

March 12, 1984 46

From: Chief, Production Branch

Subj: Attached "Arthropod-borne Virus Information Exchange"

To: Recipients of the "Arthropod-borne Virus Information Exchange"

Your copy of the most recent "Arthropod-borne Virus Information Exchange" is attached.

Those of you working with nonhuman primates will be interested in the note included (see page 2) from Dr. S. S. Kalter of the NIH and WHO Collaborating Center for Reference and Research in Simian Viruses.

Page 3 contains an important announcement concerning a Conference on Laboratory Rodents at NIH scheduled for October 24-26.

Starting on page 7 is a group of abstracts of papers presented at the Working Conference, Viral Diseases Panels, United States - Japan Cooperative Medical Sciences Program, held in San Diego, California last September. These abstracts were submitted by Dr. Thomas Yuill.

Your attention is called to the "Guide for Authors" on page 1. Please read and observe the request concerning space limitations in paragraph 5. Your following these suggestions would help me considerably in getting the "Information Exchange" ready for publication.

Please address all communications to the undersigned.

W. Adrian Chappell

W. Adrian Chappell, Ph.D.
Chief, Production Branch
Biological Products Program
Center for Infectious Diseases
Centers for Disease Control
Atlanta, Georgia 30333, U.S.A.

March 1984

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

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GUIDE FOR AUTHORS

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Deadlines for articles to be published are March 1 and September 1.

The following format should be used for all articles submitted:

1. Heading

The heading should be typed with capital letters, including name of laboratory and address. For example:

REPORT FROM THE BIOLOGICAL PRODUCTS PRODUCTION BRANCH, CENTER FOR INFECTIOUS DISEASES, CENTERS FOR DISEASE CONTROL, ATLANTA, GA 30333

2. Body of Report

The text of the report should be as brief as possible to convey the intended message and should make reference to tables and figures included in the report. The text should be single spaced with double spacing between paragraphs.

3. Authors' Names

The names of authors should be in parentheses following the text.

4. Tables and Figures

Tables and figures should be numbered and titled if appropriate. Tables and figures should not be submitted without some description or explanation.

5. Size of Pages

Since there are specific space limitations, the typed material on each page should not exceed 7-1/8" x 9-1/4". The same dimensions apply to tables and figures. If tables and figures are larger than these dimensions, they have to be reduced before being printed. The block shown on this page represents the maximum space available for each page of your report.

Reports should be typed only on one side of each page since they have to be photographed for reproduction. Each page should be numbered. Only the original typed report should be submitted.

Southwest Foundation for Biomedical Research

West Loop 410 at Military Drive
P.O. Box 28147
San Antonio, Texas 78284
(512) 674-1410

MICROBIOLOGY DEPARTMENT



WORLD HEALTH ORGANIZATION
COLLABORATING CENTER
FOR REFERENCE AND RESEARCH
IN SIMIAN VIRUSES

NIH AND WHO COLLABORATING CENTER FOR REFERENCE AND RESEARCH IN SIMIAN VIRUSES

Nonhuman primates continue to be used in biomedical research and pharmaceutical testing. Although a number are provided from breeding stocks, a large number are still imported, principally from areas known to harbor arthropod-borne viruses. This center will continue to provide virologic support to those investigators working with nonhuman primates when virus infections are suspected. Procedures employed for the handling of specimens are those applicable to any diagnostic situation. It is best, however, that problems be first discussed with Center personnel prior to collection and shipping of specimens.

S. S. Kalter, Ph.D.
Head, NIH and WHO Collaborating
Center for Reference and Research
in Simian Viruses

Conference on Laboratory Rodents at NIH

Mice, rats, hamsters and guinea pigs are used extensively in biomedical research. Naturally occurring infections of these rodents, such as Sendai virus, mouse hepatitis virus, ectromelia virus or mycoplasma to name a few, can adversely affect research results and/or contaminate the product prepared in animals. Medical literature is full of such reports. To address this timely issue, a conference entitled: "Viral and Mycoplasma Infections of Laboratory Rodents: Effect on Biomedical Research" will be held at the National Institutes of Health, Bethesda, Maryland, on October 24-26, 1984.

The conference is funded by the National Institutes of Health and the objectives of the conference are to 1) critically evaluate current information about the biology, pathogenesis and eipzootiology of these agents in light of their potential for interference with research and 2) discuss and evaluate rational approaches for their early detection, prevention and control. Invited speakers include scientists (virologists, immunologists, molecular biologists, toxicologists and veterinarians who are knowledgeable in their respective fields of laboratory rodent biology. This forum will facilitate dialogue among these groups and focus attention on unsolved problems.

There is no registration fee, but the conference is open on a "first come - first served" basis. Please contact Dr. Pravin N. Bhatt, Section of Comparative Medicine, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, CT, 06510; telephone number (203) 785-2538, with the following information if you wish to register: name, title, affiliation, address, telephone number and field(s) of interest.

REPORT FROM THE SUBCOMMITTEE ON EVALUATION OF ARTHROPOD-BORNE
STATUS (SEAS) FOR 1983

During 1983, 27 newly registered viruses were evaluated by this subcommittee. In addition, the status of two previously registered viruses were updated. Of the new registrations, 5 were classified as "probable arboviruses", 20 as "possible arboviruses" and 2 as "probably not arboviruses":

<u>Virus</u>	<u>Serogroup</u>	<u>Country</u>	<u>Source</u>	<u>SEAS Rating</u>
Almeirim	CGL	Brazil	Phlebotomine Fly	possible arbovirus
Arbia	PHL	Italy	Phlebotomine Fly	probable arbovirus
Bunyip Creek	PAL	Australia	Bovine	probable arbovirus
Cacipacore	B	Brazil	Bird	possible arbovirus
CSIRO Village	PAL	Australia	<u>Culicoides</u> sp.	probable arbovirus
Cuiaba	MOS	Brazil	Frog	possible arbovirus
Flexal	TCR	Brazil	Rodent	probably not
Iaco	BUN	Brazil	Mosquito	possible arbovirus
Itupiranga		Brazil	Mosquito	possible arbovirus
Jacareacanga	COR	Brazil	Mosquito	possible arbovirus
Jari	CGL	Brazil	Sloth	possible arbovirus
Macaua	BUN	Brazil	Mosquito	probable arbovirus
Mapuera		Brazil	Bat	probably not
Marrakai	PAL	Australia	<u>Culicoides</u> sp.	possible arbovirus
Mojui dos Campos		Brazil	Bat	possible arbovirus
Monte Doutado	CGL	Brazil	Armadillo	possible arbovirus
Munguba	PHL	Brazil	Phlebotomine Fly	possible arbovirus
Oriximina	PHL	Brazil	Phlebotomine Fly	possible arbovirus
Para		Brazil	Sentinel Mouse	possible arbovirus
Purus	CGL	Brazil	Mosquito	possible arbovirus
Santarem		Brazil	Rodent	possible arbovirus
Saraca	CGL	Brazil	Phlebotomine Fly	possible arbovirus
Sena Madureira	TIM	Brazil	Lizard	possible arbovirus
Shokwe	BUN	S. Africa	Mosquito	probable arbovirus
Sripur		India	Phlebotomine Fly	possible arbovirus
Tibrogargan		Australia	<u>Culicoides</u> sp.	possible arbovirus
Xiburema		Brazil	Mosquito	possible arbovirus

Isfahan virus was reclassified as a "probable arbovirus" and Issyk-Kul was upgraded to full arbovirus status.

Dr. Thomas Aitken stepped down as chairman of SEAS this year. The members of this subcommittee would like to express their appreciation to him for his decade of dedication to this position. Tommy will continue his service as a member of the subcommittee. The other members include Dr. Donald M. McLean (since 1970), Dr. D. Bruce Francy (since 1973), Dr. James L. Hardy (since 1973), Dr. Duane J. Gubler (since 1981), and Dr. Edward W. Cupp (since 1982).

Respectively submitted,



Andrew J. Main, Jr., Chairman

January 14, 1984

December 7, 1983

Report of Subcommittee on Interrelationships of Catalogued Arboviruses
(SIRACA)

SIRACA considered means to deal with the rapidly increasing numbers of arbovirus hybridomas and their monoclonal products which have been developed by the arbovirus community. The monoclonal antibodies are useful as group-, complex-, type- and subtype-specific reagents. Their use has already led to a more sophisticated means of arbovirus identification and classification. They have great potential in epidemiologic studies and for specialized research.

The needs identified included:

1. A repository or repositories for both fully-characterized and partly-characterized hybridomas. At present multiple U.S. and overseas laboratories have hybridomas for arenaviruses, alphaviruses, flaviviruses, bunyaviruses, phleboviruses, nairoviruses, orbiviruses, rhabdoviruses, and HFRS virus. CDC, Atlanta for instance was reported to have about 600 hybridomas in storage.
2. A computerized registry of monoclonal and characterization data. NIH and CDC have existing systems which might be modified to serve the requirements of arbovirus monoclonal antibodies.
3. A facility or facilities to test candidate antibodies for the full range of group-reactive antibodies. At present, the Yale Arbovirus Research Unit, and for some arbovirus families, CDC, Fort Collins have existing antigens for comprehensive testing.
4. A listing in priority order of the classes of data needed for acceptance and/or maintenance of a hybridoma in the repository. This would include such data as test results to certify the monoclonality of the antibody and immunoglobulin characterization.
5. A standardized nomenclature for hybridomas which would describe the antigenic determinant-reactive and relationship characteristics of their monoclonal products.

It was pointed out that U.S. Government regulations currently control and inhibit both exportation (Department of Commerce) and importation (APHIS-USDA).

A SIRACA working group was appointed. The group includes Joel Dalrymple (Chairman), Joseph McCormick, Mary Kay Gentry, Alan Schmaljohn, John Roehrig, and James Meegan. The working group at its first meeting set objectives to investigate the possibility of obtaining alphavirus and/or flavivirus group-reactive monoclonal antibodies that, once characterized, will be prepared in quantity, stored in some repository and distributed to arbovirus investigators upon request.

SIRACA also discussed its possible role in recombinant plasmid storage, testing, use, and distribution. Although now there apparently are

not enough arbovirus plasmids to initiate an activity similar to the hybridoma working group, it was agreed to discuss plasmids again in 1984 at the American Society of Tropical Medicine and Hygiene meeting in Baltimore.

The traditional SIRACA activities continued during the year. The collaborative plaque reduction neutralization test study of flavivirus relationships was completed and a manuscript is in preparation. The existing Bunyavirus manuscript will be rewritten to include genera of the entire family Bunyaviridae, and also to include morphology, genetics, and biochemistry to attempt to bring it into line with ICTV taxonomy.

Submitted by R.E. Shope, Chairman, SIRACA

THE REPLICATION STRATEGY OF THE TOGAVIRUSES

Dr. James H. Strauss
Division of Biology, California Institute of Technology
Pasadena

We have obtained the complete nucleotide sequence of the genome of Sindbis virus, the type alphavirus. Limited amino acid sequencing of virus specific proteins has been used to align these proteins with the RNA genome, and thus the amino acid sequences of all the virus encoded proteins have been deduced. Four nonstructural proteins are produced by cleavage of a polyprotein precursor, which we call nsP1, nsP2, nsP3, and nsP4 in order of translation. nsP4 is produced in relatively smaller amounts because readthrough of an opal (UGA) stop codon is required for its synthesis. These four proteins are thought to form components of the virus replicase; because nsP4 is produced in smaller quantities it may have a regulatory roll.

The structural proteins include a nucleocapsid protein and two envelope glycoproteins. Translation of these proteins involves a second level of regulation; they are translated from a subgenomic mRNA which is the major messenger in virus infected cells and structural proteins are thus produced in great excess over the nonstructural proteins.

We have performed comparative sequence studies of alphaviruses at both the RNA level and at the protein level. These studies have revealed a number of conserved regions in the alphavirus genome which we believe are important for replication of the virus RNA. We suggest that components of the virus replicase recognize these sequences as specific promoter sequences during replication. Comparative studies of protein sequences have shown that all of the alphaviruses are derived from a common ancestor and share 30-90% amino acid sequence homology in the glycoprotein region. An evolutionary tree can be constructed which illustrates the relationship of these viruses to one another more precisely than the current grouping on the basis of serological crossreactions. These data show that glycoprotein E1 is more highly conserved than E2, suggesting that E2 functions during virus evolution to generate strain diversity.

The details of alphavirus replication strategy as deduced from the complete nucleotide sequence and the results of these comparative studies will be discussed.

With: Ellen G. Strauss, Charles M. Rice, Jung-Hsuing Ou and John R. Bell

ANALYSIS OF THE ANTIGENIC STRUCTURE OF ALPHAVIRUS ENVELOPE
GLYCOPROTEINS USING MONOCLONAL ANTIBODY AND rDNA TECHNOLOGIES

Dr. John T. Roehrig
Centers for Disease Control, Fort Collins

We have isolated and characterized monoclonal antibodies specific for the envelope glycoproteins of a number of alphaviruses. These antibodies have been used to characterize the antigenic structure of the glycoproteins. At least nine epitopes have been identified on the E2 (gp56^{a-b}) of Venezuelan equine encephalomyelitis (VEE) virus, and at least seven epitopes have been identified on the E1 (gp55^{al-e2}) of Western equine encephalitis (WEE) virus. On VEE, biological functions of neutralization (N) and hemagglutination-inhibition (HI) are primarily associated with one VEE epitope, gp56^c. Only HI-activity but no N-activity could be associated with several WEE gp55 epitopes. In vivo protection from virus infection is most efficiently provided by passive immunization with high avidity anti-gp56^c antibody. Highly avid non-neutralizing antibodies can also protect, but usually 100-fold more antibody is needed when compared to neutralizing antibody. These antibodies have been used to map antigenic drift between closely related VEE viruses and to probe the antigenic changes related to the processing and maturation of the virus glycoproteins. In addition, cDNA has been synthesized from the genome RNA of VEE virus. Regions coding for the VEE viral glycoproteins have been localized in the sequence using data available from the amino-terminal amino acid sequencing of purified proteins. The clones are being used to define the fine structure of the glycoprotein epitope.

With: D.W. Trent

BIOLOGY OF HFRS VIRUSES AND NATURAL HISTORY OF DISEASE

Dr. Karl M. Johnson

U.S. Army Medical Research Institute of Infectious Diseases

Fort Detrick, Frederick, Maryland

The syndrome of hemorrhagic fever with renal syndrome (HFRS) has been known in Asia, and in milder form, in Scandinavia, for decades. It is rural with at least 3 patterns: (a) agricultural with disease peaks in late spring (planting) and late fall (harvesting), (b) forest invasive; roads, dams, mines, oil drilling, camping, military maneuvers, and (c) suburban/urban with winter invasions of field or forest rodents. It was not until the 1960's that a putative true urban form of the disease was recognized in Osaka, Japan.

More than 60 synonyms for HFRS have been recorded in the literature, many of them erroneous. Until the 1978 report of isolation of the causative virus, moreover, knowledge of this syndrome was largely circumstantial and fraught with much wish and whimsy. The best supported concept was that this was a rodent-borne disease, and candidate species were many. A viral etiology was postulated although fungi had their partisans, and there were many who strongly believed that rodent ectoparasites were the vectors for transmission to man.

During the 1960's a few of us came to suspect that HFRS might be caused by an arenavirus(es). Persistent focality of infection and rodent association were the key facts and the prediction was that things would turn out to be one virus, one rodent as for most of the arenaviruses. When the initial HFRS (Hantaan) strains from nature were all derived from Apodemus agrarius in Korea and this rodent was found to exhibit chronic viruria but no need for ectoparasites to spread within cages, the arenavirus hypothesis appeared highly plausible.

It has not turned out that way. A partial catalogue of recent unique findings may be summarized as follows:

1. There are several rodent reservoirs, or many different viruses, or both.
2. The agents are members of the Bunyaviridae, and form a distinct new genus within the family.
3. Chronic infection is immunocompetent, noncytolytic, and there is no vertical transmission, probably none via milk.
4. Agents and probable transmission to man are world-wide. Only Asia appears to have the severe, hemorrhagic form of disease.

This paper will attempt to document all of these points, and suggest future directions for investigation of these agents, their rodent hosts, and the diseases which they cause in man.

CHANGING PATTERNS OF WESTERN EQUINE AND ST. LOUIS
ENCEPHALITIS IN CALIFORNIA

Dr. William C. Reeves
School of Public Health
University of California, Berkeley

In the 25-year period from 1943 to 1968, Western equine encephalitis (WEE) and St. Louis encephalitis (SLE) viruses were highly endemic and periodically were epidemic in California. From 1969 through 1977, these viruses were almost undetectable, but since 1978, WEE virus has resurged and has been readily detectable in its basic cycles in vectors and avian hosts over much of the state each summer. SLE virus is less prevalent but present. Epidemiological studies have identified significant changes that may be related to the preceding changes in viral endemicity. These changes are the state-wide development of dams on the major river systems for flood control and to provide water for agricultural, industrial and domestic use, major extensions of vector control programs to encompass most of the heavily populated areas, the wide-spread use of organophosphorus and other insecticides for control of agricultural pests, shifts in the seasonal peaks of vector populations from early to mid or late summer and shifts in human populations from rural to urban and suburban areas. The current concern is whether the resurgence of viruses in their basic maintenance cycles will lead to epidemics in a human population that is non-immune and that includes increasing numbers of very young and old individuals which are the ages at greatest risk of developing clinical rather than inapparent infection with WEE and SLE, respectively. It has become increasingly apparent that it will be difficult to control adult vector populations, as they have developed genetic resistance to almost all licensed insecticides. A state-wide surveillance system has been developed to provide an early warning of epidemic threats and to provide guidance for control programs.

ANALYSIS OF THE PROTEIN COMPONENTS OF CHIKUNGUNYA VIRUS

Dr. Bunsiti Simizu
National Institute of Health, Tokyo

Chikungunya (CHIK) virus belongs to a member of the Semliki Forest complex in alphaviruses. CHIK virus causes a disease clinically similar to dengue, first observed in epidemic form in Africa and later in southeast Asia. To investigate the biological function of CHIK virus glycoproteins, we tried to isolate two envelope glycoproteins of the virus separately without denaturing. It is difficult to separate them completely, so we think that the two glycoprotein association in CHIK virus is much stronger than in Sindbis or WEE viruses.

Three strains of CHIK virus, one African and two Asian strains, were used and grown in Vero cells. Infected culture fluids were concentrated and purified by differential centrifugation and sucrose density gradient centrifugation. For radioisotope labeling experiments, the infected culture was labeled with ^3H -leucine for 15 min at 8 hrs after infection. 1) CHIK virus contains three structural proteins: the capsid of 33,000 dalton and two envelope glycoproteins, E1 and E2 of about 53,000 dalton, respectively. Both E1 and E2 migrated to the position very closely in SDS-PAGE. 2) A pulse-chase experiment revealed that the PE2 (E3 + E2) protein is converted to E2 which migrates faster than E1 like the PE2 protein of Sindbis virus. The E3 protein was released into culture fluid of CHIK virus infected cells as an approximately 11,000 dalton glycoprotein. 3) To obtain separately native E1 and E2 from CHIK virion lysate, we tried several ways and finally could separate both proteins by glass wool chromatography. 4) Isolated E1 showed hemagglutinating and hemolytic activities.

These data will be discussed in reference to other alphaviruses.

CELLULAR IMMUNITY IN EXPERIMENTAL JAPANESE ENCEPHALITIS

Dr. Akira Oya

National Institute of Health, Tokyo

In vitro cytotoxic activity and protective activity in vivo by passive transfer were compared with cells from spleens of immunized mice. Eight to 13 week old C3H/He mice were infected with a wild strain of Japanese encephalitis (JE) virus and spleens were taken at appropriate days after the infection. Cell-suspension was made from those spleens and was fractioned by nylon-wool column or treated by anti-IgG serum to provide a T cell rich population. A B cell population was provided by treatment with anti-Thy1.2 serum plus complement.

Cytotoxic cells were observed from day 5 and reached a peak 7 days after infection, then gradually declined. Cytotoxic activity was clearly demonstrated in T cells but little in B cells. On the contrary, protective activity was noted in B cells but was scarce in T cells. Protection was not successful when mice were treated 4 days after infection either by antiserum or by transfer of immune spleen cells. However, apparent protection was shown when both treatments were combined.

Cells from spleen of mice immunized with inactivated virus showed protective activity with no cytotoxic activity. These results suggest that B cells with cooperation of specific antibody seems to play an important role in cellular immunity in JE virus infection.

DETECTION OF JAPANESE ENCEPHALITIS ANTIBODIES BY ELISA
USING THE BIOTIN-AVIDIN

Dr. Nobuo Hashimoto
Hokkaido University, Sapporo

Biotin is a vitamin B, and avidin is a glycoprotein found in egg white which binds specifically to biotin. The biotin-avidin system was successfully adapted to measure JE antibodies in three different ELISA: labelled avidin-biotin ELISA (LAB-ELISA), biotin labelled Protein A ELISA (BLPA-ELISA) and biotin labelled antigen-sandwich ELISA (BLA-s-ELISA).

JaGAR-01 vaccine of JE virus (BIKEN), polystyrene microplate (Nunc, 96U), avidin labelled horseradish peroxidase (E.Y. Lab.) for detection of biotin and ABTS (Sigma) as a substrate were used throughout the experiment.

LAB-ELISA Biotin-labelled anti-IgM or IgG sheep IgG was prepared instead of enzyme labelled anti-immunoglobulin antibody. Anti-JE IgM or IgG antibody titers in human or swine sera were approximately 20 times higher in LAB-ELISA than in the HI test. The degree of sensitivity was essentially the same both in LAB-ELISA and ordinary ELISA, but the level of non-specific reaction of serum was four times lower in LAB-ELISA than in ordinary ELISA.

BLPA-ELISA This was applied to detect IgG antibody in the sera from different animals using one kind of reagent.

Biotin-labelled Protein A instead of anti-IgG antibody was employed. BLPA-ELISA could measure JE antibody in human, swine, horse, dog, rabbit and mouse sera, but not in cattle, sheep and rat sera. The antibody titers in BLPA-ELISA were equivalent to 2-ME resistant HI titers. The level of non-specific reaction was as low as 1:2.5 serum dilution.

BLA-s-ELISA This was also evaluated to detect JE antibodies in sera of different species of animals by using the same conjugate under the same testing condition.

After binding of JE antibody to the antigen plate, the biotin-labelled JaGAR-01 antigen was distributed into the wells of antigen-plate. The antibodies were detected by BLA-s-ELISA in all the HI positive sera from the different animal species employed (human, swine, cattle, horse, dog, rabbit, rat and mouse). The sensitivity of BLA-s-ELISA, however, was four time higher with IgM antibody than with IgG antibody, even though the titers corresponded with HI titers approximately. The level of non-specific reaction in the sera was 1:2.5 or less.

Results demonstrated that: ELISAs using biotin-avidin system had less non-specific reaction than in ordinary ELISA, and BLA-s-ELISA was superior to the other methods in measuring JE antibodies over the species and the class specificity of immunoglobulin using a single conjugate.

With: Hsi-Chi Chang and Ikuo Takashima

PATHOGENESIS OF CALIFORNIA ENCEPHALITIS IN MICE: VIRAL GENES AND PROTEINS WHICH DETERMINE VIRULENCE

Dr. Neal Nathanson
University of Pennsylvania

To analyze mechanisms of virulence in California serogroup bunyaviruses, the virulent LaCrosse/original strain was compared with the avirulent Tahyna/181-57 strain. In suckling mice, both viruses were lethal upon intracerebral (ic) injection, but differed markedly in their neuroinvasiveness following subcutaneous (sc) injection; 20 and 20,000 pfu, respectively were equivalent to 1 subcutaneous LD50. The sequential course of infection was followed after sc injection of 700 pfu; LaCrosse/original replicated in striated muscle, caused a high titer plasma viremia, invaded the CNS, and killed all mice; the same dose of avirulent Tahyna/181-57 failed to replicate in extraneural tissues, did not invade the CNS, and caused an inapparent infection. Immunofluorescent examination of peripheral and CNS tissues showed the same distinctions between virulent and avirulent viruses, and pinpointed striated muscle as the major extraneural target of virulent LaCrosse/original virus.

Preliminary studies of reassortants between these two viruses indicated that virulence co-segregates with the middle RNA segment of the viral genome, which encodes the two virion glycoproteins. This suggests that the G1 glycoprotein, which carries a domain for attachment to cellular receptors, may be responsible for the observed differences in neuroinvasiveness.

Independent evidence that the G1 protein is an important determinant of virulence comes from the selection and analysis of viral variants. Variants were selected by plaquing parent LaCrosse virus in the presence of an excess of anti-G1 neutralizing monoclonal antibody. Ten monoclonal antibodies were used to select 10 groups of variants. When tested in suckling mice, 2 of these 10 variants showed reduced virulence. Variant 22 (selected with monoclonal antibody 807-22) has been studied in detail.

V22 shows a slight reduction in ic virulence and a considerable reduction (at least 100-fold) in peripheral subcutaneous virulence. Several observations indicate that V22 is altered in fusion activity, one of the important biological functions of the virion glycoproteins. Fusion from without (FFWO) and fusion from within (FFWI) have recently been demonstrated in this laboratory for bunyaviruses, and shown to occur at pH6.3 and lower. Association of epitope 22 with fusion is indicated by several observations: (i) antibody 807-22 shows reduced binding to virions pre-treated at pH5.8; (ii) V22 fails to mediate FFWO at pH5.8; (iii) V22 has reduced ability to mediate FFWI at pH6.3 and below; (iv) V22 produces small plaques. These data suggest that the G1 protein plays a key role in the fusion activity of the virion, and that alteration in this specific glycoprotein function can be associated with reduced virulence.

With: Francisco Gonzalez-Scarano, Robert Janssen, Nadine Pobjecky, and Jon Gentsch

EPIDEMIOLOGICAL SURVEY OF ARBOVIRUS INFECTIONS IN
NORTHERN THAILAND IN THE YEAR 1982

Dr. Akira Igarashi

Institute for Tropical Medicine, Nagasaki University

From July 13 to August 17, 1982, studies were made in Chiang Mai Area, Northern Thailand, in order to obtain current information on the arbovirus infections. Virus isolation was performed from clinical materials (177 peripheral blood, 3 postmortum brains, 1 postmortem liver), 125 pools of mosquitoes (*Culex tritaeniorhynchus*, *C. gelidus*, *C. fuscocephals*), 23 swine sera without detectable HI antibodies, and 50 normal human sera, using *Aedes albopictus*, clone C6/36, cells. Japanese encephalitis (JE) virus was isolated from one of the postmortem brains of encephalitis patient dying 8 days after onset. The patient's sera showed typical primary infection of JE virus by the HI and ELISA. Eleven strains of dengue viruses were isolated from peripheral blood of patients (9 dengue hemorrhagic fever, 1 encephalitis, 1 aseptic meningitis), and typing by monoclonal antibodies showed that 8 were type 1, 2 were type 2 and 1 was type 3 virus, respectively. Two strains of unidentified flavivirus were isolated from mosquito specimens, however, they were not neutralized efficiently by anti-JE serum. Swine and normal human sera and postmortem liver specimen did not give virus isolates.

Patients' sera were examined by hemagglutination inhibition (HI) against JE and dengue, as well as by the enzyme-linked immunosorbent assay (ELISA) against JE antigen. IgM-ELISA seemed to give some help in the serodiagnosis on recent JE infection, even when HI did not give positive results.

A seroepidemiological survey was performed on healthy inhabitants in 5 locations (Sarapee, Doi Saket, Fang, and Mae Taeng in Chiang Mai Province, and Pasang in Lamphoon Province). Age distribution of anti-JE and anti-dengue HI antibodies indicated that dengue infection is more prevalent than JE in Sarapee, Doi Saket and Mae Taeng. The antibody prevalence in Fang, which belongs to Maekong Valley, was lower than those in other places belonging to Chiang Mai Valley. Prevalence of IgM-ELISA was quite different among these 5 locations, relatively high in Pasang and Mae Taeng, and low in Sarapee and Doi Saket. The results indicate that recent infection by JE virus is not uniform throughout Chiang Mai Valley at the time of our sampling. Antibody survey on several vertebrates by the HI showed that swine, horses, mules, sheep, and dogs possessed anti-JE antibodies at high rates. However, antibody prevalence was low in monkeys, ducks, and sparrows, and negative in chicken and lizards. IgM-ELISA on swine sera showed that approximately one-third of the swine population was recently infected with JE virus.

Examination of blood meals indicated that the 3 *Culex* species mentioned above showed host preference to swine, followed by bovines.

MODULATION OF WEE VIRUS IN CULEX TARSALIS AND ITS POTENTIAL
RELATIONSHIP TO TRANSMISSION IN NATURE

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Considerable epidemiological evidence has been compiled during the last 40 years to indicate that the summertime transmission cycle of western equine encephalomyelitis (WEE) virus is adversely affected by high environmental temperatures. The reason for this was unknown. However, recent field and laboratory studies suggest that this may be related, at least in part, to the adverse effect that high environmental temperatures have on the vector competence of Cx. tarsalis for WEE virus.

We reported previously that the susceptibility of Cx. tarsalis females to oral infection with WEE virus is a genetically inherited trait. Recently, we have found that the replication of WEE virus in parenterally infected Cx. tarsalis males and females is also controlled by host factors (i.e., some mosquitoes have the innate ability to modulate WEE viral titers). WEE viral high producer (HP) and low producer (LP) lines of Cx. tarsalis have been genetically selected. The mean viral titer is $10^{7.5}$ PFU/HP mosquito, as compared to $\approx 10^{1.0}$ PFU/LP mosquito, when they are inoculated intrathoracically with 100 PFU and incubated for 72 hrs at 32°C. HP females are also highly refractory to oral infection. The genetic basis of WEE viral modulation in Cx. tarsalis has been established by genetic mating experiments. The expression of the LP trait is enhanced by high extrinsic incubation temperatures.

A field population of Cx. tarsalis females was found to be polymorphic in its ability to modulate WEE viral titers after oral infection and only 33% of the infected females allowed the virus to multiply to sufficient titers to be orally transmitted to chickens. Further, WEE viral susceptibility of Cx. tarsalis females at this field site decreased as daytime ambient temperatures increased during the summer. Continued rearing of immature Cx. tarsalis at 32°C, rather than the usual 27°C, selects for a more refractory female. Thus, WEE viral transmission is adversely affected by high environmental temperatures because these temperatures select for the refractory component of the Cx. tarsalis population as well as enhance WEE viral modulation in the infected female.

EPIDEMIOLOGICAL CONSIDERATIONS OF GETAH VIRUS ISOLATES
BY OLIGONUCLEOTIDE FINGERPRINT ANALYSIS

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More than 20 strains of Getah virus which were isolated from mosquitoes, swine, and horses in Japan (1956-1981), and one prototype strain from Malaysia (AMM 2021) were analyzed by T1-RNase-resistant oligonucleotide fingerprint. The pattern of AMM 2021 is quite different from those of Japanese isolates. Although most Japanese isolates shared many large oligonucleotides in common, their patterns were not identical even among the isolates from the same place in the same year. The result indicates that Getah virus genome is easily mutable.

Several host-dependent temperature-sensitive mutants were detected from strains isolated in C6/36 cells from field-caught Culex tritaeniorhynchus by plaque formation on C6/36 cells, and their fingerprint patterns were different from the parent strain. Also some differences from the prototype strain were observed with the strain 123A, which was isolated by suckling mouse brain from C. tritaeniorhynchus pool. Host-dependent ts mutants were easily detected from 123A strain, but difficult to detect from 123M strain. Growing the 123M strain in a laboratory strain of Aedes togoi showed that the mutant viruses became easily detectable after 3 weeks of infection.

The data indicate that Getah virus mutants were generated in infected mosquitoes, and virus replication in vertebrate hosts appears to clear out some of these mutants.

ANTIGENIC ANALYSIS OF HFRS VIRUSES BY IAHA

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Various forms of hemorrhagic fever with renal syndrome (HFRS) have been reported from many parts of the world. In 1978, Korean hemorrhagic fever (KHF) agent in the lung of Apodemus agrarius coreae was first demonstrated by Lee et al. by IFA test, leading to the isolation of a HFRS virus, Hantaan virus 76-118 strain. Since 1976, laboratory type HFRS outbreaks have been reported in Japan from various medical institutions with more than 100 cases with one death. The illness was milder and laboratory rats were confirmed to be the reservoir. We have isolated two strains of HFRS virus, named SR-11 and SR-14, from lung specimens of laboratory rats associated with laboratory type HFRS outbreak in Japan through a cell culture system. So long as examined by IFA, there was antigenic difference between 76-118 and SR-11 strains, whereas virological difference has been anticipated due to their difference in clinical severity, epidemiological background and animal reservoir.

We examined the applicability of immune adherence hemagglutination (IAHA) and CF tests to the detection of HFRS antibody. VERO-E6 cells infected with HFRS virus were used as antigens in IAHA, CF and IFA tests. IAHA and CF antibody titers of HFRS-related human and rat sera correlated with those of IFA.

The etiological agent of Chinese epidemic hemorrhagic fever (EHF) seems to be closely related to Hantaan virus 76-118 strain. Comparative titrations of Chinese EHF patient sera demonstrated a clear difference between IAHA antibody titers, against 76-118 and SR-11 strains. They were negative or of lower titer against SR-11 strain. IAHA test could discriminate antibodies against two different types of HFRS virus. Similar results were obtained by checkerboard titrations using rat antisera against these strains. Furthermore, we have isolated HFRS-related virus, named TR-352, from Rattus norvegicus captured on reclaimed land in the Tokyo Bay. IAHA reactivity of TR-352 strain was very similar to that of SR-11 strain; more than that of 76-118. Antigenic difference between HFRS viruses were suggested by IAHA test. Pathogenicity in suckling mice and neutralizing activities of these three strains are now under study.

Yoshiharu Matsuura, Chiharu Morita, Sadashi Shiga, Shigeru Morikawa, Toshihiko Komatsu, Yoriyuki Akao, Akira Oya, Takashi Kitamura

SERO-EPIDEMIOLOGICAL STUDY OF HFRS IN RATTUS NORVEGICUS BY
IFA, CF AND ELISA

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For the serological diagnosis of HFRS virus infection, the IFA method has been available for use with infected cells or lung sections from infected rodents as antigen. In our sero-epidemiological study of urban rats, the CF test and ELISA were adopted in addition to the routine IFA test. This multiple assay system produced more reliable results than one system used above. In addition, the high sensitivity of ELISA was confirmed.

HFRS virus, SR-11 strain, which was isolated from infected laboratory rat lung tissue using Vero cell, was used in this study. This virus was antigenically closely related to Hantaan virus. A IFA test was performed with FITC labeled anti-rat IgG and SR-11 strain infected Vero cell. Viral antigen extracted from the infected cells for CF and ELISA were prepared as follows. The infected Vero cells were inactivated with B-propiolactone. After sonication and centrifugation at low speed, the supernatant was used as antigen. CF test was performed according to the 50% hemolysis method. ELISA was performed using a polystyrene microplate (Nunc, 96U), horseradish peroxidase labeled anti-rat IgG and ABTS.

A total of 355 serum specimens were obtained from R. n. rats captured in a reclaimed ground area of Tokyo Bay, Japan. Twenty-eight percent of the sera contained antibody to SR-11 strain in IFA test. Viral antigen was also examined in lung tissue in an antibody positive rat using Vero cell. These results indicated that an endemic focus of HFRS related virus was present among the rats of the area examined.

About 90% of the sera exhibited similar results in 3 different assay systems (IFA, CF and ELISA). Antibody titers to SR-11 strain were about 100 times higher in ELISA than in the CF and IFA tests. In addition, antibody titers in ELISA were more closely correlated to the titers obtained in CF than in IFA, $r=0.87$ ($p=0.001$) and $r=0.61$ ($p=0.001$), respectively. These results also confirmed the high sensitivity and high quantitativity of ELISA by the ordinary IFA test.

However, false positive samples, which may be caused by a non-specific reaction, were not eliminated in ELISA. Introduction of ELISA using a purified antigen and an avidin-biotin system significantly reduced the non-specific reaction.

Ikuo Takashima, Nobuo Hashimoto, Chiharu Morita, Kazuyoshi Sugiyama, Yoshihara Matsuura, Sadahi Shiga and Takasi Kitamura



The AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

1983 ANNUAL REPORT ON THE CATALOGUE OF ARTHROPOD-BORNE
AND SELECTED VERTEBRATE VIRUSES OF THE WORLD*

By
THE SUBCOMMITTEE ON ARTHROPOD-BORNE VIRUS
INFORMATION EXCHANGE

SUBCOMMITTEE ON INFORMATION EXCHANGE

I. Objectives:

The objectives of the Catalogue are to register data concerning occurrence and characteristics of newly recognized arthropod-borne viruses and other viruses of vertebrates of demonstrated or potential zoonotic importance and to disseminate this information at quarterly intervals to participating scientists in all parts of the world; to collect, reproduce, collate and distribute current information regarding registered viruses from published materials, laboratory reports and personal communications; and to prepare and distribute an annual summary of data extracted from catalogued virus registrations.

II. Materials and Methods:

Viruses are registered and information supplied on a voluntary basis, usually by scientists responsible for their isolation and identification. New registration cards, information concerning registered viruses and pertinent abstracts of published literature are distributed at quarterly intervals to participating laboratories. Abstracts of published articles dealing with catalogued viruses are reproduced by special arrangements with the editors of Biological Abstracts, Abstracts on Hygiene and the Tropical Diseases Bulletin.

* The Catalogue is supported by the Centers for Disease Control, Atlanta, Georgia.

Note: This report is not a publication and should not be used as a reference source in published bibliographies.

Distribution of Catalogue Materials: At the start of 1983, 177 mailings of Catalogue material were being made. During the year, seven addresses were dropped and four new participants were added to the mailing list. At the end of the year, 174 mailings of Catalogue material were being made, including 56 within the U.S.A. and 118 to foreign addresses. Distribution by continent was: Africa 18, Asia 22, Australasia 8, Europe 40, North America 70 and South America 16.

Abstracts and Current Information: A total of 682 abstracts or references were coded by subject matter and distributed to participants during 1983. Of this total, 282 were obtained from Biological Abstracts, 112 from Abstracts on Hygiene and the Tropical Diseases Bulletin, 284 from Medline and four from current journals, personal communications, or other sources. A total of 14,508 references or units of information have been issued since the start of the program.

Registration of New Viruses: Twenty-seven new viruses were registered during 1983. As of December 1983, the Catalogue contained 460 registered viruses. With the acceptance of 27 new virus registrations during 1983, the total number of registered viruses increased to 487 as of December 1983. The viruses registered during 1983 are listed below:

<u>Virus Name</u>	<u>Recommended Abbreviation</u>	<u>Country</u>	<u>Source</u>	<u>Antigenic Group</u>
Shokwe	SHO	S. Africa	Mosquitoes	BUN
Tibrogargan	TIB	Australia	Culicoides sp.	
Almeirim	AMR	Brazil	Phlebotomines	CGL
Cacipacore	CPC	Brazil	Bird	B
Iaco	IACO	Brazil	Mosquitoes	BUN
Mapuera	MPR	Brazil	Bat	
Munguba	MUN	Brazil	Phlebotomines	PHL
Purus	PUR	Brazil	Mosquitoes	CGL
Santarem	STM	Brazil	Rodent	
Saraca	SRA	Brazil	Phlebotomines	CGL
Sena Madureira	SM	Brazil	Lizard	TIM
Bunyip Creek	BC	Australia	Cattle	PAL
Csiro Village	CVG	Australia	Culicoides sp.	PAL
Marrakai	MAR	Australia	Culicoides sp.	PAL
Cuiaba	CUI	Brazil	Toad	MOS
Flexal	FLE	Brazil	Rodent	TCR
Itupiranga	ITU	Brazil	Mosquitoes	
Jacareacanga	JAC	Brazil	Mosquitoes	COR
Jari	JARI	Brazil	Sloth	CGL
Macaua	MCA	Brazil	Mosquitoes	BUN
Mojui dos Campos	MDC	Brazil	Bat	
Monte Dourado	MDO	Brazil	Armadillo	CGL
Para	PARA	Brazil	Sentinel mouse	
Oriximina	ORX	Brazil	Phlebotomines	PHL
Xiburema	XIB	Brazil	Mosquitoes	
Sripur	SRI	India	Phlebotomines	
Arbia	ARB	Italy	Phlebotomines	PHL

These registered viruses were isolated between 1962 and 1980. SHO was isolated in 1962, STM, CUI and SRI in 1973, CVG in 1974, FLE and PARA in 1975, TIB, BC, MAR, ITU, JAC, MCA and MDC in 1976, CPC, IACO and SM in 1977, MPR in 1979 and AMR, MUN, PUR, SRA, JARI, MDO, ORX, XIB and ARB in 1980.

SHO, BC, CVG, MCA and ARB were evaluated as Probable Arbovirus, FLE and MPR as Probably Not Arbovirus by the Subcommittee on Evaluation of Arthropod-Borne Status (SEAS)*, while all others were evaluated as Possible Arbovirus.

Only Shokwe virus has been isolated from man. In addition, Flexal virus was shown to have produced a febrile illness in a laboratory worker who was infected as a result of a laboratory accident.

Antigenic Grouping: Two additional serogroups have been formed. Firstly, the previously described Midway virus was shown to be related to, but distinct from Nyamanini virus (1). Several isolations of Midway virus were made from two species of Ornithodoros (Alectorobius) ticks parasitizing seabirds on islands of the Central Pacific and Japan. Midway and Nyamanini viruses were differentiated in CF and immunofluorescent assays (IFA) and showed one-way differences in neutralization tests. Although the first isolation of Midway virus was made in 1966, specific details of its discovery and characteristics were only published recently (1). At present, Midway virus remains unregistered.

Secondly, Kismayo virus has been shown to share an HI relationship with Bhanja virus (2). The two viruses do not appear to cross-react by CF, agar gel precipitin and neutralization tests. The HI relationship between Kismayo and Bhanja viruses also has been reproduced in another laboratory (3). Both viruses now comprise the Bhanja serogroup in which Bhanja virus is the sole registered member. Kismayo virus was isolated in 1974 from Rhipicephalus pulchellus ticks collected in Somalia (2).

Previously published studies have demonstrated that Ibaraki virus is reciprocally related to EHD virus serotypes 1 (New Jersey strain) and 2 (Alberta strain) by agar gel precipitin tests and IFA (4). Neutralization tests have indicated that Ibaraki virus is more closely related to EHD virus, serotype 2 than to EHD virus, serotype 1. As a result of these findings, Ibaraki virus now has been incorporated into the EHD serogroup as an additional member.

A considerable number of newly registered viruses have been incorporated into previously established serogroups. Please see the recently issued virus registration cards for details. New members have been placed in the Mossuril (one new member), Tacaribe (one), Corriparta (one), Changuinola (five), Bunyamwera (three), Phlebotomus fever (three), Palyam (three), Timbo (one) and B (one) serogroups.

* A.J. Main (Chairman), T.H.G. Aitken, E.W. Cupp, D.B. Francys, D.J. Gubler, J.L. Hardy and D.M. McLean.

Barmah Forest virus has been removed from the Turlock serogroup and provisionally it is being listed with the alphaviruses. Barmah Forest virus will be listed in Table 5 but its listing in Table 1 will be without a designation for its "Taxonomic Status" or its "Antigenic Group". That was done to emphasize the somewhat unsettled nature of its status. Recent molecular studies have clearly indicated that the present Barmah Forest virus is an alphavirus (5). In addition, a hemagglutinin of Barmah Forest virus has cross-reacted by HI with several members of serogroup A (6). It is puzzling that Barmah Forest virus originally was linked to the Turlock serogroup through its CF relationship with Umbre virus.

It is possible that the original "Barmah Forest virus" was actually a mixture of a bunyavirus and an alphavirus. Perhaps, after several passages the bunyavirus was lost. Several other speculative explanations have been proposed as well.

Recent serologic studies have demonstrated that Khasan virus is related to Crimean hemorrhagic fever (CHF) virus (7). The latter virus is a member of the CHF-CON serogroup. Antigenic relationships between the two viruses were detected both by CF and neutralization tests.

Taxonomic Status of Registered Viruses: Reported changes in the taxonomic classification of registered arboviruses are of a provisional nature; and in some instances, new taxonomic placements are based on very slight evidence.

By the use of thin-section electron microscopy, Le Dantec virus was shown to possess typical rhabdovirus morphology (8). Prior to these observations, Le Dantec virus was listed as an antigenically ungrouped registered virus. In light of its morphologic characteristics, its antigenic status was reexamined. Thus far, Le Dantec virus has not been found to be antigenically related to any of the other established rhabdoviruses.

Five other recently registered viruses have been shown to be rhabdoviruses. These viruses were registered in 1983, and three of the five were antigenically ungrouped. Sripur virus was isolated from phlebotomines collected in India, Tibrogargan virus was isolated from Culicoides sp. insects collected in Australia, while Xiburema virus was recovered from mosquitoes collected in Brazil. The remaining two, Cuiaba and Sena Madureira viruses were recovered from a toad and a lizard respectively. Cuiaba virus has been placed in the Mossuril serogroup while Sena Madureira virus was antigenically linked to the Timbo serogroup.

The taxonomic status of Chobar Gorge virus has been changed from unclassified to provisional placement in the Orbivirus genus. Information available on the registration card for Chobar Gorge virus indicates that the morphology and morphogenesis for this virus are typical of an orbivirus.

Synopsis of Information in Catalogue: This synopsis has been compiled primarily to provide a short review of the viruses included in the Catalogue. The following tabulations are designed to draw together groups of viruses showing certain characteristics in common, listing viruses according to their known taxonomic status and by their demonstrated serological relationships, and where appropriate, by principal arthropod vector. Isolations from arthropod and animal hosts, continental distribution, involvement in human

disease, and arbovirus status are indicated. Information is also given concerning the recommended level of practice and containment assigned to registered viruses and the basis for assignment to a level. Most of this information was published previously by the Subcommittee on Arbovirus Laboratory Safety (SALS)* (9). Thirty-eight registered viruses listed in Tables 5 through 33 have not been rated by SALS yet. Appendices I and II, following Table 38, will provide a description of recommended levels and an explanation of symbols used to define basis. Other tables summarize the taxonomic status of registered viruses; the antigenic groups comprising a given taxon to which registered viruses have been assigned; the numbers of registered viruses assigned to presently recognized antigenic groups; chronology and areas of isolations of registered viruses; continental distribution by groups; numbers of viruses recovered from naturally infected arthropods and vertebrates; association with human disease; and evaluation of arthropod-borne status of members in various serogroups.

Table 1. Alphabetical and taxonomic listing of registered viruses: Table 1 presents an alphabetical listing of the 487 viruses registered in the Catalogue as of December 1983. An official or provisional taxonomic classification is shown for each registered virus. If taxonomic status is not indicated, the registered virus is presently unclassified. Also, a recommended abbreviation is given for each virus, which has been formulated according to the guidelines established by the American Committee on Arthropod-Borne Viruses (10). All too often, abbreviations are employed in publications which are of the author's choosing and which do not conform to the recommended abbreviations. Their use is confusing, contrary to established guidelines, and erodes a portion of the effort of the Arbovirus Information Exchange program. All arbovirologists who plan to employ abbreviations in print should make every effort to use the recommended abbreviations.

