ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

(2)

December, 1993

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

HANTAVIRUSES

NOTE: As noted in "PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE", which is found on the front page of each issue, you are encouraged to submit a brief summary of your work. The summary need not be in manuscript style, the results do not have to be definitive, you need not include tables (unless you want to). This is not a peer-reviewed publication. The intent is to communicate among ourselves and to let each other know what we are doing.

The next issue will likely be mailed June 1, 1994 (probable deadline for submissions: May 15, 1994). There is nothing that requires you to wait until the last minute. If you have something to communicate in January, February, March or April, please send it. Also, there is nothing that prevents you from submitting a report to <u>every issue</u>. There are no pages charges either but, then again, this is not a publication.

<u>PLEASE</u> !!!

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

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Editor's comments

If hantaviruses (family Bunyaviridae, genus *Hantavirus*) are not arboviruses, at the least they are cousins of our old arbovirus friends, bunyaviruses (genus *Bunyavirus*), nairoviruses (genus *Nairovirus*), phleboviruses (genus *Phlebovirus*), and even tospoviruses (tomato spotted wilt virus; genus *Tospovirus*). Thus, it was with interest in May of this year that we learned of the outbreak of human Acute Respiratory Distress Syndrome (ARDS), also called Hantavirus Pulmonary Syndrome (HPS) occurring on the Navajo Reservation in the Four Corners area of the U.S. (where New Mexico, Arizona, Utah, and Colorado meet). It soon became clear that this was a much more widespread epidemic than it first appeared to be. At the time of this writing, 42 persons are know to be affected and 26 (61.9%) of them have died. The U.S. Centers for Disease Control and Prevention in Atlanta reports that confirmed HPS cases have occurred in 12 states: Arizona (10), California (2), Colorado (5), Idaho (1), Louisiana (1), Montana (1), Nevada (2), New Mexico (15), North Dakota (1), Oregon (1), South Dakota (2), and Texas (1). The earliest retrospectively confirmed case occurred in July 1991. Ages of patients have ranged from 12 to 69 years (median: 32 years); 22 (52%) were male. Twenty-three (55%) patients were American Indians, 15 (36%) were non-Hispanic whites, 3 (7%) were Hispanic, and 1 (2%) was black.

No hantavirus has been isolated from the Four Corners region but a hantavirus has been isolated from a rodent in Mississippi. This virus does not appear to have the characteristics of the virus that first reared its ugly head in the Four Corners area. The rodent host there and in certain other areas is the deer mouse (*Peromyscus maniculatus*). However, this mammal does not occur in some other areas where there have been proven HPS cases. Therefore, there must be at least two viruses and at least two distinct natural cycles. Laboratory diagnosis has been (serologically) by IgM antibody capture ELISA and (genetically) by fishing out viral gene sequences from the lungs of patients. It is very sophisticated work (polymerase chain reaction).

Initial symptoms of the disease are simple-- fever, myalgia, and mild respiratory symptoms. However, this initial stage is deceptive, and patients may evidence an abrupt onset of acute respiratory distress and die a week or so after onset. The cause of death is not perfectly clear but there is a catastrophic failure of the lungs. Capillaries leak profusely, flooding air spaces with fluid. Thus the patients are drowning in their own fluids. Survivors recover without sequelae. There does not seem to be significant renal involvement but the pathophysiology is similar to that of hemorrhagic fever with renal syndrome, except, of course, that the affected organ is the lung, not the kidney. The etiologic agent of classical hemorrhagic fever with renal syndrome (Korean hemorrhagic fever, epidemic hemorrhagic fever) is Hantaan virus, the prototype virus of the genus *Hantavirus*. Obviously, the above is a capsular description; the illness is much more complex and much more is known than is reported here.

As extensive as this outbreak appears to be, the numbers of cases are few and the geographic distribution is sparse. Indications are that these viruses have been here for a very long time and that the concentration of cases in the Four Corners area served to cast attention on them. Indeed, had it not been for very observant clinicians in the Four Corners area, this epidemic might not have been recognized at all. Many additional studies are needed to understand the epidemiologies, epizoologies, molecular biologies, evolution, and, of course, prevention and control of these viruses.

A hantavirus symposium was held at the Open Meeting of the American Committee on Arthropod-borne Viruses, held during the recent (October 31-November 4, Atlanta, Georgia) annual meeting of the American Society for Tropical Medicine and Hygiene. The program is shown below.

HANTAVIRUS ACUTE RESPIRATORY DISTRESS SYNDROME (HARDS) IN NORTH AMERICA: AN EXPLOSIVE EMERGENCE (Karl M. Johnson, Organizer and Moderator)

I. Background

World-wide status of hantaviral disease: the path from discovery to treatment and prevention. (Ho Wang Lee, Institute for Viral Diseases, Korea University, Seoul, Korea)

Serological and phylogenetic analyses of hantaviruses. (Connie Schmaljohn, Shu-Yuan Xiao, Yong-Kyu Chu, and Joel M. Dalrymple, U.S. Army Medical Research Institute for Infectious Diseases, Frederick, Maryland)

II. The new hantavirus

Immunological and diagnostic characterization of infection: from a heterologous toe-hold to the search for homologous definition. (Thomas Ksiazek and Colleagues, Centers for Disease Control and Prevention, Atlanta, Georgia)

Genotypic identification and classification using existent hantavirus sequences and those from the new agent. (Stuart Nichol and Colleagues, Centers for Disease Control and Prevention, Atlanta, Georgia)

Immunohistochemical localization of viral replication in humans and correlations among three methods for identification of infection in humans and rodents. (Sherif Zaki and Colleagues, Centers for Disease Control and Prevention, Atlanta, Georgia)

III. The new disease

Case definition and epidemiology. (Jay C. Butler, Centers for Disease Control and Prevention, Atlanta, Georgia)

Clinical features of the HARDS syndrome (Fred Koster and Colleagues, University of New Mexico School of Medicine, Albuquerque, New Mexico)

Pathology of HARDS. (Kurt Nolte, Rich Fedderson, Kathy Foucar, Edith Umland, and Ross Zumwalt, University of New Mexico School of Medicine, Albuquerque, New Mexico)

Rodent reservoir/vectors of infection. (James Childs and many Colleagues, Centers for Disease Control and Prevention, Atlanta, Georgia)

IV. Now what?

National goals and plans. (C.J. Peters, Centers for Disease Control and Prevention, Atlanta, Georgia)

PROFESSOR M. P. CHUMAKOV: A REMEMBRANCE

Professor Mikhail Petrovich Chumakov died in Moscow on June 11, 1993 in the 83rd year of a remarkable life. His awe-inspiring personal and professional achievements were all the more extraordinary when viewed against the background of political and economic turmoil in Russia -- from the Revolution and the Civil War to Perestroika and the Commonwealth of Independent States:

- A promising medical researcher who at 28 years of age was permanently crippled while investigating a mysterious new disease, but overcame the handicap (paralysis of right arm and bilateral nerve deafness) to become a renowned medical investigator;

- A scientific leader and administrator of absolute personal integrity and of the highest moral standards who possessed the civil courage to stand up against his government's edicts when they violated human dignity and human rights. For instance, in early 1953, after Stalin's death, the witch hunt in connection with the so-called "Doctors's Plot" was in full swing and institute directors were ordered to dismiss professional staff members with Jewish surnames; Chumakov refused and lost his job as director of the Ivanovskyi Institute of Virology. He was politically rehabilitated in late 1955 and was appointed director of the new Institute of Poliomyelitis and Viral Encephalitides. He held this post until 1972 when he resigned (the complex political and scientific circumstances of this event would take more time and space than we have available). From 1972 until his death this year he held a succession of positions at the Institute: deputy director for science, laboratory chief, and consultant to the Institute. (It is rumored that the name Chumakov will soon be included in the official designation of the Institute).

