ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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Harald Norlin Johnson, M.D. (1907-1996)

Harald Norlin Johnson died August 28, 1996, in a hospital near his home in Scituate, Massachusetts, following months of failing health. We have lost a dedicated scientist, researcher and arbovirologist and a fine human being. He brought special skills and distinction to the study of diseases in nature.

Harald was born March 31, 1907, in Loomis, Nebraska, a descendent of Swedish immigrant grandparents who settled in Nebraska in the 1880's. Their traditions of hard work, practical skills, self-sufficiency, close family ties, love of learning, culture and music influenced him profoundly. He was raised with five brothers and a sister. He was schooled in Nebraska until 1924, the McPhail Conservatory of Music, Minneapolis, 1924-25 (piano and pipe organ), and the University of Minnesota, Minneapolis, and the University of Nebraska, where he was awarded the B.Sc. in 1930 and the M.A. (Anatomy) in 1932. During his Masters studies he was one of the first to develop the newly discovered lymphocyte and tissue culture methods. The M.D. from the University of Nebraska School of Medicine followed in 1933. In 1936 he married Frances Maxfield Alexander, a nurse and also an accomplished pianist, who was a lifelong devoted partner and support both at home and in his extensive professional travels and research work. They raised four children to whom they passed their love of science and music; Marion Noble, R.N., Susan Robison, John Johnson, Ph.D., and Michael Johnson, M.D. There were six grandchildren to enjoy before he died.

Following his medical degree, he undertook a series of rigorous training assignments, including internship and residency at Harvard's Peter Bent Brigham Hospital; House Officer in Medicine; Assistant Resident Physician, Contagious Service, Cleveland City Hospital; House Officer in Pathology at the Brigham; Resident Pathologist, Boston Children's Hospital; and Resident Physician at the Brigham again. In 1938, a Fellowship at the Rockefeller Foundation Laboratories, Harvard School of Public Health, and introduction to the intriguing new field of virology in the Yellow Fever Laboratory steered him towards the career for which we know him best. The Rockefeller Foundation was his niche the rest of his career, which involved close association with many of the famous and pioneering scientists of the time, and wide travel throughout the world in search of disease sources and cycles. He learned Russian, French, Spanish, and Hindi, in addition to his native English and Swedish, in order to help in his travels.

Called to do important studies of rabies in Alabama from 1938 to 1945, he demonstrated the value of Semple-type killed rabies vaccine, developed a live, attenuated vaccine (LEP Flury) for dogs, and pioneered the methods to eliminate dog rabies in the U.S. He was soon recognized as a world expert and author on laboratory methods for rabies, and was on the World Health Organization Expert Panel on Rabies from 1950 to 1986. During a field expedition to Mexico in 1944 to study the phenomenon of paralytic rabies in cattle ("derriengue"), he showed that vampire bats were the source of the disease. During this study, he was bitten by a vampire bat, and despite previous immunizations, five months later he developed a nearly fatal, ascending paralytic illness, most likely a form of non-fatal rabies, although the laboratory methods available at the time could not prove this. After 5 months of intensive care at Warm Springs, GA, he gradually recovered, involving a strenous battle, to relearn to walk, write, play piano, hold a syringe and inoculating needle, and regain control of his body – a victory achieved by the intense focus of mind and will which characterized his entire life.

In 1949, Harald was able to return to the Rockefeller Foundation where he dove into the

important studies on malaria and the new field of arthropod-borne viruses just being developed. In 1951, the Foundation sent him, his wife and four young children to Poona, India as Scientific Director of the new Virus Research Centre, a joint project of the Rockefeller Foundation and the Indian Council of Medical Research. After successfully training staff and launching significant research studies there, he and his family returned to the U.S. and he was assigned in 1954 to collaborate with the California State Department of Health's Viral and Rickettsial Diseases Laboratory to develop an arbovirus study project, also cooperating with the arbovirus program of the School of Public Health, University of California, Berkeley. Though formally retiring from the Foundation when its support of the project ended in 1972, he remained "emeritus" until his death, continuing to lecture, mentor students and staff, and maintain an active laboratory and field study base in the Viral and Rickettsial Diseases Laboratory. He spent part of each year at his family home in Scituate, studying field aspects of arboviruses. An extensive autobiographical oral history was completed in 1991 by the Bancroft Library, University of California, Berkeley. He published numerous reports in the scientific literature, but preferred to emphasize lectures, teaching by example and personal contact, and training staff in his laboratory rather than by extensive publication. He was little concerned with status in science, rather emphasizing the message, not himself as messenger.

Harald's skills in anticipating where viruses might occur in nature, and how to detect them, resulted in his personal discovery of many new ones: Hart Park, Huacho (with Jordi Casals), Kern Canyon, Klamath, Modoc, Mono Lake, Punta Salinas, and Rio Bravo; and he assisted with the discovery of Farallon and Upolu viruses and others as well. He made important contributions to the understanding of Colorado tick fever, Powassan, western equine encephalomyelitis, St. Louis encephalitis, Turlock, and many more. He was noted and widely admired for his remarkable memory and his meticulous, detailed laboratory and field research records documented in his distinctive handwriting, and he could promptly locate any item about his wealth of experience and knowledge recorded over the decades. He emphasized the importance of studying nature, birds and mammals, ticks and other vectors, the need for field work, techniques for cell culture from organ suspensions of suspected virus hosts, the necessity for careful laboratory records and techniques to avoid cross-contamination and misinterpretation of results; the need to use non-neuroadapted viruses for pathogenesis studies so as to maintain the true, natural tropisms of agents. He was fascinated with how viruses got "in and out" of hosts, and stressed the need to discover the true, natural hosts of viruses, particularly small mammals, rather than focussing on the aberrant, epidemic hosts, so as to understand the true basic cycles and ultimately the best way to control diseases. He was intrgued by the natural variation of viruses, the process of natural selection, and how hybridization of viruses might confound and mislead scientific research.

Harald had a rich and warm relationship with many people in science, and constantly urged them to follow up on his insights and findings. His interests involved not only science, arboviruses, and music, his main passions, but history, world events, anthropology, religion and his own strong religious faith, bird migrations, diet and health, astronomy, and many more. He picked hard things to do, disciplined and challenged himself, and did them well, despite – or perhaps in part because of – significant adversity. The day before he died, he was asking about colleagues and spoke urgently about studies he hoped would be carried further: engaged, concerned, dedicated to the last.

R.W. Emmons, M.D. 10/21/96

INTERNATIONAL COMMITTEE ON TAXONOMY OF VIRUSES

VIROLOGY DIVISION
INTERNATIONAL UNION OF MICROBIOLOGICAL SOCIETIES

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Strasbourg, September 18, 1996

Dear Dr Casals,

It is with great pleasure that I inform you that at its annual meeting in Jerusalem last month, the Executive Committee of the ICTV, unanimously elected you as a Life Member of ICTV. The Committee wanted in this manner to pay tribute to the outstanding contributions you have made over many years to the field of viral taxonomy and classification.

I would like to add my personal congratulations and thanks for your exceptional input in this important area of Virology.

M.H.V. Van Regenmortel
President of ICTV

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F.A.S.

FEDERATION OF AMERICAN SCIENTISTS
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Animal Health/Emerging Animal Diseases (AHEAD)

AHEAD is the animal disease component of the FAS project to promote the establishment of a Program for Monitoring Emerging Diseases. The Federation of American Scientists, founded in 1945, is a center of public policy research, analysis and education on issues of global security.

AHEAD was begun in late 1995 to increase veterinary participation in the ProMED Mail network; to assess, where possible, the role played by environmental changes, population shifts and climatic anomalies on reported animal disease outbreaks; and to assist ProMED's Working Group on Animal and Zoonotic Diseases, a distinguished panel of experts headed by the World Health Organization's chief of veterinary health, Dr. Francois Meslin.

To date AHEAD has added almost 300 subscribers to the ProMED Mail network, raising the total number of participating veterinarians worldwide to more than 750. Through cross-posting of reports to other Internet lists, particularly WildlifeHealth, WildlifeRehabilitation and EpiVet (veterinary epidemiology), an additional 1000 veterinarians are reached. In an effort to raise public awareness of the interplay between the diseases of wildlife and domesticated animals and human diseases, AHEAD is constructing its own sub-section in the ProMED Web Site:

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AHEAD is eager to expand its disease reporting base, both in the number of participants and countries represented, to increase contact with field study programs worldwide, and to build a broad-based Internet repository of information and electronic links. It welcomes the suggestions and participation of all interested colleagues. For guidance in subscribing to ProMED AHEAD, visit our Web Site or go to the second page of the attached ProMED Fact Sheet.

March 1996

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Facsimile: (202) 675-1010   E-Mail: fas@fas.org

Editor's comments

The featured reports in this issue [Rescue of infectious bunyaviruses entirely from cloned cDNAs (A. Bridgen and R.M. Elliott); Summary of known and proposed hantaviruses [Bunyaviridae: *Hantavirus*] (B. Hjelle); Summary of known and proposed Arenaviridae (J.N. Mills, J.E. Childs, M.D. Bowen, T.G. Ksiazek, S.T. Nichol, C.J. Peters); and El Niño/Southern oscillation (ENSO) and arboviral disease surveillance in Australia (C. Guest)] contain some remarkable findings, information, and visions. I am most appreciative of the efforts the authors put in get these reports to the readers of the Arbovirus Information Exchange. In the aggregate they exemplify the vitality and wide interests of our subscribers. The subjects also demonstrate just how far classical arbovirology has moved from center stage. Although arboviruses continue to plague humankind, and anyone who does not see them as a significant medical problem must simply not want to see, there is no doubt that other viruses are of far greater current importance and fascination and likely will continue to be important and to fascinate for some time to come. Most of the people involved in studies of rodent-borne viruses obtained their training and primary field experiences during their days as arbovirologists.

On a completely different note -- As you might know, I left CDC more than four years ago. At that time I could not have predicted that in 1996 I would be doing field work on hantaviruses and have all sorts of other interesting involvements. I am busier now than I have ever been and the crush of responsibilities makes obsolete any reasonable definition of "retired".

Problem is, I do not have time to continue some of the tasks for which I have been volunteering. It is not, of course, that I am no longer interested in these worthwhile activities but one must match personal priorities with time available to do them. I have been editing the Arbovirus Information Exchange for more than seven years. It is not only time for me to lighten my load but time for someone with a different view to take on this pleasant and important task. Therefore, I have requested that the Executive Committee of the American Committee on Arthropod-borne Viruses (ACAV) find a replacement for me as Editor of the Arbovirus Information Exchange. They are working on it and I believe they will soon be successful.

This has been less a job than an effort of love. The marvelous people with whom I have communicated all over the world, the insights gained, discussions of arbovirology held, and jokes exchanged have made this position one I enjoyed. I suspect that whoever succeeds me will find it the same. Sure it takes some work, but it is sporadic and the end product is worthwhile. The Arbovirus Information Exchange has been in continuous publication for more than three decades and is the most conspicuous of ACAV's activities. It is the glue that holds together this great community of people.

I trust that those of you with whom I have been in contact will remain in contact with me. Until a new Editor is chosen, I will hold down the fort. Please give my successor as much support as you have given me.

With kindest personal regards to you all,

Charlie Calisher   vi

Previous Editors of the <u>Arbovirus</u> <u>Information</u> <u>Exchange</u>

Telford H. Work	1960-1972
Roy W. Chamberlain	1972-1981
W. Adrian Chappell	1981-1984
Barry R. Miller	1984-1989

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.

Until you hear otherwise, please send reports to:
Charles H. Calisher, Ph.D.
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You also may send reports by **e-mail**: ccalisher@vines.colostate.edu

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

C.H. Calisher

NEXT ISSUE

It is likely that the next issue will be mailed 1 June, 1997 (probable deadline for submissions: 15 May, 1997). There is nothing that requires you to wait until the last minute. If you have something to communicate in January, February, March or April please send it. Some people have been doing that and I can assure you it has saved the Editor considerable effort; thanks to them. Also, there is nothing that prevents you from submitting a report to every issue. There are no page charges either but, then again, this is not a publication.

RESCUE OF INFECTIOUS BUNYAVIRUSES ENTIRELY FROM CLONED cDNAS

Anne Bridgen and Richard M. Elliott,
Institute of Virology, University of Glasgow, Church Street, Glasgow G11 5JR, Scotland.

One of the major goals of our bunyavirus research has been to develop methodology to recover infectious bunyaviruses from cloned cDNAs, thus allowing full application of recombinant DNA techniques to manipulate the genomes of these viruses; this goal has now been achieved. Briefly, BHK cells were infected with a recombinant vaccinia virus, vTF7-3 (which produces bacteriophage T7 RNA polymerase), and then transfected with three plasmids containing the Bunyamwera (BUN) bunyavirus open reading frames under T7 promoter control, thus expressing all the BUN proteins. Three hours later the cells were transfected with three 'transcription' plasmids; these contain exact cDNA copies of the BUN L, M and S genome segments flanked by T7 promoter and hepatitis delta ribozyme sequences. Transcription by T7 RNA polymerase and self cleavage by the ribozyme in the cell yielded full-length viral-complementary RNA segments which were assembled into nucleocapsids, replicated into genomic sense RNA and packaged into virions. 'Transfectant' bunyavirus in lysates of these cells was amplified by passage in C6/36 mosquito cells (which do not permit replication of the helper vaccinia virus) and then bunyavirus plaques were isolated on BHK cell monolayers. The L and S segment cDNAs in the transcription plasmids were tagged with silent mutations, which created new restriction enzyme sites, and these were shown to be present in the genome RNAs of transfectant viruses by RT-PCR. The transfectant virus grew to the same titre and with the same kinetics as authentic BUN virus. Further, we recovered a reassortant virus containing the L and M segments of BUN and the S segment derived from the related Maguari (MAG) bunyavirus using appropriate transcription plasmids. Interestingly a virus with this genotype (BUN/BUN/MAG) was not obtained from a coinfection of cells with BUN and MAG viruses (1) although it was obtained subsequently as a result of co-infection of other reassortant viruses containing BUN and MAG genome segments. Hence our rescue protocol allows the production of a reassortant virus not readily obtained through conventional virological procedures.

This achievement opens the way for a detailed molecular characterization of the replication and transcription pathways of bunyaviruses and for fine analysis of the viral gene products through genetic manipulation, as well as for future studies on biological aspects such as tissue tropism, virulence, and vector competence. It may also be possible to design modified bunyaviruses with potential as vaccine strains. These experiments should provide the impetus to devise similar rescue strategies for other genera within the *Bunyaviridae* and also other families of segmented genome negative-strand RNA viruses such as arenaviruses and orthomyxoviruses.

1. Pringle, C.R. (1996) in *The Bunyaviridae*, ed Elliott, R.M. (Plenum Press, New York) pp189-226.

1

Summary of Known and Proposed Hantaviruses (Bunyaviridae: *Hantavirus*)

Before the 1993 outbreak of hantavirus pulmonary syndrome (HPS) in the United States, the genus *Hantavirus* (family Bunyaviridae) consisted of the following distinct viral species: Hantaan, Puumala, Seoul, and Prospect Hill. Dobrava-Belgrade virus was under investigation as a possible fifth serotype. One limiting factor in the recognition of new members of the genus has been the relative difficulty of their primary in vitro cultivation.

The HPS outbreak investigation made extensive use of genetic methods, methods that led to the recognition of the etiologic agent, Sin Nombre virus (SNV), well before SNV was propagated in cell cultures. The successes of RT-PCR in the rapid detection and genetic characterization of SNV, as well as the development of diagnostic antigens through recombinant DNA expression of SNV genes, has encouraged the widespread adaptation of these methods to the study of other hantaviruses. As a consequence, the pace of discovery of new hantaviruses has greatly accelerated since 1993. A number of new hantaviruses are known only genetically, while others have been subjected to serologic comparisons with previous serotypes using either tissue culture-adapted isolates or recombinant DNA methodologies.

Each hantavirus appears to be closely adapted to a single predominant rodent host. This observation suggests an ancient relationship between virus and host. The table below lists the hantaviruses, their disease associations, their predominant hosts, and the hosts' geographic ranges. In most instances the virus has not been proved to be present throughout the host's entire habitation range. For many hantaviruses there are well documented examples of a rodent other than that listed as the predominant host playing an important, even predominant, carrier role. For example, *Microtus rossiaemeridionalis* may play a role in maintenance of Tula virus in some settings, and *Peromyscus leucopus* and *P. boylii* can be important reservoirs for SNV. There are also many examples of occasional "spillover" of viruses into hosts separated from the predominant carrier at the generic, familial or higher level, but these events are considered by some to be of little epidemiologic or evolutionary importance.

