



# ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

June 1995

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The Arbovirus Information Exchange is a newsletter prepared under the auspices of the Subcommittee on Information Exchange (Nick Karabatsos, Chairman), American Committee on Arthropod-borne Viruses. Printing and mailing costs of the Arbovirus Information Exchange are paid by the Division of Vector-Borne Infectious Diseases, Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado, USA. The purpose of the Arbovirus Information Exchange is the timely trade of information. Recipients are those who study various aspects of arbovirology. The Arbovirus Information Exchange contains preliminary reports, summaries, observations, and comments submitted voluntarily by qualified agencies and individual investigators. The appearance in the Arbovirus Information Exchange of any information, data, opinions, or views does not constitute formal publication and should not be referred to in "Reference" sections of papers or included in lists of publications. The Arbovirus Information Exchange is not a "peer reviewed" publication; in fact, it is not a publication at all. Any reference to or quotation of any part of the Arbovirus Information Exchange must be authorized directly by the agency or person submitting the text. Reports need not be in manuscript style, the results do not have to be definitive, and you need not include tables (unless you want to). The intent is to communicate among ourselves and to let others know what we are doing.



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## PLEASE READ CAREFULLY

INSTRUCTIONS FOR SUBMITTING REPORTS: **PLEASE** follow these instructions for submitting reports. We want to keep this mechanism timely and viable. Therefore, submit only recent news and summaries of your work. **PLEASE** limit the submission to 1 or a very few sheets (21.59 cm x 27.94 cm = 8.5 x 11 inches) plus a table or two; condense as much as you can (**single space** the text; double-spaced pages take twice as much space as single-spaced pages); **do not** staple pages together; **do not** number pages.

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

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You also may send reports to me by e-mail: [ccalisher@vines.colostate.edu](mailto:ccalisher@vines.colostate.edu)

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

C.H. Calisher

## NEXT ISSUE

The next issue likely will be mailed December 1, 1995 (probable deadline for submissions: November 15, 1995). There is nothing that requires you to wait until the last minute. If you have something to communicate in August, September, or October please send it. Some people have been doing that and I can assure you it saves me some efforts; thanks to them. Also, there is nothing that prevents you from submitting a report to every issue. There are no pages charges either but, then again, this is not a publication.

## Death of Telford H. Work, M.D.

On February 6, 1995, Telford Hindley Work, first Editor of the Arbovirus Information Exchange and former President of the ASTMH, died at his home of complications of congestive heart failure and the inevitable process of aging. He was born July 11, 1921 in Selma, the "raisin capital of the world", in the heart of the San Joaquin Valley of California, on a typically scorching day. His ancestry was mixed: thrifty Scot, bon vivant Irish, and self-possessed and proper English. He came from a family of enterprising adventurers and educators; stubborn men and fiercely independent women. One grandfather was the founder of Texas State College for Women in Dayton; an uncle had conceived and engineered the first snow surveys to predict the availability of Spring water for California farmers. His father, a newspaper publisher, spearheaded the computerization of his trade when he move to Pacific Palisades in Los Angeles County. Tel obtained his primary and secondary education from Los Angeles City schools and spent his teen years hunting fossils in the bluffs overlooking the Pacific Ocean, wiring the neighborhood trees to communicate with his friends, and earning money delivering on horseback the Saturday Evening Post and the Country Gentleman to such celebrities as Aldous Huxley, Thomas Mann, Elizabeth Goudge, and Linda Darnell. While in high school he developed what was to be a lifelong interest and second career in still and motion picture photography.

Telford enrolled at Stanford University and, to earn money to help pay his tuition, worked as a busboy in the women's dining hall and as a printer at night. He immersed himself in his studies of biology and the history of Western Civilization, without neglecting the fun and pranks of fraternity life, which he shared with Remy Nadeau, the historian of California lore. Tel's scientific career already was emerging, as he spent his weekends studying the nest life of the Turkey Vulture (*Cathartes aura*) in the sandstone cliffs of Alameda County. The project, supported by the Stanford Museum of Natural History, led to his first publication in Condor in 1942.

Still, there was time for long distance running in -- and winning-- the state championship, and for photography. With that skill he earned summer employment and lodging at Yosemite National Park, capturing the excitement of visitors driving through the bole of a giant sequoia.

He completed his undergraduate career in 1942 and was accepted at Stanford Medical School, matriculating year-round and graduating in 1945. Upon graduation he accepted an active commission in the U.S. Navy and spent his first months obtaining sound training in surgery and management of war-related psychiatric disorders. Busy though he was, he managed to escape the demands of hospital internship by spending his free time in the Sespe Mountains of Southern California, where 40 California condors (*Gymnogyps californianus*) were then surviving being hunted, poisoned, and crowded from their ecosystem by human civilization. His films of that period and his publication of the life history of these now endangered birds are still unique.

Tel dreamed of distant countries and exotic adventures, but his first active duty was on the U.S.S. Monongahela, a tanker carrying oil from the Persian Gulf to Japan. He shuttled between a war-ravaged country of exquisite artistry and bearing to the evasive sands of the Middle East, where sheiks living in opulent style oversaw people riddled with textbook cases of infectious diseases. This 5-year adventure was a key element in formulating a career in tropical medicine. He spent a year at the London School of Tropical Medicine, but took 15 days to follow and film the Spring roundup of reindeer by Finnish Lapps. He then spent two years with Sir Philip Manson-Bahr in Fiji to study the epidemiology and transmission of filariasis and, as he put it "to learn to think like a mosquito". Meanwhile, he had married an elegant and intelligent European whom he had met on a Grace Line ship

while earning a bit of "pin money" as surgeon.

A transition period followed his return from Fiji, a time occupied by Audubon Society lectures and slide shows and an inquiry into the workings of the Rockefeller Foundation. In 1952 Hugh Smith, co-inventor with Max Theiler and Kenneth Smithburn of the yellow fever 17D vaccine, spent time with him in New York and suggested that Tel enroll in the Master's of Public Health program at Johns Hopkins University in Baltimore, which he did. There he met Alexis Shelokov, with whom he would travel throughout Russia on a joint U.S.-U.S.S.R. mission in the 1960's. Tel was then recruited by the Rockefeller Foundation, which he characterized as "generous without frills and good at getting the most of every dollar spent." He trained briefly in the New York laboratories of the Rockefeller Foundation, with Wilbur Downs bleeding monkeys, with George Martin inoculating mice, and with Sonya Buckley, who was skillfully developing invaluable cell culture systems in a small area that Max Theiler had assigned to her.

Assigned to the Naval Medical Research Unit 3 at Cairo, Egypt, in 1953, Tel trained under Richard M. Taylor who, having retired from his European endeavors with brucellosis and influenza, had embarked on another productive career, arbovirology. Tel spent many months developing a mentor/father relationship with the tough, hard-working Taylor. When John Paul detected antibody to West Nile virus in children of Sindbis Village during a serosurvey for poliomyelitis, Taylor and Work undertook an extensive serologic survey for evidence of this virus. For the first time they isolated West Nile and Sindbis viruses from hooded crows and pigeons, indicating for the first time the role of birds in the maintenance and transport of arboviruses. Bates and Davies already had demonstrated the relationship between temperature and vector competence and Hurlbut was undertaking experimental infections of mosquitoes, which led to the concept of infection rates, later refined by William Reeves. At the same time, the memorable and cigar-smoking Harry Hoogstraal was cataloging plethora of ticks, from which, among others, Quaranfil and Chenuda viruses were to be isolated.

In 1955, Tel was assigned to Poona, India, first as a gofer, later as Director and builder of the Virus Research Center there. Cell cultures developed there by Pravin Bhat were a prelude to the development of cultured arthropod cells, including C6/36 cells, by Singh. Under Tel, the Center tackled an investigation of the New Delhi hepatitis epidemic, now believed to have been caused by hepatitis E virus, and of Jamshedpour fever, which was the first recognized outbreak of what is now known as Reyes Syndrome.

On March 27, 1957 a virus was isolated from a Black-faced Langur monkey, which had been brought moribund to the laboratories from a forest in Mysore District, southern India. It first it was speculated that yellow fever virus had at last been isolated in Asia; Tel showed otherwise, identifying the virus as the as then unknown Kyasanur Forest disease virus. With his co-workers, he isolated the virus from *Haemaphysalis intermedia* ticks and, with his collaborators in the New York laboratories, showed that it was related to Russian Spring-Summer encephalitis, Central European encephalitis, Powassan and other viruses of the tick-borne encephalitis complex of viruses, but that protection against Kyasanur Forest disease virus, which causes a fatal hemorrhagic fever, was not conferred by vaccination with Russian Spring-Summer encephalitis virus. The epidemiologic studies of Kyasanur Forest disease were recorded on 16 mm film and used extensively by Tel for the benefit of generations of medical and other public health students.

In 1962, Tel returned from India, spent a brief period at the Rockefeller laboratories, long enough to



establish the Arbovirus Information Exchange with Richard Taylor; Tel was the first Editor of this very successful newsletter. He then accepted a Congressional appointment and to direct the Virology Section at the Centers for Disease Control in Atlanta. With the assistance of Roy Chamberlain, Dan Sudia, Philip Coleman, and having recruited Fred Murphy, Bernie Fields, Brian Henderson, Blaine Hollinger, and many others, investigations of the epidemiology of St. Louis encephalitis virus in Texas, Venezuelan equine encephalitis virus in Florida, La Crosse virus in Ohio were undertaken. Numerous newly recognized viruses were isolated and identified and national and international surveillance systems were put in place. Fred Murphy began his classical electron microscopic studies and other steps were taken to understand the characteristics and biology of arboviruses, many of them done in the W.H.O. Reference Center for Arbovirus Reference and Research in the Americas, managed by Charlie Calisher.

Having detected antibodies to Venezuelan equine encephalitis virus in Native Americans living in the Everglades of south Florida, Tel established a program of arbovirus studies in that area, studies that resulted not only in the isolation and characterization of Everglades virus (Venezuelan equine encephalitis virus type II) but newly recognized viruses of the Guama, Patois, and Group C viruses, now known as members of the genus *Bunyavirus* in the family Bunyaviridae, as well as Tamiami virus, a newly recognized member of the genus *Arenavirus* and other viruses. These studies showed for the first time that the virological fauna of south Florida was related to the virological fauna of subtropical and tropical areas of Central and South Americas, laying the groundwork for future studies of virus evolution.

Organized by the great Russian virologist-epidemiologist Mikhail Chumakov, six missions to Russia were undertaken by Tel, Jordi Casals, Alexis Shelokov, Karl Johnson, Ned Wiebenga, and Harry Hoogstraal, as idiosyncratic a group of people as was ever gathered.

Tel left the Centers for Disease Control in 1967, moving to the University of California at Los Angeles, where he became Professor of Tropical Medicine and Infectious Diseases. He devoted himself to teaching and to the search for foci of St. Louis encephalitis virus in arid zones of southern California. In 1987, his thesis that *Culex pipiens quinquefasciatus* and its close relatives were likely to be the "backyard" mosquito vector of this virus, was shown to be true, once more illustrating that successful transmission under laboratory conditions do not necessarily reflect transmission in the field.

Two sabbatical years were spent in Australia (1978) where an epidemic of Murray valley encephalitis occurred in the northwest Kimberley area, and in Argentina (1988), where dengue was occurring on the border with Paraguay and yellow fever was occurring in Brazil, a country at that time with a population unprepared and unvaccinated.

One would like to summon the perfect epitaph for this man. A life characterized by accomplishments and calculated eccentricities, a tapestry of sort woven and documented with detailed field notes, photographs, and motion pictures, one can say "Too good to be true and yet too true to be forgotten." Telford Work was buried "on time", at Riverside National Cemetery, California, with military honors due to his life as a public servant to a country he loved and revered.

Martine Jozan Work, M.D.  
30540 Morning View Drive  
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## Death of Trygve O. Berge

Trygve O. Berge died at his Boulder, Colorado, home on 30 April 1995. He was born 19 August 1909 in Stoughton, Wisconsin, earned a Bachelor's degree from the University of Wisconsin, and attended the university's medical school before leaving for financial reasons. He then earned a Master's degree from that university and a Doctorate degree in virology from the University of California at Berkeley. He taught bacteriology at North Dakota State University. Tryg joined the U.S. Army Medical Services Corps and rose to the rank of colonel, serving in Japan from 1945-49.

Working under the auspices of the W.H.O. on eradicating smallpox, he served in India with Dr. Henry Kempe of the Kempe Children's Foundation in Denver. While stationed at USAMRIID, Fort Detrick, he helped develop a vaccine against Venezuelan equine encephalitis.

After retiring from the military, he became curator of viruses at the American Type Culture Collection, then a division of the National Institutes of Health in Bethesda, Maryland. Moving to Colorado for his final years, Tryg worked with the Division of Vector-Borne Diseases of the Centers for Disease Control in Fort Collins as Editor of the International Catalogue of Arboviruses and Certain Other Viruses.

Tryg was an artist, designing and crafting rugs, weaving, and building furniture, and a photographer. He was a member of the Sons of Norway and of the Retired Officers' Association. A quiet, unassuming, and gentle and unfailingly polite man, Tryg had a marvelous sense of humor and a great sensitivity for others.

He leaves Martha, to whom he was married for 64 years, three sons (Peter, David and Mark), two daughters (Patricia Muckle and Judith Dillon), a brother (Ralph Jacobsen), 10 grandchildren, and three great-grandchildren.

Memorial services were held at St. Paul's United Methodist Church in Boulder and interment was at Green Mountain Cemetery in Boulder.

Contributions may be made to the Boulder Community Hospital Foundation (P.O. Box 9019, Boulder, CO 80301-9019) or to Boulder County Hospice (2825 Marine St., Boulder, CO 80303).

(Modified by C.H. Calisher from obituaries in the Boulder Daily Camera of 2 May 1995 and the Denver Post of 8 May 1995)

## CORRECTION

In the December, 1994 issue of the Arbovirus Information Exchange I reprinted a SIRACA report on a proposal for classifying the hantaviruses. Inadvertently, I omitted Prospect Hill virus. The taxonomy of the hantaviruses is "fluid", but it is not that fluid. The incorrect portion of the report is corrected below. I am indebted to Dr. Armindo Filipe, Instituto Nacional de Saúde, Aguas de Moura, Portugal for finding this discrepancy and taking the trouble to point it out to me. I trust this has not caused anyone an inconvenience.

### REPORT OF THE SUBCOMMITTEE ON INTERRELATIONSHIPS AMONG CATALOGUED ARBOVIRUSES (SIRACA)

Current molecular taxonomic status of recognized hantaviruses.

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Type (species)	Abbreviation	Subtype (subspecies)
Hantaan	HTN	76-118, Chen, HV-114
Dobrava-Belgrade	DOB	
<b>Prospect Hill</b>	<b>PH</b>	
Seoul	SEO	80-39, SR-11, R-22
Puumala	PUU	CG-1820, Sotkamo
Sin Nombre	SN	CC-107, CC-74, SN
Harvest Mouse	HM	HM-1, HM-2

Other possible types: Black Creek Canyon (from *Sigmodon hispidus*; BCC), Tula (from *Microtus arvalis*; TUL), Thottapalayam (from *Suncus murinus*; TPM)

**Further information about an e-mail network of arbovirologists: establishment of a system for electronic transmittal and distribution of the Arbovirus Information Exchange**

The December 1994 issue of the Arbovirus Information Exchange contained an announcement to the effect that we were considering the production of an electronic version of this newsletter. Dr. Jack Woodall, the Guru of ProMed (Program for Monitoring Emerging Diseases), has agreed to cooperate with the Editor of the Arbovirus Information Exchange in such an effort. There are many recipients of the Arbovirus Information Exchange who do not have access to the internet and, for them, an exclusively electronic version would mean they would no longer be recipients of this valuable communication. No one wants that! The purpose of this newsletter, as stated in each issue is "the timely trade of information".

If you intend to participate in the electronic version of the Arbovirus Information Exchange, here is what you must do:

1. If you have not already done so, subscribe to ProMed. To sign onto this system, send an e-mail message to--

**promed-request@usa.healthnet.org**

Leave the "Subject" line blank and, in the text space, write

**subscribe promed**

You will receive an automatic reply with information on how to access past files. From then on you will receive the messages posted to the ProMed conference as they are received. You can cancel at any time. The only cost is your telephone call to your e-mail server.

2. To send a report for the electronic version of the Arbovirus Information Exchange, which will be noted as such and included in ProMed distributions, send your report to me at--

**ccalisher@vines.colostate.edu**

3. I will add a note to your report, letting Dr Woodall know it is an Arbovirus Information Exchange report, and forward it to him. **SEND YOUR REPORT TO ME AT ANY TIME DURING THE YEAR.**

4. If you send your report directly to Dr. Woodall, it will be for ProMed, not for the Arbovirus Information Exchange. You must send your Arbovirus Information Exchange reports through me. In that way, I can "intercept" your report, download it, and print it for the paper edition to be mailed.

**For those of you who have no access to the internet**, simply continue to send your reports to me on paper, continuing (or starting) to pay attention to the "Instructions for submitting reports", also found in each issue. **If you can send me your report on a disk, letting me know which standard word processing system was used to compose it, I can upload the**

file for transmission to ProMed in your name.

As a beginning for this electronic interchange of arbovirus information, I am here printing part of my e-mail "little black book". If your name and number do not appear on this list, it may be because I do not have them. If you want your name and number included in this arbovirus directory, please let me know and I will include it in future listings.

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## Editor's comments

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Earlier this year, Telford H. Work, M.D., the first Editor of the Arbovirus Information Exchange died. Tel recruited me to work at the Centers for Disease Control in Atlanta and he was my mentor. I asked his devoted wife, Martine Joza Work, M.D., to write an obituary because she knew him best and loved him most. Her brief summary of the life of this extraordinary man can be found on pages i-iii.

