the respective areas. Thus, the infection rates at the respective times of human contaminations would be estimated to be at least equal to 6.97 % and 7.61 %. Therefore, these are of the same order. We have obtained a similar value for the initial MIR (6.54 %) when fitting a line with the values estimated at 17, 41 and 52 days after the last human contamination. As for the MIR, the relative density index seems to decay linearly with time. On the other hand, the parity rate does not seem to vary linearly with time. No hypothesis is yet available to explain these phenomenons.

The role of *Hg. janthinomys* as the principal vector of sylvatic YF in Brazil, and actual responsable of most of human contaminations, is confirmed once more. The high densities and infection rates, together with the presence of infected *Sabethes*, may suggest that our field work in Mato Grosso do Sul have been done only a short time after an YF epizootic. However, various eco-epidemiological aspects remain to be studied in relation with the maintenance of the YF virus in its area of endemicity. In the southern part of this area, it is not known if the virus is periodically <u>reintroduced</u> from the north (the permanent endemic focus of the french authors) or if it is able to <u>persist</u> during the dry and cold season, either in the drought-resistant eggs of the *Haemagogus* species, or in the more resistant *Sabethes* adults, or in both. Whatever the case, the "purpose" may not be the same. The long-term survival of the virus in the eggs would be necessary to wait for the reconstitution of the local population of susceptible monkeys. On the other hand, a shorter-term survival in adult mosquitoes may be a possibility for initiating another epizootic in the case of new vertebrate hosts be entering into the area.

Eggs of *Haemagogus* would be collected with ovitraps, and sentinel monkeys would be maintained to detect any early seasonal circulation of YF. The seasonal variations of the relative densities and survival rate of the vectors, and the reconstitution of the monkey populations would be the subjects of other very interesting studies. Finally, the survey of susceptible (non-vaccinated) people would be of prime importance in relation with the risks of sporadic cases emergences.

The data obtained in the Barcarena area were not sufficient to say if there have been an epizootic or not, previously to our collecting of infected mosquitoes (i.e. during the first half of 1991). However, the low rate of HI antibodies in the human population and the absence of recent human cases, despite a close contact of these people with the forest, suggest a non-epizootic mode of transmission.

RECENT STUDIES OF CALIFORNIA AND BUNYAMWERA SEROGROUP VIRUSES IN CALIFORNIA

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Studies have continued in two very different ecological settings: high alpine environments in the Sierra Nevada and Cascade Range, and along coastal saltmarshes between San Diego and San Francisco.

Alpine studies. Since our last published report on isolations of Jamestown Canyon virus from snowpool *Aedes* mosquitoes collected in Alpine County, California, we have extended the known range of this virus in California. In 1991 we isolated JC virus from 10 pools of snowpool *Aedes* mosquitoes collected as far north as Sierra County, California (Gold Lake) and as far south as Tulare County (Kings Canyon National Forest). During this past summer we isolated JC virus from a pool of *Aedes tahoensis* collected in Yosemite National Park (Tuolumne County). To date, all of our JC isolates (1988-1992) have come from three closely related snowpool *Aedes* species: *Ae. tahoensis* (14 isolates), *Ae. cataphylla* (3), and *Ae. hexodontus* (2). All three species are in the Group G as interpreted by Edwards in his 1932 monograph of world mosquitoes. In the vernacular, these are referred to as "dark legged *Aedes*". Twelve of the 19 isolates (63%) were from mosquitoes collected as adults. The remaining seven were from mosquitoes collected as larvae or pupae. The isolation rate from adults appears to be higher from mosquitoes collected from adults than from immature stages, but because of the very low isolation rates (ranging between 0.2 per thousand to 4.5 per thousand) statistically significant differences cannot be demonstrated using conventional methods. Isolation rates do not appear to differ among the three mosquito species. This year a paper by Munstermann and Brust appeared which demonstrated, on the basis of electrophoretic evidence, that a population of *Aedes communis* from Echo Summit in California represented a distinct species: *Ae. tahoensis*. We have completed a detailed study of California populations in the *Ae. communis* complex, and found that *Ae. tahoensis* is the only member of the complex to occur in California. Our studies of JC virus in the Sierra Nevada suggest that the range of the virus in California and the range of this species conform well. We have tested samples of *Aedes nevadensis*, another member of the *Ae. communis* complex, from Nevada, Oregon, and Washington, but have not isolated virus from any of the samples.

Coastal studies. We have continued our studies of salt marsh mosquito viruses which grew out of our discovery of the CE-like virus in populations of *Ae. squamiger* collected in Morro Bay, San Luis Obispo County, California. Since the publication of those results, we have isolated this same virus from *Ae. squamiger* collected from San Diego, Orange, and Santa Barbara Counties. We have not isolated the virus from numerous collections made in more northern counties surrounding the San Francisco and Monterey Bays. Thirty-one of the isolations of CE-like virus have come from *Ae. squamiger*, all collected as larvae or pupae. One isolate was from a pool of *Ae.*

washinoi collected as adults in Morro Bay. *Ae. washinoi* is a newly described species belonging to the *Ae. increpitus* complex. This represents the first arbovirus ever isolated from a member of this complex, with species ranging over most of western North America. Two isolates of CE-like virus were from *Ae. dorsalis* collected as adults from the same area. These represent the first arbovirus isolates from the coastal form of *Ae. dorsalis*. It appears that this CE-like virus is enzootic along the entire California coast from San Luis Obispo County south to Mexico border.

In connection with these studies we have isolated an alphavirus closely related to western equine encephalomyelitis virus (WEE) three times from *Ae. dorsalis* collected as larvae and pupae at Morro Bay. If these strains prove to be indistinguishable from WEE by cross neutralization tests, they will be the first ever of WEE from naturally collected immature mosquitoes, and thus the first evidence of transovarial transmission of WEE in mosquitoes. The possible significance of these isolates in terms of the winter biology of WEE is exciting.

We have isolated a Northway-like virus from a pool of *Culiseta particeps* collected as adults in Morro Bay. This species is in the same subgenus (*Culiseta*) as *Cs. inornata* and *Cs. incidens*. This is the subgenus which is the most cold-adapted and northern-ranging in the genus *Culiseta*. In California, these species (in the subgenus) are winter mosquitoes. This isolation represents the first arbovirus from *Cs. particeps*.

Indoor collections of <u>Aedes aegypti</u> in San Juan, Puerto Rico

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The indoor distribution of <u>Aedes aegypti</u>, the epidemic vector of dengue and dengue hemorrhagic fever, was studied in an urban area of San Juan, Puerto Rico. A two-person team using a battery-powered, backpack aspirator, standard insect collection net and flashlight visited four houses one day per week (during the morning hours) for 12 months and attempted to collect all of the mosquitoes present in the house. All houses were constructed of concrete and had the same basic floor plan. Collections from each room were kept in separate cartons until the specimens had been identified to species, separated by sex, counted and the external physical appearance of the females had been recorded.

Overall, 65% (2,817/4,317) of all female <u>Ae aegypti</u> were collected from the bedrooms of these houses (Table 1). In houses 1-3, 64-82% (ave. 70%) of the females were collected from the bedrooms while 78-85% (ave. 81) of the males were found in these areas. These houses were continuously occupied by two-three adults. Although two adults lived in house 4, it was unique in that the elderly gentleman was confined to a wheelchair and spent a considerable amount of his day at the dining room/kitchen table. In contrast to the other houses studied, 31% of the females and 47% of the males were collected in the dining room/kitchen area.

In these houses, 50% (2,409/4,843) of the females were classified as fully gravid (i.e., the abdomen was fully distended) and appeared ready to deposit their eggs; 9% were "semi-gravid (i.e., the abdomen was composed about 50% eggs and 50% blood; and 24% had blood-filled abdomens (Table 2). The remaining 17% were "flat" (either recently emerged nullipars or previously oviposited females).

The presence of a significant number of females in secluded sites in the bedrooms further explains the poor results obtained from vehicle-mounted, ultralow volume (ULV) applications of insecticides in urban areas directed against this species. The high percentage of females with blood and/or eggs further reflects the strong anthropophilic behavior of this vector of dengue and yellow fever viruses in the American tropics.

Table 1. Results of weekly indoor collections of Aedes aegypti in Las Virtudes urbanization, San Juan, Puerto Rico.

House no. Sex	Bedroom 1	2	3	(% of house)	Living room	Kitchen	Bath room	Hall	Other
1									
Males	539	167	549	(81)	95	84	25	73	13
Females	538	212	275	(64)	201	74	52	231	8
2									
Males	15	146	222	(85)	18	9	20	19	1
Females	35	359	279	(82)	39	21	29	57	1
3									
Males	83	235	100	(78)	45	32	24	15	0
Females	132	238	92	(69)	81	34	40	53	0
Subtotal for three houses									
Males	637	548	871	(81)	158	125	69	107	14
Females	705	809	646	(70)	321	129	121	341	9
4									
Males	226	194	22	(38)	137	548	25	4	0
Females	282	308	67	(53)	115	383	45	36	0

Table 2. Physical appearance of female Aedes aegypti collected in Las Virtudes, San Juan, Puerto Rico.