Antigenic groups to which viruses have been assigned also are shown in this table. If no antigenic group is given, the virus is ungrouped and indicates that it has not been demonstrated to be serologically related to any other known arbovirus.

Table 2. Antigenic groups of registered viruses: The originally described antigenic groups of arboviruses were designated by letters, A, B, and C; but in present practice, the first discovered virus of a newly recognized serogroup lends its name to the antigenic cluster. Before a virus can be assigned to any antigenic group, it must be shown to be serologically related to, but clearly distinguishable from a previously isolated virus.

Table 2 lists the serogroups comprising the various taxa to which registered viruses have been assigned. Fifty-nine antigenic groups have been designated for viruses registered in the Catalogue. That includes the previously established rabies serogroup. There are several instances in which

* Composition at time of publication: W.F. Scherer (Chairman, deceased), G.A. Eddy, T.P. Monath, T.E. Walton, and J.M. Richardson (ad hoc member).

Present composition: T.E. Walton (Chairman), J.M. Dalrymple, R. Endris, J.L. Hardy, T.P. Monath, and J.M. Richardson (ad hoc member).

only a single virus is shown in an antigenic group. That is so because one or more antigenic relatives of that virus have not been registered.

It is also noted that the Bunyavirus genus represents the old Bunyamwera Supergroup to which several additional serogroups have been added. The most recent additions are the Anopheles B and Turlock serogroups. The Bunyamwera Supergroup originally was formulated to reflect low-level but reproducible intergroup relationships usually by complement-fixation and/or hemagglutination-inhibition reactions. In a somewhat analogous situation, the nairoviruses consist of six distinct serogroups which share low-level intergroup relationships among themselves. Registered viruses belonging in the Bunyamwera Supergroup constitute slightly more than one-fourth of all registered viruses.

Table 3. Initial isolations by decade and country of origin: Table 3 lists the initial isolation of specific registered viruses by the decade of discovery and according to the continent or subcontinent and country in which each was first discovered. Because of the large number of virus names involved, abbreviations are employed. These abbreviations and the associated complete names of the respective viruses may be found in Table 1.

Table 4. Initial isolation of viruses by continent, country, and chronological period: Similar data were utilized in Tables 3 and 4, though they were subjected to slightly different analyses and were presented in a different format. Periods or locations which show high numbers of virus isolation undoubtedly reflect the net effect of a number of contributing factors such as the change in emphasis of field programs from a search for viruses causing specific diseases to a systematic search for viruses, new or known, in their natural ecological niche in a given geographical area, refinements in isolation and identification techniques, improved communication between arbovirus laboratories, and more rapid dissemination of new information, as well as the presence in a given area of an arbovirus laboratory with highly active and effective field programs.

Tables 5 through 33 list registered viruses by taxon and, within taxon, by serogroup, with information regarding isolations from arthropod vectors and vertebrates, and geographic (by continent) distribution based on virus isolation. Data also are presented regarding production of disease in man in nature or by laboratory infection, evaluation of arbovirus status, and proved or provisional taxonomic status. These tables now show the biohazard level assigned to each registered virus, and the basis for assignment to a level. Where possible, sets of viruses were grouped additionally according to their actual or suspected principal arthropod vector.

The data presented in these tables clearly illustrate the salient features characteristic of each set or subset of viruses. Thus, the reader is urged to carefully examine the tables for information that may be of specific interest, or that will provide an overview of the general characteristics of a given group of viruses.

Table 5. Alphaviruses: Alphaviruses clearly are mosquito associated, although a few have been isolated from other arthropods. About one-half of the alphaviruses are associated with birds, while some of them, particularly those of the VEE complex, are associated with rodents.

Eleven alphaviruses have been isolated from man while 12 have been implicated in causing human disease either by infections acquired in nature or in the laboratory. At least seven of these 12 alphaviruses have been responsible for epidemics: chikungunya, eastern equine encephalitis, Mayaro, o'nyong-nyong, Ross River, Venezuelan equine encephalitis, and western equine encephalitis. All of the 12 alphaviruses are rated as Arbovirus (11 viruses) or Probable Arbovirus (one virus).

Barmah Forest virus provisionally has been listed with the other viruses in serogroup A. Pending a final resolution of its status, it will probably remain in this serogroup.

Sindbis virus has been recovered from the organs of insectivorous bats collected in Zimbabwe. Cabassou, chikungunya and WEE viruses represent the other alphaviruses which have been isolated from bats.

Tables 6, 7, and 8. Flaviviruses: Of the 63 registered flaviviruses, 47% have been placed in the mosquito-associated category (Table 6), 23% are considered to be tick-borne (Table 7), and 30% are categorized as not being associated with a proven arthropod vector (Table 8).

Cacipacore virus, registered during 1983, has been added to the list of flaviviruses not associated with a vector (Table 8). The virus was recovered from a wild bird captured in Brazil. Cross-neutralization studies have revealed that Cacipacore virus is not closely related to any of the known flaviviruses (11). In this investigation, the antigenic relationships of all known flaviviruses were reevaluated in a single continuous study.

Twenty-six of the 30 registered flaviviruses which are mosquito-associated (Table 6) are rated as Probable Arbovirus or Arbovirus. The tick-borne flaviviruses (Table 7) contain four registered viruses, Absettarov, Hanzalova, Hypr and Kumlinge, which are very closely related or indistinguishable by conventional serological techniques, though they are said to be clearly differentiated on the basis of clinical, epidemiological, and ecological markers from RSSE and other members of the same complex.

Eighteen of 30 (60%) mosquito-borne flaviviruses and nine of 15 (60%) tick-borne flaviviruses have been implicated in the production of human disease, either through infections acquired in nature or in the laboratory. By contrast, only four of 19 (21%) flaviviruses not associated with a vector have been implicated in the production of human disease.

With the exception of two members (Israel turkey meningoencephalitis and Koutango viruses), none of the rest of the registered flaviviruses placed in the "no arthropod vector demonstrated" category are rated above Possible Arbovirus by SEAS. Seven members are rated as Probably Not or Not Arbovirus. Most of the flaviviruses listed in Table 8 have been isolated from rodents or bats. Israel turkey meningoencephalitis virus has been isolated from domestic turkeys, Cacipacore virus from a wild bird, and Aroa virus from a sentinel hamster. Only Dakar bat and Negishi viruses have been isolated from man; that has been the sole source of recovery for Negishi virus.

Tables 9 through 16. Bunyaviruses, Family Bunyaviridae: Sixteen antigenic sets of viruses plus Kaeng Khoi virus (SBU) comprise the bunyaviruses. A total of 123 registered viruses have been placed within the Bunyavirus genus.

Table 9. Anopheles A and Anopheles B serogroup viruses: Members of the Anopheles A serogroup have been isolated either from Anopheline or Culicine mosquitoes, or both. Of the five members of this serogroup, only Tacaiuma virus has been reported to cause a febrile illness in man. In addition, this virus has been isolated from man and from a sentinel monkey. Members of this serogroup and of the ANB serogroup appear to be localized. These viruses have been found on only a single continent.

Viruses of the Anopheles B serogroup have been isolated only from mosquitoes collected in South America. Neither virus has been associated with infections in man.

Table 10. Bunyamwera serogroup viruses: All members of the Bunyamwera serogroup have been isolated from culicine or anopheline mosquitoes or both. In addition, Lokern and Main Drain viruses have been isolated from Culicoides insects. Maguari virus has been recovered from livestock, Anhembi, Germiston, Kairi, Macua and Shokwe viruses from rodents, and Lokern, Main Drain and Tensaw viruses from lagomorphs. Kairi virus also was recovered from a monkey, while Macua virus was isolated from a bird.

Bunyamwera, Germiston, Ilesha, Shokwe, and Wyeomyia viruses have been isolated from man. Except for Shokwe virus, those viruses plus Calovo virus have been shown to be associated with human disease, either through infections acquired in nature or in the laboratory, or both.

Fifteen of the 22 viruses registered in the Bunyamwera serogroup have been rated as Arbovirus or Probable Arbovirus. None are rated below Possible Arbovirus.

Members have been found most frequently in North America (eight viruses), South America (eight viruses) and Africa (five viruses). Thus far, only one virus has been recovered in Asia, two in Europe and none in Australasia.

Three new members have been added to this serogroup. They include Iaco, Macaua, and Shokwe viruses. All three viruses appear to be mosquito-associated. Iaco and Macaua viruses were isolated in Brazil, while Shokwe virus was recovered initially in South Africa and subsequently in Senegal, the Ivory Coast, and Kenya.

Table 11. Bwamba serogroup and serogroup C viruses: Both Bwamba and Pongola viruses (Bwamba serogroup) are mosquito-associated, and Bwamba virus has been isolated from man. Bwamba virus has been reported to produce a febrile illness in man as a result of infections acquired in nature. Thus far, these two viruses have been found in Africa only.

The Group C viruses have been closely associated with mosquito vectors and small animals, particularly rodents. Ten of the twelve viruses have been isolated from sentinel animals, mostly mice. Only Gumbo Limbo and Vinces viruses have not been isolated from man and, with the exception of those two

viruses, all other members have been associated with cases of human febrile illness. In addition, Apeu and Oriboca viruses have been reported to infect man as a result of laboratory mishaps. Ten of these viruses have been classified as Arbovirus and two as Probable Arbovirus.

Table 12. California and Capim serogroup viruses: All the California group viruses are associated with mosquito vectors and four members have been recovered from naturally infected rodents. La Crosse, Guaroa, and Tahyna viruses have been isolated from man and, along with California encephalitis and Inkoo viruses, have been associated with disease as a result of infections acquired in nature. In addition, Jamestown Canyon and snowshoe hare viruses recently have been serologically associated with disease in man. Only Inkoo and Tahyna viruses have been isolated outside the continents of North and South America. On the basis of virus isolation, the geographic distribution of Tahyna now includes Asia as well as Africa and Europe.

Viruses of the Capim serogroup are associated with mosquito vectors, and four of the members have been isolated from rodents. None of these eight viruses have been associated with disease in man. Capim group members have been recovered only in North and South America. Six of the eight Capim serogroup viruses have been rated as Arbovirus (four viruses) or Probable Arbovirus (two viruses).

Table 13. Gamboa, Guama and Koongol serogroup viruses: In addition to Gamboa virus, the serogroup contains Pueblo Viejo and San Juan viruses. All virus members have been isolated exclusively from Aedeomyia squamipennis mosquitoes. The viruses appear to have a limited geographic distribution, and they have not been implicated in human infections.

Guama serogroup viruses have been found only in the western hemisphere. Catu and Guama viruses have been isolated from man and have been associated with disease in man as a result of infections acquired in nature. Nine of the 12 Guama group viruses have been rated as Arbovirus or Probable Arbovirus. Viruses of this serogroup clearly are mosquito-associated and the majority of them appear to be associated with rodents. Ten viruses have been isolated from sentinel animals, primarily mice.

Both Koongol group viruses were isolated in Australia and very little is known about them.

Table 14. Minatitlan, Olifantsvlei and Patois serogroup viruses: The Minatitlan serogroup now contains two registered members. In addition to Minatitlan virus, the group also includes Palestina virus. Several isolations of Palestina virus have been made from Culex sp. mosquitoes collected in Ecuador, and from sentinel hamsters. Minatitlan virus was isolated from a sentinel hamster exposed near Minatitlan, Mexico. Little is known concerning its role in nature.

The Olifantsvlei group consists of three members, and all three were isolated in Africa from mosquitoes. Information on the properties of these viruses has not been readily available.

Viruses of the Patois group now have been isolated in North and South America, and most appear to be associated with mosquito vectors and some with

rodent hosts. Babahoyo, Patois, Shark River, and Zegla viruses also were isolated from sentinel hamsters.

Table 15. Simbu serogroup viruses: Almost equal numbers of Simbu group viruses have been isolated from Culicoides insects and from mosquitoes. None have been recovered from rodents. Eight Simbu serogroup viruses have been isolated from livestock. These include Sabo, Sango, Shamonda and Shuni viruses (Nigeria), Douglas and Peaton viruses (Australia), Akabane virus (Japan and Australia) and Sathuperi virus (India and Africa). Oropouche and Shuni viruses are the only members that have been isolated from man. Oropouche virus has caused frequent large outbreaks of disease among the human population in Brazil.

Simbu group viruses have a wide distribution. Approximately 50% have been found in Africa or Africa and Asia, while others have been isolated in Asia or Asia and Australasia and North or South America.

Table 16. Tete and Turlock serogroups and unassigned (SBU) viruses: All Tete group viruses have been recovered from birds; only two of them (Bahig and Matruh viruses) have been recovered from an arthropod vector (ixodid ticks). None of these viruses have been associated with human infections.

All viruses of the Turlock serogroup are associated with mosquito vectors. In addition, Turlock and Umbre viruses appear to be associated with birds. Turlock virus has been found in both North and South America. All the other members have been found in a single continent (Africa, Asia, and Europe). Barmah Forest virus has been deleted from the listing of those viruses which belong in the Turlock serogroup. It is highly unlikely that it will ever again become a member in the Turlock serogroup.

Only Kaeng Khoi virus remains as a serologically unassigned bunyavirus. Kaeng Khoi virus was isolated from bats, sentinel mice and rats, and cimicid bugs.

Table 17. Phlebotomus fever serogroup viruses: At present, the PHL antigenic group comprises the Phlebovirus genus within the Bunyaviridae family. Sicilian sandfly fever virus is the type virus for this genus.

The majority of the group members are associated with phlebotomine flies, while eight of these viruses have been isolated from man or have been implicated in the production of disease in man.

Three additional viruses have been added to the PHL serogroup. Two of the new members, Munguba and Oriximina viruses, were isolated from phlebotomines collected in Brazil. The third new member, Arbia virus, was recovered from phlebotomines collected in Italy. Arbia virus has been evaluated as Probable Arbovirus by SEAS while the other two have been designated as Possible Arbovirus.

Rift Valley fever virus causes serious and extensive disease in domestic animals such as sheep and cattle, and may cause disease in veterinary personnel, field and laboratory workers, as well as herdsmen who handle infected animals. Previous serological studies have indicated that Zinga virus is closely related or identical to Rift Valley fever virus.

Consequently Zinga virus has been placed in the Phlebotomus fever serogroup although it may be just another strain of RVF virus. Previously it was listed as an antigenically ungrouped virus.

Table 18. Tick-borne serogroups other than serogroup B viruses.

Nairoviruses: Members of the six antigenic groups shown in Tables 18 and 19 constitute the Nairovirus genus in the Bunyaviridae family. CHF-Congo virus was designated the type virus for this genus. Furthermore, reproducible intergroup antigenic relationships have been demonstrated for the six sets of viruses. Only members of the CHF-Congo and NSD serogroups have been associated with the production of disease in man.

Both Congo and Crimean hemorrhagic fever viruses are registered in the Catalogue. It must be reiterated that the agent of Crimean hemorrhagic fever (CHF) is antigenically indistinguishable from Congo virus. The CHF virus has been implicated in more than two thousand cases of human disease in the USSR. Congo virus also has been associated with the production of disease in man, either as a result of infections acquired in nature or in the laboratory. Thus far, Hazara virus has not been known to be involved in infections of man, and little is known of this antigenic relative of CHF-Congo virus. Because of its recently described antigenic relationship to CHF virus (7), Khasan virus has been included as a new member in the CHF-CON serogroup. Khasan virus was originally isolated in 1971 from ixodid ticks collected in the USSR.

Members of the DGK serogroup have not been isolated from vertebrate hosts, nor from arthropod vectors other than ticks. The majority of the viruses appear to be associated with argasid ticks. These viruses have been found in Africa, Asia and Australasia.

Only Hughes virus of the Hughes serogroup has been isolated from birds. It has been found in both North and South America while Soldado virus has been isolated in Africa, Asia and Australasia. All Hughes serogroup members have been associated with argasid ticks. A new antigenic member of the Hughes serogroup has been described (12). This virus has been called Puffin Island, and it has not been registered as of this moment.

Table 19. Tick-borne serogroups other than serogroup B viruses.

Nairoviruses: Nairobi sheep disease virus is an important cause of veterinary disease, while both Dugbe and Ganjam viruses have been isolated repeatedly from ticks taken off of domestic animals. Dugbe and Ganjam viruses have caused febrile illnesses in man. In the case of NSD virus, one infection in man resulted in a febrile illness, while three others resulted in subclinical serologic conversions. Pending further clarification of antigenic relationships, SIRACA considers Ganjam virus to be a variety of NSD virus.

Both Qalyub group viruses were found only in Africa, and both have been isolated from ticks. In addition, Bandia virus has been isolated from rodents.

Except for Avalon virus, members of the Sakhalin antigenic set were isolated only from ixodid ticks. Avalon virus also was recovered from a bird. Sakhalin serogroup viruses are distributed in Asia (PMR,SAK), Australasia (TAG), Europe (CM), and North America (AVA,SAK). Antigenic studies indicate that Avalon and Paramushir viruses are strains of the same virus.

Table 20. Tick-borne serogroups other than serogroup B viruses: At present, Uukuniemi serogroup viruses constitute the Uukuvirus genus in the Bunyaviridae family. Other serogroups listed in that table remain provisionally classified as bunyavirus-like, family Orthomyxoviridae (THO serogroup), or are unclassified.

Except for Uukuniemi virus, all members of the Uukuniemi serogroup have been isolated only from ticks. Uukuniemi virus also has been recovered from both rodents and birds. Two of the viruses in this serogroup were found in Asia while the other three were discovered in Europe. Hemagglutination-inhibition antibodies to Uukuniemi virus have been detected in the sera of human beings residing in Europe.

At present, Bhanja virus is the sole registered virus member of the new Bhanja serogroup. Kismayo virus is the unregistered member of the serogroup, and has been demonstrated to share an antigenic relationship with Bhanja virus (2). Bhanja virus has been isolated from man and has been implicated in a laboratory-acquired human infection.

Two of the Kaisodi group viruses were isolated from ticks collected in Asia while the third was isolated in North America. None of these viruses have been found to infect man. The previous two Annual Reports have referred to unpublished studies which had indicated that the RNA species and polypeptides of Silverwater virus resembled those of uukuviruses. Additional confirming or clarifying information is still not available.

The Upolu serogroup consists of Upolu and Aransas Bay viruses. Both viruses were isolated only from argasid ticks. Neither virus has been associated with infections in man. One virus has been found in Australia (UPO), and the other in North America (AB).

Thogoto virus has been isolated from man and has been involved in the production of disease in man. An unregistered antigenic relative of Thogoto virus has been isolated in Sicily. Molecular analysis of a Thogoto group virus has indicated that its virion RNA species and structural polypeptides resemble those of members of the family Orthomyxoviridae.

Nyamanini and Midway viruses now constitute the Nyamanini serogroup (1). Midway virus presently is unregistered. Nyamanini virus was isolated from argasid ticks and birds. It has not been associated with the production of disease in man.

Quaranfil virus has been isolated from both man and rodents, and has been associated with the production of disease in man as the result of infections acquired in nature. Preliminary molecular studies conducted with Quaranfil virus indicated that this virus may resemble viruses of the family Orthomyxoviridae. At this point, further verification is required. Little is known concerning the behavior of Johnston Atoll virus in nature.

Table 21. Minor antigenic groups of viruses: All the viruses listed in this table are members of minor antigenic groups, and are classified taxonomically as bunyavirus-like members of the Family Bunyaviridae. Viruses of the Matariya and Nyando serogroups were provisionally classified as bunyavirus-like relatively recently. Most virus members of these minor serogroups have been primarily associated with mosquito vectors.

Bakau group viruses have been recovered only in Asia. Bakau virus has been isolated from both mosquitoes and ticks, and also rodents. Additional information concerning these viruses is not available.

Thus far, all four viruses of the Mapputta group have been found only in Australia. Maprik virus is rated as a Probable Arbovirus while the other three virus members are classified as Possible Arbovirus.

All three Matariya group viruses have been recovered from birds collected in Africa. Nothing is known concerning their possible vector association.

Nyando virus has been isolated from man and from mosquitoes collected in Africa. The disease which resulted from that human infection was characterized as a febrile illness.

Table 22. Tick-borne serogroups other than serogroup B viruses: While the viruses listed in Table 22 also are tick-borne agents, they differ taxonomically from those in Tables 18-21 in that they have been classified as orbiviruses in the family Reoviridae. The orbiviruses are relatively resistant to lipid solvents, are inactivated at an acid pH, and possess multiple segments of a double-stranded RNA genome. It is likely that members of the genus Orbivirus, and that the criteria used to define this genus, will be reevaluated in the near future.

Only Colorado tick fever virus of the CTF serogroup and Kemerovo virus of the KEM serogroup have produced disease in man and have been isolated from man.

Members of the Kemerovo group are widely distributed with at least one virus being found in each of the listed continents. Kemerovo virus has been found in both Africa and Asia while Wad Medani virus has been discovered in Africa, Asia and North America.

Tables 23, 24. Minor antigenic groups of viruses: Members of these minor antigenic groups have been characterized and taxonomically classified as orbiviruses.

Several of the viruses in these minor antigenic groups are important in causing disease in large animals. BLU virus causes disease in both wild and domestic ruminants; AHS virus in mules, donkeys and horses; EHD virus in deer and Ibaraki virus in cattle. Both BLU and AHS viruses have a wide geographic distribution.

Changuinola virus is the only member from these minor antigenic groups which has been isolated from man, and has been reported to produce disease in man. Of the present twelve serogroup members, only Irituia, Jari, and Monte Dourado viruses have not been isolated from an arthropod. All others, including Changuinola virus, appear to be associated with phlebotomine insects. Five newly registered viruses have been placed in the Changuinola serogroup. These are Almeirim, Jari, Monte Dourado, Purus and Saraca viruses. All five were recovered in Brazil and only three of these viruses were isolated from arthropods. Almeirim and Saraca viruses were obtained from phlebotomines, while Purus virus was recovered from Psorophora sp. mosqui-

toes. As for the other two viruses, Jari virus was isolated from a sloth, and Monte Dourado virus was recovered from an armadillo.

The recently registered Jacareacanga virus has been placed in the Corriparta serogroup. This virus was isolated from Culex (Mel.) sp. mosquitoes collected in Brazil. Neither Jacareacanga virus nor the five new additions to the Changuinola serogroup have been known to produce disease in man.

Virus members of the Corriparta, Eubenangee, and Palyam serogroups appear to be primarily mosquito-associated, while members of the Wallal and Warrego serogroup appear to be associated with Culicoides insects. Vector associations appear to be less clear for Eubenangee virus of the EUB serogroup, and for Warrego virus of the WAR serogroup.

Bunyip Creek, Csiro Village and Marrakai viruses are new members of the Palyam serogroup. All three viruses were recovered in Australia, from both Culicoides spp. insects and cattle. Bunyip Creek and Csiro Village viruses were judged as Probable Arboviruses by SEAS.

Table 25. Minor antigenic groups of viruses: Members of the serogroups listed in this table and in Table 26 possess a "bullet-shaped" morphology and are classified as members of the family Rhabdoviridae. Table 25 contains the Hart Park group viruses, a Kwatta group virus, an expanded Mossuril group consisting of eight members and a rabies serogroup consisting of two rabies-related viruses.

All of the present Hart Park serogroup members are associated with a mosquito vector and two of the viruses (Hart Park and Flanders) have been isolated from birds. None of these viruses have been associated with disease in man. Thus far, their distribution includes only North and South America.

The Kwatta virus was isolated only once from mosquitoes collected in Surinan. The antigenic relative of Kwatta virus remains unregistered. This unregistered virus was recovered from a bird collected in Brazil.

Cuiaba virus represents a new member of the Mossuril serogroup. The virus was isolated from the blood of a toad captured in Brazil. Three of the members of this serogroup have not been isolated from arthropods. These include Cuiaba, Kern Canyon, and Marco viruses. Kern Canyon virus has been rated as Probably not Arbovirus by SEAS. Previous studies have demonstrated that Kern Canyon virus could be propagated in an Aedes dorsalis cell culture line.

The rabies serogroup consists of kotonkan virus and Lagos bat virus. Kotonkan virus was isolated from Culiocides spp. collected in Nigeria. It was rated as Probable Arbovirus by SEAS. Lagos bat virus has been isolated from bats only.

Table 26. Minor antigenic groups of viruses: All three viruses of the Sawgrass serogroup were isolated from ticks collected in North America. All viruses of the Timbo serogroup, including the new Sena Madureira virus, were isolated from lizards, and none of these viruses ever were isolated from arthropods.

Three VSV group viruses have been isolated from phlebotomine flies, and three others have been recovered from mosquitoes. A fourth, VS-Indiana virus has been isolated from both types of vectors. Keuraliba, Piry and VS-Alagoas viruses have not been recovered from arthropods. Of the serogroups listed in this and the preceding table, only members of the VSV serogroup have been shown to infect man. Chandipura, Piry, VS-Indiana and VS-New Jersey viruses have been isolated from man. These viruses, plus VS-Alagoas virus, have been found to produce disease in man during infections acquired in nature or in the laboratory. Both VS-Indiana and VS-New Jersey viruses readily infect livestock, while Cocal virus has been recovered from a horse and VS-Alagoas virus from a mule.

Table 27. Minor antigenic groups of viruses: These antigenic groups consist of members which are taxonomically unclassified.

Both Boteke group viruses have been isolated in Africa only. Zingilamo virus was recovered from a bird and Boteke virus was isolated from mosquitoes. Previously published studies have indicated that Zingilamo virus resembles viruses of the family Togaviridae. Pending further information, both viruses of this serogroup will be listed as unclassified in this Annual Report.

Malakal and Puchong viruses (Malakal serogroup) have been isolated from mosquitoes only. Malakal virus was recovered from mosquitoes collected in Africa, while Puchong virus was found in Asia.

Both Marburg and Ebola viruses have caused human disease as a result of infections acquired in nature and have been associated with laboratory-acquired infections. Ebola virus was found to possess a single-stranded RNA which was noninfectious upon extraction. Recent evidence indicates that there might be different serotypes of Ebola virus (13).

The two viruses of the Tanjong Rabok serogroup have been isolated in Malaysia and neither has been associated with a vector. Telok Forest virus was isolated from a wild monkey and Tanjong Rabok virus from a sentinel monkey.

Table 28. Tacaribe group viruses: Tacaribe group viruses are serologically related to lymphocytic choriomeningitis virus, and they are classified taxonomically in the Arenavirus genus. They are primarily rodent viruses, and there is little or no evidence which suggests that they are associated with an arthropod vector in nature. SEAS has judged all members to be Not Arbovirus.

Three members of this group have been implicated in the production of severe, often fatal, human disease. These include Junin (Argentine hemorrhagic fever), Machupo (Bolivian hemorrhagic fever), and Lassa (Lassa disease). In addition to causing clinically frank laboratory-acquired infections, Junin virus also has been reported to cause subclinical laboratory-acquired infections. A subclinical seroconversion to Tacaribe virus has been documented in a laboratory worker handling large quantities of Tacaribe virus. In addition, Pichinde virus has produced subclinical infections in laboratory workers. Finally, the newly registered Flexal virus has produced a febrile illness in a laboratory worker following a laboratory accident. Flexal virus was recovered from rodents trapped in Brazil.

Table 29. Ungrouped mosquito-associated viruses: The viruses in this table are serologically ungrouped, though they have been clustered together on the basis of their association with a mosquito vector and placed into subsets according to their taxonomic classification. Tataguine virus has been isolated from man, and has been reported to produce disease in man during the course of infections acquired in nature.

Bocas virus was formerly included in the CAL serogroup until it was demonstrated that it was identical to or closely related to mouse hepatitis virus.

Of the ungrouped orbiviruses associated with mosquito vectors, two viruses have been found in Africa (LEB, ORU), two in Australasia (JAP, PR) and three in North America (IERI, LLS, UMA). Llano Seco virus is antigenically related to Umatilla virus but its relationship to other established orbivirus groups has not been resolved. Thus it and Umatilla virus have been placed with the ungrouped viruses pending a clarification of their antigenic relationships.

Orungo virus has caused human disease as a result of infections acquired in nature; and Lebombo virus, or a closely related virus, has been isolated from human plasma, although it has not been associated with the production of disease in man thus far. The isolation of Orungo virus from an adult male mosquito has been reported (14). These findings would indicate that Orungo virus undergoes transovarial transmission in nature.

Nodamura virus was isolated from wild-caught mosquitoes in Japan, and it has been demonstrated to produce disease in moths and honey bees. It also has been shown that it replicates in mosquitoes and is experimentally transmitted by mosquitoes. Nodamura virus is now the type species for a previously established genus within the family Nodaviridae. Both the family and the genus Nodavirus were established by ICTV during meetings held at the time of the Fifth International Congress of Virology in 1981.

Cotia virus, a poxvirus, has been reported to produce disease in man.

Table 30. Ungrouped mosquito-associated viruses: These serologically ungrouped viruses have been associated with mosquito vectors, and the majority of them remain taxonomically unclassified. Only Gomoka and Para viruses have been recovered from another source in addition to mosquitoes. Two isolates of Gomoka virus were obtained from birds collected in the Central African Republic. Para virus was isolated from sentinel mice.

Xiburema, Itupiranga, and Para viruses are new additions to the listings in Table 30. Xiburema virus was shown to possess typical "bullet-shaped" morphology following electron microscopic examination of this virus. All three viruses were isolated from mosquitoes, while Para virus also was isolated from sentinel mice.

Table 31. Ungrouped tick-, Culicoides-, or Phlebotomus-associated viruses: Slightly less than one-half of the listed viruses are taxonomically unclassified. Except for bovine ephemeral fever, Inhangapi, Ngaingan, Sripur, and Tibrogargan viruses, all other agents listed in Table 31 are associated with tick vectors. Inhangapi and Sripur viruses, both classified as

rhabdoviruses, are associated with phlebotomine flies. Ngaingan and Tibrogargan viruses are associated with Culicoides insects. Bovine ephemeral fever virus has been isolated from both mosquitoes and Culicoides insects. Only Issyk-Kul and Wanowrie viruses in Table 31 have been isolated from man. Wanowrie virus has not been associated with human disease either as a result of a laboratory accident or as a result of an infection acquired in nature.

Chobar Gorge virus provisionally has been placed in the Orbivirus genus as a result of information originally present on the registration card which previously had been overlooked.

Tettnang virus was shown to cross-react in CF tests with mouse hepatitis virus (MHV). Recently, three isolates of Tettnang virus were compared to prototype strains of MHV by neutralization tests (15). The relationship of Tettnang virus to MHV was confirmed; however, the precise relationship of the Tettnang virus isolates to MHV strains remained unclear because of the past passage history of the Tettnang isolates. Further, the question of whether the Tettnang isolates were, in fact, arthropod-borne remains unanswered.

Formerly, the Bunyaviridae study group of the ICTV had classified Dhori virus as a member of the then newly defined Nairovirus genus. Subsequently, molecular studies indicated that Dhori virus possessed seven virion polypeptides and seven single-stranded RNA segments which were comparable to those of viruses of the family Orthomyxoviridae.

Sripur and Tibrogargan viruses represent new additions to the listings in Table 31. Both viruses have been found to possess rhabdovirus morphology. Sripur virus was recovered in India, while Tibrogargan virus was isolated in Australia.

Issyk-Kul and Keterah viruses have been shown to be closely related or identical by complement-fixation. Cross-neutralization testing will determine whether they are the same virus or antigenic relatives. Pending the results of that testing, these viruses are being listed in the ungrouped category. Issyk-Kul virus has been isolated from the blood of man infected in nature on at least 3 occasions. The infections were classified as febrile illnesses.

Tables 32, 33. Ungrouped viruses, no arthropod vector known: None of the listed viruses have been isolated from an arthropod vector, and only Almpiwir virus is rated higher than Possible Arbovirus. Several of the viruses are rated Probably not Arbovirus or Not Arbovirus. More than 50% have been isolated from rodents or birds. Of the viruses listed in these two tables, only Bangui, Hantaan and Le Dantec viruses were isolated from man. All three viruses have been associated with the production of human disease as a result of infections acquired in nature. In addition, Le Dantec virus recently has been observed to possess rhabdovirus morphology (8).

Approximately thirty-nine percent of the viruses listed in Tables 32 and 33 have been assigned a provisional taxonomic classification. Hantaan virus has been included with those ungrouped viruses in Table 32 which provisionally have been classified as "bunyavirus-like". The taxonomic classification of Hantaan virus was based on unequivocal electron microscopic evidence presented in three previous publications. These publications showed that Hantaan virus particles had morphological features identical to those of viruses in the family Bunyaviridae.

Hantaan virus is the etiologic agent of Korean hemorrhagic fever (KHF), and either is responsible for or is antigenically closely related to the agent(s) responsible for clinically similar diseases in the U.S.S.R., Japan, Manchuria, and Eastern and Northern Europe. More than 10,000 cases have occurred in Korea alone since the disease was first recognized in that country in 1951.

Simian hemorrhagic fever virus has produced severe disease in rhesus monkeys imported from India. Other monkey species developed disease following contact with the recently imported sick rhesus monkeys. Simian hemorrhagic fever virus has been classified as Not Arbovirus by SEAS. This virus has been shown to resemble the flaviviruses morphologically and structurally, although an antigenic relationship has not been demonstrated.

A majority of the unclassified viruses shown in Table 33 appear to be bird-associated viruses. Five viruses have been recovered from rodents, four from bats, and two others from other vertebrates. Fifteen of these viruses were recovered in Africa and Asia. The remaining five viruses were found in South America.

Three newly registered viruses appear in this listing. Mapuera, Mojui dos Campos, and Santarem viruses were recovered in Brazil. Mapuera and Mojui dos Campos viruses were isolated from bats while Santarem virus was recovered from a rodent. Mojui dos Campos and Santarem viruses were obtained from blood of their respective hosts while Mapuera virus was obtained from the salivary glands of a bat. Neutralizing and CF antibodies to Santarem virus were detected in various rodents, but not in man or other animals.

Table 34 gives continental distribution of viruses in different antigenic groups on the basis of virus isolation. Most of the registered viruses are very limited in their distribution. Approximately 86% have been isolated on a single continent only, while 20 or 4.1% have been found on three or more continents. The largest number of viruses have been isolated in South America and Africa.

Table 35 shows the number of viruses, according to antigenic group, which have been isolated from various classes of arthropods. About 50% have been recovered from mosquitoes, 20% from ticks, and 17% from all other classes. One hundred and three registered viruses have never been recovered from any arthropod vector. The largest number of viruses which have been isolated from any arthropod, have been recovered from a single class only (350 of 384, 91.1%).

Table 36 presents a similar type of analysis in terms of virus isolations from various classes of vertebrates. Man and rodents have provided the largest number of virus isolations. Most of the viruses isolated from vertebrates have been recovered from a single class only (193 of 274, 70.4%).

Table 37 lists the viruses in each antigenic group which cause disease in man. Approximately 23% of all registered viruses have been associated with human disease, either as a result of infections acquired in nature or from laboratory accidents, or both. Members of serogroups A and B and those in the Bunyamwera Supergroup constitute 44% of all registered viruses. These viruses also account for 65% of the instances in which registered viruses are associated with disease production in man.

An analysis of the SEAS ratings for all registered viruses is presented in Table 38, and it shows that 258 registrations (53%) are rated as Possible Arbovirus. Clearly, additional data are required if we are to have a more precise rating of the arthropod-borne status of these viruses. Sufficient data are available for about 47% of all registered viruses so that 41% are rated Probable Arbovirus or Arbovirus, while 6% are rated Probably not Arbovirus or Not Arbovirus.

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Table 1

ALPHABETICAL AND TAXONOMIC LISTING OF 487 VIRUSES REGISTERED
AS OF 31 DEC. 1983 WITH RECOMMENDED ABBREVIATIONS
AND ANTIGENIC GROUPINGS

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
ABRAS	ABR	Bunyaviridae	<u>Bunyavirus</u>	PAT
ABSETTAROV	ABS	Togaviridae	<u>Flavivirus</u>	B
ABU HAMMAD	AH	Bunyaviridae	<u>Nairovirus</u>	DGK
ACADO	ACD	Reoviridae	<u>Orbivirus</u>	COR
ACARA	ACA	Bunyaviridae	<u>Bunyavirus</u>	CAP
AFRICAN HORSESICKNESS	AHS	Reoviridae	<u>Orbivirus</u>	AHS
AFRICAN SWINE FEVER	ASF	Iridoviridae		
AGUACATE	AGU	Bunyaviridae	<u>Phlebovirus</u>	PHL
AGUA PRETA	AP	Herpesviridae		
AINO	AINO	Bunyaviridae	<u>Bunyavirus</u>	SIM
AKABANE	AKA	Bunyaviridae	<u>Bunyavirus</u>	SIM
ALENQUER	ALE	Bunyaviridae	<u>Phlebovirus</u>	PHL
ALFUY	ALF	Togaviridae	<u>Flavivirus</u>	B
ALMEIRIM	AMR	Reoviridae	<u>Orbivirus</u>	CGL
ALMPIWAR	ALM	Rhabdoviridae		
ALTAMIRA	ALT	Reoviridae	<u>Orbivirus</u>	CGL
AMAPARI	AMA	Arenaviridae	<u>Arenavirus</u>	TCR
ANANINDEUA	ANU	Bunyaviridae	<u>Bunyavirus</u>	GMA
ANHANGA	ANH	Bunyaviridae	<u>Phlebovirus</u>	PHL
ANHEMBI	AMB	Bunyaviridae	<u>Bunyavirus</u>	BUN
ANOPHELES A	ANA	Bunyaviridae	<u>Bunyavirus</u>	ANA

NAME	ABBR.	<u>TAXONOMIC STATUS</u>		ANTI-GENIC GROUP
		FAMILY	GENUS	
ANOPHELES B	ANB	Bunyaviridae	<u>Bunyavirus</u>	ANB
APEU	APEU	Bunyaviridae	<u>Bunyavirus</u>	C
APOI	APOI	Togaviridae	<u>Flavivirus</u>	B
ARAGUARI	ARA			
ARANSAS BAY	AB	Bunyaviridae	Bunyavirus-like	UPO
ARBIA	ARB	Bunyaviridae	<u>Phlebovirus</u>	PHL
ARIDE	ARI			
ARKONAM	ARK			
AROA	AROA	Togaviridae	<u>Flavivirus</u>	B
ARUAC	ARU	Rhabdoviridae		
ARUMOWOT	AMT	Bunyaviridae	<u>Phlebovirus</u>	PHL
AURA	AURA	Togaviridae	<u>Alphavirus</u>	A
AVALON	AVA	Bunyaviridae	<u>Nairovirus</u>	SAK
BABAHOYO	BAB	Bunyaviridae	<u>Bunyavirus</u>	PAT
BAGAZA	BAG	Togaviridae	<u>Flavivirus</u>	B
BAHIG	BAH	Bunyaviridae	<u>Bunyavirus</u>	TETE
BAKAU	BAK	Bunyaviridae	Bunyavirus-like	BAK
BAKU	BAKU	Reoviridae	<u>Orbivirus</u>	KEM
BANDIA	BDA	Bunyaviridae	<u>Nairovirus</u>	QYB
BANGORAN	BGN	Rhabdoviridae		MOS
BANGUI	BGI	Bunyaviridae	Bunyavirus-like	
BANZI	BAN	Togaviridae	<u>Flavivirus</u>	B
BARMAH FOREST	BF			
BARUR	BAR	Rhabdoviridae		MOS
BATAI	BAT	Bunyaviridae	<u>Bunyavirus</u>	BUN

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
BATAMA	BMA	Bunyaviridae	<u>Bunyavirus</u>	TETE
BATKEN	BKN			
BAULINE	BAU	Reoviridae	<u>Orbivirus</u>	KEM
BEBARU	BEB	Togaviridae	<u>Alphavirus</u>	A
BELEM	BLM			
BELMONT	BEL	Bunyaviridae	Bunyavirus-like	
BENEVIDES	BVS	Bunyaviridae	<u>Bunyavirus</u>	CAP
BENFICA	BEN	Bunyaviridae	<u>Bunyavirus</u>	CAP
BERTIOGA	BER	Bunyaviridae	<u>Bunyavirus</u>	GMA
BHANJA	BHA	Bunyaviridae	Bunyavirus-like	BHA
BIMBO	BBO			
BIMITI	BIM	Bunyaviridae	<u>Bunyavirus</u>	GMA
BIRAO	BIR	Bunyaviridae	<u>Bunyavirus</u>	BUN
BLUETONGUE	BLU	Reoviridae	<u>Orbivirus</u>	BLU
BOBAYA	BOB	Bunyaviridae	Bunyavirus-like	
BOBIA	BIA	Bunyaviridae	<u>Bunyavirus</u>	OLI
BOCAS	BOC	Coronaviridae	<u>Coronavirus</u>	
BORACEIA	BOR	Bunyaviridae	<u>Bunyavirus</u>	ANB
BOTAMBI	BOT	Bunyaviridae	<u>Bunyavirus</u>	OLI
BOTEKE	BTK			BTK
BOUBOUI	BOU	Togaviridae	<u>Flavivirus</u>	B
BOVINE EPHEMERAL FEVER	BEF	Rhabdoviridae		
BUENAVENTURA	BUE	Bunyaviridae	<u>Phlebovirus</u>	PHL
BUJARU	BUJ	Bunyaviridae	<u>Phlebovirus</u>	PHL

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
BUNYAMWERA	BUN	Bunyaviridae	<u>Bunyavirus</u>	BUN
BUNYIP CREEK	BC	Reoviridae	<u>Orbivirus</u>	PAL
BURG EL ARAB	BEA	Bunyaviridae	Bunyavirus-like	MTY
BUSHBUSH	BSB	Bunyaviridae	<u>Bunyavirus</u>	CAP
BUSSUQUARA	BSQ	Togaviridae	<u>Flavivirus</u>	B
BUTTONWILLOW	BUT	Bunyaviridae	<u>Bunyavirus</u>	SIM
BWAMBA	BWA	Bunyaviridae	<u>Bunyavirus</u>	BWA
CABASSOU	CAB	Togaviridae	<u>Alphavirus</u>	A
CACAO	CAC	Bunyaviridae	<u>Phlebovirus</u>	PHL
CACHE VALLEY	CV	Bunyaviridae	<u>Bunyavirus</u>	BUN
CACIPACORE	CPC	Togaviridae	<u>Flavivirus</u>	B
CAIMITO	CAI	Bunyaviridae	<u>Phlebovirus</u>	PHL
CALIFORNIA ENC.	CE	Bunyaviridae	<u>Bunyavirus</u>	CAL
CALOVO	CVO	Bunyaviridae	<u>Bunyavirus</u>	BUN
CANANEIA	CNA	Bunyaviridae	<u>Bunyavirus</u>	GMA
CANDIRU	CDU	Bunyaviridae	<u>Phlebovirus</u>	PHL
CANINDE	CAN	Reoviridae	<u>Orbivirus</u>	CGL
CAPE WRATH	CW	Reoviridae	<u>Orbivirus</u>	KEM
CAPIM	CAP	Bunyaviridae	<u>Bunyavirus</u>	CAP
CARAPARU	CAR	Bunyaviridae	<u>Bunyavirus</u>	C
CAREY ISLAND	CI	Togaviridae	<u>Flavivirus</u>	B
CATU	CATU	Bunyaviridae	<u>Bunyavirus</u>	GMA
CHACO	CHO	Rhabdoviridae		TIM
CHAGRES	CHG	Bunyaviridae	<u>Phlebovirus</u>	PHL
CHANDIPURA	CHP	Rhabdoviridae	<u>Vesiculovirus</u>	VSV

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
CHANGUINOLA	CGL	Reoviridae	<u>Orbivirus</u>	CGL
CHARLEVILLE	CHV	Rhabdoviridae		MOS
CHENUDA	CNU	Reoviridae	<u>Orbivirus</u>	KEM
CHIKUNGUNYA	CHIK	Togaviridae	<u>Alphavirus</u>	A
CHILIBRE	CHI	Bunyaviridae	<u>Phlebovirus</u>	PHL
CHIM	CHIM			
CHOBAR GORGE	CG	Reoviridae	<u>Orbivirus</u>	
CLO MOR	CM	Bunyaviridae	<u>Nairovirus</u>	SAK
COCAL	COC	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
COLORADO TICK FEVER	CTF	Reoviridae	<u>Orbivirus</u>	CTF
CONGO	CON	Bunyaviridae	<u>Nairovirus</u>	CHF-CON
CONNECTICUT	CNT	Rhabdoviridae		SAW
CORRIPARTA	COR	Reoviridae	<u>Orbivirus</u>	COR
COTIA	COT	Poxviridae		
COWBONE RIDGE	CR	Togaviridae	<u>Flavivirus</u>	B
CRIMEAN HEM. FEVER	CHF	Bunyaviridae	<u>Nairovirus</u>	CHF-CON
CSIRO VILLAGE	CVG	Reoviridae	<u>Orbivirus</u>	PAL
CUIABA	CUI	Rhabdoviridae		MOS
D'AGUILAR	DAG	Reoviridae	<u>Orbivirus</u>	PAL
DAKAR BAT	DB	Togaviridae	<u>Flavivirus</u>	B
DENGUE-1	DEN-1	Togaviridae	<u>Flavivirus</u>	B
DENGUE-2	DEN-2	Togaviridae	<u>Flavivirus</u>	B
DENGUE-3	DEN-3	Togaviridae	<u>Flavivirus</u>	B
DENGUE-4	DEN-4	Togaviridae	<u>Flavivirus</u>	B
DERA GHAZI KHAN	DGK	Bunyaviridae	<u>Nairovirus</u>	DGK
DHORI	DHO	Orthomyxoviridae		

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
DOUGLAS	DOU	Bunyaviridae	<u>Bunyavirus</u>	SIM
DUGBE	DUG	Bunyaviridae	<u>Nairovirus</u>	NSD
EAST. EQUINE ENC.	EEE	Togaviridae	<u>Alphavirus</u>	A
EBOLA	EBO			MBG
EDGE HILL	EH	Togaviridae	<u>Flavivirus</u>	B
ENSEADA	ENS	Bunyaviridae	Bunyavirus-like	
ENTEBBE BAT	ENT	Togaviridae	<u>Flavivirus</u>	B
EP. HEM. DIS.	EHD	Reoviridae	<u>Orbivirus</u>	EHD
EUBENANGEE	EUB	Reoviridae	<u>Orbivirus</u>	EUB
EVERGLADES	EVE	Togaviridae	<u>Alphavirus</u>	A
EYACH	EYA	Reoviridae	<u>Orbivirus</u>	CTF
FLANDERS	FLA	Rhabdoviridae		HP
FLEXAL	FLE	Arenaviridae	<u>Arenavirus</u>	TCR
FORT MORGAN	FM	Togaviridae	<u>Alphavirus</u>	A
FRIJOLES	FRI	Bunyaviridae	<u>Phlebovirus</u>	PHL
GAMBOA	GAM	Bunyaviridae	<u>Bunyavirus</u>	GAM
GAN GAN	GG	Bunyaviridae	Bunyavirus-like	MAP
GANJAM	GAN	Bunyaviridae	<u>Nairovirus</u>	NSD
GARBA	GAR	Bunyaviridae	Bunyavirus-like	MTY
GERMISTON	GER	Bunyaviridae	<u>Bunyavirus</u>	BUN
GETAH	GET	Togaviridae	<u>Alphavirus</u>	A
GOMOKA	GOM			
GORDIL	GOR	Bunyaviridae	<u>Phlebovirus</u>	PHL
GOSSAS	GOS			
GRAND ARBAUD	GA	Bunyaviridae	<u>Uukuvirus</u>	UUK
GRAY LODGE	GLO	Rhabdoviridae		