All hantaviruses except for Thottapalayam (TPM) have been isolated or detected in murid rodents. Since TPM virus was isolated on only a single occasion from a shrew (Order Insectivora) the host assignment must be considered tentative.

(Brian Hjelle, M.D., University of New Mexico, Albuquerque, New Mexico)

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Virus	Abbreviation	Host Type	Original isolation	Distribution of Host	Disease
Hantaan	HTN	*Apodemus agrarius*	Korea	C Europe south to Thrace, Caucasus, and Tien Shan Mtns; Amur River through Korea to E Xizang and E Yunnan, W Sichuan, Fujiau, and Taiwan (China)	HFRS[a]
Seoul	SEO	*Rattus norvegicus, R. rattus*	Korea	Worldwide; commensal rat hosts	HFRS
Dobrava/Belgrade	DOB	*A. flavicollis*	Slovenia	fmr Yugoslavia, England & Wales, from NW Spain, France,, S Scandinavia through European Russia to Urals, S Italy, the Balkans, Syria, Lebanon, and Israel	HFRS
Puumala	PUU	*Clethrionomys glareolus*	Finland	W Palaearctic from France and Scandinavia to Lake Baikal, south to N Spain, N Italy, Balkans, W Turkey, N Kazakhstan, Altai & Sayan Mtns; Britain & SW Ireland	HFRS/NE[b]

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Sin Nombre	SN	*Peromyscus maniculatus*	New Mexico	Alaska Panhandle across N Canada, south through most of continental USA, excluding SE and E seaboard, to southernmost Baja California Sur and to NC Oaxaca, Mexico	HPS[c]
Black Creek Canal	BCC	*Sigmodon hispidus*	Florida	SE USA, from S Nebraska to C Virginia south to SE Arizona and peninsular Florida; interior and E Mexico through Middle America to C Panama; in South America to N Colombia and N Venezuela	HPS
Muleshoe	MULE	*S. hispidus*[d]	west Texas (sequence only)	See Black Creek Canal	unknown
New York	NY	*P. leucopus*	Long Island, New York	C and E USA to S Alberta and S Ontario, Quebec and Nova Scotia, Canada; to N Durango and along Caribbean coast to Isthmus of Tehuantepec and Yucatan Peninsula, Mexico	HPS
Bayou	BAY	*Oryzomys palustris*	Louisiana,	SE Kansas to E Texas, eastwards to S New Jersey and peninsular Florida	HPS

Thottapalayam	TPM	*Suncus murinus*	India	Afghanistan, Pakistan, India, Sri Lanka, Nepal, Bhutan, Burma, China, Taiwan, Japan, Indomalayan Region	unknown
Tula	TUL	*Microtus arvalis*	Russia	Throughout Europe to Black Sea and NE to Kirov reg., Russia	unknown
Thai	THAI	*Bandicota indica*	Thailand	Sri Lanka, peninsular India to Nepal, NE India, Burma, S China, Taiwan, Thailand, Laos, Vietnam	unknown
Prospect Hill	PH	*M. pennsylvanicus*	Maryland	C Alaska to Labrador, incl. Newfoundland and Prince Edwards Island, Canada; Rocky Mountains to N New Mexico, in Great Plains to N Kansas, and in Appalachians to N Georgia, USA	unknown
Bloodland Lake	BLLL	*M. ochrogaster* (sequence only)	Missouri	N & C Great Plains, EC Alberta to S Manitoba, Canada, S to N Oklahoma & Arkansas, E to C Tennessee & W West Virginia, USA; relic populations elsewhere in US & Mexico	unknown

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Isla Vista	ISLA	*M. californicus*	California (sequence only)	Pacific coast, from SW Oregon through California, USA, to N Baja California, Mexico	unknown
El Moro Canyon	ELMC	*Reithrodontomys megalotis*	California (sequence only)	SC British Columbia & SE Alberta, Canada; W and NC USA,S to N Baja California & interior Mexico to central Oaxaca	unknown
Khabarovsk	KBR	*M. fortis*	Russia	Transbaikalia Amur region; E. China	unknown
Rio Segundo	RIOS	*R. mexicana*	Costa Rica (sequence only)	S Tamaulipas & WC Michoacan, Mexico, S through Middle American highlands to W Panama; Andes of W Colombia & N Ecuador	unknown
Rio Mamore	RM	*Oligoryzomys microtus*	Bolivia	C Brazil south of Rios Solimes-Amazon, contiguous lowlands of Peru, Bolivia, Paraguay, and Argentina	unknown
Andes		unknown (human derived)	Patagonia, Argentina (sequence only)		HPS

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Topografov	TOP	*Lemmus sibiricus*	Siberia	Palearctic, from White Sea, W Russia, to Chukotski Peninsula, NE Siberia, and Kamchatka; Nearctic, from W Alaska E to Baffin Is. & Hudson Bay, S in Rocky Mtns to C British Columbia, Canada	unknown

[a] HFRS = Hemorrhagic Fever with Renal Syndrome

[b] NE = Nephropathia Epidemica

[c] HPS = Hantavirus Pulmonary Syndrome

[d] Taxonomic status of *S. hispidus* throughout its range is uncertain. Host for MULEV may be another species of *Sigmodon.*

Summary of Known and Proposed Viruses of the family Arenaviridae

A hallmark of viruses of the family the Arenaviridae is that they are generally associated with a single species of host of the rodent family Muridae, in which they establish chronic infections involving the shedding of infectious virus in urine and saliva. Human infection is thought to most frequently result from the inhalation of aerosols of secreta or excreta from infected hosts. The prototype meber of the genus *Arenavirus*, lymphocytic choriomeningitis virus (LCMV), was isolated in 1933 and subsequently shown to be a cause of aseptic meningitis in humans and of commonly occurring, asymptomatic infections in laboratory mice (*Mus musculus*). The other arenaviruses that are human pathogens are responsible for severe hemorrhagic fever in humans on two continents. These include Lassa fever in West Africa and certain South American hemorrhagic fevers. In addition, a large number of arenaviruses are not associated with human disease. At present, the total number of described arenaviruses is 19.

The arenaviruses are divided into two complexes (see accompanying table), the Old-World LCMV/Lassa complex, associated with the murid subfamily Murinae, and the New-World Tacaribe Complex associated with the murid subfamily Sigmodontinae. The association of arenaviruses with both groups of murids suggests that an ancestral arenavirus was associated with the ancestor of both murid rodent subfamilies before the two groups diverged over 20 million years ago. This provides for the potential existence of a coevolved virus/host association for each species of murid rodent. Doubtless, many more arenaviruses remain to be discovered. Many of the known arenaviruses have come to light in the aftermath of outbreaks of disease where human activities have resulted in conditions creating close contact with a reservoir host. We hope that future novel arenaviruses will be identified through active, systematic searches involving collaboration between virologists and mammalogists.

The following table was prepared through the efforts of J.N. Mills, J.E. Childs, M.D. Bowen, T.G. Ksiazek, S.T. Nichol, and C.J. Peters, Special Pathogens Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Currently recognized arenaviruses

Virus	Abbr.	Host type[a]	Original isolation	Distribution of Host[a]	Disease
A. LCMV/Lassa Complex					
Lymphocytic choriomeningitis	LCM	*Mus musculus*	Missouri, USA	Most of world, in association with humans	Lymphocytic choriomeningitis
Lassa	LAS	*Mastomys* sp.[b]	Nigeria	Africa south of the Sahara[b]	Lassa fever
Ippy	IPPY	*Arvicanthus* sp.[b]?	Central African Republic	S Mauritiania, Senegal, Gambia, east through Sierra Leone, Ivory Coast, Ghana, Burkina Faso, Togo, Benin, Nigeria, S Niger, S Chad, Sudan, Egypt, to Ethiopia; south through NE Zaire, Uganda, S Burundi, Kenya, S Somalia and Tanzania, into E Zambia	None recognized
Mopeia	MOP	*Mastomys natalensis*	Mozambique	S Africa as far north as Angola, S Zaire, and Tanzania; Senegal?	None recognized
Mobala	MOB	*Praomys* sp.[b]	Central African Republic	C Nigeria through Cameroon and Central African Republic, S. Sudan, Zaire, N Angola, Uganda, Rwanda, Kenya, south through E Tanzania to N and E Zambia	None recognized

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B. Tacaribe Complex

Junín	JUN	*Calomys musculinus*	Argentina	N and C Argentina, E Paraguay	Argentine hemorrhagic fever (AHF)
Machupo	MAC	*Calomys callosus*	Bolivia	N Argentina, E Bolivia, W Paraguay, WC to EC Brazil	Bolivian hemorrhagic fever (BHF)
Guanarito	GUA	*Zygodontomys brevicauda*	Venezuela	Savannas from SE Costa Rica through Panama, Colombia, Venezuela, Guianas, to Brazil north of the Amazon River; including Trinidad and Tobago and smaller continental shelf islands adjacent Panama and Venezuela	Venezuelan hemorrhagic fever (VHF)
Sabiá	SAB	Unknown	Brazil	Unknown	Not named
Amaparí	AMA	*Neacomys guianae*	Brazil	Guianas, S Venezuela, N Brazil	None recognized
Flexal	FLE	*Oryzomys* sp.?[c]	Brazil	Unknown	None recognized[d]
Latino	LAT	*Calomys callosus*	Bolivia	N Argentina, E Bolivia, W Paraguay, WC to EC Brazil	None recognized
Oliveros	OLV	*Bolomys obscurus*	Argentina	S Uruguay and EC Argentina	None recognized
Paraná	PAR	*Oryzomys buccinatus*?[c]	Paraguay	E Paraguay and NE Argentina	None recognized

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Pichindé	PIC	*Oryzomys albigularis*	Colombia	N and W Venezuela, E Panama, Andes of Colombia and Ecuador to N Peru	None recognized
Pirital	PIR	*Sigmodon alstoni*	Venezuela	NE Colombia, N and E Venezuela, Guyana, Surinam, and N Brazil	None recognized
Tacaribe	TCR	*Artibeus* (bats)?[e]	Trinidad	Unknown	None recognized[d]
Tamiami	TAM	*Sigmodon hispidus*	Florida, USA	SE USA from S Nebraska to C Virginia, south to SE Arizona Florida; anterior and E Mexico through middle America to C Panama; in S America to N Colombia and N Venezuela	None recognized
Whitewater Arroyo	WWA	*Neotoma albigula*	New Mexico, USA	SE California to S Colorado to W Texas, USA, south to Michoacan and W Hidalgo, Mexico	None recognized

[a] Rodent Taxonomy and distribution follows D.E. Wilson and D.M. Reeder, 1993, Mammal Species of the World, Smithsonian Institution Press, Washington, D.C.

[b] Taxonomy of these genera and distribution of species are poorly known. Mastomys is currently recognized as a genus of at least 8 sibling species. Although *M. natalensis* was originally described as the reservoir of LASV in West Africa, recent taxonomic studies describe those species implicated in the maintenance of LASV in West Africa as *M. erythroleucus* (2N=38) and *M. hildebrandtii* (=*M. huberti*; 2N=32, FN=44) which are distinct from *M. natalensis* (2N=32, FN=54).

[c] Host not well established

[d] Laboratory infections have been documented for Flexal and Tacaribe viruses

[e] Original report lists 19 isolates from bats of the genus *Artibeus*; subsequent attempts to isolate virus from *Artibeus* bats have been unsuccessful

El Nino/Southern Oscillation (ENSO) and arboviral disease surveillance in Australia

An outline for a research project appears below. I would be pleased to hear from any readers of the Arbovirus Information Exchange, particularly those
- undertaking climate-arbovirus research
- using ENSO as a variable for climatological, entomological or arboviral prediction
- involved in mosquito or arbovirus surveillance programs
- interested in developing an international collaboration on this topic.
    Please send me an email (Charles.Guest@nceph.anu.edu.au), or contact me at
    the address below.

Background:

The understanding of ENSO has grown remarkably since the 1982-83 drought, with increasing possibility of prediction of this phenomenon during the current decade. We propose to use data relating to the ENSO phenomenon itself for the prediction of disease outbreaks in Australia, in the first place examining the occurrence of Ross River virus disease (RRVD).

Exposure measurements:

We will include long-period historical mean sea level pressure and sea surface temperature in the categorization of ENSO events according to new high-resolution climatic methods. The southern oscillation index (SOI) will also be used, although this may not provide as many months warning of the development of outbreaks. We will also obtain mosquito data for analysis wherever possible.

Availability and quality of data:

RRVD disease surveillance data before the 1990s is imprecise, but it would be useful to examine whatever data are available to establish an association over a longer period.

The climate data are available from point locations (weather stations). Demographic data are obtained annually at the level of Statistical Local Area. More detailed census data, to the level of postcode area, are available on a five-yearly basis.

Power calculation:

Initial calculations were based on the national RRVD notifications, January 1993-April 1996 (National Notifiable Disease Surveillance 1996), contrasting El Nino years (drought, 1991-1995) with the La Nina period (flood, 1996). The rate of RRV infections in the 6 months to April 1996 (per national population) is approximately 352 per million which is considerably more than the corresponding figures for the El Nino summers 1994 and 1995, 89 and 352, respectively (average 138). Assuming a Poisson distribution for the 6 month national notifications rates, 90% power to detect a rate ratio (RR) of at least 1.15 (for La Nina vs El Nino periods) assuming a baseline rate of 138 per million at a standard 5% two sided significance level has been demonstrated. So one has ample power to detect increases in the infection rates much

smaller than those seen in the crude national figures (RR = 1.6).

Data analysis:

As an intitial approach, observed and expected disease reports by area will be compared after standardising for demographic factors, to determine whether the spatial pattern of the disease is explainable by population effects alone. Linear and logistic regression will be used to build explanatory models of disease occurrence, using climatic and other variables. Thorough ecological analysis will be essential with maps of disease distribution according to the timecourse of ENSO, paying careful attention to the categorization of risk estimates. Use of EPI-INFO/EPI-MAP combinations will be examined.

We expect to use time series analyses to characterise the component of temporal variations in crude disease rates. These will enable the observed variations to be broken down into various components of time, including an overall linear trend, a cyclical component, a seasonal component and an irregular random component.

Spatial analysis is a new field, particularly in relation to disease data. This project provides an important opportunity for exploration of these methods in relation to disease surveillance in Australia.

Conclusion:

We believe that this project will add significantly to the global surveillance of infectious diseases. Building on data collected at national, state and local levels, it will lead to improvements in the recognition and response to arboviral diseases in the region. A multidisciplinary and geographically representative team has been developed with the broad base of essential expertise. Of developed nations, Australia may be the most strongly affected by ENSO; this exploratory project has the potential to contribute to public health interventions critical for many parts of the world. International collaboration is invited.

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VERTICAL TRANSMISSION OF DENGUE

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The current dengue epidemic in Malaysia started in May and reached a peak in July. Up till 5 October 1996, 10,479 cases have been reported to the Ministry of Health, of which 419 were dengue haemorrhagic fever with 26 deaths. This was an increase of 97% over the corresponding period in 1995, the highest number of notified dengue cases in the country since 1973. The major serotypes involved were dengue-2 and dengue-1 viruses, with dengue-2 being the more important serotype responsible for the severe cases.

The clinical manifestations were not unlike that of previous outbreaks and the majority of cases were seen in urban settings among older children and young adults. However, there were two cases in late pregnancy which resulted in dengue infection in the newborns and these are detailed below.

Case 1.

R.A., a 20 year old Malay lady, was admitted to the Obstetric ward, University Hospital, with complications of diabetes mellitus and toxemia of pregnancy. During the 38th week of pregnancy, she developed high fever associated with gum bleeding and epistaxis. On examination, the liver was found to be markedly enlarged, soft and tender. On Day 3 of illness, the liver enzymes started to rise and a mild ting of jaundice was noticed. The platelet counts started to fall abruptly. Counts as low as 8 E9/L and 6 E9/L were recorded on the 3rd and 7th day. The liver enzyme level of 1747 AST/IU was recorded on the 7th day, with a total bilirubin level of 121 μmol/litre. A full term baby was delivered normally on Day 4 of illness. The child was ill with grunting respiration and the APGAR score was only 6/7. There was intercostal recession with clinical and x-ray evidence of pneumonia. The liver was also enlarged 3 cpm below the costal margin. At this stage, the dengue serology did not demonstrate the presence of dengue specific IgM and the baby was suspected to have severe septicaemia and high doses of antibiotics were started. The mother's IgM to dengue was positive on Day 6 of illness, two days after the baby was delivered. The baby's platelet count started dropping rapidly from Day 3 of life. An ultrasound of the ventricles done on that day did not show any evidence of haemorrhage. However, the baby's condition deteriorated rapidly and a repeat ultrasound the next day showed massive intracranial bleed. The baby expired on Day 4 of life. The final diagnosis was DHF with liver failure, renal failure and pneumonia. Dengue-2 virus was isolated from the baby's blood sample collected on Day 4 and this was confirmed by polymerase chain reaction. The mother recovered and was discharged well. In this case, the diagnosis of dengue was not suspected in the baby since the mother's first sample was negative. There was no specific management of DHF till Day 3 of life when the platelet fell rapidly and intracranial bleeding occurred.