House no.	Fully gravid*		Semi-gravid		Full of blood		Flat		Total
1	938	(55)	162	(9)	388	(23)	226	(13)	1714
2	440	(47)	92	(10)	221	(24)	173	(19)	926
3	376	(47)	58	(7)	160	(20)	200	(25)	794
4	655	(46)	114	(8)	396	(28)	244	(17)	1409

* No. (% of total from this house).

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LARVAL DIET, ADULT SIZE AND SUSCEPTIBILITY OF *AEDES AEGYPTI* TO INFECTION WITH ROSS RIVER VIRUS.

Adult mosquito body size appears to be related to vector competence in certain mosquito and virus combinations. Small *Ae. triseriatus* are more susceptible to La Crosse virus and transmit the virus more frequently than do larger adults from well-fed larvae (Grimstad & Haramis. 1984. J. Med. Entomol. 21:249-65). A similar effect, though not significant, has been suggested with *Cx. tritaeniorhynchus* and West Nile virus (Baqar et al. 1984. Mosq. News 40:165-71) and with Japanese encephalitis virus (Takahashi. 1976. J. Med. Entomol. 13:275-84). Murray Valley encephalitis virus infection and transmission rates in *Cx. annulirostris* do not vary with larval diet or adult size (Kay et al. 1989. J. Med. Entomol. 26:488-87). In this study, we examined the relationship of nutrition-induced body size differences in adult *Ae. aegypti* to susceptibility to infection with Ross River virus (RRV).

Large and small *Ae. aegypti* were reared by controlling larval diet. The adults fed on hamsters with varying RRV viremias. A sample of freshly-fed mosquitoes was tested to determine the volume of virus consumed. The remaining blood-engorged specimens were incubated for 14 days at 27 °C, and tested for the presence of RRV by plaque assay in Vero cells . Large adult mosquitoes, as indicated by larger wing lengths, consumed significantly more virus particles than did smaller mosquitoes, and volume of virus consumed decreased proportionally with size. However, correction for size indicated that smaller mosquitoes consumed significantly more virus per 0.1 mg body weight (Table 1). Low host viremia ($2.4 \log_{10}$ PFU) failed to infect mosquitoes of any size. Large mosquitoes were significantly more susceptible than small mosquitoes that fed on hamsters with viremias of $4.8 \log_{10}$ PFU (90% of large and 0% of small infected), $5.7 \log_{10}$ PFU (100% of large and 66.7% of small infected), and $6.4 \log_{10}$ PFU (59% of large and 29% of small infected). Differences in susceptibility were less apparent at higher viremias. Results indicated that large *Ae. aegypti* females are significantly more susceptible to infection with Ross River virus than are smaller females, and provide further supporting evidence that the relationship between size and infection varies among virus-vector systems.

Table 1.

Hamster	Titer in blood[1]	Size class	N	Wing length mm	Dry weight mg	Log_{10} PFU total virus consumed	Log_{10} PFU virus consumed per 0.1 mg dry weight
						Average (SD)[2]	
3	6.4	Small	4	2.48 (0.15)	0.31 (0.09)	3.9 (0.18)	1.3 (0.42)*
		Large	6	3.26 (0.10)*	0.86 (0.10)*	4.5 (0.26)*	0.5 (0.06)
4	2.4	Small	4	2.48 (0.13)	0.36 (0.07)	1.3 (0.09)	0.4 (0.11)*
		Large	5	3.17 (0.09)*	0.78 (0.08)*	1.7 (0.16)*	0.2 (0.02)

1. log_{10} Vero cell PFU/ml in hamster blood.
2. Within each treatment group (hamster) average value followed by an asterisk significantly greater (ANOVA, $P < 0.05$).

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Isolation of Ross River virus from field-caught male *Aedes* mosquitoes

by

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In this report we describe the isolation of the Australian alphavirus, Ross River (RR) virus, from pools of male mosquitoes of two different *Aedes* species. Although this is not absolute proof of vertical transmission of RR virus, we believe it is strong evidence to implicate vertical transmission as a mechanism of persistence of RR virus in arid parts of Australia.

The means by which Australian arboviruses survive in arid regions during periods which are adverse for their transmission are not well understood. There are very few regions in Australia where year round vector-host interaction would be sufficient to enable continuous transmission of arboviruses. One of the most plausible explanations for the reappearance of mosquito-borne viruses in such regions is the survival of the virus in desiccation-resistant eggs of some *Aedes* mosquito species. This transmission of virus from adult female mosquito to her offspring, known as transovarial or more recently as vertical transmission, has been demonstrated unequivocally for some bunyaviruses of the California serogroup. There is also some evidence that mosquito-borne flaviviruses may overwinter by vertical transmission but there is little or no evidence to implicate this mechanism as a means of persistence of alphaviruses (for reviews see Rosen, 1987; Turell, 1988)

Ross River virus, the aetiological agent of epidemic polyarthritis, is the single most common cause of human arboviral infection in Australia. Cases are reported from all mainland states in most years. The vertebrate hosts of RR virus are thought to be non-migratory animals such as kangaroos and other marsupials. Birds are not considered suitable hosts. Therefore it is unlikely that the virus is re-introduced from areas of endemic activity, unless viraemic humans travelling by air can initiate new cycles of virus activity (as was demonstrated during the 1979-80 epidemic in the Pacific Islands).

RR virus activity in the arid north and interior of Western Australia occurs whenever sufficient rains enable mosquito vectors, in particular those in the subgenus *Ochlerotatus* within the genus *Aedes*, to breed. Members of this subgenus, generally temporary ground pool breeders, are the first species to emerge after rains and RR virus has been isolated from adult females of various members (*Ae. camptorhynchus, Ae. vigilax, Ae. normanensis, Ae. sagax, Ae.* species #85, *Ae clelandi*). Furthermore, the date of onset of symptoms of the first human cases of RR virus in arid regions is often within three to four weeks of heavy rains. The *Ochlerotatus* subgenus mosquitoes are the only species which are abundant at this time.

Kay (1982) reported a low rate of vertical transmission of Ross River virus in *Ae. vigilax* but this work has not been repeated. Since then, the only work which suggests that vertical transmission of RR virus may occur was that of Broom *et al.,* (1989) They isolated RR virus from *Ae. normanensis* mosquitoes trapped at the beginning of the wet season in the Kimberley region in Western Australia.

During May, June and July of 1992, large outbreaks of RR virus infection occurred in towns throughout the arid Pilbara, Gascoyne and Interior regions of WA immediately following

unusually heavy rainfall. A field team from this laboratory took this opportunity to collect large numbers of adult and larval mosquitoes which bred as a result of the rainfall. Collections were made at several different locations in the Pilbara and Gascoyne regions.

The larvae were reared to adulthood but have not yet been processed for virus isolation. However, the field-caught adult mosquitoes have been processed and yielded 53 arbovirus isolates from 8 different mosquito species. Importantly, two of the isolations were made from pools of male mosquitoes. One was from a single *Ae (Macleaya) tremulus* male trapped in May at Marble Bar (the hottest town in the world) approximately 250km inland in the Pilbara region. The other was from a pool of six *Ae. vigilax* males trapped in June at Exmouth on the coast in the Gascoyne region. Both of these isolates were identified as RR virus.

We believe that these isolations are a true indication of male infection because great care was taken during processing to ensure that the pools of male mosquitoes were not contaminated by legs or other body parts of female mosquitoes. The only means, other than vertical transmission, by which these male mosquitoes could become infected is venereal transmission. However we do not know of any reports of sexual transmission of arboviruses from female to male mosquitoes.

The two species from which these isolates were obtained are ecologically suitable for 'overwintering' of RR virus in their respective regions. *Ae. tremulus* is a tree hole breeding species, the eggs of which can remain dormant on the inner surfaces of rivergum tree holes waiting for the next rains. *Ae. vigilax*, a known vector of RR virus throughout much of Australia, is a saltmarsh breeder. The eggs of this species remain dormant around the margins of saltmarsh pools and hatch following inundation by high tides or heavy rainfall. Also, the time at which the infected male mosquitoes were collected (soon after heavy rains) would have been appropriate for initiation of new cycles of RR virus activity. Indeed, several cases of RR virus infection were reported from these regions soon after these mosquitoes were caught.

The implications of these findings for public health may be substantial. If, as these results suggest, female mosquitoes can transmit RR virus to their progeny, even at a low rate, then there is potential for RR virus activity whenever unusually heavy rains occur in regions with sufficiently high daily temperatures. Unfortunately there are now numerous aspects of the interaction of RR virus with *Ae. vigilax* and *Ae. tremulus* which need to be addressed before such conclusions can be made.

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Report from the Virus Laboratory

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"Tabanid spiroplasmas from France : characterization, ecology and experimental study"

Tabanidae are considered to be among the major dipteran pests of man and animals worlwide" (Foil, 1989) They are able to transmit to animals and man a great number of pathogenic agents including viruses (notably arboviruses and lentviruses), bacteria, protozoa and helminths (Krinsky, 1976) Theferfore the study of any symbiont or pathogen encountered in these dangerous haematophagous diptera deserves attention. This is true from a theoretical point of view but also with the aim of future strategies for their control.