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
GREAT ISLAND	GI	Reoviridae	<u>Orbivirus</u>	KEM
GUAJARA	GJA	Bunyaviridae	<u>Bunyavirus</u>	CAP
GUAMA	GMA	Bunyaviridae	<u>Bunyavirus</u>	GMA
GUARATUBA	GTB	Bunyaviridae	<u>Bunyavirus</u>	GMA
GUAROA	GRO	Bunyaviridae	<u>Bunyavirus</u>	CAL
GUMBO LIMBO	GL	Bunyaviridae	<u>Bunyavirus</u>	C
GURUPI	GUR	Reoviridae	<u>Orbivirus</u>	CGL
HANTAAN	HTN	Bunyaviridae	Bunyavirus-like	
HANZALOVA	HAN	Togaviridae	<u>Flavivirus</u>	B
HART PARK	HP	Rhabdoviridae		HP
HAZARA	HAZ	Bunyaviridae	<u>Nairovirus</u>	CHF-CON
HIGHLANDS J	HJ	Togaviridae	<u>Alphavirus</u>	A
HUACHO	HUA	Reoviridae	<u>Orbivirus</u>	KEM
HUGHES	HUG	Bunyaviridae	<u>Nairovirus</u>	HUG
HYPR	HYPR	Togaviridae	<u>Flavivirus</u>	B
IACO	IACO	Bunyaviridae	<u>Bunyavirus</u>	BUN
IBARAKI	IBA	Reoviridae	<u>Orbivirus</u>	EHD
ICOARACI	ICO	Bunyaviridae	<u>Phlebovirus</u>	PHL
IERI	IERI	Reoviridae	<u>Orbivirus</u>	
IFE	IFE	Reoviridae	<u>Orbivirus</u>	
ILESHA	ILE	Bunyaviridae	<u>Bunyavirus</u>	BUN
ILHEUS	ILH	Togaviridae	<u>Flavivirus</u>	B
INGWAVUMA	ING	Bunyaviridae	<u>Bunyavirus</u>	SIM
INHANGAPI	INH	Rhabdoviridae		
ININI	INI	Bunyaviridae	<u>Bunyavirus</u>	SIM

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
INKOO	INK	Bunyaviridae	<u>Bunyavirus</u>	CAL
IPPY	IPPY			
IRITUIA	IRI	Reoviridae	<u>Orbivirus</u>	CGL
ISFAHAN	ISF	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
ISRAEL TURKEY MEN.	IT	Togaviridae	<u>Flavivirus</u>	B
ISSYK-KUL	IK			
ITAITUBA	ITA	Bunyaviridae	<u>Phlebovirus</u>	PHL
ITAPORANGA	ITP	Bunyaviridae	<u>Phlebovirus</u>	PHL
ITAQUI	ITQ	Bunyaviridae	<u>Bunyavirus</u>	C
ITIMIRIM	ITI	Bunyaviridae	<u>bunyavirus</u>	GMA
ITUPIRANGA	ITU			
JACAREACANGA	JAC	Reoviridae	<u>Orbivirus</u>	COR
JAMANXI	JAM	Reoviridae	<u>Orbivirus</u>	CGL
JAMESTOWN CANYON	JC	Bunyaviridae	<u>Bunyavirus</u>	CAL
JAPANAUT	JAP	Reoviridae	<u>Orbivirus</u>	
JAPANESE ENC.	JE	Togaviridae	<u>Flavivirus</u>	B
JARI	JARI	Reoviridae	<u>Orbivirus</u>	CGL
JERRY SLOUGH	JS	Bunyaviridae	<u>Bunyavirus</u>	CAL
JOHNSTON ATOLL	JA			QRF
JOINJAKAKA	JOI	Rhabdoviridae		
JUAN DIAZ	JD	Bunyaviridae	<u>Bunyavirus</u>	CAP
JUGRA	JUG	Togaviridae	<u>Flavivirus</u>	B
JUNIN	JUN	Arenaviridae	<u>Arenavirus</u>	TCR
JURONA	JUR	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
JUTIAPA	JUT	Togaviridae	<u>Flavivirus</u>	B

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
KADAM	KAD	Togaviridae	<u>Flavivirus</u>	B
KAENG KHOI	KK	Bunyaviridae	<u>Bunyavirus</u>	SBU
KAIKALUR	KAI	Bunyaviridae	<u>Bunyavirus</u>	SIM
KAIRI	KRI	Bunyaviridae	<u>Bunyavirus</u>	BUN
KAISODI	KSO	Bunyaviridae	Bunyavirus-like	KSO
KAMESE	KAM	Rhabdoviridae		MOS
KAMMAVANPETTAI	KMP			
KANNAMANGALAM	KAN			
KAO SHUAN	KS	Bunyaviridae	<u>Nairovirus</u>	DGK
KARIMABAD	KAR	Bunyaviridae	<u>Phlebovirus</u>	PHL
KARSHI	KSI	Togaviridae	<u>Flavivirus</u>	B
KASBA	KAS	Reoviridae	<u>Orbivirus</u>	PAL
KEMEROVO	KEM	Reoviridae	<u>Orbivirus</u>	KEM
KERN CANYON	KC	Rhabdoviridae		MOS
KETAPANG	KET	Bunyaviridae	Bunyavirus-like	BAK
KETERAH	KTR			
KEURALIBA	KEU	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
KEYSTONE	KEY	Bunyaviridae	<u>Bunyavirus</u>	CAL
KHASAN	KHA	Bunyaviridae	Nairovirus	CHF-CON
KLAMATH	KLA	Rhabdoviridae		
KOKOBERA	KOK	Togaviridae	<u>Flavivirus</u>	B
KOLONGO	KOL			
KOONGOL	KOO	Bunyaviridae	<u>Bunyavirus</u>	KOO
KOTONKAN	KOT	Rhabdoviridae	<u>Lyssavirus</u>	RABIES
KOUTANGO	KOU	Togaviridae	<u>Flavivirus</u>	B
KOWANYAMA	KOW	Bunyaviridae	Bunyavirus-like	

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
KUMLINGE	KUM	Togaviridae	<u>Flavivirus</u>	B
KUNJIN	KUN	Togaviridae	<u>Flavivirus</u>	B
KUNUNURRA	KNA	Rhabdoviridae		
KWATTA	KWA	Rhabdoviridae		KWA
KYASANUR FOR. DIS.	KFD	Togaviridae	<u>Flavivirus</u>	B
KYZYLAGACH	KYZ	Togaviridae	<u>Alphavirus</u>	A
LA CROSSE	LAC	Bunyaviridae	<u>Bunyavirus</u>	CAL
LAGOS BAT	LB	Rhabdoviridae	<u>Lyssavirus</u>	RABIES
LA JOYA	LJ	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
LANDJIA	LJA			
LANGAT	LGT	Togaviridae	<u>Flavivirus</u>	B
LANJAN	LJN	Bunyaviridae	Bunyavirus-like	KSO
LAS MALOYAS	LM	Bunyaviridae	<u>Bunyavirus</u>	ANA
LASSA	LAS	Arenaviridae	<u>Arenavirus</u>	TCR
LATINO	LAT	Arenaviridae	<u>Arenavirus</u>	TCR
LEBOMBO	LEB	Reoviridae	<u>Orbivirus</u>	
LE DANTEC	LD	Rhabdoviridae		
LEDNICE	LED	Bunyaviridae	<u>Bunyavirus</u>	TUR
LIPOVNIK	LIP	Reoviridae	<u>Orbivirus</u>	KEM
LLANO SECO	LLS	Reoviridae	<u>Orbivirus</u>	*
LOKERN	LOK	Bunyaviridae	<u>Bunyavirus</u>	BUN

* Llano Seco virus is related to Umatilla virus. Its relationship to other orbivirus serogroups has not been determined.

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
LONE STAR	LS	Bunyaviridae	Bunyavirus-like	
LOUPING ILL	LI	Togaviridae	<u>Flavivirus</u>	B
LUKUNI	LUK	Bunyaviridae	<u>Bunyavirus</u>	ANA
MACAUA	MCA	Bunyaviridae	<u>Bunyavirus</u>	BUN
MACHUPO	MAC	Arenaviridae	<u>Arenavirus</u>	TCR
MADRID	MAD	Bunyaviridae	<u>Bunyavirus</u>	C
MAGUARI	MAG	Bunyaviridae	<u>Bunyavirus</u>	BUN
MAHOGANY HAMMOCK	MH	Bunyaviridae	<u>Bunyavirus</u>	GMA
MAIN DRAIN	MD	Bunyaviridae	<u>Bunyavirus</u>	BUN
MALAKAL	MAL			MAL
MANAWA	MWA	Bunyaviridae	<u>Uukuvirus</u>	UUK
MANZANILLA	MAN	Bunyaviridae	<u>Bunyavirus</u>	SIM
MAPPUTTA	MAP	Bunyaviridae	Bunyavirus-like	MAP
MAPRIK	MPK	Bunyaviridae	Bunyavirus-like	MAP
MAPUERA	MPR			
MARBURG	MBG			MBG
MARCO	MCO	Rhabdoviridae		MOS
MARITUBA	MTB	Bunyaviridae	<u>Bunyavirus</u>	C
MARRAKAI	MAR	Reoviridae	<u>Orbivirus</u>	PAL
MATARIYA	MTY	Bunyaviridae	Bunyavirus-like	MTY
MATRUH	MTR	Bunyaviridae	<u>Bunyavirus</u>	TETE
MATUCARE	MAT			
MAYARO	MAY	Togaviridae	<u>Alphavirus</u>	A
MELAO	MEL	Bunyaviridae	<u>Bunyavirus</u>	CAL
MERMET	MER	Bunyaviridae	<u>Bunyavirus</u>	SIM
MIDDELBURG	MID	Togaviridae	<u>Alphavirus</u>	A

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
MINATITLAN	MNT	Bunyaviridae	<u>Bunyavirus</u>	MNT
MINNAL	MIN			
MIRIM	MIR	Bunyaviridae	<u>Bunyavirus</u>	GMA
MITCHELL RIVER	MR	Reoviridae	<u>Orbivirus</u>	WAR
MODOC	MOD	Togaviridae	<u>Flavivirus</u>	B
MOJU	MOJU	Bunyaviridae	<u>Bunyavirus</u>	GMA
MOJUI DOS CAMPOS	MDC			
MONO LAKE	ML	Reoviridae	<u>Orbivirus</u>	KEM
MONT. MYOTIS LEUK.	MML	Togaviridae	<u>Flavivirus</u>	B
MONTE DOURADO	MDO	Reoviridae	<u>Orbivirus</u>	CGL
MORICHE	MOR	Bunyaviridae	<u>Bunyavirus</u>	CAP
MOSQUEIRO	MQO	Rhabdoviridae		HP
MOSSURIL	MOS	Rhabdoviridae		MOS
MOUNT ELGON BAT	MEB	Rhabdoviridae		
M'POKO	MPO	Bunyaviridae	<u>Bunyavirus</u>	TUR
MUCAMBO	MUC	Togaviridae	<u>Alphavirus</u>	A
MUNGUBA	MUN	Bunyaviridae	<u>Phlebovirus</u>	PHL
MURRAY VALLEY ENC.	MVE	Togaviridae	<u>Flavivirus</u>	B
MURUTUCU	MUR	Bunyaviridae	<u>Bunyavirus</u>	C
NAIROBI SHEEP DIS.	NSD	Bunyaviridae	<u>Nairovirus</u>	NSD
NARANJAL	NJL	Togaviridae	<u>Flavivirus</u>	B
NARIVA	NAR	Paramyxoviridae	<u>Paramyxovirus</u>	
NAVARRO	NAV	Rhabdoviridae		
NDUMU	NDU	Togaviridae	<u>Alphavirus</u>	A
NEGISHI	NEG	Togaviridae	<u>Flavivirus</u>	B

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
NEPUYO	NEP	Bunyaviridae	<u>Bunyavirus</u>	C
NEW MINTO	NM	Rhabdoviridae		SAW
NGAINGAN	NGA			
NIQUE	NIQ	Bunyaviridae	<u>Phlebovirus</u>	PHL
NKOLBISSON	NKO			
NODAMURA	NOD	Nodaviridae	<u>Nodavirus</u>	
NOLA	NOLA	Bunyaviridae	<u>Bunyavirus</u>	SIM
NORTHWAY	NOR	Bunyaviridae	<u>Bunyavirus</u>	BUN
NTAYA	NTA	Togaviridae	<u>Flavivirus</u>	B
NUGGET	NUG	Reoviridae	<u>Orbivirus</u>	KEM
NYAMANINI	NYM			NYM
NYANDO	NDO	Bunyaviridae	Bunyavirus-like	NDO
OKHOTSKIY	OKH	Reoviridae	<u>Orbivirus</u>	KEM
OKOLA	OKO			
OLIFANTSVLEI	OLI	Bunyaviridae	<u>Bunyavirus</u>	OLI
OMSK HEM. FEVER	OMSK	Togaviridae	<u>Flavivirus</u>	B
O'NYONG-NYONG	ONN	Togaviridae	<u>Alphavirus</u>	A
ORIBOCA	ORI	Bunyaviridae	<u>Bunyavirus</u>	C
ORIXIMINA	ORX	Bunyaviridae	<u>Phlebovirus</u>	PHL
OROPOUCHE	ORO	Bunyaviridae	<u>Bunyavirus</u>	SIM
ORUNGO	ORU	Reoviridae	<u>Orbivirus</u>	
OSSA	OSSA	Bunyaviridae	<u>Bunyavirus</u>	C
OUANGO	OUA			
OUBANGUI	OUB	Poxviridae		
OUREM	OUR	Reoviridae	<u>Orbivirus</u>	CGL

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
PACORA	PCA	Bunyaviridae	Bunyavirus-like	
PACUI	PAC	Bunyaviridae	<u>Phlebovirus</u>	PHL
PAHAYOKEE	PAH	Bunyaviridae	<u>Bunyavirus</u>	PAT
PALESTINA	PLS	Bunyaviridae	<u>Bunyavirus</u>	MNT
PALYAM	PAL	Reoviridae	<u>Orbivirus</u>	PAL
PARA	PARA			
PARAMUSHIR	PMR	Bunyaviridae	<u>Nairovirus</u>	SAK
PARANA	PAR	Arenaviridae	<u>Arenavirus</u>	TCR
PAROO RIVER	PR	Reoviridae	<u>Orbivirus</u>	
PATA	PATA	Reoviridae	<u>Orbivirus</u>	EUB
PATHUM THANI	PTH	Bunyaviridae	<u>Nairovirus</u>	DGK
PATOIS	PAT	Bunyaviridae	<u>Bunyavirus</u>	PAT
PEATON	PEA	Bunyaviridae	<u>Bunyavirus</u>	SIM
PHNOM-PENH BAT	PPB	Togaviridae	<u>Flavivirus</u>	B
PICHINDE	PIC	Arenaviridae	<u>Arenavirus</u>	TCR
PICOLA	PIA			
PIRY	PIRY	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
PIXUNA	PIX	Togaviridae	<u>Alphavirus</u>	A
PLAYAS	PLA	Bunyaviridae	<u>Bunyavirus</u>	BUN
PONGOLA	PGA	Bunyaviridae	<u>Bunyavirus</u>	BWA
PONTEVES	PTV	Bunyaviridae	<u>Uukuvirus</u>	UUK
POWASSAN	POW	Togaviridae	<u>Flavivirus</u>	B
PRETORIA	PRE	Bunyaviridae	<u>Nairovirus</u>	DGK
PUCHONG	PUC			MAL
PUEBLO VIEJO	PV	Bunyaviridae	<u>Bunyavirus</u>	GAM

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
PUNTA SALINAS	PS	Bunyaviridae	<u>Nairovirus</u>	HUG
PUNTA TORO	PT	Bunyaviridae	<u>Phlebovirus</u>	PHL
PURUS	PUR	Reoviridae	<u>Orbivirus</u>	CGL
QALYUB	QYB	Bunyaviridae	<u>Nairovirus</u>	QYB
QUARANFIL	QRF			QRF
RAZDAN	RAZ	Bunyaviridae	Bunyavirus-like	
RESTAN	RES	Bunyaviridae	<u>Bunyavirus</u>	C
RIFT VALLEY FEVER	RVF	Bunyaviridae	<u>Phlebovirus</u>	PHL
RIO BRAVO	RB	Togaviridae	<u>Flavivirus</u>	B
RIO GRANDE	RG	Bunyaviridae	<u>Phlebovirus</u>	PHL
ROCHAMBEAU	RBU			
ROCIO	ROC	Togaviridae	<u>Flavivirus</u>	B
ROSS RIVER	RR	Togaviridae	<u>Alphavirus</u>	A
ROYAL FARM	RF	Togaviridae	<u>Flavivirus</u>	B
RUSS. SPR. SUM. ENC.	RSSE	Togaviridae	<u>Flavivirus</u>	B
SABO	SABO	Bunyaviridae	<u>Bunyavirus</u>	SIM
SABOYA	SAB	Togaviridae	<u>Flavivirus</u>	B
SAGIYAMA	SAG	Togaviridae	<u>Alphavirus</u>	A
SAINT-FLORIS	SAF	Bunyaviridae	<u>Phlebovirus</u>	PHL
SAKHALIN	SAK	Bunyaviridae	<u>Nairovirus</u>	SAK
SAKPA	SPA			
SALANGA	SGA	Poxviridae		
SALEHABAD	SAL	Bunyaviridae	<u>Phlebovirus</u>	PHL
SAL VIEJA	SV	Togaviridae	<u>Flavivirus</u>	B
SAN ANGELO	SA	Bunyaviridae	<u>Bunyavirus</u>	CAL

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
SANDFLY F. (NAPLES)	SFN	Bunyaviridae	<u>Phlebovirus</u>	PHL
SANDFLY F. (SICILIAN)	SFS	Bunyaviridae	<u>Phlebovirus</u>	PHL
SANDJIMBA	SJA			
SANGO	SAN	Bunyaviridae	<u>Bunyavirus</u>	SIM
SAN JUAN	SJ	Bunyaviridae	<u>Bunyavirus</u>	GAM
SAN PERLITA	SP	Togaviridae	<u>Flavivirus</u>	B
SANTAREM	STM			
SANTA ROSA	SAR	Bunyaviridae	<u>Bunyavirus</u>	BUN
SARACA	SRA	Reoviridae	<u>Orbivirus</u>	CGL
SATHUPERI	SAT	Bunyaviridae	<u>Bunyavirus</u>	SIM
SAUMAREZ REEF	SRE	Togaviridae	<u>Flavivirus</u>	B
SAWGRASS	SAW	Rhabdoviridae		SAW
SEBOKELE	SEB			
SELETAR	SEL	Reoviridae	<u>Orbivirus</u>	KEM
SEMBALAM	SEM			
SEMLIKI FOREST	SF	Togaviridae	<u>Alphavirus</u>	A
SENA MADUREIRA	SM	Rhabdoviridae		TIM
SEPIK	SEP	Togaviridae	<u>Flavivirus</u>	B
SERRA DO NAVIO	SDN	Bunyaviridae	<u>Bunyavirus</u>	CAL
SHAMONDA	SHA	Bunyaviridae	<u>Bunyavirus</u>	SIM
SHARK RIVER	SR	Bunyaviridae	<u>Bunyavirus</u>	PAT
SHOKWE	SHO	Bunyaviridae	<u>Bunyavirus</u>	BUN
SHUNI	SHU	Bunyaviridae	<u>Bunyavirus</u>	SIM
SILVERWATER	SIL	Bunyaviridae	Bunyavirus-like	KSO
SIMBU	SIM	Bunyaviridae	<u>Bunyavirus</u>	SIM

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
SIMIAN HEM. FEVER	SHF	Togaviridae		
SINDBIS	SIN	Togaviridae	<u>Alphavirus</u>	A
SIXGUN CITY	SC	Reoviridae	<u>Orbivirus</u>	KEM
SLOVAKIA	SLO			
SNOWSHOE HARE	SSH	Bunyaviridae	<u>Bunyavirus</u>	CAL
SOKULUK	SOK	Togaviridae	<u>Flavivirus</u>	B
SOLDADO	SOL	Bunyaviridae	<u>Nairovirus</u>	HUG
SOROROCA	SOR	Bunyaviridae	<u>Bunyavirus</u>	BUN
SPONDWENI	SPO	Togaviridae	<u>Flavivirus</u>	B
SRIPUR	SRI	Rhabdoviridae		
ST. LOUIS ENC.	SLE	Togaviridae	<u>Flavivirus</u>	B
STRATFORD	STR	Togaviridae	<u>Flavivirus</u>	B
SUNDAY CANYON	SCA	Bunyaviridae	Bunyavirus-like	
TACAIUMA	TCM	Bunyaviridae	<u>Bunyavirus</u>	ANA
TACARIBE	TCR	Arenaviridae	<u>Arenavirus</u>	TCR
TAGGERT	TAG	Bunyaviridae	<u>Nairovirus</u>	SAK
TAHYNA	TAH	Bunyaviridae	<u>Bunyavirus</u>	CAL
TAMDY	TDY	Bunyaviridae	Bunyavirus-like	
TAMIAMI	TAM	Arenaviridae	<u>Arenavirus</u>	TCR
TANGA	TAN			
TANJONG RABOK	TR			TR
TATAGUINE	TAT	Bunyaviridae	Bunyavirus-like	
TEHRAN	TEH	Bunyaviridae	<u>Phlebovirus</u>	PHL
TELOK FOREST	TF			TR
TEMBE	TME			

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
TEMBUSU	TMU	Togaviridae	<u>Flavivirus</u>	B
TENSAW	TEN	Bunyaviridae	<u>Bunyavirus</u>	BUN
TERMEIL	TER			
TETE	TETE	Bunyaviridae	<u>Bunyavirus</u>	TETE
TETTNANG	TET	Coronaviridae		
THIMIRI	THI	Bunyaviridae	<u>Bunyavirus</u>	SIM
THOGOTO	THO	Orthomyxoviridae		THO
THOTTAPALAYAM	TPM			
TIBROGARGAN	TIB	Rhabdoviridae		
TILLIGERRY	TIL	Reoviridae	<u>Orbivirus</u>	EUB
TIMBO	TIM	Rhabdoviridae		TIM
TIMBOTEUA	TBT	Bunyaviridae	<u>Bunyavirus</u>	GMA
TINAROO	TIN	Bunyaviridae	<u>Bunyavirus</u>	SIM
TLACOTALPAN	TLA	Bunyaviridae	<u>Bunyavirus</u>	BUN
TONATE	TON	Togaviridae	<u>Alphavirus</u>	A
TOSCANA	TOS	Bunyaviridae	<u>Phlebovirus</u>	PHL
TOURE	TOU			
TRIBEC	TRB	Reoviridae	<u>Orbivirus</u>	KEM
TRINITI	TNT	Togaviridae		
TRIVITTATUS	TVT	Bunyaviridae	<u>Bunyavirus</u>	CAL
TRUBANAMAN	TRU	Bunyaviridae	Bunyavirus-like	MAP
TSURUSE	TSU	Bunyaviridae	<u>Bunyavirus</u>	TETE
TURLOCK	TUR	Bunyaviridae	<u>Bunyavirus</u>	TUR
TURUNA	TUA	Bunyaviridae	<u>Phlebovirus</u>	PHL
TYULENIY	TYU	Togaviridae	<u>Flavivirus</u>	B

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
UGANDA S	UGS	Togaviridae	<u>Flavivirus</u>	B
UMATILLA	UMA	Reoviridae	<u>Orbivirus</u>	
UMBRE	UMB	Bunyaviridae	<u>Bunyavirus</u>	TUR
UNA	UNA	Togaviridae	<u>Alphavirus</u>	A
UPOLU	UPO	Bunyaviridae	Bunyavirus-like	UPO
URUCURI	URU	Bunyaviridae	<u>Phlebovirus</u>	PHL
USUTU	USU	Togaviridae	<u>Flavivirus</u>	B
UTINGA	UTI	Bunyaviridae	<u>Bunyavirus</u>	SIM
UUKUNIEMI	UUK	Bunyaviridae	<u>Uukuvirus</u>	UUK
VELLORE	VEL	Reoviridae	<u>Orbivirus</u>	PAL
VEN. EQUINE ENC.	VEE	Togaviridae	<u>Alphavirus</u>	A
VENKATAPURAM	VKT			
VINCES	VIN	Bunyaviridae	<u>Bunyavirus</u>	C
VIRGIN RIVER	VR	Bunyaviridae	<u>Bunyavirus</u>	ANA
VS-ALAGOAS	VSA	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
VS-INDIANA	VSI	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
VS-NEW JERSEY	VSNJ	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
WAD MEDANI	WM	Reoviridae	<u>Orbivirus</u>	KEM
WALLAL	WAL	Reoviridae	<u>Orbivirus</u>	WAL
WANOWRIE	WAN			
WARREGO	WAR	Reoviridae	<u>Orbivirus</u>	WAR
WESSELSBRON	WSL	Togaviridae	<u>Flavivirus</u>	B
WEST. EQUINE ENC.	WEE	Togaviridae	<u>Alphavirus</u>	A
WEST NILE	WN	Togaviridae	<u>Flavivirus</u>	B
WHATAROA	WHA	Togaviridae	<u>Alphavirus</u>	A

NAME	ABBR.	TAXONOMIC STATUS		ANTI-GENIC GROUP
		FAMILY	GENUS	
WITWATERSRAND	WIT	Bunyaviridae	Bunyavirus-like	
WONGAL	WON	Bunyaviridae	<u>Bunyavirus</u>	KOO
WONGORR	WGR			
WYEOMYIA	WYO	Bunyaviridae	<u>Bunyavirus</u>	BUN
XIBUREMA	XIB	Rhabdoviridae		
YACAABA	YAC			
YAQUINA HEAD	YH	Reoviridae	<u>Orbivirus</u>	KEM
YATA	YATA	Rhabdoviridae		
YELLOW FEVER	YF	Togaviridae	<u>Flavivirus</u>	B
YOGUE	YOG			
YUG BOGDANOVAC	YB	Rhabdoviridae	<u>Vesiculovirus</u>	VSV
ZALIV TERPENIYA	ZT	Bunyaviridae	<u>Uukuvirus</u>	UUK
ZEGLA	ZEG	Bunyaviridae	<u>Bunyavirus</u>	PAT
ZIKA	ZIKA	Togaviridae	<u>Flavivirus</u>	B
ZINGA	ZGA	Bunyaviridae	<u>Phlebovirus</u>	PHL
ZINGILAMO	ZGO			BTK
ZIRQA	ZIR	Bunyaviridae	<u>Nairovirus</u>	HUG

Table 2. Antigenic Groups of 487 Viruses Registered in Catalogue

Virus Family and Genus	Antigenic Group	Abbreviation	No. Registered Viruses in Group	Percent
<u>ARENAVIRIDAE</u> <u>Arenavirus</u>	Tacaribe	TCR	10	2.1
<u>BUNYAVIRIDAE</u> <u>Bunyavirus</u>	Bunyamwera Supergroup		123	25.3
	Anopheles A	ANA	5	
	Anopheles B	ANB	2	
	Bunyamwera	BUN	22	
	Bwamba	BWA	2	
	C	C	12	
	California	CAL	13	
	Capim	CAP	8	
	Gamboa	GAM	3	
	Guama	GMA	12	
	Koongol	KOO	2	
	Minatitlan	MNT	2	
	Olifantsvlei	OLI	3	
	Patois	PAT	6	
	Simbu	SIM	21	
	Tete	TETE	5	
	Turlock	TUR	4	
	Unassigned	SBU	1	
<u>Nairovirus</u>	CHF-Congo	CHF-CON	4	0.8
	Dera Ghazi Khan	DGK	5	1.0
	Hughes	HUG	4	0.8
	Nairobi sheep disease	NSD	3	0.6
	Qalyub	QYB	2	0.4
	Sakhalin	SAK	5	1.0
<u>Phlebovirus</u>	Phlebotomus fever	PHL	34	7.0

Table 2 (Continued)

Virus Family and Genus	Antigenic Group	Abbreviation	No. Registered Viruses in Group	Percent
BUNYAVIRIDAE				
<u>Uukuvirus</u>	Uukuniemi	UUK	5	1.0
"Bunyavirus-like" (Unassigned, probable or possible members)	Bakau	BAK	2	0.4
	Bhanja	BHA	1	0.2
	Kaisodi	KSO	3	0.6
	Mapputta	MAP	4	0.8
	Matariya	MTY	3	0.6
	Nyando	NDO	1	0.2
	Upolu	UPO	2	0.4
	Ungrouped		13	2.7
REOVIRIDAE				
<u>Orbivirus</u>	African horsesickness	AHS	1	0.2
	Bluetongue	BLU	1	0.2
	Changuinola	CGL	12	2.5
	Colorado tick fever	CTF	2	0.4
	Corriparta	COR	3	0.6
	Epizootic hemorrhagic dis.	EHD	2	0.4
	Eubenangee	EUB	3	0.6
	Kemerovo	KEM	16	3.3
	Palyam	PAL	7	1.4
	Wallal	WAL	1	0.2
	Warrego	WAR	2	0.4
	Ungrouped		9	1.8
RHABDOVIRIDAE				
<u>Vesiculovirus</u>	Vesicular stomatitis	VSV	11	2.3
<u>Lyssavirus</u>	Rabies		2	0.4
Unassigned or possible members	Hart Park	HP	3	0.6
	Kwatta	KWA	1	0.2
	Mossuril	MOS	8	1.6
	Sawgrass	SAW	3	0.6
	Timbo	TIM	3	0.6
	Ungrouped		15	3.1

Table 2 (Continued)

Virus Family and Genus	Antigenic Group	Abbreviation	No. Registered Viruses in Group	Percent
TOGAVIRIDAE				
<u>Alphavirus</u>	A	A	26	5.3
<u>Flavivirus</u>	B	B	64	13.1
Possible members	Ungrouped		2	0.4
CORONAVIRIDAE	Ungrouped		1	0.2
<u>Coronavirus</u>	Ungrouped		1	0.2
HERPESVIRIDAE	Ungrouped		1	0.2
IRIDOVIRIDAE	Ungrouped		1	0.2
NODAVIRIDAE				
<u>Nodavirus</u>	Ungrouped		1	0.2
ORTHOMYXOVIRIDAE				
	Thogoto	THO	1	0.2
	Ungrouped		1	0.2
PARAMYXOVIRIDAE				
<u>Paramyxovirus</u>	Ungrouped		1	0.2
POXVIRIDAE	Ungrouped		3	0.6
UNCLASSIFIED				
	Boteke	BTK	2	0.4
	Malakal	MAL	2	0.4
	Marburg	MBG	2	0.4
	Nyamanini	NYM	1	0.2
	Tanjong Rabok	TR	2	0.4
	Quaranfil	QRF	2	0.4
	Ungrouped		44	9.0
	TOTAL		487	

Table 3. Initial Isolations of Viruses by Decade and Country of Origin

<u>Decade</u>	<u>Continent</u>	<u>Country</u>	<u>Virus</u>	
1900-09	Africa	S. Africa	BLU	
1910-19	Africa	Kenya	ASF, NSD	
1920-29	Africa	Nigeria	YF	
	Europe	Scotland	LI	
	N. America	U.S.A.	VSI	
1930-39	Africa	Kenya	RVF	
		S. Africa	AHS	
		Uganda	BWA, WN	
	Asia	Japan	JE	
		U.S.S.R.	RSSE	
		U.S.A.	EEE, SLE, WEE	
	S. America	Venezuela	VEE	
1940-49	Africa	Uganda	BUN, NTA, SF, UGS, ZIKA	
	Asia	Japan	NEG	
		U.S.S.R.	OMSK	
		Hawaii	DEN-1*	
	Australasia	New Guinea	DEN-2*	
		Czechoslovakia	HAN	
	Europe	Italy	SFN*, SFS*	
		U.S.A.	CE, CTF, TVT	
	N. America	U.S.A.	ILH	
	S. America	Brazil	ANA, ANB, WYO	
		Colombia		
	1950-59	Africa	Egypt	CNU, QRF, QYB, SIN
			Nigeria	ILE, LB
S. Africa			BAN, GER, ING, LEB, MID, MOS, NDU, NYM, PGA, SIM, SPO, TETE, USU, WIT, WSL	
		Sudan	WM**	
		Uganda	CHIK, CON, ENT, NDO, ONN, ORU	
Asia		India	ARK, BHA, GAN, KAS, KSO, KFD, MIN, PAL, SAT, VKT, UMB, WAN	
		Israel	IT	
		Japan	AKA, APOI, IBA, NOD, SAG, TSU	
		Malaya	BAK, BAT, BEB, GET, KET, LGT, TMU	
		Australasia	Australia	MVE
Europe		Philippines	DEN-3*, DEN-4*	
		Czechoslovakia	HYPR, TAH	
		Finland	KUM	
		U.S.S.R.	ABS	
N. America		Canada	POW	
		Panama	BUC, LJ, PCA	
		U.S.A.	CV, EHD, HP, MML, MOD, RB, SA, SSH, TUR, VSNJ	
S. America		Argentina	JUN	
		Brazil	APEU, AURA, BSQ, CAP, CAR, CATU, GJA, GMA, ITQ, MAG, MIR, MOJU, MTB, MUC, MUR, ORI, TCM, UNA	
		Colombia	GRO, NAV	
		Trinidad	ARU, BIM, BSB, IERI, KRI, LUK, MAN, MAY, MEL, NEP, ORO, TCR, TNT	

* Isolated in U.S.A. laboratory

** Isolated in Egypt laboratory

Table 3 (Continued)

<u>Decade</u>	<u>Continent</u>	<u>Country</u>	<u>Virus</u>	
1960-69	Africa	Cameroon	NKO, OKO	
		Cent. Afr. Rep.	BAG, BGN, BIA, BIR, BOT, BOU, BTK, MPO, PATA, YATA, ZGA	
		Egypt	ACD, AMT, BAH*, BEA, MTR, MTY, RF	
		Kenya	THO	
		Nigeria	DUG, KOT, LAS*, SABO, SAN, SHA, SHU	
		Senegal	BDA, DB, GOS, KEU, KOU, LD, SAB, TAT, TOU, YOG	
		South Africa	OLI, SHO	
		Sudan	MAL***	
		Uganda	KAD, KAM, MEB, TAN	
		Asia	Cambodia	PPB
			India	BAR, CHP, DHO, KAN, KMP, SEM, THI, TPM, VEL
			Iran	KAR*, SAL*, TEH*
			Japan	AINO
			Malaysia	JUG, KTR, LJN, PUC, TR
			Pakistan (West)	DGK, HAZ, MWA
			Persian Gulf	ZIR
			Singapore	SEL
	Thailand		KK	
	U.S.S.R.		CHF, KYZ, OKH, SAK, TYU, ZT	
	Australasia		Australia	ALF, ALM, BEF, BEL, CHV, COR, DAG, EH, EUB, JAP, JOI, KOK, KOO, KOW, KUN, MAP, MPK, MR, RR, SEP, STR, TRU, UPO, WAR, WON
			New Zealand	WHA
			Pacific Island	JA*
			Europe	Czechoslovakia
		Finland		INK, UUK
	France	GA, PTV		
	West Germany	MBG		
	N. America	Canada	SIL	
		Guatemala	JUT*	
		Mexico	MNT, TLA*	
		Panama	AGU, CHG, CHI, CGL, FRI, GAM, JD, LAT, MAD, MAT, OSSA, PAR, PAT, PT*, ZEG	
		U.S.A.		BUT, CR, EVE, FLA, GL, HJ, HUG, JC, JS, KC, KEY, KLA, LAC, LOK, LS, MER, MD, MH, ML, PAH, SAW, SC, SHF, SR, TAM, TEN, UMA
				MAC**
	S. America	Bolivia		
		Brazil		ACA, AMA, AMB, ANH, ANU, AP, ARA, BEN, BER, BLM, BOR, BUJ, BVS, CAN, CDU, CHO, COT, GTB, GUR, ICO, INH, IRI, ITP, JUR, MCO, OUR, PAC, PIRY, PIX, SDN, SOR, TBT, TIM, TME, URU, UTI, VSA
				BUE, PIC
				CAB
		Colombia		
		French Guiana		

* Isolated in U.S.A. laboratory

** Isolated in Panama laboratory

*** Isolated in Egypt laboratory

Table 3 (Continued)

<u>Decade</u>	<u>Continent</u>	<u>Country</u>	<u>Virus</u>	
1960-69	S. America	Peru	HUA*,PS*	
		Surinam	KWA	
		Trinidad	COC,MOR,NAR,RES,SOL	
1970-79	Africa	Cent. Afr. Rep.	BBO,BGI,BMA,BOB,GAR,GOM,GOR,IPPY,KOL, LJA,NOLA,OUA,OUB,SAF,SEB,SGA,SJA,SPA, ZGO	
		Egypt	AH,KS,PTH	
		Nigeria	IFE	
		Seychelles	ARI***	
		S. Africa	PRE***	
		Zaire	EBO	
		Asia	India	CG,KAI,SRI
			Iran	ISF*
			Korea	HTN
			Malaysia	CI,TF
	U.S.S.R.		BKN,CHIM,IK,KHA,KSI,PMR,RAZ,SOK,TDY	
	Australasia	Australia	BC,BF,CVG,DOU,GG,KNA,MAR,NGA,NUG,PEA, PIA,PR,SRE,TAG,TER,TIB,TIL,TIN,WAL, WGR,YAC	
		Europe	Czechoslovakia	SLO
			Germany	EYA,TET
			Italy	TOS
			Scotland	CM,CW
			U.S.S.R.	BAKU
	Yugoslavia	YB		
	N. America	Canada	AVA,BAU*,GI*	
		Mexico	SAR*	
		Panama	CAC,CAI,NIQ	
		U.S.A.	AB,CNT,FM,GLO,LLS,NM,NOR,RG,SCA,SP, SV,VR,YH	
	S. America	Brazil	ALE,ALT,CNA,CPC,CUI,ENS,FLE,IACO,ITA, ITI,ITU,JAC,JAM,MCA,MDC,MPR,MQO,PARA, ROC,SM,STM,TUA	
		Ecuador	ABR,BAB,NJL,PLA,PLS,PV,SJ,VIN	
		French Guiana	INI,RBU,TON	
		Venezuela	AROA	
		Italy	ARB	
1980-83	Europe	Italy	ARB	
	S. America	Argentina	LM	
		Brazil	AMR,JARI,MDO,MUN,ORX,PUR,SRA,XIB	

* Isolated in U.S.A. laboratory

** Isolated in Panama laboratory

*** Isolated in Egypt laboratory

Table 4. Initial Isolation of 487 Registered Viruses By
Continent, Country, and Chronological Period

Continent	Country or Area	Before 1930	1930 -39	1940 -49	1950 -59	1960 -69	1970 -79	1980 -83	Totals
AFRICA	Cameroon					2			2
	Cent. Afr. Rep.					11	19		30
	Egypt				5	7	3		15
	Kenya	2	1			1			4
	Nigeria	1			2	7	1		11
	Senegal					10			10
	Seychelles						1		1
	S. Africa	1	1		15	2	1		20
	Sudan					1			1
	Uganda		2	5	6	4			17
	Zaire							1	1
	Totals	4	4	5	28	45	26	0	112
ASIA	Cambodia					1			1
	India				12	9	3		24
	Iran					3	1		4
	Israel				1				1
	Japan		1	1	6	1			9
	Korea						1		1
	Malaysia				7	5	2		14
	W. Pakistan					3			3
	Persian Gulf					1			1
	Singapore					1			1
	Thailand					1			1
	U.S.S.R. (East)		1	1		6	9		17
	Totals	0	2	2	26	31	16	0	77
AUSTRAL- ASIA and PACIFIC ISLANDS	Australia				1	25	21		47
	Hawaii			1					1
	Johnston Island					1			1
	New Guinea			1					1
	New Zealand					1			1
	Philippines				2				2
	Totals	0	0	2	3	27	21	0	53
EUROPE	Czechoslovakia			1	2	5	1		9
	Finland				1	2			3
	France					2			2
	West Germany					1	2		3
	Italy			2			1	1	4
	Scotland	1					2		3
	U.S.S.R. (West)				1		1		2
	Yugoslavia						1		1
	Totals	1	0	3	4	10	8	1	27
NORTH AMERICA	Canada				1	1	3		5
	Guatemala					1			1
	Mexico					2	1		3
	Panama				3	15	3		21
	U.S.A.	1	3	3	10	27	13		57
	Totals	1	3	3	14	46	20	0	87

Table 4 (Continued)

Continent	Country or Area	Before 1930	1930 -39	1940 -49	1950 -59	1960 -69	1970 -79	1980 -83	Totals
SOUTH AMERICA	Argentina				1			1	2
	Bolivia					1			1
	Brazil			1	18	37	22	8	86
	Colombia			3	2	2			7
	Ecuador						8		8
	French Guiana					1	3		4
	Peru					2			2
	Surinam					1			1
	Trinidad				13	5			18
	Venezuela		1					1	2
	Totals	0	1	4	34	49	34	9	131
	GRAND TOTALS	6	10	19	109	208	125	10	487

Table 5. Alphaviruses, Family Togaviridae

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS				
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis			
	Mosq.	Ticks		Phlebotomine	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials	Other	Sentinels
		Culicine	Anopheline																						
Aura	+																	2	S	22	Alphavirus				
Barmah Forest	+																	2	A7	22	"				
Bebaru	+																	2	S	22	"				
Cabassou	+																	2	1E	21	"				
Chikungunya	+																	3*	S	20	"				
Eastern equine enc.	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	2V	S	20	"				
Everglades	+	+																3*V	S	20	"				
Fort Morgan																		2	S	20	"				
Getah	+	+																3	A1	20	"				
Highlands J	+																	2	S	20	"				
Kyzylagach	+																	3	1E	22	"				
Mayaro	+																	3	S	20	"				
Middelburg	+																	3	A1	20	"				
Mucambo	+																	3*V	S	20	"				
Ndumu	+																	3	A1	21	"				
O'nyong-nyong		+																2	S	20	"				
Pixuna	+	+																2	S	22	"				
Ross River	+																	2	S	20	"				
Sagiyama	+																	3	A1	21	"				
Semliki Forest	+	+																3	A2	20	"				
Sindbis	+	+	+															2	S	20	"				
Tonate	+	+																3*V	1E	21	"				
Una	+	+																2	S	21	"				
Ven. equine enc.	+	+																3*V	S	20	"				
Western equine enc.	+	+																2V	S	20	"				
Whataroa	+																	2	S	20	"				

* Work with these viruses at containment level 3 requires HEPA filtration of all exhaust air prior to discharge to the outside.

** 20 = Arbovirus
 21 = Probable Arbovirus
 22 = Possible Arbovirus
 23 = Probably not Arbovirus
 24 = Not Arbovirus

V = Vaccination with demonstration of antibody development; without such vaccination, the next higher containment level is recommended.

Table 6. Mosquito-Borne Flaviviruses, Family Togaviridae

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					Africa	Asia	Aus tralasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq. Culicine	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds												
Alfuy	+																	2	S	20	Flavivirus	
Bagaza	+																	2	S	22	"	
Banzi	+					+												2	S	20	"	
Bouboui	+	+					+											2	S	22	"	
Bussuquara	+						+	+										2	S	20	"	
Dengue-1	+					+												2	S	20	"	
Dengue-2	+					+	+											2	S	20	"	
Dengue-3	+					+												2	S	20	"	
Dengue-4	+					+												2	S	20	"	
Edge Hill	+	+																2	S	20	"	
Ilheus	+																	2	S	20	"	
Japanese enc.	+	+																3	S	20	"	
Jugra	+																	2	S	21	"	
Kokobera	+									+								2	S	21	"	
Kunjin	+																	2	S	20	"	
Murray Valley enc.	+																	3	S	20	"	
Naranjal	+																	3	1E	21	"	
Ntaya	+																	2	S	21	"	
Rocio	+																	2	S	20	"	
Sepik	+																	3*	S	21	"	
St. Louis enc.	+	+				+												3	1E	21	"	
Spondweni	+																	3	S	20	"	
Stratford	+																	3	S	20	"	
Tembusu	+	+																2	S	22	"	
Uganda S	+																	2	S	21	"	
Usutu	+																	2	S	20	"	
Wesselsbron	+	+																2	S	22	"	
West Nile	+	+	+															3*	S	20	"	
Yellow fever	+		+															3	S	20	"	
Zika	+																	2	S	20	"	

* See footnote Table 5
 ** See footnote Table 5

V: See footnote Table 5
 X: Arboviruses restricted by U.S. Department of Agriculture regulations or policy.