Case 2

S.M., a 31 year old Malay lady, was admitted with high fever in the 38th week of pregnancy. She had gum bleeding and pervaginal bleeding on Day 7. The platelet count started to fall on Day 6 but the haematocrit was within normal range. The dengue specific IgM was positive on Day 6 and petechial haemorrhages also started appearing on the same day. There was no marked hepatomegaly. However, a diagnosis of dengue fever (normal PCV) was made after a positive IgM on Day 6 and isolation of dengue-2 virus. A full term baby was born on Day 8 of illness. There was generalized flushing at birth and the baby became febrile from Day 2 of life. The liver was palpable 3 cm below the costal margin. The platelet count started to drop from Day 3 of life and the haematocrit showed a significant rise. There was no fall in haemoglobin level and no evidence of bleeding. The dengue specific IgM was negative initially but became positive on Day 5 of life. From the previous experience (Case 1) and as the mother's serology was positive before delivery, careful monitoring of the baby was done, and as soon as the platelets started to drop, FFP and platelet concentrates were transfused. The baby was discharged well on Day 9 of life. It is interesting to note that although the mother was diagnosed as dengue fever, the baby showed evidence of DHF.

CONCLUSION

Although vertical transmission of dengue virus has been reported in a number of countries, we believe this is the first time this has been reported in Malaysia. It is important for obstetricians and paediatricians to take note of this phenomenon and to monitor babies born from mothers who have DF or DHF during the final week of pregnancy.

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DHF outbreak in the paediatric age group at Delhi (India) in the 2-3rd week of September 1996

Report from National Institute of Virology
Pune, Maharashtra (India)

An outbreak of suspected dengue haemorrhagic fever was reported in Delhi around the 2nd week of September 1996. Twenty nine acute-phase serum samples collected from typical clinically diagnosed cases were referred to the National Institute of Virology, Pune, Maharashtra, India, for serodiagnosis.

The patients had haemorrhagic manifestations in the form of epistaxis (3); haemetemesis (5); GI tract bleeding (2); gum bleeding (1); rash (4) and melena (2). The symptoms suggestive of shock viz. low blood pressure and cold extremities were recorded in four patients. Thrombocytopenia was present in nine patients.

The youngest patient was a two-month-old infant while the oldest was of 32 years. Seventy five percent (22/29) of the patients were between 0-15 years of age; of which 3 were infants.

Serological investigations revealed recent infection of dengue virus in 48% (14/29) of cases as indicated by the detection of IgM type of antibodies to dengue viral antigens in the MAC-ELISA test.

An investigative report of clinically suspected denguefever outbreak in rural areas of West Bengal (India) - an unusual event with important epidemiological bearing.

Dr. N. Bhattacharya, Dr. D.K. Neogi and Dr. K.K. Mukherjee

Deugue had been considered since long to be an urban problem with seldom making its inroads to the rural population. The city of Calcutta, for example is experiencing dengue episode since 1963-64 and is considered to be endemic for dengue.

A distinct departure from this set pattern was noticed during the later half of August 1995, when two rural districts of West Bengal experienced an outbreak of dengue episode - clinically suspected and subsequently proved Virologically. Dengue virus could be isolated from 3 acute phase sera and two mosquito pools of Aedes aegypti and Aedes albopictus, collected from the local areas. Seroevidence of dengue infection was observed by HAI testing and IgM detection by MAC ELISA. A few thousand persons were affected and the infection was self limiting with uneventful recovery. There was no incidence of DHF and/or DSS and no report of case fatality. All age group was affected though maximum incidences were observed between 15-20 years of age group.

This recent happenings of dengue outbreak in rural areas as proved by its epidemic pattern in some small villages in West Bengal, has brought about a new dimension in dengue epidemiology in the state. It is likely that rapid urbanization associated with profound changes in eco system has resulted in proliferation of Aedes mosquitoes with the incidence of dengue in rural areas. This rural shifting in dengue epidemiology warrants extensive dengue surveillance in rural areas along with an extensive and in-depth research at the molecular and genetic level to ascertain the probability of changing pattern of susceptibility of Aedes aegypti to dengue virus.

Rapid characterization of genetic diversity among 12 dengue-2 virus isolates by single-strand conformation polymorphism analysis. (Jose A.Farfan[‡], Kenneth E. Olson, William C. Black IV, Duane J. Gubler[†] and Barry J. Beaty*)

Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Colorado State University, Fort Collins, CO. 80523. [‡]Univerity of Yucatan, Mexico, [†] Division of Vector-Borne Diseases, National Center for Infectious Diseases, Centers for Disease Control, Fort Collins, CO. 80523.

Single-strand conformation polymorphism (SSCP) analysis was used to characterize genetic polymorphisms among 12 isolates of dengue-2 virus, which had been genetically characterized by RNase T1 oligonucleotide mapping and by sequencing the viral envelope (E) gene. Specific cDNA fragments from the dengue-2 virus isolates were amplified by reverse transcriptase-polymerase chain reaction (RT/PCR). The viral E, premembrane (prM), and NS5 gene cDNAs of 291 bp, 291 bp, and 201 bp, respectively, were denatured, rapidly chilled to promote intrastrand reassociation, electrophoretically separated on nondenaturing polyacrylamide gels, and SSCP patterns were observed by silver staining. SSCP analysis revealed polymorphisms among a number of dengue-2 virus isolates from the same topotype, and these were markedly different between isolates of different topotypes. Comparison of nucleotide sequence and SSCP analyses of the 291 bp E cDNA demonstrated that virus isolates that produced identical SSCP patterns contained 0 to 7 nucleotide substitutions, whereas isolates that showed different SSCP patterns contained 4 to 25 nucleotide substitutions (see table). Positive predictive value (PPV) and negative predictive value (NPV) as measures of certainty for predicting identical and different sequences were 26 and 100%, respectively. The SSCP patterns of the 12 dengue-2 virus isolates suggested greater genetic variation in the prM gene region than in either the E or NS5 gene regions. SSCP analyses should allow easy, sensitive, and rapid genotyping of dengue viruses and allow the assessment of variation at a number of sites in the virus genome.

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Table 1. Number of nucleotide substitutions among the strains of dengue-2 virus analyzed by SSCP

Strain	D80-38	PUO-218	D80-100	16681	1409	D80-141	206-714	271-235	1583	1592	10
D80-38	0										
PUO-218	8[a],1[b],+[c]	0									
D80-100	6,1,-	6,0,-	0								
16681	5,1,+	4,0,-	4,0,+	0							
1409	7,2,+	13,1,-	13,1,-	11,1,-	0						
D80-141	6,3,+	11,2,-	11,2,-	9,2,-	4,1,-	0					
206-714	15,2,-	16,1,-	16,1,-	12,1,-	17,0,-	16,1,-	0				
271-235	15,2,-	16,1,-	16,1,-	12,1,-	17,0,-	16,1,-	0,0,+	0			
1583	16,2,-	19,1,-	19,1,-	15,1,-	20,0,-	17,1,-	11,0,-	11,0,-	0		
1592	16,2,-	19,1,-	19,1,-	15,1,-	20,0,-	17,1,-	11,0,-	11,0,-	4,0,+	0	
10	14,3,-	14,2,-	14,2,-	14,2,-	15,1,-	14,2,-	12,1,-	12,1,-	13,1,-	13,1,-	0
Tonga	23,2,-	25,1,-	23,1,-	23,1,-	24,0,-	23,1,-	19,0,-	19,0,-	24,0,-	26,0,-	23,1,-

a=transitions   b=transversions   c=band similarity (+), dissimilar (-)

Susceptibility of Chinese strains of *Aedes albopictus* and *Ae. aegypti* mosquitoes to infection with chikungunya virus

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Susceptibility of mosquitoes to infection with mosquito-borne viruses and the ability of mosquitoes to transmit viruses are of essential importance for prediction and control of mosquito-borne diseases. In particular, susceptibility of mosquitoes from different geographic areas is one of the problems to be investigated from an epidemiological point of view. We have examined several geographic strains of *Aedes albopictus* and *Ae. aegypti* mosquitoes for their susceptibility to infection with chikungunya virus (CHIKV). In this communication, we report data concerning the susceptibility of *Ae. albopictus* and *Ae. aegypti* originated from China to infection with CHIKV.

Examined in this study were laboratory-colonized strains of *Ae. albopictus* (from Dianbai and Chengdu) and *Ae. aegypti* (from Shanghai and Haikou), all of which were kindly provided by the Department of Vector Biology and Control, Institute of Microbiology and Epidemiology, Beijing, China. Haikou is located in the southern part of China and the other three are in the middle China. Adult females of these strains were orally infected with CHIKV at an infective titer of 6.5×10^7 PFU per ml. Fully engorged mosquitoes were maintained for 14 days at 26-28°C and 70-80% relative humidity and were examined for virus titer on Vero cells.

Results are summarized in Table. The Shanghai strain of *Ae. aegypti* showed the highest infective titer and the highest infection rate among 4 strains. The Haikou strain of *Ae. aegypti* had the lowest infection rate, but its infective titer was relatively high (10^5-10^6 PFU per mosquito). Although we did not investigate the virus in saliva, these high titers were possibly related to the ability to transmit the virus. In China, CHIKV is distributed in the southern part. In this study, though the number of mosquitoes examined were limited in number, it was to be noted that mosquitoes originated not only from southern areas but also from middle areas of China were susceptible to infection with CHIKV.
(Reported by Susumu Hotta)

Table CHIKV growth in Chinese strains of *Aedes albopictus* and *Ae. aegypti* mosquitoes orally infected 14 days previously

Strain	Mathematical Mean of infective titers (PFU per mosquito)	Frequency distribution of infective titers (PFU)							Infection rate (%)
		$<10^1$	10^1	10^2	10^3	10^4	10^5	10^6	
(*Ae. albopictus*)									
Dianbai	2.1×10^4	13	0	1	1	0	1	0	18.8
Chengdu	1.6×10^3	6	0	1	5	0	0	0	50.0
(*Ae. aegypti*)									
Shanghai	2.0×10^5	2	0	1	6	3	2	1	86.7
Haikou	2.7×10^4	10	0	0	0	0	1	1	16.7

CHARACTERISATION OF KOKOBERA VIRUS

M Poidinger[1], RA Hall[1], PJ Turley[1], MD Lindsay[2], AK Broom[2] and JS Mackenzie[1]

We have studied the molecular epidemiology and phylogeny of Kokobera (KOK) virus, a flavivirus found in Australia and Papua New Guinea. We sequenced a region encompassing the 200 nucleotides of the 3' terminus of the NS5 gene, and the first 300 nucleotides of the 3' untranslated region (UTR). The study includes 31 isolates of the virus, including 10 isolates recently isolated for the first time from the south-west of Western Australia (WA). We found that the KOK isolates clustered based on geographic location and time of isolation, within two distinct topotypes: one covering Queensland and New South Wales; and the other the Northern Territory and WA. This latter group was further subdivided into northern and south-west isolates. This phylogeny is quite different from other Australian flaviviruses, such as Murray Valley encephalitis (MVE) and Kunjin (KUN) viruses, which exist as single genetic types across the entire Australian continent. However, it is similar to the phylogeny of the alphavirus Ross River (RR) virus. This may be explained by the fact that MVE and KUN viruses are known to have birds as their main vertebrate hosts, whilst RR virus utilises macropods, which have been suggested as the vertebrate host for KOK virus. In addition, the south-west isolates exhibited a degree of sequence heterogeneity, including one isolate that has a 9 nucleotide deletion in the 3'UTR. The data suggest KOK virus has been in the south-west of WA for some time, and has not been recently introduced into the area.

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(The Sea Sponge uses its brain to locate a nice spot to settle down. Having found one, it has no further use for its brain, so it eats it...which is a lot like gaining tenure.)

Repeated Emergence of Epidemic/Epizootic Venezuelan Equine Encephalomyelitis from a Single Genotype of Enzootic Subtype ID Virus

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Venezuelan equine encephalomyelitis (VEE) viruses (Togaviridae; *Alphavirus*) cause a wide range of diseases in humans and equines varying from inapparent infection to acute encephalitis. Major epidemics have only been associated with VEE viruses serologically classified into subtype I, varieties AB and C. The remaining serotypes (subtypes II - VI and subtype I, varieties D, E, and F) are designated enzootic because they circulate in sylvatic or swamp habitats and only rarely cause human or domestic animal disease (Johnson and Martin, 1974; Walton and Grayson, 1988).

Since the first recognized VEE epidemic/epizootic in 1936, sporadic outbreaks occurred in South and Central America through the early 1970's. However, between 1973 and 1992, no epidemic/epizootic VEE activity was observed. After speculation that IAB and IC variety VEE viruses had become extinct, outbreaks occurred in South America in 1992-1993 (Rico-Hesse *et al.*, 1995) and 1995 (Weaver *et al.*, 1996) prompting further investigation into the mechanism of persistence of these viruses between outbreaks.

To delineate the relationships of variety ID viruses to epidemic/epizootic VEE viruses, we conducted detailed phylogenetic analyses using isolates covering both the geographical and temporal range of strains available. Each VEE viral RNA was reverse transcribed and the resulting cDNA's were amplified using PCR. The 857 bp products, colinear with the E3 gene and the N-terminus of the E2 gene, were sequenced. Phylogenetic analyses were conducted with both parsimony and distance matrix methods (Felsenstein, 1993; Swofford, 1991). As in previously published trees (Weaver *et al.*, 1992), the closest relatives of the epidemic/epizootic strains were the subtype ID and II viruses (Fig. 1). These results support the hypothesis that epidemic/epizootic VEE viruses emerge from enzootic, subtype ID viruses. Four distinct ID-like groups or genotypes were identified: 1) ID isolates from Tumaco, in southwestern Colombia, and adjacent coastal Ecuador; 2) Everglades virus (subtype II) isolates from southern Florida; 3) ID viruses from eastern Panama and; 4) ID viruses from a variety of locations in Colombia, Peru and Venezuela (Fig. 1). Within the Colombia-Peru-Venezuela group, three separate monophyletic groups of epidemic/epizootic viruses were found; the IAB strains fell

into a single group, while representatives of the 1992-93 IC outbreak were in a group distinct from those isolated during the 1962-64 and 1995 IC outbreaks. These results indicate that VEE epidemic/epizootic emergence has occurred at least three times, but that only one (the Colombia-Peru-Venezuela genotype; see Fig. 1) of four ID-like lineages has been responsible for all the outbreaks examined here. The lack of VEE emergence from genotypes circulating in southwestern Colombia/Ecuador, Florida or Panama has several possible explanations including 1) a chance lack of occurrence in these locations of the combination of mutations and ecological conditions required for VEE emergence, or 2) genetic differences in these viruses, with respect to the Colombia-Peru-Venezuela ID genotype, requiring more mutations to produce equine viremia sufficient for epizootic emergence.

Because changes in the VEE E2 glycoprotein region have previously been implicated in decreased virulence in mice (Davis *et al.*, 1991; Kinney *et al.*, 1993), additional E2 region nucleotides were sequenced to generate complete PE2-6K sequences for representatives of each major group from our original phylogenetic analysis (Fig. 1). Phylogenetic relationships among these longer sequences were completely consistent with those depicted in Fig. 1. The E2 amino acid sequences were derived and compared with those of laboratory attenuated strains to detect potential determinants of natural equine virulence (Fig. 2). Those mutations involved in mouse attenuation of vaccine and laboratory strains (Davis *et al.*, 1991; Kinney *et al.*, 1993) do not appear to be involved in natural equine virulence of epizootic vs. enzootic strains, as they generally do not vary in our E2 sequences of natural isolates. While several informative (for a given residue, two or more different amino acids each found in two or more isolates) amino acid differences were identified, most correlated with phylogenetic relationships rather than phenotypic (equine virulence or antigenic) traits. These single changes are therefore probably not directly responsible for differences in antigenic variety or virulence. These results suggest that emergence of epidemic/epizootic VEE relies on several different combinations of mutations that result in similar antigenic and virulence phenotypes. This conclusion agrees with the convergent evolution of epidemic/epizootic viruses depicted in our phylogenetic tree (Fig. 1).