One of Professor Chumakov's most far-reaching achievements was his pivotal role in making Sabin's oral poliovaccine (OPV) available to the world. In 1956 he learned from Jonas Salk how to make the inactivated poliovaccine (IPV) and from Albert Sabin about the prospects for a live virus vaccine (OPV). Soon his Institute was producing a large quantity of IPV for routine immunization of Soviet children. But, because he believed in the advantages of OPV, once Sabin successfully concluded a small clinical trial, Chumakov obtained 300,000 doses of the experimental vaccine. He proceeded with a large scale safety and efficacy trial in spite of initially powerful opposition from bureaucrats and colleagues. In the meantime, using Sabin's production strains, his Institute manufactured millions of doses of their own brand of OPV. Fortunately, the trial was successful (what would have happened to him if the vaccine were not safe?) and Chumakov was allowed to mount a massive immunization program in the Soviet Union and Eastern Europe with approximately 100,000,000 children soon immunized. The skeptics everywhere were reassured and the live virus vaccine was licensed in the United States and other countries.

The Chumakov poliovaccine history would not be complete without recognizing the contribution of his late wife and closest scientific collaborator, Dr. Marina K. Voroshilova. He depended on her through the years as his confidante and alter ego, sometimes his "ears" (when the hearing aid failed), and always his faithful companion.

Professor Chumakov has been described as a contemporary giant in the field of zoonotic virology. Indeed, most readers of the Newsletter are familiar with his research on arbovirus infections and viral hemorrhagic fevers. Beginning with the isolation of tick-borne encephalitis (TBE) virus in 1937, he and his associates studied literally hundreds of arthropod-borne viruses -- from their molecular biology to public health significance. After Chumakov isolated the viruses of the Crimean-Congo and Omsk hemorrhagic fevers in the 1940s, his laboratories became the center for hemorrhagic fever research, with much work dedicated to "hemorrhagic fever with renal syndrome" (the term he coined in place of the once popular but more restrictive "hemorrhagic nephrosonephritis"). His pioneering concepts and approaches allowed other investigators to work out the natural history of hemorrhagic fevers encountered in South America, Africa, and elsewhere. Chumakov's interest in the subject was keen until the end -- when we last met in June 1992, a year before his death, he suggested (with recent journals having become difficult to obtain in Moscow) American collaboration in up-dating and publishing a new encyclopedic monograph on all the viral hemorrhagic fevers!

Professor Chumakov was an inspiration and a role model for Russian virologists for more than 50 years. The world virologic community respected him as a great scientist; those of us who were fortunate to know him recognized the greatness of his spirit as well as of his intellect.

Alexis Shelokov, M. D.
San Antonio, Texas

OBITUARY

Louis Strathmore Grant

1913 - 1993

Jamaican Professor Emeritus Louis S. Grant unexpectedly passed away from a heart attack 13 June 1993 while visiting in Canada. Louie, as he fondly was known to his many friends, will be remembered as a happy, friendly person, energetic, resourceful and with a strong determination to succeed in any endeavor he pursued.

Louis Strathmore Grant was born 6 March 1913 in Vere, Clarendon, Jamaica, son of Henry Augustus and Catherine (Clucas) Grant. Following early schooling in Jamaica, Louis studied at Edinburgh University (1934-39), obtaining his M.B. and Ch.B.. During this period (summers 1935-37) he studied German language, history, philosophy, art and music at the University of Munich. Thereafter, graduate and professional experience included the following: (1) Medical Officer, Government Bacteriology and Pathology Laboratory, Kingston (1939-41); (2) Michigan Univ., M.P.H. (1940-41); (3) Assnt. Govt. Bacteriologist/Pathologist, Jamaica (1943-51); (4) London Univ., Diploma in Bacteriology (1948-49); (5) Senior Lecturer in Bacteriology, Univ. West Indies, Jamaica (1951-60); (6) Edinburgh Univ., M.D. (1964, Thesis: A Survey of Arthropod Transmitted Virus Infections in Jamaica.); (7) Professor of Microbiology, U.W.I. (1964-73); (8) Med. Advisor, Ministry of Health, Jamaica (1973-74); (9) Chair, Univ. Hosp. Board of Management (1973-74); (10) Professor Emeritus, U.W.I.(1977); Assoc. Health Officer, Niagara and Simcoe Regional Health Units, Ontario, Canada (1977-84).

Grant's interests in Jamaican health were broad. Besides the general field of preventive medicine and public health (including organization of health services and hospital laboratory services), he involved himself with investigating enteric and fungus infections, helminths, Rickettsioses, leptospires and many other agents (about 70 publications). In the early 50's Louie became interested in the field of arboviruses and called on the Trinidad Regional Virus Laboratory for help in instituting an arbovirus research program in Jamaica. Thereafter, from time to time, personnel from that laboratory visited Jamaica as consultants or as instructors in field and laboratory techniques. The Jamaican program was a successful one, yielding useful information on the existence of a number of arboviruses (EEE, SLE, Dengue, Cache Valley and Wad Medani) and also providing expertise and experience in combating arthropod-borne epidemics.

In 1987 the University of the West Indies conferred on Louie an Honorary Doctor of Laws degree and the following year the Government of Jamaica awarded him the Commander of the Order of Distinction (CD) for services of health in Jamaica.

Perhaps Dr. Grant's greatest contribution to the people of Jamaica was his inspiration to create (with the help of distinguished friends and organizations) the Foundation for International Self Help (F.I.S.H.), establish the FISH Clinic in the Papine quarter of Kingston and which he directed over the last nine years of his life. His dream was to provide the best medical care for the poor at the lowest possible price. Professor Grant is survived by his wife, Pauline, two daughters and two sons as well as other relatives and a host of friends both in Jamaica and elsewhere in the world.

Thomas H.G. Aitken
Leslie Spence

Memories of Carl M. Eklund

For those of you who remember or may have had the pleasure of knowing him, Carl Eklund (born 5 Dec. 1903 in Moorhead, MN) was a noted arbovirologist/microbiologist of the 50's and 60's who spent much of his professional life at the Rocky Mountain Laboratory in Hamilton, Montana. There he studied the infectious agents of such diseases as scrapie, Colorado tick fever, western and eastern encephalomyelitis, St. Louis encephalitis, and many others. A little known facet of Carl's life is revealed in the March 1993 issue of National Geographic magazine in the article "Reclaiming a Lost Antarctic Base" by Michael Parfit.

Eklund was a member of the 1940-41 expedition (Finn Ronne, leader) organized to establish the first permanent United States station in Antarctica. Called East Base, it was built on Stonington Island, contiguous with the west coast of the Antarctic Peninsula (Palmer Land). Author Parfit describes an episode __ "The journeys were prodigious. Ronne and a biologist named Carl Eklund left Stonington on November 6, 1940, and returned January 28, 1941 __ 84 days on the trail. They sledged a total of 1,264 miles, one of the longest journeys on foot in Antarctic history, mapping as they went. They left home with 24 dogs and returned with 7."

(Thomas H.G. Aitken, YARU, New Haven, Connecticut, August 1993)

GUIDELINES FOR SUBMITTING REPORTS

We want to keep this mechanism timely and viable. Therefore, submit only recent news and summaries of your work. PLEASE limit the submission to 1 or a very few sheets (21.59 cm x 27.94 cm = 8.5x11 inches) plus 1 or 2 tables; condense as much as you can (single space the text); do not staple pages together; do not number pages.

FUTURE MEETINGS
THE AMERICAN SOCIETY FOR TROPICAL MEDICINE AND HYGIENE

1994 13-17 November	Cincinnati, Ohio
1995 5-9 November	San Antonio, Texas
1995 (to be announced)	San Diego, California

MEMBERSHIPS AVAILABLE

AMERICAN SOCIETY FOR TROPICAL MEDICINE AND HYGIENE

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

If any of the readers of the Arbovirus Information Exchange would care to join either of the above societies, I have applications and would be delighted to nominate you. Dues for each society includes a subscription to its journal (American Journal of Tropical Medicine and Hygiene; Transactions of the Royal Society of Tropical Medicine and Hygiene)

Let me know.