Our studies in France, although yet preliminary, have already established a number of important facts.

During 1989 and 1990, tabanidae were collected from two differents areas (see map) in Western France and assayed for spiroplasmas. From Central Britanny 62 flies of 9 different species yielded 20 spiroplasma isolates belonging to 6 different serogroups : VIII, XIV, XXIII, HYOS, TABS1, TAAS. From Atlantic biotopes 81 flies of three different species yielded 58 isolates belonging mainly to serogroup IV, but also TABS1, HYOS and XIV. All the 78 French isolates were able to multiply in vitro at 37° C.

In additon, 240 specimens of flowers from "Brière", were collected in 1990 and tested for spiroplasmas : all were negative.

Finnally, in 1990, from "Brière" 81 animals sera were collected and examined using Deformation test and 11 different spiroplasmas from honey bees, mosquitoes and tabanids. 61 bovines sera (or 80.3 %) were found positive but exclusively for Ar1357 spiroplasma, a

mosquito Spiroplasma belonging to serogroup XVI_3 and previously isolated in this area from <u>Aedes</u> mosquitoes.

At present we have no explanation for the paradoxical absence of antibody to tabanid spiroplasmas in cattle since all French tabanid spiroplasmas are able to multiply <u>in vitro</u> at 37° C and, at least for three isolates, in suckling mice

(Le Goff F.[1], Leclercq M.[2], Marjolet M.[3], Humphery-Smith I.[1], Supplisson F.[3] and Chastel C.[1]. [1] Brest and [3] Nantes, France, and [2] Gembloux, Belgium).

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BREST

FORET DE PAIMPONT

●RENNES

①

PARC REGIONAL
DE BRIERE

②

NANTES

France
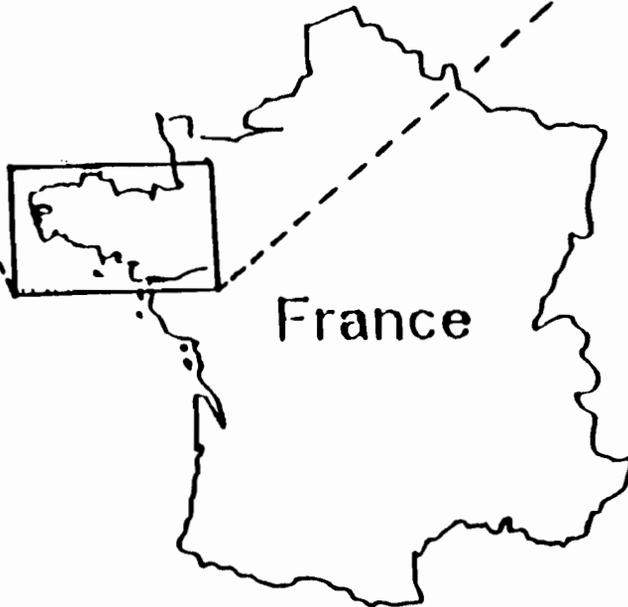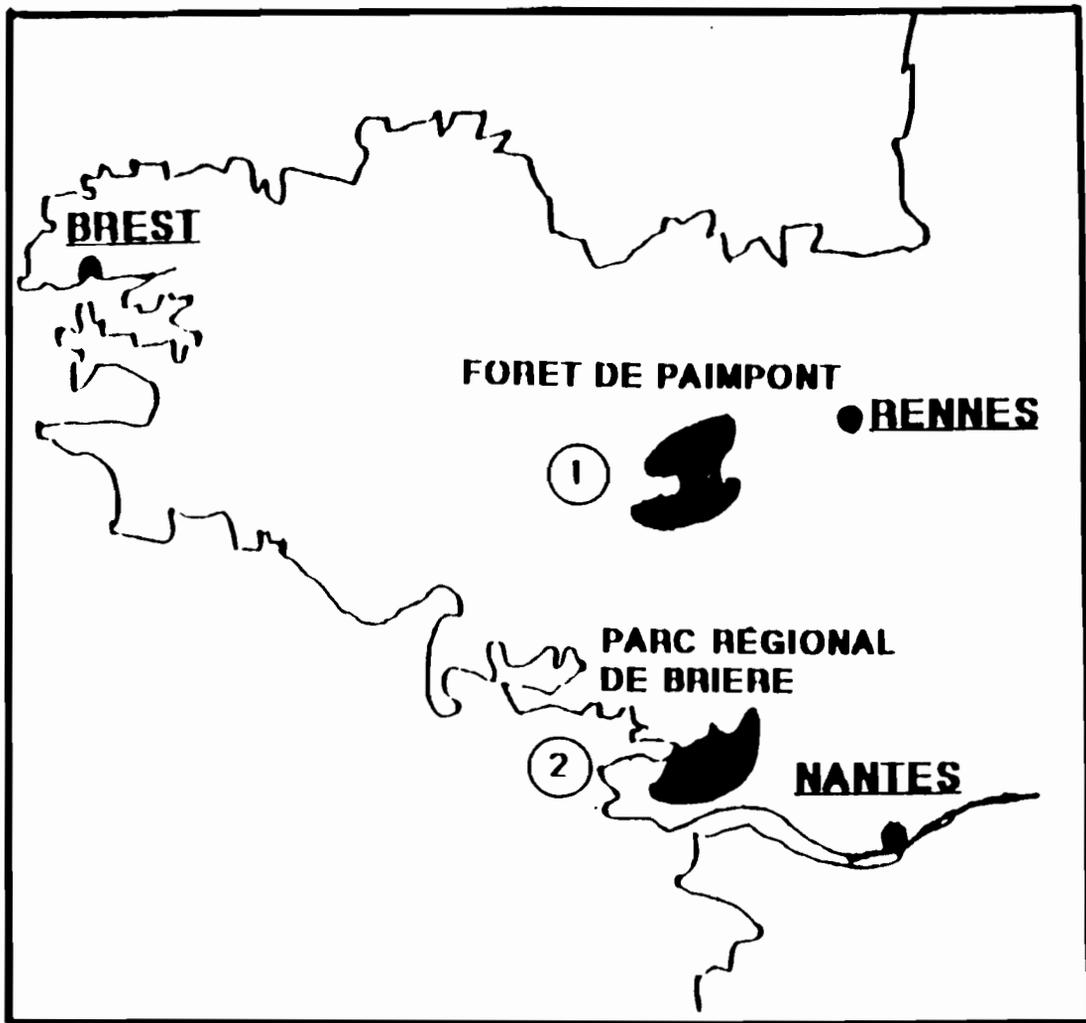
An Outbreak of Japanese Encephalitis Among US Forces Stationed on Okinawa

Dept of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Dept of Internal Medicine and the Consolidated Preventive Medicine Unit, U.S. Naval Hospital, Okinawa; Naval Environmental & Preventive Medicine Unit Six, Pearl Harbor, HI; U.S. Navy Environmental Health Center, Norfolk, VA

More than 45,000 U.S. military personnel and their dependents are stationed on the island of Okinawa, a prefecture of Japan. Japanese encephalitis virus circulates among swine raised on the island. Nevertheless, the absence of cases of encephalitis among U.S. persons living there resulted in an assessment that the risk of infection with Japanese encephalitis virus was negligible. Accordingly, U.S. persons stationed on Okinawa were not routinely immunized with inactivated Japanese encephalitis virus vaccine. That vaccine is currently available in the United States only as an investigational drug.

Yet between June and October of 1991, 3 soldiers developed Japanese encephalitis. Health authorities responded by initiating an outbreak investigation to reassess the risk of infection among U.S. personnel. Preliminary results of that investigation indicated that inapparent infection with Japanese encephalitis virus was widespread. Therefore, a massive immunization program was initiated. Early in the immunization program, side effects of vaccine administration were noted. A case control study to elucidate factors associated with adverse vaccine reactions was begun. This report briefly presents the 3 index cases, preliminary serologic results from the epidemiologic investigation, and some comments about vaccine reactions.

Cases of Japanese encephalitis

Patient	Age (years)	Sex	Onset Date	Diagnostic Test	Outcome of Infection
SL	20	M	14 Jun 91	Anti-JEV IgM detected in convalescent serum	Severely disabled
DK	35	M	26 Sep 91	Anti-JEV IgM detected in acute and convalescent cerebrospinal fluid	Severely disabled
MH	28	M	6 Oct 91	Anti-JEV IgM in convalescent cerebrospinal fluid	Recovered

Epidemiologic investigations

More than 2,000 sera were collected from members of Marine and Air Force units, adult civilians and secondary school students from several U.S. communities on Okinawa. Many of the donors completed a questionnaire designed to elucidate risk factors for infection with Japanese encephalitis virus. All sera were tested for anti-Japanese encephalitis virus and anti-dengue virus (types 1-4) hemagglutination-inhibiting (HI) antibody; many were also tested for anti-Japanese encephalitis virus and anti-dengue virus IgM and IgG antibody in an isotype-capture enzyme immunoassay.