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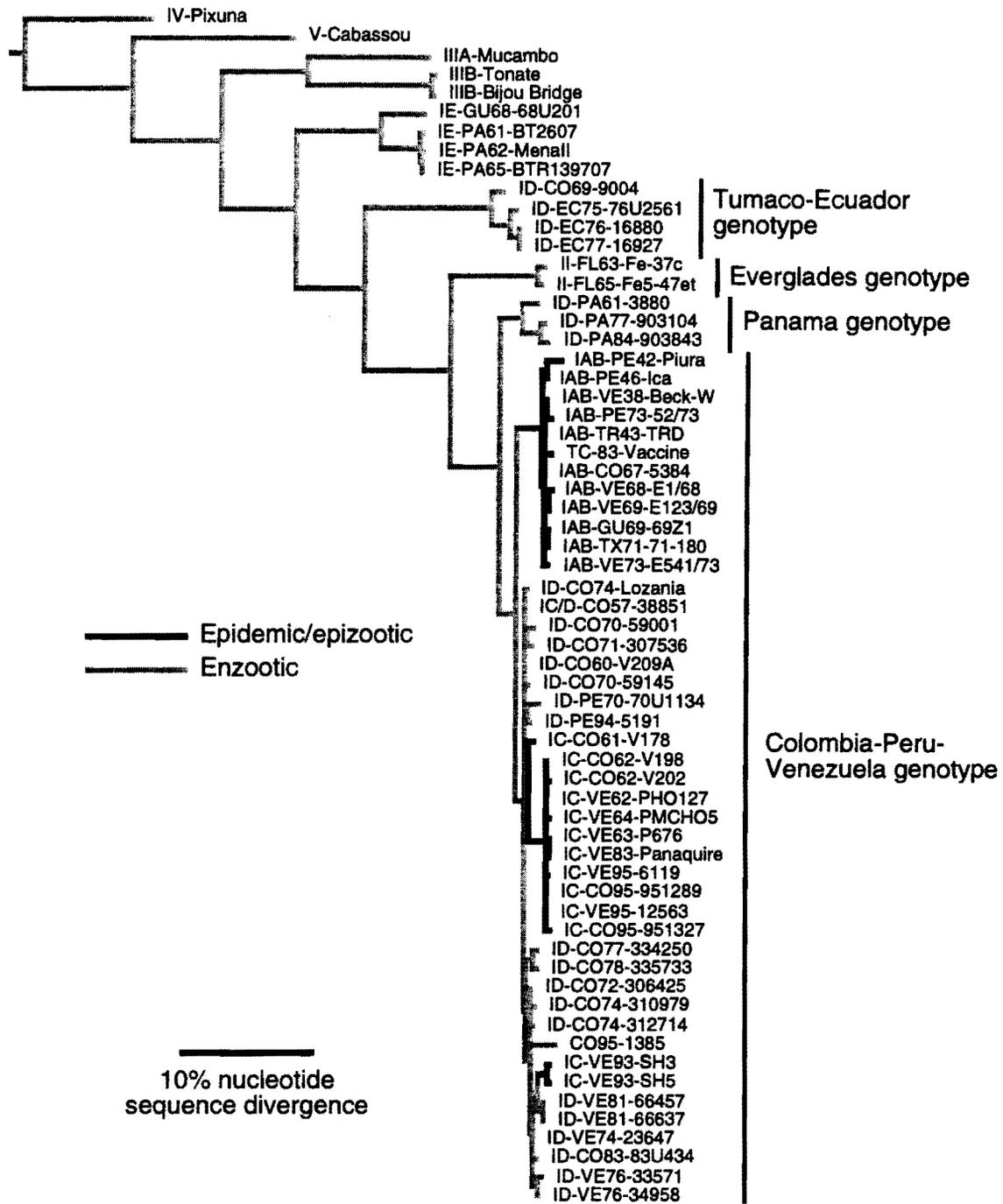
Fig. 1. Phylogenetic tree generated by maximum parsimony, depicting relationships among enzootic and epidemic/epizootic strains of VEE virus. Branch shading reflects predicted epidemiological status of ancestral lineages, based on minimizing the number of changes between enzootic and epidemic/epizootic serotypes. Four representative strains of N. American and S. American antigenic varieties of eastern equine encephalomyelitis (EEE) virus were used as an outgroup to root the tree.

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                                                                                                        100
IAB-TR43-TRD       STRELFKEYK LTRPYMARCI RCAVGSCHSP IAIEAVKSDG HDGYVRLQTS SQYGLDSSGN LKGRTMRYDM HGTIKEIPLH QVSLHTSRPC HIVDGHGYFL
IAB-VE38-B-W       .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-CO67-CoAn5384  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-GU69-69Z1      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-TX71-71-180    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-PE73-52/73     .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IC-CO61-V178       .......... .......... .......... .......... .......... .......... .......... V......... .......... ..........
IC-CO62-V198       .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
IC-VE63-P676       .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
IC-VE83-Panaquire  .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
IC-VE95-6119       ...L..... .......... .......... .......... .......... .......... .......... E......... .......... ..........
IC-VE93-SH3        .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
IC-VE93-SH5        .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
ID-PA61-3880       .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
ID-PE94-DEI5191    .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
PE94-IQT1724       .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
ID-CO60-V209A      .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
ID-VE81-66457      .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
ID-CO83-83U434     .......... .......... .......... .......... .......... .......... .......... E......... .......... ..........
II-FL63-Fe37c      .......... .......... ........V. .......... .......... ......P... ..S.....N. Y...E..... .......... ..........
II-FL65-Fe5-47et   .......... .......... ........V. .......... .......... ......P... ..S.....N. Y...E..... .......... ..........
ID-CO69-CoAn9004   .......... .......... ..K.....M. .......... .......... .......... .......... E......... .......... ..........
IE-PA62-MenaII     .......... .......... .......... .......... .R.E...... ......P... .......... E......... .......... ..I.......
IE-GU68-68U201     .......... .......... .......... .......... .R.E...... ......P... .......... E......... ...V...... ..I.......


                                                                                                        200
IAB-TR43-TRD       LARCPAGDSI TMEFKKDSVI HSCSVPYEVK FNPVGRELYT HPPEHGVEQA CQVYAHDAQN RGAYVEMHLP GSEVDSSLVS LSGSSVTVTP PVGTSALVEC
IAB-VE38-B-W       .......... .......... .......... .......... .......... .......... .......... .......... .R........ ..........
IAB-CO67-CoAn5384  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-GU69-69Z1      .......... .......... .......... ...A...... .......... .......... .......... .......... .......... ..........
IAB-TX71-71-180    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-PE73-52/73     .......... .......... .......... .......... .......... .......... .......... .......... .R........ ..........
IC-CO61-V178       .......... ......G... .......... .......... .......A... .......... .......... .......... .......... ..........
IC-CO62-V198       .......... ......G... .......... .......... .......A... .......... .......... I......... .......... ..........
IC-VE63-P676       .......... ......G... .......... .......... .......A... .......... .......... I......... .......... .......K.
IC-VE83-Panaquire  .......... ......G... .......... .......... .......A... .......... .......... I......... .......... .......K.
IC-VE95-6119       .......... ......G... .......... .......... .......A... .......... .......... I......... .......... ..........
IC-VE93-SH3        .......... ......N... .......... .......... .......A... .......... .......... .......... .......... .AR.......
IC-VE93-SH5        .......... ......N... .......... .......... .......A... .......... .......... .......... .......... .AR.......
ID-PA61-3880       .......... .......A.. .......... .......... .......A... .......... .......... .......... .A........ ..........
ID-PE94-DEI5191    .......... .......... .......... .......... .......A... .......... .......... .......... .A........ ..........
PE94-IQT1724       .......... .......... .......... .......... .......A... .......... .......... I......... .A........ ..........
ID-CO60-V209A      .......... .......... .......... .......... .......A... .......... .......... .......... .A........ ..........
ID-VE81-66457      .......... ......NL.. .......... .......... .......A... .......... .......... .......... .A........ ..........
ID-CO83-83U434     .......... .......... .......... .......... .......A... .......... .......... .......... .A........ ..........
II-FL63-Fe37c      .......... .......... .......... .......... .......A... .......... .......... .SGL.S... .A........ ..........
II-FL65-Fe5-47et   .......... .......... .......... .......... .......A... .......... .......... .SG..S... .A........ ..........
ID-CO69-CoAn9004   .......... .....E... .......... .T........ ....E.LP.. .......... .......... I....... ......S... .A.I.V....
IE-PA62-MenaII     .......... .....E... .......... .......... ....A..P .H........ .......... .T.L. T.....H... .A.Q.V....
IE-GU68-68U201     .......... .....E... .......... ...A...... ....A..P .H........ .......... .T.L. M.....H... .A.Q.VQ...
```

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Fig. 2. Aligned E2 amino acid sequences for representatives of major VEE virus groups depicted in Fig. 1. Shaded boxes show amino acids shown in previous studies to be involved in laboratory mouse attenuation.

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                                                                                                                    300
IAB-TR43-TRD       ECGGTKISET INKTKQFSQC TKKEQCRAYR LQNDKWVYNS DKLPKAAGAT LKGKLHVPFL LADGKCTVPL APEPMITFGF RSVSLKLHPK NPTYLTTRQL
IAB-VE38-B-W       .........  .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-CO67-CoAn5384  .........  .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-GU69-69Z1      .........  .......... .......... .......... .....V.... .......... .......... .......... .......... ..........
IAB-TX71-71-180    ....K...   ..R....... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-PE73-52/73     .........  .......... .......... .......... .......... .......... .......... .......... .......... ..........
IC-CO61-V178       .........  ...A...... .......... .......... .......... .......... .......... .......... .......... ..........
IC-CO62-V198       K........  ...A...... .......... .......... .......... .......... .......... .......... .......... ..........
IC-VE63-P676       K........  ...A...... .......... .......... .......... .......... .......... .......... .......... ..........
IC-VE83-Panaquire  K........  ...A...... .......... .......... .......... .......... .......... .......... .......... ..........
IC-VE95-6119       K........  ...A...... .......... .......... .......... .......... .......... .......... .......... ..........
IC-VE93-SH3        .........  ..RA...... .......... .......... .......... .......... ....IL.... .....N.... .......... ..........
IC-VE93-SH5        .........  ..RA...... .......... .......... .......... .......... .......... .......... .......... ..........
ID-PA61-3880       .........  ..TA...... .......... .......... .......... .......... .......... .......... .......... ..........
ID-PE94-DEI5191    .........  ..TA...... .......... .......... .......... .......... .......... .......... .......... ..........
PE94-IQT1724       .........  ..TA...... .......... .......... .......... .......... .......... .......... .......... ..........
ID-CO60-V209A      .........  ..TA...... .......... .......... .......... .......... .......... .......... .......... ..........
ID-VE81-66457      .........  ..TA...... .......... .......... .......... .......... .......... .......... .......... ..........
ID-CO83-83U434     .........  ..TA...... .......... .......... .......... .......... .......... .......... .......... ..........
II-FL63-Fe37c      ..S..T..   .......... .......... .......... .......... .......... .......... .......... .......... Y......E.
II-FL65-Fe5-47et   ..S..T..   .......... .......... .......... .......... .......... .......... .......... .......... Y......E.
ID-CO69-CoAn9004   ...S....   ..TA...... .......... .......... ......S... .......... .......... ....I..H.. ..I....... ..........
IE-PA62-MenaII     .........  ..SA..Y... S.TA...... T......... ........E. .........V .TEA...... ....I..... .......... ...F.....
IE-GU68-68U201     .........  ..SA..Y... S.TS...... T......... ......S.E. .........V .TEA...... ....I..... .......... ...F.....
```

Fig. 2, continued

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                                                                                                                  400
IAB-TR43-TRD      ADEPHYTHEL ISEPAVRNFT VTEKGWEFVW GNHPPKRFWA QETAPGNPHG LPHEVITHYY HRYPMSTILG LSICAAIATV SVAASTWLFC RSRVACLTPY
IAB-VE38-B-W      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-CO67-CoAn5384 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-GU69-69Z1     .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-TX71-71-180   .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IAB-PE73-52/73    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
IC-CO61-V178      .......... .......... .......... .......... .......... .......... .......... .......V.. .......... K...S.....
IC-CO62-V198      .......... .......... .......... .......... .......... .......... .......... .......V.. .......... K...S.....
IC-VE63-P676      .......... .......... .......... .......... .......... .......... .......... .......V.. .......... K...S.....
IC-VE83-Panaquire .......... .......... .......... .......... .......... .......... .......... .......V.. .......... K...S.....
IC-VE95-6119      .......... .......... .......... .......... .......... .......... .......... .......V.. .......... K...S.....
IC-VE93-SH3       .......... .......... .......... .......... .......... .......... .......... .......V.. .......... K...S.....
IC-VE93-SH5       .......... .......... .......... .......... .......... .......... .......... .......V.. .......... K...S.....
ID-PA61-3880      .......... ....V....S .......... .......... .......... .......... .......... .......V.. .I......L. K...S.....
ID-PE94-DEI5191   .......... .......... .......... .......... .......... .......... .......... .......VI. .......... K...S.....
PE94-IQT1724      T......... .........S .......... .......... .......... .......... .......... .......V.. .I......L. K...S.....
ID-CO60-V209A     .......... .......... .......... .......... .......... .......... .......... .......V.. .......... K...S.....
ID-VE81-66457     .......... .......... .......... .......... .......... .......... .......... .......V.. .......... K...S.....
ID-CO83-83U434    .......... .......... ......I... .......... .......... .......... .......... .......V.. .......... K...S.....
II-FL63-Fe37c     .......... ....S....S ..A....... .......... .......... ......V... .......T. .....VA. .I.....L. ...AS.....
II-FL65-Fe5-47et  .......... ....S....S ..A....... .......... .......... ......V... .......T. .....V.. .I.....L. ...AS.....
ID-CO69-CoAn9004  DG..N..... ..Q....... .......... ....Q..... .......... W......... ........V. ...VVVT .L.....L. K...S.....
IE-PA62-MenaII    DG..A..... .TN.V....S .......... ....Q.Y.S .......... .......... .......... ...V.T .I...V.... K..IS.....
IE-GU68-68U201    DG..A..... .TH.V....S .......... ....Q.Y.S .......... .......... .......... ...V.T .I...V.... K..IS.....