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EVIDENCE SUPPORTING VERTICAL TRANSMISSION OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS IN *AEDES DORSALIS* MOSQUITOES

Report from the Arbovirus Research Unit, Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720

LD Kramer, CF Fulhorst, MD Bowen, RC Chiles, JL Hardy, MM Milby, SB Presser, WC Reeves, WK Reisen

The mechanism which allows alphaviruses to persist in North America during periods of vector inactivity has yet to be elucidated. Recently, one of the doctoral students in our program, Charles F. Fulhorst, isolated three strains of western equine encephalomyelitis (WEE) virus from 29,841 *Aedes dorsalis* (in 666 pools) that were collected from a coastal salt marsh habitat near Morro Bay in central California. These WEE viral isolates are unique in that they came from two pools of males and one pool of females that were collected in the field as immatures and reared to adults in the laboratory. One isolate was made from specimens collected in August, 1991, and the other two isolates came from specimens collected during August, 1992, from the same collection site within the Morro Bay tidal salt marsh. This observation potentially represents the first definitive evidence that WEE virus may be maintained in North America by vertical transmission in *Ae. dorsalis* Complex mosquitoes. Members of this complex (*Ae. dorsalis* Meigen, *Ae. melanimon* Dyar and *Ae. campestris* Dyar & Knab) have been reported in thirty states in the United States as well as in Canada and Mexico, and WEE virus has been isolated from each of these species in the Unites States and from *Ae. campestris* in Canada.

To further confirm the field observations, horizontal and vertical transmision studies were conducted in the laboratory with the DAV 3340 strain of WEE virus from Morro Bay and female *Ae. dorsalis* either trapped in the Morro Bay area or reared from the Fort Baker laboratory colony. To evaluate horizontal transmisison, females ingested 3.1 $\log_{10}$ plaque forming units (PFU) of virus from pledgets soaked with a virus-rabbit blood-sucrose suspension. Engorged mosquitoes were incubated for 7-14 days at 20°C before peroral transmission rates were determined by the capillary tube method. No significant differences were noted in horizontal transmission rates between field females from Morro Bay and females from the Fort Baker colony. Overall, 98% of 56 females became infected, 96% of the infected females had disseminated infections and 33% transmitted virus. In vertical transmission studies, *Ae. dorsalis* females from Morro Bay were

inoculated intrathoracically with 100-1,000 PFU of virus and maintained at 20°C for 10 days until fed on normal chickens for egg production. After conditioning, eggs were hatched and larvae were reared to adults at 10-12°C. The F_1 adults were held for 5-8 days at 25°C before being frozen for viral tests. Second ovarian cycle eggs were collected from pledget-fed Morro Bay females and processed similarly. Surprisingly, no infectious virus could be detected in 28 pools (297 F_1 males and 392 F_1 females) from inoculated female *Ae. dorsalis* or in 9 pools (68 F_1 males and 102 F_1 females) from pledget-fed females by plaque assay in Vero cells. In an attempt to help resolve the discrepancy between the apparent vertical transmission of WEE virus by *Ae. dorsalis* in nature and the lack thereof in the laboratory, we used molecular virologic methods to determine if WEE viral RNA was transmitted vertically to F_1 larval and adult progeny of virus-inoculated parents from the Fort Baker colony. Extracts from triturated pools of 10 larvae or 10 adults each were examined for WEE viral RNA by reverse transcription (RT) followed by polymerase chain reaction (PCR) using specific primers from the conserved sequences in the E1 region of the WEE viral genome. The Southern blot was used to confirm that the RT/PCR products were derived from WEE viral RNA. Approximately 33% of 30 pools of F_1 larval progeny and 20 pools of F_1 adult progeny were positive for WEE viral RNA whereas none of these pools was positive for infectious virus. Nucleotide sequences of the amplified portion of the Vero cell stock DAV 3340 virus and the cDNA amplified following viral replication in *Ae. dorsalis* parental females differed by <10 nucleotides from the published sequence of the BFS 1703 strain of WEE virus isolated from *Culex tarsalis* in California in 1953. Further studies are in progress to confirm that WEE viral RNA can be transmitted vertically in *Ae. dorsalis* without the production of infectious virus.

Economic impact of eastern equine encephalomyelitis virus on a south Florida emu farm: 1991-1992

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Flocks of emus (Dromaius novaehollandiae) have been introduced recently to Florida as part of a speculative market to supply prospective farmers with emu breeding stock. Many of the emu farms are located in rural agricultural areas that were formally used for chicken and turkey farming.

Eastern equine encephalomyelitis (EEE) virus is found throughout Florida with the exception of the southern tip and the Florida Keys. However, the main focus of EEE transmission is from Orange County north through the panhandle.

In 1991 an abandoned chicken farm in central Volusia County, Florida was purchased and modified to raise and breed emus. The farm is adjacent to a bald cypress (Taxodium distichum (L.)) wetland and close to sites where EEE transmission is periodically documented in domestic horses and sentinel chickens.

The starting flock consisted of 108 yearling emus valued at $4,000 apiece making the initial investment in emus $432,000 (Table 1). Between November 1991 and March 1992, 23 emus (21.3 percent of the flock) succumbed to an acute infection that was later confirmed as EEE virus by viral isolation from blood, serum, and tissues (brain, spleen, liver, and intestine). In addition, serology from surviving birds indicated recent EEE infections. Between March and June 1992, an additional 11 birds died as a result of EEE infection. As the emus approach breeding age, they increase in value. This accounts for the increasing value of birds that died between November 1991 and June 1992 shown in Table 1. A total of 34 emus (31.5 percent of the initial flock) died as a result of EEE infection accounting for a financial loss of $206,000. Of the 34 fatalities, 11 were confirmed and 23 were presumptive EEE cases. A total of 12 emus are known to have survived EEE infection.

Symptoms of EEE infection in emus included, anorexia for up to a week, hemorrhagic enteritis, central nervous system disfunction, and sudden death with no other notable symptoms. Infected birds often first displayed clinical symptoms within 24 hr of death. The mortality rate of emus showing clinical symptoms was >60 percent.

Table 1. Cost of the 1992 EEE outbreak at the Volusia County emu farm.

Date	**Emu Population**	**EEE Mortality (No./%)**	**Other Mortality**	**Value of Birds (Each)**	**Estimated Investment or Loss**
Fall 1991	108			$4000	$432,000
Nov.'91/ Feb. '92	85	23(21.3)		$5000	-115,000
Mar.'92/ Jun. '92	72	11(13.0)	2(2.4)	$7000	-91,000
Totals		**34(31.5)**			**-206,000**

Intervention by vaccination beginning in July 1992 seemed to terminate EEE transmission. Yearling birds were given 1.0 cc of a killed eastern, western and Venezuelan vaccine (Solvay Triple E) via intramuscular inoculation. Significant levels of neutralizing antibody were not always detected after immunization. There was no indication of further EEE transmission at the emu farm after July 1992. However, this may also represent a reduction in natural EEE transmission in the area.

Colorimetric Detection of RT-PCR Products in the Differential Diagnosis of Dengue Virus Serotypes

P.Young, M. Law and C. Bletchly

Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Herston, Brisbane. 4029 AUSTRALIA

The laboratory diagnosis of dengue virus infection in most cases still relies on the isolation of infectious virus or the detection of a rise in virus-specific serum antibodies. However poor virus isolation rates and cross-reactive antigenic determinants shared by all members of the flaviviruses have lead to significant problems in specific diagnosis. In addition, the time it takes to obtain these results mean that they are often of little use in the clinical management of the disease.

In order to address these problems, several groups have turned to the sensitivity and specificity of the PCR technique to develop a more rapid diagnostic assay for dengue infections (Deubel et al. [1990] J Virol Meth **30**:41-54; Henchal et al. [1991] Am J Trop Med Hyg **45**:418-428; Laille et al. [1991] J Med Virol **34**:51-54; Lanciotti et al. [1992] J Clin Micro **30**:545-551). Although these reports have confirmed the usefulness of the PCR approach in achieving the sensitive and serotype-specific detection of dengue viruses in clinical samples, the analysis of PCR products by gel electrophoresis and/or hybridization means that in this form, the assay will remain the province of the specialist laboratory. In order to successfully transfer PCR to pathology laboratories routinely dealing with large numbers of samples, more appropriate methods for the analysis of PCR products need to be developed.

We adapted primers designed by Lanciotti et al (J Clin Micro **30**:545, 1992) to a semi-nested RT-PCR whose products could be identified in an "ELISA" style format. This was achieved by the incorporation of a DNA binding motif (specific for the yeast DNA binding protein GCN4) into each of the serotype specific primers and digoxygenin (DIG) labelling of the dengue group consensus upstream primer. First round PCR generates a 511 bp product for each of the dengue virus serotypes while the nested second round PCR results in products of varying size. The products of 1st and 2nd round amplifications of a DEN2 harvest are shown in Figure 1.