Table 7. Tick-Borne Flaviviruses, Family Togaviridae

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS				
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials	Other	Sentinels
		Culicine	Anophelinae																							
Absettarov		+				+						+					+	+	4	A4	20	Flavivirus				
Hanzalova		+				+											+	+	4	A4	20	"				
Hypr		+				+											+	+	4	S	20	"				
Kadam		+						+	+	+								+	2	S	21	"				
Karshi		+																+	2	S	22	"				
Kumlinge		+				+		+	+	+		+					+	+	4	A4	20	"				
Kyasanur Forest dis.		+			+	+		+	+	+		+					+	+	4	S	20	"				
Langat		+																+	2	S	20	"				
Louping ill		+				+		+	+	+		+					+	+	3*	S	20	"				
Omsk hem. fever		+				+		+	+	+		+					+	+	4	S	20	"				
Powassan		+				+		+	+	+		+					+	+	3	S	20	"				
Royal Farm																		+	2	S	22	"				
RSSE		+				+		+	+	+								+	4	S	20	"				
Saumarez Reef		+				+		+	+	+								+	4	S	20	"				
Tyuleniy		+																	3	1E	22	"				
																			2	S	21	"				

* See footnote Table 5

** See footnote Table 5

X: See footnote Table 6

Table 8. Flaviviruses, Family Togaviridae:
No Arthropod Vector Demonstrated

VIRUS	ISOLATED FROM							ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS				VERTEBRATES			Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq.	Ticks	Phlebotomine	Culicoides	Other	Man	Other Primates												
Culicine	Anopheline	Ixodid	Argasid																
Apoi							+								+	2	S	22	Flavivirus
Aroa																2	S	22	"
Cacipacore																2	S	22	"
Carey Island																2	S	22	"
Cowbone Ridge							+									2	S	23	"
Dakar bat						+										2	S	24	"
Entebbe bat																2	S	24	"
Israel turkey men.																3	S	21	"
Jutiapa																2	S	22	"
Koutango																3	S	21	"
Modoc																2	S	24	"
Montana myotis leuk.																2	S	24	"
Negishi																3	S	22	"
Phnom-Penh bat						+										2	S	23	"
Rio Bravo																2	S	24	"
Saboya																2	S	22	"
Sal Vieja																3	A7	22	"
San Perlita																3	A7	22	"
Sokuluk																2	S	22	"

** See footnote Table 5

Table 9. Bunyaviruses, Family Bunyaviridae:
Bunyamwera Supergroup, Anopheles A and Anopheles B Serogroup Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq.	Ticks	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats												
Culicine	Anopheline	Ixodid	Argasid																			
<u>ANOPHELES A GR.</u>																						
Anopheles A																		2	S	21	Bunyavirus	
Las Maloyas																		2	A7	22	"	
Lukuni	+	+																2	S	22	"	
Tacaiuma	+	+																2	S	21	"	
Virgin River	+	+				+	+					+					+	2	A7	22	"	
<u>ANOPHELES B GR.</u>																						
Anopheles B																		2	S	22	Bunyavirus	
Boraceia	+	+																2	S	22	"	

** See footnote Table 5

Table 10. Bunyaviruses, Family Bunyaviridae:
Bunyamwera Supergroup, Bunyamwera Serogroup Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS					
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis				
	Mosq.	Ticks		Phlebotomine	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials	Other	Sentinels	
		Anopheline	Ixodid																							Argasid
Culicine																										
<u>BUNYAMWERA GR.</u>																										
Anhembi	+						+									+						2	S	22	Bunyavirus	
Batai	+																					2	S	21	"	
Birao	+																					2	S	22	"	
Bunyamwera	+					+																2	S	20	"	
Cache Valley	+																					2	S	20	"	
Calovot	+																					2	S	21	"	
Germiston	+					+	+					+	+					+				3*	S	20	"	
Iaco	+																					2	S	22	"	
Ilesha	+					+																2	S	21	"	
Kairi	+						+				+	+										3	AE	20	"	
Lokern	+																					2	S	20	"	
Macaua	+						+				+											2	S	21	"	
Maguari	+																					2	S	20	"	
Main Drain	+																					2	S	20	"	
Northway	+																					3	1E	21	"	
Playas	+																					3	1E	22	"	
Santa Rosa	+																					3	1E	22	"	
Shokwe	+					+	+															2	S	21	"	
Sororoca	+																					2	S	22	"	
Tensaw	+											+	+									2	S	20	"	
Tlacotalpan	+																					3	1E	22	"	
Wyeomyia	+					+																2	S	21	"	

* See footnote Table 5
** See footnote Table 5
† May be strain of Batai

Table 11. Bunyaviruses, Family Bunyviridae:
Bunyamwera Supergroup, Bwamba Serogroup and Serogroup C Viruses

VIRUS	ISOLATED FROM								ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS							
	ARTHROPODS				VERTEBRATES				Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis						
	Mosq.	Ticks		Phlebotomine	Cuticoides	Other	Man	Other Primates													Rodents	Birds	Bats	Marsupials	Other	Sentinels
		Anopheline	Ixodid																							
Cuticine																										
BWAMBA GR.																										
Bwamba						+												S	21							
Pongola	+																	S	20							
GROUP C																										
Apeu	+					+												S	20							
Caraparu	+					+												S	20							
Gumbo Limbo	+					+												S	21							
Itaqui	+					+												S	20							
Madrid	+					+												S	20							
Marituba	+					+												S	20							
Murutucu	+					+												S	20							
Nepuyo	+					+												S	20							
Oriboca	+					+												S	20							
Ossa	+					+												S	20							
Restan	+					+												S	20							
Vinces	+					+												S	21							

** See footnote Table 5

Table 12. Bunyaviruses, Family Bunyaviridae:
Bunyamvera Supergroup, California and Capim Serogroup Viruses

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS						
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis					
	Mosq.	Ticks		Phlebotomine	Cuticoides	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials	Other	Sentinels		
	Cuticine	Anopheline	Ixodid																								Argasid	
CALIFORNIA GR.																												
California enc.	+																						2	S	20	Bunyavirus		
Guarua		+																					2	S	20	"		
Inkoo	+																						2	S	22	"		
Jamestown Canyon	+						+																2	S	22	"		
Jerry Slough	+																						2	S	20	"		
Keystone	+	+																					2	S	20	"		
La Crosse	+							+	+														2	S	20	"		
Melao	+																						2	S	21	"		
San Angelo	+																						2	S	20	"		
Serra do Navio	+	+																					3	1E	22	"		
Snowshoe hare	+												+	+									2	S	20	"		
Tahyna	+	+																					2	S	20	"		
Trivittatus	+																						2	S	20	"		
CAPIM GR.																												
Acara	+																						2	S	21	Bunyavirus		
Benevides	+																						2	A7	21	"		
Benfica	+																						2	A7	20	"		
Bushbush	+																						2	S	20	"		
Capim	+																						2	S	20	"		
Guajara	+																						2	S	20	"		
Juan Diaz	+																						2	S	22	"		
Moriche.	+																						2	S	22	"		

** See footnote Table 5

Table 13. Bunyaviruses, Family Bunyaviridae:
Bunyamwera Supergroup, Gamboa, Guama and Koongol Serogroup Viruses

VIRUS	ISOLATED FROM								ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS				VERTEBRATES				Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates												
Culicine	Anopheline	Ixodid	Argasid																	
GAMBOA GR. Gamboa Pueblo Viejo San Juan	+	+	+														2	S	22 22 22	Bunyavirus " "
GUAMA GR. Ananindeua Bertioga Bimiti Cananea Catu Guama Guaratuba Itimirim Mahogany Hammock Mirim Moju Timboteua	+							+	+		+						2 2 2 2 2 2 2 2 2 2 2 2	A7 S S S S S S S S S S S S A7	21 22 20 21 20 20 21 22 22 20 20 21	Bunyavirus " " " " " " " " " " " " "
KOONGOL GR. Koongol Wongal	+	?											+				2 2	S S	21 21	Bunyavirus "

** See footnote Table 5

Table 14. Bunyaviruses, Family Bunyaviridae:
Bunyamwera Supergroup, Minatitlan, Olifantsvlei and Patois Serogroup Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq.	Ticks	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats												
Culicine	Anophelinae	Ixodid									Argasid											
<u>MINATITLAN GR.</u> Minatitlan Palestina	+											++						2	S	22 21	Bunyavirus "	
<u>OLIFANTSVLEI GR.</u> Bobia Botambi Olifantsvlei	+	+											+++					3 2 2	1E S S	22 22 22	Bunyavirus " "	
<u>PATOIS GR.</u> Abrás Babahoyo Pahayokey Patois Shark River Zegla	+	+														+	+	2 2 2 2	S S S S	22 21 22 20 21 22	Bunyavirus " " " " "	

** See footnote Table 5

Table 15. Bunyaviruses, Family Bunyaviridae:
Bunyamwera Supergroup, Simbu Serogroup Viruses

VIRUS	ISOLATED FROM							ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS				VERTEBRATES			Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq.	Ticks		Other	Man	Other Primates	Rodents												
Culicine	Anophelinae	Ixodid	Argasid					Phlebotomine	Culicoides										
Aino	+																22	Bunyavirus	
Akabane	+																	21	"
Buttonwillow	+																	20	"
Douglas																		21	"
Ingwavuma	+																	20	"
Inini																		22	"
Kaikalur	+																	22	"
Manzanilla																		22	"
Mermet																		22	"
Nola	+																	20	"
Oropouche	+																	21	"
Peaton	+																	21	"
Sabo																		22	"
Sango	+																	22	"
Sathuperi	+																	22	"
Shamonda																		22	"
Shuni	+																	22	"
Simbu	+																	21	"
Thimiri																		22	"
Tinaroo																		22	"
Utinga																		22	"

* See footnote Table 5

** See footnote Table 5

Table 16. Bunyaviruses, Family Bunyaviridae:
Bunyamwera Supergroup, Tete and Turlock Serogroups and Unassigned Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq. Culicine	Ticks Ixodid Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats												
<u>TETE GR.</u> Bahig Batama Matruh Tete Tsuruse		+						+	+	+								2	S	21	Bunyavirus	
								+	+	+								3	TE	22	"	
		+						+	+	+								2	S	22	"	
								+	+	+								2	S	22	"	
								+	+	+								2	S	22	"	
<u>TURLOCK GR.</u> Lednice M'Poko (=Yaba-1) Turlock Umbre	+																	2	A7	21	Bunyavirus	
	+																	2	S	22	"	
	+																	2	S	20	"	
	+																	2	S	21	"	
<u>UNASSIGNED - "SBU"</u> Kaeng Khoi					+													2	S	22	Bunyavirus	

** See footnote Table 5

Table 17. Phleboviruses, Family Bunyaviridae:
Phlebotomus Fever Serogroup Viruses

VIRUS	ISOLATED FROM												ISOLATED IN						HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS
	ARTHROPODS						VERTEBRATES						Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level	Basis		
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials												
Culicine	Anopheline	Ixodid	Argasid																					
Aguacate																				2	S	21	Phlebovirus	
Alenquer						+														3	TE	22	"	
Anhanga																				2	S	22	"	
Arbia																				2	S	21	"	
Arumowot	+																			2	S	22	"	
Buenaventura																				3	TE	22	"	
Bujaru																				2	S	22	"	
Cacao																				2	S	21	"	
Caimito																				2	S	22	"	
Candiru																				2	S	22	"	
Chagres	+																			2	S	21	"	
Chilibre																				2	S	21	"	
Frijoles																				2	S	22	"	
Gordil																				2	TE	22	"	
Icoaraci	+	+																		2	S	21	"	
Itaituba																				3	TE	22	"	
Itaporanga	+																			2	S	20	"	
Karimabad																				2	S	21	"	
Munguba																						22	"	
Nique																				2	S	22	"	
Oriximina																						22	"	
Pacui																				2	S	21	"	
Punto Toro																				2	S	21	"	
Rift Valley fever	+																			3*VX	S	20	"	
Rio Grande																				2	S	21	"	
Saint-Floris																				2	S	22	"	
Salehabad																				2	S	22	"	

* See footnote Table 5
** See footnote Table 5

V: See footnote Table 5
X: See footnote Table 6

Table 17. Phleboviruses, Family Bunyaviridae:
Phlebotomus Fever Serogroup Viruses
(Continued)

VIRUS	ISOLATED FROM				ISOLATED IN						HUMAN DISEASE	SALS RATING		SEAS RATING**	TAXONOMIC STATUS		
	ARTHROPODS		VERTEBRATES		South America	North America	Europe	Australasia	Asia	Africa		Natural Infection	Lab Infection			Level	Basis
	Mosq.	Ticks	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels				
SF-Naples			+			+								2	S	20	Phlebovirus
SF-Sicilian			+			+								2	S	20	"
Tehran			+											2	A7	22	"
Toscana			+											2	S	21	"
Turuna			+											3	1E	22	"
Urucuri			+											2	S	22	"
Zinga			+											3	S	22	"

* See footnote Table 5
** See footnote Table 5

V: See footnote Table 5
X: See footnote Table 6

Table 18. Nairoviruses, Family Bunyaviridae:
Tick-Borne Serogroups Other Than Serogroup B Viruses

VIRUS	ISOLATED FROM									ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS					
	ARTHROPODS				VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level*			Basis				
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents													Birds	Bats	Marsupials	Other
Culicine		Anopheline	Ixodid							Argasid															
<u>CHF-CONGO GR.</u>																									
Congo				+		+												4	A6	20		Nairovirus			
Crimean hem. fever						+												4	A6	20		"			
Hazara																		2	S	22		"			
Khasan																		3	IE	22		"			
<u>DERA GHAZI KHAN GR.</u>																									
Abu Hammad				+														2	S	22		Nairovirus			
Dera Ghazi Khan																		2	S	22		"			
Kao Shuan																+		2	S	22		"			
Pathum Thani																		2	S	22		"			
Pretoria																		2	S	22		"			
<u>HUGHES GR.</u>																									
Hughes																									
Punta Salinas																									
Soldado																									
Zirga																									
																		2	S	21		Nairovirus			
																		2	S	22		"			
																		2	S	20		"			
																		2	S	22		"			

** See footnote Table 5

Table 19. Nairoviruses, Family Bunyaviridae:
Tick-Borne Serogroups Other Than Serogroup B Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds												
Cuticine	Ar:ophelinae	Ixodid	Argasid																			
<u>NAIROBI SHEEP DIS.</u> Dugbe Ganjam Nairobi sheep dis.	+	+				+	+	+										3 X X	S	21 22 20	Nairovirus " "	
<u>QALYUB GR.</u> Bandia Qalyub				+				+										2 2	S S	22 22	Nairovirus "	
<u>SAKHALIN GR.</u> Avalon Clo Mor Paramushir Sakhalin Taggart									+									2 2 3 2 2	S S E S S	21 22 22 22 22	Nairovirus " " " "	

** See footnote Table 5
X: See footnote Table 6

Table 20. Uukuviruses, Bunyavirus-Like, Family
 Bunyaviridae; Family Orthomyxoviridae; Unclassified
 Viruses: Tick-Borne Serogroups Other Than Serogroup B Viruses

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE	SALS RATING		SEAS RATING**	TAXONOMIC STATUS				
	ARTHROPODS					VERTEBRATES						Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection			Level	Basis		
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials	Other
Culicifne	Anophele	Ixodid	Argasid																						
<u>UUKUNIEMI GR.</u> Grand Arbaud Manawa Ponteves Uukuniemi Zaliv Terpeniya			+	+																		2 2 3 2 2	S S 1E S S	20 22 22 21 22	Uukuvirus " " " "
<u>BHANJA GR.</u> Bhanja			+			+		+				+							+			3	S	21	Bunyavirus-like
<u>KAISODI GR.</u> Kaisodi Lanjan Silverwater			+						+													2 2 2	S S S	21 22 21	Bunyavirus-like " "
<u>UPOLU GR.</u> Aransas Bay Upolu				+																		3 2	1E S	22 22	Bunyavirus-like "
<u>THOGOTO GR.</u> Thogoto			+				+					+										3	S	21	Orthomyxoviridae
<u>NYAMANINI GR.</u> Nyamanini				+																		2	S	21	Unclassified
<u>QUARANFIL GR.</u> Johnston Atoll Qaranfil				+																		2 2	S S	20 20	Unclassified "

** See footnote Table 5

Table 21. Bunyavirus-Like Viruses, Family Bunyviridae:
Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS							
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis						
	Mosq. Culicine	Anopheline	Ticks Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds													Bats	Marsupials	Other	Sentinals		
BAKAU GR. Bakau Ketapang	+			+					+											2	S	22	21	Bunyavirus-like "					
MAPPUTTA GR. Gan Gan Mapputta Maprik Trubanaman	+																			2	A7	22	22	21	22	Bunyavirus-like " " "			
MATARIYA GR. Burg el Arab Garba Matariya																				2	S	22	22	22	22	Bunyavirus-like " "			
NYANDO GR. Nyando																									+	2	S	21	Bunyavirus-like

** See footnote Table 5

Table 22. Orbiviruses, Family Reoviridae:
Tick-Borne Serogroups Other Than Serogroup B Viruses

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE	SALS RATING		SEAS RATING**	TAXONOMIC STATUS					
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection			Level	Basis			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials	Other	Sentinels
		Culicine	Anopheline																							
COLO. TICK FEVER GR.																										
Colorado tick fever																										
Eyach																										
KEMEROVO GR.																										
Baku																										
Bauline																										
Cape Wrath																										
Chenuda																										
Great Island																										
Huacho																										
Kemerovo																										
Lipovnik																										
Mono Lake																										
Nugget																										
Okhotskiy																										
Seletar																										
Sixgun City																										
Tribec																										
Wad Medani																										
Yaquina Head																										

** See footnote Table 5

Table 23. Orbiviruses, Family Reoviridae:
Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE	SALS RATING		SEAS RATING**	TAXONOMIC STATUS				
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America		South America	Natural Infection			Lab Infection	Level	Basis	
	Mosq.	Ticks	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats						Marsupials			Other	Sentinels				
<u>AFR. HORSESICKNESS</u> Afr. horsesickness				+							+									X		20	Orbivirus	
<u>BLUETONGUE GR.</u> Bluetongue				+							+				+					2	S	20	Orbivirus	
<u>CHANGUINOLA GR.</u>																								
Almeirim				+													+			3	1E	22	Orbivirus	
Altamira				+																3	1E	22	"	
Caninde				+																2	S	21	"	
Changuinola				+		+									+					3	1E	22	"	
Gurupi				+																3	S	22	"	
Irituia							+													2	S	22	"	
Jamanxi				+																3	1E	22	"	
Jari											+											22	"	
Monte Dourado											+											22	"	
Ourem				+																3	1E	22	"	
Purus																						22	"	
Saraca				+																		22	"	
<u>CORRIPARTA GR.</u>																								
Acado	+													+						2	S	22	Orbivirus	
Corriparta	+														+					2	S	21	"	
Jacareacanga	+																+					22	"	
<u>EHD GR.</u>																								
Epizootic hem.dis.																				2	S	21	Orbivirus	
Ibaraki														+						3	1E	22	"	

** See footnote Table 5

X: See footnote Table 6

Table 24. Orbiviruses, Family Reoviridae:
Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM											ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS				
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis			
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials	Other	Sentinels
		Culicine	Anophelline																							
<u>EUBENANGEE GR.</u> Eubenangee Pata Iilligerry	+	+	+	+															2 2 3	S S E	22 22 22	Orbivirus " "				
<u>PALYAM GR.</u> Bunyip Creek Csiro Village D'Aguilar Kasba Marrakai Palyam Vellore				+								+							2 2 2 2	S S S S	21 21 22 22 22 22	Orbivirus " " " " "				
<u>WALLAL GR.</u> Wallal				+															2	S	22	Orbivirus				
<u>WARREGO GR.</u> Mitchell River Warrego	+	+		+															2 2	S S	22 22	Orbivirus "				

** See footnote Table 5

Table 25. Family Rhabdoviridae; Lyssaviruses, Family Rhabdoviridae:
Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM											ISOLATED IN						HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS			
	ARTHROPODS						VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level	Basis					
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials	Other	Sentinels
		Culicine	Anopheline																							
<u>HART PARK GR.</u>																										
Flanders	+								+											2	S	22				
Hart Park	+								+											2	S	21				
Mosqueiro	+																			3	1E	22				
<u>KWATTA GR.</u>																										
Kwatta	+																			2	S	22				
<u>MOSSURIL GR.</u>																										
Bangoran	+								+											2	S	22				
Barur									+											2	S	22				
Charleville		+																		2	S	22				
Cuiaba				+																						
Kamese	+																			2	S	22				
Kern Canyon										+										2	S	23				
Marco											+									2	S	22				
Mossuril	+								+											2	S	22				
<u>RABIES SEROGROUP</u>																										
Kotonkan																				2	S	21				
Lagos bat				+							+									2	S	24				

** See footnote Table 5

Table 26. Family Rhabdoviridae; Vesiculoviruses, Family Rhabdoviridae:
Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq. Culicine	Anopheline	Ixodid	Argasid	Plebotomine	Culicoides	Other	Man	Other Primates	Rodents												
<u>SAWGRASS GR.</u> Connecticut New Minto Sawgrass			+																3	1E	22	Rhabdoviridae
			+																2	1E	22	"
			+																2	S	22	"
<u>TIMBO GR.</u> Chaco Sena Madureira Timbo																			2	S	22	Rhabdoviridae
																			2	S	22	"
<u>VES. STOMATITIS GR.</u> Chandipura Cocal Isfahan Jurona Keuraliba La Joya Piry VS-Alagoas VS-Indiana VS-New Jersey Yug Bogdanovac	+				+		+		+					+					2	S	20	Vesiculovirus
																			3	A1	20	"
					+														2	S	21	"
																			2	S	22	"
																			2	S	22	"
																			2	S	22	"
																			3	S	22	"
																			3	S	22	"
					+		+												2	A3	20	"
							+												2	A3	22	"
					+														3	1E	22	"

** See footnote Table 5

Table 27. Taxonomically Unclassified Viruses:
Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS		
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis	
	Mosq.	Ticks		Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents													Birds
<u>BOTEKE GR.</u> Boteke Zingilamo	+									+									2	S	22	22	Unclassified "
<u>MALAKAL GR.</u> Malaka Puchong	+	+																	2	S	22	22	Unclassified "
<u>MARBURG GR.</u> Ebola Marburg								+	+									+	4	S	23	23	Unclassified "
<u>TANJONG RABOK GR.</u> Tanjong Rabok Telok Forest									+										2	S	22	22	Unclassified "

** See footnote Table 5

Table 28. Arenaviruses, Family Arenaviridae:
Tacaribe (LCM) Serogroup Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS		
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis	
	Mosq.	Ticks	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials
Amapari					+																24	Arenavirus	
Flexal																					23	"	
Junin					+							+									4	A6	"
Lassa																					4	S	"
Latino																					2	A5	"
Machupo																					4	S	"
Parana																					2	A5	"
Pichinde					+																2	A5	"
Tacaribe	?	?																			2	A5	"
Tamiami																+					2	A5	"

** See footnote Table 5

Table 29. Families Bunyviridae, Coronaviridae, Reoviridae, Nodaviridae, Poxviridae:
Antigenically Ungrouped Mosquito-Associated Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq.	Ticks	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats												
Culicine	Anopheline	Ixodid	Argasid																			
Belmont	+																	2	S	22	Bunyavirus-like	
Enseada	+																	2	S	22	"	
Kowanyama		+																2	S	22	"	
Pacora	+																	2	S	22	"	
Tataguine	+	+				+												2	S	21	"	
Witwatersrand	+						+					+						2	S	20	"	
Bocas	+									+					+					22	Coronavirus	
Ieri	+																	3	1E	22	Orbivirus	
Japanaut	+																	2	S	21	"	
Lebombo	+					+	+											2	S	21	"	
Llano Seco††	+																	3	1E	21	"	
Orungo	+	+																3	S	21	"	
Paroo River	+																	3	1E	22	"	
Umatilla	+							+										2	S	20	"	
Nodamura	+																	3	1E	23	Nodavirus	
Cotia	+					+												2	S	24	Poxvirus	
Oubangui	+											+						3	1E	22	"	

** See footnote Table 5

†† Although it has been demonstrated that Llano Seco virus is antigenically related to Umatilla virus, its antigenic relationship to other established orbivirus serogroups is uncertain.

Table 30. Families Rhabdoviridae, Togaviridae; Taxonomically Unclassified Viruses:
Antigenically Ungrouped Mosquito-Associated Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS					
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis				
	Mosq.	Ticks		Phlebotomine	Cuticoles	Other	Man	Other Primates	Rodents	Birds													Bats	Marsupials	Other	Sentinels
		Cuticine	Anopheline																							
Aruac	+																	2	S	21	Rhabdoviridae					
Gray Lodge	+																	3	1E	22	"					
Joinjakaka	+																	2	S	22	"					
Kununurra	+																	2	S	22	"					
Xiburema	+																			22	"					
Yata	+																	2	S	22	"					
Trinititi	+																	2	S	21	Togaviridae					
Arkonam	+	+																2	S	22	Unclassified					
Gomoka	+	+																2	S	22	"					
Itupiranga	+							+										2	S	22	"					
Minnal	+																	2	S	22	"					
Nkolbisson	+																	2	S	22	"					
Okola	+																	2	S	22	"					
Para	+																	2	S	22	"					
Picola	+																	3	1E	22	"					
Rochambeau	+																	3	1E	22	"					
Tanga		+																2	S	22	"					
Tembe		+																2	S	22	"					
Termeil	+																	3	1E	21	"					
Venkatapuram	+																	2	S	22	"					
Wongorr	+																	2	S	22	"					
Yacaaba	+																	3	1E	22	"					

** See footnote Table 5

Table 31. Families Bunyaviridae, Coronaviridae, Iridoviridae, Orthomyxoviridae, Reoviridae, Rhabdoviridae;
Taxonomically Unclassified Viruses:
Antigenically Ungrouped Tick-, Culicoides-, or Phlebotomus-Associated Viruses

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS	
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis
	Mosq.	Ticks	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials												
Lone Star																			2	S	22	Bunyavirus-like
Razdan		+													+				3	1E	22	"
Sunday Canyon		+																	2	S	22	"
Tamdy		+													+				3	1E	22	"
Tettnang															+				2	S	22	Coronaviridae
Afr. swine fever										+					+				X		20	Iridoviridae
Chobar Gorge															+				2	S	22	Orbivirus
Dhori															+	+			3	S	22	Orthomyxoviridae
Bovine eph. fever											+				+	+			X		22	Rhabdoviridae
Inhangapi		+													+				3	1E	22	"
Sripur															+						22	"
Tibrogargan															+						22	"
Aride															+				2	S	22	Unclassified
Batken	+														+				3	1E	22	"
Chim															+				3	1E	22	"
Issyk-Kul															+				3	1E	20	"
Keterah															+				2	S	21	"
Matucare															+				2	S	22	"
Ngaingan															+				2	S	22	"
Slovakia															+				3	1E	24	"
Wanowrie	+														+				2	S	22	"

** See footnote Table 5

X: See footnote Table 6

§ Cuba

Table 32. Families Bunyaviridae, Herpesviridae, Reoviridae, Paramyxoviridae, Poxviridae, Rhabdoviridae, Togaviridae: Antigenically Ungrouped Viruses - No Arthropod Vector Known

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS					
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis				
	Mosq.	Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds													Bats	Marsupials	Other	Sentinels
		Culicine	Anopheline																							
Bangui						+											+		2	S	22	Bunyavirus-like				
Bobaya																			3	1E	22	"				
Hantaan						+		+										+	3§§	S	22	"				
Agua Preta										+									3	1E	22	Herpesvirus				
Ife																			3	1E	22	Orbivirus				
Nariva									+										3	1E	23	Paramyxovirus				
Salanga									+										3	1E	22	Poxvirus				
Almpiwar																			2	S	21	Rhabdoviridae				
Klamath									+										2	S	22	"				
Le Dantec																	+		2	S	22	"				
Mount Elgon bat						+													2	S	23	"				
Navarro										+									2	S	22	"				
Simian hem. fever								+											2	S	24	Togaviridae				

** See footnote Table 5

§§ If virus is handled in very high concentrations or in animals, then level 4.

Table 33. Taxonomically Unclassified Viruses:
Antigenically Ungrouped Viruses - No Arthropod Vector Known

VIRUS	ISOLATED FROM										ISOLATED IN					HUMAN DISEASE		SALS RATING		SEAS RATING**	TAXONOMIC STATUS				
	ARTHROPODS					VERTEBRATES					Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection	Level			Basis			
	Mosq.	Ticks		Phlebotomine	Other	Man	Other Primates	Rodents	Birds	Bats													Marsupials	Other	Sentinels
		Anopheline	Ixodid																						
Cuticine																									
Araguari										+								3	1E	22	Unclassified				
Belem								+							+			3	1E	22	"				
Bimbo								+										3	1E	22	"				
Gossas									+									2	S	23	"				
Ippy							+											2	S	22	"				
Kammavanpettai								+										2	S	22	"				
Kannamangalam								+										2	S	22	"				
Kolongo								+							+			2	S	22	"				
Landjia								+							+			2	S	22	"				
Mapuera										+						+					23	"			
Mojui dos Campos										+						+					22	"			
Quango								+										3	1E	22	"				
Sakpa							+											3	1E	22	"				
Sandjimba								+										2	S	22	"				
Santarem								+								+					22	"			
Sebokele								+										2	S	22	"				
Sembalam									+									2	S	22	"				
Thottapalayam											+							2	S	22	"				
Toure							+											2	S	22	"				
Yogue										+								2	S	22	"				

** See footnote Table 5

Table 34. Continental Distribution of Grouped and Ungrouped Viruses.

Antigenic Group	Total in Group	Africa	Asia	Aus-tral- asia	Eur- ope	North Amer- ica	South Amer- ica	No. of Conti- nents involved					
								1	2	3	4	5	6
A	26	6	8	6	2	7	10	18	6	0	2	0	0
AHS	1	1	1	0	1	0	0	0	0	1	0	0	0
B	64	19	23	13	7	12	10	49	11	3	1	0	0
BAK	2	0	2	0	0	0	0	2	0	0	0	0	0
BHA	1	1	1	0	1	0	0	0	0	1	0	0	0
BLU	1	1	1	1	1	1	0	0	0	0	0	1	0
BTK	2	2	0	0	0	0	0	2	0	0	0	0	0
Bunyamwera Supergroup	ANA	5	0	0	0	0	1	4	5	0	0	0	0
	ANB	2	0	0	0	0	0	2	2	0	0	0	0
	BUN	22	5	1	0	2	8	8	20	2	0	0	0
	BWA	2	2	0	0	0	0	0	2	0	0	0	0
	C	12	0	0	0	0	5	9	10	2	0	0	0
	CAL	13	1	1	0	2	9	3	11	1	1	0	0
	CAP	8	0	0	0	0	3	7	6	2	0	0	0
	GAM	3	0	0	0	0	1	2	3	0	0	0	0
	GMA	12	0	0	0	0	2	11	11	1	0	0	0
	KOO	2	0	0	2	0	0	0	2	0	0	0	0
	MNT	2	0	0	0	0	1	1	2	0	0	0	0
	OLI	3	3	0	0	0	0	0	3	0	0	0	0
	PAT	6	0	0	0	0	4	2	6	0	0	0	0
	SIM	21	9	6	5	0	2	4	16	5	0	0	0
	TETE	5	4	1	0	2	0	0	3	2	0	0	0
TUR	4	1	1	0	1	1	1	3	1	0	0	0	
SBU	1	0	1	0	0	0	0	1	0	0	0	0	
CGL	12	0	0	0	0	1	11	12	0	0	0	0	
CTF	2	0	0	0	1	1	0	2	0	0	0	0	
COR	3	1	0	1	0	0	1	3	0	0	0	0	
EHD	2	1	1	0	0	1	0	1	1	0	0	0	
EUB	3	1	0	2	0	0	0	3	0	0	0	0	
HP	3	0	0	0	0	2	1	3	0	0	0	0	
KSO	3	0	2	0	0	1	0	3	0	0	0	0	
KEM	16	3	4	1	4	6	1	14	1	1	0	0	
KWA	1	0	0	0	0	0	1	1	0	0	0	0	
MAL	2	1	1	0	0	0	0	2	0	0	0	0	
MAP	4	0	0	4	0	0	0	4	0	0	0	0	
MBG	2	2	0	0	1	0	0	1	1	0	0	0	
MOS	8	3	1	1	0	1	2	8	0	0	0	0	
MTY	3	3	0	0	0	0	0	3	0	0	0	0	
Nairo- viruses	CHF-CON	4	2	4	0	2	0	2	0	2	0	0	0
	DGK	5	2	4	1	0	0	3	2	0	0	0	0
	HUG	4	1	1	0	1	1	3	2	1	1	0	0
	NSD	3	2	1	0	0	0	0	3	0	0	0	0
	QYB	2	2	0	0	0	0	0	2	0	0	0	0
	SAK	5	0	2	1	1	2	0	4	1	0	0	0
NDC	1	1	0	0	0	0	0	1	0	0	0	0	
NYM	1	1	0	0	0	0	0	1	0	0	0	0	
PAL	7	0	3	4	0	0	0	7	0	0	0	0	
PHL	34	7	5	0	4	10	14	30	2	2	0	0	

Table 34. (Continued) Continental Distribution of Grouped and Ungrouped Viruses

Antigenic Group	Total in Group	Africa	Asia	Aus-tral- asia	Eur- ope	North Amer- ica	South Amer- ica	No. of Conti- ents involved						
								1	2	3	4	5	6	
QRF	2	1	0	1	0	0	0	2	0	0	0	0	0	0
RABIES	2	2	0	0	0	0	0	2	0	0	0	0	0	0
SAW	3	0	0	0	0	3	0	3	0	0	0	0	0	0
TCR	10	1	0	0	0	1	8	10	0	0	0	0	0	0
THO	1	1	0	0	1	0	0	0	1	0	0	0	0	0
TIM	3	0	0	0	0	0	3	3	0	0	0	0	0	0
TR	2	0	2	0	0	0	0	2	0	0	0	0	0	0
UPO	2	0	0	1	0	1	0	2	0	0	0	0	0	0
UUK	5	0	2	0	3	0	0	5	0	0	0	0	0	0
VSV	11	2	2	0	1	3	6	8	3	0	0	0	0	0
WAL	1	0	0	1	0	0	0	1	0	0	0	0	0	0
WAR	2	0	0	2	0	0	0	2	0	0	0	0	0	0
Ungrouped	93	32	20	14	4	10	21	88	2	4	0	0	0	0
Totals	487	127	101	61	42	101	146	420	48	16	3	1	0	0

Table 35. Number of Viruses Isolated From Wild Caught Arthropods

Antigenic Group	Total in Group	Isolated From:					No. of Classes Involved		
		Mosq.	Ticks	Phlebotomine Flies	Culicoides	Other	1	2	3
A	26	25	1	0	1	5	22	2	2
AHS	1	0	0	0	1	0	1	0	0
B	64	30	17	0	0	2	41	4	0
BAK	2	2	1	0	0	0	1	1	0
BHA	1	0	1	0	0	0	1	0	0
BLU	1	0	0	0	1	0	1	0	0
BTK	2	1	0	0	0	0	1	0	0
Bunyamwera Supergroup	ANA	5	5	0	0	0	5	0	0
	ANB	2	2	0	0	0	2	0	0
	BUN	22	22	0	0	2	20	2	0
	BWA	2	2	0	0	0	2	0	0
	C	12	12	0	0	0	12	0	0
	CAL	13	13	0	0	0	12	1	0
	CAP	8	7	0	0	0	7	0	0
	GAM	3	3	0	0	0	3	0	0
	GMA	12	9	0	1	0	8	1	0
	KOO	2	2	0	0	0	2	0	0
	MNT	2	1	0	0	0	1	0	0
	OLI	3	3	0	0	0	3	0	0
	PAT	6	6	0	0	0	6	0	0
	SIM	21	10	0	0	11	11	5	0
TETE	5	0	2	0	0	2	0	0	
TUR	4	4	0	0	0	4	0	0	
SBU	1	0	0	0	0	1	0	0	
CGL	12	1	0	8	0	0	9	0	0
CTF	2	0	2	0	0	0	2	0	0
COR	3	3	0	0	0	0	3	0	0
EHD	2	0	0	0	0	0	0	0	0
EUB	3	3	0	0	1	0	2	1	0
HP	3	3	0	0	0	0	3	0	0
KSO	3	0	3	0	0	0	3	0	0
KEM	16	0	16	0	0	0	16	0	0
KWA	1	1	0	0	0	0	1	0	0
MAL	2	2	0	0	0	0	2	0	0
MAP	4	4	0	0	0	0	4	0	0
MBG	2	0	0	0	0	0	0	0	0
MOS	8	3	1	1	0	0	5	0	0
MTY	3	0	0	0	0	0	0	0	0
Nairoviruses	CHF-CON	4	0	4	0	1	3	1	0
	DGK	5	0	5	0	0	5	0	0
	HUG	4	0	4	0	0	4	0	0
	NSD	3	2	3	0	1	1	1	1
	QYB	2	0	2	0	0	2	0	0
SAK	5	0	5	0	0	0	5	0	0
NDO	1	1	0	0	0	0	1	0	0
NYM	1	0	1	0	0	0	1	0	0
PAL	7	3	0	0	4	0	7	0	0
PHL	34	6	0	21	0	0	23	2	0
QRF	2	0	2	0	0	0	2	0	0

Table 35. (Continued) Number of Viruses Isolated From Wild Caught Arthropods

Antigenic Group	Total in Group	Isolated From:					No. of Class- es. Involved		
		Mosq.	Ticks	Phleboto- mine Flies	Culi- coides	Other	1	2	3
RABIES	2	0	0	0	1	0	1	0	0
SAW	3	0	3	0	0	0	3	0	0
TCR	10	1	1	0	0	3	3	1	0
THO	1	0	1	0	0	0	1	0	0
TIM	3	0	0	0	0	0	0	0	0
TR	2	0	0	0	0	0	0	0	0
UPO	2	0	2	0	0	0	2	0	0
UUK	5	0	5	0	0	0	5	0	0
VSV	11	5	0	4	0	2	5	3	0
WAL	1	0	0	0	1	0	1	0	0
WAR	2	1	0	0	2	0	1	1	0
Ungrouped	93	42	16	3	3	1	55	5	0
Totals	487	240	98	38	30	15	350	31	3

Table 36. Number of Viruses Isolated From Naturally Infected Vertebrates

Anti- genic- Group	Total in Group	Other Pri- mates	Rod- ents	Birds	Bats	Marsu- pials	Live- stock	All others	No. of Classes Involved							
									1	2	3	4	5	6		
A	26	11	2	6	11	4	6	6	3	7	4	3	3	1	1	
AHS	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	
B	64	28	4	19	16	13	1	5	6	29	8	5	4	2	1	
BAK	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0	
BHA	1	1	0	1	0	0	0	1	1	0	0	0	1	0	0	
BLU	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	
BTK	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0	
Bunyamwera Supergroup	ANA	5	1	1	0	0	0	0	0	0	1	0	0	0	0	
	ANB	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
	BUN	22	5	1	5	1	0	0	1	3	8	4	0	0	0	0
	BWA	2	1	0	0	0	0	0	0	0	1	0	0	0	0	0
	C	12	10	0	8	0	1	5	0	1	2	5	3	1	0	0
	CAL	13	3	0	4	0	0	0	0	1	4	2	0	0	0	0
	CAP	8	0	0	4	0	0	1	0	0	3	1	0	0	0	0
	GAM	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	GMA	12	2	0	8	2	2	4	0	0	5	1	1	2	0	0
	KOO	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	MNT	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OLI	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	PAT	6	0	0	3	0	0	0	0	0	3	0	0	0	0	0
	SIM	21	2	1	0	4	0	0	8	4	13	3	0	0	0	0
TETE	5	0	0	0	5	0	0	0	0	5	0	0	0	0	0	
TUR	4	0	0	0	2	0	0	0	1	1	1	0	0	0	0	
SBU	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	
CGL	12	1	0	1	0	0	0	0	2	4	0	0	0	0	0	
CTF	2	1	0	1	0	0	0	0	1	0	0	1	0	0	0	
COR	3	0	0	0	1	0	0	0	0	1	0	0	0	0	0	
EHD	2	0	0	0	0	0	0	1	1	2	0	0	0	0	0	
EUB	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
HP	3	0	0	0	2	0	0	0	0	2	0	0	0	0	0	
KSO	3	0	1	0	1	0	0	0	1	3	0	0	0	0	0	
KEM	16	1	0	1	1	0	0	1	0	0	2	0	0	0	0	
KWA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
MAL	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
MAP	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
MBG	2	2	0	0	0	0	0	0	0	2	0	0	0	0	0	
MOS	8	0	0	1	2	1	0	0	3	7	0	0	0	0	0	
MTY	3	0	0	0	3	0	0	0	0	3	0	0	0	0	0	
Nairo- viruses	CHF-CON	4	2	0	0	0	0	1	1	1	1	0	0	0	0	
	DGK	5	0	0	0	0	0	0	0	0	0	0	0	0	0	
	HUG	4	0	0	0	1	0	0	0	1	0	0	0	0	0	
	NSD	3	3	0	1	0	0	0	2	1	1	1	1	0	0	0
	QYB	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0
SAK	5	0	0	0	1	0	0	0	0	1	0	0	0	0	0	
NDO	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	
NYM	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	
PAL	7	0	0	0	0	0	0	4	0	4	0	0	0	0	0	
PHL	34	8	0	8	2	0	2	1	2	15	4	0	0	0	0	
QRF	2	1	0	0	1	0	0	0	0	0	1	0	0	0	0	
RABIES	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	

Table 36. (Continued) Number of Viruses Isolated From Naturally Infected Vertebrates

Anti- genic- Group	Total in Group	Other Pri- mates	Rod- ents	Birds	Bats	Marsu- pials	Live- stock	All others	No. of Classes Involved						
									1	2	3	4	5	6	
SAW	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TCR	10	3	0	9	0	1	0	1	7	2	1	0	0	0	0
THO	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0
TIM	3	0	0	0	0	0	0	3	3	0	0	0	0	0	0
TR	2	0	1	0	0	0	0	0	1	0	0	0	0	0	0
UPO	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UUK	5	0	0	1	1	0	0	0	0	1	0	0	0	0	0
VSV	11	4	0	2	0	0	1	3	2	2	5	0	0	0	0
WAL	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WAR	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ungrouped	93	8	1	10	13	11	1	2	2	43	2	0	0	0	0
Totals	487	100	13	94	72	35	21	39	40	193	50	15	11	3	2

Table 37. Number of Viruses Associated with Naturally or Laboratory Acquired Disease in Man

Antigenic Group	Total in Group	In Nature	Lab. Infection	Either or Both Number	Percent
Group A	26	11	8	12	46.2
Afr. horsesickness	1	0	0	0	
Group B	64	28	25	31	48.4
Bakau	2	0	0	0	
Bhanja	1	0	1	1	100.0
Bluetongue	1	0	0	0	
Boteke	2	0	0	0	
Anopheles A	5	1	0	1	20.0
Anopheles B	2	0	0	0	
Bunyamwera	22	5	2	6	27.3
Bwamba	2	1	0	1	50.0
C	12	10	2	10	83.3
California	13	7	0	7	53.8
Capim	8	0	0	0	
Gamboia	3	0	0	0	
Guama	12	2	0	2	16.7
Koongol	2	0	0	0	
Minatitlan	2	0	0	0	
Olifantsvlei	3	0	0	0	
Patios	6	0	0	0	
Simbu	21	2	1	2	9.5
Tete	5	0	0	0	
Turlock	4	0	0	0	
SBU	1	0	0	0	
Changuinola	12	1	0	1	8.3
Colorado tick fever	2	1	1	1	50.0
Corriparta	3	0	0	0	
Epizoot. hem. dis.	2	0	0	0	
Eubenangee	3	0	0	0	
Hart Park	3	0	0	0	
Kaisodi	3	0	0	0	
Kemerovo	16	1	1	1	6.3
Kwatta	1	0	0	0	
Malakal	2	0	0	0	
Mapputta	4	0	0	0	
Marburg	2	2	2	2	100.0
Matariya	3	0	0	0	
Mossuril	8	0	0	0	
CHF-Congo	4	2	2	2	50.0
Dera Ghazi Khan	5	0	0	0	
Hughes	4	0	0	0	
Nairobi sheep dis.	3	3	2	3	100.0
Qalyub	2	0	0	0	
Sakhalin	5	0	0	0	
Nyando	1	1	0	1	100.0
Nyamanini	1	0	0	0	
Palyam	7	0	0	0	
Phlebotomus fever	34	7	1	7	20.6
Quaranfil	2	1	0	1	50.0
Rabies	2	0	0	0	

Table 37. (Continued) Number of Viruses Associated with Naturally or Laboratory Acquired Disease in Man

Antigenic Group	Total in Group	In Nature	Lab. Infection	Either or Both Number	Percent
Sawgrass	3	0	0	0	
Tacaribe	10	3	6	6	60.0
Tanjong Rabok	2	0	0	0	
Thogoto	1	1	0	1	100.0
Timbo	3	0	0	0	
Upolu	2	0	0	0	
Uukuniemi	5	0	0	0	
Vesicular stom.	11	4	3	5	45.5
Wallal	1	0	0	0	
Warrego	2	0	0	0	
Ungrouped	93	7	0	7	7.5
Totals	487	101	57	111	22.8

Table 38. Evaluation of Arthropod-Borne Status of 487 Registered Viruses (SEAS)

Anti- genic Group	Total in Group	Arbo- virus	Prob- ably Arbo- virus	Pos- sible Arbo- virus	Prob- ably not Arbo- virus	Not Arbo- virus	Arbo or Probably Arbo		Not or Probably Not Arbo	
							No.	%	No.	%
A	26	16	5	5	0	0	21	84.0	0	
AHS	1	1	0	0	0	0	1	100.0	0	
B	64	30	10	17	2	5	40	62.5	7	10.9
BAK	2	0	1	1	0	0	1	50.0	0	
BHA	1	0	1	0	0	0	1	100.0	0	
BLU	1	1	0	0	0	0	1	100.0	0	
BTK	2	0	0	2	0	0	0		0	
Bunyamwera Supergroup	ANA	5	0	2	3	0	2	40.0	0	
	ANB	2	0	0	2	0	0		0	
	BUN	22	8	7	7	0	0	15	68.2	0
	BWA	2	1	1	0	0	0	2	100.0	0
	C	12	10	2	0	0	0	12	100.0	0
	CAL	13	10	1	2	0	0	11	84.6	0
	CAP	8	4	2	2	0	0	6	75.0	0
	GAM	3	0	0	3	0	0	0		0
	GMA	12	5	4	3	0	0	9	75.0	0
	KOO	2	0	2	0	0	0	2	100.0	0
	MNT	2	0	1	1	0	0	1	50.0	0
	OLI	3	0	0	3	0	0	0		0
	PAT	6	1	2	3	0	0	3	50.0	0
	SIM	21	3	5	13	0	0	8	38.1	0
	TETE	5	0	1	4	0	0	1	20.0	0
TUR	4	1	2	1	0	0	3	75.0	0	
SBU	1	0	0	1	0	0	0		0	
CGL	12	0	1	11	0	0	1	8.3	0	
CTF	2	1	0	1	0	0	1	50.0	0	
COR	3	0	1	2	0	0	1	33.3	0	
EHD	2	0	1	1	0	0	1	50.0	0	
EUB	3	0	0	3	0	0	0		0	
HP	3	0	1	2	0	0	1	33.3	0	
KSO	3	0	2	1	0	0	2	66.7	0	
KEM	16	0	3	13	0	0	3	18.8	0	
KWA	1	0	0	1	0	0	0		0	
MAL	2	0	0	2	0	0	0		0	
MAP	4	0	1	3	0	0	1	25.0	0	
MBG	2	0	0	0	2	0	0		2	100.0
MOS	8	0	0	7	1	0	0		1	12.5
MTY	3	0	0	3	0	0	0		0	
Nairo- viruses	CHF-CON	4	2	0	2	0	2	50.0	0	
	DGK	5	0	0	5	0	0		0	
	HUG	4	1	1	2	0	0	2	50.0	0
	NSD	3	1	1	1	0	0	2	66.7	0
	QYB	2	0	0	2	0	0	0		0
	SAK	5	0	1	4	0	0	1	20.0	0

Table 38 (Continued)

Anti- genic Group	Total in Group	Arbo- virus	Prob- ably Arbo- virus	Pos- sible Arbo- virus	Prob- ably not Arbo- virus	Not Arbo- virus	Arbo or Probably Arbo		Not or Probably Not Arbo	
							No.	%	No.	%
NDO	1	0	1	0	0	0	1	100.0	0	
NYM	1	0	1	0	0	0	1	100.0	0	
PAL	7	0	2	5	0	0	2	28.6	0	
PHL	34	4	11	19	0	0	15	44.1	0	
QRF	2	2	0	0	0	0	2	100.0	0	
RABIES	2	0	1	0	0	1	1	50.0	1	50.0
SAW	3	0	0	3	0	0	0		0	
TCR	10	0	0	0	1	9	0		10	100.0
THO	1	0	1	0	0	0	1	100.0	0	
TIM	3	0	0	3	0	0	0		0	
TR	2	0	0	2	0	0	0		0	
UPO	2	0	0	2	0	0	0		0	
UUK	5	1	1	3	0	0	2	40.0	0	
VSV	11	3	1	7	0	0	3	27.3	0	
WAL	1	0	0	1	0	0	0		0	
WAR	2	0	0	2	0	0	0		0	
Ungrouped	93	4	10	71	5	3	13	14.0	8	8.6
TOTALS	487	110	91	257	11	18	199	40.9	29	6.0

APPENDIX I

Summary Description of Recommended Practice and Containment Levels for Arboviruses and Certain Other Viruses of Vertebrates^a (6).