                                    423
IAB-TR43-TRD      RLTPNARIPF CLAVLCCART ARA
IAB-VE38-B-W      .......... .......... ...
IAB-CO67-CoAn5384 .......... .......... ...
IAB-GU69-69Z1     .......... .......... ...
IAB-TX71-71-180   .......... .......... ...
IAB-PE73-52/73    .......... .......... ...
IC-CO61-V178      .......M.L .......... ...
IC-CO62-V198      .......M.L .......... ...
IC-VE63-P676      .......M.L .......... ...
IC-VE83-Panaquire .......M.L .......... ...
IC-VE95-6119      .......M.L .......... ...
IC-VE93-SH3       .......M.L .......... ...
IC-VE93-SH5       .......M.L .......... ...
ID-PA61-3880      .......M.L .......... ...
ID-PE94-DEI5191   .......M.L .......... ...
PE94-IQT1724      .......M.L .......... ...
ID-CO60-V209A     .......M.L .......... ...
ID-VE81-66457     .......M.L .......... ...
ID-CO83-83U434    .......M.L .......... ...
II-FL63-Fe37c     ......KM.L .........S ...
II-FL65-Fe5-47et  ......KM.L .........S ...
ID-CO69-CoAn9004  .....V.L.L .......... ...
IE-PA62-MenaII    .......M.L .......... ...
IE-GU68-68U201    ......SM.L .......... .K.
```

Fig. 2, continued

GEOGRAPHICAL EXPANSION OF OROPOUCHE FEVER IN THE AMAZON REGION OF BRAZIL

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Oropouche (ORO) virus, *Bunyavirus* genus of the family *Bunyaviridae* and one of at least 21 Simbu group viruses in the Bunyamwera supergroup, has been recognized as a mayor cause of human febrile illness in the Brazilian Amazon. Between 1961 and 1980, several outbreaks occurred in urban and rural center's of Pará state, in the eastern part of Amazon. It is estimate that at least, 165,000 persons were infected (figure 1).

From May 1980 to February 1981, the first outbreaks of ORO were recorded in Amazonas state (Barcelos and Manaus). Nearly 97,000 out of the 650,000 inhabitants of Manaus were infected. Based on serological studies, we were also able to detect, in 1980, an outbreak of ORO in Mazagão, a town in Amapá state. In 1988, other outbreaks were studied in Maranhão state (Porto Franco) and Tocantins state (Tocantinópolis) with thousands of persons infected.

During the first quarter of 1991 an extensive outbreak broke out in the villages of Ariquemes and Ouro Preto do Oeste, in the Rondonia state. A randomized survey carried out in Ariquemes by Health Secretary of Rondonia estimated in 58,874 cases of ORO infections. In December 1994, a new epidemic of human illness caused by ORO virus was recognized in the Serra Pelada gold mine (5°35's, 49°30'w) in Southeast region of Pará state. Based on the serological data, we conclude that in this epidemic occurred 5,085 cases or a prevalence of 83 %. From the beginning of 1996, another epidemics caused by ORO virus has been occurring in Pará state (Vitória do Xingú, Brazil Novo and Oriximiná), Amazonas state (Novo Airão) and for the first time in Acre state (Xapuri). Numerous virus strains were isolated from blood of febrile patients and hundreds cases of recent infections were diagnosed by MAC ELISA (Figure 2).

One must be concerned about the spread of the ORO fever to urban and rural centers, infested with the biting midge *Culicoides paraensis*, that seems to play a major role in virus transmission. In fact, outbreaks have already been reported outside of the Amazon region, in Panama (1989) and in Peru (1991 - 1992).

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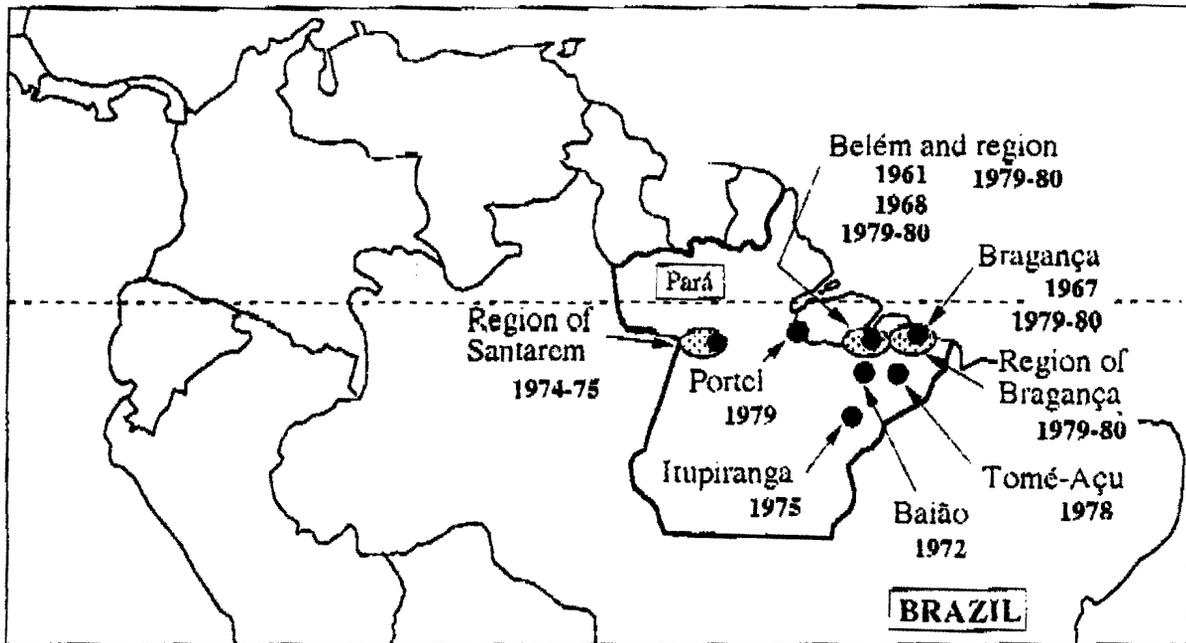
Figure 1 : OROPOUCHE outbreaks between 1961 and 1980.
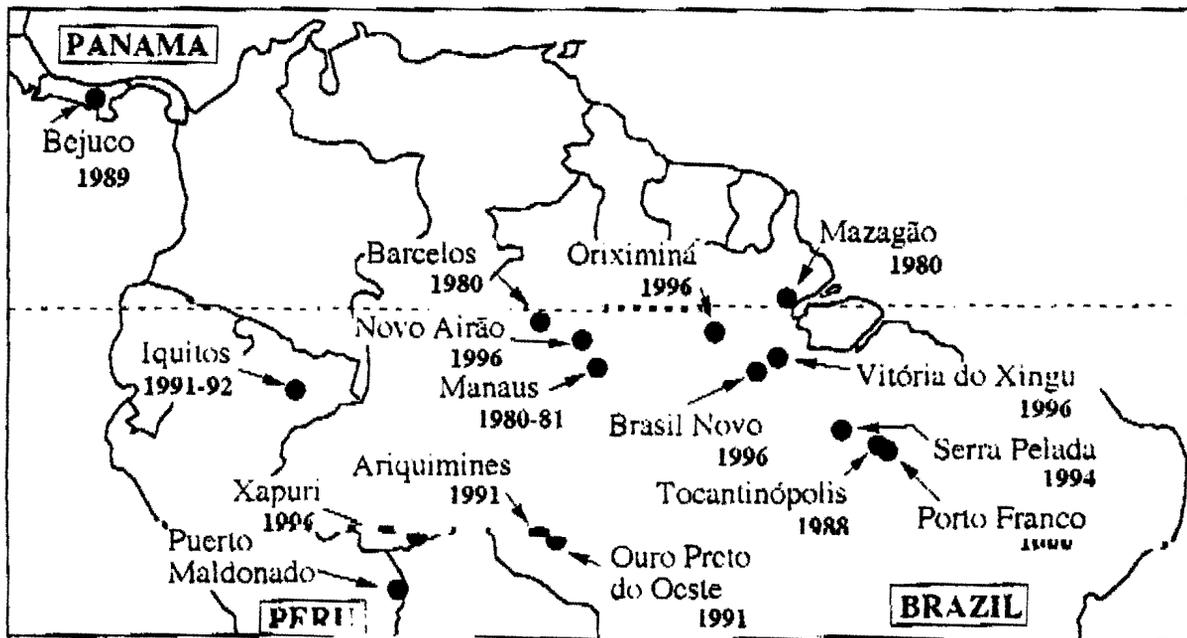
Figure 2 : OROPOUCHE outbreaks between 1980 and 1996.

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SURVEILLANCE FOR ARBOVIRUSES IN MOSQUITOES IN NEW SOUTH WALES, AUSTRALIA, 1995-96.

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Arbovirus surveillance by our laboratory for the state of New South Wales (NSW) has continued for the period of November 1995 through to April 1996. Methodology has been detailed in earlier issues of *Arbovirus-Borne Virus Information Exchange* (December 1991, pp. 40-44, and June 1995, pp. 33-36), although there were no sentinel chicken flocks used for flavivirus detection this season.

Table 1 presents the arbovirus isolates for 1995-96. The overall number of viruses was well below that of recent years and they were widely distributed across the state. The most common arbovirus was Ross River (RR) virus (18 isolates) and RR was also the most widespread, being isolated from 9 localities. Sindbis (SIN) was the next most common with 4 isolations and there were two isolates each of the flaviviruses, Stratford (STR) and Edge Hill. Additionally, there were 4 untyped isolates (neither alphaviruses nor flaviviruses). Griffith, within the irrigation area of the southwest, again produced the most viruses (14) and *Culex annulirostris* was the mosquito that yielded the highest number (22). Two new records for Australia were made; for the first time SIN was isolated from *Coquillettidia linealis* and STR was recovered from *Aedes procax*. This STR isolate isthe first flavivirus recovered from the Sydney region. Interestingly, there were no isolates of Barmah Forest (BF) virus for 1995-96, following the major outbreak in 1994-95 along the south coast of NSW (*Arbovirus-Borne Virus Information Exchange*, June 1995, pp. 33-36).

Prior to the 1995-96 season, mosquito numbers across NSW have been generally well down on average, and associated arbovirus activity has been minimal (the notable exception being the BF outbreak mentioned above). The reduced mosquito numbers have followed several years of rainfall deficiencies associated with recurrent El Niño episodes. With the end of the El Niño episode in 1995, rainfall was well above average towards late 1995 and early 1996, but thereafter was well below average. These weather patterns lead to mosquito populations that were generally about average in number for the 1995-96 season, with little arbovirus activity occurring. The exception to this was in the far north of the state where increased rainfall activity in inland areas and coastal areas (combined with high tides along the coast), led to increased mosquito populations with some RR virus activity. Ross River virus was the most common arbovirus infecting humans in NSW for 1995-96.

Table 1. Arbovirus isolates collected in New South Wales, 1995–96.

Mosquito Collections			Virus Isolates					
Date	Locality	Species	RR	SIN	EH	STR	V?	Total
26/11/95	Leeton	*Cx annulirostris*					1	1
15/1/96	Griffith	*Cx annulirostris*	2					2
22/1/96	Griffith	*Cx annulirostris*	3					3
29/1/96	Boggabilla	*Cx annulirostris*	1					1
29/1/96	Deniliquin	*Cx annulirostris*	2					2
29/1/96	Griffith	*Cx annulirostris*	4					4
5/2/96	Menindee	*Cx annulirostris*	1					1
5/2/96	Warren	*Cx annulirostris*	1					1
6/2/96	Forbes	*Cx annulirostris*	1					1
6/2/96	Griffith	*Cx annulirostris*	1				1	2
6/2/96	Griffith	*An annulipes*	1					1
18/2/96	Bourke	*Cx annulirostris*		1				1
19/2/96	Griffith	*Cx annulirostris*		1				1
19/2/96	Port Stephens	*Ae vigilax*			1	1		2
21/2/96	Batemans Bay	*Cq linealis*		1				1
21/2/96	Sydney	*Ae procax*				1		1
4/3/96	Griffith	*An annulipes*					1	1
4/3/96	Moree	*Cx australicus*					1	1
11/3/96	Boggabilla	*Cx annulirostris*			1			1
11/3/96	Port Stephens	*Ae vigilax*	1					1
12/3/96	Condobolin	*Cx annulirostris*		1				1
TOTALS			18	4	2	2	4	30

Ae = Aedes, An = Anopheles, Cq = Coquillettidia, Cx = Culex, RR = Ross River, SIN = Sindbis,
EH = Edge Hill, STR = Stratford, V? = Unidentified virus (not alphavirus or flavivirus).

Identification of *Aedes aegypti* in Arizona for the first time in fifty years.

David M. Engelthaler, Craig E. Levy, T. Michael Fink, and Mira Leslie

In Arizona, the last documented *Aedes aegypti* populations were found in 1943 (Murphy 1953) and 1946 (Bequaert 1946) in the Tucson region of Pima County. Elsewhere in the western United States, *Ae. aegypti* has been conspicuously absent, except for reports of populations in New Mexico from 1946 (Bequaert 1946) and 1951-2 (Ferguson 1954).

In October 1994 five adult *Ae. aegypti* were found in a Tucson backyard and identified by the Arizona Department Health Services (ADHS) and the Centers for Disease Control and Prevention (CDC).

During September 1995, *Ae. aegypti* adults were found both in Nogales (Santa Cruz County) and again in Tucson (Pima County). The adult mosquitoes were collected using CO_2 traps in various locations in these two cities. In Tucson, trapping from four of five different sites yielded a total of 43 adult *Ae. aegypti*. In Nogales, trapping from two of four different sites yielded 122 adults. Trapping done earlier, in 1995, at these sites yielded no *Ae. aegypti* adults. All trapping reflects 2-3 trapnights per site. Monthly arbovirus surveillance trapping yielded an additional 32 adults from Tucson during September and October, 1995.

In late March 1996, ADHS responded to a report of "ankle-biting" mosquitoes in central Tucson. Subsequently, two adult *Ae. aegypti* were trapped in the complainant's home. Since then, adult *Ae. aegypti* have been found in several new areas in and around Tucson, and have been found for the first time in southern Cochise County, along the Arizona - Mexico border.

While oviposition trapping is used extensively for *Ae. aegypti* surveillance (Service 1976), it was not very productive in the southern deserts of Arizona. ADHS used hay infusion enhanced oviposition traps as described in Reiter et al. 1991. The traps probably did not maintain enough water for a long enough period of time for the hay infusion to be a suitable attractant for egg laying. Future oviposition trapping attempts will use variations of this trap to determine a more appropriate *Ae. aegypti* trap for use in the arid deserts of Arizona.

The identification of new populations of *Ae. aegypti* in Arizona is of particular concern to ADHS, due to the presence of dengue and dengue hemorrhagic fever in the bordering Mexico state of Sonora (C.Levy, unpublished data). While no endemic cases of dengue have been found in Arizona, two imported cases were identified in 1994. ADHS records show that, between 1941 and 1946, nine cases of dengue fever were reported in Arizona's residents, eight of which were from Pima and Santa Cruz counties. No exposure or travel history for those cases are available.[1]

Whether or not any of these earlier cases were endemically acquired is not known. Their mere presence allowed for possible disease spread, due to the simultaneous presence of *Ae. aegypti* populations. If, or better stated, when new cases of dengue are identified in Arizona residents this same predicament will again exist. ADHS will expand *Ae. aegypti* surveillance throughout Southern Arizona in the coming years and will maintain active vigilance for new dengue cases.

[1] It should be noted that cases of yellow fever have also been reported in southern Arizona. Two fatal imported cases occurred southeast of Tucson in the early 1880's. Both were associated with a yellow fever epidemic in Sonora, Mexico in 1883 (Quebbeman 1966). These historic cases of dengue and yellow fever illustrate the susceptibility of the southwestern United States to tropical arboviral introductions.

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ARBOVIRUS SURVEILLANCE-LABORATORY TESTINGS IN DELAWARE

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The Delaware Public Health Laboratory collaborates with the Mosquito Control Section of The Department of Natural Resources and Environmental Control of Delaware to provide laboratory testing for arbovirus surveillance in the State of Delaware.

The mosquito pools and chicken bloods are collected from strategically defined areas throughout Delaware.

The mosquito pools are processed for arbovirus isolation by inoculating into cell cultures (Vero cells). Eastern Equine Encephalitis (EEE) virus was isolated from 3 of 112 mosquito pools.

Serologically pretested negative chickens, four each are placed in 12 sites (shown on map). At weekly intervals, two of four birds are bled alternatively and tested for detecting antibody to arbovirus.

The rainfall and warm temperature this spring and summer produced a bountiful population of mosquitoes. Our fears of finding arboviral activity sooner than usual in the season became a reality when we detected seroconversions in the 6 of 48 sentinel birds in July.

In addition to the surveillance testing, EEE virus was isolated from a brain specimen of a horse with symptoms shown in central nervous system. Our test results clearly indicated that EEE activity was found in 9 locations all over the state (please see table).

Once the arboviral activity is located by the laboratory testing, the Mosquito Control officials stepped up more aggressive mosquito spraying to prevent the spread of the EEE in the human population.

EEE virus is obviously maintained in a cycle involving many species of wild birds, horses and mosquito vectors primarily _Culiseta melanura_ that breeds in inland water swamps in Delaware.

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ARBOVIRAL ACTIVITIES DETECTED
IN DELAWARE, 1996

TABLE

SPECIMEN	DATE RECEIVED	LOCATION	EEE ISOLATED OR SEROCONVERSION
Blood of Sentinel Birds:			
#199	07/09/96	Cypress Swamp	Seroconversion
#233	07/09/96	Kenton	" "
#197	07/16/96	Cool Spring	" "
#212	07/16/96	Paradise Alley	" "
#235	07/22/96	Glasgow	" "
#202	07/22/96	Woodland Ferry	" "
#204	08/13/96	Cypress Swamp	" "
#216	08/27/96	Hazletville	" "
#244	10/08/96	Cypress Swamp	" "
Mosquito Pools:			
# 24	08/01/96	Beaver Dam	EEE Virus
# 29	08/01/96	Kenton	" "
# 80	09/12/96	Cool Spring	" "
Horse Brain:			
#991	09/18/96	Millsboro	EEE Virus
EEE = Eastern Equine Encephalitis			

MAP

Locations of EEE Virus Activity Detected in Delaware, 1996
ⓔ = Seroconversion of Antibody Titer to EEE Virus
▲ = EEE virus isolated
● = Negative

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b:\Exchange.AR1 (JHJ)
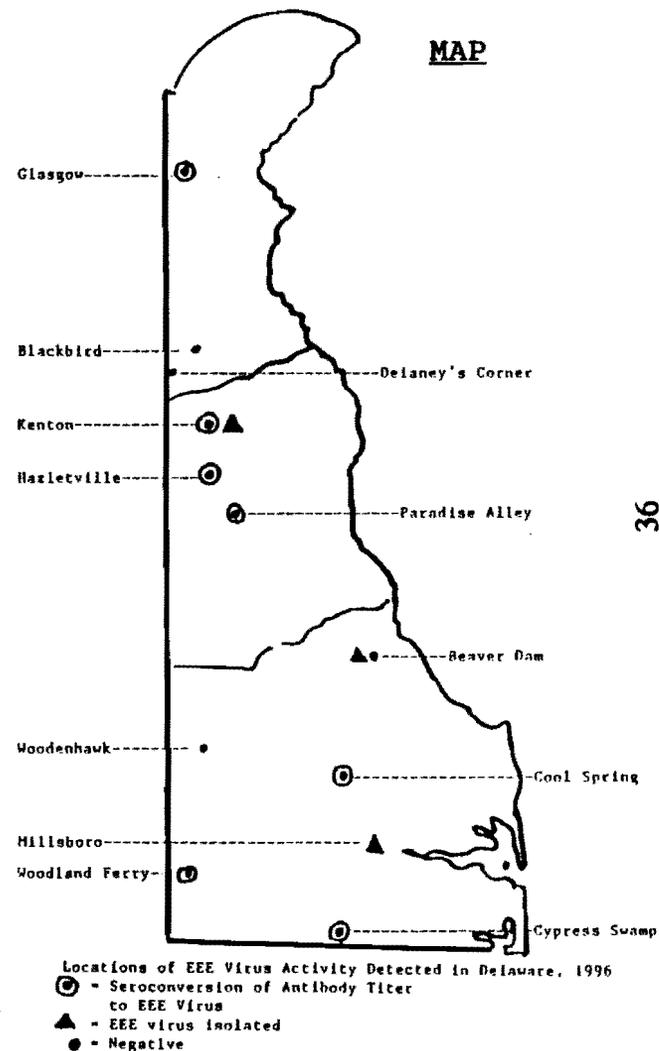
# MISSOURI MOSQUITO SURVEILLANCE - 1996

Christina Lee Frazier, Department of Biology Southeast Missouri State University, Bill Kottcamp
St. Louis County Department of Health, and F Thomas Satalowich Missouri Department of Health

In a continuation of a multi year project, mosquitoes were collected in areas of Missouri effected by flooding of the Mississippi and Missouri Rivers during the summer of 1993 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 and Culex tarsalis mosquitoes were assayed for SLE and WEE, Coquillettidia perturbans and Aedes albopictus were tested for EEE and Aedes albopictus and Aedes triseriatus were assayed for LAC antigen.

During the summers of 1994 mosquitoes were routinely collected in the Kansas City area (Region 4: Atchison, Andrews, Clay, Buchanan, Platte, Jackson, and Lafayette counties) St. Louis area (Region 5: St. Louis (5.1) and Jefferson counties and St. Louis City (5.3)), and the northeast (Region 1: Marion, Ralls, St. Charles, and Lincoln counties), southeast (Region 2: Cape Girardeau, Scott, Perry, Ste. Genevieve and Stoddard counties) and central (Region 3: Boone, Calloway, Cole and Pettis counties) areas of the state. During the summer of 1995 collections were again made in Clay, Buchanan, Jackson, St. Louis, Jefferson, St. Charles, Cape Girardeau, Scott, Perry, Ste. Genevieve and Stoddard counties and St. Louis City . Collections were also made in Butler, Stone and Greene (region 6) counties. In 1996 collections were made in Clay, St. Louis, Cape Girardeau, and Scott counties and St. Louis City.

CO$_2$ traps and gravid traps were used with varying frequencies and mosquitoes were aspirated from resting places such as the walls of culverts and sanitary sewers in some area. Mosquitoes were trapped from the week of May 27, 1996 (week 5) to the week of October 14, 1996 (week 25).

Culex pipiens complex collected by Week
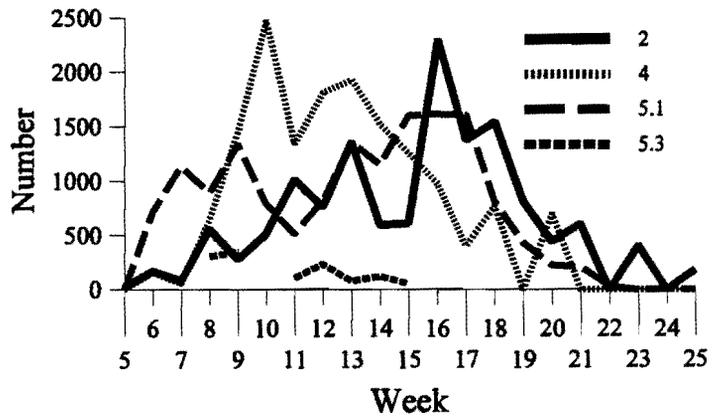