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Tryge Berge  
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Joel Dalrymple  
Bernie Fields  
Andre Lwoff  
Dick Matthews  
Nils Oker-Blom  
Helio Pereira  
Igor Tamm  
Howard Temin  
Tel Work

'nuf said....

We can define as "emerging" those diseases or pathogens that are newly appeared in the population, or that are rapidly increasing in incidence or geographic range (1, 2). Recent examples of emerging diseases in various parts of the world include HIV/AIDS, classic cholera in South America and Africa, cholera due to *Vibrio cholerae* O139, Rift Valley fever, Hantavirus Pulmonary Syndrome, Lyme disease (3), and hemolytic uremic syndrome, a foodborne infection caused by certain strains of *E. coli* (in the United States, serotype O157:H7). A considerable number of emerging infections, such as dengue, Rift Valley fever, and Lyme disease, are vector borne, therefore the expertise of arbovirologists and medical entomologists is critical.

However inexplicable they may seem, rarely if ever do emerging infections actually appear at random. It is crucial to note that specific factors responsible for disease emergence can be identified in virtually all cases studied (2, 4, 5). These include ecological, environmental or demographic factors that place people in increased contact with a previously unfamiliar microbe or its natural host or that promote dissemination. I have suggested (5) that infectious disease emergence can be viewed operationally as a two-step process: [1] Introduction of the agent into a new host population (whether the pathogen originated in the environment, possibly in another species, or as a variant of an existing human infection), followed by [2] Establishment and further dissemination within the new host population ("adoption"). Whatever its origin, the infection "emerges" when it reaches a new population. Factors that promote one, or both, of these steps will therefore tend to precipitate disease emergence. Most emerging infections, and even antibiotic resistant strains of common bacterial pathogens, usually originate in one geographic location and then disseminate to new places.

Categorizing factors responsible for emergence is, of course, somewhat arbitrary, but any classification should represent the underlying processes that cause emergence. The categories devised for the Institute of Medicine report on emerging infections (6) appear a reasonable starting point. Responsible factors include ecological changes, such as those due to agricultural or economic development or to natural anomalies in climate; human demographic changes and behavior (such as movement from rural areas to cities); travel and commerce; technology and industry (such as changes in food processing that may increase the chances of contamination); microbial adaptation and change (such as the emergence of antibiotic-resistant bacteria); and breakdown of public health measures. Deficiencies or breakdowns in public health measures can allow the re-emergence of diseases once thought under control, including many vector borne diseases that increase when vector control efforts are relaxed.

Thus, it appears that most emerging infections are caused by pathogens already present in the environment, brought out of obscurity or given a selective advantage by changing conditions and afforded an opportunity to infect new host populations (on rare occasions, a new variant may also evolve and cause a new disease) (2, 5). This process, by which infectious agents may transfer from animals to humans, or disseminate from isolated groups into new populations, can be called "microbial traffic" (4, 5). A number of activities increase microbial traffic and, as a result, promote emergence and epidemics. In some cases, including many of the most novel infections, the agents are zoonotic, crossing from their natural hosts into the human population (because of the many similarities, I include here vector-borne diseases). In other cases, pathogens already present in geographically isolated

populations are given an opportunity to disseminate further. Surprisingly often, disease emergence (not only in humans but even in other species as well) is caused, inadvertently, by human actions in the world; natural causes such as changes in climate can also at times be responsible (7), as for example with the outbreak of Hantavirus Pulmonary Syndrome in the Southwestern United States in the spring and summer of 1993 (8). Arbovirologists have long been sensitive to the importance of ecological factors, which are increasingly becoming appreciated as a key factor in many emerging infections.

Where do new introductions come from? The numerous examples of infections originating as zoonoses (2, 9, 10) suggest that the "zoonotic pool"--introductions of infections from other species--is an important and potentially rich source of emerging diseases; periodic discoveries of "new" zoonoses suggest that this pool is by no means exhausted. Once introduced, an infection might then be disseminated through other factors, although rapid course and high mortality combined with low transmissibility are often limiting. However, even if a zoonotic agent is not able to spread readily from person to person, and so establish itself, other factors (nosocomial transmission, for example) might serve to transmit the infection. Additionally, if the reservoir host or vector becomes more widely disseminated, the microbe can appear in new places. The dissemination (over the last three centuries) of *Aedes aegypti*, and with it yellow fever and dengue, is one example.

The history of infectious diseases has been a history of microbes that have taken advantage of the rich opportunities offered them to thrive, prosper, and spread. The historical processes that have given rise to the emergence of "new" infections throughout history continue today with unabated force; indeed, they are accelerating, as the conditions of modern life, including massive and rapid global mobility, ensure that the factors responsible for disease emergence are more prevalent than ever before. Humans are not powerless, however, against this relentless march of microbes. Knowledge of the factors underlying disease emergence can help to focus resources on the key situations and areas worldwide (4, 5), and to develop more effective prevention strategies. If we are to protect ourselves against emerging diseases, the essential first step (of many) is effective global disease surveillance to give early warning of emerging infections (4, 6, 11, 12). This must be tied to incentives such as national development and eventually be backed by a system to provide an appropriate rapid response. World surveillance capabilities are critically deficient (6, 12, 13). Efforts to remedy this situation, such as those proposed by the U.S. Centers for Disease Control and Prevention (11) and the World Health Organization, and now underway in the United States and internationally, are the essential first steps and deserve strong support. Shortages of personnel in many of the disciplines that will be essential, including medical (as well as general) entomology and field ecology, should be matter of great concern for the future. Research, both basic and applied, will also be vital, and will require the participation of many disciplines.



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## Japanese Encephalitis - Across the Wallace Line

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Three cases of encephalitis in residents of one island in the Torres Strait between mainland Australia and Papua New Guinea were reported to the Laboratory of Microbiology and Pathology in early April. Two of the patients died as a result of the infection; the other has been released from hospital with some mild neurological impairment.

Serological results (Table 1) on convalescent specimens collected from these patients suggested that Japanese encephalitis (JE) virus was the causative agent. Virus isolation was attempted in suckling mice and C6-36 (*Aedes albopictus*) culture from acute phase serum and from brain biopsies taken from the deceased patients. To date no virus isolate has been recovered from these clinical specimens.

Sera and brain specimens were forwarded to AFRIMS, Bangkok, for further serological and polymerase chain reaction tests (Table 2).

The Tropical Public Health Unit has made considerable effort to control the outbreak. Education of the community has highlighted the importance of personal mosquito protection measures. Mosquito breeding sites and the associated larval species were identified, and a residual larvacide (*s*-methoprene pellets) applied. Adult mosquitoes were collected using CDC-type light-traps, speciated and sent for virus isolation to the WHO Laboratory in Brisbane. High numbers of *Aedes kochi*, *Aedes culiciformis* and *Culex annulirostris* were collected. Fogging, using a pyrethrum preparation (Drift) was carried out to kill adult mosquitoes. A flavivirus has been isolated from two pools of *Culex annulirostris*. These isolates have been identified as Japanese encephalitis virus using a panel of monoclonal antibodies in an indirect immunofluorescence assay (IFA).

With permission, blood was taken from 212 asymptomatic island residents. Recent or current infection with Japanese encephalitis virus was demonstrated in 22 of the residents by the detection of IgM using an IgM capture ELISA. A flavivirus has been isolated from the sera of each of two asymptomatic island residents. These viruses have typed as Japanese encephalitis virus using a panel of monoclonal antibodies in the IFA test.

A veterinarian collected blood from 10 horses and 12 domestic pigs living near the human habitation on the island. All 12 pigs and 9 horses had high JE titres in the HAI assay. Neutralising antibody to JE virus was detectable in the 12 pigs and in 6 horses.

Nucleotide sequencing of these viral isolates is underway to determine the possible route of entry of JE into this region. Surveys are in progress to determine if persons or animals elsewhere in the Torres Strait have been infected. In addition, retrospective serological studies on stored sera will be carried out.

**Table 1. Serological results for three encephalitic patients**

	Days post hospitalisation	HAI titres		IgM capture ELISA
		MVE	JE	
Patient 1	10	<20	40	Positive
	15	40	640	Positive
Patient 2	1	<20	<20	Negative
	4	40	640	Positive
Patient 3	1	<20	<20	Negative
	8	<20	80	Positive

**Table 2. Results of assays performed at AFRIMS, Bangkok**

	Days post hospitalisation	EIA (JE virus IgM)	RT-PCR
Patient 1	10	Positive	Positive
	15	Positive	Not tested
Patient 2	1	Not tested	Not tested
	4	Positive	Negative
Patient 3	1	Positive	Positive
	8	Positive	Negative

## **VIRULENT DENGUE 3 OUTBREAK IN MALAYSIA**

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The World Health Organization believes that dengue is one of the most under-reported diseases in the world and that millions of cases occur each year. It has been estimated that over 2 billion people are at risk to dengue and the disease poses a threat to over 85 countries. The increasing disease trend in tropical and sub-tropical areas of the world is due to rapid population growth, expanding urbanization, inadequate water supplies, and difficulties in refuse disposal. With frequent air travels, dengue is now becoming an important disease of tourism.

In Malaysia, all four dengue serotypes are present, but only one serotype predominates in any one year. Dengue 3 was responsible for the outbreaks in 1985 and 1986, followed by Dengue 1 outbreaks in 1987 and 1988 and dengue 2 in 1989 to 1991.

In 1992, dengue 3 re-emerged as the predominant strain and 5,628 dengue cases were reported of which 649 were DHF with 25 deaths. The case fatality rate (CFR) was 3.85%. Some of these cases have been associated with unusual clinical manifestations, including dengue encephalitis, as evidenced by virus isolation and polymerase chain reaction.

The WHO Centre confirmed 39 DHF/DSS cases which clinically satisfied the case definition of DHF based on WHO criteria. Among these 39 cases, 26 were in children under 12 years of age. In addition to serological confirmation, 17 were virologically confirmed as dengue 3. There were nine deaths among this group of patients, 5 in children aged between 6-11 years and four in adults aged between 22-46 years. Four of the cases in children and one in an adult were confirmed to be caused by dengue 3. Four of the fatal cases were in females.

Among the 3 major ethnic races in Malaysia, there were four deaths each among the Malays and Chinese and one in an Indian, which is roughly proportional to the racial composition of the country. It has previously been observed that all ethnic groups in Malaysia are equally susceptible to severe dengue infection irrespective of the serotype involved.

The dengue 3 outbreak in Malaysia has continued into 1993 and 1994. In 1993, there were 5,589 dengue cases of which 554 were DHF, with 23 deaths and in 1994, there were 3,130 dengue cases, a reduction of 44%, of which 255 were DHF with 13 deaths. The CFR for DHF remained high at 4.15% in 1993 and 5.10% in 1994.

Table 1 shows the CFR of DHF based on major dengue serotypes between 1985 to 1994. The CFR for dengue 1 is low as compared to the other two strains with dengue 3 giving rise to the highest CFR.

There is evidence to show that dengue 3 has also spread to other countries in the region. The report of dengue 3 outbreak in Central America in 1994 is indeed a cause of concern since the last outbreak by this serotype was in 1977.

There is little doubt that dengue 3 is associated with severe dengue infection, similar to dengue 2 in Thailand and Cuba. The virus is highly virulent and has been shown to affect all age and ethnic groups, leading to fatal cases in both children and adults. Countries which are receptive to the introduction of this virus should be aware of the circulation of this virulent strain of dengue 3 in South-East Asia and the South-West Pacific.

**Table 1 Case Fatality Rate Based on Major Dengue Serotypes**

Major Serotype	Years	DHF	Death	CFR%
Dengue 1	1987-88	537	10	1.86
Dengue 2	1989-91	1903	76	3.99
Dengue 3	1985-86	422	20	4.74
	1992-94	1458	61	4.18

# EFFECT OF RIBAVIRIN ON A MOSQUITO CELL LINE INFECTED WITH DENGUE VIRUS

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Dengue viruses are widely distributed in tropical and subtropical regions of the world and are the cause of dengue fever and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS); and transmitted by the mosquito Aedes sp. Recently, major epidemics of dengue fever and DHF/DSS have been occurred around the world; in America, several countries including Mexico have reported cases of DHF/DSS.

To now, the basic strategy to limit the virus transmission is by means of mosquito control, however this procedure is only partially effective. On the other hand, in spite of encouraging early clinical trials with live-attenuated vaccines, there is no currently licensed dengue vaccines. Furthermore, therapy for dengue is limited to support care.

Ribavirin is a nucleoside analog which has a broad spectrum activity against both DNA (p.e. adenoviruses, herpesviruses, and poxviruses) and RNA viruses (p.e. bunyaviruses, alphaviruses, flaviviruses, and arenaviruses)<sup>1-5</sup>. This antiviral agent was approved for use as an aerosol for infants with serious infections due to respiratory syncytial virus and is under clinical investigation for activity against a variety of viral illnesses, including those due to influenza virus, Lassa fever virus, Hantaan virus and human immunodeficiency virus (HIV). Treatment of alphaviruses has been unsuccessful, as has been the treatment of encephalitic flavivirus infections except for West Nile. Rift Valley Fever can be successfully treated in mice and hamsters. Korean Hemorrhagic Fever virus can be inhibited in vitro and lung infection can be prevented in the asymptomatic natural host. Ribavirin can ameliorate the hemorrhagic symptoms of Junin and Machupo infections and the most dramatic success, however is against Lassa infection<sup>4,6</sup>. The precise mechanism of action of the drug against most of these viruses is not well known. Ribavirin has been found to be inhibitory in vitro to a variety of viruses; its activity is dependent upon cell line used, virus concentration, virus strain and time of addition to virus-infected cells<sup>1, 2</sup>.

In this work we report the effect of Ribavirin on a mosquito cell line infected with dengue virus.

The mosquito cell line (TRA-284) was kindly provided by Dr. Duane J. Gubler (CDC, Fort Collins, Colorado, USA); it was grown in culture medium Leibovitz supplemented with tryptose phosphate broth (50% vol/vol) and 1% of inactivated fetal calf serum.

Cell monolayers were infected with a MOI of 0.1 of Dengue-2 (NGC strain); infection was monitored by direct immunofluorescence (IF). The IF assay was done blind twice by two independent researchers at days 3, 5 and 7 postinfection (pi).

Ribavirin (Virazide; 1- $\beta$ -D-ribofuranosyl-1,2,4-triazole-3-carboxamide, Grossman Laboratories, Mexico City) was used at final concentrations of 10, 30, 60 and 100  $\mu$ g/ml. The drug was added at the time of infection and in some experiments it was added at 72 hr pi. The cytotoxic effect of Ribavirin on infected and non-infected cells was determined by trypan blue exclusion. All experiments were carried out by duplicate and repeated at least twice. When different concentrations of Ribavirin were assayed on cell viability, only



100 ug/ml had a mild cytotoxic effect. For this reason, the IF experiments were carried out with 60 ug/ml of the drug.

In Figure 1, is shown the effect of Ribavirin on the viability of non-infected and infected cells assayed at days 3 and 7; in the presence of the drug, the effect on cell cytotoxicity was less evident in infected cells (7 days pi).

The Table I shows the detection of dengue virus by immunofluorescence in mosquito cells infected with dengue virus and treated with different concentrations of Ribavirin . When 60 ug/ml of the drug was added at time of infection, very few fluorescent cells were observed at 7 days pi when compared with untreated infected cells. This experiment indicates that the Ribavirin used under the described conditions, inhibits the virus replication. When the drug was added 72 hr pi, this phenomenon was not observed. These results are similar to those reported by Ussery<sup>7</sup> in LLC-MK2 cells infected with dengue-1.

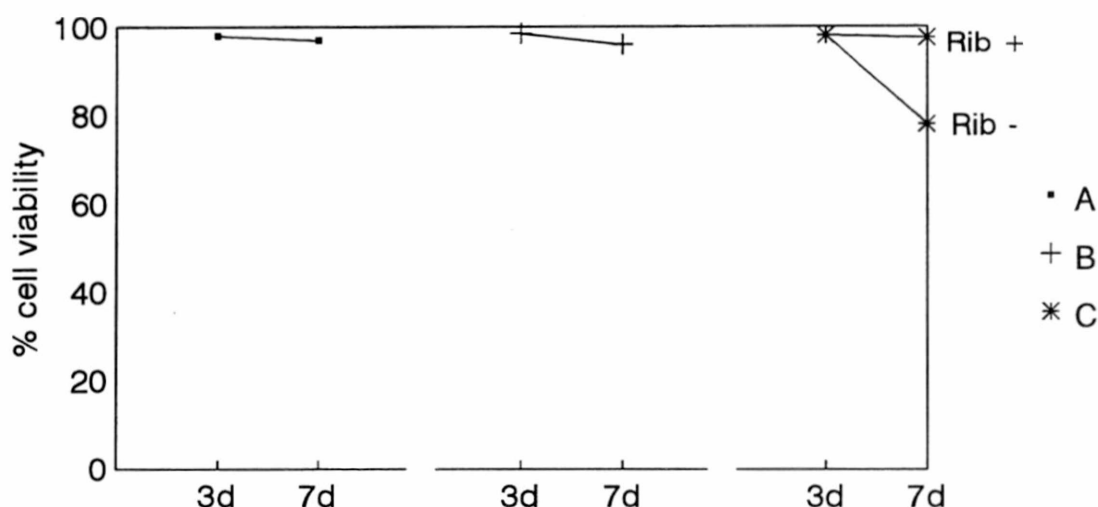
Additionally, we showed that Ribavirin treatment of infected cells decreased the synthesis of dengue virus proteins, mainly the E protein (data not shown).