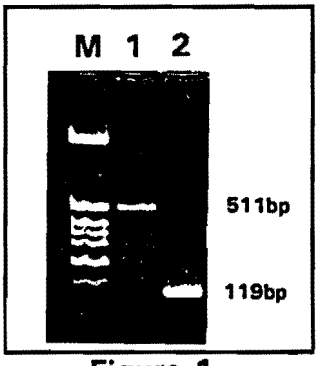

Figure 1

The products of the second round PCR could also be monitored in an enzyme-linked colorimetric assay. PCR products incorporating the GCN4 binding motif were bound to GCN4 coated microtitre plates (Kemp et al. [1989] PNAS **86**:2423-2427) and their presence detected

with anti-DIG peroxidase and OPD. Figure 2 shows the absorbance readings obtained following the detection of GCN4-bound PCR products generated with the DEN 4 specific primers with cellular RNA isolated from mock and DEN 4 infected C6/36 cells. As little as 0.1μl of the PCR mix has given a positive result. The type specificity of all four sets of primers has been confirmed in this assay with no cross-reactive amplification being observed. We have however, encountered problems with the GCN4 detection of DEN 1 specific RNA with the negative control samples showing a strong positive absorbance reading in the absence of any identifiable product on gels. It is likely that this set of primers is generating primer-dimer artifacts which are detected in this assay. This problem is currently being addressed and may necessitate the generation of a new primer pair.

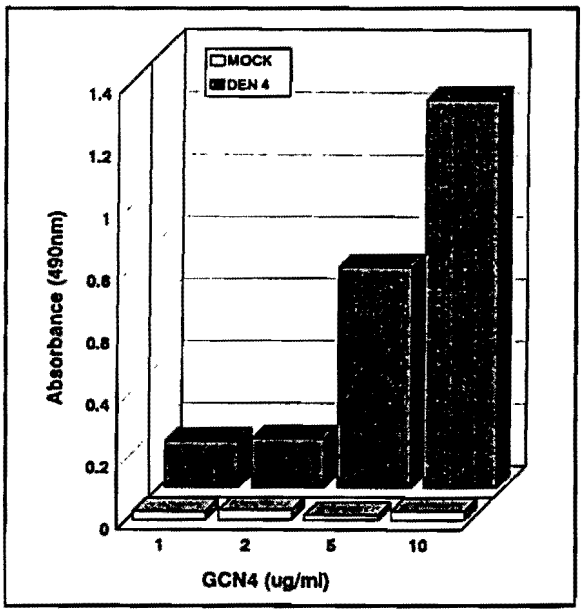

Figure 2

We believe that this assay has the potential for being adapted to large scale screening of serum samples for dengue virus serotype detection.

INVESTIGATIONS OF A DISEASE OUTBREAK DUE TO DENGUE TYPE-2 VIRUS IN CHIRIMIRI AREA, MADHYA PRADESH, INDIA

BY

Mahadev, P.V.M., Prasad, S.R., Ilkal, M.A., Thakare, J.P., Mavale, M.S., Gangadhar Rao, T.L., Mohanty, S.K.* and Banerjee K.

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Following an outbreak of fever with suspected dengue viral aetiology, virological, serological and entomological investigations were carried out in August, 1992 at S.E. Colliery Ltd., Chirimiri. The Chirimiri area comprises of miners settlements amidst Korea hills of Vindhya range (23°.05' - .10'N; 82°.15' - .20'E) and is situated at 2000' M.S.L. As per 1991 census this area constitutes an agglomeration of non-municipal settlements with an overall 87000 population (provisional figure). Three of these settlements abut sylvan environment and the rest of the settlements are devoid of any sylvan cover. Hence, although Chirimiri is presently classified under 'Kurasia urban agglomeration', but ecologically it belongs to a fringe area between rural and urban classes of settlements.

A total of 100 sera collected during 1990 outbreak were tested in MAC ELISA and HI tests. Recent infection to flavivirus as revealed in MAC ELISA was recorded in 37% (37/100) of samples. Out of these 37 samples, 26 were positive only for DEN infection. Post flavivirus infection was recorded in 23% of samples.

During July-August 1992 a fresh batch of 40 sera was collected and tested in MAC ELISA HI tests. Recent infection to flavivirus was recorded in total of 52.5% (21/40) of samples. Out of these 21 samples, 18 were positive only for DEN-2 infection. Past flavivirus infection was recorded in 38% (15/40) of samples).

One larva per container surveys were conducted in various residential quarters of the Chirimiri area to assess the relative prevalence of the vector mosquitoes.

A total of 1387 containers in 301 houses were examined for presence of mosquito larvae, of which 184 containers were positive. During the study both the important vector species of dengue viz., Ae. aegypti and Ae. albopictus were collected. The relative prevalence of Ae. aegypti varied between 9.09 to 125.00 whereas Ae. albopictus ranged between 5.0 to 36.36. The details are summarised in the table.

Adult Aedes species mosquitoes were also collected from these areas for dengue virus isolation. The collections were transported live to Pune laboratory for testing. A total of 22 M and 99 F Ae. aegypti and 3 F Ae. albopictus were tested for the presence of dengue antigen by indirect immunofluorescent technique. Among these 3 F Ae. aegypti collected at NCPH and 1 F Ae. aegypti collected at north Chirimiri colliery were positive for dengue antigen. After establishing the isolates by making further passages, two of the isolates were identified as dengue type-2 by complement fixation test.

This shows continued activity of dengue virus in Chirimiri area.

Relative prevalence of Aedes species and virus isolations from adult collections in Chirimiri area during August 1992.

Colliery	No.of Houses visited	No.of containers examined	No.of containers per 100 houses with				Adult Ae. aegypti	
			Ae. aegypti	Ae. albopictus	Ae. vittatus	Others*	House index	No.DEN +ve / No.tested
Doman hill	30	171	60.00	-	3.33	16.67	26.67	0/5F
Chirimiri @	40	175	22.5	5.00	10.00	2.5	20.00	0/2F
West Chirimiri @	40	193	2.5	5.0	17.5	15.00	5.0	-
Gadabuda @	40	163	2.5 **	17.5	10.00	7.5	0	-
G.M. Complex	11	44	9.09	36.36	100.00	0	0	-
North Chirimiri	40	176	27.5	15.00	10.00	30.00	17.5	2/10F
Korea @@@	40	152	7.5	-	5.00	22.5	5.00	-
NCPH	40	186	22.5	27.5	5.00	7.5	35.00	3/20F 0/16M
Godripada	20	127	125.00	-	5.00	25.00	60.00	0/42F 0/5M

* Others include Aedes (Christophersomia) thomsoni, Cx. quinquefasciatus, Anopheles sp., Armigeres sp.

** Aedes aegypti could not be collected by usual single larva survey method, but the adults emerged from a pool of larvae collected in this area. This suggests that Aedes aegypti shared its breeding places with more predominant Ae. albopictus and Ae. vittatus.

@ M - Males, F - Females,

@@ 1 M Ae aegypti and 3 F Ae. albopictus were, -ve for DEN virus antigen at a coal mine area.

@ Settlement adjoining a sylvan area.

Experimental oral infection of Aedes aegypti mosquitoes against dengue type 2 viruses

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Recently, all four types of dengue viruses have been found to be associated with dengue haemorraghic fever (DHF) and dengue shock syndrome (DSS). These constitute a major public health problem where Aedes aegypti mosquito and dengue virus of more than one type are prevalent. Our purpose was to know whether Ae. aegypti female mosquitoes can replicate and transmit different dengue type 2 viruses, which were isolated from patients of classical dengue fever symptom (DF), DHF and DSS, respectively.

Two laboratory mosquito colonies of Ae. aegypti collected from Samutsongkram province, central part of Thailand, and from Chumporn province, southern part of Thailand in 1990 and field collected-Ae. aegypti mosquitoes collected as the 3rd and 4th instar larvae at Wat Makok village near Faculty of Tropical Medicine, Mahidol University, Bangkok were used for comparison of the susceptibility against the four different origin dengue type 2 viruses. They were derived from patients of DF, DHF, and DSS, respectively, and New Guinea B strain (NGB) was used as standard type 2 virus.