Trap Index for Culex pipiens complex by Region by Week
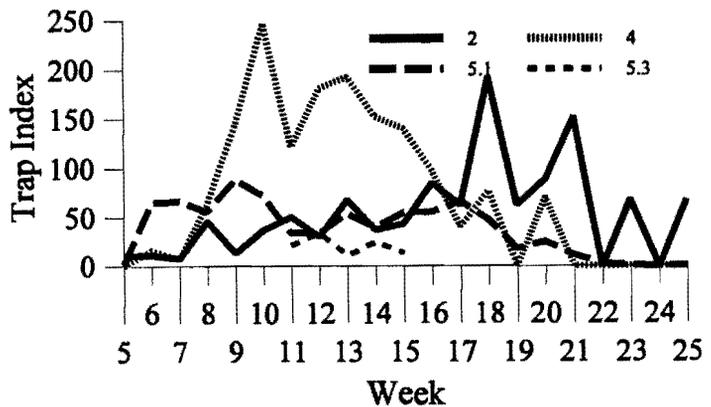
Fewer collections were made in all regions except for St. Louis County (Region 5.1) and the number of Culex complex mosquitoes collected (45676) was lower than 1995(87725) and 1994 (82549). However in two regions the number of mosquitoes per collection was greater than in previous years. Aedes albopictus was collected for the first time at 7 sites that had been collected in previous years and 11 sites collected only in 1996.

Rgn	Yr	Col	Culex pipiens	Trap Index
2	94	521	35773	68.7
	95	459	35135	76.5
	96	256	13694	54.9
4	94	740	24446	33.0
	95	331	12010	36.3
	96	218	15534	71.3
5.1	94	447	10187	22.7
	95	239	10341	43.3
	96	306	15329	50.1
5.3	94	86	4140	48.1
	95	80	7998	100
	96	34	1469	43.2

Summary of Vectors Collected by Region
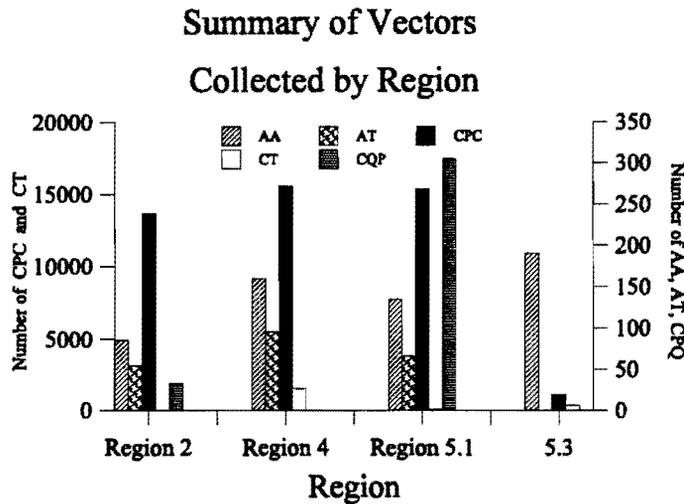
Mosquitoes were sent to the laboratory at Southeast Missouri State University. The ELISAs for SLE, WEE and EEE were performed using reagents provided by CDCP following the protocol outlined by Tsai and colleagues (1) with the addition of a sonication step. Pools tested for WEE and EEE were treated with PBS Tween immediately before testing. 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.

SUMMATION of 1996 POOLS TESTED

Mosquito	Antigen	Pools
Aedes albopictus	LAC	147
Aedes albopictus	EEE	147
Aedes triseriatus	LAC	117
Culex pipiens complex	SLE/WEE	1380
Culex tarsalis	SLE/WEE	85
Coquillettidia perturbans	EEE	55

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New records of arboviruses isolated from mosquitoes in the Northern Territory of Australia, 1982-1992.

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During the 10-year period between 1982 and 1992, 342,737 mosquitoes were collected and tested for virus in the Northern Territory (NT). Viruses representing 10 serogroups were isolated from 12 species of mosquitoes (alphavirus and flavivirus reported previously; Whelan, P.I. and Weir, R.P., 1993. The isolation of alpha and flaviviruses from mosquitoes in the Northern Territory, 1982-1992. Arbovirus Research in Australia. Proceedings of the Sixth Symposium, pp: 270-277). Collection sites were concentrated in the vicinity of major population centres of the NT during peak vector activity or potential periods of virus activity (January - July) or in response to disease outbreaks. Species collected initially were those of human pest species in relatively high numbers, but later collections concentrated on species from which human disease-causing viruses had been isolated in the NT or in other parts of Australia.

Identification of the viral isolates (14 members of the family Bunyaviridae and seven bunyavirus-like viruses, 176 orbiviruses and 24 orbivirus-like viruses, one member of the family Rhabdoviridae and one rhabdovirus-like virus, and six unidentified viruses) has extended the known distribution of several viruses to include the NT as well as increasing the number of mosquito species from which members of genera *Bunyavirus* (family Bunyaviridae) and *Orbivirus* (family Reoviridae) and various rhabdoviruses have been isolated. A total of 38 viruses which did not fall into any recognised serogroup were isolated from seven mosquito species. Preliminary electron microscopic and serological testing have placed the isolates into four groups a) bunyavirus-like, seven isolates of which 5 are serologically identical, b) orbivirus-like, 24 isolates of which two are serologically related, c) a rhabdovirus-like virus unrelated to any Australian rhabdovirus and d) nine as yet unclassified, possibly new, viruses.

More detailed results (source mosquitoes, locations, dates, etc.) will be reported at the Seventh Australian Arbovirus Symposium, Brisbane, and will be published in scientific journals.

Table 1. Summarized results of identifications of viruses from mosquitoes collected in the Northern Territory, Australia, 1982-1992.

Orbiviruses

Wongorr serogroup: 129 isolates, including Wongorr, Paroo River, and Picola viruses.

Wallal serogroup: 9 isolates, including Wallal and Mudjinbarry viruses.

Warrego serogroup: 10 isolates of Warrego virus

Eubenangee serogroup: four isolates of Eubenangee virus

Corriparta serogroup: 18 isolates of Corriparta virus

Bluetongue serogroup: two isolates of BLU-16 virus

Palyam serogroup: one isolate of CSIRO Village virus

Bunyaviridae

Mapputta serogroup: 10 isolates, including Mapputta, Trubanaman, Gan Gan, and a Mapputta serogroup virus

Leanyer virus: one isolate

Other: four isolates of Kowanyama and one of Yacaaba viruses.

Rhabdoviridae

Oakvale: one isolate

INTERIM REPORT FROM THE NEW YORK STATE HEALTH DEPARTMENT'S ARBOVIRUS LABORATORY

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During the 1996 mosquito season the laboratory has processed more than 238,000 mosquitoes in more than 4,300 pools. Species tested in greatest numbers were *Aedes canadensis, Ae. stimulans, Ae. trivittatus, Ae. vexans, Coquillettidia perturbans, Culex pipiens-restuans and Culiseta melanura*. They were collected in 10 counties of western and central New York State and Long Island.

The first strains of eastern equine encephalitis (EEE) virus of the year were isolated from *Cs. melanura* and *Cx. pipiens-restuans* mosquitoes collected on 30 July 1996 in Suffolk County, Long Island. A total of 26 isolates of EEE virus was made from that county, the last being from *Cs. melanura* collected on 24 September 1996. All but three of the isolates were from that species, the remainder being from *Cx. pipiens-restuans*.

A single isolation of a California group virus was obtained from *Ae. cantator*, and one of Highlands J (HJ) virus from *Cs. melanura*, both collected at the extreme eastern end of Long Island on 1 August and 13 September, respectively. This year was unusual in that when EEE virus is abundant here, HJ virus is often abundant also, but that was not the case this time, even though Rhode Island and Massachusetts had many isolates of HJ virus.

The only virus isolations from mosquitoes from central NY State were one of EEE virus from *Cs. morsitans* and two of EEE virus from *Cs. melanura*, collected from three different sites in the same county from 5-7 August 1996.

There were six isolations of virus from western NY State, all of the California group. The first was from *Ae. canadensis* collected on 13 June, the second from *Ae. stimulans* collected 19 June, and the rest from *Ae. trivittatus*. The last positive mosquito pool was collected on 19 July.

The California group isolates were tentatively identified by the immunofluorescence test as Jamestown Canyon and trivittatus viruses, pending confirmation by PCR and sequencing.

There were no confirmed cases of arbovirus encephalitis in humans, horses, or other sick animals or birds, and no seroconversions in sentinel pheasant flocks kept in central NY State.

Effect of Interferon on the synthesis of Mayaro Virus Glycoproteins

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We have investigated the effect of recombinant interferon (IFN) α 2b on the Mayaro virus protein synthesis. TC7 cells (monkey kidney cells) were pretreated with IFN (10^3 IU/mL) and infected with Mayaro virus. Cells were pulse-labelled with ^{35}S methionine at 20 hours post infection and processed for radio immuno precipitation. The structural proteins were analysed by SDS-PAGE and the autorradiogram submitted to a densitometric scan. We observed an inhibition of 28% of the capsid protein (p34) while the glycoprotein precursors p110 and p62 were inhibited 84% and 77% respectively. The glycoprotein E1 (p52) was inhibited 99% and E2 (p50) 79%. These data show that the inhibition of virus structural protein synthesis is selective, that is, the glycoproteins and their precursors are most drastically inhibited by the IFN treatment than the capsid protein.

As described by Maheshwari et al (1, 2) the IFN increases the intracellular pH and this effect interferes with the glycoproteins transport. The authors also showed a potentialization of virus yield inhibition when the cells were treated simultaneously with IFN and weak bases. Based upon these observations, we pre treated or not TC7 cells with increasing doses of IFN and posteriously infected with Mayaro virus. After the adsorption period, fresh culture medium containing 20mM of amonium chloride or chloroquine 0.2 mM was added to the cells. After 20 hours post infection, the supernatant was recovered for yield assay and the monolayer incubated with ^{35}S methionine for 1 hour and processed for SDS-PAGE. We observed an inhibition of the virus yield in 66.2% after treatment with weak-bases alone. The pre-treatement with a low dose of IFN (10 IU/mL) inhibited virus yield in 74.3%. When the cells were pre treated with 10 IU/mL of IFN and co-treated with the weak base the inhibition reached values of 97.6%, an enhancement of nearly 90%.

When we analyse the profile of the protein synthesis obtained from infected treated cells we observe that weak bases in the used concentration do not inhibit virus protein synthesis. If, however, these weak-bases treated and infected cells are pre- treated with a low dose of IFN (10 IU/mL) we notice that the virus glycoproteins (p50/p52) and their precursors are inhibited. The capsid protein, however, is still visible even when we increase the IFN dose. These results suggest that weak bases enhance the effect of IFN on the synthesis of glycoproteins and their precursors indicating that the intracellular pH may play a role in the process.

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INHIBITION OF MAYARO VIRUS REPLICATION BY PROSTAGLANDINS A_1 AND B_2 IN VERO CELLS.

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Prostaglandins (Pgs) are a group of cyclic 20-carbon fatty acids that are synthesized by most types of cells and are involved in the control of many physiological phenomena including cell growth and differentiation, immune function, inflamation and virus replication. In the present report we describe the effect of PGA_1 and PGB_2 on the replication of Mayaro virus in Vero cells. Prostaglandin A_1 and B_2 were found to potently suppress the replication of Mayaro virus. Vero cell cultures treated with Pgs reduced viral yield when compared to untreated controls 24h after infection with Mayaro virus. We found a reduction of 95% in progeny virus at the concentration of 10 ug/ml of PGA_1. At this concentration PGB_2 was somewhat less effective resulting in 58% inhibition of virus yield. Under conditions described, no toxic effects on the cells were observed as demonstrated by microscopic examination and vital (trypan blue) dye uptake. The effect of PGA_1 on macromolecular synthesis was studied in infected and non infected cells. In both infected and non infected cells PGA_1 did not suppress the incorporation of $[^3H]$ thymidine, $[^3H]$ uridine and $[^{35}S]$ methionine into macromolecules. When the time of PGA_1 and PGB_2 addition post infection was increasingly delayed, an antiviral effect though more and more reduced could still be observed as late as 6h post infection However, removal of PGA_1 at earlier times post infection led to a decrease in inhibition. This suggests that in order to be inhibitory, PGA_1 had to be present during the initial times of viral replicative cycle. SDS-PAGE analysis of ^{35}S-methionine labeled proteins showed that PGA_1 moderately inhibited the synthesis of protein C (34KDa). However, we found a more effective inhibition in the synthesis of glycoproteins E_1, and E_2 (52 and 54 KDa), suggesting an action of PGA_1 at the glycosylation process.

INTERFERENCE WITH YELLOW FEVER VIRUS REPLICATION IN CELLS INFECTED WITH SINDBIS VIRUSES EXPRESSING FLAVIVIRUS SEQUENCES.

S.Higgs, J.Rayner, K.E.Olson, B.S.Davis, B.J.Beaty and C.D.Blair.

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Double subgenomic Sindbis (dsSIN) expression viruses were engineered to transduce mosquito cells with sense and antisense RNAs derived from yellow fever virus (YFV, strain 17D). cDNAs encoding the flavivirus premembrane (prM) proteins were inserted, in sense and antisense orientations into the dsSIN plasmid pTE/3'2J. C6/36 (*Aedes albopictus*) cells infected with the recombinant dsSIN viruses containing the PrM sequences were less susceptible to subsequent infection with YFV (strains 17D and Asibi) (Table 1) and produced significantly less YFV than did cells infected with control dsSIN viruses lacking the YFV sequences (Table 2). These data indicate that antisense sequences targetting the PrM region of the YFV genome may be applicable to reducing transmission of all YFV strains.

Table 1. Interference with YFV replication in C6/36 mosquito cells infected with dsSIN viruses.

Challenge	% of cells positive for SIN E1 protein at 72h pi.	% of cells positive for YFV E protein at 120h post-challenge
Asibi		
No dsSIN	0	100
TE/3'2J	100	100
YFV PrMa	100	40
YFV PrMs	100	0.3
17D		
No dsSIN	0	93
TE/3'2J	100	70
YFV PrMa	100	2.3
YFV PrMs	100	0.1

Table 2. Interference in C6/36 cells to YFV (Asibi)

Titer ($\log_{10}$ TCID$_{50}$) of YFV After Inoculation With

Days PI	YFV.As Only	YFV.As+ TE/3'2J*	YFV.As+ YFprMa	YFV.As+ YFprMs
0	2.5	2.5	1.5	1.5
1	2.5	2.5	0.0	0.0
2	4.5	4.5	1.5	1.5
3	4.5	4.5	1.5	1.5
4	7.0	6.0	3.0	2.5

*TE/3'2J=control dsSIN virus containing no insert.

INTERFERENCE WITH YELLOW FEVER VIRUS REPLICATION AND TRANSMISSION IN MOSQUITOES USING SINDBIS VIRUSES EXPRESSING FLAVIVIRUS SEQUENCES.

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Double subgenomic Sindbis (dsSIN) expression viruses were engineered to transduce mosquito cells with RNAs derived from yellow fever (YF) virus. cDNAs encoding the flavivirus premembrane (prM) and putative polymerase (NS5) proteins were inserted, in sense and antisense orientations, into the dsSIN plasmid pTE/3'2J. These viruses and wild-type YF viruses were inoculated into *Aedes aegypti* mosquitoes. Dually infected mosquitoes did not transmit African YF virus and poorly transmitted South American YF virus (Table 1). Thus targeting antisense sequences to PrM or NS5 regions of the YF virus genome may be applicable to controlling transmission of all YF virus strains.

Table 1. Transmission data for mosquitoes coinoculated with dsSIN constructs and YFV (either BA-55 or 1899/81). "?" is used to indicate that although a specimen was probably negative, a small area of weak fluorescence was observed. No reduction of DEN-2 strain 16681 infection and transmission rates were observed in mosquitoes coinoculated with TE/3'2J/YFV NS5a (data not shown).

| dsSIN construct | YFV BA-55 | | | YFV 1899/81 | | |
	Infected	Transmitting	(%)	Infected	Transmitting	(%)
None (L-15)	5/5	3/5	60%	11/11	8/10	80%
2J	10/10	7/10	70%	11/11	9/10	90%
CAT	10/10	7/9	78%	nd	nd	nd
DEN-2 PrMa	4/4	4/4	100%	nd	nd	nd
YFV PrMa	1?/4	0/4	0%	1/9	1-3?/8	12.5-37.5%
YFV PrMs	9?/10	1/10	10%	6/8	4-5?/9	44-55%
YFV GDDa	0/10	0/10	0%	2/9	1/10	10%
YFV GDDs	8/8	3/8	38%	9/9	7/10	70%

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EXPRESSION OF TUMOR NECROSIS FACTOR- ALPHA (TNF-α) mRNA IN MACROPHAGES INFECTED WITH DENGUE VIRUS.

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Soluble mediators such as cytokines (IL-1, IL-6, and TNF-α) produced by macrophages infected with dengue virus have been implicated as a trigger mechanism in the pathogenesis of Dengue Hemorrhagic Fever/Dengue Shock Syndrome, DHF/DSS (1,2).

Tumor Necrosis Factor (TNF-α) causes hypotension, hemorrhagic necrosis, disseminated intravascular coagulation, fever headache, myalgia, vascular injury, capillary leakage and the promotion of thrombocytopenia; a similar phenomenon is observed in DHF/DSS patients. Additionally, TNF-α has effects on cytokine and lipid mediator production and additive or synergistic interactions with other cytokines (2, 3).