Of 802 sera collected in October-November 1991, 70 (8.7%) were positive for anti-Japanese encephalitis virus HI or IgM EIA antibody. Of the 70 persons with antibody, 26%

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(18/70) had elevated levels of anti-Japanese encephalitis virus IgM, suggesting many infections were acquired during Sep-Oct 91. IgM resulting from inapparent infections with Japanese encephalitis virus is detectable in the AFRIMS assay for less than 2 months.

Of 1222 sera collected from soldiers and adult or adolescent civilians between Dec 91 and Jan 92, 47 (3.8%) were positive for anti-Japanese encephalitis virus HI antibody. Those with HI antibody included adult and adolescent civilians. All sera positive for HI antibody were tested for anti-Japanese encephalitis virus IgM. None were positive, implying no donor had acquired antibody recently. Yet 3/218 sera from soldiers lacking anti-Japanese encephalitis virus HI antibody did contain anti-Japanese encephalitis virus IgM, suggesting virus was transmitted to a few soldiers possibly as late as Dec 91.

Risk factors associated with serologic evidence of infection are under analysis. Based on the above preliminary data, we infer U.S. military and civilian personnel living on Okinawa have been infected with Japanese encephalitis virus. Many of those infections were presumably acquired on Okinawa, especially in 1991.

Immunization program

When the diagnoses of the index cases of encephalitis were established and serologic evidence of additional inapparent infection was acquired, U.S. Naval health authorities commenced a program of immunization with inactivated Japanese encephalitis virus (Nakayama strain). The vaccine used was an investigational drug obtained under a permit held by Dr. T. Tsai, Division of Vector-Borne Viral Diseases, CDC, Fort Collins, CO. So far, more than 80,000 doses of vaccine have been administered to soldiers and adult and child residents of U.S. military communities. A small proportion of vaccine recipients have experienced adverse vaccine effects. Approximately 200 cases of reactions including 10 cases requiring hospitalization are the subject of a case-control study to elucidate risk factors for vaccine-related side effects. Reactions, including urticaria and angioedema, occurred among those receiving either a first or subsequent vaccine dose. Those following an initial vaccine dose generally appeared to occur within 24 hours; those following a subsequent vaccine dose generally appeared to occur after an interval of up to 4 days.

Conclusions

Data presented here are preliminary. Nevertheless, several facts have been established. In 1991, Japanese encephalitis virus infected US military personnel and their dependents on Okinawa. Data are in hand to evaluate risk factors for infection; those data are expected to contribute to improved recommendations for immunization of U.S. Forces assigned to areas of Asia where Japanese encephalitis is endemic or epidemic. Additionally, administration of inactivated Japanese encephalitis virus vaccine was associated with a small proportion of adverse effects, including some that required parenteral corticosteroid therapy. An on-going case control study of factors linked to vaccine reactions is expected to contribute to deliberations of the U.S. Food and Drug Administration on licensure of the vaccine, and to safer use of the vaccine.

Implication of the Cotton Rat <u>Sigmodon</u> <u>alstoni</u> as the Probable Rodent Reservoir of Guanarito Virus, Etiologic Agent of Venezuelan Hemorrhagic Fever

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During February 1992, field studies on the epidemiology of Venezuelan hemorrhagic fever (VHF) were carried out in a rural area of Portuguesa State in central Venezuela. The objective of this work was to determine the prevalence of infection with Guanarito virus, the etiologic agent of VHF, among wild rodents and humans living within an endemic focus of the disease. A total of 234 rodents, representing 9 different species, were collected; and their spleens were cultured for virus. Thirty-one Guanarito virus isolates were made from 2 rodent species: 19 from 40 <u>Sigmodon</u> <u>alstoni</u> and 12 from 106 <u>Zygodontomys</u> <u>brevicauda</u> (Table 1). Guanarito virus antibody rates among these 2 species were 5.1 and 15.0%, respectively. Nine of the 12 <u>Z</u>. <u>brevicauda</u> that yielded virus from their spleens also had Guanarito virus antibodies in their sera. In contrast, none of the 19 Guanarito virus-positive <u>S</u>. <u>alstoni</u> had antibodies to the virus. These data suggest that <u>S</u>. <u>alstoni</u> usually develops a persistent non-immunizing infection with Guanarito virus, while <u>Z</u>. <u>brevicauda</u> develops an immunizing infection. Based on knowledge of the behavior of other human pathogenic arenaviruses, these results imply that <u>S</u>. <u>alstoni</u> is the principal rodent reservoir of Guanarito virus in nature.

To determine the prevalence of Guanarito virus infection among humans in the same region, 195 people living near one of the rodent collecting sites were bled; and their sera were tested for antibodies to the virus. Five individuals (2.6%) had Guanarito virus antibodies; all were adults, and 2 had been diagnosed previously as having VHF. Results of these preliminary serologic studies suggest that the prevalence of human infection with Guanarito virus in the VHF endemic region is relatively low, but that the percentage of infected people who develop severe disease is relatively high.

Table 1

Frequency of Guanarito virus isolations and antibody prevalence
among small mammals collected in Portuguesa State,
Venezuela in February 1992

Species	Virus isolation		Guanarito antibody	
	No.tested	percent positive	No.tested	Percent* positive
Zygodontomys brevicauda	106	11.3	100	15.0
Sigmodon alstoni	40	47.5	39	5.1
Rattus rattus	34	0	32	6.3
Proechimys guairae	25	0	24	4.2
Oryzomys fulvescens	22	0	21	9.5
Holochilus brasiliensis	3	0	3	0
Oecomys speciosus	2	0	2	0
Oecomys flavicans	1	0	1	0
Heteromys anomalus	1	0	1	0
Marmosa robinsoni	16	0	–	–
Didelphis marsupialis	2	0	–	–

*Serum gave positive reaction in IFAT at 1:20 screening dilution.

REPORT FROM THE ARBOVIRUS UNIT

Institut Pasteur Bangui

RCA

During the year 1992, Arbovirus surveillance has led to the isolation, from January to October, from homogenized mosquito pools, of 42 strains of Arboviruses, among which the following viruses were identified or confirmed by the CRORA (Dakar): Chikungunya: 2; Middleburg: 2; Bagaza: 2; Bouboui: 2; Wesselsbron: 2; Bozo: 4; Mossuril/Kamese: 3.

Out of 50 human sera, 10 viruses were isolated among which 1 strain of Zika, 2 strains of Babanki, and 2 strains of Wesselsbron were identified. Although the other strains have been identified in our laboratory, they are not yet confirmed as Usutu, Ilesha and Bozo. Only two human strains: Zika and Usutu, could be directly isolated in tissue culture.

A serological survey on horse sera yielded positive reactions to Wesselsbron confirming the relative importance of this virus, but the serological reactions could not be related to defined clinical symptoms.

As compared with the previous results, it seems that there had been a greater activity of Arbovirus in 1992 than in 1991.

The presence of Rift Valley Fever in cattle was detected by Elisa, the antigen used being a generous gift of the "Laboratoire des Fièvres hémorragiques, Institute Pasteur Paris" and prepared according to the protocol proposed by TG Ksiazek.

The results obtained confirm and extend those previously found that RVF virus is present practically through the whole territory of Central African Republic, but with a rather low prevalence in cattle: out of 583 sera of Zebu, 22 gave a strong positive reaction and 53 were very weakly positive or indetermined.

Emergency Mosquito Surveillance and Control Associated with Hurricane Andrew in Florida and Louisiana

Hurricane Andrew crossed south Florida on August 24, entered the Gulf of Mexico, and hit the Louisiana coast on August 26. Rainfall and the storm surge associated with the hurricane flooded coastal and inland areas and quickly produced large populations of mosquitoes. As a result of the destroyed and damaged housing, people staying in the affected areas or returning after the initial evacuation were exposed to mosquito biting for prolonged periods. High densities of nuisance mosquitoes hampered recovery efforts and the prolonged exposure to mosquitoes increased the potential for mosquito-transmitted diseases among recovery workers and displaced residents. Numerous incidents of secondary infection of mosquito bites in children were reported in both states.

Florida

The majority of damaged housing was contained in a 200,000 acre area in the southern portion of Dade county. An estimated 25,000 housing units were destroyed and 37,000 severely damaged.

Mosquito densities were monitored by taking landing rates at 27 sites and with CO_2-baited traps placed at 8 locations. *Aedes taeniorhynchus* and *Culex nigripalpus* were the most prevalent mosquito species in the area. Densities increased within the week after the storm. Daytime landing rates of nuisance mosquitoes exceeded 20 per minute in several areas. Dade County Mosquito Control surveillance data indicated that mosquito densities were not significantly above normal levels for the time of year. However, mosquito densities that were tolerable prior to the storm were unacceptable when human exposure increased.