Mosquitoes were given a chance to feed orally virus mixture within 2-3 hours at 28-30C through cotton pledge with virus mixture which contained equal volume of virus culture and whole rabbit blood in 2% sucrose (VBS). Fully-fed female mosquitoes were transferred to small cup containers covered with nylon mesh. They were reared with 5% sugar solution for 9 to 10 days at 28 - 32C, until they were checked the viral antigen by the head squash of the mosquitoes using indirect fluorescent antibody (IFA) technique. Remnants of the mosquitoes (thorax, abdomen of the mosquitoes) were kept at -20C, and they may be also checked by IFA.

Present data of the mosquito head squash showed that no cleared differences were founded on the susceptibility of mosquito colonies and field collected mosquitoes against the viruses. Viruses used may be equally replicated in the mosquitoes. However, low titer of each virus replication in the mosquitoes are considered for further experiment.

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ISOLATION OF DENGUE VIRUS TYPE 1 FROM LARVAE OF AE. ALBOPICTUS IN THE STATE OF MINAS GERAIS, BRAZIL.

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In the Municipality of Campos Altos, located at 20º S 46º W, State of Minas Gerais, with a population of 11,100 inhabitants, laboratory confirmed cases of dengue have been observed, starting by April, 1993. No Ae. aegypti had been reported in the city, since December, 1991.

A dengue surveillance system had been then stablished, including the collection of mosquitoes (adults and larvae) during May and June this year in 14 breeding sites in the periurban area of the city.

About 1,120 larvae and 93 adults of Ae. albopictus have been collected in two spots, one a water tank, formely used to supply water to animals and another one in old tires, founded in a canyon close to the urban area. From this last one, 732 larvae were collected and distributed in pools containing ca. 30 specimens each. Dengue virus type 1 has been isolated from two of these pools by inoculation in C6/36 tissue culture cell line, at Ezequiel Dias Foundation. One of the positive pools have been sent to the Flavivirus Laboratory/FIOCRUZ Rio de Janeiro where the virus could be reisolated and dengue type 1 genome demonstrated in the original larvae pool by PCR technique. No virus could be detected in the adult mosquitoes.

Ae. albopictus has been found in the city, since March 1993 but no Ae. aegypti could be encountered in the breeding sites or houses searched in the urban areas.

Eleven human cases of dengue have been confirmed after April 1993 in the city, all of them near to the two breeding sites described (maximal distance about 300 meters).

These data suggest a transmission of dengue type 1 by Ae. albopictus as sporadic cases in the area, in the absence of Ae. aegypti as well as the transovarial transmission of virus by the Ae. albopictus strain stablished in Brazil, confirming laboratory data obtained by Mitchell et al., 1990.

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USE OF THE AGGREGATION-ATTACHMENT PHEROMONE TRAP TO IDENTIFY HABITATS AND COLLECT WILD ADULTS OF *AMBLYOMMA HEBRAEUM*

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African species of *Amblyomma* (Acari: Ixodidae) are actually or potentially involved in a wide variety of pathogenic infections and disease conditions affecting man and animals. One species alone, *A. variegatum*, has been asssociated with 9 arboviruses (Bhanja, CCHF, Dugbe, Jos, Kadam, Nairobi Sheep Disease, Somone, Thogoto, yellow fever), 4 rickettsias (*Rickettsia conori, Coxiella burnetii, Cowdria ruminantium, Ehrlichia bovis*), a number of other bacteria (*Pasteurella multocida, Corynebacterium* spp., *Dermatophilus congolensis, Nocardia farcinica*) and protozoa (*Theileria* spp.), as well as with tick paralysis and significant skin damage. Estimates of natural infection rates of ixodid ticks with pathogenic agents provide the basis for an understanding of the endemic stability of disease in a given area, which, in turn, often influences control strategy.

In order to accurately estimate infection rates, wild (unnattached) ticks must be collected. In the case of species that ascend vegetation and quest while awaiting chance contact with a host, wild ticks are easily collected on drags; some species are also attracted to CO_2. However, post-larval stages of the important amblyommine vectors of human and animal disease, *A, variegatum* and *A. hebraeum*, neither quest on vegetation nor are readily attracted to CO_2. Instead, they secrete themselves beneath ground litter, to emerge only when chemical signals indicate the presence of a suitable host nearby. These signals consist of CO_2, which stimulates the ticks into activity (but provides no long range directional attraction), and the aggregation-attraction pheromone (AAP) produced by male ticks already parasitizing the host, which attracts activated ticks to the host (Norval et al., 1989. *Science* 243: 364-365). Previous work has revealed a significant attractiveness of the AAPs of *A. variegatum* and *A. hebraeum* for marked, released adults of either species (Yunker et al., 1990. *J. Insect Behav.* 3: 557-565). Large proportions of both males and females were attracted over long distances to sources of pheromone and CO_2, where they were recaptured. However, use of this technique to collect wild amblyommas has not been reported. We tested the AAP of *A. hebraeum* in combination with CO_2 vapors, as described by Yunker et al. (1990), as a means to identify suitable tick habitats and to collect wild forms in heartwater-endemic areas of southern Africa.

Trapping was done in two communal grazing areas of rural Bophuthatswana (Rietgat and Madinyane) late in the cool, dry season of 1992. Blocks of dry ice (0.5-1.0 kg) were set at ecologically different areas on leaf litter. Filter papers soaked with pheromone extract were positioned next to each block of dry ice. Traps were visited regularly and ticks moving to traps were collected. Most ticks could be caught within the first 15 minutes. Areas differing ecologically differed also in trap yield. Most successful traps were those placed near cattle kraals surrounded by dense bush,

Euclea crispa ("mokgwelekgwele"). Using this method, as many as 78 adults (35 males, 43 females) could be trapped in one day. This capture method has since been successfully used at other heartwater-endemic areas in southern Africa. Ticks collected are returned to the laboratory where their midguts and salivary glands are removed and frozen in preparation for testing for *Cowdria ruminantium* by means of a DNA probe.

These findings indicate that habitats suitable for *A. hebraeum*, and possibly also *A. variegatum*, can be identified and wild ticks easily collected for assessment of infection with arboviruses, rickettsias or other infectious agents.

INHERITANCE OF ORAL SUSCEPTIBILITY OF AEDES AEGYPTI TO CHIKUNGUNYA VIRUS

By

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While rearing Ae. aegypti in the laboratory we came across a rosy eye (ry) mutant. After crossing it with wild type (black eye "+") and inbreeding of F1 hybrids a pure colony of rosy eyed Ae. aegypti was established. We wished to determine whether there was any differential susceptibility of this mutant to a few arboviruses. Susceptibility studies with Chikungunya virus showed that the head squash positivity of the wild strain was significantly higher than the mutant strain ($\theta = 0.0919$, $P < 0.01$). The average of a few replicative experiments showed that the difference of susceptibility was about 35.02%. The difference was consistent with different CHIK virus strains even when experiments were conducted at a different filial generations.

When wild type and mutant strains were inoculated with CHIK virus intrathoracically, both the strains of mosquitoes supported the multiplication of virus and there was no significant difference in the head squash positivity ($\theta = 0.0069$, $P > 0.1$). These results suggest the differential susceptibility was due to gut/messentral barrier.

Results of oral susceptibility of the parent strains and F1 crosses of rosy eye and wild type mosquitoes suggested that refractory gene(s) were dominant over susceptibility gene(s). Percent head squash positivity of hybrids was almost closer to the percent positivity of rosy eye parents. When the hybrid (M-ry/m-+) males were back crossed with rosy eye (m-ry/m-ry) the ratio of rosy and black eye was 1:1, and rosy eye mosquitoes were refractory to CHIK virus as compared to wild type parents (Table-1). Thus, it is apparent from the results that CHIK refractory genes are situated on linkage Group III, since they showed association with rosy eye mutant gene(s) which are located on chromosome III (Munstermann, 1990).

Experiments were also performed to determine whether these refractory gene(s) also control oral susceptibility to DEN and Sagiyama viruses. Results showed that there was no difference in oral susceptibility of rosy eye and wild type mosquitoes ($\theta = 0.0018$, $P > 0.1$) to dengue virus. With the Sagiyama virus it was observed that the rosy eye strain was comparatively less susceptibility then the wild type ($\theta = 0.064$, $0.2 > P > 0.1$).

Oral refractoriness of rosy eye strain to CHIK and Sagiyama virus may be due to the specificity of receptor sites of gut epithelial cells. Further studies are required to find out exact mechanism for refractoriness in this mutant strain.