Level	Laboratory Practices	Primary Containment	Secondary Containment
1	Standard microbiological practices are required.	None. Open bench.	None required.
2	Care required to limit aerosols and contamination. Limited access. ^c	Class I or II BSC ^b required for aerosol producing procedures.	Designed to facilitate cleaning and disinfection.
3	All virus materials contained. Special lab gowns required.	Class I or II BSC or equivalent required for all manipulations of infectious materials.	Restricted access, ^d air lock facility, controlled unidirectional air flow. Exhaust air discharged away from building. Work with certain viruses indicated by an * requires HEPA filtration of exhaust air.
4	Rigorous containment of all virus manipulations. Change of clothing and shower required.	Class I or II BSC adequate for work with infectious materials if all laboratory personnel are immune or insusceptible. Otherwise, Class III BSC or one-piece positive pressure suits are required.	Facility equivalent to separate building. Includes shower facilities, heat-treated biowaste, HEPA filtration of all exhaust air, double-door autoclaves.

^a There are also SALS recommendations concerning vector and vertebrate studies.

^b BSC = Biological Safety Cabinets.

^c Access limited to persons with knowledge of the biohazard potential.

^d Access restricted to persons with programmatic or support requirements for entry.

APPENDIX II

Explanation of Symbols Used to Define Basis for Assignment of Viruses to Levels of Practice and Containment (6).

- S = Results of SALS surveys and information from the Catalogue.
- IE = Insufficient experience with virus; i.e., experience factor from SALS surveys was less than 500 in laboratory facilities with low biocontainment.
- A = Additional criteria 1, 2, 3, 4, etc.
1. Disease in sheep, cattle or horses.
 2. Fatal human laboratory infection, 1978, probably aerosol (14). This is recognized to be a unique incident in a long history of work with SFV under minimal biocontainment conditions. However, since the virulence characteristics of the strain responsible in this case require further study and the prevalence of subclinical infections in laboratories working with SFV remains unknown, the committee recommends Level 3 until further information is available warranting reconsideration at a lower level.
 3. Extensive laboratory experience and mild nature of aerosol laboratory infections justifies Level 2.
 4. Placed in Level 4 based on the close antigenic relationship with a known Level 4 agent, Russian spring-summer encephalitis, plus insufficient laboratory experience.
 5. Level 2 arenaviruses are not known to cause serious acute disease in man and are not acutely pathogenic for laboratory animals, including primates. Survey experience is sufficient to conclude that laboratory aerosol infection does not occur in the course of routine work with cell cultures and animals not subject to chronic infection. In view of a reported high frequency of laboratory aerosol infection that occurred in workers manipulating high concentrations of Pichinde virus, it is strongly recommended that work with high concentrations of Level 2 arenaviruses be done at Level 3.
 6. Level assigned to prototype or wild-type virus. A lower level may be recommended for laboratory strains or geographic variants of the virus with well-defined reduced virulence characteristics, as mentioned in the text.

REPORT FROM THE MEDICAL RESEARCH COUNCIL OF NEW ZEALAND VIRUS
RESEARCH UNIT, MICROBIOLOGY DEPARTMENT, UNIVERSITY OF OTAGO,
DUNEDIN, NEW ZEALAND.

Studies on strains of Ross River virus

Two strains of Ross River virus, one (F9073) isolated early and the other (F9006) isolated late in the large epidemic which occurred in Fiji from April 1979 until January 1981, were compared by a variety of tests.

No differences could be found between the two strains by standard serological tests including neutralization, kinetic haemagglutination-inhibition, and reciprocal titrations with an enzyme immunoassay. The strains appeared to be identical by electron microscopy.

In suckling mice, Ross River virus produces a severe myositis which manifests as a flaccid hindlimb paralysis and eventual death. Examination of infected suckling mice using indirect fluorescent antibody staining and titration of selected tissues showed that higher levels of virus F9073 were found in the striated muscle tissue ($10^{7.5}$ TCID₅₀ per g) than in the brain ($10^{6.2}$ TCID₅₀ per g). When the two strains were titrated simultaneously in cell culture and in litters of suckling mice, F9006 was found to be nearly 500 times more virulent for mice than was F9073, when the titres were based on TCID₅₀ standards. A single passage of F9073 through tissue cultures (Vero) was sufficient to produce this reduction in virulence for mice.

A time-course study showed that F9006 produced an earlier viraemia and a higher level of virus in brain and muscle than did F9073, the greatest difference being in the rate of increase of virus in the brain tissue.

Preliminary oligonucleotide fingerprint mapping of RNA extracted from these two strains indicates that a small number of fragment differences do exist, particularly among the largest fragments.

The significance of the differences found between the early and late epidemic strains is difficult to assess, but the fact that differences, which are not detectable by standard laboratory serology, do occur, is worthy of further study.

(C.W.Bell, T.Maguire, F.J.Austin)

Report from the Chinese Academy of Medicine Sciences, Institute of Virology and Beijing The First Infectious Hospital, Beijing, People's Republic of China

USE OF McAb AGAINST JE VIRUS FOR EXPERIMENTAL TREATMENT OF MICE INFECTED BY JE VIRUS

Three-week-old mice were infected subcutaneously and intraperitoneally by JE virus. The infected mice were treated with 51-8 McAb against JE virus once with 1/2-1/5 dilution before and after infection.

In before 2 hr. and after 4 hr. infection group, 100% protection rate was observed; in 24 hr. postinfection group 60-100% protection rate; in 48 hr. postinfection group 60-83% protection rate; in 5 days postinfection group 20-29% protection rate was observed. At that time some of mice became ill and died. All of the results in the different treatment groups are significant (P 0.05).

It is interesting to find when the same group of mice was treated with immune rabbit sera, only before 2 hr. and after 4 hr. infection gave as good protection rate as 51-8 did. Insignificant results (P 0.05) were observed with another group.

From the growth curve of the virus in the brain of the mice when infected by the i.p. route, no virus could be detected by the plaque assay method in 2 days postinfection, but 3 days after infection the virus in the control group grew rapidly, on 5 days and 8 days postinfection the titer of virus reached $\text{Log } 10^{5.6}$ and 9.0 PFU respectively. But in 48 hr. and 72 hr. postinfection treatment group with 51-8 McAb, the peak of viral growth curve had disappeared. Fourteen days postinfection no virus could be found in survival mice treated by 51-8 McAb. In 5 days postinfection, the virus could be isolated in each brain of infected mice and the titer of the virus was around 5.0-10.7 Log_{10} PFU and the mean titer was 8.25 Log_{10} PFU. It seems McAb may kill the virus in the cell.

(Chen Bo Quan¹, Zhou Guo Fang¹, Liu Qin Zhi¹, Li Guo Chui², Pu Xiu Zhen²)

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

JAPANESE ENCEPHALITIS VIRUS (JEV) ISOLATION REPORT FROM THE BLOOD OF A CHILD WITH FEATURES OF ACUTE ENCEPHALITIS.

During the course of virological investigation of an encephalitis case admitted in the Infectious Disease Hospital (I.D), Calcutta, India, a strain of JEV could be recovered in infant swiss mice and vero cell culture from the serum both on the 10th and 20th day of illness. The isolation of JEV in the blood along with the prolonged viraemia are extremely rare events; the commonest source of isolation being post mortem human brains & mosquitoes.

The patient, a 12 year old boy living in rural area of West Bengal had history of muscle and joint pains followed by remittent type of fever (99° - 102°F). Gradually, he developed headache with severe attacks of tonic spasms of limbs and subsequently passing into a state of confusion and general deterioration and was admitted to the hospital, on 6th day of his illness. The clinical examinations revealed; the patient was unconscious with bizarre type of movement of the limbs, neck rigidity and positive kerning's sign. The patient's condition further worsened by the next 3-4 weeks with development of bed sores and extreme dehydration inspite of supportive therapy; and finally he died of pulmonary oedema.

C.S.F was clear and normotensive with biochemical values within normal limits. Malarial parasite was absent in peripheral blood smear which revealed leucocytosis with preponderance of polymorphs. A mouse pathogenic as well as cytopathogenic agent could be isolated from 10th and 20th day of serum samples which could be identified as JE virus by quick C.F. and finally by neutralization tests. The blood sample collected on 30th day of illness however yielded no virus.

The results of sero investigation are provided in the following table.

Table

Results of serological tests (HI,CF & N) with sequential serum samples.

Day of illness	HI				CF				N	
	Chik.	Den-2	JE	WN	Chik	Den-2	JE	WN	JE	WN
10th day	< 20*	< 20	40	40*	< 4*	< 4	< 4	< 4	< 10*	< 10
20th day	< 20	< 20	< 20	< 20	< 4	< 4	< 4	< 4	< 10	< 10
30th day	< 20	< 20	< 20	< 20	< 4	< 4	< 4	< 4	< 10	< 10

* Titres, expressed as the reciprocal of highest serum dilution inhibiting haemagglutination, showing positive CF test and neutralizing 100 TCID₅₀ virus.

The isolation of JE virus from the 10th and 20th day serum samples indicate the persistence of viraemia in this particular case which is a rare event. It is interesting to note that the virus was present in the 10th day sample along with HI antibody in it, although there was no CF and neutralizing antibody in the sample. This seems to suggest that HI antibody alone could not probably arrest the viraemic phase in the patient. The uniform absence of CF and N antibodies in the circulation and HI antibody from 20th day onwards might probably be one of the factors for non clearance of the virus from the circulation. The uniform absence of N and CF antibodies is difficult to explain. The subsequent fall of HI antibody however might be related to gross impairment of nutritional status of the patient during the unconscious state and also due to heavy loss of protein and fluids through extensive bed sores. The presence of an immunodeficient state in the patient could be another possibility.

(M.S. Chakraborty, J. Chandra, S.K. Chakravarti, K.K. Mukherjee, M. Bhattacharjee and A. Nag)

REPORT FROM THE UNITED STATES ARMY INSTITUTE OF INFECTIOUS DISEASES,
FORT DETRICK, FREDERICK, MARYLAND, USA, AND THE DEPARTMENT OF
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Cell Culture Isolation of Puumala Virus
The Etiologic Agent of Nephropathia Epidemica

Hemorrhagic fever with renal syndrome (HFRS) is the collective term applied to a large group of related diseases with world-wide distribution (1). Although Korean hemorrhagic fever (KHF) is perhaps the best known among these syndromes, the Fennoscandian type, Nephropathia epidemica (NE), is a well-recognized clinical entity throughout Scandinavian countries (2). Following the initial discovery of an HFRS antigen in the lungs of infected *Apodemus agrarius coreae* in Korea (3), the relationship of NE to HFRS was clearly established in that the sera from convalescent NE patients was shown to cross-react with the KHF antigen (4). Subsequent demonstration of an NE antigen in the lungs of the rodent host in Finland, *Clethrionomys glareolus* (5), confirmed that the etiologic agents of NE and KHF are antigenically related but not identical (6).

Hantaan virus, strain 76-118, is registered as the prototype virus of a group of recently discovered related agents believed to be associated with HFRS (7). This virus has been adapted to replication in cell culture (8) as have other agents belonging to this group. Repeated attempts to isolate the etiologic agent of NE in cell culture have been uniformly unsuccessful; however, animal passage by injecting antigen-positive rodent lung material into sero-negative *Clethrionomys glareolus* has been possible. In this report we describe the isolation of Puumala virus in cell culture and demonstrate virus characteristics relating it to NE as well as to other members of the HFRS group of agents.

The infectious material used in all isolation attempts consisted of lung tissue from a female *Clethrionomys glareolus* collected November 1981 near Sotkamo, Finland. Lung sections from this bank vole were found positive by immunofluorescence using convalescent sera from NE patients. Homogenates of these positive lungs were inoculated into sero-negative laboratory bank voles and lung sections from animals sacrificed at various time intervals examined by immunofluorescence. Antigen could be detected in the lungs of 10-40% of the voles at day 30 post-injection and beyond. A second passage of positive lung material was similarly positive beyond day 37, but the percentage of infected animals did not increase significantly (9). Positive lung suspensions from this second passage through *Clethrionomys glareolus* were inoculated directly into the lungs of colonized *Clethrionomys gapperi*. Approximately 80% of the *Clethrionomys gapperi* were infected as indicated by the presence of immunofluorescent antigen in the lungs. It was this positive lung material that was used for all subsequent cell culture adaptation studies. Three similar lung suspensions with comparable passage histories were also examined in cell culture, but virus isolation was unsuccessful.

All cell culture passage was performed using the VERO E6 cell line. This cell is listed as the VERO C1008 clone of VERO 76 cells and is available from the American Type Culture Collection as ATCC CRL 1586. The initial infection was accomplished by mixing equal volumes of a 10% positive vole lung suspension with approximately 10^6 freshly trypsinized VERO E6 cells. Following an initial 2-hour incubation with continuous mixing, the suspension was diluted 1:2 with growth medium and incubated as a stationary culture in a sealed T-25 flask at 37°C for 48 hours and the media changed. The medium used for all of these studies consisted of Eagle's Minimum Essential Medium supplemented with non-essential amino acids, 10% heated fetal bovine serum and antibiotics. At various intervals following this infection, the culture was washed, trypsinized and the cells "split" 1:3. Each of the resultant subcultures received 10^4 freshly trypsinized VERO E6 cells from an uninoculated culture at every passage. An aliquot of the "infected" cells was examined for NE antigen by indirect immunofluorescence. The cell culture passages and immunofluorescence results are detailed in Table 1.

TABLE 1

PASSAGE OF NEPHROPATHIA EPIDEMICA (PUUMALA VIRUS) IN CELL CULTURE

Passage No.	Day	Cell Split No.	Fluorescence	% Pos Cells
1	10	1	+++	50
1	16	2	++	20
1	21	3	+	<1
1	30	4	-	0
1	43	5	+	1
1	52	6	++	5
1	60	7	++	7
Day 60 Supernatant Fluids Used to Infect VERO E6 Monolayers				
2	66	1	+++	75
2	72	2	+++	80
Day 72 Supernatant Fluids Used to Infect VERO E6 Monolayers				
3	78	1	+++	90
3	84	2	+++	100

Following the adaptation procedure outlined above, virus containing supernatant fluids could be used as infectious inocula for confluent monolayers of VERO E6 cells and maximum titers of infectious virus harvested between 8-13 days post-infection. Virtually all cells were infected as indicated by the indirect fluorescent antibody test, yet no microscopic evidence of cytopathology could be observed. The virus appears stable upon storage at -70°C and repeated passage of this virus through these cells does not appear to result in a decrease in virus titer or the

percentage of infected cells. Seed virus preparations appear to be free of contaminating Reovirus as indicated by the absence of fluorescence using polyvalent Reovirus antisera and are apparently free of mycoplasma contamination as screened by electron microscopy.

Cell culture adapted Puumala virus has been shown to produce plaques on confluent monolayers of VERO E6 cells using procedures established for the plaque assay of Hantaan virus. Briefly, infected monolayers in T-25 flasks are covered with an overlay containing Minimal Essential Medium (Earles' Salts), 0.6% agarose, 10% heated fetal bovine serum, glutamine, non-essential amino acids and antibiotics. Following 8-13 days incubation at 37°C, the cells are stained with an overlay containing neutral red and the cells incubated for an additional 24-96 hours. Plaque development may be slow, often requiring 13-18 days total incubation, but distinct 1-2 mm plaques can be easily observed in detail sufficient for accurate titration of virus titers and plaque reduction neutralization assays. Virus titers approaching 10⁶ PFU/ml are routinely obtained using the procedures outlined above.

Puumala virus in cell culture appears antigenically identical to the Nephropathia epidemica antigen in wild vole lung sections in that immunofluorescence titers of convalescent NE patients are high, while those of KHF convalescent patients are very low but detectable. The fluorescence pattern of intracellular granules is indistinguishable from that of Hantaan virus infected cells. Plaque reduction neutralization data using both Hantaan and Puumala viruses and homologous antisera obtained by the infection of laboratory Wistar rats are shown in Table 2.

TABLE 2

PLAQUE REDUCTION NEUTRALIZATION OF HANTAAN AND PUUMALA VIRUSES

Antisera	Hantaan Virus	Puumala Virus
Rat anti-Puumala	<10*	1280
Rat anti-Hantaan	4000	<10
NE convalescent human	<20	1280
KHF convalescent human	1280	<10
Hantaan HMAF	2560	10

*Dilution of antisera resulting in >80% reduction of a virus dose of approximately 100 plaques.

Preliminary data on the characterization of Puumala virus suggest that it is similar to other Hantaan agents of the HFRS group. This virus appears to contain three segments of RNA and a conserved 3' terminal nucleotide sequence that is identical to other members of the group. A unique oligonucleotide fingerprint and the antigenic characteristics described above serve to emphasize that Puumala virus represents a new virus isolate that is related to Nephropathia epidemica. The adaptation

of this agent to cell culture should greatly increase the capability of the diagnostic laboratory with an interest in NE and HFRS.

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- (S. Hasty, J. Dalrymple, C. Schmaljohn, M. Brummer-Korvenkontio, A. Vaheri, and C.-H. von Bonsdorff)

The replication and eclipse-phase of the tick-borne encephalitis (TBE) virus in Dermacentor reticulatus nymphs

Survival of TBE virus in the epidermis of ticks is a common phenomenon for viruses as well as rickettsiae. After hatching, an eclipse-phase occurs which may last for several days or weeks. It is quite different in tick genera.

The aim of our study is to confirm the development of TBE virus and the length of eclipse-phase in D. reticulatus nymphs on electron micrographs.

Virological Procedures

D. reticulatus larvae used in experiment were fed on viraemic suckling white mice. Suckling mice 8-10 days old were used in experiment. To induce viraemia white mice were infected by a dose of 0.01 ml of 10 per cent brain suspension of TBE virus (strain 198). Titer of suspension reached the value of $10^{8.5}$ per 0.03 ml.

Engorged larvae (April 6, 1983) were kept in Erlenmeyer flasks in thermostat at room temperature. The titer of virus in engorged larvae ranged from 10^1 - 10^3 per 0.03 ml. The nymphs hatched April 14 to 16, 1983. Ten days later these nymphs were placed into feeding capsules. After 33 hours their feeding was interrupted and the nymphs were prepared for electron microscopy.

Electron Microscopy

The half fed nymphs were dissected longitudinally for fixation and transferred to fixative solution. Halves were fixed in 2.5 per cent glutaraldehyde in 0.2 M sodium cacodylate buffer pH 7.2 at temperature of 4°C for 2 hours. Postfixation lasted 4 hours in 1% osmium tetroxide in the same buffer at room temperature. Dehydration was carried out in increasing acetone concentrations and embedded in Araldit CY 212.

Ultrathin sections and micrographs were prepared in the laboratory of electron microscopy at the Institute of Poliomyelitis and Viral Encephalitides, Academy of Medical Sciences of the USSR, Moscow. Ultrathin sections were cut on a OM - Reichert ultramicrotome, and stained with uranyl acetate and lead citrate. Specimens were observed in a JEM - 100 B electron microscope.

TBE virus was found in D. reticulatus nymphs 10 days after hatching and 33 hours feeding on white suckling mice. The virus particles were localized in the lumen of vacuoli in epidermal cells and vacuoli in region of Golgi complex of e cells of the salivary gland. The virus particles occurring in vacuoli of epidermal cells were of 42-50 μ m in diameter. The virus titer in nymphs reached 10^2 - 10^4 per 0.03 ml.

The eclipse-phase in D. reticulatus viruliferous nymphs lasted approximately as long as their metamorphosis, 8-11 days.

(Nosek, J., Korelev, M. B., Čunichin, S. P., Kožuck, O., Čiampor, F.)

Haemorrhagic fever virus with renal syndrome
/HFRS/ in small rodents in Czechoslovakia

The disease that appeared 30 years ago in Czechoslovakia was found to be very similar to nephroso-nephritis and/or to haemorrhagic fever with renal syndrome. In 1954 three fatal cases have been reported in Eastern Slovakia. The clinical symptoms of the diseases were: high fever, hyperaemia of the skin, melaena, hematuria, albuminuria, oliguria and uraemia. Morphologically, the typical picture of nephroso-nephritis was found.

No etiologic agent however was detected so far by means of virus isolation attempts in Czechoslovakia.

Up today some small rodents, eg. Apodemus agrarius in Korea and Clethrionomys glareolus in Europe, have been proved to be natural hosts of HFRS.

Therefore we investigated small rodents as the hosts of haemorrhagic fever with renal syndrome in Eastern Slovakia. Small rodents /Clethrionomys glareolus, Apodemus flavicollis, Apodemus agrarius and Microtus arvalis/ were trapped alive into the traps of swedish type. In the laboratory, small rodents were autopsied and the lung specimens were collected. Cryostat sections of lung from small rodents were prepared for immunofluorescence. The sections were fixed with acetone and overlayed with serial dilution of serum. After 30 minutes at 37°C, slide glasses were washed, than overlayed with fluorescein isothiocyanate conjugates and kept 30 minutes at 37°C.

In 1982 and in 1983 years 124 lungs specimens of small rodents were collected in Eastern Slovakia /Dolica, Tarnava and Ruská Poruba localities/. We have continued our search for evidence of virus serologically related to haemorrhagic fever with renal syndrome in rodents in Slovakia.

By immunofluorescence method the etiologic agents of haemorrhagic fever with renal syndrome were detected in the lungs of small rodents: Clethrionomys glareolus, Apodemus agrarius and Apodemus flavicollis /Table 1/.

Two strains were isolated from Cl. glareolus, 6 strains from A. agrarius and 1 strains from A. flavicollis. The highest proportion of infected animals was found in Clethrionomys glareolus species trapped in the vicinity of Dolica cave.

Specific granular immunofluorescence was detected in the lungs of Cl. glareolus, A. flavicollis and A. agrarius by indirect immunofluorescence. Specific fluorescence was confirmed by the complement-fixation reaction. In the complement-fixation reaction, a higher cross-reactivity with antiserum to Epidemic nephropathy of Scandinavia than with antiserum to Korean haemorrhagic fever was observed.

/Grešíková, M., Rajčáni, J., Sekeyová, M.,
Brummer-Korvenkontio, M., Kožuch, O., La-
buda, M., Turek, R., Weissmann, P., Nosek,
J., Lysý, J./

Table 1

Detection of specific immunofluorescence /IF/ in the lungs
of small rodents caught in Eastern Slovakia

Species	Locality	No of examined species	No of positive by IF	% of positive
<u>Microtus</u> <u>arvalis</u>	Domica	2	0	0
<u>Clethrionomys</u> <u>glareolus</u>	Domica	7	2	28,5
<u>Apodemus</u> <u>flavicollis</u>	Domica	7	0	0
<u>Apodemus</u> <u>agrarius</u>	Tarnava	26	3	11,5
<u>Apodemus</u> <u>flavicollis</u>	Tarnava	24	0	0
<u>Apodemus</u> <u>agrarius</u>	Ruská Poruba	40	3	7,5
<u>Apodemus</u> <u>flavicollis</u>	Ruská Poruba	17	1	5,8
<u>Microtus</u> <u>arvalis</u>	Ruská Poruba	1	0	0

REPORT FROM THE INSTITUTE FOR VERTEBRATE RESEARCH,
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CZECHOSLOVAKIA

A serological survey for Bhanja virus in Czechoslovakia

Following the detection of antibodies against Bhanja virus in goats, sheep (1) and man (2) in Czechoslovakia, we examined a larger number of vertebrate blood sera for the virus antibodies in various regions of this country. Serum samples were collected in the years 1975-1983 and stored at -20°C . Human sera were selected from: (i) adult patients with meningoencephalitis or "virosis", (ii) goat breeders, and (iii) forest workers. Neutralization tests (TNT) were carried out in tube cultures of Vero cells with ca.100 (50-300) TCD₅₀ of Bhanja virus (strain Bg 326), hemagglutination-inhibition tests (HIT) were performed on microplates with 4 HA units of saccharose-acetone antigen of the strain Bg 335/336. Prior to examination, the sera were either inactivated for 30 m. at 56°C (TNT) or acetone-extracted and absorbed with goose erythrocytes (HIT). For the purpose of this survey, sera were scored "positive" when their antibody titres reached at least 1:16 in TNT or 1:10 in HIT.

There was a very good agreement in the results between both tests (TNT, HIT). Antibodies against Bhanja virus were detected frequently in the district Rožňava (eastern Slovakia), while only sporadically in the other districts (Tab.). The reason might be that the tick Haemaphysalis punctata (primary vector of the virus in Europe) occurs in Rožňava district but is absent from the other districts examined. The highest seropositivity in that district was found in goats (38.5%), followed by sheep (26.7%), cows (16.7%) and humans (9.5% - mostly goat breeders). Recent circulation of Bhanja virus is indicated by the presence of antibodies even among 1-yr-old sheep and calves.

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Tab. Antibodies against Bhanja virus in Czechoslovakia

Sera	District	No. tested	Positive			
			TNT	%	HIT	%
Man	Rožňava	74	7	9.5	5	6.8
	Svidník	11	0	-	0	-
	Znojmo	112	0	-	0	-
	Břeclav	62	0	-	0	-
	Total	259	7	2.7	5	1.9
Sheep	Trebišov	137	3	2.2	4	2.9
	Michalovce	259	1	0.4	0	-
	Humenné	239	4	1.7	4	1.7
	Rožňava	120	32	26.7	30	25.0
	Olomouc	35	1	2.9	1	2.9
	Hradec Králové	11	0	-	0	-
	Total	801	41	5.1	39	4.9
Goat	Rožňava	26	10	38.5	10	38.5
	Svidník	28	0	-	0	-
	Total	54	10	18.5	10	18.5
Cow	Rožňava	60	10	16.7	7	11.7
Grand Total		1174	68	5.8	61	5.2

(Z. Hubálek, Z. Juřicová)

Cooperative effects between monoclonal antibodies to different epitopes on the tick-borne encephalitis (TBE) virus glycoprotein.

By the use of monoclonal antibodies we have defined eight distinct epitopes on the structural glycoprotein of TBE virus. Mutual blocking assays of antibody pairs to define overlapping and nonoverlapping determinants revealed a clustering of 7 of the 8 epitopes into two major antigenic domains (A and B). These domains have different structural properties, domain A being strongly conformation-dependent in contrast to domain B, which is resistant to denaturing agents like SDS or guanidine-HCl. Epitopes of this domain could also be located on proteolytic fragments with molecular weights of approximately 9000 daltons. In the course of simultaneous antibody binding studies it became apparent that enhancement of antibody binding by a second antibody to a distant site was a frequent event, revealing a complex network of interactions between antibodies directed against distinct nonoverlapping epitopes. This cooperative effect can be either unidirectional or bidirectional. Overall, antibodies to the conformation-dependent domain A are enhanced by antibodies to domain B but by themselves do not enhance the binding of other antibodies. Most epitopes of the denaturation-resistant domain B cannot be enhanced and therefore represent unidirectional enhancers. Bidirectional enhancement was observed within domain A and between certain other antibody pairs. The binding data were quantitatively evaluated by Scatchard analysis which revealed that the observed phenomenon is due to a two- to sixfold increase of antibody avidity. This could also be demonstrated with Fab fragments purified after papain digestion thus excluding that the cooperative effect is dependent on antibody bivalency. We therefore assume that binding of antibodies to certain epitopes on the TBE virus glycoprotein induces conformational changes in distant parts of the molecule which can result in increased avidity of antibodies to conformationally changed epitopes.

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(F. X. Heinz)

UK

METHODS OF ISOLATING AND CHARACTERISING ARBOVIRUSES

Mice and various cell lines were used to isolate orbiviruses and viruses of the bunyaviridae from ticks collected from a seabird colony on Great Saltee Island, Eire.

Three methods of virus isolation were compared: inoculation of tick homogenates into mice, into various cell lines, and into mice followed by passage in various cell lines. None of the methods produced all 13 of the viruses isolated (Table 1). Inoculation of tick homogenates into various cell lines was the most successful, yielding 9 virus isolations. Mice appeared to be the least sensitive system, producing only 3 isolations based on clinical evidence of infection. However, when inoculated mouse brain was then passaged in various cell lines, 7 of 12 isolations were made. Thus virus in 4 tick homogenates replicated in mouse brain without inducing overt clinical signs. The successes of the different methods of virus isolation appeared to vary depending on virus type. Whereas all Kemerovo and Uukuniemi serogroup viruses except one were obtained by inoculation of cell cultures, the virus of the Hughes serogroup (GS80-3) and the two ungrouped viruses (in GS80-7 and -8) were only isolated by inoculation of mice followed by passage in cell culture.

Although BHK cell cultures produced the greatest number of virus isolations from tick homogenates (Table 2), Xenopus cell cultures yielded the highest number from inoculated mouse brain (Table 3). This may relate to the type of virus isolated by this method since Xenopus cells were particularly sensitive to viruses of the bunyaviridae.

A useful early indication as to the identity of isolated viruses was obtained by comparative plaquing in Vero and Xenopus cells. Viruses of the Uukuniemi serogroup did not produce plaques in Vero cells; the Hughes group virus produced plaques more consistently and at higher titres in Xenopus cells compared with Vero, and Kemerovo group viruses in Vero compared with Xenopus cells.

Electron microscopic examination of inoculated cell cultures and mouse brain was not a sensitive method of detecting viruses, generally requiring titres of at least $5.0 \log_{10}$ pfu/ml to ensure that virus was detected. However, this method was useful in substantiating results of complement fixation tests, and showed greater versatility in detecting two putative members of the bunyaviridae in GS80-7 and -8 that were not detected by CFT. Attempts to further isolate these two viruses were unsuccessful.

The results show that methods used for isolating viruses from field material vary in efficiency of isolating viruses and are selective in respect to the type of virus(es) isolated. Thus the results indicate that in order to screen field material for the presence of viruses the use of more than one method of isolating viruses is advisable.

(P. A. Nuttall, S. R. Moss and D. Carey)

Table 1. Details of viruses isolated from ticks

Code no. GS80-	Tick pool	Virus*	Method of Isolation**		
			mice	cell	mice & cell
<u>Ixodes uriae</u>					
4a	5 ♀♀	KEM	+	-	-
b	♀♀	UUK	+	+	+
7	5 ♂♂	KEM	-	+	-
		bunya	-	-	+
8	10 ♀♀	KEM	-	+	+
		bunya	-	-	+
9	3 ♀♀	KEM	-	+	-
10	1 ♀	UUK	+	+	+
13	5 ♂♂	UUK	-	+	+
<u>Ixodes rothschildi</u>					
11	9 nymphs	UUK	-	+	ND
<u>Ornithodoros maritimus</u>					
3	5 ♂♂	HUG	-	-	+
5	100 eggs (approx.)	KEM	-	+	-
6	5 ♂♂	KEM	-	+	-

* Type of virus isolated from tick pool. Kemerovo (KEM), Uukuniemi (UUK) and Hughes (HUG) serogroups as indicated by complement fixation tests; morphology of bunyaviridae (bunya) observed by electron microscopy. All viruses except GS80-3, -5 and -6 were re-isolated from the original tick pools (stored at -70°C) up to 6 months after the initial isolations. Tick pools GS80-4, -7 and -8 contained two types of viruses; the two viruses in GS80-4 were separated and designated GS80-4a and -4b.

** Virus isolation (+) by inoculation of tick pool into either mice, cell culture, or mice followed by passage in cell culture. -, virus not isolated; ND, not done.

Table 2. Results of inoculating mice and cell lines with tick pools

tick pool GS80-	mice* (day sick)	cell lines**				
		BHK	Vero	CEF	CEL	<u>Xenopus</u>
3	healthy	-	-	-	-	-
4a	9	-	-	-	-	-
4b	10-12	-	-	+	+	+
5	healthy	+	-	-	-	-
6	healthy	+	-	-	-	-
7	healthy	+	-	-	-	-
8	healthy	+	-	-	-	-
9	healthy	+	-	-	-	-
10	5	+	+	+	+	+
11	healthy	+	+	+	+	+
13	healthy	-	+	-	+	-

* intra-cerebral inoculation of 2 day-old mice with clarified tick pool homogenates; observed for a period of 21 days after inoculation.

** inoculation of various cell lines with clarified tick homogenates. +, virus(es) isolated; -, virus not isolated.

Table 3. Virus isolation from inoculated mouse brain

MB + tick pool GS80-*	cell line**			
	BHK	Vero	CEF	<u>Xenopus</u>
3	+	-	-	+
4a	-	-	-	-
4b	-	-	+	+
5	-	-	-	-
6	-	-	-	-
7	+	-	-	+
8	+	-	+	+
10	-	+	+	+
11	-	-	-	-
13	+	-	-	+

* inoculation of various cell lines with mouse brain (MB) from mice inoculated intracerebrally with clarified tick pool homogenate.

** +, virus(es) isolated; -, virus not isolated.

Report from the Neurovirology Unit, Rayne Institute, St. Thomas' Hospital, London SE1, 7EH, U.K.

Our main interest is the study of the pathogenesis of central nervous system (CNS) virus diseases using different arboviruses. We are particularly concerned with the effects of the avirulent strain (A774) of the togavirus Semliki Forest (SF) in adult mice following an intraperitoneal inoculation. This infection produces a subacute meningoencephalomyelitis with focal demyelination throughout the CNS, which subsequently recovers (Webb et al 1979).

The work is concentrated on attempting to define the mechanism by which the demyelination is produced. To date it appears to be immunologically mediated.

Studies involving immunosuppression by total body irradiation have shown that despite high and persistent virus titres in the brain no demyelination results. Infection of athymic nude mice does not result in CNS demyelination although persistent virus titres in the brain have been shown up to 70 days post infection. Transfer of spleen cells from immuno-competent litter-mates to these nude mice prior to or post SFV infection results in demyelination. The demyelination may be brought forward in time by transfer of spleen cells from mice sensitised to SFV (Fazakerley et al 1982). The spleen cells from these reconstituted nude mice can be transferred to a second SFV infected nude mouse, again resulting in demyelination suggesting that this feature is T-cell dependent. Electron microscopical studies have shown before demyelination takes place lymphoblastic type cells invade the CNS and can be seen lying close to oligodendrocytes and myelin. Demyelination starts and macrophages take up the debris. (Pathak et al 1983). It is possible that these lymphoblastic cells initiate the myelin damage.

The nude mice produce IgM which terminates the viraemia, as in normal mice, but only on reconstitution is significant IgG synthesised (Suckling et al 1982). IgG against SFV in normal immunocompetent mice is known to be synthesised locally within the blood brain barrier and may be damaging. Experiments have shown that IgG levels in cerebrospinal fluid (CSF) remain raised for many weeks after infection (Parsons and Webb 1982a). The white cell count in the CSF also remains elevated (Parsons and Webb 1982b). Any role which IgG may have in the demyelination mechanism is uncertain though inoculations of mouse anti-SFV IgG peripherally and intracerebrally does not result in demyelination.

Many arboviruses are known to persist in the mammalian CNS tissue for long periods of time and in certain situations produce chronic changes such as gliosis seen up to 2 years following the tick-borne encephalitis virus Langat. Gliosis is also a chronic feature following SFV infection (Zlotnik et al 1971) and attempts to determine any latent infection of this

virus are being made.

SFV is a budding virus which incorporates host cell membrane components into the envelope during replication (Renkonen et al 1971; Richardson and Vance 1976). Of particular interest is the glycolipid content of the envelope. Brain derived SFV reacts in the elisa with immune serum made against the purified CNS glycolipids - galactocerebroside, glucocerebroside and ganglioside. SFV budding from brain cell cultures has been labelled with gold and ferritin using immune electron microscopy techniques, with immune serum against glucocerebroside. This suggests that the glycolipid antigens displayed on the SFV envelope are immunogenic. We are interested in whether the glycolipid in the envelope produces an autoimmune reaction against the glia from which it was derived. Such a reaction could be of importance in diseases such as multiple sclerosis which may be an autoimmune disease with a viral "triggering" mechanism.

Dual infections using arboviruses have shown that mice infected with the alphaviruses SFV or Sindbis are protected against a subsequent, normally lethal challenge with either the tick-borne encephalitis virus (Langat TP21 strain) and West Nile virus: both flaviviruses (Oaten et al 1976, 1980a). It is possible that the protection conferred results from cross immunity, not to viral antigens, but to host brain cell antigens, and possibly glycolipids common to the envelopes of these viruses as a result of replication in the same tissues. This may provide further evidence for the involvement of the immune reaction to glycolipids in arbovirus infections.

The effect of various drugs on the pathogenesis of SFV infection have been investigated. These include various antiviral drugs; Cyclosporin A which selectively suppresses the reaction of T-lymphocytes and Myocrisin (sodium aurothiomalate) a soluble gold immunomodulator which causes the avirulent strain of SFV to become virulent (Oaten et al 1980b). It also increases the virulence of other encephalitogenic arboviruses. The peritoneal macrophages which take up the myocrisin become sites of intense virus replication with depressed lysosomal enzyme production which is thought to promote increased titres and thus virulence (Mehta and Webb 1982). More recently electron microscopic studies have shown that myocrisin enhances membrane proliferation in the cells of the CNS. Normally the avirulent strain of SFV produces high titres of virus in the brains of adult mice but cannot be seen electromicroscopically whereas the virulent strain, L10, is seen. The virulent strain stimulates good membrane proliferation whereas the avirulent strain produces little membrane production (Pathak and Webb 1983). When myocrisin, which stimulates membrane proliferation, is given prior to the avirulent virus this virus becomes virulent. This suggests that the proliferation of cell membranes may be a requirement to the development of virulence.

We are trying to raise monoclonal antibodies to glycolipids in the envelope of SFV derived from different cell types in

order to study these antigens with a greater specificity.

The SFV infection in mice has produced a very good model to study virus induced demyelination as the virus does not have to be inoculated intracerebrally for this to occur as in the case of most other demyelinating models. Intracerebral inoculation changes the pattern of infection as it artificially breaks the blood brain barrier. It may also be a model in which virus induced CNS autoimmune pathology occurs. If this is the case then it will be an even more fascinating model.

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(Dr. H.E. Webb, Sandra Amor, Sandra Couldrick, Nicholas Evans, John Fazakerley, Shirin Illavia, Azy Khalili-Shirazi, Surekha Mehta, Linda Parsons, Snehlata Pathak).

Comparison of Yellow Fever Virus-Specified Proteins and Glycoproteins in Mosquito and Mammalian Cells.

The synthesis of viral proteins was observed in two cell lines infected with a wild strain of yellow fever virus (YFV), PM 24553 (CORNET *et al.*, 1978), isolated in Senegal from *Aedes furcifer taylori*. In absence of shut-off in host cell protein synthesis, this work was performed after immunoprecipitation of virus-specified proteins (WECHSLER *et al.*, 1979) using mouse hyperimmune ascitic fluid prepared with the PM 24553 YFV strain.

The pulse labeling with ^{35}S methionine at different times after infection allowed to determine the maximum of viral protein synthesis; it was found as 35 hr post infection in AP61 mosquito cells (*Aedes pseudoscutellaris*) and 50 hr post infection in SW13 cells (human adenocarcinome).

At least seven virus-specified proteins were shown after migration in SDS-polyacrylamide gel electrophoresis with the same molecular weights for virus propagated in SW13 or AP61 cells: p98 (NV5), p77 (NV4), gp53 (V3), gp49 (NV3), gp26 (NV2), p19 (NV1 1/2), and p17 (NV1). The viral structural membrane (M) and core (C) proteins are not shown.

The analysis of virus-specified glycoproteins was first performed using 4 hr pulse labeling with ^3H mannose or ^3H glucosamine at the maximum of the viral protein synthesis. The mannose was not incorporated in the envelope glycoprotein gp53, but in gp49 and gp26 in the two cell systems and the labeled glucosamine was found in the three viral glycoproteins.

We have confirmed the carbohydrate content of these glycoproteins by affinity-chromatography on concanavalin A-Sepharose (Stohlman *et al.*, 1976). The three glycoproteins were adsorbed on the gel and eluted with α methyl D glucoside. The tunicamycine is an inhibitor of the glycosylation by blocking the transfer of the N acetylglucosamine (TAKATSUKI *et al.*, 1975). The glycosylation of the three proteins was affected by this treatment in SW13 and AP61 cells.

A comparative study has been done with the vaccinal 17D yellow fever virus. Two differences in the protein structure were observed: the molecular weights of the envelope glycoprotein V3 and the complement fixing antigen NV3 were slightly higher, about 1000 daltons, in the vaccinal strain comparing to the wild strain. These proteins were each subjected to peptide mapping by limited proteolysis with V8 protease or α chymotrypsine and the fragments showed after gel electrophoresis similar but distinguishable patterns.

The replication of yellow fever virus in mammalian or in mosquito cells seemed to be very similar. Little differences were recorded when a vaccinal and a wild strain were compared, particularly in the structure of the two glycoproteins V3 and NV3. These differences confirm those observed in the RNA structure by oligonucleotide fingerprints.

(V. DEUBEL, J.P. PAILLIEZ and J.P. DIGOUTTE)

REPORT FROM EVANDRO CHAGAS INSTITUTE, F.S.E.S.P., BELÉM, PARÁ, BRAZIL, ADOLFO LUTZ INSTITUTE, HEALTH DEPARTMENT OF STATE OF SÃO PAULO, BRAZIL AND DEPARTMENT OF EPIDEMIOLOGY, PUBLIC HEALTH SCHOOL, UNIVERSITY OF SÃO PAULO, SÃO PAULO, BRAZIL.

Human sera collected in the period ranging from 1977 to 1983, from five different groups living in Ribeira Valley, São Paulo, Brazil, a forested area where an arbovirus encephalitis epidemic first occurred in 1975, were tested by hemagglutination inhibition against Mucambo (An 10967), Pixuna (Be Ar 35645) and SP An 50783 and by neutralization against Mucambo and SP An 50783. The last is one of the two strains isolated by Calisher et al (1982) from pools of Culex (Melanoconion) and a bat (Carollia perspicillata) collected in Iguape County, Ribeira Valley, in 1976. Based on biological, serological and biochemical characteristics, Calisher et al postulate that these strains should be considered as a newly recognized variant (IF) of VEE sub-type I.

The results of the present research show that all the investigated sera which react with Mucambo also do so with An 50783 (Table 1). Titers to An 50783 are higher than to Mucambo and Pixuna virus. The neutralization tests in Vero cells performed in sera of human groups I, II, III and IV demonstrate higher titers to An 50783 in 28 out of 32 sera, lower titers in 3 sera and similar values in one sera.

In the group of fishermen, people who seem to present the highest exposition to arboviroses (Iversson et al, 1981), 20,8% (63/303) have monotypic reaction to An 50783, which suggests intensive circulation of this new virus in human population. Neutralization tests are being carried out in these sera. In the first eleven tested sera the positive results have been confirmed.

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(A.P.A. Travassos da Rosa, I.M. Rocco and L.B. Iversson)

Table 1

Prevalence of VEE strains (An 10967 , Be Ar 35645 and SP An 50783) hemagglutination inhibition antibodies in sera of five human groups residing in Ribeira Valley, São Paulo, Brazil.

Human groups	An 10967	SP An 50783	Be Ar 35645
	<u>no. positive</u> <u>no. tested</u>	<u>no. positive</u> <u>no. tested</u>	<u>no. positive</u> <u>no. tested</u>
I	14/83	8/8*	0/8*
II	34/516	17/17*	2/17*
III	3/82	3/3*	0/3*
IV	6/337	4/4*	1/4*
V	12/303	79/303	...

- I - Road construction workers, living in three camp sites close to forest area.
- II - Non-encephalitis patients of the major hospital in Ribeira Valley, residents in urban and rural zones.
- III - Households of cases of Rocio encephalitis, living in urban zone of four cities of the region.
- IV - School children from 6 to 14 years old, living in urban and rural zones of Iguape County.
- V - Fishermen from Iguape and Cananeia, coastal counties.
- * - Tested sera that have HI antibodies against Mucambo (An 10967)
- ... - not tested.

Table 2

Comparative results between VEE strains (An 10967 and SP An 50783) HI tests performed in sera of fishermen from Ribeira Valley, São Paulo, Brazil.

Results	Sera	
	Number	%
Twofold difference in titer*	3	3,8
Fourfold difference in titer*	6	7,6
Cross reactions with another alphavirus (EEE and WEE)**	7	8,9
Monotypic reaction (SP An 50783)	63	79,7
Total	79	100,0

* An 50783 has the highest titer.

** Two sera react with An 10967 e seven sera with SP An 50783

REPORT FROM THE CARIBBEAN EPIDEMIOLOGY CENTRE (CAREC)
(PAHO/WHO), TRINIDAD, WEST INDIES

Dengue type 4 virus activity in Trinidad, 1981 - 1983

In May, 1981, dengue type 4 virus was first detected in the Caribbean causing outbreaks in countries to the north of Trinidad. Strains were isolated at the CAREC laboratory from sera referred from patients in Jamaica, St. Kitts/Nevis, Dominica and Grenada. Viruses were isolated in AP-61 tissue culture and identification was done by CFT (in 1981) or by immunofluorescence using dengue type-specific monoclonal antibodies developed at the WRAIR.

Following the initial dengue 4 reports in 1981, active airport surveillance was instituted in Trinidad, resulting in the detection of imported cases from 4 Caribbean countries. At this time, indigenous dengue type 1 transmission was occurring in Trinidad.

In March, 1982, a regular programme of fever surveillance yielded the first dengue 4 isolate from a Trinidadian resident with no travel history, and 11 strains of this type were isolated in that year in the absence of a reported outbreak.

Dengue 4 activity increased sharply in November, 1982, accompanied by clinical reporting, and cases were detected from all sampled areas throughout 1983, the largest number of virus isolates being made in September. (Figure 1).