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# FIGURE 1

Effect of Ribavirin on cell viability of non-infected and infected cells



A:non-infected cells

B:non-infected+ Rib(60 ug/ml)

C:infected cells

## TABLE I

Detection of dengue virus by immunofluorescence in mosquito cells infected and treated with Ribavirin

Cultured cells	Ribavirin (ug/ml)	IF <sup>a</sup>		
		Day 3 pi	Day 5 pi	Day 7 pi
Non-infected	none	-	-	-
Infected <sup>b</sup>	none	+	++	++++
Infected	10	+	++	+++
Infected	30	+	++	+++
Infected	60	+	+	+

a: Arbitrary criterion to estimate the immunofluorescence grade:

+ =scarce fluorescent cells/average of 10 observed microscopic fields, 480X

++ =few fluorescent cells/as above

+++ =many fluorescent cells/as above

++++ =too many fluorescent cells/as above

b:MOI=0.1

**REPORT OF THE WHO COLLABORATING CENTER FOR VIRAL DISEASES DENGUE VIRUS BRANCH, INSTITUTO "PEDRO KOURI", HAVANA, CUBA.**

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During 1994 the Dengue reference activities were intense, mainly in countries in the Central American sub-region. These activities included consulting, training as well as serological performing and virological studies in Nicaragua, Panama, Costa Rica, Venezuela, Ecuador and Guatemala .

The director of the collaborating center was appointed by PAHO for the evaluation of the Dengue Control Programme in Guatemala. In the "Instituto de Medicina Tropical, Universidad Central de Venezuela" two of our staff members participated in a seminar organized by the Venezuelan Society of Microbiology about Dengue and DHF. One staff member attended as expert the meeting organized by PAHO in Maracay Venezuela to discuss the Guidelines for the Control of Dengue and DHF in the American Region. One member of the group worked for 6 weeks in the Instituto Izquieta Pérez, Guayaquil, Ecuador, collaborating with the virology department in Dengue related techniques, and two others in INCIENSA in Costa Rica, one in Maracay, Venezuela and one in Managua, Nicaragua.

The situation in Central America is critical considering that both Panama and Costa Rica which were dengue-free countries suffered outbreaks beginning by the end of last year. The most interesting features of our collaborative work are as follows:

**IN PANAMA:** Fourteen cases were detected early by the surveillance system established by the Gorgas Memorial Laboratory. This lab could isolate Den-2 virus, this isolation was also confirmed by our center. Sixty four percent of the cases showed a pattern of secondary serological response. The outbreak was apparently controlled and a seroepidemiological study performed in our center with 770 samples, showed a 5.8 % of positivity in individuals of 45 or more years old. This study demonstrated that the first manifestation of this outbreak was limited to Santa Librada in the San Miguelito district in Panama city. After this initial phase of the outbreak low level transmission was maintained in the city and by December 1994 they reported more than three hundred cases, also in Panama city. Dengue 3 has been reported recently in Panama.

**COSTA RICA:** The first Dengue Fever Epidemic was reported in the country starting on October 1993. In 170 sera received through the serological surveillance system during 1994, IgM antibodies were detected in 39% of the samples and 18 cases showed a secondary type serological response indicating a previous infection with a Group B Arbovirus (HI test). All individuals with secondary infection were 40 or more years old. In two cases with secondary infection minor bleeding manifestations were recorded (petechiae, ecchymosis nasal bleeding). Dengue 1 was reisolated in our center although Den-2 and Den-4 have been also isolated, showing that as is usual three serotypes are involved in this outbreak.

**NICARAGUA:** In 1985 Nicaragua suffered a large Dengue epidemic, with 17483 reported cases and 7 deaths associated to the outbreak, since then dengue has been considered endemic in the country. Dengue 1,2 and 4 have been previously isolated. In September 1994, an increase in the number of reported cases was observed, mainly in Managua and Leon.

The number of hospitalized children and adult's dengue cases also increased, some of them with hemorrhagic manifestations and hemoconcentration. The clinical picture of these cases should be carefully evaluated to confirm the presence of a DHF epidemic in the country, although this situation is highly suggestive. Forty two per cent of 356 sera samples showed the presence of IgM antibodies. These specimens came from different departments in the country. Also IgM antibodies and high titers of HI antibodies were detected in hospitalized patients. Two viral isolations identified as Dengue 3 were done, documenting the re-introduction of Den-3 in the American region after 17 years of absence. This finding is of tremendous importance for the region, making more complex the Dengue situation.

The results obtained by our center in the last 12 months clearly show the importance and complexity of the "Dengue Problem" in the American Region and that the efforts of PAHO and other international agencies should be continued and increased, and the national health authorities should take more action in preventing Dengue and DHF epidemics.

Considering this uncertain future our center is organizing a training course with PAHO and two other collaborating centers (YARU and Institut Pasteur) for next August in Havana in which we intend to cover epidemiological, clinical, control and laboratory aspects following the Guidelines for the Control of Dengue and DHF that by that time should be edited by PAHO.

**PATHOGEN DERIVED RESISTANCE TO DENGUE-2 VIRUS IN MOSQUITO CELLS BY  
EXPRESSION OF THE CAPSID AND PREMEMBRANE CODING REGIONS OF THE VIRAL  
GENOME.**

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The full-length capsid (C) and premembrane (prM) coding regions of dengue virus type 2 (DEN-2; Jamaica) genome were expressed in C6/36 (*Aedes albopictus*) cells in either sense or antisense orientations from double subgenomic Sindbis (dsSIN) viruses. Northern blot analysis confirmed the expression of sense or antisense DEN-2 prM or C RNA in infected C6/36 cells. C and prM proteins were demonstrated in cells infected with dsSIN viruses expressing DEN-2 sense RNAs by radiolabeling and immunofluorescent assays. C6/36 cells were infected with each dsSIN virus at a multiplicity of infection (MOI) of 50 and challenged 48 h later with DEN-2 NGC (New Guinea C) at 0.1 MOI. Whereas C6/36 cells infected with control dsSIN viruses supported high levels of DEN-2 replication, C6/36 cells infected with the dsSIN viruses expressing C or prM antisense RNA were completely resistant to DEN-2 challenge. The expression of full-length C protein was toxic to C6/36 cells. Cells expressing prM protein were completely resistant to DEN-2 challenge; however, resistance was due to RNA expression since dsSIN viruses expressing untranslatable prM sense RNAs were also resistant to challenge with DEN-2 virus. Cells expressing prM protein were not resistant to DEN-2 virus when challenged at an MOI of 10. However, untranslatable sense prM RNA was completely protective when challenged at high MOIs. Heterologous interference was not observed in any dsSIN infected C6/36 cells after challenge with DEN-3 or DEN-4 viruses.

## Synanthropy and epidemiological vector role of *Aedes scapularis* in South-Eastern Brazil.

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During the fulfilment of investigation program in the Ribeira Valley region, São Paulo State, Brazil, the *Aedes scapularis* behaviour was focused. Deserved special attention to study that in the human dominated environment regarding the biting activities and the mosquito occurrence at several different places. Thus, during 1992-1993 period adults were collected through the use of human bait and Shannon type traps.

Among *Aedes* species *Ae. scapularis* and *Ae. serratus* predominated but differently split between them. As a general pattern that first one was particularly frequent outside the forest environment while the other it was inside.

Regarding the frequency at the dwellings *Ae. scapularis* was significantly more frequent as comparing with *Ae. serratus*. Relating to the three sampled environments (remnant forest, cultivated open land and dwelling) the results obtained with the Nuorteva synanthropic indices were as follows:

<i>Ae. scapularis</i>	+24.5
<i>Ae. serratus</i>	-74.5

Considering the behavioural succession estimated through the index of change (IC) of Amerasinghe and Ariyasena (1990, 1991) the values obtained were as follows:

<i>Ae. scapularis</i>	0.8846
<i>Ae. serratus</i>	-0.9930

This indicating major changes for both mosquitoes. Comparing these changes, it was concluded that *Ae. scapularis* increased 16.33 times with the natural habitat transformation to anthropic one, while *Ae. serratus* decreased 284.0 times under that circumstance.



As consequence of these studies it was concluded that *Ae. scapularis* represents an epidemiologically significant vector in the anthropic environment. It was concluded too that quite probably it fulfilled the role of Rocio encephalitis vector when this infection occurred on an epidemic pattern in the Ribeira Valley region, some time ago.

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## The adult behavior of Spissipes Section species of *Culex* (*Melanoconion*) in South-Eastern Brazil.

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It is well known that several species of the Spissipes Section of *Culex* (*Melanoconion*) were found naturally infected through arboviruses isolations. In São Paulo State, southern Brazil, strains of VEE and a number of bunyavirus were isolated from *Cx. ribeirensis* (formerly confounded with *Cx. crybda* and *Cx. epanastasis*), *Cx. sacchettae* and *Culex* sp. VR (hitherto misidentified as *Cx. taeniopus*) (Calisher et al 1982, 1983). So that isolations presages the incrimination of these *Melanoconion* mosquitoes as vectors of arboviruses.

During the fulfilment of investigation program in the Ribeira Valley region of São Paulo State, observations about mosquitoes behaviour were carried out. As results *Cx. ribeirensis* and *Cx. sacchettae* appeared widespread in the region. Considering the general anthropic and the dwelling environments, these species seems adapted and quite attracted to the human bait. Continuing that investigation program, adults mosquitoes collections were performed through the use of human bait and the Shannon type traps. Four environments types were sampled, the remnant forests (two), the open land, the dwelling and a testimony area. All of them represented the anthropic environment with several disturbing degrees regarding the natural one. The results are presented in the table 1. The human bait was used indoor and outdoor simultaneously and the results are presented in Table 2(4).

The Nuorteva indices<sup>(s)</sup> were -44.7 for *Cx. ribeirensis* and -42.0 for *Cx. sacchettae*. So, considering the general over-view of this man-made environment, both mosquitoes appeared as retaining a large part of its sylvatic habits. Nevertheless, the human dominated environment as a whole, it included several different features each one with its peculiar characteristics. Thus the present studies allowed to consider *Cx. ribeirensis* as *Cx. sacchettae* as potentially synanthropic at least in the Ribeira Valley region. As its competence for virus transmission as been reported, they deserve attention in the epidemiological studies to be effectuate in the anthropic environment.

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# **TYPING OF LACROSSE, SNOWSHOE HARE, AND TAHYÑA VIRUSES BY ANALYSES OF SINGLE STRAND CONFORMATIONAL POLYMORPHISMS OF THE S RNA SEGMENTS**

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Single strand conformational polymorphism (SSCP) analysis was developed to differentiate the small (S) RNA segments of the California serogroup bunyaviruses LaCrosse, snowshoe hare, and Tahyña. Their S RNA segments were reverse transcribed and amplified by polymerase chain reaction. The cDNAs then were denatured, rapidly chilled to prevent reassociation, and separated electrophoretically. The resulting SSCP patterns were specific for the respective viruses, distinguishing not only one prototype from another but also distinguishing multiple isolates or strains of each virus. This molecular technique offers great potential for rapidly identifying and genotyping viruses and will facilitate studies of virus evolution by identifying virus genotypes for subsequent molecular or biological characterization and, by delineating polymorphisms in regions of virus genomes, for sequence analyses.

(Acknowledgment: This work was supported in part by NIH grant AI 34454 and in part by the MacArthur Foundation)

## Expression of Heterologous Genes in Mosquitoes and Mosquito Cells using a Sindbis Replicon System

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Sindbis (SIN) replicon viruses were used to express the full length La Crosse (LAC) virus medium (M) genome segment, truncated versions of the LAC M segment coding for the individual glycoproteins (G1, truncated G1 (T.G1), and G2), and the chloramphenicol acetyltransferase (CAT) gene in mosquito cells. RNA *in vitro* transcribed from SIN replicon plasmids (pSINrep5/M, pSINrep5/G2, pSINrep5/G1, pSINrep5/T.G1, pSINrep5/CAT) and from SIN virus helper constructs (pDH-EB or pDH(26S)5'SIN) were co-electroporated into BHK-21 cells to generate replicon viruses, designated rep5/X/EB and rep5/X/26S (where X = heterologous gene).

*Aedes albopictus* (C6/36) cells infected with rep5/CAT/EB or rep5/CAT/26S virus at a multiplicity of infection (MOI) of 3, expressed  $3.8 \times 10^6$  and  $6.0 \times 10^6$  CAT trimers per cell, respectively, at 2 days post infection (pi). Both rep5/CAT viruses attained peak titers by day 2 pi. Adult female *Aedes triseriatus* mosquitoes were intrathoracically inoculated with rep5/CAT/EB or rep5/CAT/26S virus. Virus titers of approximately  $10^5$  IFU/ml were detected through day 2 pi and then decreased roughly 1 log by day 10 pi. CAT enzyme activity in whole mosquitoes was detected 2 days pi and remained nearly unchanged through day 10 pi. CAT was detected in the head, salivary glands, midgut, and ovaries of inoculated mosquitoes by indirect immunofluorescence (IFA) and CAT activity assays. In addition, pools of saliva collected from *Culex pipiens* mosquitoes infected with rep5/CAT/26S virus showed CAT enzyme activity.

C6/36 or BHK-21 cells infected with replicon viruses containing LAC virus M segment sequences expressed LAC proteins indistinguishable from wild type LAC proteins by immunoprecipitation and IFA. C6/36 cells infected with a replicon virus containing a truncated form of the LAC G1 gene (rep5/T.G1/EB) secreted G1 protein into the media of infected cells as detected by antigen capture ELISA. In addition, a protein of the predicted molecular weight of T.G1 (approximately 95 kD) was detected in the saliva of *Culex pipiens* mosquitoes infected with rep5/T.G1/EB virus.

**INTRACELLULAR IMMUNIZATION OF C6/36 MOSQUITO CELLS AND *AEDES TRISERIATUS* MOSQUITOES TO BUNYAVIRUSES USING A SINDBIS VIRUS EXPRESSION SYSTEM.**

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A double subgenomic Sindbis (dsSIN) virus expression system was utilized to transcribe sequences of LaCrosse (LAC) virus small (S) or medium (M) segment RNA in either sense or antisense orientation. The recombinant viruses that were generated were used to infect C6/36 (*Aedes albopictus*) mosquito cells. These cells were challenged with wild type LAC virus 48 hours later then examined by EIA to determine their ability to inhibit replication of the LAC challenge virus. One virus, designated TE/3'2J/ANTI-S, expresses the full length, negative sense S RNA of LAC virus. This virus can be used to intracellularly immunize C6/36 cells against LAC virus. Cells infected with TE/3'2J/ANTI-S virus prior to challenge with LAC virus yielded at least 4 log<sub>10</sub>TCID<sub>50</sub>/ml less LAC virus than cells infected with a dsSIN virus containing no LAC insert. When C6/36 cells infected with TE/3'2J/ANTI-S were challenged with a heterologous bunyavirus, a similar inhibitory effect was seen particularly when the challenge virus was closely related to LAC virus. The ability of this recombinant virus to provide the same protective effect *in vivo* was subsequently examined. *Aedes triseriatus* mosquitoes were intrathoracically inoculated with TE/3'2J/ANTI-S and orally challenged with wild type LAC virus. Tissues productively infected by the dsSIN virus were refractory to LAC infection. In cephalic tissue, a 1-2 log reduction in LAC titer was seen in mosquitoes inoculated with TE/3'2J/ANTI-S. These studies demonstrate the usefulness of the dsSIN expression system for evaluation of molecular mechanisms of viral interference in mosquitoes.



# THE GENERATION OF MOSQUITOES RESISTANT TO FLAVIVIRUS INFECTION VIA EXPRESSION OF FLAVIVIRUS SEQUENCES USING SINDBIS VIRUS VECTORS

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Complementary DNA derived from the premembrane (prM) coding regions of dengue type 2 (DEN-2, Jamaica) virus genome has been inserted in either sense or antisense orientations downstream of the second internal initiation site of double subgenomic Sindbis (dsSIN) virus expression vectors (pTE/3'2J). Northern blot analysis of C6/36 (*Aedes albopictus*) cells infected with the dsSIN viruses confirmed the presence of DEN-2 prM sense and antisense RNA. Protein analysis has also confirmed the translational expression of prM protein from a dsSIN virus that produces prM sense RNA. These dsSIN viruses induced pathogen derived resistance (PDR) in C6/36 cells to the homologous DEN-2 virus.

Experiments to demonstrate PDR in *Aedes aegypti* have shown that dsSIN viruses can knock out DEN-2 and YFV virus replication in mosquito salivary glands. Whole body DEN-2 titers in mosquitoes concurrently infected with TE/3'2J/antiPrM were up to 100 fold less than in those infected with DEN-2 only, or concurrently infected with a control TE/3'2J virus. Knock out of flavivirus replication in salivary glands may effectively prevent transmission. We are currently investigating sequences (e.g. NS5) which may generate more broadly protective PDR.

This research using dsSIN has identified important sequences that might be utilized to produce transgenic mosquitoes lines that are genetically resistant to viral infection. Additionally, dsSIN viruses may be important molecular tools for knocking out expression of endogenous mosquito genes.

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## **An update on the use of a blocking ELISA to distinguish between Murray Valley encephalitis and Kunjin virus antibodies in chicken sera**

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Murray Valley encephalitis (MVE) and Kunjin viruses are two mosquito-borne flaviviruses found in Australia that cause the disease Australian encephalitis (AE) in humans. Infection with MVE virus has been responsible for severe and fatal cases of AE whereas Kunjin virus has only been associated with a mild form of the disease. Both viruses appear to have enzootic foci in the far north Kimberley region of Western Australia and possibly in the Northern Territory. These viruses are however epizootic in the Pilbara region of Western Australia and northern Queensland and cause occasional epidemics in south-eastern Australia. In nature both viruses normally circulate between mosquitoes and birds, particularly herons, while man is only an incidental host.