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Table-1

Comparative susceptibility of Aedes aegypti strains (Parent, F1 hybrid and back crosses) to CHIK virus (Senegal strain) after oral infection

Titre of feeding suspension	Parents		F1 progeny of		Backcross		
	Rosy	Black	B(F) x R(M)	R(F) x B(M)	F1(M) x R(F) Phenotype		F1(M) x B(F)
					Rosy	Black	Black
4.6	2/60 (3.33)	28/60 (46.66)	5/84 (5.95)	12/84 (14.28)	ND	ND	ND
4.6	5/64 (7.81)	25/80 (31.25)	11/96 (11.45)	6/96 (6.25)	ND	ND	ND
4.5	6/76 (7.89)	35/56 (58.72)	ND	ND	11/84 (13.84)	33/84 39.28	ND
4.2	20/78 (25.64)	65/84 (77.38)	ND	ND	18/66 (22.27)	31/84 36.93	ND
4.5	4/26 (15.38)	28/60 (46.66)	14/72 (19.44)	12/72 (16.66)	ND	ND	24/72 (33.33)
Total	37/304 (12.17)	181/340 (53.23)	30/252 (11.90)	30/252 (11.90)	29/150 (19.33)	64/168 (38.09)	24/72 (33.33)

B = Wild type, black eye

R = Rosy eye

F1 = Hybrid (M-ry/m-+) (Male Rosy x Female wild type)

ND = Not done

Percentages in parenthesis.

Susceptibility and Transmissibility of *Aedes albopictus* and *Aedes aegypti* Mosquitoes to Chikungunya Virus

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Aedes albopictus and *Ae. aegypti* mosquitoes were tested by oral infection with four strains of chikungunya(CHIK) virus isolated from Yunnan, China and Africa, for their susceptibility and transmissibility. Studies showed that these two species of mosquitoes were susceptible to CHIK virus. Either *Ae. albopictus* or *Ae. aegypti* for 6th day after infection were capable of transmitting CHIK virus by bite to suckling mice. Their transmission rates were 55.55%-100% on the 8th to 13th day post-infection. In another experiment, infected mosquitoes were capable of transmitting CHIK virus by bite to chickens. Infection and transmission rates were higher *Ae. aegypti* tested than *Ae. albopictus* tested. The susceptibility and transmission potentials of the different strains of CHIK virus were some difference from each other, such as the M81 strain isolated from *Ae. albopictus* in Yunnan apparently was more virulent than the other strains. These results indicate that *Ae. albopictus* and *Ae. aegypti* from China have the potential to play a role in the maintenance and transmission of CHIK virus in nature.

Transovarial Transmission of Chikungunya Virus in *Aedes albopictus* and *Aedes aegypti* Mosquitoes

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Aedes albopictus and *Ae. aegypti* mosquitoes from mainland China were tested for their ability for transovarial transmission of chikungunya (CHIK) virus. The strains of CHIK virus employed for B8635, M81 and Ross isolated from Yunnan, China or Africa. Studies showed that these two species of mosquitoes were capable of transmitting CHIKvirus by bite to susceptible hosts. Parental females of *Ae. albopictus* and *Ae. aegypti* were infected orally with CHIK virus, and 3070 first generation progeny from infected mothers were pooled into 55 pools and examined for the virus. Positive rate of pools from *Ae. albopictus* were 46.15%(6/13) for larvae, 33.33%(4/12) for adult females and 62.50%(5/8) for adult males. Positive rate of pools from *Ae. aegypti* were 18.18%(2/11) for larvae and 22.22%(2/9) for adult females. In another experiment, CHIK viruses were detected in second and third generations progeny obtained from individual infected *Ae. albopictus* and *Ae. aegypti*. Based on these studies, infected females were capable of transmitting CHIK virus vertically to their offspring. There was no apparent difference in transovarial transmission capabilities of these two species in orally infected mosquitoes. These results indicate that *Ae. albopictus* and *Ae. aegypti* have the potential to play a role in the maintenance of CHIK virus in nature.

REPORT FROM THE VECTOR BIOLOGY LABORATORY
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La Crosse virus transmission by *Aedes albopictus*

Columbus, Ohio is one of the more recent U.S. urban areas to suffer infestation with *Aedes albopictus*. As Ohio is one of several midwestern states with locales endemic for La Crosse virus (LACV), the possibility that *Ae. albopictus* might pick up and vector LACV remains of growing interest. Moreover, should this occur, LACV (for the most part, a rural and suburban arbovirus) could be spread into the damaged and urban environments for which *Ae. albopictus* has shown considerable affinity. We have been examining some of the parameters which affect laboratory LACV transmission by *Ae. albopictus*.

Our studies have included olfactometric tests to estimate the avidity of *Ae. albopictus* for LACV amplifying hosts such as eastern chipmunks (*Tamias striatus*) and southern flying squirrels (*Glaucomys volans*). Experiments employing an olfactometer have yielded results which suggest that *Ae. albopictus* responds to amplifying host stimuli faster and in greater numbers than the natural LACV vector *Aedes triseriatus* (Table 1).

Furthermore, when we released *Ae. albopictus* and *Ae. triseriatus* into a 1.5 cubic meter cage equipped with a flying squirrel-baited treehole habitat, *Ae. albopictus* appeared more aggressive toward the amplifying host animals. *Ae. albopictus* was found to be more successful than *Ae. triseriatus* at both entering the treehole and at obtaining a bloodmeal within 23 hours of release.

Ae. albopictus is capable of transmitting LACV to eastern chipmunks (Cully et al. 1992) and flying squirrels. These mammals, when viremic, are in turn infectious for *Ae. albopictus*. A dose effect is observed, with *Ae. albopictus* showing greater susceptibility to LACV than *Ae. triseriatus*. For one chipmunk, a waning virus titer sufficient to infect two of 49 *Ae. albopictus* (280 pfu/mL) failed to infect any of 75 *Ae. triseriatus*. In artificial feeding experiments, the low infectious LACV dose required to infect *Ae. albopictus* suggested that during the course of viremia, amplifying rodent hosts are infectious to *Ae. albopictus* for up to five hours beyond the period of infectivity for *Ae. triseriatus*.

Horizontal transmission of LACV to suckling mice by orally infected *Ae. albopictus* has been demonstrated (Grimstad et al. 1989). In *Ae. albopictus*, transovarial transmission (TOT) of LACV from *per os* infected females to F_1 progeny occurs at a relatively low 2.7% filial infection rate (FIR) (Tesh & Gubler 1975). We have observed some variation in such TOT rates across geographic strains of *Ae. albopictus* (Streit & Grimstad 1990), with a maximum of 17% FIR in one southern U.S. strain.

Diapausing F_1 eggs, the progeny of *per os* LACV-infected female *Ae. albopictus* and *Ae. triseriatus* were placed out of doors for the winter of 1992-1993. The eggs were sealed in zip-lock bags which were themselves in turn placed in a discarded tire located in a controlled access area of our South Bend, Indiana university campus (latitude 41°42'N). The eggs remained outside from December to April. LACV was detected in adult *Ae. albopictus* males and females as well as *Ae. triseriatus* reared from the overwintered eggs.

Stabilized TOT akin to that reported for San Angelo virus in *Ae. albopictus* (Tesh & Shroyer 1980, Shroyer 1986) occurs with LACV as well. Of seven geographic strains of *Ae. albopictus* tested, some *per os* infected isofemale lines from each of five of the strains transmitted LACV to F_2 progeny at FIRs exceeding 90%. Select lines have been carried out to the F_7 generation while maintaining efficient virus transmission. It is interesting to note that the CHICAGO-92 and COLUMBUS strains of *Ae. albopictus* were collected from urban areas in states endemic for La Crosse virus encephalitis.

Ae. albopictus shows some autogenous egg production (Hawley 1988). Two isofemale lines of the ALGIERS *Ae. albopictus* strain were maintained for seven generations autogenously. Virus transmission was efficient in these isolines, with FIRs exceeding 95% in every generation beyond the F_1. LACV can be maintained in mosquitoes, then, for seven generations without vertebrate amplification; indeed without even so much as a single vertebrate bloodmeal.

TABLE 1. Olfactometric response of *Aedes spp.* to La Crosse virus amplifying hosts: *Tamias striatus* and *Glaucomys volans* (12 trials).