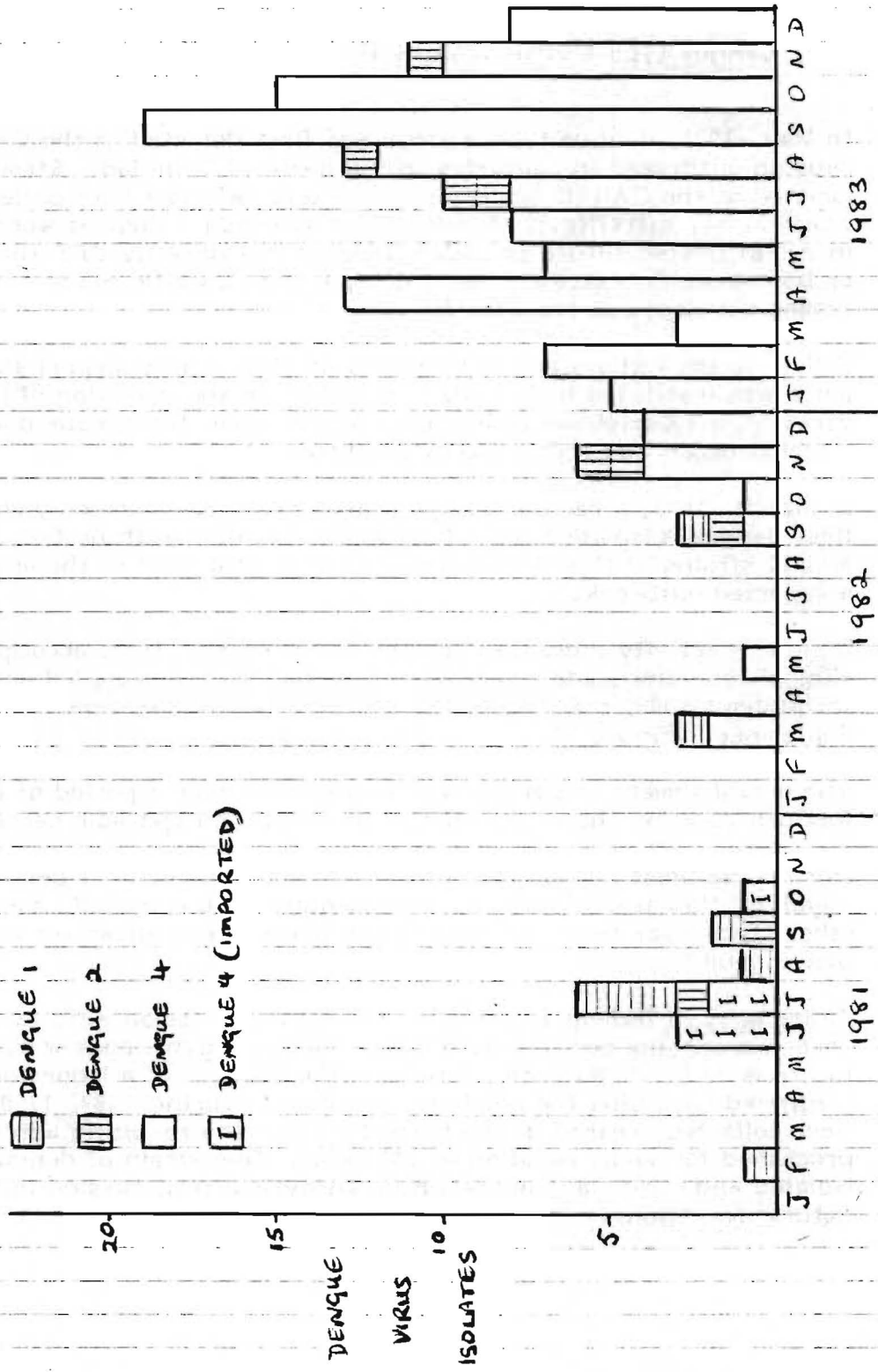
The establishment of dengue 4 thus occurred over a period of time and was preceded by the occurrence of imported and sporadic cases.

During the period 1981 - 83, there were also isolations of dengue types 1 and 2. No cases of haemorrhagic dengue were reported, and symptoms other than fever were reported in the percentages shown on the accompanying table.

Aedes aegypti indices in areas of dengue transmission were as high as 34.8 (percentage positive premises). Aedes aegypti eggs were collected from 17 locations each comprising the address of a laboratory - confirmed case plus the adjoining premises. During 1983, 17,857 eggs were collected, reared in the laboratory and the resulting adults were processed for virus isolation in 290 pools. One strain of dengue 4 was isolated and reisolated, suggesting transovarial transmission under natural conditions.

Figure 1

DENQUE CASES CONFIRMED BY VIRUS ISOLATION: TRINIDAD, 1981-1983.



CLINICAL DATA ON 130 DENGUE 4 CASES

Fever plus-:

Myalgia	33.1%
Anthralgia	28.5%
Headache	27.7%
Gastrointestinal Symptoms	13.8%
Rash	10.0%
Malaise	6.9%
Weakness of limbs	4.6%
Respiratory Tract Symptoms	3.8%
Meningitis) Convulsions) Encephalitis)	3.8%

(B. Hull, M. de Souza, E. S. Tikasingh, D. Chadee)

Report from San Juan Laboratories, Dengue Branch
DVBVD, CID, CDC, San Juan, Puerto Rico

Peripheral Blood Leukocytes (PBL) for Isolation of Dengue Viruses

Investigations of dengue hemorrhagic fever patients in Thailand have reported improved isolation of dengue viruses from peripheral blood leukocytes compared to plasma, and successful specific fluorescent antibody (FA) staining of dengue antigen on the surface of lymphocytes. This has been used to support the hypothesis that mononuclear cells are an important site of dengue virus replication. If true, detection of viral antigen in these cells could provide a method for rapid diagnosis of dengue and DHF. A blind study was therefore designed to evaluate FA testing of peripheral blood leukocytes from Puerto Rican patients with dengue fever and to compare dengue isolation rates from peripheral blood leukocytes with those from serum or plasma.

Acute blood samples were collected in heparinized tubes from patients suspected of having dengue. PBL were separated from plasma by density gradient centrifugation in Ficoll-Hypaque and washed 3 times in complete RPMI-1640 medium containing 10% fetal bovine serum. The pelleted PBLs were suspended in the same medium. One aliquot of the PBL suspension was spotted on 12-well slides to test directly for virus infection by DFAT. A second aliquot was incubated in 24-well plastic tissue culture plates at 37°C for 3 days to determine whether prolonged incubation would increase the amount of virus antigen, facilitating detection by means of direct immunofluorescence. A third aliquot was stored at -70°C for virus isolation. After incubation, the 3-day cultures of PBL were also spotted on slides. All slides were air-dried, fixed in cold acetone for 10 minutes and observed for virus infection by DFAT.

Acute and convalescent sera or plasma from each patient were tested by HI and the acute plasmas of all serologically positive samples were tested for virus isolation, first by tissue culture, and if negative, by mosquito inoculation. PBL from patients who were confirmed as dengue serologically were disrupted by sonic energy and inoculated into mosquitoes for virus isolation.

A total of 19 serologically confirmed dengue patients were studied. The results are summarized in Table 1. Of 16 patients whose PBL were smeared directly for DFAT, none were positive for dengue antigen. Only 1 of 15 patients whose PBL were cultured for 3 days was positive. The direct smear of PBL from this patient was negative, but dengue 4 virus was isolated from the frozen PBL by mosquito inoculation and from the plasma in mosquito cell cultures. Dengue 4 virus was isolated from the frozen PBL of another 2 patients by mosquito inoculation, in one of whom, virus isolation from the plasma was negative. PBL smears of both of these patients were negative by DFAT. By contrast, dengue virus was isolated from the plasma of 6 patients including the 1 patient from whom virus was detected in PBL by DFAT.

Of the 3 patients for whom dengue virus was detectable in the PBL, 2 were experiencing secondary infections and 1 a primary infection. This distribution fits the total number of primary and secondary cases studied.

Although only 19 confirmed dengue patients were studied, the blood samples were all taken and processed under ideal conditions. The data would suggest that dengue virus detection or isolation from PBL is less sensitive than isolation from plasma or sera. Furthermore, fluorescent antibody staining of PBL appears to be of little or no value for the rapid diagnosis of dengue.

(S. H. Waterman, G. Kuno, D. J. Gubler, N. A. Colon, E. Vergne, A. Oliver, M. Velez, G. E. Sather, and I. Rios).

Table 1

Comparative Dengue Virus Isolation and/or Detection by
Immunofluorescence on Human Peripheral Blood Leucocytes and from Plasma

Patient	Serologic Response	Days after onset blood sample taken	Isolation or Detection From PBL ^a			Isolation from Plasma	
			DFAT		Mosquito Inoculation	TC ^b	Mosquito Inoculation
			Direct	3-day Culture			
1	Secondary	8	-	C ^c	-	-	-
2	Secondary	?	ND ^d	-	-	-	-
3	Secondary	6	ND	-	-	-	-
4	Primary	6	-	-	-	-	-
5	Primary	3	-	-	-	-	DEN 4
6	Primary	2	-	-	DEN 4	DEN-4	DEN 4
7	Secondary	3	-	-	-	-	DEN 4
8	Secondary	7	-	-	-	-	-
9	Secondary	7	-	-	-	-	-
10	Secondary	1	-	+	DEN 4	DEN 4	ND
11	Secondary	7	-	-	-	-	-
12	Secondary	4	-	-	-	-	-
13	Inconclusive	2	-	-	-	DEN 4	ND
14	Primary	3	-	ND	-	DEN 4	DEN 4
15	Primary	5	-	-	-	-	-
16	Secondary	6	-	ND	-	ND	-
17	Secondary	6	ND	-	-	-	-
18	Secondary	5	-	-	-	-	-
19	Secondary	4	-	ND	DEN 4	-	-
TOTAL			0/16	1/15	3/19	4/18	4/17

^a Peripheral blood leucocytes (PBL)

^b TRA-284-SF mosquito cell cultures

^c Contaminated - unsatisfactory

^d ND = Not done

Studies on Dissemination and Salivary Gland Barriers to Dengue Infection in Aedes Aegypti

Recent studies with several arboviruses and their mosquito vectors have suggested that barriers exist which prevent the dissemination of virus from the midgut to other tissues of the mosquito. In other words, the midgut epithelial cells may become infected, but the virus is contained and does not spread to the nervous tissue, ovaries, salivary glands and other tissues.

Assay for dengue infection in our experimental mosquitoes is based on direct fluorescent antibody tests (DFAT) on brain and/or salivary glands to detect the presence or absence of viral antigen. Infection rates in our mosquitoes, therefore, represent mosquitoes with disseminated infections. While there were no data to suggest that a dissemination barrier occurred in Ae. aegypti infected with dengue virus, it was of interest to determine if one existed.

Bodies (thorax and abdomen) of experimental mosquitoes which had been fed on dengue 1 or 2 virus suspensions were stored in 1 dram vials after the head and salivary glands had been processed for the presence or absence of viral antigen by the DFAT. These bodies were processed for dengue virus infection in 2 ways. First, individual bodies were disrupted by sonic energy in 1.0 ml PBS containing 30% FCS, centrifuged at 3000rpm for 30 min at 4°C, the supernatant fluid decanted and inoculated into mosquitoes. These mosquitoes were incubated for 10 days at 30°C and tested for dengue virus infection by DFAT on brain squashes. Secondly, after blind trials had established the reliability of the technique, whole body squashes of individual mosquitoes were processed for virus infection by DFAT directly. In both cases, a direct comparison was made of dengue virus infection in the brain and the body (midgut ?) of Ae. aegypti which had fed on dengue 1 or dengue 2 virus suspensions and had been incubated for 14 days at 30°C.

Table 2 shows results of comparative infection rates determined by brain squash and by inoculation of sonicated bodies from the same mosquitoes. Several geographic strains of mosquito and 2 virus serotypes were used. It will be noted that there was no instance where infection rates determined by the 2 methods were significantly different. In several of the strains, there were occasional infected mosquitoes detected by processing the body, which had been missed by the brain squash, but in no case did this change interpretation of the results.

Table 3 shows comparative infection rates in mosquitoes by DFAT on brain and body squashes of the same mosquitoes. With the exception of the Bansang strain, there were no marked differences in infection rates in brain tissue and whole bodies of mosquitoes infected orally. With the Bansang strain, twice as many infected mosquitoes were observed with the body squash (6) than with the head squash (3). However, only 16 mosquitoes were tested and, therefore, it is difficult to draw conclusions at this time. It should be noted that there was no difference between brain and body infections in this strain when the prototype virus was used (Table 2).

These data, with the exception of the Bansang strain, fail to show any evidence of a dissemination barrier to dengue viruses in Ae. aegypti.

Furthermore, there was no convincing evidence that this phenomenon may be associated with some mosquito strains and not others. Differences such as those observed with the African strains of Ae. aegypti are most likely due to slower replication of the virus in some strains, not to a dissemination barrier. On the other hand, lack of virus dissemination may be associated with the infecting virus strain rather than the mosquito host. Since only 2 strains (and 2 serotypes) were studied, conclusions cannot be made on this at the present time.

Recent studies with yellow fever and western equine encephalitis viruses have suggested that mosquitoes may become infected with a virus orally, but cannot transmit that virus because there is a barrier to salivary gland infection. As noted above, dengue virus infection in our experimental Ae. aegypti is routinely determined by DFAT on both the brain and dissected salivary glands of the same mosquitoes. Since dissection of salivary glands is time consuming and it is often not possible to dissect the glands of all experimental mosquitoes, it is important to determine whether brain infection can be used to represent salivary gland infection. In other words, is there, or is there not a salivary gland barrier to dengue virus infection in Ae. aegypti.

Geographic strains of Ae. aegypti were infected orally with a Puerto Rican strain of dengue 2 virus and incubated for 14 days at 30°C. For each experiment, a portion of the mosquitoes were dissected for salivary gland infections and these plus the head from the same mosquito were placed on a microscope slide side by side and processed for virus infection by DFAT. The results, comparing brain and salivary gland infection in the same mosquito, are presented in Tables 4 and 5. Table 4 represents experiments carried out with feeding suspensions prepared from frozen aliquots of dengue 2 virus whereas Table 5 represents experiments done with feeding suspensions prepared from fresh virus. It will be noted that there were no marked differences in numbers of infected mosquitoes with either virus when DFAT was done on the brain or the salivary glands. With both the frozen and the fresh virus, there were a few mosquitoes which had detectable virus in the brain and not in the salivary glands, but in general, the viral antigen in the brain tissue of these particular mosquitoes was barely detectable. Overall, 596 mosquitoes were tested and 195 (33%) had detectable antigen in the brain tissue whereas 189 (32%) had detectable antigen in the salivary glands. The data do not support the hypothesis that there is a salivary gland barrier to dengue 2 infection in the geographic strains of Ae. aegypti tested. Furthermore, the data show that there is good correlation between dengue virus infection of brain tissue and salivary glands. This suggests that DFAT on brain squashes would provide reliable data on salivary gland infection and thus potential transmission of dengue viruses by Ae. aegypti.

(D. J. Gubler, E. Vergne, N. A. Colon and M. Velez).

Table 2

Oral Infection Rates in Geographic Strains of Ae. aegypti Determined by DFAT on Brain Squashes and Passage of Sonicated Mosquito Bodies*

Mosquito Strain	Number infected/Number tested		Virus Serotype
	Brain squash	Whole body	
New Orleans (Magazine), La.	6/23	7/23	DEN 1
New Orleans (Airport), La.	3/17	3/17	DEN 1
Jakarta, Indonesia	4/16	4/16	DEN 1
St. Martin, Antilles Is.	0/20	1/19	DEN 2
Antigua, Antilles Is.	0/8	1/8	DEN 2
St. Lucia, Antilles Is.	3/15	3/15	DEN 2
Martinique, Antilles Is.	0/5	0/5	DEN 2
Paramaribo, Suriname	5/21	4/20	DEN 2
Rexville, Puerto Rico	11/37	7/29	DEN 2
Bansang, The Gambia	0/14	0/11	DEN 2
Diourbel, Senegal	26/38	24/35	DEN 2

*All mosquitoes incubated 14 days at 30°C after infection with Mexican dengue 1 (1298) or the prototype dengue 2 viruses.

Table 3

Oral Infection Rates in Geographic Strains of Aedes aegypti
 Determined by DFAT on Brain and Body Squashes*

Mosquito Strain	Number infected/Number tested	
	Brain squash	Body squash
Rexville, Puerto Rico	11/48	11/48
Kedougou, Senegal	2/12	2/12
Bansang, The Gambia	3/16	6/16
Kongolikan, Upper Volta	21/64	25/64
Koumbri, Upper Volta	19/86	21/87
Tou, Upper Volta	10/27	13/27

*All mosquitoes incubated 14 days at 30°C after infection with Puerto Rico dengue 2 (1328).

Table 4

Comparative Infection Rates in Brain Tissue and Salivary Glands of Geographic Strains of Aedes aegypti and Aedes mediovittatus after Oral Infection with Dengue 2 Virus*

Mosquito Strain	Number infected/Number tested	
	Brain	Salivary Glands
<u>Aedes aegypti</u>		
Rexville, Puerto Rico	7/73**	10/73**
Coconut, Puerto Rico	3/17	3/17
Bromeliad, Puerto Rico	1/19	1/19
Rex-yellow, Puerto Rico	1/16	1/16
St. Lucia, Antilles Is.	8/28	7/28
St. Kitts, Antilles Is.	1/8	1/8
Malaga, Colombia	8/18	0/18
San Pedro Sula, Honduras	0/7	0/7
Matamoras, Mexico	0/8	0/8
Brownsville, Texas	0/4	0/4
Corpus Christi, Texas	0/15	0/15
San Antonio, Texas	0/10	0/10
New Orleans, Louisiana	2/27	2/27
Rocky Mount, SC.	2/19	2/19
Colombo, Sri Lanka-New	5/30	5/30
Colombo, Sri Lanka-Old	4/15	5/15
Bansang, The Gambia	0/24	0/24
Bangui, Central Africa Rep.	0/9	0/9
Minteh Kunda, The Gambia	0/10	0/10
<u>Ae. mediovittatus, Puerto Rico</u>	24/38	22/38
Totals	58/395	59/395

*Feeding Suspension prepared from aliquoted frozen mosquito virus (PR Dengue 2, 1328).

**Number infected/number tested. Mosquitoes incubated 14 days at 30°C.

Table 5

Comparative Infection Rates in Brain Tissue and Salivary Glands of
Geographic Strains of *Ae. aegypti* after Oral Infection
with Dengue 2 Virus*

Mosquito Strain	Number infected/Number tested	
	Brain	Salivary Glands
Rex-yellow, Puerto Rico	17/20**	17/20**
Bromeliad, Puerto Rico	15/19	15/19
St. Lucia, Antilles Is.	24/25	22/25
Antigua, Antilles Is.	4/15	4/15
St. Martin, Antilles Is.	9/10	8/10
Martinique, Antilles Is.	5/9	4/9
St. Kitts, Antilles Is.	6/10	6/10
Tortola, BVI	10/12	10/12
Port of Spain, Trinidad	5/8	5/8
Brownsville, Texas	12/17	12/17
Acapulco, New Mexico	10/18	10/18
Colombo, Sri Lanka-New	14/20	13/19
Minteh Kunda, The Gambia	6/18	4/18
Totals	137/201	130/200

*Feeding Suspension prepared from fresh mosquito virus (PR Dengue 2, 1328).

**Number infected/number tested. Mosquitoes incubated 14 days at 30°C.

Report from the Department of Arbovirology, Institute of Tropical
Medicine "Pedro Kouri," Havana, Cuba

Clinical Study of Dengue Hemorrhagic Fever Fatal Cases, Cuba, 1981

The clinical charts of 120 fatal cases occurred during the Dengue hemorrhagic fever epidemic of 1981 in Cuba, have been studied. Deaths were more frequent in patients under 15 years of age (60%). There was no significant difference (p 0,01) in relation to sex, or race, according to the structure of our population in the 1981 Census. In over 50% of the cases, the reason for hospitalization was fever and/or vomiting, and about half of them were hospitalized with the diagnosis of hemorrhagic dengue.

Over 60% were reported as seriously or very seriously ill at the moment of hospitalization. Only in 10% of them a previous Dengue-like illness was recorded. In 40% of the cases, family or personal antecedents of Asthma or Diabetes were reported.

The predominant signs and symptoms were: fever, vomiting, hepatomegaly, abdominal pain and asthenia, haematesis and petechia were the most relevant hemorrhagic manifestations.

Shock was frequently observed at the beginning of the disease.

(G. Kourí, J. Bravo, María G. Guzmán, A. Díaz, and A. Ruiz)

The Identification of Various Dengue Virus Strains Using Monoclonal Antibodies in the Immunofluorescence Technique

Several strains isolated during the Cuban Dengue Hemorrhagic Fever epidemic, were identified by the indirect immunofluorescence technique: 8 strains using hyperimmune ascitic fluid and 7 with monoclonal antibodies. Both were prepared against 4 serotypes of Dengue virus. All the strains belonged to the Dengue 2 serotype.

The use of monoclonal antibodies for their identification was quicker and more economic since serial dilutions required with the use of hyperimmune ascitic fluid was unnecessary.

As has been referred by E. A. Henschel et al 1983, the work in our laboratory demonstrated that monoclonal antibodies raised against high passage prototype strains of different Dengue virus serotypes were capable of recognizing low passage strains and even wild ones. Thus, the highly specific determinants recognized by the monoclonal antibodies do not vary in dependence to the number of passages suffered by the strain.

In our case, they were very useful for the identification of isolates and, therefore, recommended for their use with this purpose.

(Maritza Soler, Ma. Ga. Guzman, and G. Kourí)

Comparison between Enzyme Linked Immunosorbent Assay (ELISA) and Hemagglutination Inhibition Test (HI) for the Serological Diagnosis of Dengue

The usefulness of ELISA for the serological diagnosis of Dengue was evaluated applying the test to a group of 43 paired sera from patients with clinical symptoms of Dengue fever, collected during the epidemic of Dengue Hemorrhagic fever which occurred in our country in 1981. As a reference test we used the hemagglutination inhibition test (HI) as described by Clark and Casals. The antibody levels were detected against Dengue 1 (DEN 1) and Dengue 2 (DEN 2) antigens obtained from suckling mouse brain infected with the standard strains Hawaii for DEN 1 and New Guinea C for DEN 2 and extracted by the sucrose-acetone method. For indirect ELISA this crude antigen was partially purified by gel filtration according to the method described by Yoshinaka y Hotta in 1971.

The indirect ELISA test was carried out with a working dilution of 1:40 for the antigen; 1:100 for the sera and 1:5000 for the anti-human Ig serum conjugate with peroxidase. The seroconversion criteria adopted was the increase of the optical density in the second sample to a value more than the mean of the optical density of the first sample plus two standard deviations. For HI the criteria of seroconversion adopted was the fourfold increase of the antibody titer.

Sixty-three percent of the samples were positive for both tests while 28% were negative. ELISA detected 9% more of positivity than HI and no sample was HI positive and ELISA negative. These results show that ELISA seems to be more sensitive than HI for the detection of seroconversion in paired sera from Dengue patients. Neither ELISA nor HI were able to detect serotype specific antibodies for DEN 1 and DEN 2 although ELISA has some advantages over HI. ELISA is faster, easier to perform and the antigens and the reagents are more stable than in HI.

(F. de la Cruz, R. J. Fernández, and G. Kouri)

EEE Virus Isolation in Cuban Natural Foci

For over two decades the Cuban archipelago has been studied since several zones are natural foci of the Eastern Equine Encephalitis virus. One such isolation was obtained recently in this zone from the "jutia conga", Capromys (Capromys) pirolides pirolides. The isolation was performed by intracerebral inoculation of suckling mice and identified by the Neutralization Test in primary cultures of chicken embryo.

The strain isolated from Capromys (Capromys) pirolides pirolides is the first report of EEE virus in this species which is endemic of the Caribbean. This finding is also proof of the possible role of the large rodents as reservoirs of EEE virus.

(Luis Morier, A. Fernández, and M. Soler)

Clinical and Serological Study of Children with DHF/DDS, Cuba, 1981

The clinical charts of a sample of children suffering DHF/DDS during the hemorrhagic dengue epidemic of 1981 in Cuba, was studied. There was a higher frequency of the disease in female. In relation to race, however, there was no significant difference ($p = 0,01$) according to the race structure of our population in the 1981 Census. The most frequent age for the disease was in the 4 to 10 years range. In more than 80% of the cases fever and/or vomiting were the reason for hospitalization. More than 90% of the cases were diagnosed as dengue and, of these, approximately 25% were diagnosed as "DHF", or "complicated dengue", and more than 80% were classified as seriously or very seriously ill, upon admission to the hospital. Duration of the disease was about 12 days and shock appeared on an average of 4 days after the first symptoms were referred. More than 20% were asthmatic and only 12% referred to a previous dengue-like illness.

The predominant signs and symptoms were fever, vomiting, hepatomegaly, abdominal pain and ascites. Petechia and haematesis were the most relevant hemorrhagic manifestations.

(María M. Guzmán, G. Kourí, J. Bravo, S. Vázquez, and M. Soler)

Use of the Radial Hemolysis Technique for the Determination of Antibodies to Dengue, Ilheus, and St. Louis Encephalitis Viruses

The World Health Organization has recommended the use of several techniques for serological diagnosis including the Radial Hemolysis (RH) technique. This method was standardized in our laboratory and compared to the Hemagglutination Inhibition technique for the determination of antibodies to Dengue virus, Ilheus and St. Louis Encephalytis (SLE) in a group of Dengue patients affected during the DHF epidemic of 1981 in our country and against hyperimmune ascitic fluid for each virus studied.

Both techniques behaved in a similar manner as to the capacity for antibody detection against Dengue virus. The RH technique demonstrated a higher specificity than HI to differentiate between Dengue virus antibodies and those against SLE and Ilheus, although in relation to the Dengue complex high cross reaction was observed.

Tables 1, 2 and 3 demonstrate the results observed with both techniques.

HI

	D ₁	D ₂	SLE	IL
D ₁	+	+	+	+
D ₂	+	+	+	+
SLE	+	+	+	+
IL	+	+	+	+

RH

	D ₁	D ₂	SLE	IL
D ₁	+	+	-	-
D ₂	+	+	-	-
SLE	-	-	+	-
IL	-	-	-	+

Table 1 and 2: Results obtained by the HI and RH techniques using hyperimmune ascitic fluid against the 4 viruses studied.

HI

RH

	Positive cases	%	Positive cases	%
D ₁	63	60	58	55,24
D ₂	55	52,38	57	54,29
SLE	63	60	6	5,71
IL	52	49,52	30	28,57

Table 3: Results obtained with patients sera by the HI and RH techniques.

(Susana Vazquez, Ma. G. Guzman, and G. Kourí)

REPORT FROM THE CENTER FOR TROPICAL ANIMAL HEALTH
COLLEGE OF VETERINARY MEDICINE
UNIVERSITY OF FLORIDA
GAINESVILLE, FLORIDA, USA

Between 1980 and 1983, 45,484 Culicoides spp. collected in Florida near cattle have been examined for orbiviruses by attempted isolation in cell cultures and intravenous inoculation of embryonating chicken eggs. Bluetongue serotype 2 was isolated from a pool of Culicoides insignis trapped near Ona. This is the first recovery of a bluetongue virus from this species representing the second new world species of Culicoides from which bluetongue viruses have been isolated. Culicoides insignis is a neotropical form which extends from northern Florida through the Caribbean region and much of South America. This species is commonly collected in large numbers near cattle, it is distributed in areas where the principal bluetongue vector in the new world is absent, but where antibodies to these viruses are prevalent, it is known to feed on cattle and it has now been demonstrated to be infected with bluetongue virus in nature. All of the evidence points to the concept that this species is a second vector of bluetongue virus, but should remain in the suspect category until transmission has been effected. Epizootic hemorrhagic disease virus serotype 2 has been isolated from Culicoides variipennis at two sites in Florida.

(Ellis C. Greiner and E. Paul J. Gibbs)

Antigenic and Molecular Properties of Eight Viruses in
the Newly Proposed Hantavirus Genus of Bunyaviridae

Hantaan virus is the prototype of a group of serologically related viruses believed to be associated with similar diseases which have collectively been termed "hemorrhagic fever with renal syndrome." Korean hemorrhagic fever, epidemic hemorrhagic fever and Nephropathia epidemica are probably the most well-known diseases included in the syndrome. Various forms of these diseases have been endemic throughout Korea, Scandinavia, Europe, Russia and China for many years, but only in the last few years has a viral etiology been recognized.

No serological relationship between Hantaan-like viruses and viruses in any family of animal viruses has yet been documented. Molecularly, however, Hantaan virus appears to be similar to viruses in the Bunyaviridae family in that it possesses a tripartite, single-stranded RNA genome which is contained in virion particles composed of three ribonuclease-sensitive nucleocapsids and an envelope with two glycoproteins. Viruses within each of the four existing genera of Bunyaviridae can be classified not only serologically, but also on the basis of a conserved 3'-nucleotide sequence, which differs from that of viruses in the other three genera. Comparison of the 3'-terminal nucleotide sequence of Hantaan virus to those of viruses in each of the four Bunyaviridae genera demonstrated that Hantaan virus cannot be classified with any genus molecularly. Consequently, a new genus (*Hantavirus*) has been proposed to accommodate Hantaan and related viruses.

To more completely describe the biochemical and antigenic variation among viruses in this new group, eight Hantaan-like viruses were examined in detail. Viruses selected represented the currently recognized host range of these agents, as well as diverse geographical areas of isolation (Table 1).

All eight viruses were found to be genetically unique by fingerprint analysis (not shown), but similar in that they each possessed the same 3'-terminal nucleotide sequence on each of their 3 RNA segments (Table 2).

Serological comparisons utilizing immune sera obtained from experimentally infected rats demonstrated an antigenic similarity of all isolates by fluorescent antibody staining. A solid-phase radioimmune assay, using infected cell culture lysates as antigens, revealed close antigenic relationships among the *Rattus* isolates and a clear differentiation of the *Microtus* and *Clethrionomys* isolates from the other members of this group (Table 3). The more specific plaque reduction neutralization test readily distinguished these latter two viruses, but still suggested antigenic similarities among the *Rattus* isolates.

This combination of biochemical and serological techniques has established the shared and unique characteristics of these viruses and confirms the requirement for the newly proposed genus status of *Hantaviruses*.

(C. Schmaljohn, S. Hasty, and J. Dalrymple)

Table 1

VIRUS ISOLATES RELATED TO HEMORRHAGIC FEVER WITH RENAL SYNDROME

<u>VIRUS</u>	<u>HOST</u>	<u>LOCATION</u>	<u>DISEASE</u>
HANTAAN ¹	FIELD MOUSE	KOREA	?
LEE ²	HUMAN	KOREA	KHF
URBAN RAT ³	RAT	KOREA	?
TCHOUPITOULAS ⁴	WHARF RAT	NEW ORLEANS	?
GIRARD POINT ⁵	WHARF RAT	PHILADELPHIA	?
PROSPECT HILL ⁶	VOLE	FREDERICK, MD	?
SAPPORO RAT ⁷	LAB RAT	JAPAN	EHF
PUUMALA ⁸	VOLE/HUMAN	FINLAND	NE

Viruses were isolated by: ^{1,2,3} H.W. Lee et al., Seoul, Korea; ⁴ T. Tsai et al., CDC Atlanta; ⁵ J. LeDuc, USAMRIID; ⁶ C. Gajdusek et al. NIH; ⁷ Kitamura et al., Tokyo, Japan; ⁸ J. Dalrymple et al., USAMRIID.

Table 2
 3' NUCLEOTIDE SEQUENCES OF HANTAVIRUS L, M AND S RNA SPECIES

L RNA

VIRUS

		5	10	15	20	25
HANTAAN	3'	AUCAUCAUCUGAGGG	AUUUAUUGAU			
LEE		CU	CAUG	UUG
URBAN RAT		CC	CUC	U
TCHOUPITOULAS		CU	C	C
GIRARD POINT		CU	CAUG	UGG
PROSPECT HILL		XCUC	AUXXXX	
PUUMALA (NE)		CU	C	C
SAPPORO RAT		CC	CUC	CG

M RNA

HANTAAN	3'	AUCAUCAUCUGAGGG	CGUUUCUUUG			
LEE		A	C	
URBAN RAT		C	U	G
TCHOUPITOULAS		C	U	G
GIRARD POINT				C
PROSPECT HILL		A	CU	CX
PUUMALA (NE)		C	U	G
SAPPORO RAT		C	U	G

S RNA

HANTAAN	3'	AUCAUCAUCUGAGGG	AUUUCUCGAU			
LEE				
URBAN RAT				
TCHOUPITOULAS				
GIRARD POINT			U	X
PROSPECT HILL		A	C	
PUUMALA (NE)				
SAPPORO RAT				

Table 3

ANTIGENIC RELATIONSHIPS OF HFRS AGENTS
(RADIOIMMUNE ASSAY OF RAT SERA)

Serum Pools	Infected cell lysate								
	Hantaan 76-118	Lee	Urban Rat	Sap. Rat	Tchou-pitou.	Gir. Point	Pros. Hill	Puum-ala	Con-trol
Hantaan (76118)	100	30	30	30	30	30	10	3	.03
Lee (human)	100	100	30	30	30	30	10	10	.03
Urban Rat	100	10	100	100	100	100	10	10	.03
Sapporo Rat	30	30	100	100	100	100	3	10	.03
Tchoup-itoulas	100	30	100	100	100	100	10	10	.01
Girard Point	100	100	100	100	100	100	10	10	.03
Prospect Hill	3	3	3	10	3	3	100	10	1.0
Puumala (NE)	30	10	10	10	10	10	100	100	1.0
Control	0	0	0	0	0	0	0	0	0

For convenience, values have been expressed as % of the highest titer obtained with each antiserum (i.e. homologous = 100%).

TABLE 4

PLAQUE REDUCTION NEUTRALIZATION OF HFRS AGENTS

Serum Pools	Virus							
	Hantaan 76-118	Lee	Urban Rat	Sap. Rat	Tchou- pitou.	Gir. Point	Pros. Hill	Puum- ala
Hantaan (76-118)	<u>4000</u>	4000	-	40	160	ND	-	-
Lee (human)	4000	<u>2000</u>	20	200	160	ND	-	-
Urban Rat	2000	80	<u>4000</u>	8000	16,000	ND	160	-
Sapporo Rat	2000	20	2000	<u>2000</u>	2000	ND	200	-
Tchoup- itoulas	2000	200	4000	8000	<u>32,000</u>	ND	200	-
Girard Point	200	80	2000	8000	8000	ND	200	-
Prospect Hill	-	-	-	20	-	ND	<u>4000</u>	-
Puumala (NE)	-	-	-	-	-	ND	-	<u>1280</u>

Titers expressed as reciprocal of the highest dilution of antisera with greater than 80% reduction of approximately 100 plaques.

- = Titer less than 20.

ND = Not done.

REPORT FROM THE DIVISION OF CLINICAL MICROBIOLOGY
BUREAU OF LABORATORIES
PENNSYLVANIA DEPARTMENT OF HEALTH
LIONVILLE, PENNSYLVANIA

Arbovirus Surveillance in Pennsylvania, 1983

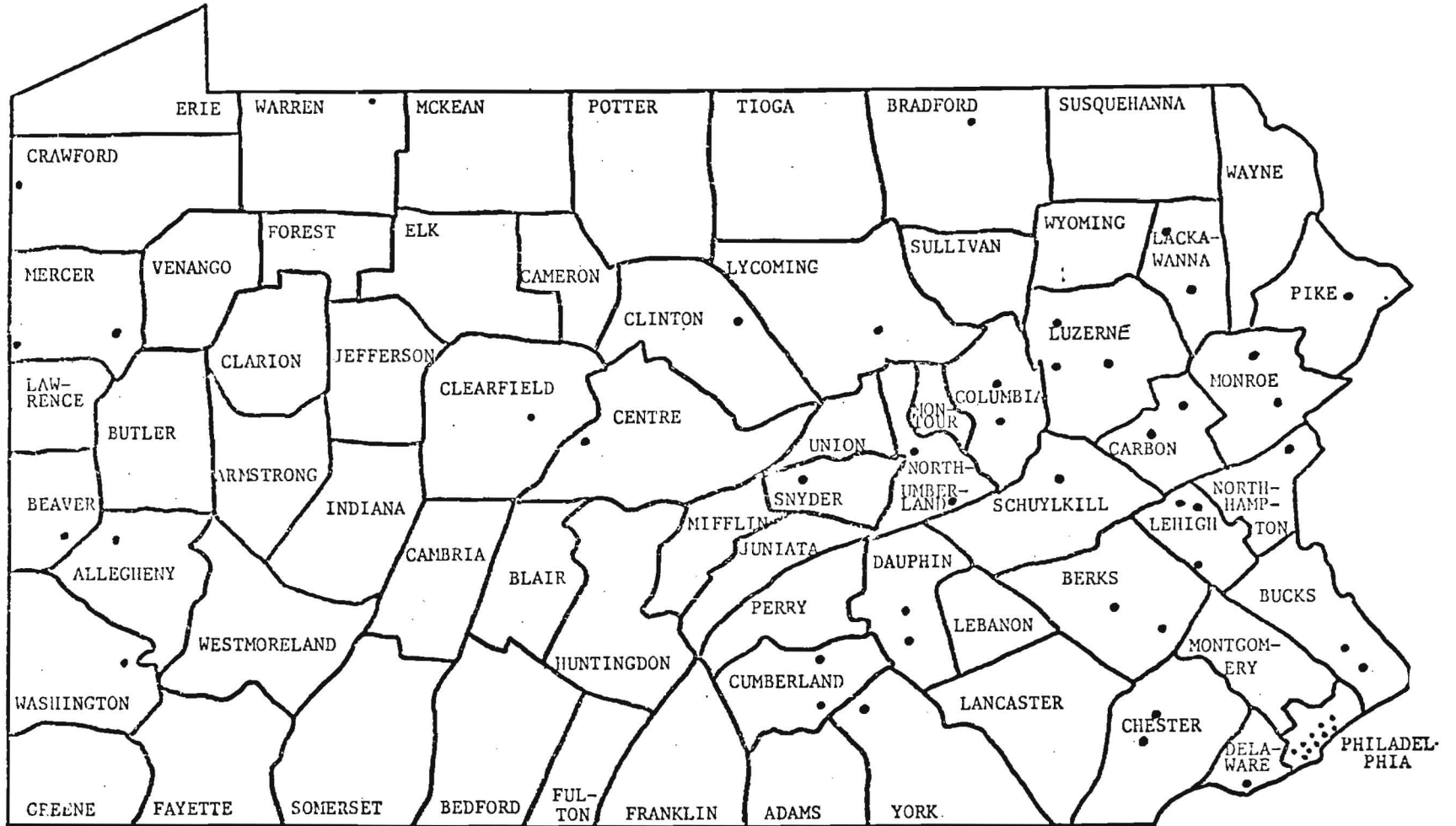
The Pennsylvania Departments of Health and Environmental Resources reinstated an arbovirus surveillance program similar to ones conducted during the summers of 1978 - 1981.

Sentinel flocks of three (3) chickens, mostly hens, were placed at 57 sites throughout the state at the locations shown in the Figure. This compares with 65 sentinel sites in 1981. There was at least one (1) sentinel flock in 31 of the 67 counties as compared with coverage in 32 counties in 1981. The chickens were bled weekly and the sera tested, after protamine sulfate-acetone extraction, for hemagglutination-inhibition (HI) antibodies against St. Louis Encephalitis (SLE) and Eastern Equine Encephalitis (EEE) viruses. Two thousand six hundred thirty (2,630) HI tests were performed. Two (2) chickens from a site in Monroe County, located in the eastern part of the state, seroconverted to EEE virus, exhibiting rises in serum antibodies to titers of 1:1280 and 1:2560.

The surveillance program was conducted from July 10 through September 25, 1983.

Through October, 1983, 32 patients with a clinical diagnosis of central nervous disease were tested for serologic evidence of infection with SLE and EEE viruses. There was no evidence of recent or current infection with arboviruses in any of these cases.

(Bruce Kleger, Gisela Fischer and Vern Pidcoe)



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

Arbovirus Surveillance in New Jersey, 1983

During the 1983 surveillance period, from June through October, 2117 mosquito pools containing up to 100 mosquitoes each were tested for viruses in day old chicks. There were seventeen (17) mosquito pools positive for Eastern encephalitis (EE) and Western encephalitis (WE) was isolated from twenty-five (25).

Table I summarized the collection area totals, species of mosquito and time of collection for the EE isolates. Activity began with the late July collection and continued into October. There were fifteen (15) isolates from Culiseta melanura pools at five (5) sites and two (2) Coquillettidia perturbans at a single site.

WE mosquito activity is summarized in Table II. The August collections yielded the first isolates with continued observation of WE activity into October. All the isolates were from Culiseta melanura pools at five collection sites.

EE isolates were also made in July and August from three horses (3) in Ocean and Sussex counties and from a pheasant flock in Ocean county in August and October.

Sentinel chicken flocks of ten (10) cockerals were placed at four (4) sites in June. The flocks were bled bi-weekly on a rotating schedule and St. Louis encephalitis hemagglutination inhibition tests were conducted. There were no conversions in the 170 sera tested.

(David Kirsh, Bernard Taylor and Wayne Pizzuti)

TABLE I

AREA COLLECTED	MOSQUITO SPECIES	1983 EE MOSQUITO POOL ISOLATES FOR WEEK ENDING											AREA TOTALS	
		7/29	8/05	8/12	8/19	8/26	9/02	9/09	9/16	9/23	9/30	10/07		10/14
Bass River	Cs. melanura								1			1		2
Dennsville	Cs. melanura						1							1
Green Bank	Cs. melanura	1	1	1	1		1				1	1	1	8
Jackson	Cs. melanura								1	2				3
Jackson	Cq. perturbans	1						1						2
New Gretna	Cs. melanura				1									1
Weekly Totals		2	1	1	2	0	2	1	2	2	1	2	1	17

TABLE II

AREA COLLECTED	MOSQUITO SPECIES	1983 WE MOSQUITO POOL ISOLATES FOR WEEK ENDING										AREA TOTALS		
		8/05	8/12	8/19	8/26	9/02	9/09	9/16	9/23	9/30	10/07		10/14	
Bass River	Cs. melanura				1									1
Dennsville	Cs. melanura	2		3	3	3	1	1		1	1	1		16
Green Bank	Cs. melanura			1		1								2
Jackson	Cs. melanura							1		1				2
Woodbine	Cs. melanura		2	1	1									4
Weekly Totals		2	2	5	5	4	1	2	0	2	1	1		25

Report from the Department of Veterinary Science and the School of Veterinary
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PATHOGEN-TOXICANT INTERACTIONS

Plant growth regulators (PGRs), a new class of agricultural chemicals that may soon exceed the use of insecticides, have very low LD₅₀s in conventional laboratory white mice and rats. Recent studies by colleagues R. Hinsdill and L.J. Olson have demonstrated immunomodulatory properties for several of these agents. Glyphosine (GLY), chlorocholine chloride (CCC) and daminozide (DA) all decreased splenic plaque forming cells, number of circulating white blood cells, hematocrit and thymus weight in deer mice (*Peromyscus maniculatus*) and Swiss-Webster white mice fed small amounts (100mg/kg) daily. Gibberillic acid (GA) increased these parameters. Deer mice were used to represent a natural, outbred population and were found to be more sensitive to the PGRs' effects than were the white mice.

We tested the response of PRG-exposed deer mice to challenge with an acute arboviral infection and measured changes in mortality and the immune response. Venezuelan equine encephalitis virus (vaccine strain TC-83) was chosen as the antigenic challenge because it is safe and easy to work with in the laboratory and produces relatively high, reproducible viremia and antibody titers in deer mice. Enzootic strains of this virus naturally infect species of Peromyscus in the Everglades.

Adult (>3 months) deer mice from our outbred colony were divided into 3 groups: old (>2 years) female mice, old male mice, and young (3 months to 2 years) female mice. Only young female mice were used to measure viremia and antibody titers. Unless otherwise specified, 10 mice were used in each group. PGRs were added to the feed in the following doses (in mg/kg/day): CCC -- 1, 10, 20, 40; GLY -- 20, 80; GA -- 25, 100; DAM -- 25, 100. (GA and DAM were not used in the mortality experiments). Saline was added to the feed of one group as a known negative control. Cyclophosphamide (CP), a known immunosuppressant, was used as a positive control at 20 mg/kg/day. Mice were fed the appropriate toxicant for 23 days prior to viral challenge to allow the immunomodulatory properties to take effect. Mice on the mortality experiment were inoculated intraperitoneally with 3×10^5 pfu of virus (an LD₁₀) and the remaining mice were inoculated with 3×10^4 pfu of virus divided half intraperitoneally and half subcutaneously (LD₀ but ID₁₀₀). Mortality was monitored for 21 days post inoculation (PI). Brains from all dead mice were assayed for virus. Serum was tested at 1:10 for the presence of VEEV antibody. Mice were bled daily for 7 days to collect whole blood for viremia determinations and at days 8, 10, 13, 21, 24, 32, 38, 51 and 63 PI to collect sera for antibody assay.

The results from the mortality experiments are shown in Table 1. It appears that both test compounds (GLY and CCC) significantly increased mortality in old females while only GLY increased mortality in old males. Young females apparently had no increase in mortality due to the test compounds. CP increased mortality in all groups. The percent mortality of both young and old females fed GLY and CCC was the same. Therefore, we believe that there is no effect of age on an animal's response to VEEV

infection following the consumption of either of these 2 PGRs. However, an animal's sex appears to affect mortality caused by VEEV while eating CCC with females having greater mortality than males.

Table 1. Mortality of deer mice challenged with Venezuelan equine encephalitis virus after being fed two plant growth regulators for 23 days.

Compound	Old ♀			Old ♂			Young ♀		
	No. Dead	MDT		No. Dead	MDT		No. Dead	MDT	
	N	(%)	(days)	N	(%)	(days)	N	(%)	(days)
Saline	6	0	-	9	0*	-	8	1 (12.5)	7
Glyphosine	10	3 (30)	4	10	3 (30)	9.3	10	2 (20)	7.5
Chlorocholine Cl	10	3 (30)*	2.8	10	1 (10)	3	9	3 (33.3)	8
Cyclophosphamide	8§	2 (25)	9.5	10§	2 (20)	10.5	6	3 (50)	8

* 1 additional mouse died -- virus not isolated from brain

§ 1 mouse had no antibody titer

Viremia onset in untreated mice occurred prior to 6 hours PI and lasted until 4 days PI. Titers were highest day 1 PI at $4 \log_{10}/\text{ml}$ with a secondary peak of $3 \log_{10}/\text{ml}$ on day 3 PI. CP, our known immunosuppressant, significantly decreased viremia titers days 1 and 3 PI and then increased and prolonged the viremia until 7 days PI in some individuals. None of the test compounds affected either the magnitude or duration of the viremia. Neutralizing antibodies were present by day 8 PI and reached the maximum measured titer by day 30 for all compounds. CP significantly decreased antibody titers by about $1.5 \log_{10}$ on days 8 through 32. By day 38, antibody titers of CP-treated mice had reached their maximum but were still lower than titers of saline control mice. In general, all the PGRs seemed to slightly depress the initial antibody response until day 21 PI, but to a lesser degree than the CP control. Thereafter, the antibody titers of PGR-fed mice were no different from those of saline control mice. Exceptions were the lowest dose of DAM which had no effect on antibody titers and the highest dose of GLY which elevated antibody titers on day 21 PI.

In summary, the plant growth regulators that we investigated may increase mortality in animals challenged with high doses of VEE virus. PGRs given in these low doses do affect the immune response but not enough to significantly affect an animal's ability to respond to challenge by low doses of an acute viral infection that is terminated primarily by an antibody-mediated response.

(A. Fairbrother, T.M. Yuill)

SUBLETHAL EFFECTS OF VEEV INFECTION

We were interested in determining what effect an acute but sublethal viral infection might have on the mass and energy balances of wild mammal hosts to see if the virus might affect reproduction, one of the demographic

parameters important in population regulation. Venezuelan equine encephalitis virus (VEEV) was selected as our test virus. It naturally infects wild cotton mice (Peromyscus gossypinus) and other small forest rodents and the vaccine (TC-83) strain produces a sublethal infection in rodent hosts but is safe to use in the laboratory. We selected the deer mouse (Peromyscus maniculatus) as our test rodent. High doses (6 - 8 log₁₀ pfu) of virus cause almost 100% mortality but doses of 4 log₁₀ pfu or less produce measurable viremia and antibody responses with little or no mortality.