Mosquito control agencies from Dade, Monroe, Lee and Broward counties applied mosquito adulticides to a total of 273,339 acres during the period from Aug. 30-Sept. 5. The Air Force Reserve 910th Airlift Group, Aerial Spray Branch treated 142,080 acres on Sept. 6 and 7. *Aedes taeniorhynchus* populations were reduced by 97-99%. Additional adulticide applications were made to 98,960 additional acres on Sept. 11, 73,280 acres on Sept. 29, and 69,530 acres from Oct. 5-13. The adulticide applications resulted in a >98% reduction in trap counts and landing rate counts.

While *Cx. nigripalpus*, the vector of St. Louis encephalitis virus, is present in Dade county, SLE activity in the area is traditionally very low. During the 1990 SLE outbreak in Florida, 223 cases were reported in the

state while only 2 were reported from Dade county. SLE activity throughout the state was low prior to and following the hurricane, and the potential for SLE in the area was minimal. However, SLE surveillance was initiated because of the increased exposure of the displaced residents and recovery workers to mosquito biting. Mosquito-based surveillance for St. Louis encephalitis was conducted from Sept. 8 to Oct. 15. A total of 28,369 specimens (primarily *Cx. nigripalpus*) in 402 pools was tested with an antigen capture ELISA. No SLE antigen was detected.

Louisiana

The disaster area covered 36 parishes, of which 9 were considered candidates for large buildup of pest mosquitoes or mosquito-borne disease. An estimated 25,000 housing units were destroyed or severely damaged by the storm. Emergency surveillance programs were established in Lafourche, Terrebonne, St. Martin, St. Mary, Iberville, Point Coupee, Livingston and Lafayette parishes, and ongoing programs in E. Baton Rouge, Iberia, and Vermilion parishes were augmented. In the affected Louisiana parishes, storm-associated rainfall significantly increased mosquito populations and displaced people were exposed to higher than normal mosquito densities.

Densities of nuisance species were estimated with CO_2-baited CDC light traps, by landing/biting counts, and by numbers of telephone complaints received by health departments or vector control agencies. In East Baton Rouge Parish, September mosquito indices were 0.23, 212.6, and 19.6 times the long-term September averages for light traps, landing/biting counts, and telephone complaints, respectively. The most common pest species collected were *Psorophora columbiae*, *Culex salinarius*, *Psorophora ferox* and *Aedes sollicitans*.

Light trap collections were processed for virus detection by the state public health laboratory. A total of 2,738 mosquitoes (131 pools) of known or suspected vector species (*Culiseta melanura*, *Coquillettidia perturbans*, *Culex pipiens quinquefasciatus*, *Cx. salinarius*) were processed. No arboviruses were detected.

Aedes albopictus in the United States, 1992

Aedes albopictus, is widely distributed in Asia. Until its discovery in Houston, Texas, in August 1985, this species was unknown in the New World. It now has been reported from 351 counties in 25 states in the continental U.S.; currently, it is believed to be established in 347 counties in 22 states (Figure 1). The northernmost infestation in the U.S. is Chicago, Illinois. It has been found as far south as Cameron County, Texas, and Broward County, Florida. In the past year, *Ae. albopictus* has extended its westward range to Del Rio (Val Verde County), Texas, and Omaha (Douglas County), Nebraska. Limited focal infestations in at least three northern states, Indiana, Minnesota, and Ohio, apparently have been eliminated by persistent control efforts by state and local agencies, perhaps coupled with severe winter temperatures. Nonetheless, other areas in Indiana and Ohio continue to be infested. Although found on one occasion in Matamoros, Mexico, there is no evidence that it has become established there. The species was independently introduced into Brazil and occurs in at least four Brazilian states. In September 1991, *Ae. albopictus* eggs were collected in three separate locations in Delta State, Nigeria, suggesting that this species has colonized urban and rural forested sites. In September 1991, an infestation was reported in Padua and surrounding communities in Italy. Since its introduction into the U.S., four arboviruses [Potosi (POT), Tensaw (TEN), Keystone (KEY) and eastern equine encephalitis (EEE)] have been isolated from *Ae. albopictus*. KEY, POT, and TEN viruses are not considered to be pathogenic to humans, but EEE causes severe, often fatal disease in humans, domestic animals (equines, pheasants, emus), and at least one endangered species (whooping crane). (Reported by Field Services Section, Medical Entomology-Ecology Branch, DVBID, NCID, CDC, Fort Collins, Colorado 80522-2087, USA.)
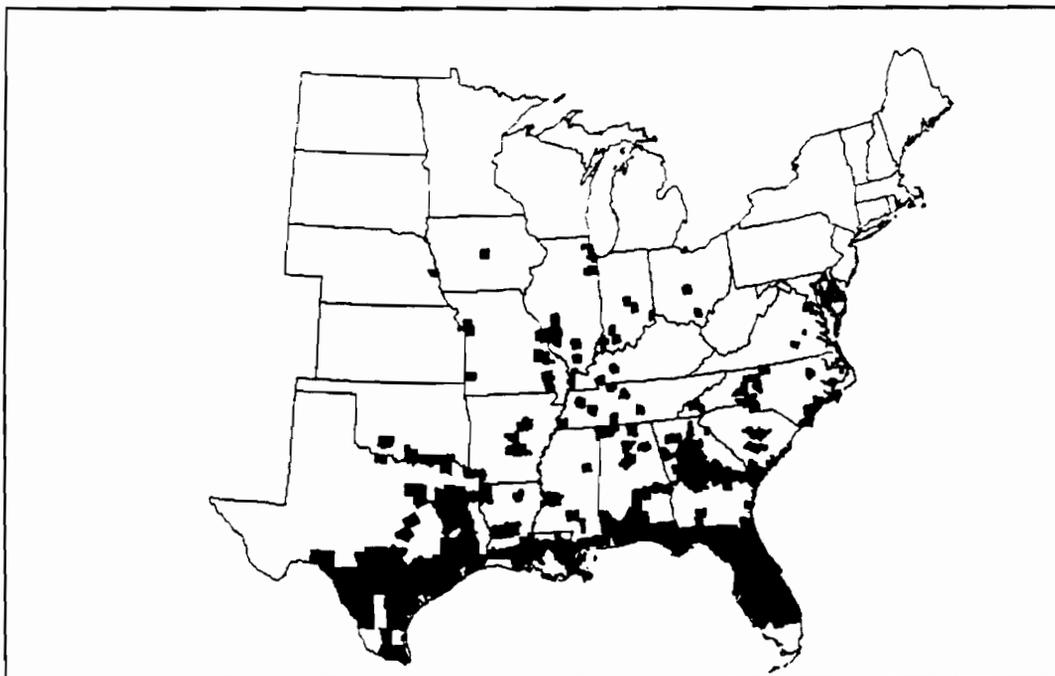

Figure 1. Reported distribution of *Aedes albopictus* in the continental U.S., 1992.

PARTIAL MOLECULAR CHARACTERIZATION OF ARAGUARI VIRUS, AN AS-YET UNCLASSIFED VIRUS.

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Several viruses have not yet been definitively assigned to a recognized virus family. Among such "unclassified" viruses are Araguari virus, originally isolated from an opossum in Brazil, and Quaranfil and Johnston Atoll viruses, originally isolated from ticks (different genera) in Egypt and Johnston Atoll, respectively. Utilizing electron microscopic evaluation and serological assays, Zeller *et. al.* (1989) found that the morphological and morphogenetic features of these three viruses were similar to those characteristically displayed by the Arenaviridae. While an antigenic relationship exists between Quaranfil and Johnston Atoll viruses, no significant serological reactivity could be demonstrated between any one of these viruses and antibodies to recognized members of the Arenaviridae. These viruses were similarly non-reactive with antibodies prepared against viruses from other virus families. To determine whether Araguari, Quaranfil, and Johnston Atoll virions share molecular similarities with the Arenaviridae, studies have been initiated in our laboratory to describe the nucleic acid and protein components of these viruses. We report here the preliminary results of our investigations with Araguari virus.

Araguari, Quaranfil, and Johnston Atoll viruses were obtained from Drs. Charles Calisher and Nick Karabatsos (CDC, Ft. Collins, CO). Araguari virus was propagated both in VERO and in BHK-21 cells, the culture fluids harvested 5-6 days post-inoculation, and the virus precipitated with polyethylene glycol prior to purification by rate-zonal centrifugation through a continuous gradient of 20-70% sucrose as described by Flamand and Bishop (1974). The well-defined virus band was collected from the gradient, diluted and pelleted through a cushion of 30% sucrose, and resuspended in a Tris/EDTA buffer. Araguari virus was highly stable and retained infectivity throughout the course of this purification protocol, in contrast to Quaranfil virus, which was labile and lost infectivity during purification.

SDS-PAGE analysis of unlabeled or [^{35}S]methionine-labeled purified Araguari virions, from both VERO and BHK-21 cells, revealed the presence of 3 major polypeptides with molecular weights of 67 KDa, 58 KDa, and 30 KDa. Visual inspection of Coomassie Blue - stained gels indicated that these proteins were present in a molar ratio of approximately 1:2:3, respectively. Two minor polypeptides with molecular weights of 43.5 KDa and 35 KDa were also detected. Both the 67 KDa and the 30 KDa proteins could be radiolabeled with [3H]glucosamine, and thus appear to be glycoproteins. Although further study will be needed before definitive conclusions can be drawn, the virion polypeptides of Araguari virus do not appear to closely resemble those of recognized arenaviruses (Buchmeier and Parekh, 1987).