	Aedes albopictus	*Aedes triseriatus*
Average time to 1st response	7.4 sec.	21.7 sec.
Avg. proportion of 200 ♀♀ mosquitoes responding within 5 minutes	47.6%	38.6%

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Seasonal Distribution of the Ticks of Coastal South Carolina

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The lack of knowledge on the seasonal abundance of ticks in South Carolina was justification for conducting this research. The data presented here represents more than 2 years of tick collection from small- and medium-sized hosts.

Small- and medium-sized hosts in coastal South Carolina examined yielded 3 genera and 8 species of hard ticks (Ixodidae). They include the following: *Amblyomma americanum* (Linnaeus), *A. maculatum* Koch, *Dermacentor variabilis* (Say), *Ixodes brunneus* Koch, *I. cookei* Packard, *I. minor* Neumann, *I. scapularis* Say, and *I. texanus* Banks. All species were collected from mammals except *I. brunneus* which was taken from a white-throated sparrow, *Zonotrichia albicollis*. Hosts included raccoons, *Procyon lotor* (Linnaeus) (n = 190), opossums, *Didelphis virginiana* Kerr (n = 98), foxes, *Urocyon cinereoargenteus* (Schreber) (n = 6) and *Vulpes fulva* (Desmarest) (n = 1), cotton mice, *Peromyscus gossypinus* (LeConte) (n = 70), house mice, *Mus musculus* Linnaeus (n = 5), Florida wood rat, *Neotoma floridana* (Ord) (n = 1), roof rat, *Rattus rattus* (Linnaeus) (n = 3), cotton rat, *Sigmodon hispidus* Say and Ord (n = 3), and southern gray squirrel, *Sciurus carolinensis* Gmelin (n = 4). Table 1 indicates the seasonal distribution of life stages of ticks collected from small- and medium-sized mammals in coastal South Carolina.

Dermacentor variabilis, the American dog tick, was the most frequently encountered tick in coastal South Carolina and most specimens were recovered from raccoons. These specimens were almost exclusively adults whereas immature stages were mostly found on cotton mice. Adults appear to exhibit a unimodal June peak. The American dog tick is a known vector of Rocky Mountain Spotted Fever (RMSF) in the eastern United States (Sonenshine, 1991). There were 37 reported cases of RMSF in South Carolina in 1991 (MMWR, 1992) but only 8 were reported in 1992 (MMWR, 1993).

Ixodes texanus was the second most abundant tick and all stages were found exclusively on raccoons. Seasonal distribution of adult *I. texanus* (almost entirely female) appeared erratic, however, a bimodal curve was observed with peaks in February and again in June. Nymphs also exhibited bimodality with a strong peak in February and again in October. Larvae peaked twice, in June and November.

Immature *A. americanum*, the lone star tick, were most abundant on raccoons but very few adults were collected from any of the hosts examined. Lone star ticks are known to vector RMSF (Harwood and James, 1979), are capable of acquiring the Lyme disease spirochete (Piesman and Sinsky, 1988), and are the presumed vector of human ehrlichiosis (Anderson et al., 1993). Peak activity for larvae and nymphs appeared to be from June to July. Additional collection techniques which involved a comparison of flagging and dragging methods indicated that this species was the most abundant questing tick collected (unpublished, 1993). Furthermore, observations in coastal South Carolina showed that recreationists and outdoorsmen are much more likely to encounter all stages of *A. americanum* during the summer months.

Adult *I. scapularis* were most prevalent on opossums while immatures were found only on cotton mice, Florida wood rats, and house mice. Peak activity for adults appeared to be November - January. The numbers of immatures collected were not sufficient enough to draw conclusions regarding seasonal abundance. Oliver et al. (1993) demonstrated conspecificity of *I. scapularis* and *I. dammini* based on morphological, karyotypical, and hybridization studies, therefore, this would indicate the presence of the Lyme disease vector. On the other hand, Telford (1993)

presented evidence to support retaining *I. dammini* as a valid species.

The other tick species recovered from hosts were collected in only small numbers therefore discussion with regards to seasonal activity is limited.

Raccoons yielded the greatest overall abundance of ticks with an average of 24.7/animal. The majority of these ticks were *D. variabilis* which accounted for 35% of the total number collected. Collections of *I. texanus, A. americanum, I. scapularis, I. cookei*, and *A. maculatum* from this host accounted for 32.2, 31.4, 0.7, 0.6, and 0.1% of the total numbers respectively.

Opossums were the second greatest source of ticks with an average of 7.0/animal. *Dermacentor variabilis* collections represented 65.3% of the total number of ticks recovered from these animals. *Ixodes scapularis, A. americanum*, and *I. cookei* accounted for 31.6, 2.9, and 0.2% of the total number of ticks collected from opossums, respectively.

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Table 1: Seasonal distribution of life stages of ticks collected from hosts trapped in coastal South Carolina.

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
A. americanum													
	Male				+	+	+	+		+			
	Female				+	+	+	+					
	Nymph	+	+	+	+	+	+	+	+	+	+	+	+
	Larvae	+			+	+	+	+	+	+	+	+	
A. maculatum													
	M					+							
	F									+			
	N												
	L												
D. variabilis													
	M			+	+	+	+	+	+	+			
	F		+	+	+	+	+	+	+	+		+	
	N			+	+	+	+	+		+	+	+	+
	L			+	+			+	+	+	+	+	+
I. cookei													
	M												
	F	+	+									+	
	N	+	+								+	+	+
	L		+	+									
I. minor													
	M												
	F										+		
	N			+	+	+	+				+		+
	L			+							+		+
I. scapularis													
	M	+	+	+	+						+	+	+
	F	+	+	+	+	+					+	+	+
	N	+	+	+	+	+			+			+	
	L			+		+		+			+	+	+
I. texanus													
	M		+			+	+						
	F	+	+	+	+	+	+	+	+	+	+	+	+
	N	+	+	+	+	+	+	+	+	+	+	+	+
	L	+	+	+					+		+	+	+

A Concern for the Use of Aedes pseudoscutellaris (AP-61) Cell Cultures in the Studies of Arbovirus RNA

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Since the AP-61 cell line was established (1), it was used for the isolation of a variety of arboviruses, including dengue, yellow fever, and Rift Valley Fever viruses (2,3,4,5). Because of the wide spectrum of susceptibility to arbovirus infections (6), the cell line will continue to enjoy its popularity for virus isolation and identification. In fact, the cell line was selected as one of the principal means of arbovirus isolation in a WHO Reference Center (7). Further, it has been used for various areas of research ranging from preparatin of immunogens for immunologic investigations (8) to a base-sequencing study of dengue virus by PCR (9).

As reported by a member of the group that established the cell line (10), the AP-61 cell line had been contaminated from the inception with at least a few uncharacterized or poorly characterized viruses, including Kawino virus, a picorna-like RNA virus (11). According to the report, the mosquito colony from which the larvae were obtained for primary culture was apparently contaminated with the viruses; and all attempts to free the AP-61 cell line of the viral contaminants were unsuccessful. Further, other cell line established later from the mosquito was similarly contaminated (10). Thus, if the authors of publications used or if any readers are aware of any AP-61 culture free of viral contaminants, it should be made available for the benefit of arbovirus community through this Information Exchange.

Viral contamination of mosquito cell cultures has been reported before, but the exact source of contamination has been often unknown. The greater concern, however, is the lack of or inadequate characterization of those contaminants. The recent identification of a viral contaminant of a cell line from Aedes aegypti as a new Flavivirus based on genomic structure and base sequence homology (12) is an important reminder for those working with Flaviviruses.

Although RNA extracted from the AP-61 cells infected with arboviruses was used for molecular or biochemical studies in the past, fortunately no interference by the viral contaminants has been reported to date. Nevertheless, when information on the base sequence or other characteristics of the RNAs of viral contaminants are hardly known, there is no complete guarantee that cross reaction will not be observed in the future when other arboviruses are grown in the cells and directly used for RNA analyses. The same precaution also applies to the PCR studies using directly mosquito specimens caught in the field, since detection of virus inhabitants (natural or accidental) in field-caught mosquitoes is not rare, as illustrated in the above case of Ae. pseudoscutellaris from Fiji.