We tested the effect of infection on food consumption and weight gain. Daily weights proved to be too variable to be able to meaningfully interpret the results. Food consumption was reduced during the week of viremia. Increased bleeding and weighing frequencies each increased food consumption while the presence of virus decreased food consumption.

Another experiment measured daily body temperatures of deer mice infected with VEEV. In people and horses infection with VEEV produces fever. We expected this to occur in deer mice also. One gram AM transmitters were implanted in the abdomens of 3 mice. The number of pulses per minute emitted by the transmitters is a function of temperature. The transmissions were received by standard AM radios and impulses sent to an HP personal computer which converted the pulse frequency into degrees centigrade and recorded the time and temperature. Readings were taken every 10 minutes for each mouse. Temperatures were monitored for 7 days to obtain normal temperatures for each mouse. The mice were then inoculated with 3×10^4 pfu virus intraperitoneally and their temperatures monitored for another 7 days or until death. The experiment was repeated with 2 additional mice on reduced food rations (80% of ad lib. consumption). Preliminary data analysis showed that infected mice had a decreased body temperature. Temperature slowly decreased 2 to 3 days before death. Mice on reduced rations torpored for 2 to 3 hours every morning; their body temperatures declined as much as 7 to 8 °C. One mouse on reduced rations had a prolonged torpor for almost 24 hours 2 days post inoculation with VEEV. We intend to use a mathematical model to look at this data in combination with the data showing reduced food consumption when viremic to describe the energy costs and savings of sublethal infection by VEEV. Deer mice are not stubborn homeotherms and may use their ability to decrease body temperature to overcome the energy loss due to decreased food consumption.

A last experiment has shown that the energy drain from infection with VEEV indirectly produces population effects. In a collaborative experiment with W.P. Porter and R.D. Hinsdill (Dept. of Zoology and Dept. of Environmental Toxicology, respectively), we have shown that infection of lactating deer mice with VEEV (3×10^4 pfu) significantly reduces the weaning weight of their offspring. This has important biological implications as there is some evidence that smaller young remain smaller as adults and are not as successful breeders as the larger individuals. Therefore, it is possible for sublethal infections of VEEV to adversely affect the energy balance of small rodents and ultimately result in changes in at least one of the demographic parameters of the population.

(A. Fairbrother and T.M. Yuill)

CALIFORNIA SEROGROUP VIRUS INFECTIONS IN WISCONSIN DOMESTIC ANIMALS

Since recognition of California (CAL) serogroup viruses in Wisconsin in the early 1960's, extensive research has identified the primary vector and wild mammal reservoir hosts of the most important CAL group member, La Crosse (LAC) virus. However, the role of domestic mammals as amplifier hosts or as sentinels of virus transmission has gone unstudied. We have undertaken a serological survey to ascertain the prevalence of LAC virus antibodies in various domestic species and are conducting studies to assess the capacity of domestic animals to amplify virus transmission.

Blood serum from bovines, equines, canines, swine, felines and caprines was collected by cooperating veterinarians in the LAC endemic region of southwestern Wisconsin. Additional sera from this area was obtained from the Wisconsin State Animal Health Laboratory, Madison, or collected by members of the Department of Veterinary Science. All sera were screened by tissue culture neutralization test in 96 well plates at a 1:10 dilution against 30-300 TCID₅₀/0.205ml of each of the 4 CAL group viruses found in Wisconsin: LAC, Jamestown Canyon (JC), trivittatus (TVT), and snowshoe hare (SSH) viruses. Antibody titers of positive sera were determined by constant virus-serum dilution neutralization tests (SDNT) using 2-fold dilutions of serum. Cross-reactive sera were tested against all 4 viruses by SDNT. The virus producing the highest antibody titer was assumed to be the virus which infected the animal. Sera neutralizing more than 1 virus at the same titer were considered uninterpretable and are not included here.

Results of the serosurvey are shown in Table 1. Prevalence of LAC virus antibody was low in all species tested; equine animals had the highest antibody rate. The lack of LAC virus antibody-positive canines was surprising, since 16% of 57 canine sera in an earlier study from Iowa County, Wisconsin (in the endemic area), neutralized LAC virus. However, these sera have not been tested against all 4 viruses, so cross-reactions cannot be ruled out. Red foxes (*Vulpes fulva*) are naturally infected with LAC virus, and experimentally can provide infectious blood meals for *A. triseriatus*. More intensive sampling will be necessary to gain an accurate picture of LAC antibody prevalence in domestic canines.

Except for felines, the highest antibody prevalence in all species was to JC virus. This is of some interest due to recent reports of human encephalitis caused by JC virus. Our results indicate active JC virus transmission to domestic animals in Wisconsin, but human illness due to this virus has not been confirmed. Trivittatus virus antibodies were found only in 1 horse and 4 canines, while SSH virus antibodies were found only in 1 cow, indicating that domestic animals are unimportant in the natural cycles of these viruses.

Since antibody presence alone does not indicate host status, experimental infections of calves, ponies, and dogs with LAC virus were carried out by 1 of us (F.A.) Each animal was inoculated with a suspension containing $6.5 \log_{10}$ SMICLD₅₀/0.25ml of LAC virus by the combined IV, IM and SC routes. Post-inoculation blood samples were drawn on days 1-5 for viremia determinations. In addition, tracheal swabs were obtained on days 1-5 post-inoculation from canines for virus isolation attempts. This was prompted by the report of oral infection of red foxes ingesting LAC viremic chipmunks and suckling mice. All 4 ponies and 3 of 4 calves developed LAC neutralizing antibodies, but no viremia was detected. Results of the canine infection are pending.

In summary, our results suggest that two common domestic animals, cattle and horses, are infrequently infected with LAC, TVT, and SSH viruses, and appear to play no role in virus maintenance or amplification. However, cattle and horses may be useful sentinels of JC virus transmission, and experimental studies to assess JC virus host status are planned for the near future.

(Marvin S. Godsey, Jr., Fulurunso Amoo, and Thomas M. Yu111)

Table 1: Prevalence in Wisconsin Domestic Animals of CAL Group
Virus Antibodies Neutralizing 30-300 TCID₅₀/0.025 ml of Virus

Virus	Bovine	Canine	Equine	Porcine	Caprine	Feline
LAC	4/261 (1.5)*	0/25 (0)	8/120 (6.7)	2/48 (4.2)	0/10 (0)	0/4 (0)
TVT	0/261 (0)	4/35 (11.4)	1/120 (0.8)	0.48 (0)	0/10 (0)	0/4 (0)
JC	36/261 (13.8)	4/35 (11.4)	18/120 (15.0)	5/48 (10.4)	1/10 (10.0)	0/4 (0)
SSH	1/161 (0.1)	0/35 (0)	0/120 (0)	0/48 (0)	0/10 (0)	0/4 (0)

* No. positive/no. tested (%)

ARBOVIRUS SURVEILLANCE IN IOWA

Studies of arbovirus activity in Iowa for 1983 concentrated on the detection of viruses which have the potential of causing human disease. This effort began in late spring and terminated in late fall, the period when insect vectors for these viruses are most active. The surveillance program consisted of three main activities. The first was the establishment of sentinel flocks of domestic chickens in two locations in the state, one in the east in Davenport, Scott County, and a second in the west in Council Bluffs, Pottawattamie County. These chickens were bled two to three times per month and sera were examined for the presence of antibody to western equine encephalitis virus (WEE) and St. Louis encephalitis virus (SLE).

The second main activity was concerned with the trapping of wild birds to obtain sera for antibody studies. At the time of bleeding the birds were identified and examined for an estimate of age. This activity was carried out from June to early August in Des Moines and in Davenport.

The third effort in the surveillance program was involved with the capture of mosquitos throughout the state which were identified and sorted into pools for virus isolation and identification. A summary of all surveillance activities and the location of each within the state are presented in figure 1.

Results of Surveillance Activities

Serological studies conducted on sera obtained from sentinel flocks are summarized in Table 1. Antibody to SLE was detected in two of nine birds in August in the Davenport area, whereas both SLE and WEE antibody were detected in the flock in Council Bluffs. Sera were titrated beginning at a 1:10 dilution using the hemagglutination-inhibition technique. No studies were performed to account for the fluctuation observed in these antibody titers.

A list of the wild birds examined in these serological studies is presented in Table 2. The number of each avian species studied and the species in which virus antibody was detected are indicated. Antibody was detected to SLE in sera obtained from the American goldfinch, the house sparrow, and the red-headed blackbird. Antibody was detected in early June and in July in the Davenport area and only in June in the Des Moines area (Table 3). It is believed that the long period of drought experienced during the summer played a significant role in decreasing the mosquito population and thus the transmission of arboviruses within the avian population.

The mosquito species examined for the presence of infectious virus are listed in Table 4. Indicated also are the average number of mosquitos in each pool, the number of pools prepared, and a summary of the viruses isolated. Virus isolation studies were performed by intracerebral inoculation of suckling mice with extracts of each mosquito pool. The identity of each virus was confirmed serologically. Mosquitos were

collected in nine population centers in Iowa and the results of virus isolation studies are presented in Table 5. Virus was isolated in all areas studied except for the Cedar Rapids area, probably due to the low number of mosquitos examined. In the Sioux City area there were three WEE isolations and an isolate of Turlock virus which was isolated with Flanders virus from the same pool of mosquitos. Viruses were only isolated from pools of Aedes trivittatus, Culex pipiens, and Culex tarsalis.

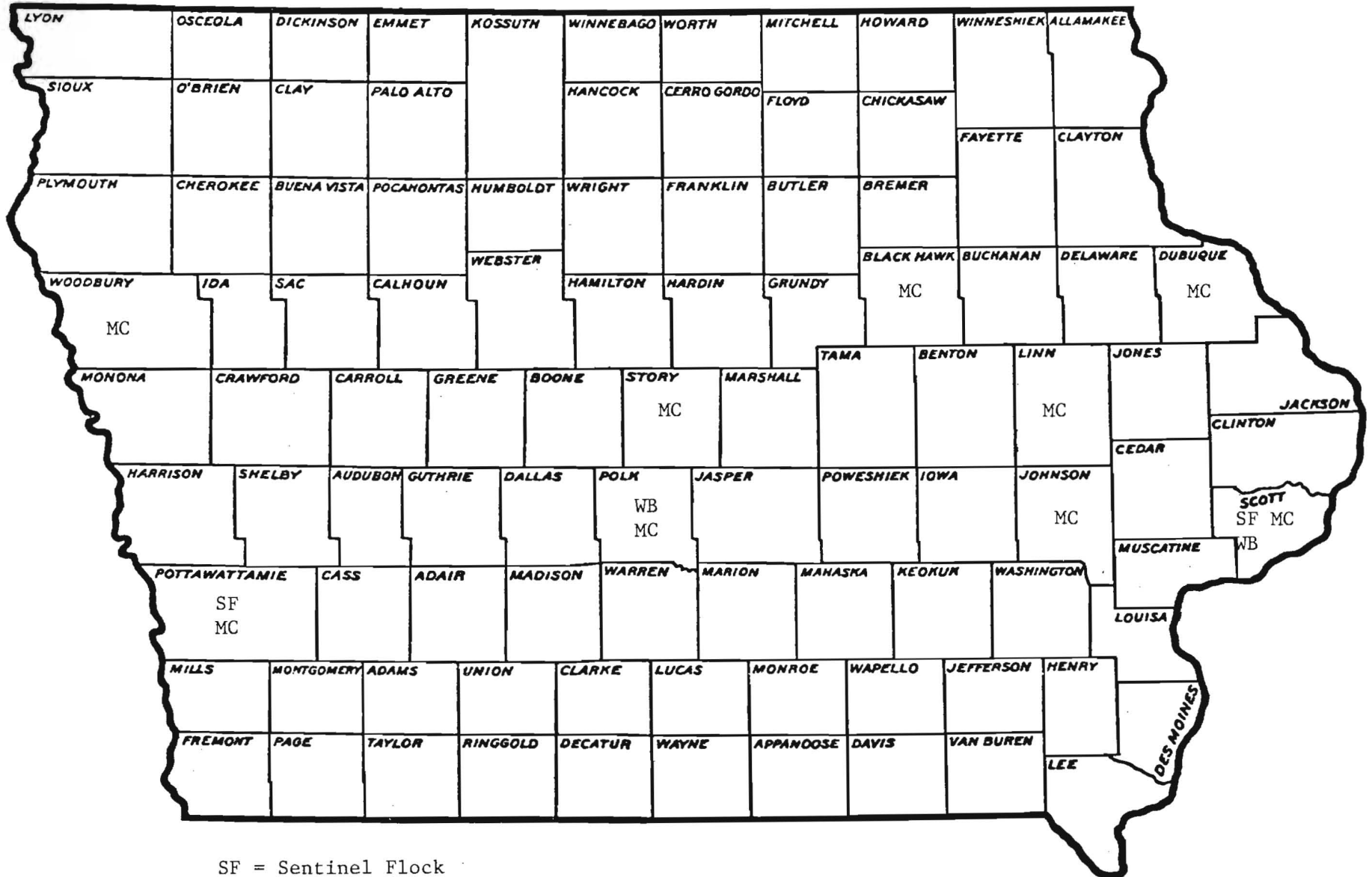
Summary

In 1983, Arbovirus Surveillance in Iowa was conducted by serological studies of avian populations and virus isolation studies on populations of mosquitos. Activity due to WEE was detected by both techniques in western Iowa. In western, central, and eastern Iowa activity due to SLE was detected serologically. Other viruses detected by isolation and identification included Flanders, Trivittatus, and Turlock viruses. A summary of all viruses detected in Iowa during the entire surveillance program is presented in Figure 2.

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Figure 1. SITES OF ARBOVIRUS SURVEILLANCE ACTIVITIES - 1983



SF = Sentinel Flock
 WB = Wild Bird Sera
 MC = Mosquito Collections

Table 1.

ARBOVIRUS SURVEILLANCE BY SEROLOGICAL
STUDIES OF SENTINEL CHICKEN FLOCKS

Location	Date Tested	<u>No. Pos.</u> <u>No. Tested</u>	Virus Detected
Davenport	6/14	0/11	
	6/24	0/10	
	7/18	0/10	
	8/8	2/9	St. Louis Encephalitis
Council Bluffs	6/9	1/10	St. Louis Encephalitis
	6/21	0/10	
	6/30	1/10	Western Encephalitis
	7/11	0/10	
	7/20	1/10	St. Louis Encephalitis
	8/9	0/10	

Table 2. WILD BIRD POPULATION STUDIED BY SEROLOGICAL MONITORING FOR ARBOVIRUS SURVEILLANCE

Bird Species Studied	No. Studied	Virus Antibody Detected ^(a)		
		No.	Type	
American Goldfinch	10	1	SLE	(Adult)
American Robin	2			
Black-Crested Chickadee	1			
Blue Jay	2			
Boat-tailed Grackle	1			
Brewers Blackbird	3			
Cardinal	2			
Catbird	2			
Cedar Waxwing	1			
Chickadee	1			
Goldfinch	5			
Grackle	1			
House Sparrow	292	5	SLE	(Adult-2) (Immature-3)
Indigo Bunting	1			
Killdeer	3			
Philadelphia Vireo	4			
Red-shafted Flicker	1			
Red-winged Blackbird	19	1	SLE	(Immature)
Red-winged Woodpecker	1			
Robin	1			
Sparrow	14			
Sparrow Hawk	2			
Starling	64			
Swamp Sparrow	3			
Total	436	7		

(a)SLE = St. Louis Encephalitis virus

Table 3. ARBOVIRUS SURVEILLANCE BY SEROLOGICAL STUDIES
OF THE WILD BIRD POPULATION

Location	Date Tested	<u>No. Positive</u> <u>No. Tested</u>	Bird Species	Virus Detected
Davenport	6/7-6/12	1/103	American Goldfinch	SLE ^a
	7/25-7/29	3/111	Red-winged Blackbird-1	SLE
			House Sparrow-2	SLE
Des Moines	6/7	3/34	House Sparrow	SLE
	6/22	0/49		
	7/6	0/17		
	7/20	0/51		
	8/4	0/71		

a.SLE = St. Louis Encephalitis

Table 4.

MOSQUITO POPULATION STUDIED BY VIRUS
ISOLATION FOR ARBOVIRUS SURVEILLANCE

Mosquito Species	Average No. Per. Pool	No. Pools Studied	Virus (a) Isolated
<u>Aedes nigromaculis</u>	19	1	
<u>Aedes triseriatus</u>	27	26	
<u>Aedes trivittatus</u>	159	46	TVT (14)
<u>Anopheles punctipennis</u>	30	15	
<u>Anopheles quadrimaculatus</u>	7	1	
<u>Anopheles walkeri</u>	42	4	
<u>Coquilletidia perturbans</u>	61	35	
<u>Culex pipiens</u>	48	146	FLAN (7)
<u>Culex tarsalis</u>	43	40	FLAN (4) WEE (3) FLAN+TURL (1)
<u>Psorophora ciliata</u>	6	1	
<u>Psorophora horrida</u>	39	3	

(a) Number isolated in parenthesis

Table 5.

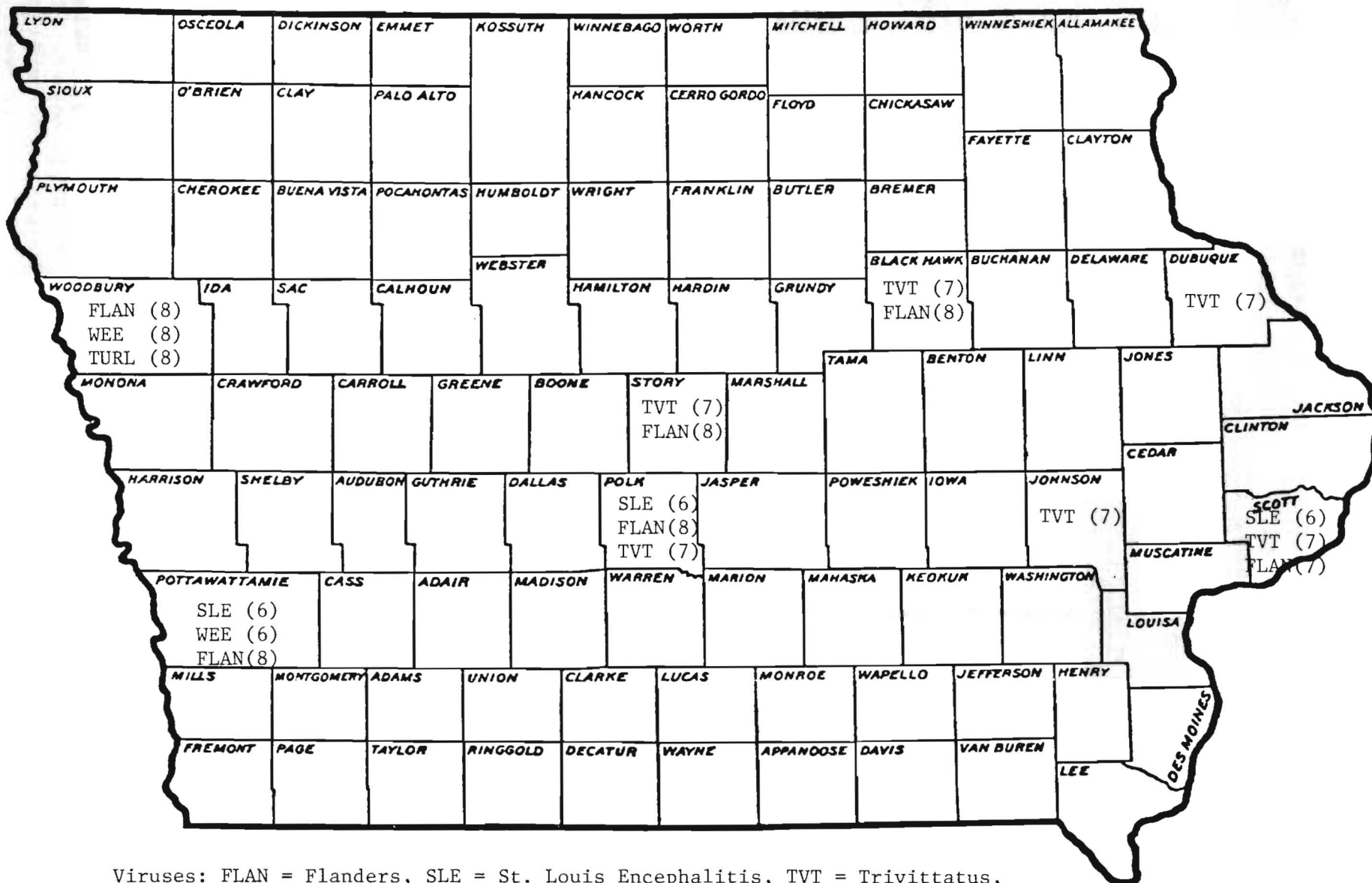
ARBOVIRUS SURVEILLANCE BY ISOLATION OF
VIRUS FROM MOSQUITO POOLS

Location	Date Collected	<u>No. Isolates</u> No. Pools Tested	Mosquito Species	Virus Isolated ^(a)
Sioux City	8/1	6/26	<u>Culex tarsalis</u>	FLAN (4) WEE FLAN+TURL ^(b)
	8/22-23	4/31	<u>Culex tarsalis</u> <u>Culex pipiens</u>	FLAN, WEE(2) FLAN
Council Bluffs	8/2	1/68	<u>Culex pipiens</u>	FLAN
	8/22-23	0/18		
Ames	7/29	2/24	<u>Aedes trivittatus</u> <u>Culex pipiens</u>	TVT FLAN
	8/24	0/16		
	8/30	0/5		
Des Moines	8/4	2/11	<u>Culex pipiens</u>	FLAN
	8/31	1/8	<u>Aedes trivittatus</u>	TVT
Waterloo	7/18	3/21	<u>Aedes trivittatus</u>	TVT
	8/27	2/7	<u>Aedes trivittatus</u> <u>Culex pipiens</u>	TVT FLAN
Cedar Rapids	7/21	0/5		
Iowa City	7/21	3/26	<u>Aedes trivittatus</u>	TVT
	9/2	0/3		
Dubuque	7/28	1/7	<u>Aedes trivittatus</u>	TVT
	8/19	0/4		
Davenport	7/25	4/35	<u>Aedes trivittatus</u> <u>Culex pipiens</u>	TVT FLAN
	8/17	1/7	<u>Aedes trivittatus</u>	TVT

(a) Number isolated in parenthesis; FLAN=Flanders, WEE=Western Equine Encephalitis, TVT=Trivittatus

(b) Two virus isolates from the same pool

Figure 2. LOCATION AND MONTH OF FIRST DETECTION OF ARBOVIRUS ACTIVITY IN IOWA IN 1983



Viruses: FLAN = Flanders, SLE = St. Louis Encephalitis, TVT = Trivittatus,
 TURL = Turlock, WEE = Western Encephalitis
 Months: (6) = June, (7) = July, (8) = August

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY AND ENVIRONMENTAL HEALTH, COLLEGE OF VETERINARY AND BIOMEDICAL SCIENCES, COLORADO STATE UNIVERSITY, FORT COLLINS, COLORADO 80523 USA, AND AGRICULTURAL RESEARCH SERVICE, U.S.D.A, DENVER, COLORADO 80225 USA

The Virogenesis of Bluetongue Virus in Culicoides variipennis

Culicoides variipennis is the principal arthropod vector of bluetongue virus (BTV) in the United States. Substantial knowledge has accrued concerning basic virus-fly interactions, such as thresholds of infection, extrinsic incubation (EI) periods, transmission rates, and the genetic basis of BTV oral infection of C. variipennis. However, little has been known about the mode of development or virogenesis of BTV in the vector. The purpose of these studies was to develop a direct immunofluorescence (IF) technique to detect BTV antigen in tissues of C. variipennis and to elucidate the virogenesis of BTV in the fly.

EXPERIMENTAL DESIGN: Three basic experiments were conducted.

1. In the first study, the IF technique was compared with virus titration in BHK-21 cells⁵ for detection of BTV in C. variipennis. Flies were permitted to engorge an infectious blood meal and held for 14 days. They were then separated into two groups; infection rates were determined by virus isolation for one group and by IF for the other.

To assay flies by IF, heads were severed and squashed on glass slides.⁴ These head squash preparations were then stained with FITC-conjugated¹ hamster anti-BTV immunoglobulins² for 30 min at 37C^o, washed twice in PBS (pH 7.6), and once in distilled water. Coverslips were mounted using PBS-glycerin (1:1) and the slides were examined using an epifluorescence microscope.

2. In the second study, the relative sensitivity of the IF technique was determined. Flies were exposed to an infectious blood meal and were harvested on predetermined days post-engorgement. For each sample day, flies were processed by both IF and virus isolation.

3. In the final studies, the virogenesis of BTV in the vector was investigated. Flies were exposed to an infectious blood meal, then harvested, dissected, and organ systems processed by IF on predetermined days post-engorgement. Also each sample day, five of the flies were titrated in BHK-21 cells.

RESULT: The direct IF and virus isolation techniques were similar in their respective abilities to detect infected culicoids after 14 days extrinsic incubation (EI). Infected flies contained a geometric mean titer of 3.3 log₁₀ TCID₅₀ per fly. Relative fluorescence readings varied from +1 to +4; however, 64% were scored as either +3 or +4. Relative fluorescence readings were directly correlated with the amount of virus in the vector

and the length of the EI period.

Similar results were obtained in the virogenesis studies, however, the length of EI which resulted in the majority of arthropods having disseminated infections was reduced to 10 days, perhaps due to differences in virus titer in the meal or to changes in ambient temperature during the EI. Approximately 50% of dissected midguts contained detectable antigen by six days post-engorgement. In contrast, 50% of head squash preparations were not positive until 10 days post-engorgement.

Results of the virogenesis studies indicated minimal involvement of secondary target organs. (Table 1) The only secondary organ that somewhat regularly contained detectable viral antigen was fat body. Other secondary organ systems, such as heart and pericardial cells infrequently contained BTV antigen. Abdominal ganglia, hindgut, and malphigian tubules were almost invariably antigen free. Virus antigen was infrequently associated with the ovarian sheath, but never with ovarian follicles. This is consistent with the reported lack of transovarial transmission of BTV in C. variipennis.³ BTV antigen was not detectable in salivary glands. This was principally due to the presence of nonspecific staining (NSS) associated with the glands which was frequently so intense that it would undoubtedly obscure the presence of specific fluorescence. NSS was not associated with the accessory salivary glands.

CONCLUSIONS: The virogenesis of BTV in C. variipennis seems generally similar to that of arboviruses in mosquito vectors.¹ After infection and replication in midgut cells, BTV escapes from the midgut and disseminates throughout the vector. In these studies, dissemination of virus apparently occurred between 8 and 10 days EI, with antigen first being detected in midguts between 6 and 8 days EI. The apparent minimal involvement of secondary target organs in virogenesis was surprising. This is in contrast to other virus-vector systems where large quantities of antigen are detectable in secondary target organs.¹ Additional studies are in progress using organ titrations and cryostat sections to delineate further the role of secondary organ systems in the overall virogenesis of BTV in C. variipennis.

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Table 1

Virogenesis of BTV-11 in *C. variipennis* as determined by IF and virus titration.

EI (days)	Immunofluorescence Results					Virus titration	
	Headsquash		Midgut		Secondary Organs	# Pos/# examined (%)	Mean Titer
	# Pos/# examined (%)	Mean IF Reading	# Pos/# examined (%)	Mean IF Reading			
6	0/10 (0)*	-	5/10 (50)	++	1**	1/5 (20)	2.3
8	2/10 (20)	++	5/10 (50)	++	1	3/5 (60)	2.5
10	6/10 (60)	+++	7/10 (70)	++	5	2/5 (40)	3.7
12	5/10 (50)	++++	8/10 (80)	+++	6	3/5 (60)	2.9
14	6/10 (60)	++++	6/10 (60)	++	10	3/5 (60)	3.3
17	1/4 (25)	++++	1/4 (25)	+++	1	0/2 (0)	

* Percent of preparations or suspensions that contained detectable virus antigen or infectious virus.

** Total number of secondary organs systems containing detectable antigen in all flies examined.

Secondary organ systems included salivary glands, ovaries, ovarian duct, malphigian tubules, abdominal ganglia, thoracic muscle, and heart and pericardial cells. The vast majority of the listed positive results were fat body. Almost invariably the other organ systems contained no or minimal detectable antigen.

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY AND ENVIRONMENTAL HEALTH, COLLEGE OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES, COLORADO STATE UNIVERSITY, FORT COLLINS, COLORADO 80523 USA

Titration of Dengue Viruses by Immunofluorescence in Microtiter Plates: A Fast, Reliable, and Inexpensive Alternative

Dengue viruses are some of the most important arboviruses which infect man and are among the most difficult to assay for. A variety of techniques are used to assay infectivity of dengue viruses, which include plaque formation, mosquito inoculations, and determination of endpoints in cell cultures by immunofluorescence. Each of these techniques have their shortcomings. Plaque formation is difficult, mosquito inoculation is sensitive but requires the maintenance of a mosquito colony, and determinations of immunofluorescent endpoints often employ chambered slides that are expensive and may not be easily obtained around the world. This report describes a fast, reliable, and inexpensive alternative to these methods using microtiter plates.

Our laboratory is presently evaluating dengue candidate vaccine viruses in mosquito vectors. This work entails titrations of a large number of mosquitoes orally infected with dengue virus. Currently we assay for the presence of infectious virus by detection of fluorescent foci in mosquito cell cultures using an indirect fluorescent antibody technique [IFAT]. We have developed a titration technique using commonly obtained 96-well, flat bottom, microtiter plates.

The procedures are as follows:

Titration: Chambered slides

Serial 10-fold dilutions of infectious virus suspensions [Table 1] were inoculated into eight chambered Lab-Tek^R slides [Miles Laboratories, Inc., 30W475 North Aurora Road, Naperville, Illinois, 60540] [0.10 ml per chamber]. Four replicates were done for each dilution. Each well was seeded with 0.35 ml of a suspension of C6/36 cells containing 2×10^6 cells per ml. The slides were kept in a humid incubator at 27°C for 7 days. After incubation the slides were wet fixed in cold acetone for at least 10 min and stored at -20°C until examination for viral antigen by IFAT.

Titration: Microtiter plates

Serial 10-fold dilutions of infectious virus suspensions [Table 1] were inoculated into 96-well, flat bottom, microtiter plates [0.025 or 0.050 ml per well]. Eight to sixteen replicates were done of each dilution. Each well was seeded with 0.15 ml of a suspension of C6/36 cells containing 1×10^6 cells per ml. The plates were kept in a humid incubator at 27°C for 7 days. After incubation, cold buffered acetone [1:3 ratio of 0.10M phosphate buffered saline [PBS], pH 7.5 and acetone] was added to each well and incubated at room temperature for 30 min. The plates were examined for viral antigen by IFAT.

Table 1. Samples titrated in both chambered slides and microtiter plates.

Sample	Description of the Sample
1-4	<u>Aedes albopictus</u> mosquitoes ingesting dengue-4 parent virus [H-241] and extrinsically incubated for 21 days at 33°C.
5-8	<u>Aedes albopictus</u> mosquitoes ingesting dengue-4 candidate vaccine virus [H-241, Lot 1] and extrinsically incubated for 21 days at 33°C.
9	Blood meal preparation of dengue-1 parent virus [P8D6].
10	Blood meal preparation of dengue-1 candidate vaccine virus [45AZ5].
11	Working stock virus dengue-3 parent virus [CH53489, PGMK-4, d9].
12	Working stock virus dengue-3 candidate vaccine virus [CH53489, Clone 24/28].

Indirect Fluorescent Antibody Technique :

The chambered slides were stained with a 1:100 dilution of the specific hyperimmune mouse ascitic fluid [HMAF] and incubated for 40 min at 37°C in a humidified chamber. After incubation, the slides were washed 2 times in PBS, pH 7.5 for 10 min each, rinsed in distilled H₂O, and air dried. Then a 1:100 dilution of a commercial, fluorescein-conjugated, goat anti-mouse gamma globulin was added to each slide and incubated again for 40 min at 37°C in a humidified chamber. Cells were counter stained by adding 0.0005% Evans blue to the conjugate. After incubation the slides were washed as above and were then mounted using a 1:1 mixture of PBS and glycerol.

Microtiter plates were stained before storage at -20°C. After fixation the buffered acetone was aspirated using a 14 gauge, 1 1/2 inch needle attached to a vacuum system. The bevel of the needle was placed against the side of the well to prevent loss of cells during aspiration. The plates were air dried and stained by the same procedure as described above. Washes were done using an eight-channeled pipettor or an ELISA plate washer. After washing, 0.10-0.20 ml of a mixture of PBS and glycerol [1:3] was added to each well and incubated at room temperature for 30 min. The PBS-glycerol mixture was aspirated out of each well leaving a thin film to cover the cells. The cover was sealed to the microtiter plate with tape. The plates were inverted and examined using an epifluorescence microscope [Olympus Model BH-2] equipped with a 20X long working distance objective, an HBO 100W high pressure mercury burner, and a IF-490 exciter filter. Negative and positive controls were included in all tests.

Table 2. Comparison of virus titers obtained in titrations using chambered slides and microtiter plates.

Sample	Virus Titer ¹	
	Chambered Slides	Microtiter Plates
1	≥5.25	5.1
2	≥5.5	5.4
3	≥5.5	4.8
4	5.5	≥5.1
5	0	0
6	0	0
7	4.5	4.4
8	0	0
9	7.25	6.7
10	7.50	7.2
11	6.75	7.1
12	6.25	6.5

¹ Log₁₀TCID₅₀ per ml

Virus titers obtained in the microtiter system were comparable to those in the chambered slide system [Table 2]. Unfortunately end points were not reached in three samples using the slides. However, titrations of previous samples have indicated that these titers are very near the endpoints. Further comparisons of the two systems are in progress.

The microtitration system had three basic advantages over the chambered slide system. First, the cost of supplies was higher for titrations using slides. Titration of a single mosquito suspension required two slides at a cost of \$2.50 each [American Scientific Products Catalog 1984-85]. In a microtiter plate that costs approximately \$1.80 [Costar^R Price List, July 15, 1983], three mosquito suspensions could be run with twice as many replicates. Second, the biggest savings were in the time spent by laboratory personnel in setting up the plates and during the staining procedure. It was considerably easier working with three mosquito titrations as a single unit than six individual slides when adding samples, HMAF, or conjugate. There was no need for gasket removal, which at times can be a difficult process. In addition, washings during the staining procedure were greatly facilitated by the use of the ELISA plate washer. Third, valuable reagents were conserved; the wells of the microtiter plate greatly reduced the amount of immune reagents needed to stain the cells.

[Randal J. Schoepp and Barry J Beaty]

Report from Division of Vector-borne Viral Diseases, CID, CDC,
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VERTICAL TRANSMISSION OF ST. LOUIS ENCEPHALITIS (SLE) VIRUS FOR SEVERAL
GEOGRAPHIC STRAINS OF CULEX PIPIENS COMPLEX MOSQUITOES

Vertical transmission of SLE virus was successfully demonstrated recently for Culex pipiens complex mosquitoes (1). The current study was initiated to look at variation in rates of vertical transmission (VT) of SLE virus among several geographic strains of this mosquito complex. The mosquito strains and their history are summarized in Table 1. The seven U.S. strains included three of Cx. pipiens pipiens, three of Cx. p. quinquefasciatus, and a hybrid population from Memphis, Tennessee. A single strain from Guayaquil, Ecuador was also included in the study.

The SLE virus strain used in the study was one originally isolated from Culex mosquitoes from Memphis, Tennessee, in 1976. Stock virus was prepared by intrathoracic inoculation of mosquitoes from a laboratory colony of Cx. p. pipiens. The virus had a titer of $10^{6.6}$ /ml in Vero cell culture. Colonization of mosquitoes was conducted in a growth chamber at a temperature of $27 \pm 1^\circ\text{C}$, relative humidity of $80\% \pm 5\%$, and a L:D photophase of 16:8. Mated female mosquitoes were fed on chicks inoculated 36 hrs. previously with the stock virus. Only fully engorged mosquitoes were used for the study. Females were allowed to oviposit, and eggs from the first ovarian cycle were discarded. In the first experiment, intervals of 10, 14, 18, and 22 days were established between the initial infectious blood meal and a subsequent second blood feeding on normal chicks. In the main study, a period of 14 days between the two blood feedings was selected for comparison of vertical transmission among the geographic mosquito strains.

Mosquitoes were allowed to oviposit after the second blood meal on a noninfected chick, and second ovarian cycle eggs were immediately transferred to a growth chamber with a temperature of $18 \pm 1^\circ\text{C}$, RH of 80%, and a L:D cycle of 16:8. Emerged adults were held for 10 days before they were separated by sex into pools of ≤ 100 and held at -60°C for testing. Pools were triturated in 1 ml of diluent, centrifuged, and the suspension inoculated onto cell culture for assay. Suspensions from the first experiment were tested in primary embryonic Pekin duck, LLCMK₂ cells, and Aedes albopictus C6/36, while those from the main experiment were tested in LLCMK₂ cell culture only.

Chicks used to infect mosquitoes were shown to have viremias ranging from $10^{4.8}$ to $10^{6.0}$ PFU/ml in the first experiment and $10^{4.3}$ to $10^{5.5}$ PFU/ml in the second trial. These titers were above the threshold necessary to infect virtually 100% of the feeding mosquitoes.

In the first experiment, summarized in Table 2, 10 to 14 days of parental extrinsic incubation were observed to produce the highest rates of infected progeny. Since the second trial included a larger sample and because VT rates for female progeny concurred at 14 days, this was the extrinsic incubation period chosen to be used for the main experiments.

Results for the experiments comparing the eight strains of Cx. pipiens complex mosquitoes are summarized in Table 3. In comparing the strains representing Cx. p. pipiens (Colorado, Ohio, Illinois) vs. those representing Cx. p. quinquefasciatus (Texas, Missouri, Florida), VT rates in the Cx. p. quinquefasciatus were statistically significantly higher. In making comparisons within the groups, the three strains of Cx. p. pipiens were found to be similar in their ability to vertically transmit SLE virus. The Missouri and Texas Cx. p. quinquefasciatus were equally capable of vertically infecting progeny; however, the Florida Cx. p. quinquefasciatus were less efficient and was indistinguishable from the Cx. p. pipiens populations in this respect. Likewise, the Ecuador Cx. p. quinquefasciatus were similar to the North American Cx. p. pipiens populations.

The variation in vertical transmission rates exhibited by these mosquito populations seems to indicate that the unique genetic makeup of a population constitutes at least one factor that determines frequency of vertical virus transmission to progeny. The failure of the Florida Cx. p. quinquefasciatus to infect its progeny with the same efficiency as the Missouri and Texas strains may reflect the vector status of this subspecies in Florida where Cx. nigripalpus has replaced Cx. p. quinquefasciatus as a vector. Perhaps, the association between SLE virus and Cx. p. quinquefasciatus is a weak one and has presented little opportunity for selecting for a trait such as vertical transmission. It could also be hypothesized that an analogous situation has evolved in Colorado where Cx. tarsalis is the principal vector of SLE virus and the Cx. p. pipiens play an insignificant role in transmission.

The vector of SLE virus in Ecuador is not known with certainty; however, the only isolate of this virus to date from Ecuador is from Cx. nigripalpus, a situation analogous to Florida. If Cx. nigripalpus is the vector in Ecuador, then it could again be conjectured that the relationship that exists between SLE virus and Cx. p. quinquefasciatus is tenuous and that no opportunity to select for vertical transmission efficiency has occurred.

The superior ability for VT to progeny in the Memphis hybrid population of Cx. pipiens complex mosquitoes is somewhat surprising. It is possible that vertical transmission efficiency has somehow been selected for in the interbreeding and hybridization of the two subspecies. Perhaps, a more plausible explanation is the fact that the virus strain used in the study was isolated from field collected Memphis mosquitoes. This hypothesis can be easily explored in the laboratory by using SLE virus strains that are geographically associated with each of the populations.

The rates of vertical transmission to progeny in these experiments are generally low, and the question still remains as to whether or not this phenomenon is of importance in the field. The proof of its value in the natural history of SLE virus can most convincingly be shown by isolation of the virus from field collected eggs or larval mosquitoes. It has been experimentally demonstrated that virus is efficiently transmitted to a host by bite from vertically infected females (1). Other considerations that may increase the importance of relatively low vertical transmission rates are venereal transmission, which has been demonstrated for Ae. triseriatus and La Crosse virus (2), and a stabilized infection in vertically infected progeny, demonstrated to exist for California encephalitis virus in Ae. dorsalis (3).

(Susan A. Taylor and D. Bruce Francy, Arbovirus Ecology Branch, Division of Vector-Borne Viral Diseases, Fort Collins, Colorado)

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Table 1. Colonization history of Culex pipiens subspecies.

Subspecies	Geographical origin	Date of colonization
<u>Cx. p. pipiens</u>	Ft. Collins, Colorado	1973
<u>Cx. p. pipiens</u>	Chicago, Illinois	1980
<u>Cx. p. pipiens</u>	Dayton, Ohio	1975
<u>Cx. p. quinquefasciatus</u>	Houston, Texas	1981
<u>Cx. p. quinquefasciatus</u>	St. Louis, Missouri	1975
<u>Cx. p. quinquefasciatus</u>	Tampa, Florida	1977
<u>Cx. p. quinquefasciatus</u>	Guayaquill, Ecuador	1976
<u>Cx. pipiens</u> complex	Memphis, Tennessee	1976

Table 2. Comparison of four extrinsic incubation periods by orally infected (SLE virus) *Culex pipiens* complex females on transovarial infection rates in second ovarian progeny.

	10 days		14 days		18 days		22 days	
	Male	Female	Male	Female	Male	Female	Male	Female
Infection rate ¹⁾								
1st trial	4/818 (4.9)	5/469 (10.1)	0/664 (---)	2/483 (4.1)	0/225 (---)	1/191 (5.2)	0/69 (---)	0/43 (---)
2nd trial	12/7951 (1.5)	21/7357 (2.9)	12/5222 (2.3)	14/3386 (4.1)	6/3598 (1.7)	5/2573 (1.9)	3/2737 (1.1)	6/2351 (2.6)
TOTAL	16/8769 (1.8)	26/7626 (3.4)	12/5886 (2.0)	16/3869 (4.1)	6/3823 (1.6)	6/2764 (2.2)	3/2806 (1.1)	6/2394 (2.5)

¹⁾Minimum infection rate per thousand, tested in pools of ≤ 100 , DECC, Vero, and C6/36 cell culture.

Table 3. Transovarial infection rates¹⁾ in second ovarian progeny from eight geographical subspecies of Culex pipiens females orally infected with St. Louis encephalitis virus.

Mosquito	Chick viremia/ml	Male	Female	Total
<u>Cx. p. pipiens</u>				
Dayton, Ohio	pre- 4.7-5.7 post- 4.6-5.7	3/7220 (.4)	2/6493 (.3)	5/13663 (.4)
Chicago, Illinois	pre- 4.2-6.0 post- 4.4-4.6	1/5666 (.2)	1/4065 (.3)	2/9731 (.2)
Ft. Collins, Colorado	pre- 5.5-6.3 post- 5.3-6.0	0/926 (---)	0/779 (---)	0/1705 (---)
<u>Cx. p. quinquefasciatus</u>				
Tampa, Florida	pre- 4.7-5.8 post- 4.6-5.4	8/11645 (.7)	7/8467 (.8)	15/20112 (.8)
St. Louis, Missouri	pre- 3.9-5.8 post- 4.7-5.6	6/2091 (2.9)	3/1844 (1.6)	9/3935 (2.3)
Houston, Texas	pre- 5.5-6.3 post- 5.3-6.0	6/2853 (2.1)	9/2351 (3.8)	15/5204 (2.9)
Ecuador	pre- 3.9-5.8 post- 4.7-5.6	0/6100 (---)	1/5079 (.2)	1/11179 (.1)
<u>Cx. pipiens complex</u>				
Memphis, Tennessee	pre- 4.2-6.0 post- 4.4-4.6	81/18318 (4.4)	97/12715 (7.6)	178/31033 (5.7)

¹⁾Minimum infection rate per thousand, tested in pools of ≤ 100 , LLC-MK₂ cell culture.

Arboviral Encephalitides,
United States, 1983

One hundred and 2 cases of encephalitis caused by arboviruses were reported in the United States in 1983 (Figures 1 and 2). A major outbreak of Cx. pipiens borne SLE that was expected this year did not materialize. Although minimal evidence of SLE activity was observed in the central United States, 2 sporadic cases occurred as far north as Cook County, Illinois. Sporadic SLE was reported from Bernalillo County, N. Mexico, El Paso County, Texas, and Jackson County, Indiana. SLE was also documented in a Florida man who travelled widely in the month before onset of illness. An outbreak of Cx. tarsalis borne SLE, localised in the southwestern United States, occurred in association with flooding along the Colorado river. The crude attack rate for towns and census subdivisions bordering the river was 5.1/100,000 residents (8/155,928). The attack rate in the greater Yuma area, including Winterhaven and Bard, California, was estimated to be 7.2/100,000 (5/70,649).

Hyperendemic transmission of EEE in the Taunton Valley in Massachusetts and in nearby Rhode Island and Connecticut led to cases in 8 persons and 14 horses. An outbreak of EEE in northeastern Indiana and southern Michigan resulted in 1 human and 16 equine cases.