MVE and Kunjin viruses are active in Western Australia each wet season (December to May) and virus activity is monitored on a year round basis using sentinel chicken flocks. In the wet season the mosquito numbers increase and it is important for health reasons to know if these large mosquito populations are carrying MVE or Kunjin viruses. Chicken flocks consisting of 12 birds are maintained at most towns and some mine sites in the Kimberley and Pilbara regions of Western Australia. These flocks are bled every two weeks from December to May and monthly at other times and the sera is sent to the laboratory in Perth to be tested for the presence of antibodies to flaviviruses. This surveillance program is used to give an early warning of an increase in MVE activity in the north of the state. The program is run by the Department of Microbiology at the University of Western Australia and is funded by the Health Department of Western Australia..

Chicken sera are tested for the presence of antibodies to flaviviruses in a blocking ELISA using monoclonal antibodies (Hall *et al.*, 1995). Briefly test sera and specific monoclonal antibodies compete for sites on bound MVE or Kunjin antigens. Specifically bound monoclonal antibodies are detected by goat anti-mouse IgG conjugated to peroxidase. Initially the sera are screened against a flavivirus group specific monoclonal antibody 3H6. Any flavivirus positive sera are then tested against an MVE specific monoclonal antibody (10C6) and a Kunjin specific monoclonal antibody (31112) to determine the identity of the infecting virus. These specific monoclonal antibodies are reactive to the NS1 protein of MVE and Kunjin viruses respectively (Hall *et al.*, 1995). This type of ELISA is particularly useful as it enables us to test sera collected from any species except mice.

This test has been used routinely in our laboratory since 1992 with 3H6 and 10C6 monoclonal antibodies and the 31112 Kunjin specific monoclonal antibody was included in early 1993. Over the two year period from January 1993 to December 1994 a total of 5350 chicken sera were tested. There were 119 flavi-positive sera from the Kimberley and 73 from the Pilbara. Of the 119 sera from the Kimberley 90 were positive for MVE antibodies alone, one for Kunjin alone and 28 for both MVE and Kunjin antibodies. The majority of the chickens with dual infections were

infected with both viruses at about the same time although a small number were positive for MVE virus for a period before they developed antibodies to Kunjin virus. The picture is different in the Pilbara region. We have shown that flavivirus activity is epizootic in the Pilbara region of Western Australia and that there is a reduced level of flavivirus activity in this area. MVE or Kunjin virus activity can be demonstrated at one location each wet season, although the location may vary from year to year. Of the 73 flavi-positive sera from the Pilbara from 1993 to 1994, 50 were positive for MVE, 4 for Kunjin and 16 for both MVE and Kunjin. The seroconversions in the Pilbara usually occur later in the wet season than those in the Kimberley. It has been suggested that flavivirus activity in the Pilbara is caused by MVE and Kunjin virus movement from enzootic areas in the Kimberley to epizootic areas probably as a result of the movement of viraemic waterbirds from wetter areas farther north.

The sentinel chicken program was tested during the 1992/93 wet season when there were record rains and flooding in the north east Kimberley region of Western Australia. There was a large number of seroconversions in the sentinel chicken flocks, mainly to MVE virus from early March 1993. The Health department issued a number of warnings of the threat of an outbreak of AE to the residents of the Kimberley and the first case of AE from Fitzroy Crossing (a small town in the Kimberley) was reported in late March. There was a total of 9 cases of AE from WA and 6 from the Northern Territory with dates of onset from March to June 1993. This is the most cases ever confirmed from both states in a single wet season. The first of the chicken seroconversions was reported from the Kununurra flock in the east Kimberley in early March 1993, only 2 weeks before the first human case of AE. Therefore this shows that the program does provide an early warning of an increased risk of an outbreak of AE and the ELISA test allows the rapid and specific identification of antibodies to the infecting agent. During 1993 the seroconversions quickly spread to other areas of the Kimberley and in April the first seroconversions were reported from the Pilbara. However no cases of AE were reported from the epizootic Pilbara region in this year.

This ELISA system is also used to test human sera for the presence of flavivirus antibodies and has been used in a number of serosurveys and also as a confirmatory test to determine the infecting agent in suspected cases of AE. Our laboratory is also looking to develop similar competitive ELISA tests for other Australian alpha and flaviviruses. We would be interested to hear from anyone who has access to specific monoclonal antibodies to Australian arboviruses who would be willing to let us test them in our system.

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Comparison of Rabbit Kidney Cell Line to Newborn Mice for  
Primary Isolation of Eastern and Western Equine Encephalitis Virus

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A set of equine tissues submitted to the Diagnostic Virology Laboratory, National Veterinary Services Laboratories, Ames, Iowa, resulted in the isolation of western equine encephalomyelitis (WEE) virus from tissue suspensions inoculated in rabbit kidney (RK-13Ky) cells.<sup>a</sup> No isolation was made from the same tissue suspensions intracranially (IC) inoculated into newborn mice. As WEE is reported to be difficult to isolate from clinical specimens,<sup>1,2</sup> a project was undertaken to compare RK-13Ky cell cultures to IC inoculation of newborn mice for primary isolation of equine encephalitis viruses.

From June 1991 to September 1993, 257 field specimens from 9 genera were inoculated into both newborn mice and RK-13Ky cells for primary virus isolation (Table 1). These included 239 brain suspensions; 9 other tissue suspensions; and 9 blood, sera, or cerebrospinal fluid inoculations. From these 257 specimens, 91 virus isolations were made consisting of 85 eastern (EEE), 3 WEE, 2 Venezuelan (VEE), and 1 Highlands J (HJ) viruses (Table 1). All 91 viruses were isolated in RK-13Ky cells. Only 62 were recovered in newborn mice (Table 1). Of the 29 virus isolations recovered only in RK-13Ky cells, 20 caused no mortality in newborn mice and 9 caused less than 30 percent mortality with no sample available for laboratory diagnosis.

Other studies have indicated both Vero-M and baby hamster kidney (BHK 21) cells are suitable continuous cell lines for arbovirus isolation.<sup>3,4,5</sup> This study compared only the RK-13Ky cells and IC inoculation of 2- to 5-day-old newborn mice for the primary isolation of equine encephalomyelitis viruses from field-submitted specimens. The results indicated that the RK-13Ky cell line is an excellent cell culture system for the isolation of EEE, VEE, and WEE viruses.

Table 1. Field specimens used to compare frequency of equine encephalomyelitis virus isolation between cell cultures (CC) and newborn mice (NBM).

Source	Brain	Other tissue	Blood sera, CSF*	Total isolates	Isolates in CC	Isolates in NBM
Equine	195		7	73 EEE 1 WEE 2 VEE	73 1 2	47 0 0
Avian	34	9		10 EEE 2 WEE 1 HJ†	10 2 1	10 2 1
Porcine	2			2 EEE	2	2
Other	8‡		2§	0	0	0
Total	239	9	9	91	91	62

\* CSF=1

† Highlands J virus

‡ 1 Bovine, 2 canine, 2 caprine, 1 feline, 1 camelid, 1 ovine

§ Macropodine (kangaroo)

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Among the arboviral encephalitides known to occur, the most serious is the highly fatal and severe disease caused by eastern equine encephalitis (EEE) virus. Two varieties of the virus are known to exist, North American (NA) and South American (SA), which differ in biological and molecular features. Individuals at risk for infection (e.g. laboratory and field personnel), can be immunized with the investigational formalin-inactivated whole virus EEE vaccine, derived from the PE-6 strain. Unfortunately, the pedigree of the PE-6 strain has not been fully documented. Only recently have molecular analyses of the RNA and proteins of the PE-6 strain been performed, for comparison with field isolates of EEE virus. Genetic analyses of the PE-6 genome by RNA fingerprinting in our laboratory, and nucleic acid sequencing by M. Parker and R. Schoepp at USAMRIID, Ft. Detrick, MD (GenBank Accession No. L37662) have revealed that the genotype of the vaccine strain shares a high degree of homology with NA variety EEE strains. We report here the results of SDS-PAGE and endoglycosidase analyses of the PE-6 virion proteins.

Comparison of the PE-6 strain with other EEE strains by SDS-PAGE revealed that, with the exception of the E1 protein, the polypeptide profile of the PE-6 strain was identical to that reported previously for NA variety EEE strains (Strizki and Repik, *J. Gen. Virol.* 75:2897-2909, 1994). The major E2 and C proteins of the PE-6 strain were found to have apparent molecular weights of 43,500 and 33,000, respectively, while the minor high molecular weight (HMW) protein (mol. wt. 91,000 in NA strains) was also evident in the PE-6 sample. The E1 protein of the PE-6 strain migrated faster, with an apparent mol. wt. of 51,000, in comparison with other NA strains of EEE virus, the vast majority of which possess an E1 protein with an apparent mol. wt. of 53,000. The molecular weight of the E1 protein of the PE-6 vaccine strain is, so far, unique among the NA strains examined, although two other strains (Sanchez and Williams) have also been found to possess E1 proteins having unique molecular weights (56,000 and 57,500, respectively).

Although deduced amino acid sequence analysis of the PE-6 strain revealed the presence of a single potential N-linked glycosylation site in the E1 protein, endoglycosidase digestion analyses were performed to determine whether this site was, in fact, glycosylated, or whether the lower apparent molecular weight was indicative of a non-glycosylated protein. The E1 protein of the PE-6 strain, like the E1 proteins of all other EEE strains examined, was resistant to digestion with Endoglycosidase H and Endoglycosidase F, but was susceptible to treatment with N-Glycosidase-F (PNGase F), as evidenced by an increase in electrophoretic mobility relative to that of untreated samples. The molecular weight of the E1 protein of the PE-6 strain was reduced by 2000 following treatment with PNGase F, an enzyme which cleaves the oligosaccharide component from the polypeptide backbone of a glycoprotein. Like other EEE virus strains, the E2 and HMW proteins of the PE-6 vaccine strain were susceptible to cleavage with all three endoglycosidases. These results verify that the E1 protein of the PE-6 strain is glycosylated, and that the unique apparent molecular weight of this protein is likely due to variations in the amino acid structure of the protein.



# MOLECULAR EVOLUTION OF WESTERN EQUINE ENCEPHALITIS VIRUSES

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Western equine encephalitis (WEE) virus is an alphavirus (Togaviridae: *Alphavirus*) and a member of the WEE antigenic complex (1). In North America, the virus is transmitted among passerine birds by mosquito vectors, primarily *Culex tarsalis*. The virus has also been isolated from South America and Cuba, where transmission cycles have not been described. A single isolate was also made in Russia (Table 1). WEE virus has been responsible for periodic, extensive equine epizootics and epidemics in North America since it was first isolated in 1930 (7).

Serologic analyses of several WEE virus strains revealed considerable antigenic diversity. Strains McMillan, R-43738, AG80-646, BeAr102091 and Y-62-33 were categorized as subtypes or varieties of the Fleming strain (2). Several strains of WEE virus were also characterized genetically (9) using oligonucleotide fingerprint analyses. All strains isolated from 1941-1975 were remarkably homogeneous, with an estimated 90% or greater sequence identity. Later, Hahn et al. (5) sequenced the 26S structural portion of the genome of WEE virus strain BFS1703, a 1953 isolate from California. They reported that the WEE capsid protein amino acid sequence was most like that of eastern equine encephalitis (EEE) virus, while the E1 and E2 envelope glycoproteins were more like Sindbis, another member of the WEE complex. Hahn et al. therefore concluded that this strain of WEE virus had a recombinant genome derived from EEE and Sindbis-like ancestors. Levinson et al. (6) confirmed these structural gene relationships using phylogenetic analyses, and the nonstructural genes were also confirmed to be EEE-like by Weaver et al., (11) who placed the recombination event before divergence of the North and South American antigenic varieties of EEE virus.

To provide more detailed information on the diversity and molecular evolution of WEE viruses, 34 representative strains (Table 1) were examined using limited genome sequencing. A portion of the E1 (C-terminus) envelope glycoprotein gene, as well as the 3' untranslated region, were amplified using reverse transcription and polymerase chain reaction (RT-PCR) to yield sequences of 791-865 nucleotides. Previously published, homologous E1 sequences of Sindbis, Ockelbo, Semliki Forest, Ross River, Venezuelan equine encephalitis (VEE) and eastern equine encephalitis (EEE) viruses were included in the analysis. All sequences were aligned using the PILEUP program of the Genetics Computer Group (3), and a parsimony analysis was conducted using the PAUP algorithm (8) with unordered characters.

Parsimony analysis yielded 320 trees of minimum (220 steps) length, all with the same branching pattern when zero length branches were collapsed to yield polytomies. This tree is shown in Fig. 1. Many of the antigenic subtypes described by Calisher et al. (2) do appear to represent distinct lineages or genotypes of WEE virus. The AR80 strain isolated in Argentina from *Culex (Melanoconion) ocoosa* mosquitoes was the most distantly related to the remaining strains examined,

followed by another Argentina (1933) isolate and a 1966 Brazil strain. Only single isolates representing these lineages are available. However, the lack of closely related isolates from relatively well sampled North America suggests that these genotypes may be restricted to South America. Isolates from Trinidad and Argentina (1982, 1983) also appeared to represent lineages distinct from those containing North American isolates. Because the exact locations where most of the Argentina strains were isolated is not known, it was not possible to determine whether the distinct genotypes circulating there are geographically defined.

All of the remaining WEE viruses clustered into two major groups. Group A (Fig. 1) included strains from California, Ontario, Brazil, Mexico, Cuba and Russia, while group B was comprised of viruses isolated in a wide variety of North American locations, as well as Brazil and Argentina. The lack of recent isolates in group A indicates that this lineage may have become extinct sometime after 1972; the other North American genotype (group B) was still circulating in 1993 (AZ93). In both groups, the oldest isolates occupied basal positions while the most recent occurred near the terminal branches, indicating that these two genotypes evolved overall as single lineages since the 1930-1940's. However, smaller regionally-based groupings also were observed, such as the CA53-CA54-CA61-CA78 group from Kern Co., California. This group excluded other strains isolated at different locations during the same time period, such as MT67, CA68, CA71, OR71, TX73 and MN75. This suggests that more than one regional lineage evolved independently for periods of several years to a few decades. The presence of both North and South American isolates in groups A and B indicates that some WEE viruses are readily dispersed between the continents. This contrasts with other New World Alphaviruses such as EEE and VEE which have genetically and antigenically distinct viruses restricted to North America.

To estimate rates of WEE virus evolution, isolates in the best sampled group (B, Fig. 1) were analyzed by regression, with year of isolation plotted vs. nucleotide substitutions from the hypothetical ancestor (Fig. 2, node B). The slope, 0.028%/yr., represented the average rate of sequence change for this lineage. There was no evidence of appreciable change in this rate from 1946-1993. Slightly higher rates of evolution have been reported for EEE (0.043%/yr)(10) and VEE viruses (<0.05%/yr)(12) in South and Central America. The slower rate estimated for EEE virus in North America (0.016%/yr)(10) may reflect the limited transmission season of viruses limited to temperate and subtropical vs. tropical locations.

Finally, we used this evolutionary rate to estimate ancestral divergence events for several WEE virus lineages. Using the oldest isolate, CA30 as a starting point, and internal branch lengths using the one parameter formula (4), we estimated that the AR80 lineage diverged roughly 300 years ago, followed by the AR33 lineage about 180 years ago. Groups A and B which included North American isolates probably diverged around the turn of this century.

Table 1. WEE virus strains used in phylogenetic analysis

Code	Strain	Location	Date	Host	Passage <sup>1</sup>
CA30	California	San Joaquin Valley	7-30	horse	gp?,sm27
TR	Fleming	California	??	human	sm5
AR33	TR25717	Trinidad	??	??	p?,sm3
ON41	Ar Enc MV	Argentina	1933	horse	sm2, v1
CA46	McMillan	Ontario	1941	human	m2,sm2
MO50	BFS932	Bakersfield, CA	1946	<i>Culex tarsalis</i>	sm1
CA53	EP-6	Missouri	1950	mosquito	cel
CA54	BFS1703	Kern Co., CA	7-53	<i>Culex tarsalis</i>	sm1,C6/36-1
AR58	BFS2005	Kern Co., CA	1954	<i>Culex tarsalis</i>	de1
BR61	CBA87	Argentina	1958	horse	sm1
CA61	Rio-1257	Brazil	1961	horse	p8,wc1,de1,sm1
CA62	A7712	Kern Co., CA	3-24-61	<i>Amnosperus nelsoni</i>	hk5,de1
RU62	BFS 4348	Kern Co., CA	8-2-62	<i>Culex tarsalis</i>	sm1
BR66A	Y62-33	Urdmurt, Russia	1962	<i>Ae. cantans/ciner</i>	sm2,de1
BR66B	BeAr102091	Brazil	1966	<i>Culex (Mel.) portesi</i>	p3,sm1
MT67	BeAn112509	Brazil	1966	sentinel mouse	de1
TX67	Montana 64	Montana	1967	horse	de1
CA68	67V5009	Hale City, Texas	8-23-67	<i>Culex tarsalis</i>	cel
CA71	S8 1-22	Paradise(Butte Co.)	8-2-68	<i>Sciurus griseus</i>	sm1
CU71	BFN 3060	Butte Co., CA	7-19-71	<i>Culex tarsalis</i>	CK1, sm1
OR71	UPA	Cuba	1971	??	wc1,de1,sm1
TX71	71V1658	Oregon	8-13-71	horse	p2, sm1
MX72	TBT-235	Texas	1971	<i>Gopherus berland.</i>	wc1,de1,sm1
CO72	M2-958	Mexico	1972	<i>Culex tarsalis</i>	v2,sm3
MN75	72V4768	Morgan City, CO	7-18-72	<i>Culex tarsalis</i>	sm1
CA78	75V9291	Wilkin City, MN	7-26-75	<i>Culex tarsalis</i>	v2
AR80	BFS 09997	Kern Co., CA	6-30-78	<i>Culex tarsalis</i>	vero1
AR82A	AG80-646	Argentina	1980	<i>Culex (Mel.) ocosa</i>	v2,sm1
AR82B	AG83-356	Argentina	1982	<i>Mansonia sp.</i>	p2,sm1
AR83	AG83-367	Argentina	1982	<i>An. albitarsis</i>	p3,sm1
CA83	CBA-C1V26A	Argentina	1983	horse	v2,sm1
SD83	CHLV 53	Riverside Co., CA	7-19-83	<i>Culex tarsalis</i>	vero1
NM85	R-43738	South Dakota	1983	human	p1,sm2
TN87	85-452-NM	New Mexico	1985	<i>Culex tarsalis</i>	sm2
CA92	TN87-3918	Tennessee	1987	<i>Culex pipiens-quinx</i>	v1
CO92	IMPR 441	Imperial Co., CA	7-21-92	<i>Culex tarsalis</i>	vero1
AZ93	CO92-1356	Larimer City, CO	7-30-92	<i>Culex tarsalis</i>	v1
	93A-27	Parker, Arizona	6-9-93	mosquito	v1

<sup>1</sup>C6/36 - *Aedes albopictus* mosquito cells; ch - chicken; de - duck embryo cell; gp - guinea pig; m - mouse; mq - mosquito; p - unknown passage; rd - human rhabdomyosarcoma; sm - suckling mouse; v - Vero cell.