Nucleic acid extracted from purified Araguari virions was sensitive to digestion with pancreatic ribonuclease (RNase A), indicating that the genome consists of single-stranded RNA. Eight species of RNA, ranging in size from approximately 0.3 Kb to 4.4 Kb, were consistently detected by agarose gel electrophoresis. Although two of the RNA species were similar in size to 28S and 18S ribosomal RNAs, which are also found in RNAs extracted from arenavirus virions, no Araguari viral RNA species corresponding in size to the L and S RNAs of arenaviruses were detected (by comparison to Tacaribe and Pichinde viral RNAs run in the same gel). Determination of genome polarity and analysis of the eight individual species of RNA will be required before definitive conclusions can be made concerning the structure of the genome.

Araguari-infected VERO cells were also re-examined by electron microscopy, and the morphology of the intracellular virions were confirmed to be arenavirus-like (P. Jahrling, USAMRIID, personal communication) as previously described (Zeller *et.al.*, 1989).

In summary, while Araguari virions appear to be morphologically similar to those of the Arenaviridae, molecular analyses of the viral nucleic acid and protein performed to date suggest a distant relationship, if any, to the Arenaviridae. The polypeptide characteristics of this virus more closely resemble those of Thogoto and Dhori viruses, which are orthomyxovirus-like (Clerx *et. al.*, 1983), rather than those of the Arenaviridae. However, no serological cross-reactivity was detected between Araguari virus and antibodies to Thogoto and Dhori viruses (C. Calisher, personal communication). The nucleic acid characteristics of Araguari virus also differ from those of two recognized arenaviruses (Tacaribe and Pichinde) which were analyzed in parallel. The classification of Araguari virus based upon morphological and molecular features therefore remains enigmatic, and further studies will be required to determine its position within viral taxa.

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Genetic Relationships among the Structural Genes of VEE Viruses

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Our laboratory has determined the nucleotide sequences of the genomes of epizootic VEE viruses TRD, 71-180, and P676 as well as the genomes of the TC-83 vaccine virus and enzootic strain 3880. The structural gene region of the enzootic VEE viruses EVE and MENA have also been determined. These data are published or "in press" in Virology and J. Gen. Virol.

Figure 1 illustrates the number and relative positions of the nucleotide differences detected between the 26S RNA region of the genome of TRD virus and the 26S RNAs of TC-83, 71-180, P676, 3880, EVE, and MENA viruses. These results demonstrate clearly that enzootic VEE subtype I-E and II viruses are genetically very different from the epizootic I-AB virus responsible for the widespread VEE outbreak of 1969-1972. The close genetic relationship between TRD virus, isolated in Trinidad in 1943, and 71-180 virus, a 1971 Texas isolate, provide the most definitive evidence to date that the equine-virulent, epizootic VEE I-AB virus responsible for the 1969-1972 epizootic in Middle America and the USA (Texas), and perhaps earlier outbreaks, originated from incompletely-inactivated formalized vaccine prepared with epizootic VEE subtype I-AB virus. No epizootics of VEE virus have been reported since the introduction of the live attenuated TC-83 vaccine virus during the 1969-1972 epizootic.

Figure 1: Nucleotide Differences among the Structural Genes of VEE Viruses
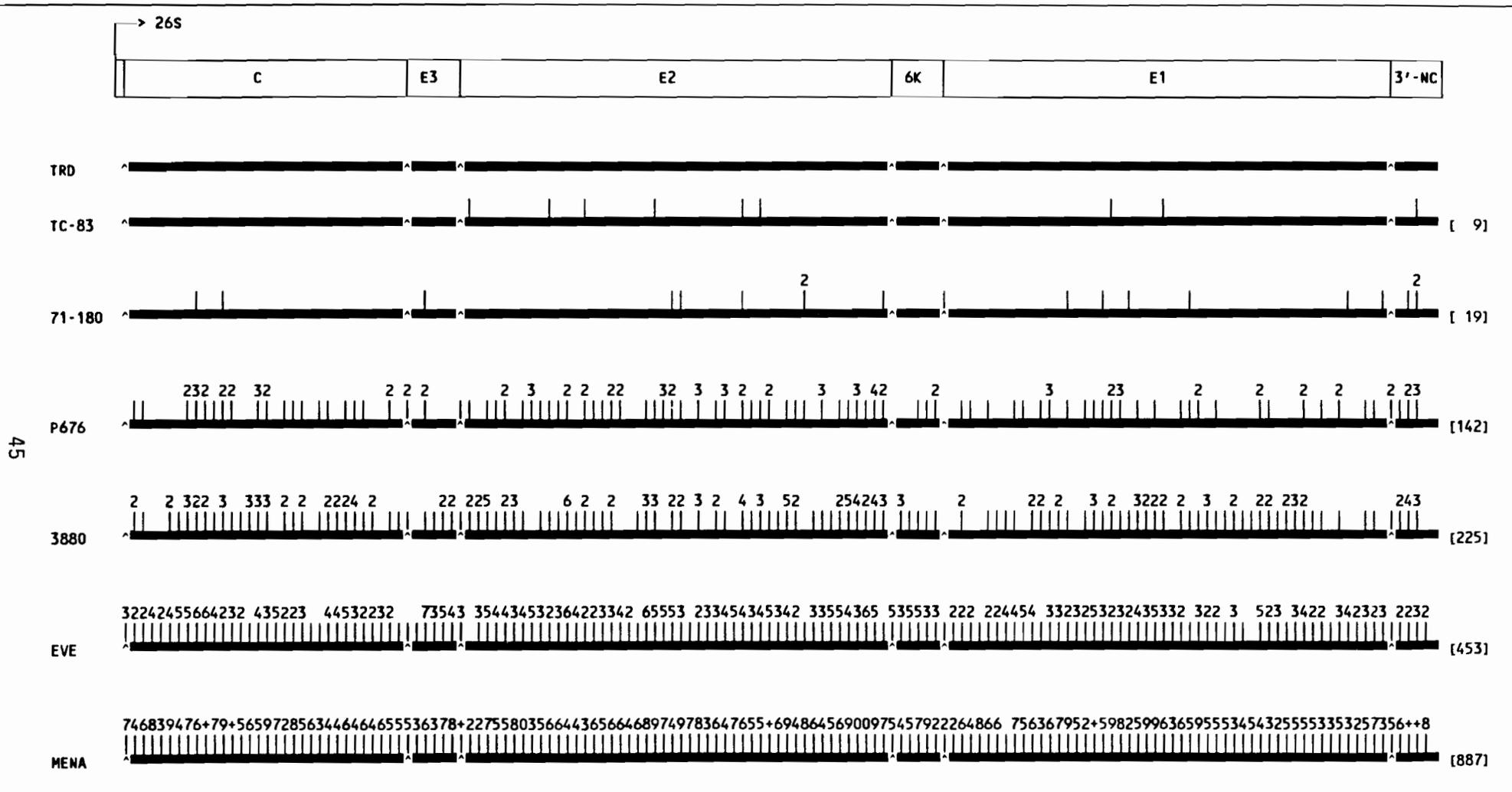
A schematic diagram of the structural genes encoded within the 26S RNA region of the VEE virus genome is shown at top. Cleavage sites in the polyprotein precursor molecule are shown as carrot marks in the baseline graphs. The vertical tick marks above the baseline graphs indicate relative positions of nucleotide differences relative to the VEE Trinidad donkey (TRD)(subtype I-AB) reference sequence, of the vaccine TC-83 strain, epizootic strains 71-180 (I-AB)(Texas, 1971) and P676 (I-C) (Venezuela, 1963), and enzootic VEE strains 3880 (I-D)(Panama, 1961), Everglades Fe3-7c (EVE)(II)(Florida, USA, 1963), and Mena II (MENA)(I-E)(Panama, 1962). Numbers appearing above these tick marks indicate more than one (ten for "0", greater than ten for "+") difference per tick mark resolution (26 nucleotides/graph column). The total number of nucleotide differences in the 26S RNA region is shown in brackets to the right of each graph.

Nucleotide sequence of the envelope glycoprotein of Kyasanur Forest Disease (KFD) virus

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In recent years the nucleotide sequences of several flaviviruses have been published. Among the tick-borne flaviviruses, the sequences of western European and far eastern subtypes of TBE, louping ill, Langat, Negishi, Omsk haemorrhagic fever (OHF) and Powassan have been determined. Alignment of the deduced amino acid sequences of these viruses has enabled us to identify genetic markers for these viruses.

Kyasanur Forest Disease (KFD) virus is an important member of this group causing haemorrhagic disease in man. KFD was first reported in 1957 as a human epidemic in the Shimoga district of Karnataka in India. The human disease was preceded by an outbreak of disease in monkeys. Since then, KFD has been reported every year with an average of 400-500 cases. Although the disease was localized initially to small areas, possibly from ecological imbalances resulting from deforestation and intensive agricultural practices, there has been an increase in the number of foci of disease outbreaks and in 1983 there was an incidence of 1555 cases with 150 deaths. Apart from the human danger, KFD has almost decimated two species of monkeys *Presbytis entellus* and *Macaca radiata* in this area and the possibility of other primate species being affected is now considerable.