In the present work we report the expression of TNF-α mRNA in a human macrophage cell line infected with dengue virus.

The human macrophage cell line U-937 was obtained from the American Type Culture Collection (Rockville,Md) and cultured in RPMI 1640 supplemented with 10% FCS (GIBCO); 1×10^6 cells/culture were infected with a moi=0.1 of dengue virus serotype 2 (NGC strain). A non-infected culture stimulated with an inducer of TNF-α (TPA, 10 nM) was included as a control. Dengue virus was detected in infected macrophages by RT-PCR according to the procedure described by Lanciotti et al (4).

Cellular RNA was extracted using the TRIzol reagent (GIBCO, BRL) at 2 and 8 hr postinfection; cDNA was produced by reverse transcriptase (RT) reaction at 42°C, 1 hr using an oligo $(dT)_{18}$ as a primer. The RT products were amplified during 35 cycles by PCR (94°C, 45 sec., 60°C, 45 sec., 72°C, 2 min), using specific primers for TNF-α (5´- GAGTGACAAGCCTGTAGCCCATGTTGTAGCA-3´ and 5´- GCAATGATCCCAAAGTAGACCTGCCCAGACT-3´) to generate an amplimer of   444 pb. The PCR amplified products were separated in 6% PAGE, stained with ethidium bromide and visualized under a UV lamp.

At 2 and 8 hr postinfection, there was an increment in the expression of mRNA for TNF-α similar to that observed in macrophages treated with TPA. Similar results were obtained in two independent experiments. The increased level of mRNA for TNF-α in macrophages infected with dengue virus could support the role of TNF-α in the physiopathology of dengue virus infection in humans. This preliminary result may be supported by findings published by Hober et al who reported high levels of TNF-α in children and adult patients with grades III and IV of DHF (5). Additionally, Ingkaran et al (6) detected an augmented level of TNF-α in children who had primary dengue virus infection with clinical features of Reye's syndrome.

Detection of mRNA for other inflammatory cytokines such as IL-1α and IL-6 is in progress.

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EPIDEMIC OF HAEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) IN CROATIA IN 1995. PRELIMINARY REPORT.

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In 1995, we recorded the largest HFRS epidemic in Croatia to date. Among 203 suspected HFRS patients, we confirmed approximately 42% of them by enzyme-linked immunosorbent assay (ELISA) IgM and IgG tests and Hantaan (HTN) and Puumala (PUU) antigens[1]. Our finding of specific antibodies against HTN and PUU antigens in HFRS patients is in accordance with previous findings in Croatia[2]. It seems that both, HTN- and PUU-like viruses circulate in this region. Sera of approximately one third of our patients were tested for specific IgG against Dobrava antigen. We did not find any of them to be positive. This does not mean that in some other cases we may not find Dobrava infections[3]. On the north-west border, Croatia has possibility to exchange Dobrava virus with Slovenia[4]. Most of the patients were soldiers of Croatian Army and the disease occurred as an epidemic at no less than three particular spots in Croatia[5]. Patients developed severe but also moderate and mild clinical features with renal, but also pulmonary and some gastrointestinal and neurological symptoms. Deaths were recorded as well. It is interesting that among 50 patients hospitalised in the University Hospital for Infectious Diseases Zagreb, 50% of them developed severe course of the disease with hemorrhagic manifestations, acute renal failure and severe complications[5,6]. The disease has been reported to have usually moderate and rarely severe course in Croatian HFRS patients until now[7].

In 22 of our patients we looked for changes of peripheral blood mononuclear cell phenotype. The results of double- and triple-colour immunofluorescence analysis showed increased percentage of cytotoxic T-cells (CD3+CD8+) and activated T-cells. The percentage of naive (CD4RA+) cells were decreased, while that of memory (CD45RO+) T-cells were elevated. CD23+ and CD21+ B-lymphocytes were also lower in seropositive patients[8].

Croatia and two neighbouring countries, Bosnia and Herzegovina (BH) and Slovenia are known as endemic HFRS regions. The war, together with other epidemiological and ecological factors, precipitated the greatest HFRS epidemic in Croatia. Although, the first report of HFRS in the former Yugoslavia was done in Croatia[9], we still have a lot of unknown facts about HFRS here[10]. This requires an additional effort in HFRS research in Croatia. However, no matter what steps we take, we can never ignore the threat from hantaviruses.

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HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) IN NORTH-WESTERN CROATIA

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The north-western Croatia (Medimurje) has not been recognised as an HFRS area until now. The first HFRS case was reported in 1989 [1]. However, the leptospirosis has been endemical to Medimurje. Five patients with developed acute renal failure (Table 1) were tested on Leptospira in acute and early convalescent phase. Four of them found to be positive on different Leptospira strains with antibodies titer between 1:500 and 1:4000. Three to fourteen months later, all of them were tested on Hantaan (HTN) and Puumala (PUU) antigens by ELISA IgM and IgG tests. We found specific IgG antibodies with cross-reaction to both antigens in each patient. The first patient had also specific IgM antibodies to both antigens (Table 1). The last patient in table 1 had no antibodies against Leptospira, but positive anti-HBc IgM associated with acute hepatitis B.

As the major reservoir of both infecions (leptospirosis, HFRS) are rodents, there is a possibility that people become infected simultaneously with the hantaviruses (HTV) and Leptospira [2]. The possibility of dual infection with HTV and hepatitis B virus shuld be additionaly researched. However, an additional case with acute HFRS and hepatitis B was reported in the northern Croatia during 1995. (Dr.B.Križanović, personal communication).

Our results show that in the north-western part of Croatia we should expect some HFRS cases.

Table 1. Patients with developed acute renal failure in the north-western part of Croatia

Patients	Urea (μmol/l)	Creatinine (μmol/l)	Haemodialysis (days)	Serology (positive)
1.	19.09	304.6	N.D.	LPT, HTV
2.	24.60	196.9	N.D.	LPT, HTV
3.	25.9	691.4	4	LPT, HTV
4.	31.2	751.3	5	LPT, HTV
5.	34.4	976.5	9	HBV, HTV

LPT - Leptospira, HTV - hantaviruses, HBV - hepatitis B virus

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ONGOING LONGITUDINAL STUDIES OF SIN NOMBRE AND OTHER HANTAVIRUSES IN DIVERSE ECOSYSTEMS IN COLORADO, 1994-96

Longitudinal capture-release studies of transseasonal transmission of Sin Nombre virus (SNV; family Bunyaviridae, genus *Hantavirus*, the etiologic agent of hantavirus pulmonary syndrome, were continued at three ecologically diverse sites in Colorado.

Trapping of small mammals at each site each six weeks has revealed the presence of antibody (IgG ELISA) to a hantavirus, presumably SNV at Fort Lewis (montane shrubland; west of Durango in southwestern Colorado = FL) and Molina (montane shrubland and semidesert shrubland; east of Grand Junction in west central Colorado = MOLINA) and both SNV and El Moro Canyon (ELMC) virus at Piñon Canyon (short grass prairie, canyon, and piñon-juniper ecosystems; south of Rocky Ford in southeastern Colorado = PC).

In six months of trapping at the FL site in 1994, seroprevalence ranged from 0% to 15.6% (mean=8.6%) in deer mice (*Peromyscus maniculatus*, the ostensible principal vertebrate host of SNV). Many fewer deer mice were trapped at the FL site in 1995 but antibody prevalence in these rodents remained between 0% and 12.5% (mean=10.4%). None of 46 deer mice at this site had antibody in 1996. At MOLINA, prevalence of antibody to SNV in deer mice was 0% in October 1994, ranged from 2.3% to 33% (mean=16.1%) in 1995, and has declined from a high of 24% in April of this year to 0 in October, with peak prevalences in early summer of 1995 and 1996. Both the FL and MOLINA study sites are ecologically disturbed areas with a limited variety and number of rodents (5.9 deer mice/145 Sherman traps/night at FL; 9.6 deer mice/145 Sherman traps/night at MOLINA).

In contrast, since January 1995 individuals belonging to 16 rodent species have been trapped at the more ecologically diverse PC sites. Western harvest mice (*Reithrodontomys megalotis*), piñon mice (*P. truei*), and deer mice have been the most common species and the only ones with antibody; 25/251 (10%) of western harvest mice, 6/370 (1.6%) piñon mice, and 4/338 (1.2%) deer mice. Antibody prevalence in harvest mice peaked in the springs of 1995 and 1996 and antibody prevalence varied with size of the rodent population and season of the year.

Although male deer mice, piñon mice, and western harvest mice represent about 50% of the populations of those species, 62%-90% of the rodents with antibody have been males.

Many animals have been recaptured at each site. Seroconversions have been detected in rodents at all sites. Data thus far suggest that infections can occur at essentially any time of year but more frequently occur in late summer-early autumn and early winter-early spring. This may indicate that peak populations and cold weather "huddling" of rodents may be a risk factor for acquiring infection with the hantaviruses at these sites. Certain few animals have been captured repeatedly over as long a period as 18 months and have not yet seroconverted.

Molecular characterizations (by Joan Rowe, University of Nevada, Reno) of M and S RNAs from lungs of a persistently-infected piñon mouse and a persistently-infected harvest mouse at PC identified SNV and ELMCV, respectively. These data suggest the possibility that reassortment of RNA segments between these closely related viruses could occur and that genetic

diversity of hantaviruses at these sites could ensue. However, because >50% of the animals are not recaptured more than once (at 6-week intervals) and >80% are not recaptured more than three months after they are first captured, it appears that their life spans are relatively brief. Unless unusual conditions develop (for example, high rodent populations resulting from high rainfall providing conditions suitable for production of an abundance of food supplies), the rate of transmission of hantaviruses at these sites must be low because of low rodent population densities, low infection rates, and short life spans.

We hypothesize that there are two mechanisms of transmission of hantaviruses at these sites. One, summer transmission among males competitively fighting for females, territory, food and access to other necessities, may represent epidemic period transmission. This hypothesis is supported by experimental data and field observations on Seoul virus in rats in Baltimore and in South Korea and Japan and by recent summaries of North American data regarding SNV by Jim Mills and others at CDC/Atlanta. Recently it was reported that Seoul virus infection of newborn rats (*Rattus norvegicus*) resulted in persistent infection, the rats being infected for the 180 days of the study. Rats of the same species, but infected with Seoul virus when they were seven weeks old, became infected but the infection was quenched after no more than 50 days. There is no reason to believe that everything we know about one hantavirus can be applied to all other hantaviruses. If, however, mechanisms of infections are the same, we suggest that fighting might be an important mechanism for transmission of hantaviruses when populations are high or food supplies are low or both, but that it takes infection of newborns to create conditions under which virus can persist for extended periods. This is a testable hypothesis.

The other suggested mechanism, from long-lived, persistently infected to uninfected rodents, may serve as the transseasonal mode of transmission. We have recaptured male and female infected rodents for as long as nine months after they were first shown to have antibody.

Continuing these studies should provide information useful in characterizing an interepidemic period for SNV and for determining the conditions required to initiate an epizootic/epidemic state.

(Charles H. Calisher[a], Barry J. Beaty[a], William P. Sweeney, Jr.[a], K. Max Canestorp[b], Ted Davis[c], James N. Mills[d], and Gordon Smith[a])

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Processing of LaCrosse virus envelope glycoproteins expressed by a Sindbis replicon in mosquito cells

K.I. Kamrud, K.E. Olson, S. Higgs, and B.J. Beaty (Arthropod-borne and Infectious Disease Laboratory, Colorado State University, Fort Collins, CO 80523)

Processing and trafficking of envelope proteins of viruses of the family Bunyaviridae have been studied in vertebrate cell culture. Most members of the family are transmitted by arthropods but studies of the processing and trafficking of arthropod-borne bunyavirus envelope proteins in insect cells has not been done. We investigated the processing and trafficking of La Crosse (LAC) virus (family Bunyaviridae, genus *Bunyavirus*) in *Aedes pseudoscutellaris* (AP-61) and *Aedes albopictus* (C6/36) mosquito cells. The G1 and G2 proteins of LAC virus were expressed individually using the Sindbis replicon system. Replicon-expressed LAC envelope proteins were processed through the endoplasmic reticulum (ER) and Golgi complex correctly, as compared to wild type LAC proteins by endoglycosidase H digestion. LAC G1 protein, when expressed by itself, exhibited a dispersed cellular localization but became localized to the Golgi region of AP-61 cells when G2 protein was coexpressed in the same cell, as determined by indirect immunofluorescence. In addition, expression of a truncated form of the G1 protein (lacking 270 amino acids from the carboxyl end) exhibited similar Golgi type localization when expressed with LAC G2 protein. This suggest that the region of the G1 protein associated with G2 protein for movement into the Golgi is not found in the carboxyl end of G1.

A grid-like G2 protein distribution, in addition to the Golgi type localization, was noted in AP-61 cells expressing the replicon G2 construct that was not evident in BHK-21 cells. The NSm protein of tomato spotted wilt virus (a member of the family Bunyaviridae, genus *Tospovirus*) has been postulated to be involved in cell-to-cell movement of ribonucleocapsid structures. 257 base pairs from the 5' end of the NSm gene (LAC M segment gene order: 5' G2-NSm-G1 3') were included in the G2 expression construct. Perhaps this region of the NSm protein interacts with G2 protein and together they interact with mosquito cell cyto-skeletal structures resulting in the grid-like fluorescence.

The Connecticut Agricultural Experiment Station
Yale Arbovirus Research Unit

1996 Mosquito Arbovirus Surveillance

September 9 - October 18, 1996

Theodore G. Andreadis (CAES)
John F. Anderson (CAES)
Shirley Tirrell-Peck (YARU)

Mosquito Species	No tested		No isolations	
	Specimens	**Pools**	**EEE**	**HJ**
Aedes canadensis	270	22		1
Aedes cantator	316	43	1	1
Aedes cinereus	63	11		
Aedes sollicitans	307	31	2	
Aedes taeniorhynchus	152	17		
Aedes triseriatus	126	31		
Aedes trivittatus	30	7	1	
Aedes vexans	475	74	1	3
Anopheles punctipennis	94	46		
Anopheles quadrimaculatus	4	4		
Coquilletidia perturbans	46	14	1	
Culiseta melanura	2,322	112	19	9
Culiseta morsitans	36	9	3	1
Culex pipiens	1,876	138	8	3
Psorophora ferox	1	1		
Uranotaenia sapphirina	322	60		
TOTAL	6,440	620	36	18