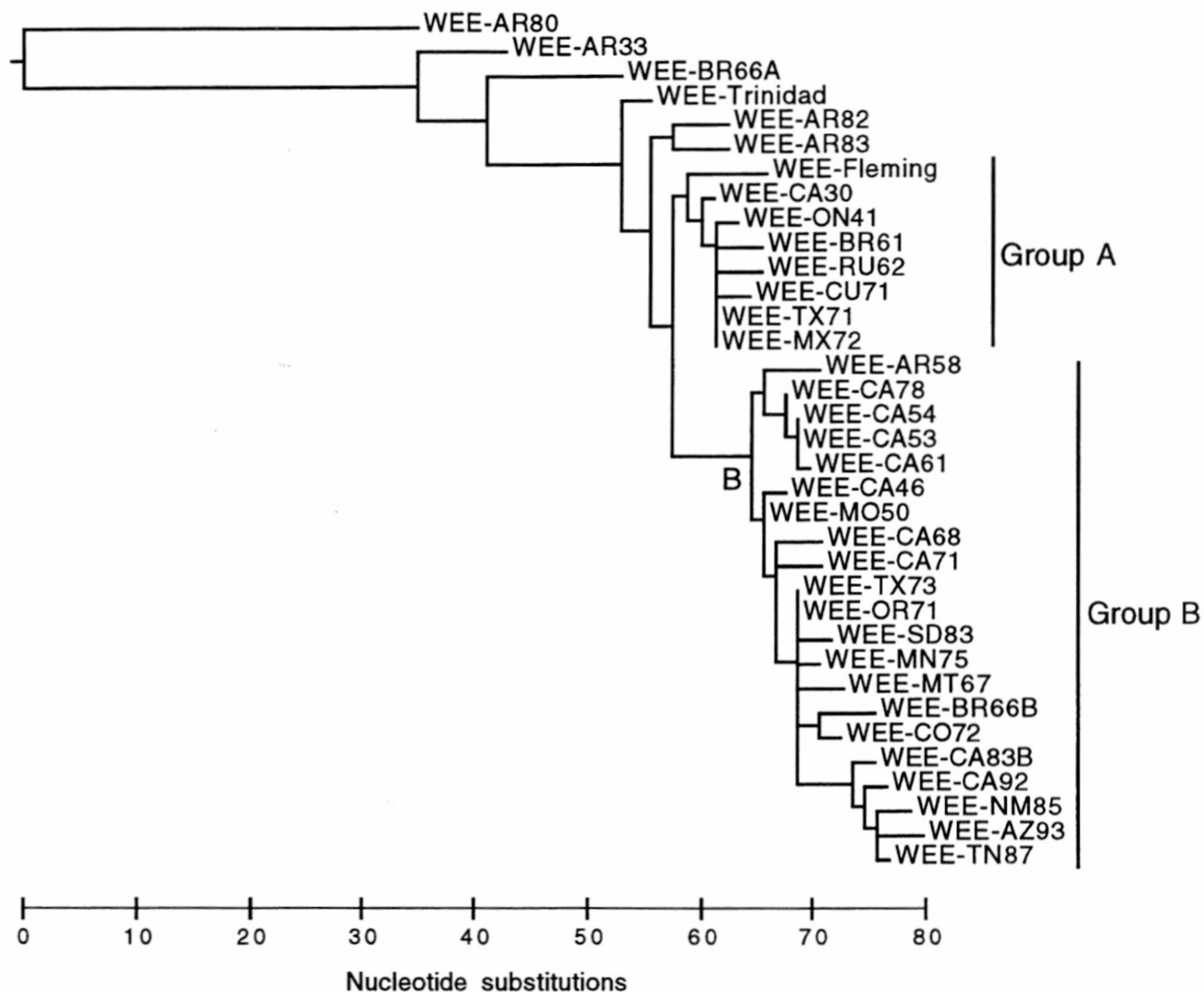


Fig. 1. Phylogenetic tree derived from E1-3' untranslated sequences of 35 strains of WEE virus. The tree was rooted using an outgroup consisting of homologous E1 sequences of Sindbis, Semliki Forest, Ross River, o'nyong nyong, Venezuelan and eastern equine encephalitis viruses. Node B represents the hypothetical ancestor used to estimate the evolutionary rate in Fig. 2.

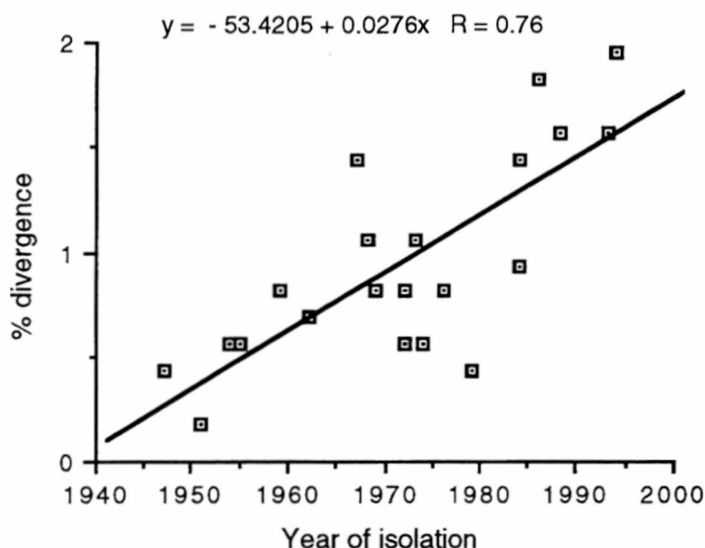


Fig. 2. Regression analysis of average evolutionary rate of WEE viruses in monophyletic group B of Fig. 1. Slope,  $0.028x$ , indicates average rate of sequence divergence from hypothetical ancestor B in Fig. 1, expressed in %/yr.

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# SURVEILLANCE FOR ARBOVIRUSES IN MOSQUITOES IN NEW SOUTH WALES, AUSTRALIA, 1991/92-1994/95

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We have previously reported on the arbovirus surveillance program for the state of NSW in southeastern Australia (see *Arthropod-Borne Virus Information Exchange*, December 1991, pp. 40-44). That report presented the methodology of the program and the general results for the three seasons 1988/89-1990/91.

We have continued to annually monitor mosquito populations and arbovirus activity, as represented by arbovirus isolation from mosquitoes, for 6 months from November, with weekly collections at two sites at each of 24-27 localities throughout the state.

The annual activity of the various viruses is presented in Table 1. The months of greatest arbovirus activity have been January through March (Table 2). Of the arboviruses identified during the four seasons, Barmah Forest virus was that most often isolated (73 of the 321 isolates) and Sindbis (SIN) was the next most common (67 of 321). There were 56 isolates of Ross River (RR) virus. RR virus was the most widespread, being recorded from 14 of the 21 localities yielding virus; the localities of Batemans Bay on the south coast and Griffith in the irrigation area of the southwest inland, provided most isolates (Table 3). *Aedes vigilax* and *Culex annulirostris* yielded most viruses; RR virus was recorded from more species of mosquito (9 of the 13) than any other virus (Table 4).

During 1995 the largest outbreak of BF virus recorded in Australia occurred in NSW, and was concentrated on the south coast (Van Buynder, P., Sam, G., Russell, R.C. *et al.* Barmah Forest virus epidemic on the south coast of New South Wales. *Communicable Diseases Intelligence*, 1995, **19**: 188-191). As well as BF virus, RR, Edge Hill (EH) and Stratford (STR) viruses were isolated from mosquitoes collected in the region at the time. These isolates are included in the accompanying Tables but the data are not yet complete.

With respect to clinical infections, RR virus remains the most prevalent arbovirus infecting humans and causes considerable morbidity throughout Australia (Russell, R.C. Ross River virus: disease trends and vector ecology in Australia. *Bulletin of the Society for Vector Ecology*, 1994, **19**: 73-81). Other arboviruses causing human disease are detected occasionally or more often in various areas of the continent (Mackenzie, J.S. *et al.* Arboviruses causing human disease in the Australian zoogeographic region. *Archives of Virology*, 1994, **136**: 447-467; Russell, R.C. Arboviruses and their vectors in Australia: an update on the ecology and epidemiology of some mosquito-borne arboviruses. *Review of Medical and Veterinary Entomology*, 1995, **83**: 141-158).

**Table 1. VIRUS ISOLATIONS BY YEAR, 1991/92-1994/95**

YEAR	VIRUS ISOLATES													TOTAL
	RR	BF	BF + EH	BF + STR	BF + Flav	SIN	GG	TRU	EH	STR	STR + EH	Flavi	Virus ?	
1991-92	18						3	1				1	27	50
1992-93	16					30	1	1	2				15	65
1993-94	9					37							20	66
1994-95	13	68	2	2	1				12	5	1	1	35	140
TOTAL	56	68	2	2	1	67	4	2	14	5	1	2	97	321

RR = Ross River virus, BF = Barmah Forest virus, BF + EH = Barmah Forest virus plus Edge Hill virus (dual mosquito infection), BF + STR = Barmah Forest virus plus Stratford virus (dual mosquito infection), BF + Flavi = Barmah Forest virus & unidentified Flavivirus (dual mosquito infection), SIN = Sindbis virus, GG = Gan Gan virus, TRU = Trubanaman, EH = Edge Hill virus, STR = Stratford virus, STR + EH = Stratford virus plus Edge Hill virus (dual mosquito infection), Flavi = Flavivirus (yet to be identified), Virus ? = Unidentified Virus not GET, RR, BF, SIN, ALF, EH, STR, MVE, KOK, KUN.

**Table 2. VIRUS ISOLATIONS BY MONTH, 1991/92-1994/95.**

MONTH	VIRUS ISOLATES													TOTAL
	RR	BF	BF + EH	BF + STR	BF + Flav	SIN	GG	TRU	EH	STR	STR + EH	Flavi	Virus ?	
November													2	2
December	4													4
January	15	56	2	2	1	10	1		7				36	130
February	21	9				49		1	6	5	1	2	32	126
March	13	3				8	3	1	1				23	52
April	3												4	7
TOTAL	56	68	2	2	1	67	4	2	14	5	1	2	97	321

**Table 3. VIRUS ISOLATIONS BY LOCALITY, 1991/2-1994/95.**

LOCALITY	VIRUS ISOLATES													TOTAL
	RR	BF	BF + EH	BF + STR	BF + Flav	SIN	GG	TRU	EH	STR	STR + EH	Flavi	Virus ?	
Ballina	3					1							6	10
Batemans Bay	10	64	2	2	1				11	5	1	1	20	117
Boggabilla	1												1	2
Bourke													3	3
Condobolin	6					1	1						3	11
Deniliquin													2	2
Griffith	16					49	1	2				1	42	111
Forster		1											1	2
Lake Cargelligo	1													1
Leeton						1							6	7
Menindee	2					12							3	17
Merimbula	1													1
Moree	1													1
Port Macquarie	1												1	2
Port Stephens	7						2						4	13
Sydney		1												1
Tamworth	1												1	2
Tathra	3	2							1				1	7
Tweed Heads													1	1
Warren									2				1	3
Wentworth	3					3							1	7
TOTAL	56	68	2	2	1	67	4	2	14	5	1	2	97	321

**Table 4. VIRUS ISOLATIONS BY MOSQUITO SPECIES, 1991/92–1994/95.**

MOSQUITO	VIRUS ISOLATES													TOTAL
	RR	BF	BF + EH	BF + STR	BF + Flav	SIN	GG	TRU	EH	STR	STR + EH	Flavi	Virus ?	
<i>Ae aculeatus</i>													1	1
<i>Ae camptorhyncus</i>	2	1												3
<i>Ae funereus</i>	2												1	3
<i>Ae notoscriptus</i>	1													1
<i>Ae procax</i>	1												1	2
<i>Ae vigilax</i>	17	66	2	2	1		2		12	5	1	1	23	132
<i>An amictus</i>													1	1
<i>An annulipes</i>	3					2		2					13	20
<i>An annulipes</i> ♂													1	1
<i>Cq linealis</i>	2	1											1	4
<i>Cx annulirostris</i>	26					63	1		2			1	41	134
<i>Cx annulirostris</i> ♂						1								1
<i>Cx australicus</i>													4	4
<i>Cx quinquefasciatus</i>	1												3	4
<i>Cx sitiens</i>						1							4	5
Mixed species pools	1						1						3	5
<b>TOTAL</b>	<b>56</b>	<b>68</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>67</b>	<b>4</b>	<b>2</b>	<b>14</b>	<b>5</b>	<b>1</b>	<b>2</b>	<b>97</b>	<b>321</b>

# THE PREVALENCE OF ANTIBODIES TO FOUR ARBOVIRUSES IN BLOOD DONORS IN THE SOUTH-WEST OF WESTERN AUSTRALIA

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This report describes the seroprevalence of Ross River (RR), Barmah Forest (BF), Sindbis (SIN) and Trubanaman (TRU) viruses in a group of 250 human sera from blood donors in the Mandurah region, approximately 80-110 km south of the capital Perth, in the south-west of Western Australia. It also describes a comparison of the sensitivities and specificities of two techniques, the Neutralisation Test (NT) and a RR virus-specific monoclonal antibody blocking ELISA developed in our laboratory.

Arbovirus activity in the south-west of Western Australia is monitored by routine collection of adult mosquitoes and their processing for virus isolation. Results of this ongoing work show that RR virus is endemic in the Mandurah region (Lindsay *et al.*, 1989). RR has caused several major outbreaks of epidemic polyarthritis in the area. Other arboviruses are also known to occur in the Mandurah region. BF virus, a closely related alphavirus has been isolated from mosquitoes collected since 1993 and caused a small outbreak of human disease between 1992 and 1994 (Lindsay *et al.*, 1995). Another alphavirus SIN and the bunyavirus TRU have also been isolated from mosquitoes in the area, though their role in human infection or disease is not known.

Four topotypes of RR virus have been identified by RNase T1 mapping. These are designated T48 (the type strain), WK20 (including isolates WK20 and SW876), SW2191 and K1503 (Lindsay *et al.*, 1993). Cross-neutralisation studies showed that antisera raised against Western Australian isolates (WK20, SW876) of RR virus did not neutralise eastern Australian isolates, including T48, as efficiently as homologous strains. Subsequent work indicated that our RR blocking ELISA detected more seropositive samples in a group of animal sera collected in the south-west than did the NT, when T48 was used as antigen. Thus, we were particularly interested in comparing the abilities of the NT and the RR blocking ELISA to detect antibodies to different strains of RR in the collection of sera from human blood donors from Mandurah. To do this the sera were assayed by NT and blocking ELISA. Isolates representing the four RR virus topotypes (isolates T48, WK20 and SW876, SW2191 and K1503) were used in the NT. T48 was the only coating antigen used in the blocking ELISA. We also screened the sera by NT against Western Australian mosquito isolates of BF, SIN and TRU viruses.

The NT method used was a modification of the method published by Rosenbaum *et al.* (1972). The RR virus-specific monoclonal antibody blocking ELISA was developed in our laboratory as an adaptation of the method of Hall *et al.* (1994) for detection of virus-specific antibodies in sentinel animal serum. In the modified method, cell culture supernatant stocks of T48 antigen were used to coat each 96 well U-bottom plates. The monoclonal antibody used was raised against the K1503 strain and was shown to be RR virus specific. Percent inhibition of monoclonal antibody was calculated for each serum using the formula from Hall *et al.* (1994). Results of both the NT and blocking ELISA are shown in Table 1. The neutralisation titre is expressed as the reciprocal of the highest serum dilution where CPE did not occur. Samples were recorded as positive in the blocking ELISA if their percentage inhibition was greater than or equal to 20%.