Recently, in collaboration with Professor Lashkevich of the Institute of Poliomyelitis and Viral Encephalitides, Moscow, we have determined the nucleotide sequence of the structural region of KFD virus. The virus showed a genomic organisation similar to flaviviruses. The nucleotide sequence data showed closer homology to tick-borne than to mosquito-borne flaviviruses. The recognised genetic markers specific for tick-borne flaviviruses were also conserved in the envelope gene sequence. Comparison of the deduced amino acid sequences of the envelope glycoprotein with other tick-borne flavivirus sequences gave the following percentage homologies.

Western TBE	81.10
Far eastern TBE	80.29
Louping ill	79.27
Langat	79.68
Powassan	77.30
Negishi	79.60
Omsk haemorrhagic fever	80.51

We conclude that KFD is a distinct member of the tick-borne flavivirues which shows its closest homology with western European strains of TBE virus.

REPORT FROM THE DEPARTMENT OF ARBOVIROLOG,INSTITUTE OF
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ISOLATION OF SOME NEW PROBABLE COLTIVIRUS MEMBERS FROM
MOSQUITOES IN CHINA

Coltivirus is a new genus which has two known serotypes—Colorado Tick
Fever virus and the European isolate, Eyach. Indonesian and Chinese
isolates are the probable members of Coltivirus(1).

Our laboratory colleagues have reported isolation of the probable Coltivirus
members in Beijing, Hainan, Yunnan, Henan, Shanxi and Xinjiang provinces
since 1985(2-7).

The present study describes the isolation and identification of some new
probable Coltivirus members from mosquitoes collected in Gansu province
and Beijing city in 1991.

Mosquitoes were collected in Wudu county and Winxian county, Gansu
province, central-western China, situated in the border between Gansu
and Sichuan province, 105° E, 33° N, during July 15 to August 14, 1991

During Octomber 24 to November 3, 1991, we collected some mosquitoes in
Taoranting park, located in Beijing city and Changping county,
approximately 20 Km north of Beijing city, 116.4° E, 40° N.

Mosquitoes were stored in liquid nitrogen for shipment to laboratory.
0.1 ml of supernatant fluid from each mosquitoes poor of about 100
individuals was inoculated onto a single C6/36 and BHK- 21 cell
monolayer separately, cultures were examined for cytopathic effects
more than 10 days(8-10).

10 viruses were isolated in Gansu province and Beijing city, they were
called WDC2, WX1, WX2, WX3, ACH, TRT2, TRT5, LY1, LY2, and LY3
respectively, after the name of the place where the virus was isolated.

The viruses had CPE on C6/36 and BHK-21 cells, pathogenic for new-born
mice, not sensitivity to ether and BUDR.

Electron micrography showed the viruses were spherical virons without
envelope about 62.7 nm in diameter.

All the above mentioned viruses and AV which was isolated by Dr. Xu in Yunnan province before, were passaged to C6/36 cell monolayers respectively, when more than 50% of cells showed CPE, each tube was centrifuged at 2,000 rpm for 10 min. , discarded o. 6 ml of the supernatant, scraped off cell monolayer with 0.4 ml of the remained supernatant, transfered the suspension into a microfuge tube, mixed with an equal volume of extraction buffer(o.o2 M Tris-HCl,PH7.4, 0. 3 M NaCl,0,01 M MgCl2, 0.1% sodium dodecyl sulphate, 0.005 M EDTA, 4% sucrose, 0.04% bromophenol blue) described by Pyndiah et al, and 0.4 ml of phenol-chloroform(1:1). After incubation for 10 min. at 56° C, the materials were centrifuged at 10,000 rpm for 30 min. at 4° C, and 20 ul of the aqueous phase were loaded onto 10% polyacrylamide gel. Gels were visualized by the silver-staining method (11-13,18).

By PAGE, the isolates were assigned to five distinct RNA electropherotypes repeatedly. The Gansu province isolates had three RNA patterns, WDC2:2-3-1-3-1-2; WX3:2-2-2-3-1-2; WX1, WX2, and ACH were similar:2-3-2-3-1-2. All of the Beijing isolates TRT2, TRT5, LY1, LY2, LY3 were similar:2-2-2-3-1-2. The Yunnan isolate AV was:2-2-2-3-1- 2 . It was easy to find AV, WX3 and Beijing isolates Different from each other.

It was very amazing that WX1,WX2 and ACH genomes comprised 13 segment ds RNA,this might due to the reassorting of WDC2 and WX3 in nature, another possibility was the coinfection of WDC2 and WX3.(14-17)

Recently, we are studying the relatedness among Chinese isolates by cross-neutralization test and comparing them with Colarado Tick Fever virus and Eyach by CF test.

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SINDBIS VIRUS ISOLATED IN CHINA FOR THE FIRST TIME

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An apparent alphavirus, XJ-160, was isolated from a pool of <u>Anopheles</u> species mosquitoes collected in the Yi Li area, Xin Jiang Province, People's Republic of China. The identification of the virus was indicated by the following data:

1. Illness and death in suckling mice; evident cytopathic effect in C6/36 and BHK cells.
2. Sensitivity to acid and ether but resistence to 5-FUDR; agglutination of sheep red blood cells; and
3. Spherical enveloped particles characteristic of alphaviruses approximately 58 nm in diameter.

The virus was antigenically related to the alphaviruses Sindbis (SIN), chikungunya (CHIK),and eastern equine encephalomyelitis (EEE) viruses, particularly to SIN. The neutralizing index of SIN antibody with the XJ-160 isolate, as measured in suckling mice, was 100,000 while the index obtained with homologous virus was lower by a factor of 10.

This is the first reported isolation of SIN virus in mainland China.

Characterization of Potosi Virus, A Bunyamwera Group Virus Originally Isolated from *Aedes Albopictus* Mosquitoes.

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A total of ten isolates of Potosi virus were obtained from pools of *Aedes albopictus* mosquitoes collected in a scrap tire yard near Potosi, Missouri, during August and September of 1989 (1). Six additional isolates were identified as Potosi virus from *Aedes albopictus* mosquito pools collected in the area of Potosi, Missouri, also in 1989 (2). This brief report summarizes characterization studies conducted with two representative isolates selected from the first ten isolates obtained. All of the virus isolates were tested initially by indirect immunofluorescence antibody (IFA) tests against NIH grouping antibody reagents and all reacted only with the Bunyamerwera grouping fluid.

Plaques on Vero cell cultures produced by Potosi virus were observed on days 3-4. Plaques were clear and measured 1.0-2.5 mm on day 3 for strain 89-3380 and 2.5-3.0 mm on day 4 for strain 89-3470. Both isolates were inoculated into Vero and BHK-21 cell cultures and were processed for hemagglutinin (HA) production. Only infected BHK-21 cultures successfully yielded HA antigens for both isolates. Similar attempts with infected Vero cells were negative. However, the resulting hemagglutinins were not very sensitive in homologous and heterologous hemagglutination-inhibition tests. This is not necessarily unusual for some bunyaviral hemagglutinins.

A Vero-3 suckling mouse-1 (V3SM1) passage of strain 89-3380 was inoculated by the intracerebral (1C) and intraperitoneael (1P) routes into 2-4 day-old SM, into 3-4 week-old weanlings and into 12-13 week-old adults. This virus preparation titered 4.6 dex IC $LD_{50}/0.02$, AST = 4.3, 1.0 dex IP $LD_{50}/0.03$, AST = 5.1, and IP inoculations in weanlings and adult mice did not kill mice. Titrations carried out by the IC route in adult mice yielded titers of 3.3 dex IC $LD_{50}/0.03$, AST = 6.6

Adult mice were hyperimmunized with infective serum-free Vero cell culture fluid prepared with the two representative isolates of Potosi virus. These antisera were used in cross-neutralization tests which compared the two representative isolates with five other members of the Bunyamerwera (BUN) antigenic group found in the United States (Table 1). The results clearly show that both isolates were distinct. In fact, these isolates appeared to be more closely related to Tensaw virus then to the other four BUN group viruses. One of the Potosi isolates (89-3380) then was tested by neutralization against antibody to 13 other BUN

group viruses recovered from Mexico, Central and South America (Table 2). These results indicate that Potosi virus is quite distinct from the other BUN group viruses, although our initial tests indicated Kairi antibody could not distinguish between homologous Kairi and Potosi viruses. Provisionally, we considered Potosi virus to be a subtype of Kairi virus. Since then, one of us (RES) has re-prepared reagents to Potosi and Kairi viruses and has repeated the cross-neutralization testing between these two viruses. It was determined that although these two viruses are closely related, they are distinguishable in both directions using hyperimmune ascitic fluids.