Among many more seriously scientific reports to the September 1960 issue of <u>Arbovirus</u> <u>Information Exchange</u>, was the one below. I reprint it for its humor. Other articles in that issue include those reporting for the first time evidence for: human infection with Everglades virus (T.H. Work), Ilheus virus from birds in Panama (A.C. Pipkin), phleboviruses from mosquitoes (A. Shelokov), viral isolates from arthropods in Pakistan and Iran (H.C. Barnett), a dengue virus as the etiologic agent of hemorrhagic fever in the Philippines and Thailand (W.McD. Hammon), Kern Canyon virus from a bat in California (H.N. Johnson and E.H. Lennette).

NOTES FROM DR. WILLIAM F. SCHERER,
UNIVERSITY OF MINNESOTA MEDICAL SCHOOL, MINNEAPOLIS,
ON THE SEITZ EK FILTER AND ITS INSIGNIFICANCE

<u>Initial inquiry</u>

April 28, 1960

Republic Seitz Filter Corporation
17 Stone Street
Newark 4, New Jersey

Dear Sirs:

We have used extensively the small Seitz EK filter pad for Swinney syringe adapters supplied by Becton-Dickinson Company. Upon writing BD Company I was informed that the pore size for Seitz EK filters is 0.1 micron. Could you please inform me whether this is the minimum pore size or the average pore size? ... Also, could you let me know...how you measure the pore size of a filter.

I shall appreciate your prompt reply.

Sincerely yours,

Rob Ra, M.D.

<u>First Reply</u>

May 2, 1960

Brochure arrives with letter stating:

"The pore size of our filter pads was determined by passing known particles of standard sizes through the pads to determine which were retained and which were passed.

We trust this information will serve you."

Very truly yours,

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Republic Seitz Filter Corp.

June 7, 1960

Republic Seitz Filter Corporation
17 Stone Street
Newark 4, New Jersey

Dear Sir:

Thank you for your letter of 2 May 1960 and accompanying brochure which describes your Seitz filter pads for Swinney adapters. I still have some further questions, however, which I would appreciate your answering.

1. "What do you mean by pore size range?" You list the "pore size range" of an EK filter as 0.1 micron. How can a single figure be a range? Is this an average pore size or a minimum pore size?

2. What kind of particles of known standard size do you use to determine pore size of your filter?

3. When you speak of pore size, do you mean the size of the hole in the filter or do you mean the size of the particle that goes through the filter?

4. How do you calculate the size of the hole in the filter from the known size particle which passes through it?

5. What diluent do you use for the particles of known size which are used for standardization of filters?

We shall greatly appreciate your prompt reply.

Sincerely yours,

Rob Ra, M.D.

Second Reply

June 16, 1960

Dr. Rob Ra
The Medical School

Dear Dr. Rob Ra:

We are replying to your letter of June 7 concerning filter pads for Swinney adapters. In answer to your inquiries, we wish to submit the following information.

1. By "pore size range" we mean to convey the information that each porosity of pad will hold back particles as large and larger than the dimension shown on the literature.

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2. The particles used for the determination of the capacity of filter sheets in the clarifying grade were known sizes of charcoal. For the "GP" and "S" grade we used Serratia marcesens. <u>The last three grades EK, S1 and S3 were evaluated by our parent company, the Seitz Company of Germany, and I do not have a record of the exact particles used by them for their determinations.</u>*

3. We believe our explanation No. 1 will answer this.

4. We do not calculate the hole size, we simply grade the pad according to the particle size which it retains. In actual operation asbestos filter pads will retain, by adsorption, particles of a smaller size than the actual interstices of the pad so that size of hole is not necessarily the determining factor.

5. Distilled water was used to hold the suspended particles in all tests.

We trust the above information will help you.

Very truly yours,

Republic Seitz Filter Corp.

Third inquiry

June 30, 1960

Republic Seitz Filter Corporation
17 Stone Street
Newark 4, New Jersey

Dear Sir:

Thank you for your letter of 16 June 1960 which answers most of our questions.

However, we are still a bit confused about the procedure for evaluating "pore size range" for EK filters. Would it be possible for you to find out from your parent company, the Seitz Company of Germany, what kind of particle is used for this determination?

Also, could you tell us whether you spot check the "pore size range" by periodically sending some of your filters to Germany? Or was this determination done merely a number of years ago and you only follow the procedure for making the filters originally worked out by the Seitz Company.

Moreover, since you do not have a record of the exact particles used for the EK filters, can I infer that you do have a record that distilled water was used to suspend the particles used to test EK filters?

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*Underlining by Editor.   59

I am sorry to keep bothering you, but this information is extremely important to us in our research.

Yours truly,

Rob Ra, M.D.

Third Reply

Dr. Rob Ra
The Medical School

Gentlemen:

We are replying to your letter of June 30 with further reference to pore size range determination.

We are sorry we gave you the wrong impression about our relationship with the Seitz Company of Germany. While it is true that they were our parent company, we were forced to part company with them during World War II. Since then, like many children who have left their parents, we are no longer on speaking terms.

You can therefore see that it is impossible for us to send to Germany for any information, so that you are correct when you state this determination was done years ago and we have been following the procedure laid down by the Seitz company. We can, however, assure you that all determinations were made with distilled water.

I would now like to ask a question concerning you research. As we are in the process of setting up new evaluating procedures, could you recommend standard uniform sizes that we could use for this purpose? We would certainly appreciate any recommendations you could offer us.

Yours truly,

Republic Seitz Filter Company

Editor's Note

The above exchange of correspondence is factual. Any resemblance to filters of known pore size is purely coincidental.

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QUOTES

Robert Frost: "The best things and best people rise out of their separateness; I'm against a homogenized society because I want the cream to rise."

Dora Russell: "We want better reasons for having children than not knowing how to prevent them."

Voltaire: "The art of medicine consists of amusing the patient while Nature cures the disease."

David Frost: "He left his body to science and science is contesting the bill."

Samuel T. Coleridge: "There is nothing insignificant."

Rucherts' Law: "There is nothing so small that it can't be blown out of proportion."

Jordi Casals: "A virus or species is a cluster of different individualities grouped around and resembling a prototype or model, rather than a number of strains all identical with a prototype." (Editor's note: He understood "quasispecies", even if that word had not then been invented.)

Rod Gilbert: "If you don't know where you are going, you might end up where you are headed."

Charles Kettering: "If you want to kill an idea, get a committee to work on it."

Mark Twain: "If you pick up a starving dog and make him prosperous, he will not bite you. This is the principal difference between a dog and a man."

Joseph Joubert: "It is better to debate a question without settling it than to settle a question without debating it."

Sydney J. Harris: "The primary purpose of a liberal education is to make one's mind a pleasant place in which to spend one's time."

Tom Stoppard: "I don't think I can be expected to take seriously any game which takes less than three days to reach its conclusion."

Reverend G. Avery Lee: "When Congressman Newt Gingrich was a graduate student at Tulane University, I baptized him by immersion into the membership of the St. Charles Avenue Baptist Church. Perhaps I didn't hold him under long enough."

S.B. Calisher (but not the first one): "Correlation is not the same as causality."

Hans Zinnser: "Nothing in the world of living things is permanently fixed."

Kurt Vonegut: "Trout once wrote a short story about a conversation between two yeast cells. Due to their limited intelligence they never knew that while they were making champagne they actually were dying in their own excrement."

Bill Vaughn: "We hope that, when the insects take over the world, they will remember with gratitude how we took them along on all our picnics."

Annie Savoy (Bull Durham): "I believe in the church of baseball. I've tried all the major religions, and most of the minor ones. I've worshipped Buddha, Allah, Vishnu, Siva, trees, mushrooms and Isadora Duncan. I know things. For instance, I know there are 108 beads in a Catholic Rosary and 108 stitches in a baseball. When I learned that, I gave Jesus a chance. But it just didn't work out between us. The Lord just laid too much guilt on me. I prefer metaphysics to theology. You see, there's no guilt in baseball. And it's never boring. It's a long season and you gotta trust it. I've tried 'em all, I really have, and the only church that truly feeds the soul, day in, day out, is the church of baseball."

Henry David Thoreau: "People talk about Bible miracles because there is no miracle in their lives. Cease to gnaw the crust. There is ripe fruit over your head."

Robert Browning: "The rest may reason and wonder, but 'tis only we bassoonists know."

Shelley Calisher: "There is a relationship between political and ethical convictions."

Dylan Thomas: "Do not go Gentle into that Good Night. Rage, rage against the Dying of the Light."

Thomas Jefferson: "I haved lived temporately. I double the doctor's recommendation of a glass and a half of wine a day, and even treble it with a friend."

Samuel Butler: "People in general are equally horrified at hearing the Christian religion doubted, and at seeing it practiced."

Red Smith: "Ninety feet between the bases is the nearest thing to perfection that man has yet achieved."

Branch Rickey: "Man may penetrate the outer reaches of the universe. He may solve the very secret of eternity itself. But for me, the ultimate human experience is to witness the flawless execution of the hit and run."

Dwight D. Eisenhower (34th President of the United States): "When I was a small boy in Kansas, a friend of mine and I went fishing. I told him I wanted to be a real major league baseball player, a genuine professional like Honus Wagner. My friend said that he'd like to be President of the United States. Neither of us got our wish."

John Ruskin: "This is the true nature of home- it is the place of Peace; the shelter, not only from all injury, but from all terror, doubt, and division. Insofar as it is not this, it is not home: so far as the anxieties of the outer life penetrate into it, and the inconsistently minded, unknown, unloved, or hostile society of the outer world is allowed by either husband or wife to cross the threshold, it ceases to be home; it is then only a part of that outer world which you have roofed over, and lighted fire in."

Fyodor Dostoyevsky: "Love animals: God has given them the rudiments of though and joy untroubled. Do not trouble their joy, don't harass them, don't deprive them of their happiness, don't work against God's intent. Man, do not pride yourself on superiority to animals; they are without sin, and you, with your greatness, defile the earth by your appearance on it, and leave the traces of your foulness after you."

Unknown: "Law school is the opposite of sex. Even when it's good it's lousy."

John Bracken (on Captain Barney Kelly, who ran the USS Enterprise into the mud of San Francisco Bay in May, 1983): "He grounds the warship he walks on."

Abraham Lincoln: "People who like this sort of thing will find this the sort of thing they like."

A.H. Weiler: "Nothing is impossible for the man who doesn't have to do it himself."

Sports Illustrated (on official timers at track meets): "These are the souls that time men's tries."

Donald O. Rickter: "Historical reminder: always put Horace before Descartes."

Bertrand Russell: "The most savage controversies are those about matters as to which there is not good evidence either way. Persecution is used in theology, not in arithmetic."

Bertrand Russell: "The fact that an opinion has been widely held is no evidence whatever that it is not utterly absurd; indeed in view of the silliness of the majority of mankind, a widespread belief is more likely to be foolish than sensible."

Ouida (Marie Louise de la Ramée): "Christianity has made of death a terror which was unknown to the gay calmness of the Pagan."

Thomas Jefferson: "There is not a single crowned head in Europe whose talents or merit would entitle him to be elected a vestryman by the people of any parish in America."

Arthur Godfrey: "I'm proud to be paying taxes in the United States. The only thing is, I could be just as proud for half the money."

Neil Simon: "If no one ever took risks, Michelangelo would have painted the Sistine floor."

Dennis Miller: "Having Al D'Amato head an ethics committee is like having Jack Kevorkian teach the Heimlich maneuver."

Will Durant: "Perhaps man, having remade his environment, will turn around at last and begin to remake himself."

Ransom K. Ferm: "With every passing hour our solar system comes 43,000 miles closer to globular cluster M13 in the constellation Hercules, and still there are some misfits who continue to insist that there is no such thing as progress."

Emma Goldman (American anarchist on the Russian revolution): "If there's no dancing, count me out."

Lefty Gomez: "I'm throwing twice as hard as I ever did. It's just not getting there as fast."

Aldo Leopold: "When we hear his call we hear no bird. We hear the trumpet in the orchestra of evolution."

Thomas H. Huxley: "Irrationally held truths may be more harmful than reasoned errors."

Bill Watterson: "Sometimes I think the surest sign that intelligent life exists elsewhere in the universe is that none of it has tried to contact us."

Eric Hoffer: "In times of change, the learners will inherit the earth while the knowers will find themselves beautifully equipped to deal with a world that no longer exists."

From Soviet Weekly: "There will be a Moscow Exhibition of Arts by 15,000 Soviet Republic painters and sculptors. These were executed over the past two years."

Ed Lahey (Chicago Daily News, on the death of Richard Loeb in prison): "Richard Loeb, despite his erudition, today ended his sentence with a proposition."

Michael Levine: "Having children makes you no more a parent than having a piano makes you a pianist."

Lewis Carroll: "Alice thought the whole thing very absurd, but they all looked so grave she did not dare to laugh."

Dave Barry: "If a woman has to choose between catching a foul fly ball and saving an infant's life, she will choose to save the infant's life without even considering if there are men on base."

Unknown: "I think animal testing is a terrible idea. They get all nervous and give the wrong answers."

Unknown: "Madness has its toll. Please have exact change."

Unknown: "On one occasion a student burst into his office. "Professor Stigler, I don't believe I deserve this 'F' you've given me." To which Stigler replied, "I agree, but unfortunately it is the lowest grade the University will allow me to award."

A. Whitney Brown: "I am not a vegetarian because I love animals. I am a vegetarian because I hate plants."

Charlie Brown: "Sometimes I lie awake at night and I ask, 'Where have I gone wrong?'. Then a voice says to me, 'This is going to take more than one night.'"