Twenty one of the 250 samples tested were RR positive in the NT against one or more of the RR strains used. The blocking ELISA detected only twenty positive sera. The single positive by NT and not ELISA reacted weakly (1/40) to K1503 but was negative to all other topotypes. All other positive sera reacted strongly (>70% inhibition) in the ELISA. The neutralisation titres of the sera were extremely variable for the different topotypes though there was no obvious pattern of reactivity. This may be due to differences in the level of recognition of different neutralising epitopes in polyclonal sera. T48 and K1503 detected 95% of the positive samples. WK20 detected 81%, SW876 and SW2191 (both south-west topotypes) detected only 71%. These results suggest that the RR blocking ELISA was at least as specific as the NT for detecting RR virus-specific antibody in human serum. Given the ease and rapidity of the ELISA and the variability between topotypes in the NT we believe the ELISA is a more useful assay. It is interesting to note that south-west strains (SW876 and SW2191) were less effective than northern and eastern Australian strains (T48 and K1503) at detecting antibodies to RR virus in sera from the south-west. The relatively high rate of detection of human anti-RR virus antibody using T48 as antigen in the NT conflicts with our previous results using animal sera. Screening of these animal sera against other RR virus topotypes is required to further define this discrepancy.

8.4% of the sera tested showed evidence of RR virus infection. The ratio of male to female was 1:1.1. Ages ranged from 19 to 67, the average age was 49. Over 76% of sero-positive individuals were older than 45. There did not appear to be an association between length of residency and likelihood of infection with RR virus. This suggests that other factors such as occupation or life-style may contribute to the risk of exposure to RR virus.

Three samples (1.2%) had neutralising antibodies to BF virus. This was not unexpected considering the very recent introduction of BF virus into the Mandurah region. This compares with an overall prevalence of 2% to BF virus in New South Wales (Hawkes *et al.*, 1987) and 0.23% in Queensland (Phillips *et al.*, 1990). There was no evidence of SIN infection in any of the sera tested by NT. Only one sample showed the presence of antibodies to TRU virus (0.4%), somewhat lower than the seroprevalence of 2.1% in New South Wales (Boughton *et al.*, 1989).

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**TABLE 1: Results of the NT and RR blocking ELISA.**

SAMPLE No.	RR <sup>†</sup>					BF <sup>†</sup>	SIN <sup>†</sup>	TRU <sup>†</sup>	RR Blocking ELISA*
	T48	K1503	WK20	SW876	SW2191				
7	40	80	20	10	20	-	-	-	72.1
11	80	>640	320	>640	20	-	-	-	74.2
39	>640	>640	>640	>640	>640	-	-	-	100.0
45	80	320	320	320	80	-	-	-	96.8
48	160	320	320	320	160	-	-	-	97.4
58	160	80	40	20	80	-	-	-	94.7
63	80	>640	320	40	40	-	-	-	100.0
69	-	40	-	-	-	-	-	-	-
84	320	>640	>640	>640	>640	-	-	-	100.0
92	160	>640	320	>640	>640	-	-	-	100.0
113	40	>640	320	80	160	-	-	-	97.5
125	80	40	40	160	160	-	-	-	88.8
138	80	40	320	160	160	-	-	-	94.7
141	160	>640	320	>640	320	-	-	-	96.0
194	>640	320	160	160	160	-	-	-	96.2
201	160	>640	80	80	160	-	-	-	97.4
242	320	160	160	40	160	-	-	-	98.0
243	320	80	40	20	20	-	-	-	86.9
252	160	-	-	-	-	-	-	-	91.5
293	40	40	10	10	20	-	-	-	86.4
301	>640	160	40	80	320	-	-	-	95.1
21	-	-	-	-	-	80	-	-	‡
59	-	-	-	-	-	320	-	-	‡
67	-	10	-	-	-	40	-	-	‡
207	-	-	-	-	-	-	-	80	‡

<sup>†</sup> NT titre expressed as the reciprocal of the highest serum dilution where CPE did not occur.

\*Numbers represent the % inhibition where a positive is > or = to 20%. ‡ not tested.



## RECENT EPIDEMICS OF ROSS RIVER VIRUS AROUND BRISBANE

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During the 1991-92 summer (i.e. December-April), approximately 4500 cases of Ross River (RR) virus occurred mainly in south east Queensland. Clinical cases were distributed throughout Brisbane (approx. 770 cases) indicating that the major saltmarsh vector *Aedes vigilax* was not solely involved.

It is well known that RR virus is capable of infecting a broad range of mosquitoes (now 6 different genera) and vertebrate hosts. During the past summer, Brisbane had a further 747 cases of RR with the peak occurring from March-April 1994.

The North East Moreton Mosquito Organization (a collection of 5 municipal authorities charged with mosquito control but mainly saltmarsh) commissioned us to collect mosquitoes from inner western suburbs of Brisbane to determine the relative roles that various mosquito species might play. From March to June 1994, 29931 mosquitoes were caught using octenol and CO<sub>2</sub> supplemented light traps.

Predictably, the freshwater breeding *Culex annulirostris* was the most common species (31.7%) while the saltmarsh mosquito *Aedes vigilax* comprised 18.4%. However rather surprisingly, numbers of the peridomestic breeding *Aedes (Finlaya) notoscriptus* were high and it was second in abundance (23.7%). This latter species was prevalent into winter and one RR isolate was recovered from this species on June 7. Although *Aedes (Ochlerotatus) procax* is known to be present in the Brisbane area, it never has been as common as in our 1994 collection (14.3%). A brackish water species, *Aedes (Verrallina) funereus*, appeared in large numbers mainly in March (9.8% of total collection) and had high RR virus carriage rates.

The mosquitoes were processed for both RR and Barmah Forest viruses using microtitre C6/36 cells and monoclonal antibody specific immunoperoxidase staining for RR and BF. This method was developed and found to be sensitive by Nidia Oliveira and Roy Hall within John Mackenzie's group at the University of Western Australia.

The virus recovery rates and the species infected proved to be rather surprising (Table 1). In total, there were 38 RR and 19 BF isolates but *Ae notoscriptus* and *Ae funereus* provided 12 and 10 of these, respectively. In general, these data point towards an increased need to control freshwater breeding and peridomestic species without diminishing efforts on *Ae vigilax*.

Table 1  
Number of mosquitoes processed, Ross River and Barmah Forest  
virus isolates and carriage rates

Species	No processed	No Pools	No infected		Infection rate/1000*
			RR	BF	
<i>An bancroftii</i>	4	1			
<i>An annulipes</i>	8	4			
<i>Ae kochi</i>	2	1			
<i>Ae notoscriptus</i>	7087	392	8	4	1.7
<i>Ae alternans</i>	1	1	1	0	-
<i>Ae procax</i>	4266	245	1	2	0.7
<i>Ae vigilax</i>	5517	305	4	4	1.5
<i>Ae vittiger</i>	43	8			
<i>Ae funereus</i>	2943	159	9	1	3.6
<i>Cx annulirostris</i>	9475	513	15	6	2.3
<i>Cx australicus</i>	160	8			
<i>Cx quinquefasciatus</i>	206	32			
<i>Cx sitiens</i>	97	10	0	1	10.5
<i>Cx pullus</i>	1	1			
<i>Cx halifaxii</i>	2	1			
<i>Cq xanthogaster</i>	36	5			
<i>Ma uniformis</i>	83	12	0	1	12.4
Total	29931	1698	38	19	

\*After Chiang and Reeves (1962).

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

Arbovirus Surveillance in New Jersey, 1994

During the 1994 surveillance period from June into October, 554 mosquito pools containing up to 100 mosquitoes each were tested for viruses in day old chicks. There were 29 mosquito pools positive for Eastern encephalitis (EE) and Highland J virus (HJ) was isolated from 11.

Table 1 summarizes the collection area totals, species of mosquito and time of collection for the EE isolates. Activity began with mid-August collections and continued into October. All of the 29 isolates were from pools containing Culiseta melanura mosquitoes at 6 sites.

HJ mosquito activity is summarized in Table 2. The mid-August collections also gave the first isolates with continued observation of HJ activity into late October. There were 11 isolates from Culiseta melanura at 5 sites.

EE isolates were also made in July and October in 2 horses in southern coastal counties.

There were, for the first time, 2 EE isolates from Emu flocks in August. These birds presented with a hemorrhagic gastroenteritis and no apparent encephalitis. Isolates were made from lung, liver, kidney and spleen.

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

		Table 1 1994 EE MOSQUITO POOL ISOLATES FOR WEEK ENDING											
AREA COLLECTED	MOSQUITO SPECIES	8/5	8/12	8/19	8/26	9/2	9/9	9/16	9/23	9/30	10/7	10/14	AREA TOTALS
Centerton	Cs. melanura	1			4	2	1			2			10
Dennisville	Cs. melanura	2			2			1		1			6
Hamonton	Cs. melanura											1	1
Ocean City	Cs. melanura							1					1
Waretown	Cs. melanura	1					1				1		3
Waterford	Cs. melanura					1				6	1		8
WEEKLY TOTALS		4	0	0	6	3	2	2	0	9	2	1	29

		Table 2 1994 HJ MOSQUITO POOL ISOLATES FOR WEEK ENDING											
AREA COLLECTED	MOSQUITO SPECIES	8/5	8/12	8/19	8/26	9/2	9/9	9/16	9/23	9/30	10/7	10/14	AREA TOTALS
Bass River	Cs. melanura	1		1				1					3
Centerton	Cs. melanura						1		1			1	3
Dennisville	Cs. melanura							1	1				2
Waretown	Cs. melanura							1				1	2
Waterford	Cs. melanura									1			1
WEEKLY TOTALS		1	0	1	0	0	1	3	2	1	0	2	11

# **Acute viral encephalitis due to tick-borne encephalitis virus in Hokkaido, Japan.**

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Human meningo-encephalitis is caused by more than a dozen flaviviruses including Japanese encephalitis (JE), St. Louis encephalitis, Murray Valley encephalitis, Russian spring-summer encephalitis (RSSE), Central European tick-borne encephalitis and Negishi viruses [1]. Most of these have been identified as arthropod-borne viruses and are confined to certain areas because of the distribution of these vector invertebrates. In Japan, only JE and Negishi viruses have been confirmed as flavivirus pathogens that cause human central nervous system infections.

In October 1993, a 37-year-old Japanese woman who was living in Hokkaido, northern Japan and who had never been overseas developed acute viral meningo-encephalitis with typical clinical manifestations (high fever, headache, stiff neck, coma, etc.) and laboratory findings of the cerebro-spinal fluid (increased lymphocytes, etc.). She recovered with paralysis of the extremities after 3 weeks. She underwent MRI and serological analysis against HSV, JE, etc. in order to determine the causative pathogen. MRI demonstrated abnormalities in the thalamus and basal ganglia, which is a typical finding of JE [2], and haemagglutination inhibition (HI) antibody against JE rose in the convalescent sera (Table 1). However, Hokkaido is a JE non-epidemic area, and moreover it was not an epidemic period of the year. Since flaviviruses are known to be widely cross-reactive in the HI test, the possibility of some other flavivirus infection was investigated by the focus reduction virus neutralization test (NT) [3], which is the most specific among a number of serological examinations on flavivirus infections. In Japan, only three local flaviviruses have been isolated so far, i.e. JE, Negishi and Apoi viruses. JE and Negishi viruses were isolated from human meningo-encephalitis cases and Apoi was isolated from a rodent. Negishi and Apoi viruses were classified into the tick-borne encephalitis group because of their immunogenicity.

Surprisingly, NT against RSSE, which has never been isolated in Japan, was extremely high compared with that against JE, Negishi and Apoi in the patient's sera (Table 1). The causative virus was not isolated and so it was impossible to identify the causative agent only by serological examination, but we can conclude at least that the patient was infected with a tick-borne flavivirus and that the anti-JE

HI antibody elevation was due to cross-reactivity between JE and RSSE or some closely related tick-borne flavivirus. It is noteworthy that the patient had a history of JE vaccination. It is supposed that the high level immune response measured by JE virus antigen (Table 1) was due to the secondary stimulation of flavivirus-common antigen carried on the infecting tick-borne virus. It is considered that patients that have not received JE vaccination may not show such a marked elevation of anti-JE antibody in tick-borne virus infection.

Two possible virus transmission routes can be considered. One is that an unknown tick-borne virus very close to RSSE exists in Hokkaido, Japan. The other is that RSSE entered Japan from eastern Russia where the virus has been circulating. After the end of the Cold War, trade between eastern Russia and the countries of the Pan-Pacific area including Japan has increased. In fact, ships sail frequently into the Hokkaido port of Hakodate close to the patient's home. We therefore cannot exclude the possibility that RSSE recently entered Hokkaido from Siberia or eastern Russia. RSSE is transmitted by the tick *Ixodes persulcatus* and infects to wild and domestic animals. Both these potential vectors (*Ixodes* tick) and animals exist widely in northern temperate regions including Japan and the western part of North America [4]. Thus, the possibility of RSSE infection should be considered when encephalitis cases of unknown origin are observed in these areas.

Table 1. Results of the serological examination of serum and CSF.

Sample (Day after onset)	anti-JE				NT			
	IgG-ELISA	IgM-ELISA	HI	HI(2ME)	JE	RSSE	Neg	Apoi
Serum (day 6)	1,600*	100>	20	20	10	640	40	10>
Serum (day 43)	16,000	100>	160	160	20	2560	320	10
CSF (day 52)	1,600	10>	20	20	nt	nt	nt	nt

JE: Japanese encephalitis, NT: neutralization test, HI: Haemagglutination inhibition test, HI(2ME): 2ME resistant HI titer, RSSE : Russian spring-summer encephalitis Neg: Negishi, CSF: cerebro-spinal fluid, \*: Numbers indicate endpoint titers.

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# MISSOURI MOSQUITO SURVEILLANCE - 1994

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Mosquitoes were collected in areas of Missouri effected by flooding of the Mississippi and Missouri Rivers during the summer of 1994 and assayed for St. Louis Encephalitis(SLE), Western Equine Encephalitis (WEE) , Eastern Equine Encephalitis (EEE) and LaCrosse Encephalitis (LAC) antigens by ELISAs. Culex pipiens complex (CC) and Culex tarsalis (CT) mosquitoes were assayed for SLE and WEE, Coquillettidia perturbans (CP) and Aedes albopictus (AA) were tested for EEE and Aedes albopictus and Aedes triseriatus (AT) were assayed for LAC antigen.

The state was divided into five Regions each of which was subdivided into two or more sections (Table I) (Figure I.) Mosquitoes were collected sporadically in Southwest Missouri which was designated region six.

Figure 1  
AREAS WHERE MOSQUITOES WERE COLLECTED

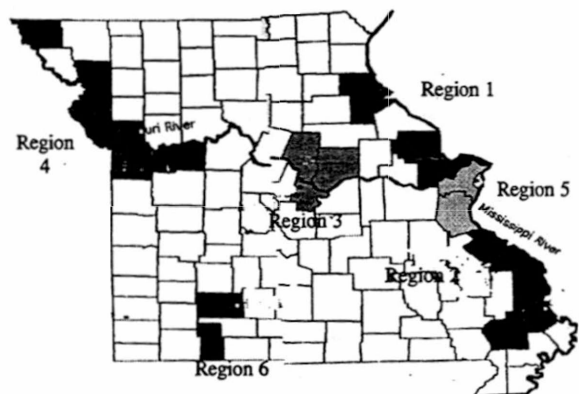


Table I  
COUNTIES WHERE MOSQUITOES WERE COLLECTED

Rgn		Counties	Dates
1	.1	Marion, Ralls	6/14-8/9
	.2	St. Charles, Lincoln	5/15-8/14
2	.1	Cape Girardeau, Scott, Perry, Ste. Genevieve	5/18-10/17
	.2	Stoddard	6/6-8/12
3	.1	Boone, Calloway, Cole	5/31-8/25
	.2	Pettis	8/8-8/11
4	.1	Atchison, Andrews, Clay, Buchanan, Platte	5/31-9/21
	.2	Jackson, Lafayette	5/24-9/20
5	.1	St. Louis	4/27-8/19
	.2	Jefferson	5/21-8/12
	.3	St. Louis City	6/24-8/11
6	.1	Greene	8/13
	.2	Stone	8/19

Sites were selected in regions one to five and a set of sites to be trapped each day was established. After the trapping of mosquitoes commenced, field personnel surveyed their areas for additional sites. Non productive sites were eliminated from the collecting cycle and new sites added. After the first few weeks there was little site changing and a regular rotation was established for each area. The majority of the trapping used light traps baited with CO<sub>2</sub> but gravid traps were used at some sites. Mosquitoes were also aspirated from resting places such as the walls of culverts and sanitary sewers.

Table II shows the number of sites, collections, and mosquitoes by species by region. State totals are in Table III. Collection indexes (mosquitoes collected/number of collections) were calculated and statistical analysis revealed that there were a significant differences between the productivity of sites. The mean and high collection indexes varied greatly by species and region (Table IV) . Many sites had collection indexes below ten while a few were above 100 ( Table V).