The two Potosi isolates also were tested in complement-fixation (CF) tests against the five other BUN group viruses from the United States, and Kairi virus. In general, the CF test results did not show a great deal of discrimination, although it was quite clear that Kairi virus showed a weak or distant relationship in CF tests with both isolates as compared to the other heterologous reactions with these isolates.

Since the intital isolations and characterizations, we have identified eight additional strains of Potosi virus from mosquito isolates submitted for identification by the Medical Entomology and Ecology Branch. These included five isolations from pools of non-*Aedes albopictus* mosquitoes collected in Ohio, 1991, one isolation from mosquitoes collected in South Carolina, 1991, and two isolations from previously ground mosquito suspensions submitted by the state of Michigan, 1992 (3).

Two independent studies have demonstrated that *Aedes albopictus* mosquitoes are competent vectors for horizontal transmission of Potosi virus but that there was no evidence of transovarial transmission (4,5).

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TABLE 1

Results of serum-dilution plaque-reduction neutralization tests (PRNT)
comparing two Potosi virus isolates (89-3380, 89-3470)
recovered from *Aedes albopictus* mosquitoes with other
Bunyamwera group viruses found in the United States

Virus	Strain	90% PRNT titer of antibody to:						
		89-3380	89-3470	MD	LOK	CV	TEN	NOR
Potsoi	89-3380	2560	2560	20	-	-	80	-
Potosi	89-3470	5120	2560	20	40	-	160	-
MD	BFS 5015	-	-	320				
LOK	FMS 4332	-	-		>5120			
CV	6V633	-	-			160		
TEN	A9-171B	20	40				1280	
NOR	0234	-	-					1280

- = < 20; blank = not done.

MD = Main Drain virus; LOK = Lokern; CV = Cache Valley; TEN = Tensaw; NOR = Northway.

TABLE 2

Results of plaque-reduction neutralization tests (PRNT) comparing Potosi virus with Batai virus and other Bunyamwera group viruses recovered in areas south of the United States

Virus	Strain	Potosi	AMB	BAT	FS	KRI	MAG	PLA	SAR	SOR	TAIA	TLA	TUC	WYO	XIN
							90% PRNT titer of antibody to:							
Potosi	98-3380	5120*	-	40	-	1280	-	-	10	40	-	10	-	-	-
AMB	SPAr2984	-	10240												
BAT	MM222	-		1280											
FS	86MSP18	-			320										
KRI	TR8900	<20				640									
MAG	BeAr7272	-					320								
PLA	75V3066	-						160							
SAR	M2-1493	10							1280						
SOR	BeAr32149	-								10240					
TAIA	BeAr671	-									1280				
TLA	61D240	-										640			
TUCU	BeAr278	-											40		
WYO	Original	-												1280	
XIN	BeH388464	-													5120
IACO	BeAr314206	-													
MCA	BeAr306329	-													

* Highest dilution of antibody producing ≥90% neutralization.

- = < 10; blank = not tested

AMB = Anhembi virus; BAT = Batai; FS = Fort Sherman; KRI = Kairi; MAG = Maguari; PLA = Playas; SAR = Santa Rosa; SOR = Sororoca; TAIA = Taiassui; TUC = Tucunduba; WYO = Wyeomia; XIN = Xingu; IACO = Iaco; MCA = Macaua .

An OspA Antigen Capture Enzyme-Linked Immunosorbent Assay Detects
North American Isolates of *Borrelia burgdorferi* in Larvae, Nymphs
and Adult Ticks

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An antigen capture enzyme-linked immunosorbent assay (ELISA)
was developed for detecting North American isolates of *Borrelia
burgdorferi* in larval, nymphal and adult ticks. The assay uses
an anti-OspA monoclonal antibody (H5332) for antigen capture and
biotin-labelled polyclonal sera with streptavidin-horseradish
peroxidase for signal generation. The assay recognized 15 of 15
low passage North American *B. burgdorferi* isolates and did not
cross-react with spirochete antigens of *B. hermsii*, *B. turicatae*,
B. coriaceae, or *B. parkeri* or with tick antigens of *Ixodes
dammini*, *I. scapularis*, *I. pacificus*, *I. cookei*, *I. angustus* or

Ambylomma americanum. The assay, with a sensitivity of 200 spirochetes, can detect infections in larval, nymphal and adult ticks. In addition to fresh ticks, *B. burgdorferi* infections in ticks stored frozen, dried or in 70% ethanol can be determined with the assay.

In studies on the growth kinetics of the JD1 strain of *B. burgdorferi*, the assay was able to detect infected larvae on the first day post-repletion. Spirochete numbers increased to over 30,000 per larvae but dropped to less than 2000 prior to taking the next blood meal as nymphs.

Epidemiological study of Lyme borreliosis in area around Paris

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Preliminary studies carried out in 1990 and 1991 in the forests of seven departments around Paris (77 : Seine et Marne, 78 Yvelines, 91 : Essonne, 92 : Hauts-de-Seine, 93 : Seine Saint Denis, 94 : Val de Marne) demonstrated the presence of *Borrelia burgdorferi* in almost each of these forests. The presence of the *Borrelia* was shown either by its isolation from ticks, or by demonstration of antibodies in humans or large wild mammals.

A serologic survey of 208 workers of the "Office National des Forêts" (ONF), revealed that 0 to 25,7% of individuals were positive by direct immunofluorescence, depending on the forest where they worked.

A serologic survey of large wild mammals living in the same forests is currently in progress. A modified Elisa technique called ELGA (in press) revealed that 19,2% of roe deers were positive. A study of sera of red deer and wild boar, by the same method, is currently in progress.

Ticks were collected in 2 of this forests, one to the west of Paris : the forest of Rambouillet, and the other to the south-east : the forest of Fontainebleau. Two species of *Borrelia* were found : *Borrelia burgdorferi* and *Borrelia garinii*. The identification carried out by the "Unité de Bactériologie Moléculaire et Médicale" Institute Pasteur (Dr Baranton), was based on :
    1 - strain reactivity with monoclonal antibodies
    2 - rRNA gene restriction pattern
    3 - restriction site polymorphism in PCR products from a inter-rRNA gene sequence

One series of results obtained by direct immunofluorescence on 569 ticks, in nymphal and adult stages showed that the percentage of infected specimens varied between 4,5 and 16,6% for nymphs, depending on the site of capture and season, and between 0 and 33,3% for the adults.

Another series of ticks, captured in the same localities, and studied by indirect immunofluorescence (ticks in alcohol), and by PCR, gave much higher percentages which we are currently in the process of interpreting.

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LYME DISEASE: STUDY Of A HYPERENDEMIC AREA IN SPAIN

We have studied human sera from Arcos de Jalon (an area of the Soria Province, Spain), using an indirect immunofluorescence test for total antibodies (IgG, IgM and IgA) and specific IgM against *Borrelia burgdorferi* (B 31 strain). The age of the studied people ranged between 13 and 89 years. The results were considered positive when the titers were the same as or above 256. Antibodies were present in 18.9% of the examined sera. The IgM was positive for 3 sera (5.7%).

The high prevalence of the *Borrelia burgdorferi* infection in this area of the country confirms the existence of endemic areas for Lyme disease. We think that it is very important to organize more epidemiological studies in order to have a better knowledge about the distribution of this disease in Spain.

REPORT FROM THE CENTER FOR ZOONOSES RESEARCH, NATIONAL INSTITUTE OF HEALTH, AGUAS DE MOURA, PORTUGAL.   (J.V. Saz, S. Nuncio, F. J. Merino, A.R. Filipe)

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LYME DISEASE NEWSLETTER FOR THE U.K.

We have initiated a "Lyme Disease Newsletter" in an attempt to record the distribution of *Borrelia burgdorferi* infected ticks in the U.K. The letter is sent out to fieldworkers, foresters, gamekeepers, general ecologists, and anyone else interested. It acts as a source of information and encouragement to potential tick collectors.

Ticks are posted to us, the collection details recorded, and then the samples stored at -70°C prior to screening using the polymerase chain reaction (PCR). The test is performed with a nested set of primers as described by Guy and Stanek (J. Clin. Pathol., 1991, 44:610-611).

Since July 1990, we have received 82 gifts of ticks (few of them rather mushy, but most were alive and kicking). These have been provided by some 30 workers in the field, with odd collections from interested landowners and the general public. Some 250 ticks (mostly *Ixodes ricinus*) have been processed individually and 15 % have yielded PCR positives, representing 17 sites. Most of these sites are located in Scotland, with others in the north and south of England; so far we have no positive sites from Ireland and Wales.

At this stage the distribution map of PCR positive ticks (see Fig.) reflects the activities of tick collectors rather than the distribution of *B. burgdorferi* infected ticks in the U.K. Most of the positive ticks were collected from deer (or questing in deer inhabited areas), but some were from dogs and sheep. The greatest proportion of positive ticks were adults (17% engorged females, 19% unfed females, and 19% unfed males), with fewer positive nymphs (7% unfed nymphs) and no positive unfed larvae (n = 12; 2 pools).

We are extending the range of our collections and sample size with the aim of producing a more representative picture of *B. burgdorferi* in its natural habitat.

From: Dot Carey and Pat Nuttall. May 1992.