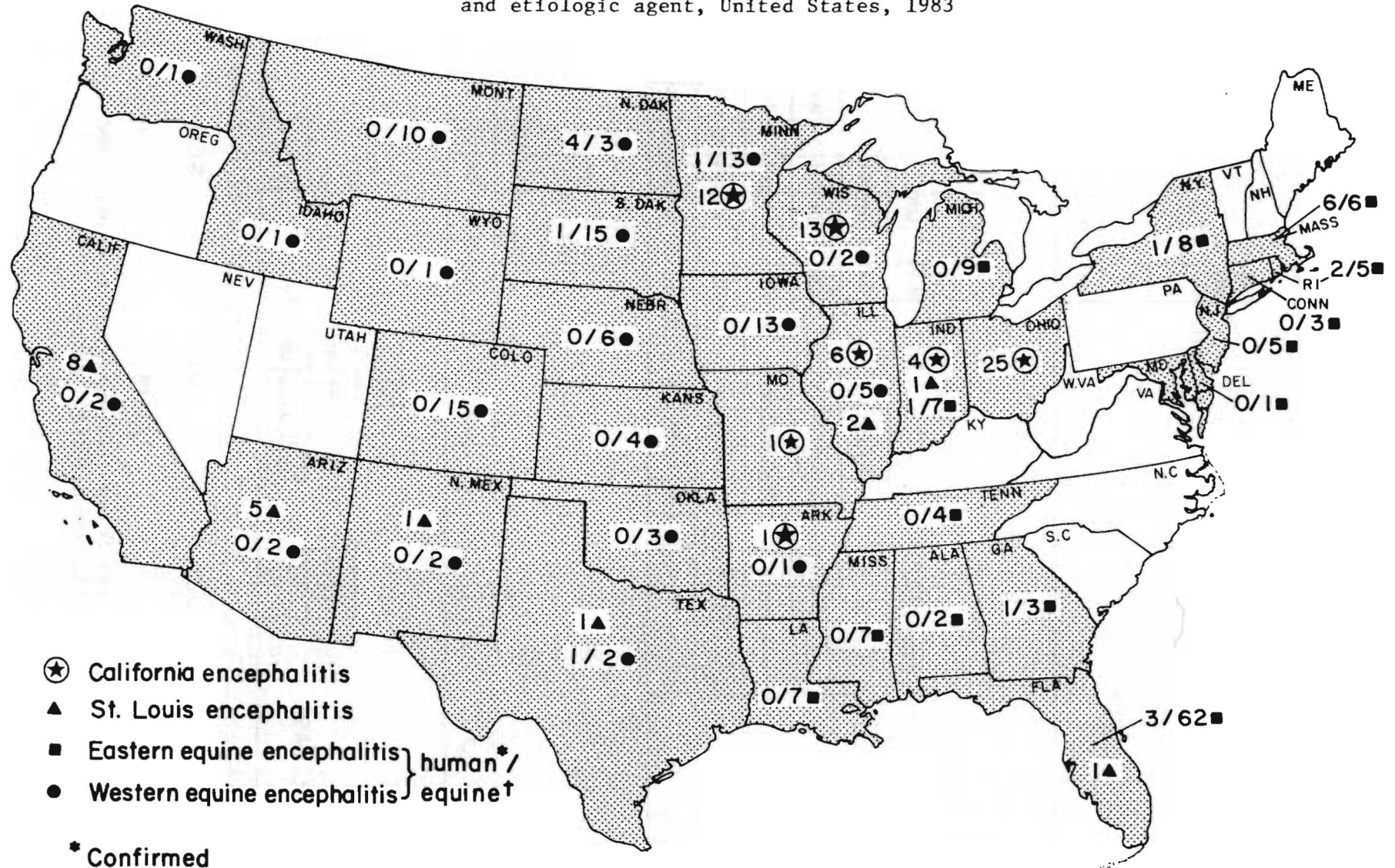
An outbreak of WEE led to 6 human cases in disparate areas of North and South Dakota and Minnesota. The cases were all in males: 3 and 7 week old infants, 6, 10, and 15 year old boys, and a 22 year old man. A single case of WEE was confirmed in a man from Hale County, Texas where WEE has been prevalent in previous years.

Sixty two confirmed cases of California encephalitis occurred primarily in states bordering the Great Lakes. Forty one cases (66%) occurred in males. All the cases but one were in children between 1 and 15 years of age. It is unknown which of the California viruses led to the case in a 70 year old man.

(T.F.Tsai, Bruce Franczy, and T.P. Monath, Division of Vector-Borne Viral Diseases, Fort Collins, Colorado)

Figure 2

Arboviral Encephalitides, by state of residence and etiologic agent, United States, 1983



Hemagglutination Inhibition Antibody in
Hemorrhagic Fever with Renal Syndrome

A pH dependent hemagglutinin was found in a sucrose acetone extracted antigen prepared from brain of suckling mice (SM) infected with Hantaan virus (HTNV), the etiologic agent of hemorrhagic fever with renal syndrome (HFRS). Suckling mice were inoculated with a mouse adapted 76-118 strain of HTNV and harvested 12 days after inoculation. The preparation of antigen deviated from the conventional procedure of Clarke & Casals in two points: the brain was irradiated from a ^{60}Co source before homogenizing and extraction, in order to minimize risk of infection to the operators; and the antigen was reconstituted in saline and sonicated at low intensity. Hemagglutination (HA) of erythrocytes from a male Chinese grey goose (Anser cygnoides) was pH dependent and optimum at pH 6.0 and 6.2. Sera were extracted with acetone and tested using a standard microtiter procedure.

Hemagglutination inhibition (HI) and immunofluorescent (IF) antibodies were compared in 64 sera from cases of HFRS, 16 control patients, and 13 animal sera. The correlation of HI and IF antibody titers was excellent ($\log \text{HI titer} = 1.0341 \log \text{IFA titer} + .1171$, $r = .887$, $p < .01$). The HI test was 97% sensitive when compared to the IFA procedure. HI antibody rises rapidly in the course of infection and was present in all acute HFRS sera tested. HI antibody was found in patients bled 15-20 years after recovery from HFRS and may be life-long. The presence of HI and IF antibodies were concordant in two Suncus murinus and three Apodemus agarius.

Disadvantages of the IFA test such as the need for expensive equipment, species specific conjugates, and difficulties in standardization are overcome by the HI test.

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Epizootic of Western Equine Encephalitis in Argentina (1982-83)

A severe epizootic of equine encephalitis occurred in Argentina during the latter part of 1982 and the first few months of 1983. The first cases were detected in Santa Fe Province during October 1982, and the last in Viedma, Rio Negro Province, during May 1983. Dra. Marta Sabattini isolated several strains of WEE virus from horses that died, and demonstrated specific fourfold or greater rises in HI antibody to WEE virus in more than 20 pairs of sera from clinically ill horses.

Extensive mosquito collections were made in Santa Fe Province during December 1982, at sites where horse cases were occurring. Sixteen virus isolates have been made from 149,348 mosquitoes tested in 2,189 pools (Table 1). These include 4 WEE, 6 VEE, 1 SLE, 1 strain of an unregistered virus (Antequera) previously isolated in Argentina by us in 1980, and 4 strains that have yet to be identified. The WEE and VEE strains have not been subtyped. The isolation of WEE virus from such a wide variety of mosquito species (An. albitarsis, Ae. albifasciatus, Ms. spp., and Ps. pallescens) and the apparent lack of involvement of Culex mosquitoes is puzzling. The possibility that horses play a role as virus donors, as in the case of epizootic VEE elsewhere in the Americas, must be considered.

Cx. (Mel.) delpontei was previously shown to be an important vector of enzootic VEE virus in Chaco Province (DVBVD Ann. Rpts. 1980, 1981). The involvement of this species as a vector of presumably enzootic VEE virus in Santa Fe Province has now been confirmed by the isolation of 3 strains of VEE virus from 526 specimens (minimum infection rate of 5.7/1,000).

(Carl J. Mitchell and Thomas P. Monath, Division of Vector-Borne Viral Diseases, Fort Collins, Colorado; Marta S. Sabattini and Jose F. Daffner, Institute of Virology, Faculty of Medical Sciences, National University of Cordoba.)

Table 1. Relative abundance of mosquitoes* in collections from Santa Fe Province during December 1982 and viruses isolated.

Species or Group	No. Collected	% of Total	No. Pools Tested for Virus	Virus Isolates
<u>Ad. squamipennis</u>	483	0.3	51	1****
<u>Ae. albifasciatus</u>	63,890	42.8	675	1 WEE
<u>Ae. scapularis</u>	1,384	0.9	49	
<u>Ae. serratus</u>	221	0.1	25	
<u>Ae. stigmaticus</u>	180	0.1	11	1 VEE
<u>Ae. spp.</u>	3,919	2.6	65	
<u>An. albitarsis</u>	2,132	1.4	47	1 WEE
<u>An. minor</u>	2	----***	2	
<u>An. oswaldoi</u>	7	---	2	
<u>An. punctimacula</u>	2	---	1	
<u>An. spp.</u>	474	0.3	26	
<u>Cx. (Car.) spp.</u>	91	0.1	13	
<u>Cx. (Mcx.) spp.</u>	108	0.1	14	
<u>Cx. (Mel.) delpontei</u>	526	0.4	23	3 VEE, 1 ANT, 1****
<u>Cx. (Mel.) spp.</u>	890	0.6	27	
<u>Cx. p. quinquefasciatus</u>	3	---	2	
<u>Cx. spp.**</u>	51,426	34.4	596	1 SLE, 1****
<u>Haemagogus spp.</u>	9	---	6	
<u>Ma. humeralis</u>	31	---	8	
<u>Ms. spp.</u>	5,289	3.5	106	1 WEE
<u>Ph. spp.</u>	166	0.1	6	
<u>Ps. ciliata</u>	849	0.6	35	
<u>Ps. confinnis</u>	19	---	11	
<u>Ps. cyanescens</u>	2,582	1.7	51	
<u>Ps. dimidiata</u>	1,132	0.8	31	
<u>Ps. discrucians</u>	525	0.4	43	
<u>Ps. ferox</u>	98	0.1	7	
<u>Ps. pallescens</u>	654	0.4	29	1 WEE
<u>Ps. paulli</u>	357	0.2	16	
<u>Ps. varinervis</u>	10,760	7.2	145	1 VEE, 1****
<u>Ps. varipes</u>	6	---	2	
<u>Ps. (Jan.) spp.</u>	837	0.6	32	
<u>Ps. spp.</u>	92	0.1	10	
<u>Ur. spp.</u>	204	0.1	22	1 VEE
TOTALS	149,348		2,189	

* In addition to mosquitoes, 9 specimens of Ceratopogonidae (2 pools) and 6 specimens of Simuliidae (1 pool) also were collected and tested for virus.

** Although not specified, the majority of the 51,426 Cx. spp. were in the subgenus Culex.

*** Less than 0.1% of total.

**** Identification pending.

Genetic and Antigenic Variation Among Geographical
Isolates of Sindbis Virus

We have examined the genetic and antigenic variation of 12 Sindbis (SIN) virus isolates from four zoogeographic regions: Paleoarctic, Ethiopian, Oriental, and Australian (Table 1). RNase T1 oligonucleotide fingerprinting of the genomes of the SIN isolates from these geographic regions revealed that the primary structure of the RNA from viruses representing each zoogeographic region was unique and that strains isolated from different areas were similar to each other and different than those from other areas (Table 2).

Tryptic peptide maps of the E1 and E2 envelope glycoproteins and the capsid proteins of two SIN virus isolates from each of the zoogeographic regions were compared with the tryptic maps of corresponding proteins of the AR-339 prototype strain (Table 3). Tryptic maps of the structural proteins of the Gresikova virus from Sicily and two Ethiopian isolates MP-684 and AR-18132 were similar to AR-339. Peptide maps of the E1 and E2 glycoproteins of SIN virus isolates from the Oriental and Australian region were quite different suggesting a distant relationship between viruses from these geographic areas and the prototype AR-339 virus.

Ten SIN virus isolates representing each of the four zoogeographic regions were analyzed antigenically by neutralization with polyclonal sera and ELISA using anti-E2 monoclonal antibody to the prototype AR-339 strain (Table 4). A clear antigenic divergence of the SIN isolates along two broad geographic lines was observed. Viruses from the Paleoarctic-Ethiopian region were antigenically similar to AR-339, whereas Oriental-Australian isolates were distinct.

Our results support the hypothesis proposed by Rentier-Delrue and Young (*Virology*, 106, 59-70, 1980) suggesting that ancestral Sindbis virus diverged into two distinct groups and indicates that the genetic changes have resulted in phenotypic divergence within each of the geographic varieties, or SIN topotypes (Table 5).

(Kenneth Olson and Dennis W. Trent, Immunochemistry Branch, Division of Vector-Borne Viral Diseases, Fort Collins, Colorado.)

Table 1. Sindbis viruses studied: strain designation, geographic origin, year of isolation, source of isolation, laboratory passage host, and number of passages.

Strain	Geographic Locality	Origin Country	Year of Isolation	Source of Isolation	Laboratory Host of Isolation	Passage Number
AR-339	Sindbis	Egypt	1952	<u>Culex univittatus</u>	mice	13
Gresikova	Eastern	Sicily	1975	<u>Hyaloma marginatum</u>	mice	1
Acrocephalus	Western	Czech.	1971	<u>Acrocephalus Scirpaceous</u>	mice	7
AR-18132	Upington	S. Africa	1976	<u>Culex univittatus</u>	mice	1
MP-684	Entebbe	Uganda	1958	<u>Mansonia fuscopennata</u>	mice	7
W32309	Rubin	Israel	1964	<u>Streptopelia turtur</u>	mice	1
A-1036	Mysore State	India	1953	<u>Bdellonyssus bursa</u>	mice	13
B-322/23/24	Poona	India	1953	<u>Motacilla alba</u>	mice	10
P-886	Luzon	Philippines	1956	<u>Culex bitaeniorhynchus</u>	mice	1
MM-2215	Kuantan	Malaysia	1955	<u>Culex tritaeniorhynchus</u>	mice	2
C-377	Cairns	Australia	1960	<u>Mansonia septempunctata</u>	mice	1
MRM-18520		Australia	1975	Mosquitoes (species unknown)	mice	6

Table 2. Summarization of RNA homologies of SIN viruses by T1 RNase oligonucleotide fingerprints.

Zoogeographic region ^{1/}	SIN strain	Geographic origin	Percent homology of SIN virus isolates ^{2/}	Missing oligonucleotides ^{3/}	No. of new oligonucleotides ^{4/}
Paleoarctic	AR-339 ^{5/}	Egypt	37/37 (100%)	None	None
	Acrocephalus	Czech.	36/37 (97%)	2	None
	Gresikova	Sicily	34/37 (92%)	2,3,5	None
	W32309	Israel	25/37 (68%)	4,6,16,18,21,24,32-37	10
Ethiopian	AR-18132 ^{5/}	S. Africa	41/41 (100%)	None	None
	MP-684	Uganda	34/41 (85%)	3,5,9,11,33,34	1
Oriental	A-1036 ^{5/}	India	40/40 (100%)	None	None
	B-322/23/24	India	39/40 (98%)	26	2
	P-886	Philippines	30/40 (75%)	1,5,9-11,17,18,26,27,30	4
Australian	MRM-18520 ^{5/}	Australia	46/46 (100%)	None	None
	C-377	Australia	38/46 (83%)	6-9,12,15,17,19,30	2
	MM-2215	Malaysia	37/46 (80%)	1,6,7,9,12,14,15,19,29	9

^{1/} Zoogeographic region determined by mammalian migration barriers and described by Darlington (1957).

^{2/} Percent homology (greater than 50%) determined by the number of comigrating oligonucleotides with a reference strain.

^{3/} The number of the oligonucleotide present in the reference strain but missing from the compared strain.

^{4/} The number of new oligonucleotides missing from the reference strain.

^{5/} Reference strain for a specific zoogeographic region.

Table 3. Summary of comparative HPLC tryptic peptide map analysis of Sindbis virus structural proteins.

Virus Strain	Geographic Origin	Different peaks ^{1/}			Similarity ratio ^{2/}		
		Capsid	E1	E2	E1	Capsid	E2
Ar-339	Africa	0	0	0	100	100	100
Gresikova	Sicily	2	2	1	96	94	96
AR-18132	S. Africa	0	5	7	100	85	69
MP-684	Uganda	4	2	5	85	97	86
A-1036	India	8	14	17	70	64	55
B-322/23/24	India	7	17	19	75	55	59
C-377	Australia	--	21	19	--	26	45
MRM-18520	Australia	12	18	14	65	50	66

^{1/} Number of non-coeluting AR-339 and comparison virus in the HPLC chromatogram of tryptic maps.

^{2/}
$$\frac{(\text{Total of } ^3\text{H-peaks and } ^{14}\text{C-peaks}) - (\text{number of different peaks})}{(\text{Total number of } ^3\text{H-peaks and } ^{14}\text{C-peaks})}$$

Table 4. Plaque-reduction neutralization (N) and ELISA analysis of Sindbis virus isolates using polyclonal antibody (AR-339) and anti-E2 monoclonal antibody to Sindbis prototype virus AR-339.

Virus	N titer ^{1/}	ELISA ^{2/}
AR-339	1280	1.11
Acrocephalus	1280	ND
Gresikova	1280	1.07
W32309	320	ND
AR-18132	320	0.82
MP-684	1280	0.69
A-1036	1280	0.29
B-322/23/24	ND	0.32
P-886	320	0.27
MM-2215	320	0.28
MRM-18520	320	0.36
C-337	640	0.34

^{1/} The N titer is expressed as the reciprocal of the antibody dilution, which neutralized 80% of the infectious virus in the test.

^{2/} Absorbance reading of ELISA reaction (400 nm).

^{3/} ND, Not done

Table 5. A comparison of geographic groupings of SIN virus isolates based on T1 oligonucleotide and structural proteins tryptic peptide homologies with RNA-RNA hybridization.^{1/}

SIN groups based on T1 oligonucleotide fingerprints, and peptide maps	SIN groups based on hybridization studies
<p>Paleoarctic (Group I)</p> <p>AR-339 Egypt Acrocephalus Czechoslovakia Gresikova Sicily W32309 Israel</p>	<p>Paleoarctic (Group I)</p> <p>AR-339 Egypt M-1855 Israel R-33 Czechoslovakia AZ-16 USSR</p>
<p>Ethiopian (Group II)</p> <p>AR-18132 South Africa MP-684 Uganda</p>	<p>Ethiopian (Group II)</p> <p>AR-86 South Africa AR-18132 South Africa AR-6071 South Africa Girdwood South Africa</p>
<p>India-Far Eastern (Group III)</p> <p>A-1036 India B-322/23/24 India P-886 Philippine</p>	<p>India-Far Eastern (Group III)</p> <p>A-1036 India B-322/23/24 India P-886 Philippines</p>
<p>Australian-Far Eastern (Group IV)</p> <p>MRM-18520 Australian C-377 Australian MM-2215 Malaysia</p>	<p>Australian-Far Eastern (Group IV)</p> <p>C-377 Australia CH-19470 Australian MM-2215 Malaysia</p>

^{1/} Rentier-Delrue and Young (1980)

Japanese Encephalitis Vaccine - Results of a Two Dose Regimen
as an Investigational New Drug in the United States

On May 17, 1983 the Division of Vector-Borne Viral Diseases (DVBVD), CID, CDC, received approval from BOB/FDA to proceed with preliminary evaluation of the Biken JE vaccine as an investigational new drug (IND). Several co-investigators were recruited to administer the vaccine to persons traveling to Asia. An approved protocol was used, following guidelines for voluntary consent. Three lots of Biken vaccine purchased by CDC were used. One lot (55-1), used only in the first group of 11 vaccinees at the World Bank, was obtained prior to a large CDC purchase of vaccine in single dose (Lot 57-1) and 10-dose (Lot 56-1) vials used for all subsequent immunizations. All lots had passed Biken standards for purity and potency. Vaccinees received 2 1.0 ml injections spaced 7-14 days apart, as specified by the manufacturer. Pre-immunization sera were obtained; 4-6 weeks after the first inoculation, vaccinees were re-bled.

This report summarizes information on 89 participants in the IND evaluation.

Serologic Responses to JE Vaccine

To date, 89 participants have been recruited from the following institutions: The World Bank, Washington, D.C. (31 persons); Colorado State University, Fort Collins, Colorado (28); Atlantic-Richfield Oil Company, Los Angeles, California (11); and the University of Washington, Seattle (19). Fifteen participants were excluded from analysis of serological response because they either were seropositive to JE virus prior to receiving the first vaccine dose (14 participants) or failed to complete the course of two vaccine doses (1 participant). Sera of vaccinees were tested at serial twofold dilutions in a plaque reduction neutralization test in Vero cells; the virus dose was 70-120 PFU; sera were heat-inactivated and fresh serum factor was included in the virus-serum mixtures. Titer was expressed as the highest dilution of serum which reduced plaque counts by $\geq 70\%$.

Table 1 shows the results of immunization of the 68 seronegative participants: 56 (82%) had a fourfold or greater antibody response to the Nakayama JE virus strain, the virus strain used to prepare the vaccine; 49 (72%) developed titers of ≥ 8 , considered to be the minimum protective titer.

Table 2-A compares serologic responses of participants to six JE virus strains - Nakayama (vaccine substrate); and strains G8924, isolated in 1956 in southern India; 826309, Goa, India, 1982; 782219, Madras State, India, 1978, and two Nakayama laboratory strains received from Walter Reed identified as

"YOKEN" and "WRAIR". There was a high degree of correlation between serologic responses to Nakayama and strains G8924, 826309, 782219, YOKEN, and WRAIR ($r = .78, .74, .60, .64, \text{ and } .72$, respectively; $p < .001$). Table 2-B shows that from 68 to 74 percent of vaccinees developed neutralizing antibody titers ≥ 8 to both Nakayama and each of the 5 comparative strains. From 91 to 93 percent developed titers of ≥ 8 to both Nakayama laboratory strains received from Walter Reed and from the 1978 isolate from India compared to 82% achieving titers in that range to the prototype Nakayama vaccine strain.

The geometric mean titers shown in Table 3 are underestimates since many sera were not titrated beyond a 1:64 dilution; a few sera were re-examined at higher dilutions. Females consistently responded with higher titers than males to all six JE strains tested. There was no evidence that the Biken vaccine, prepared from the Nakayama strain isolated in Japan 38 years ago, was less effective in inducing immunity to recent strains from a distant area, India.

A history of prior vaccination with another flavivirus (yellow fever) had no evident effect on the serological response to the JE vaccine (Table 4).

In summary, our experience is consistent with published accounts of the effects of the Biken vaccine: 82% of persons initially seronegative to JE had a fourfold increase in antibody to the vaccine. However, only 72% of the vaccinees developed a titer of ≥ 8 2.5 to 4.5 weeks after completion of the 2-dose vaccine schedule. On the basis of protection tests in animals with a related virus (SLE), a titer of ≥ 8 is considered to be the minimal level associated with 100% protection. Antibody titers would be expected to wane with time, rendering a marginally acceptable titer unacceptable. These considerations lead us to believe that a third injection should be given 1-2 weeks after the second. This is the presently recommended schedule of administration for persons over 60 years and with vaccine distributed by Biken to India and other areas of Asia.

Clinical Reactions to JE Vaccine

Reports were received on 59 participants who completed the 2 dose primary vaccination series. None had febrile responses or changes in blood pressure or pulse at followup examination 2 to 3 days after each vaccine dose. Twenty-four (41%) reported local tenderness, lasting 1 to 3 days; 2 of these persons also reported an episode of shooting pain down the arm. Nine (15%) had redness at the inoculation site and 11 complained of other symptoms including sore throat (2), stiff neck (1), diarrhea (3), headache (2), malaise (3), and nausea (2). One respondent who had transient nausea, dizziness, myalgia, and felt feverish thought her symptoms may have related to a chemical she had worked with on the day of vaccination.

One of the initial volunteers had an anaphylactic reaction while engaged in an aerobics session about 7.5 hours after her first vaccine dose. It appeared that her reaction was not causally related to the vaccine. However, she was referred to an allergist for evaluation, who concluded that she suffers from exercise induced anaphylaxis. She had a prior history of two similar episodes while swimming and, 19 days and 8 weeks after being entered into this study, she reported repeat occurrences during other aerobics sessions. Although we concluded that her post-vaccination symptoms were not vaccine induced we elected not to administer the scheduled second dose of vaccine. This experience illustrates the utility in excluding from pilot studies persons who have histories of severe allergic episodes, since a coincidence of such an episode with administration of an experimental product confuses evaluation. In fact, if a second dose had been administered to this participant 14 days after the first dose (as was done with several of her co-participants) we would have had to report allergic reaction occurring within 5 days, further confounding the evaluation of this product.

In summary, local tenderness was reported in 41% of vaccinees and redness in about 1/3 of those reporting tenderness. No severe reactions attributable to the vaccine was detected.

Current status of IND evaluation

1. Attempt to obtain 6 and 12 month post-immunization sera from individuals already vaccinated, to determine rate of decline of titers after 2 shot schedule.
2. Establish additional coinvestigators in geographically selected areas thereby making Biken vaccine more generally available to the public under the IND.
3. Have modified IND and are currently recruiting approximately 100 additional persons, who will receive 3 doses of vaccine spaced 1-2 weeks apart; determine antibody responses.
4. If analysis of a 3 dose regimen produces a more satisfactory serological response, make the vaccine available to cooperating vaccination centers for use in selected travelers to JE endemic areas.

(Jack Poland, Thomas P. Monath, and C. Bruce Cropp)

Table 1. Serologic responses to 2 doses of JE vaccine of 68 seronegative participants.*

Age Group (years)	No Vaccinated	N Antibody Titers to Nakayama Strain						
		<2	2	4	8	16	32	>64
20-29	21	2		1	1	3	3	11
30-39	16	3	1	3	1	2	1	5
40-49	14	1		2	1	2	3	5
50-59	14	3	1	1	3	2	0	4
60 +	3	1						2
Totals	68	10	2	7	6	9	7	27

* Determined 4-6 weeks after receipt of the 1st vaccine dose (2.5-4.5 weeks after receipt of the 2nd dose).

Table 2. Neutralizing antibody titers to 5 strains of Japanese encephalitis virus compared to the Nakayama strain following 2 doses of Biken JE vaccine containing inactivated Nakayama JE virus.

2-A. Number of persons in each category

JAPANESE ENCEPHALITIS STRAIN

Nakayama	G8924					826309					782219					YOKEN					WRAIR				
	<2	2-4	8-32	>64	TOT	<2	2-4	8-32	>64	TOT	<2	2-4	8-32	>64	TOT	<2	2-4	8-32	>64	TOT	<2	2-4	8-32	>64	TOT
<2	6	2	2	0	10	6	0	4	0	10	3	2	2	2	9	1	3	3	2	9	5	0	4	0	9
2-4	5	0	3	1	9	2	3	3	1	9	0	0	5	4	9	1	1	6	1	9	0	1	7	1	9
8-32	1	3	12	7	23	1	1	12	7	21	0	0	8	14	22	0	0	10	12	22	0	0	18	3	21
>64	0	0	5	24	29	0	0	4	23	27	0	0	3	26	29	0	0	4	24	28	0	0	8	18	26
TOTAL	12	5	22	32	71	9	4	23	31	67	3	2	18	46	69	2	4	23	39	68	5	1	37	22	65

2-B. PERCENT of "N" in each category

Nakayama	N = 71 G8924		N = 67 826309		N = 69 782219		N = 68 YOKEN		N = 65 WRAIR	
	<4	>8	<4	>8	<4	>8	<4	>8	<4	>8
<4	18	8	16	12	7	19	9	18	9	18
>8	6	68	3	69	-	74	-	74	-	72
TOTALS	24	76	19	81	7	93	9	91	9	91

JE Strains: NAKAYAMA - Vaccine substrate
 G8924 - 1956 Southern India
 826309 - 1982 Goa, India
 782219 - 1978 Madras State, India
 YOKEN - Laboratory stored Nakayama strains received 1983, from WRAIR

Table 3. Geometric mean titer of neutralizing antibody by sex in post vaccination sera (N = 28 to 32 females; 37 to 40 males).

JE Strain*	Males	Females	Total
Nakayama	12	21	16
G8924	16	19	17
826309	16	23	19
782219	24	30	27
YOKEN	22	29	25
WRAIR	15	23	18

* See Table 2 for description of strains.

Table 4. Correlation of prior yellow fever vaccination and neutralizing antibody response to Japanese encephalitis vaccine.

A. Number of respondents by JE titer:

History of prior YF vaccination	Number vaccinated	Post JE immunization titer						
		<2	2	4	8	16	32	64
Yes	23	3	2	0	3	3	3	9
No	22	3	0	2	1	4	1	11
Totals	45*	6	2	2	4	7	4	20

B. 2 x 2 Comparison

Post JE vaccination titer	History of prior YF vaccination		Totals
	Yes	No	
<4	5 (22%)	5 (23%)	10 (22%)
≥8	18 (78%)	17 (77%)	35 (78%)
Totals	23	22	45

* Vaccination history for 1 individual unknown.

REPORT FROM THE HEMOPARASITE LABORATORY (USDA
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A collaborative project between the Rocky Mountain Laboratory (NIH) and the Department of Veterinary Science, University of Wisconsin from 1976 through 1980 resulted in the isolation of a number of strains of arboviruses of various groups from seabirds of the Pribiloff Islands area and their ixodid tick parasites [see Arthrop. Vir. Info. Exch. No. 32 (1977) and No. 36 (1979)]. One of these strains (RML-105355) proved to be a previously unrecognized member of the genus Uukvirus (Bunyaviridae), and is briefly characterized here.

The virus was recovered from plaques formed in Xenopus laevis (XTC-2) cells that were inoculated six days previously with a triturate made from a partially engorged, female Ixodes uriae. The tick had been collected on St. Paul I., Alaska, by B. C. Easterday on 17 July 1977. A relationship of strain RML-105355 to members of the Uukvirus genus was seen in cross-complement fixation and cross-microimmunofluorescence (MIF) tests employing hyperimmune mouse ascitic fluids (Table 1). However, the isolate was not identical to any of five Uukviruses with which it was compared (Uukuniemi, Oceanside, Zaliv Terpeniya, Manawa, Grand Arbaud). When strain RML-105355 was compared to the latter entities in suckling mouse cross-neutralization tests, no significant relationships were seen (Table 1). Also, cross-MIF tests of these agents and their specific immune (mouse) sera clearly demonstrated the uniqueness of strain RML-105355 (Table 2). A recent report by Chastel et al. (Arch. Virol. 1981. 70:357-366) confirms this.

Strain RML-105355 is sensitive to DCA and diethyl ether, stable in the presence of the DNA inhibitor, BUdR, and sensitive to acid pH. It is readily deactivated upon exposure to 56°C. Average virion diameter is less than 220 but greater than 100 nm. It is lethal for suckling mice by i.c., i.p. and s.c. routes of inoculation, but kills weanlings only by the i.c. route. In XTC-2 cells it causes plaques of about 2 mm diameter, but it does not cause plaques in Vero cells. Although it survived for three weeks in mosquito cells (Singh's Aedes albopictus, Hsu's Culex quinquefasciatus), and for at least four weeks in primary nymphal tissue cultures from Dermacentor andersoni, no viral increase was apparent in any of these in vitro arthropod systems.

The serological tests of the present study also demonstrate the close relationship of Oceanside virus to Zaliv Terpeniya virus. The former, an unregistered viral strain of the UUK serogroup, was first isolated from multiple pools of Ixodes uriae taken from seabird nesting sites of offshore Oregon, USA, islands (Thomas, et al., 1973, J. Med. Ent. 10:165; Yunker, 1975, Med. Biol. 53:302). Subsequently, it or a similar virus was recorded from ticks of Flat Iron Rock, off the coast of northern California, USA (H. Johnson, pers. comm.). Our findings confirm those of R. Shope (pers. comm.) and Chastel et al. (1981) who were unable to distinguish between Oceanside and ZT viruses. Thus, the former name should be relegated to synonymy with the latter.

[C. E. Yunker (WSU), J. E. Keirans and L. A. Thomas (RML).]

Table 1. Serological relationships of virus strain RML-105355 and Uukuniemi serogroup viruses.

Hyperimmune mouse ascitic fluid						
Complement-fixation test ¹						
virus/antigen	RML	OCE	ZT	UUK	MWA	GA
RML-105355 (RML)	≥ 256	8	128	8	0	0
Oceanside (OCE)	32	1024	16	32	0	4
Zaliv Terpeniya (ZT)	32	128	32	64	0	4
Uukuniemi (UUK)	32	64	4	256	0	4
Manawa (MWA)	(tr)	4	0	0	128	4
Grand Arbaud (GA)	8	8	0	8	0	256

Microimmunofluorescence test ²						
	RML	OCE	ZT	UUK	MWA	GA
RML	1024	8	16	32	4	64
OCE	64	64	256	128	0	32
ZT	128	128	256	128	4	128
UUK	512	64	64	512	4	64
MWA	0	0	0	0	128	0
GA	128	4	16	32	0	1024

Neutralization test ³						
	RML (7.8)	OCE (6.4)	ZT (6.9)	UUK (6.6)	MWA (5.8)	GA (6.4)
RML (7.8)	2.5	0.5	0.0	0.0	0.8	0.1
OCE (6.4)	0.0	4.0	≥ 3.4	3.8	0.2	0.7
ZT (6.9)	0.0	4.7	3.9	4.7	0.3	0.7
UUK (6.6)	0.4	1.5	0.2	≥ 3.9	0.0	0.6
MWA (5.8)	0.0	2.2	0.0	0.9	≥ 3.2	0.5
GA (6.4)	0.2	0.0	0.6	0.0	0.0	≥ 3.0

¹ Serum-titer reciprocal (lowest dilution tested, 1:4) at optimal antigen dilution.

² Reciprocal of highest serum dilution giving a positive reaction.

³ Neutralization index (logs reduction after incubation of virus with immune fluid). (Figures in parentheses, control titers in suckling mouse LD₅₀/0.02 ml/i.c.)

Table 2. Relationships of virus strain RML-105355 and Uukuniemi serogroup viruses in a microimmunofluorescence test employing specific antisera.¹

Immune Serum						
Antigen	RML	OCE	ZT	UUK	MWA	GA
RML	256	0	0	0	0	(tr)
OCE	0	128	128	16	0	0
ZT	0	128	128	8	0	0
UUK	0	128	64	128	0	0
MWA	0	0	0	0	8	0
GA	4	0	0	0	0	64

¹ Reciprocal of highest serum dilution giving a positive reaction. For abbreviations, see Table 1.

REPORT FROM THE NATIONAL ARBOVIRUS REFERENCE SERVICE

DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF TORONTO

TORONTO, ONTARIO, CANADA.

Serological Evidence of California Serogroup Infections in the People's Republic of China

California (CAL) serogroup viruses are known to occur in North and South America, Europe and Africa but their distribution in the Orient is not documented. A limited study was undertaken on sera from residents of the People's Republic of China in which hemagglutination inhibiting and neutralizing antibodies were demonstrated to a CAL serogroup virus (or viruses).

Sera were obtained in May, 1982 from 126 residents of Long Hua, a suburb with a population of 20,000 located about 6 km southwest of metropolitan Shanghai. Sera were taken from individuals aged 3 to 60 years including 75 males and 51 females.

Hemagglutination inhibition tests were performed on acetone-treated sera against snowshoe hare (SSH) antigen. Neutralization tests were undertaken with the following CAL serogroup viruses: SSH Burgdorfer, California encephalitis (CE) BFS 283, Melao (MEL) strain 9375, Tahyna (TAH) strain 92 and trivittatus (TVT) strain 7941.

Neutralization tests were performed by incubating 0.1 ml volumes of heat inactivated (56°C for 30 minutes) sera with 0.1 ml containing 200 TCID₅₀ of virus at 4°C overnight and inoculating of vero cells with 0.1 ml of the mixture (100 TCID₅₀ challenge dose). Sera were considered as neutralization positive if complete inhibition of cytopathic effect was observed.

Five hemagglutination inhibition reactors (3.9%) were found to SSH antigen with titers ranging from 1:10 to \geq 1:40 (Table 1). Reactors ranged from 4 to 28 years of age and included 3 males and 2 females. Neutralizing antibodies were demonstrated in 3 of the 5 hemagglutination inhibition reactors. Highest neutralizing titers were to SSH of the California encephalitis (CE, TAH, SSH) as opposed to MEL and TVT sub-complexes (Calisher 1983).

The demonstration of 2 hemagglutination inhibition reactors including one with a titer \geq 1:40 without confirmatory neutralizing anti-

bodies may suggest that more than one CAL serogroup is circulating in this region. Alternately these hemagglutination inhibition positive, neutralization negative sera may reflect non-specific positives or signify a more specific immunological response in these individuals (both children and possibly showing a primary response) to the infecting virus.

We believe this to be the first report of CAL serogroup activity in China. Our serology indicates that the infecting serotype(s) is a member of the California encephalitis complex. It would be of interest to attempt isolation of the serotype(s) circulating in China and to monitor for possible human disease in China due to CAL serogroup viruses.

Reference

1. Calisher, C.H. (1983). Taxonomy, classification and geographic distribution of California serogroup bunyaviruses. In Calisher, C.H. and W.H. Thompson (eds). California Serogroup Viruses, pp 1 - 16, Alan R. Liss, New York.

(H. Artsob, L. Spence, C. Th'ng and V. Lampotang, National Arbovirus Reference Service in collaboration with H.X. Gu, Department of Epidemiology, First Medical College, Shanghai and W.K. Chia, Toronto General Hospital, Toronto).

TABLE 1 California serogroup reactions of residents from Long Hua, China

Reactor Number	Age	Sex	Hemagglutination Inhibition		Neutralization			
			SSH ¹	SSH	TAH ¹	CE ¹	MEL ¹	TVT ¹
12	28	F	20 ²	320	80	20	- ³	-
26	25	F	≥ 40	640	40	20	-	20
29	28	M	20	160	40	-	-	20
78	6	M	10	-	-	-	NT ⁴	NT
161	4	M	≥ 40	-	-	-	-	-

¹ SSH = snowshoe hare virus, TAH = Tahyna virus, CE = California encephalitis virus, MEL = Melao virus, TVT = Trivittatus virus.

² reciprocal of antibody dilution

³ - = < 1:20

⁴ NT = Not Tested

REPORT FROM TANDIL VIRUS RESEARCH LABORATORY, FACULTY OF VETERINARY SCIENCES,
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HI Antibodies against EEE, WEE, and VEE in Animal and Humans from Buenos Aires Province, Argentina

HI antibodies against EEE, WEE, and VEE were tested for in 2961 sera from five different species living in the southeast part of Buenos Aires Province collected from 3-22-79 to 10-11-83. Some of the horses received previous vaccination against EEE and WEE but not the other four species. The three viruses have been isolated at different times in Buenos Aires Province from horses. The EEE (Arg Enc. B) and WEE strain (Arg Enc MV) used in the test are Argentinian prototypes but the VEE is TC-80 (vaccine strain) obtained from Yale Arbovirus Reference Unit.

Results can be seen in table 1. One hundred, fourteen sera reacted against one or more of the alphaviruses tested giving a 3.85% of general positivity. Higher rates were obtained for horses but not because of vaccination since most of the vaccinees did not have HI antibodies using 8 HAI units of antigens in the test.

With WEE all the species were involved except dogs. Titers were as high as 1:320 with no reaction against EEE and VEE but most of these positive animals were from a group where a small epizootic was taking place.

The antibodies in cattle, especially against VEE removes any doubt about the existence of VEE in our environment. Even if the virus seems to produce infection without disease, it may be a natural vaccine strain (a type 6 VEE was isolated a short time ago in Argentina).

The survey showed that the three viruses under study are endemic in the southeast part of Buenos Aires Province even if the involvement of the human population seems to be low, as measured by demonstrable HI antibody.

(Norma E. Mettier, Lidia M. Gogorza and Juan O. Torres)

Table 1: HI antibodies against EEE, WEE, VEE in 2961 sera from different species (Bs. As. Province - Argentina)

SPECIE	EEE	WEE	VEE
Human	✠ 3/394 (0.76%)	3/394 (0.76%)	3/394 (0.76%)
Bovine	23/1760 (1.31%)	10/1760 (0.57%)	30/1760 (1.70%)
Equine	14/405 (3.46%)	22/405 (5.43%)	12/405 (2.96%)
Ovine	1/372 (0.27%)	0/372 (0%)	1/372 (0.27%)
Canine	1/30 (3.33%)	0/30 (0%)	0/30 (0%)

✠ Numerator: number of positive sera,
Denominator: " " sera tested.

Table 2: Titers found against Equine Encephalitis Viruses
in the sera from table 1

SPECIES	EEE	WEE	VEE	Number of sera with this pattern	TOTAL
Human	0	20	0	3	8
"	20	0	0	2	
"	20	0	40	1	
"	0	0	20	1	
"	0	0	40	1	
Canine	20	0	0	1	1
Bovine	0	0	80	2	60
"	0	0	40	4	
"	0	0	20	22	
"	20	0	20	1	
"	20	0	40	1	
"	20	0	0	20	
"	20	20	0	1	
"	0	20	0	8	
"	0	40	0	1	
Equine	20	0	0	2	43
"	40	0	0	4	
"	80	0	0	3	
"	40	20	0	1	
"	40	0	20	1	
"	80	160	0	2	
"	20	160	0	1	
"	0	320	0	2	
"	0	80	0	5	
"	0	40	0	4	
"	0	20	0	7	
"	0	0	20	10	
"	0	0	80	1	
Ovine	20	0	0	1	2
"	0	0	20	1	

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

Human patients:

In 1983, 411 sera from 289 patients with central nervous system (CNS) manifestations were screened by hemagglutination-inhibition (HI) and plaque reduction neutralization (PRN) technique for antibody to two California (CAL) serogroup viruses, LAC and JC. Specimens from 40 patients with HI reactions were subsequently assayed by PRN with LAC, SSH, JC, KEY and TVT viruses; none of the patients exhibited significant antibody rises. Infection at an undetermined time was indicated with the following subtypes: LAC (6 cases), JC (27), undetermined (6); sera from one patient did not neutralize any of the test strains. Two hundred and twenty-two sera from 100 of the patients sampled more than once were also tested for HI antibody to EEE, WEE, SLE and POW viruses with negative results.

A single serum and cerebrospinal fluid from a 7-year-old boy with clinical diagnosis of encephalitis was submitted by the hospital directly to CDC, Fort Collins, Colorado. Virus isolation attempts in cell cultures from these specimens were negative; however, HI and neutralization titers of 1:40 and 1:80, respectively, indicated EEE virus as the etiologic agent. This patient had a history of exposure to mosquitoes in Onondaga County, where an outbreak of EEE among equines was in progress, 7 to 12 days prior to onset of illness on 8.26.83. Clinical symptoms included headache, malaise and vomiting initially, followed by seizures, coma and death one week later.

Mosquitoes:

Under the auspices of the Bureau of Communicable Disease Control, 1,388 pools of 81,779 adult female mosquitoes, collected from 10 counties in 4 regions (Albany, Buffalo, Syracuse and White Plains) were submitted in 1983 for laboratory studies. Tests performed in suckling mice and/or Vero cell cultures yielded 35 isolations of the following arboviruses: 9 CAL, 23 EEE and 3 FLA. California serogroup viruses were found in 4 counties of central and western New York: Oswego (3 JC), Onondaga (1 JC, 1 SSH), Erie (1 JC, 1 SSH) and Cattaraugus (2 JC); EEE virus was encountered in 2 upstate counties in the Syracuse region: Oswego (2 isolates) and Onondaga (21). Flanders virus was detected only in Suffolk County on Long Island.

California serogroup viruses were isolated exclusively from Aedes, EEE primarily from Culiseta and FLA from Culex mosquitoes (Table 1). The minimal field infection rate (MFIR) for JC was highest in Aedes triseriatus (1:988) followed by Aedes canadensis (1:4150) and Aedes stimulans group (1:5131); the latter also produced 2 SSH isolates with an MFIR of 1:2566. The EEE MFIR was highest in Culiseta melanura (1:336) followed by Culiseta morsitans (1:941) and Aedes canadensis (1:5,533).

These findings confirm and extend previous observations on the prevalence of the JC serotype in the CAL serogroup and the regional nature of EEE virus in New York State. The importance of Culiseta melanura in EEE transmission cycles was reaffirmed. This species, which

exhibits a host preference for avians, is a primary vector of EEE virus among birds throughout its range along the eastern seaboard. Aedes canadensis, on the other hand, has a broader host range, including man and other mammals; the detection of EEE virus in this pestiferous species signifies a potential threat for epidemic spread of the virus among susceptible equine and human populations.

Table 1

Virus Isolation Attempts from Wild-Caught Mosquitoes in New York State - 1983

Genus and species	No. tested		No. of isolates			
			CAL		EEE	FLA
	Pools	Specimens	JC	SSH		
<u>Aedes canadensis</u>	202	16,599	4		3	
<u>Aedes triseriatus</u>	39	988	1			
<u>Aedes stimulans</u> gr.	104	5,131	1	2		
<u>Aedes</u> spp.	91	5,645	1			
<u>Culiseta melanura</u>	144	4,365			13	
<u>Culiseta morsitans</u>	135	6,586			7	
<u>Culex</u> spp.	127	11,726				3
Other species	546	30,739				
Totals	1,388	81,779	7	2	23	3

Equines:

A total of 64 blood (57) and brain (7) samples from 20 sick equine residents (19 horses, 1 burro) of 8 upstate counties, with onset dates from 7.13-9.25.83, were examined for virus and/or HI antibody to EEE and WEE virus. As shown in Table 2, four cases of EEE were confirmed by isolation of virus from post-mortem brain tissue and/or by 4-fold or greater rise in HI antibody levels. In five presumptive cases, two animals exhibited low-titered monotypic reactions and three horses with heterotypic reactions presented higher HI titers to EEE than to WEE virus. All of the confirmed and suspected cases were residents of three contiguous counties in central New York where EEE virus is endemic. Onset of illness in these animals occurred from 7.13-8.10.83; all exhibited signs of CNS disturbance including ataxia, circling, tooth grinding, paralysis, stupor, depression and fever to 106°F. Three horses died and five were euthanized, due to rapidly deteriorating conditions, within one to four days of onset; one equine which had been vaccinated with EEE virus in 1982 recovered.

Case #3 was a 25-year-old pregnant mare which exhibited manifestations of acute CNS infection, including a temperature of 105⁰F., on 7.24.83; blood specimens were obtained from this animal on 7.24 and 7.25 at which time her foal was delivered by cesarian section and she was euthanized. A 4-fold rise in HI antibody in these sera, drawn 24 hours apart, provided laboratory confirmation of current infection with EEE virus in the mare. The foal, which was delivered one week prematurely and appeared normal at birth, died two days later; brain tissue obtained at autopsy on 7.28.83 yielded EEE virus and blood serum drawn the same day exhibited HI antibody levels of 40 and 10, respectively, to EEE and WEE virus (Table 2). The possibility of transplacental transmission of EEE virus must be considered in this case.

In three of the 11 remaining clinical cases, HI antibody levels to WEE were equivalent to or significantly higher than those to EEE virus. Two of these animals had apparently been relocated from the western U.S. where they may have been exposed to WEE virus and one was vaccinated with both EEE and WEE virus in the spring of 1983. No evidence of infection was found in 8 other equines investigated.

Table 2

Eastern Equine Encephalitis Cases in Upstate New York - 1983

Case No.	Equine Name	County	Date of Onset	Date of Death	Sample	Date of Coll.	Virus Isol	HI Titer*	
								EEE	WEE
1.	Ginger	Oswego	7.13	7.15	Serum	7.14	Neg.	80	<10
2.	Magic	Onondaga	7.24	7.24	Serum	7.24		40	<10
3.	Candy	Oneida	7.24	7.25	Serum	7.24	Neg.	640-1280 2560-5120	40
					" Brain	7.25 7.26			40
4.	Candy's foal	Oneida	7.27	7.27	Serum	7.28	EEE	40	10
					Brain	7.28			
5.	Cricket	Onondaga	7.25	7.29	Serum	7.29		640	320
6.	Julie	Onondaga	8.2	Recovered	Serum	8.2	Neg	640	20
					"	8.3	"	1280	40
					"	8.5	"	640	40
					"	8.9	"	640	20
7.	Saffanah	Onondaga	8.3	8.6	Serum	8.4	Neg.	2560	20
					"	8.5	"	5120	40
					"	8.6	"	5120	40
8.	Babe	Oswego	8.10?	8.11	Brain	8.11	EEE		
9.	Luke	Oneida	8.10	8.12	Serum	8.10	Neg.	640	<10
					"	8.12	"	2560	<10
					Brain	8.12	EEE		

*Reciprocal of highest serum dilution inhibiting 4 units of hemagglutinin.

(Margaret A. Grayson and Rudolf Deibel)