Table II  
NUMBER OF SITES, COLLECTIONS AND MOSQUITOES BY REGION

	1.1			1.2			2.1			2.2			3.0			3.1			4.1			4.2			5.1			5.2			5.3			6.1			6
	S	C	M	S	C	M	S	C	M	S	C	M	S	C	M	S	C	M	S	C	M	S	C	M	S	C	M	S	A	M	S	C	M	S	C	M	M
AA							15	25	227				1	1	1				5	27	1542	2	9	82	8	12	37	3	13	160	3	3	79	7	8	123	372
AT	4	14	51	15	91	315	24	36	83				12	34	45				18	144	1145	15	91	1072	14	26	4250	6	22	108	3		6	18	18	437	16
CC	4	43	49	30	401	4964	80	534	35389	3	6	384	34	360	1762	8	16	258	23	437	18996	30	308	5450	15	28	10287	15	116	876	13	43	4140				4
CT				3	4	4	3	3	5				3	6	8	1	1	1	18	136	607	18	59	119	102	310	270				1	1	2				
CP				9	33	65	20	53	323	2	5	132	2	2	2				8	31	79	17	62	309													

S = SITES

C = COLLECTIONS

M = MOSQUITOES

Table IV  
MEAN AND HIGH COLLECTION INDEXES BY SPECIES AND REGION

	1.1		1.2		2.1		2.2		3.0		3.1		4.1		4.2		5.10		5.2		5.3		6.1		6.2
	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	
AA					8.6	70.0			1.0	1.0			36.5	130.9	5.1	7.1	3.1	14.0	2.5	4.0	1.4	2.0	17.75	47	NA
AT	2.1	5.	2.3	12.6	1.6	2.6			1.2	2.0			3.1	36.0	6.0	27.3	43.4	351.8	2.7	38.0	1.7	3.0	21.5	74	NA
CT			1.0	1.0	1.3	2.0			1.3	2.0	1.0	1.0	3.7	11.5	1.6	3.5	3.9			5.2	2.0	2.0			
CC	0.8	2.	21.4	138	67.1	382.2	64.0	89.3	4.7	1.5	19.1	115.0	47.2	160.6	16.6	79.8	24.3	237.5	30.3	178.0	10.6	29.0			
CP			1.7	4.5	5.4	15.0	26.4	33.3	1.0	1.0			1.8	4.0	4.2	20.2	2.5								

M = MEAN COLLECTION INDEX

H = HIGH COLLECTION INDEX

Table V  
PERCENTAGE OF CITES WITH HIGH AND LOW COLLECTION INDEXES

	1.1	1.2		2.1		2.2	3.0	3.1	4.1		4.2	5.1		5.2		5.3	6.1	6.2
	<	<	>	<	>	<	<	<	<	>	<	<	>	<	>	<	<	<
AA				80					60		100	87.5		100		100	14	NA
AT	25			100			100		94.4		80	92.9	1	100		100	33	NA
CT				100			100	100	94.4		100	86.67				100		
CC		50	7	61.3	8.8	33	94	50	47.8	13	60	57.84	4	53	20	54		
CP				90			100		100		88.9							

Mosquitoes were sent to the laboratory at Southeast Missouri State University. The ELISAs for SLE, WEE and EEE were performed using reagents provided by CDC following the protocol outlined by Tsai and colleagues (1). After the antigen was added, the test was incubated overnight at 4 °C and ABTS was chosen as the enzyme substrate. Pools giving a preliminary positive in the ELISA were tested in an inhibition assay as described by Tsai(1).None of the pools giving a preliminary positive reaction were found to be positive upon confirmatory testing. The ELISA for LAC was performed as outlined in Hildreth and Frazier (2) using reagents prepared in or obtained by our laboratory. Table VI enumerates the number of pools rested by antigen and region.

Table III  
STATE TOTALS

Aedes albopictus	2623
Aedes triseriatus	7528
Culex species	82559
Culex tarsalis	1016
Coq. perturbans	910
Aedes sp	42
NA	7
	94685
Aedes eggs& Larvae	82

Table VI  
POOLS TESTED BY REGION AND ANTIGENS

	1.1	1.2	2.1	2.2	3	3.1	4.1	4.2	5.1	5.2	5.3	6	6.2	STATE
EEE ORIGINAL		34	93	5	3		75	59	13	23	11		21	337
RETEST			2											2
TOTAL		34	95	5	3		75	59	13	23	11		21	339
LAC ORIGINAL	14	93	87		31		193	68	123	63	15	22	20	729
RETEST			4				26	3	10	4	1	5		53
TOTAL	14	93	91		31		219	71	133	67	16	27	20	782
SLE ORIGINAL	22	352	1109	13	283	42	799	366	522	141	135	1	4	3789
RETEST	2	2	12		5		11	9	16		3			60
TOTAL	24	354	1121	13	288	42	810	375	538	141	138	1	4	3849
WEE ORIGINAL	22	352	1109	13	283	42	799	366	522	141	135	1	4	3789
RETEST		1	71		9		53	13	37	1	12			197
TOTAL	22	353	1180	13	292	42	852	379	559	142	147	1	4	3986

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Uukuniemi (UUK) virus (family *Bunyaviridae*, genus *Phlebovirus*) is widely distributed in Europe where it is ecologically associated with *Ixodes ricinus* ticks. Strains of this virus have been isolated from naturally-infected *Culex modestus* mosquitoes in Azerbaijan (T.M. Skvortsova et al. 1992). Experimental transmission of UUK virus through the bite of *Aedes aegypti* has been demonstrated (T.I. Samoilova et al., 1976).

During 1990-92, four strains of UUK virus were isolated at the Anti-Plague Station, Novorossiysk, from *Ix. ricinus* ticks and another strain was isolated from *Ae. cataphylla* mosquitoes collected in a subtropical area in the Krasnodar Region (Black Sea coast of Caucasus). In 1993 UUK virus antigen was detected by ELISA in 11 pools of *Ix. ricinus* (0.5% of tested specimens). Serum samples collected in the same area in the summer months of 1991-93 from 733 febrile patients with acute illness of unknown etiology were tested for antibody to UUK, Tahyña, Inkoo, Batai, CHF-Congo, Bhanja, Sindbis, West Nile, Russian Spring-Summer encephalitis, and Dhori viruses.

IgM (1600), complement-fixing (40), and neutralizing ( $LN \geq 2.5 \log_{10}$ ) antibodies to UUK virus were detected in Patient 502. Serum samples from Patient 712, collected 12 and 26 days after onset of illness, also contained IgM (200, 200), complement-fixing (40, 40), and neutralizing ( $LN \geq 2.5 \log_{10}$ ,  $\geq 2.5 \log_{10}$ ) antibodies to UUK virus. Tests of serum from Patient 742 on the tenth day after onset of illness revealed antibody to UUK virus by IgM (1600), complement-fixing (40), neutralization ( $LN \geq 2.5 \log_{10}$ ), and precipitation in agar (positive reaction of undiluted serum).

Clinical manifestations of these three cases, diagnosed as UUK virus infection, were characterized by acute onset, fever, (to  $38^{\circ}C$ ) lasting 7-12 days, headache, muscular, joint and lumbar pains, hyperemia of the face, and abundant and clear rash all over the body (Patient 712). Patient 742 had been infected with UUK virus in the Astrakhan Region (Northern coast of the Caspian Sea).

During these studies, human illnesses caused by Sindbis (3), West Nile (4), Batai (5), Inkoo (6), and Tahyña (1) viruses also were diagnosed.

# Complete Nucleotide Sequences of the M and S Segments of Two Hantavirus Isolates from California: Evidence for Reassortment in Nature Among Viruses Related to Hantavirus Pulmonary Syndrome

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We report the complete nucleotide sequence of the M and the S genome segments and a portion of the L segments of two hantavirus isolates from *Peromyscus maniculatus* trapped in eastern California. The isolates, Convict Creek 107 and 74 (CC107 and CC74) are genetically similar to viruses known to cause hantavirus pulmonary syndrome in New Mexico. CC107 and CC74 each have an M segment consisting of 3696 nucleotides with a coding potential of 1140 amino acids in the virus complementary-sense RNA (cRNA). The S segments of CC107 and CC74 are 2083 and 2047 nucleotides long, respectively, and each has an ORF in the cRNA capable of encoding a protein of 428 amino acids. Unusually long 3' noncoding regions of 757 and 721 nucleotides follow the S segment ORF of CC107 and CC74, respectively, and include numerous imperfect repetitive sequences.

Whereas the M and S segments of any given hantavirus typically appear to diverge at comparable rates from homologous genes of any other hantavirus, CC107 and CC74 have M segments that differ by only 1% from one another but S segments that differ by 13%. After trivial explanations are rendered improbable; i.e., by consideration of the genetics of closely and distantly related hantaviruses, the most likely explanation for our data is that hantavirus genome segment reassortment occurred within rodent populations in California.

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A fatal case of hantavirus pulmonary syndrome (HPS) in northern California prompted our attempt to isolate viruses from local rodents. From tissues of two deer mice, *Peromyscus maniculatus*, two hantaviruses (Convict Creek virus #107 and #74, CC107 and CC74) were established in cell culture. Viral antigens, proteins, and RNAs of the first and archetypical isolate (CC107) were examined, and portions of the medium (M) and small (S) genome segments of both isolates were sequenced. Antigenically, CC107 virus and the second isolate, CC74 virus, were more closely related to Puumala virus than HTN virus, though distinct from both. Northern blots of viral RNAs showed the large (L) and M segments of CC107 to be the same size as those of HTN virus, whereas the S segment was larger. Protein gels did not reveal CC107 to have a substantially larger nucleocapsid protein than HTN virus. Partial nucleotide sequence comparisons of CC107 and CC74 viruses revealed their M segments to be highly similar to one another, while their S segments differed by more than 10%. Nucleotide and deduced amino acid sequence comparisons showed the California isolates to be closely related to the newfound hantaviruses first detected in the Four Corners area, and since incriminated in HPS through wide areas of the United States.

## CUBAN NEUROPATHIC EPIDEMIC.

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A virus isolation with two types of cytopathic effect from the cerebrospinal fluid (CSF) of patients with Cuban Epidemic Neuropathic was reported previously (1). One CPE was typical of enterovirus, and the other had a light and slow progression (L-CPE). Of 125 CSF 5 specimens from patients, Coxsackie A9 (CA9) with typical CPE were isolated (4%) and 100 agents with the L-CPE (84%). From 30 CSF of a control group (surgical patients) 2 agents of L-CPE were isolated (6.6%) ( $p < 0.01$ ).

CSF that gave L-CPE in tissue culture were also inoculated by intracerebral and intraperitoneal routes to Balb/C suckling mice. In 67% of the samples death or sickness was observed. The clinical symptoms and the histopathological lesions in the sick animals were compatible with those produced by Coxsackie virus.

Sera from rabbits immunized with the L-CPE agents proved to have neutralizing antibodies to CA9, CB4 and to the homologous strains. There was no neutralization with the other 8 polio and Coxsackie B enteroviruses. A serum produced against Vero cells did not show neutralization to any of the proved viruses.

The percentage of sera with neutralizing antibodies (titer  $> 1:10$ ) titer and the geometric mean titer (GMT) to CA9 was higher in patients than in healthy people from work centers in Havana city and from people living in municipalities with low rates of neuropathy.

Also the GMT of neutralizing antibodies was higher to the CA9, CB2, CB3 and CB4 reference strains in the patients than in the control group.

The correlation between the immunologic profile of the patients and the antigenic characteristics of the L-CPE agents isolated makes us think that they alone or together with other factors have participated in the etiology of the sickness.

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Emerging Diseases.....They've been Emerging for a LONG,  
LONG Time. Let's Cool It.

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Human to human transmission of diseases is long standing and well recognized by lay and scientific communities. These disease outbreaks (epidemics?) result from infection by all types of microorganisms; bacteria, parasites, fungi and viruses. Diseases from unknown causes have occurred and will continue until identified (PRIONS?). A few diseases have been conquered, others kept under control, some have reasserted themselves and "new" diseases have "emerged": AIDS and other retrovirus diseases, Lyme disease, cat scratch, Hantavirus pulmonary syndrome, additional types of hepatitis, *E. coli* (O157:H7), *Cryptosporidium*, to name a few. Will new agents of disease be recognized ("emerge") in the future? As in answer to this question, a virus disease of horses, with human involvement, has recently "emerged" in Australia. Various factors contributing to continued disease emergence as well as technological advances assures us that "new" diseases will be forthcoming.

Of equal long standing, but apparently perceived differently, are zoonoses (epizootics) or transmission of disease from animal to human. Human infection resulting from contact with one or another animal or vector (rabies, yellow fever, herpes B, salmonella, dengue, etc.) have occurred over the years with relative frequency. While certain of these outbreaks have been devastating, and even newsworthy at the time, it would appear that none compare, with the possible exception of rabies, to the attention now given the novel virus disease in monkeys (Ebola-Reston). The Australian horse disease would probably receive little world attention if the monkey Ebola-Reston disease had not been so publicized. Animal to animal diseases constantly occur and apparently are of little public interest unless food or economic losses result.

Emerging diseases, regardless of the source, have always been of public health concern. The recent occurrence in New Mexico of human deaths resulting from severe pulmonary disease is a clear example. In this instance, an association was found with rodent (Deer mouse - *Peromyscus maniculatus*) infestation and evidence suggesting the etiologic role of a hantavirus. A number of rodent hantaviruses are recognized, some not infectious for humans, others producing hemorrhagic fever with renal involvement, the Poroglia strain is responsible for cases with high mortality. Human infection in New Mexico resulted from contact with deer mouse saliva, urine and possibly their droppings.

A report by a Committee of the Institute of Medicine of the National Academy of Sciences recently concluded that emerging pathogens are "not newly evolved," but already exist in nature. "The introduction of viruses into human populations...is often the result of human activities." Transfer of animal agents to humans frequently results from a breakdown in biocontainment.



Expanded human contact with nonhuman primates occurs from the use of these animals, not only in research (phylogenetic relationship to humans make them highly desirable), but following recognition that most viruses grew in monkey cells, in vitro. Virus growth in monkey cells made many human vaccines and virus diagnosis feasible. Use of monkeys permitted such R&D objectives as: drug testing, pathogenesis of disease studies, organ donors in xenotransplantation, etc. Nonhuman primates have unquestionably markedly improved human health and well being.

Utilization and subsequent drain upon monkey sources has placed a number of simian species on the endangered list. In certain instances depletion of animals has caused the countries of origin to either limit the number of animals exported or placed a total embargo on overseas marketing. These limitations and continued need of monkeys have resulted in the development of breeding colonies in order to satisfy the demand for desired species. Animals bred in captivity have benefited as a result of proper management and husbandry, including careful monitoring and recognition of disease. However, on a negative note, the close proximity of animals of the same species or to other species frequently resulted in disease outbreaks.

Employment of nonhuman primates and their association with humans has not been without problems although these did little to alter the continued use of monkeys and apes. B virus (*Herpesvirus simiae*) in *Macaca* spp. and its ability to produce human fatalities is well known. That wild chimpanzees were able to transmit hepatitis A virus to humans, while recognized, did not modify the continued use of these animals. Marburg virus carried to Europe by African green/vervet monkeys (*Cercopithecus aethiops*) resulted initially in 31 human cases with 7 deaths. Subsequent infections in Africa resulted in at least 2 human deaths with little modification in the continuing use of monkeys. Monkeypox virus was found in 1972 to cause a human disease clinically similar to smallpox. The recognized relationship of monkeypox virus to smallpox virus did not counter the use of monkeys. The origin of Marburg disease and monkeypox is still not clear, but definitely involved monkeys. A number of arboviruses associated with monkeys, including yellow fever, dengue, Chickungunya, Kyasanur forest virus, among others, involves humans with recognized fatalities. The role of monkeys in these diseases is similar to that in humans. That the monkey may be implicated in the virus growth cycle of certain arboviruses is appreciated. The number of animals lost as a result of all these diseases will never be known.

In addition to recognized zoonoses, existence of large numbers of simian viruses, counterparts of human viruses, have been described. Human infection resulting from these simian viruses is fortunately rare. Most notable is human infection with B virus as a result of a macaque monkey bite or scratch. B virus (*Herpesvirus simiae*) is the macaque herpesvirus counterpart to human herpes simplex and to SA 8, an African simian herpesvirus. In the course of producing poliovirus and adenovirus vaccines, it became apparent that these vaccines also carried an oncogenic simian virus SV 40,

a simian papovavirus. Millions of individuals received these vaccines without any observed ill effects.

Many of the human and simian viruses are antigenically related to each other: adenoviruses, herpesviruses and others. More recently it was recognized that there are simian retroviruses (SIV, SRV, STLV) related to human retroviruses, capable of producing simian AIDS (SAIDS) in various monkey species. The origin of the human AIDS virus with regard to nonhuman primates is still questioned, but a simian lineage appears to be likely. Other viruses exist in both human and nonhuman primates as only one serotype: measles, rabies and others. Nonhuman primates do develop disease as a result of infection with simian viruses. Outbreaks are probably more frequent than suspected or identified and are generally limited to a single species. The natural host may develop disease, but usually not as severe as in alien species.

When colony outbreaks occur, the greatest concern is possible inclusion of humans. The susceptibility of humans to B virus has been indicated; macaques, the natural host, may show evidence of infection, but rarely die. B virus infection of macaques is similar to human infection with herpes simplex. Experimental infection of several animal species with B virus, including monkeys other than macaques, does result in fatalities. SA 8, another herpesvirus antigenically related to B virus and herpes simplex, but indigenous to African simians, does cause widespread disease in baboons even though this species is considered the natural host. Latency, a frequent characteristic of infection, occurs with maintenance of the agent in the colony as a result of virus shedding.

Close association of animals in colonies had the disadvantage of permitting an enhanced spread of disease within the colony. Gastroenteritis is a major problem in colony management. SA 8 disease, including infant deaths and genital lesions, occurred in a baboon colony. SAIDS and retroperitoneal fibromatosis outbreaks were seen in macaque colonies. Episodes of simian hemorrhagic fever (SHF), without human involvement, have been recognized in macaque colonies in the United States, Soviet Union and Great Britain. More recently, SHF occurred in a macaque colony with animal losses approaching 100%. In addition to concern over animal disease and deaths, it was apparent that humans were not involved. However, that occurrence set the stage for another series of monkey deaths which in many respects simulated that due to SHF.

The MORBIDITY AND MORTALITY WEEKLY REPORT, Dec. 8, 1989/Vol. 38/No. 48 reported: "In late November 1989, Ebola virus was isolated from cynomolgus monkeys (*Macaca fascicularis*) imported into the United States from the Philippines via Amsterdam and New York. During quarantine in a primate facility in Virginia, numerous macaques died, some with findings consistent with SHF. The U.S. Army Medical Research Institute of Infectious Diseases tested 10 animals and reported: "from three, isolated SHF from tissues and serum; however, five other animals of the 10 tested were positive for Ebola virus." A second group of animals also with high mortality indicated Ebola virus antigen in serum and/or tissues from 7 monkeys. Liver material exhibited "particles with typical

filovirus morphology by electron microscopy and Ebola virus antigen by immunohistochemistry." Choice of test with known antigenic deficiencies, realization that Ebola is an African disease and these animals came from the Philippines, recognition of the potential public concern over such a finding, should have suggested caution in appending an "Ebola" connotation to the isolate despite the filovirus morphology and IFA antigenic relationship. Differences in pathogenicity among Ebola viruses are known.

A MMWR (Jan.19, 1990/39/ No.2) modified the association of the isolate to Ebola by restating the finding: "a filovirus closely related to Ebola virus" was isolated. After 21 days, 149 human contacts showed no evidence of illness or development of antibody. Some 3 months later (MMWR, April 27, 1990/39/No.16), of 2200 (cynomolgus, rhesus and African green monkeys) sera tested, approximately 10% were positive by indirect immunofluorescent antibody assay to "one of four filovirus test antigens (Ebola-Zaire, Ebola-Sudan, Filovirus-Reston, Marburg)." Of 178 human sera examined by the same procedure, 6 animal handlers "had serologic evidence of recent infection." Continued testing indicated the existence of seropositives among various groups of individuals including 449 persons randomly selected from adult primary-care outpatient populations. Seropositive individuals failed to develop symptoms or show any evidence of disease. "The background seropositivity rate for persons chosen randomly from an adult primary-care outpatient population remains unexplained. One possibility is antigenic crossreactivity between the known filoviruses and another, as yet undetermined, antigen."

A Lancet 335,502-505,1990 report was captioned "Preliminary report:isolation of Ebola virus from monkeys imported into the USA." Many investigators questioned the use of the term "Ebola virus" and choice of IFA for such testing. It was felt that the term "Ebola" and its connotation was (and proved to be) misleading. A critical review of the laboratory diagnosis of filovirus infection by one of the original investigators (Lab. Animal 20,34-46,1991) indicated "Because of the uncertainty surrounding the use of IFAT in unpaired serum specimens researchers continue to define more specific serological assays and determine the specificity and significance of IFAT findings." The use of the IFA when testing acute and convalescent sera may also be questioned in view of the need to obtain a titer equal to or greater than 1:16.

A more recent study (Lab Animal Science, in press) emphasizes the need for caution when interpreting IFA results. Thirty sera previously tested by ELISA, IFA and WB at CDC were also tested by DIAdot® (VRL registered name for dot immunobinding assay [DIA], a modified EIA using nitrocellulose sheets rather than plastic beads or plates as the matrix for absorbing the viral test antigens) at VRL:

TABLE 1: Comparative testing of 30 sera by ELISA, IFA, WB and DIAdot.

Serum #	ELISA Reston	ELISA Zaire	IFA Reston	IFA Zaire	WB	DIAdot
1	+	+	+	+	+	+
2	-	-	+	-	-	-
3	-	-	+	+	+	+
4	+	+	+	+	+	+
5	-	-	+	+	-	-
6	+	+	+	+	+	+
7	+	+	+	-	+	+
8	+	+	+	-	+	+
9	-	-	+	-	-	-
10	-	-	+	+	-	-

The remaining 20 sera were all negative.

The question might be asked whether or not the IFA was more sensitive than the other procedures? The distribution of IFA positive results would not support this suggestion. These results, as well as others (data not provided) indicate the similarity between WB and DIAdot testing.

This overview of recent nonhuman primate related zoonoses, indicates none differed significantly from the others and human involvement was always a consideration. In the Ebola-Reston outbreak, questions were asked by knowledgeable scientists with regard to the choice of the IFA as the preferred diagnostic test. The original reports indicating an Ebola virus isolation did nothing to alleviate the fear and apprehension generated in those individuals informed of their "positive" test results.

The staff of VRL has a long history in laboratory diagnosis of both human and nonhuman primate viral diseases. For many years as the WHO/PAHO "COLLABORATING CENTER FOR REFERENCE AND RESEARCH IN SIMIAN VIRUSES" as well as the NIH "VIRUS REFERENCE CENTER" gives credence to the expertise in virus diagnosis. The presence of a Biosafety Level Class 4 laboratory that meets the CDC/NIH standards provides the opportunity to work with those agents included at the BSC Levels 1-4. VRL is the only non-governmental laboratory with such a biocontainment capability. Approximately 100,000 specimens, of which 20,000 were simian, were received in 1994. Of 364 human sera tested for arbovirus disease antibody (SLE, EEE and WEE) none was positive. Two hundred and eighty two human sera were tested for rickettsial disease antibody (typhus group and Q fever). Ten sera were positive for the typhus group and 16 others were seropositive for Q fever. A final evaluation of the serological and isolation studies is in preparation.

A mechanism for protecting the health and well being of the population at large is expected. The right of an individual to report on such laboratory findings is uncontested. However, it is important that such reports be carefully critiqued. The Ebola-

Reston outbreak was initially one of a continuing series of epidemics or zoonoses occurring in simians with little, if any, difference from those preceeding it. Monkeys were dying from some unknown cause and at first thought to be SHF virus which was recovered from a number of dead animals. However, a filovirus isolated from dead and dying monkeys suggested Marburg and then "identified" and publicized as Ebola. This initial confusion on the precise identity, because of the publicity it received, makes this event different from similar recent isolations of: simian hemorrhagic fever virus from the same monkey species with no human infections, recovery of a hantavirus from fatal human cases and host deer mouse, rabies virus from humans and animals or a virus from horses in Australia. The Australian handling of the isolation of a virus related to human measles from horses and humans is an outstanding example of a proper stratagy to a previously unencountered disease. Perhaps the handling of the Ebola-Reston incident suggested this more appropriate approach?

"Emerging diseases." have been emerging since the beginning of recorded time and will continue to emerge. When the etiology of each new disease is correctly defined, it is incumbent upon the scientific community to prevent and control further inroads. As long as human to human, animal to human and human to animal contact continues, epidemics and zoonoses will persist. Correct future action will undoubtedly be undertaken by appropriate laborarories and the event properly recorded, "Caveat lector."



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Organización Panamericana de la Salud  
Oficina Sanitaria Panamericana, Oficina Regional de la  
Organización Mundial de la Salud

## **DENGUE Y DENGUE HEMORRAGICO EN LAS AMERICAS**

**CURSO INTERNACIONAL**  
**14 AL 25 DE AGOSTO DE 1995**  
**INSTITUTO "PEDRO KOURI" (IPK)**  
**CIUDAD DE LA HABANA, CUBA**

### **PATROCINADORES:**

**CENTRO COLABORADOR PARA ENFERMEDADES VIRICAS  
(IPK)**  
**YALE ARBOVIRUS RESEARCH UNIT (YARU)**  
**UNITE DES ARBOVIRUS & VIRUS DES FIEVRES  
HEMORRAGIQUES ( INSTITUT PASTEUR)**  
**OFICINA PANAMERICANA DE LA SALUD (OPS)**

### **OBJETIVO:**

- ♦ Ofrecer a médicos, biólogos, epidemiólogos e investigadores los conocimientos teóricos y prácticos relacionados con el diagnóstico, clínica, epidemiología y control del dengue y el dengue hemorrágico, tomando como base las Guías de la OPS/OMS para el control de esta enfermedad en la Región de las Americas.
- ♦ Los participantes tendrán la oportunidad de presentar la situación del dengue en sus respectivos países.

*El Curso constará de conferencias, trabajos prácticos y  
discusiones en grupos pequeños.*

## **CONFERENCIAS:**

- Situación del dengue y dengue hemorrágico en la región y en el mundo. (Dr. F. Pinheiro, E.U.A.).
- Características del agente etiológico de la enfermedad. (Dra. M. G. Guzmán, Cuba).
- Diagnóstico de laboratorio. (Dra. M. G. Guzmán, Cuba).
- Cuadro clínico y manejo terapéutico del paciente. (Dr. E. Martínez, Cuba).
- Epidemiología de la enfermedad. Factores de riesgo. (Dr. G. Kourí, Cuba).
- Epidemiología molecular del dengue. (Dr. V. Deubel, Francia).
- Etiopatogenia del dengue hemorrágico. (Dr. G. Kourí, Cuba).
- Aspectos de la respuesta inmune. (Dr. V. Deubel, Francia).
- Estado actual sobre el desarrollo de vacunas para el dengue. (Dr. R. Shope, E.U.A.).
- Aspectos entomológicos. (Lic. O. Fuentes, Cuba).
- Estrategias en el control del vector. (Dr. R. Figueredo, Cuba).
- Participación comunitaria en el control del dengue. (Dra. V. de Rojas, Cuba).
- Papel de la OPS/OMS en el control del dengue y el dengue hemorrágico. (Dr. F. Pinheiro, E.U.A.).

*Los trabajos prácticos se desarrollarán en los aspectos relacionados:*

- ★ Diagnóstico de laboratorio y caracterización molecular de cepas de dengue.
- ★ Entomología y control de vectores.
- ★ Participación comunitaria.

**Las solicitudes de matrícula deben ser enviadas via Fax antes del  
30 de Mayo de 1995 y deben incluir:**

- ★ Nombre y dirección postal, número de teléfono, telex y fax.
- ★ Un corto curriculum vitae que incluya experiencia investigativa y publicaciones en los últimos tres años.

**Costo del curso: \$1000.00 USD.**

(incluye matrícula, materiales docentes, coctel de bienvenida  
y cena de despedida).

**Alojamiento y alimentación: \$ 300.00 USD**

El IPK le brinda la posibilidad de alojamiento con un precio módico en el Hotel Mariposa, en habitación doble, con otro participante del Curso y la alimentación en el Instituto (desayuno, almuerzo y comida).

**Las solicitudes de matrícula y correspondencia deben ser dirigidas a:**

**Prof. María Guadalupe Guzmán**

**Instituto "Pedro Kourí"**

**Teléf./Fax: 53-7-336051 y 53-7-215957**

**Telex: CUIPK 511902 Y 512341**

**E. mail: ciipk % infomed. sld. Cu @ gn. apc. org.**

## QUOTES

Samuel Adams (not the second U.S. President): "If you love wealth better than liberty, the tranquility of servitude better than the animating contest of freedom, go home from us in peace. We ask not your counsel or arms. Crouch down and lick the hands which feed you. May your chains set lightly upon you, and may posterity forget that ye were our countrymen."

Moshe Waldoks: "There isn't a serious Jew today, whatever denomination or affiliation, who is not still traumatized that a third of our people were destroyed so viciously and in such a short period of time. It's like the amputee who still feels the phantom pain. The leg isn't there, but the pain is always there."

Gaius Petronius Arbiter (Pro-Consul at Bithynia in the time of Nero, A.D. 65): "We trained hard, but it seemed every time we were beginning to form teams we would be reorganized. I was to learn later in life that we tend to meet every situation by reorganizing, and a wonderful method it can be for creating the illusion of progress while producing confusion, inefficiency, and demoralization."

Howard Ruff: "It was not raining when Noah built the ark."

Fred Allen: "I have just returned from Boston. It is the only thing to do if you find yourself there."

Abraham Lincoln: "It has been my experience that folks who have no vices have very few virtues."

David Frost: "Television enables you to be entertained in your home by people you wouldn't have in your home."

Unknown: "Experience teaches you to recognize a mistake when you've made it again."

Steve McQueen: "I'd rather wake up in the middle of nowhere than in any city on earth."

George Burns: "Too bad all the people who know how to run the country are busy driving taxis and cutting hair."

TIME Magazine: "It was about as controversial as shouting 'Theatre!' in a crowded fire."

George Porter: "There are only two sorts of research; research which is applied and research which is not yet applied." (courtesy of John Aaskov)

Robin Cook: "When there are too many variables, chance become a major factor."

Sydney J. Harris: "A cynic is not merely one who reads bitter lessons from the past, he is one who is prematurely disappointed in the future."

Thomas Szasz: "Happiness is an imaginary condition formerly often attributed by the living to the dead, now usually attributed by adults to children, and by children to adults."

Ann Landers: "Opportunities are usually disguised as hard work, so many people don't recognize them."

George Washington: "Government is not reason, it is not eloquence, it is force. Like fire, it is a dangerous servant and a fearful master; never for a moment should it be left to irresponsible action. Once government is unloosened from the chains of the Constitution, it cannot be controlled."

Mao Tse-Tung: "The power of government comes out of the barrel of a gun."

J.H. Comroe, Jr.: "Serendipity is looking in a haystack for a needle and finding the farmer's daughter."

Chief Seattle: "This we know; the earth does not belong to man; man belongs to the earth. Man did not weave the web of life. He is merely a strand of it. Whatever he does to the earth he does to himself."

Harry Lime (in "The Third Man" by Graham Greene): "In Italy for 30 years under the Borgias they had warfare, terror, murder, and bloodshed but they produced Michelangelo, Da Vinci, and the Renaissance. In Switzerland they had brotherly love and they had 500 years of democracy and peace and what did that produce? The cuckoo clock." (Harry Lime was not a nice guy.)

Thomas Jefferson: "I tremble for my country when I reflect that God is just."

Mort Sahl (on being asked to say something funny): "John Foster Dulles."

Paul Westhead: "If Shakespeare had been in pro basketball he never would have had time to write his soliloquies. He would always have been on a plane between Phoenix and Kansas City."

Alex Levine: "Only Irish Coffee provides, in a single glass, all four essential food groups: alcohol, caffeine, sugar and fat."

Sandra Carey: "Never mistake knowledge for wisdom. One helps you make a living; the other helps you make a life."

Marshall McLuhan: "I wouldn't have seen it if I hadn't believed it."

Anais Nin: "We don't see things as they are, we see them as we are."

Doug Larson: "The reason people blame things on previous generations is that there's only one other choice."

Oscar Wilde: "The difference between journalism and literature is that journalism is unreadable and literature is not read."

Anonymous: "Life does not begin at the moment of conception or at the moment of birth. Life begins when the children leave home."

José Simon: "In Mexico we have a word for sushi: bait."

Joan Rivers: "It's been so long since I made love I can't even remember who gets tied up."

Andy Rooney: "Phyllis Schlafly speaks for all American women who oppose equal rights for themselves."

Lord Acton: "Power tends to corrupt and absolute power corrupts absolutely."

Joseph Addison: "We are always doing something for Posterity, but I would fain see Posterity do something for us."

Kingsley Amis: "More will mean worse."

Voltaire: "The husband who decides to surprise his wife may be very much surprised himself."

Fisher Ames: "A monarchy is a merchantman which sails well, but will sometimes strike on a rock, and go to the bottom; a republic is a raft which will never sink, but then your feet are always in the water."

Tom Paine: "It is necessary to the happiness of man that he be mentally faithful to himself. Infidelity does not consist in believing, or in disbelieving, it consists in professing to believe what one does not believe."

Philip Goodheart: "Far more university graduates are becoming criminals every year than are becoming policemen."

Oliver Wendell Holmes: "Insanity often is the logic of an accurate mind overtaxed."

Arthur Conan Doyle (Sherlock Holmes): "I should have more faith", he said; "I ought to know by this time that when a fact appears opposed to a long train of deductions it invariably proves to be capable of bearing some other interpretation."

William James: "There is no worse lie than a truth misunderstood by those who hear it."

George Orwell: "Saints should always be judged guilty until they are proved innocent."

Rudyard Kipling: "Take my word for it, the silliest woman can manage a clever man; but it needs a very clever woman to manage a fool."

Garrison Keillor: "Where I come from, when a Catholic marries a Lutheran it is considered the first step on the road to Minneapolis."

Norbert Wiener: "A conscience which has been bought once will be bought twice."



Bob Cosby: "I don't know the way to succeed, but the surest way to fail is to try to please everybody."

Edward Abbey: "... the leisurely economy of Nature..."

Duc de la Rochefoucauld: "In jealousy there is more of self-love than love."

Edgar Z. Friedenberg: "The examined life has always been pretty well confined to a privileged class."

Author unidentified: "A neurotic is a man who builds castles in the air. A psychotic is the man who lives in it. And a psychiatrist is the man who collects the rent."

John Steinbeck (Cup of Gold): "But all these thoughts of his were shocked from his brain when Sir Edward appeared. It was his father as he remembered him, and yet never his father. Old Robert would never have had a mustache like an eyelash, and nothing in Robert's life could have made him pinch his lips together until they were as thin as the mustache. These two might have been born alike as beans, but each had created his own mouth."

Sigmund Freud: "The first human being who hurled an insult instead of a stone was the founder of civilization."

Finley Peter Dunne: "A fanatic is a man who does what he thinks the Lord would do if He knew the facts of the case."

E.B. White: "Analyzing humor is like dissecting a frog. Few people are interested and the frog dies of it."

Woody Allen: "I tended to place my wife under a pedestal."

G.K. Chesterton: "The word **good** has many meanings. For example, if a man were to shoot his grandmother at a range of five hundred yards, I should call him a good shot, but not necessarily a good man."

Carl Reiner: "A lot of people like snow. I find it to be an unnecessary freezing of water."

Karen Elizabeth Gordon: "Either I've been missing something or nothing has been going on."

Artemus Ward: "Why is this thus? What is the reason for this thusness?"

Susan Sontag: "Any important disease whose causality is murky, and for which treatment is ineffectual, tends to be awash in significance."

Benjamin Franklin: "There are more old drunks than old doctors."

Albert Einstein (when asked what he thought was the most powerful thing in the world): "Compound interest."