This communication was not meant to question the utility of the cell line. Undoubtedly, some arboviruses are more efficiently isolated in the AP-61 cells than in other cell lines. However, if the ultimate objective of a study is characterization or use of viral nucleic acid, perhaps it would be advisable to purify the virus by one of the available techniques, such as plaque purification, and grow in another cell line free of contaminant before undertaking a biochemical or molecular biologic study. Regardless, use of virus-contaminated cell cultures for these areas of virology does not appear scientifically prudent.

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"DETECTION OF AHSV IN INFECTED SPLEENS BY A SANDWICH ELISA USING TWO MONOCLONAL ANTIBODIES SPECIFIC FOR VP7"

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African horsesickness (AHS) is a highly fatal infectious disease of equidae caused by an orbivirus that is transmitted by arthropods of various *Culicoides* species. It produces important economical damage in affected countries because of direct costs of horse deaths and vaccination as well as some other indirect costs such as loss of trade and international competition.

Since August 1987, sporadic outbreaks of AHS have taken place in Spain, where this disease had not been detected since the first epizootic in 1966.

Effective control measures to stop the spread of the disease, in case of a new outbreak, can only be applied when detection of the virus is made in a short period of time. The current methods of diagnosis were virus isolation in tissue culture or mice, with the subsequent virus identification by seroneutralization. This proved to be very laborious and time-consuming, and the need for a quick, easy and reliable technique had to be satisfied.

Different ELISA tests for the detection of AHSV have been described (Du Pleiss *et al.*,1990, and Hamblin *et al.*,1991). However, the use of more uniform and easily obtained reagents would be desirable in order to make a more standardized test.

We have developed a new ELISA for detection of AHSV in horse spleens or cell culture supernatants by the use of two monoclonal antibodies (MAbs) that recognize two non-overlapping epitopes of the major core protein (VP7).Both 5G5 and 3D2 MAbs are used to coat the solid phase because when used together, a clear cooperative effect in capturing AHSV is observed. Since the 5G5 MAb has a high titer in binding to AHSV and labelled well with biotin, it has been chosen for conjugation.

This ELISA was evaluated for its ability to detect AHSV in infected spleens, resulting in a sensitivity of 97.4% and a specificity of 100% compared to virus isolation in cell culture. It can be used for the detection of the nine different AHSV serotypes. This assay also allows the detection of about 10nm/well of purified virus or 2.5 x 10^3 $TCID_3$/well infected cell cultures in only 3 h.

The use of MAbs that can be produced in large amounts with uniform quality will facilitate the standardization of this assay, compared to those using polyclonal antisera. The ELISA described here, therfore, provides a valuable test for use for the routine diagnosis and rapid detection of AHSV in biological samples.

A SINGLE CHAIN ANTIBODY FRAGMENT EXPRESSED IN BACTERIA NEUTRALIZES TICK-BORNE FLAVIVIRUSES

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A recombinant single heavy and light chain antibody fragment that identifies a neutralizing epitope on the envelope glycoprotein of louping ill virus has been developed using a bacteriophage expression system. The mRNA was extracted from a cloned hybridoma cell culture that produces a mouse monoclonal antibody (MAb 4.2) known to bind to amino acids 308-311 of louping ill virus (Jiang *et al.*, 1992; J Gen Virol **74**, 931-935), corresponding to domain B on the proposed two dimensional model of the tick-borne encephalitis virus envelope protein (Mandl *et al.*, 1989; J Virol. **63**, 564-571). The V-genes encoding the antibody-binding site were amplified by PCR and cloned for expression as a fusion protein to the pIII coat protein of filamentous phage. Antibody fragments displayed on the surface of the phage were selected by their binding affinities to louping ill virus antigen coated on microtitre plates. The antibody fragments were subsequently produced in a soluble form which was secreted from the bacterial cells. The nucleotide sequences of the heavy and light chain variable regions were determined and shown to resemble closely those of known immunoglobulin molecules. The characteristics of the expressed single chain antibody have been compared with the mouse monoclonal antibody derived conventionally by fusion of immune spleen and myeloma cells. The expressed antibody portrayed the antigenic specificity of the mouse monoclonal antibody and also neutralized the infectivity of louping ill and some other tick-borne flaviviruses, thus confirming its capacity to bind to the specific epitope (antibody binding site) identified by the conventionally derived monoclonal antibody. The potential of this technique for studying antigen-antibody interactions and for the development of human prophylactic agents is being investigated.

Noncoding sequences of Flavivirus Kunjin

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The previously published nucleotide sequence of the coding region and partial sequence of the noncoding regions of flavivirus Kunjin (KUN) (Coia et al., J. Gen. Virol., 1988, 69, 1-21) have now been extended and completed by the method of Mandl et al (Biotechniques, 1992, 10, 480-486). This involved decapping of the 5' end of purified viral RNA, cyclisation by RNA ligase, reverse transcription by use of KUN specific primers and amplification across the join by PCR. The complete sequence comprises 11022 nucleotides; it is the longest known flavivirus RNA sequence. The 5' and 3' noncoding regions comprise 96 and 624 nucleotides respectively. These sequence data have been deposited in GeneBank and have been given the accession numbers: L24511 for the 5' noncoding region and L24512 for the 3' noncoding region.

The 5' end sequence is similar to that of other WN virus subgroup members. Although the KUN nucleotide sequence is similar in length to the WN sequence with 78% homology, the first 46 nucleotides in the 3' noncoding sequence are not present in the WN sequence, nor in any other published flavivirus sequence. The KUN 3' noncoding sequence includes the conserved regions described by Hahn et al (J. Mol. Biol., 1987, 198, 33-41) in MVE, WN and DEN2 viruses. The reported secondary structures of 5' and 3' ends of other flaviviruses appear to be applicable to the KUN sequences.

PHENOTYPIC VARIATION AMONG KUNJIN VIRUS ISOLATES

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Kunjin (KUN) virus is a mosquito-borne flavivirus and is one of the agents causing Australian encephalitis. KUN is endemic in the tropical and monsoonal areas of northern Australia and occurs sporadically in the temperate region of the south eastern part of the continent where epidemics of encephalitis occasionally occur (Broom *et al.*, 1992). KUN isolates which have been examined using RNase T1 oligonucleotide mapping, are considered to be genetically homogeneous within Australia (Flynn *et al.*, 1989), however recent evidence has shown that phenotypic variation may exist. We investigated the extent of the antigenic variation among 33 KUN virus isolates (Table 1) using an immunoperoxidase assay and a panel of six monoclonal antibodies. The glycosylation of the E protein of each isolate was also examined using Endoglycosidase F enzyme digestion. In addition, these isolates were examined for the production of syncytia in C636 cells.

The results have shown that KUN viruses isolated in Australia are not antigenically homogeneous and occur in two major antigenic types (Table 2). A single isolate from north west Australia (WK 436) formed a third type and. an isolate from Borneo was found to be different to all the Australian isolates forming a fourth antigenic type. The endo F digestion of KUN isolates revealed that glycosylation of the E protein was also variable. Indeed, 19 of the 33 isolates tested were found to possess a glycosylated E protein. Of particular interest was the glycosylation observed on the E protein of the sequenced strain of KUN (MRM61C) which was previously found to be unglycosylated (Wright, 1982) and lack the glycosylation motif (Asn-X-Ser/Thr) on the E protein (Coia *et al.*, 1988). It was also observed that some strains of KUN produced distinctive syncytia in C6/36 cell cultures while others produced no significant effects on the cell morphology.

Further analysis of these phenotypic variations revealed a significant correlation between date of isolation and antigenic type. From 1984 onwards isolates changed from predominantly type one to predominantly type two. It is interesting to note that about this time the viral isolation procedure changed from suckling mouse inoculation to isolation in C6/36 or Vero cells. Further studies confirmed that passage in suckling mouse brain, C6-36 or Vero cells could influence the antigenic type and glycosylation status of the E protein. This was clearly demonstrated when an isolate from human serum (Phillips et al, 1992) changed antigenic type after a single passage in suckling mouse brain and lost glycosylation after serial passage in C6/36 cells and suckling mouse brain.

Table 1: Kunjin Virus Isolates Used in This Study.

Year of Isolation.	Virus Identification.	Source of Isolate.	Place of Isolation.	Passage History.
1984	35911(1)	Horse brain	Hunter, NSW	6smb/3v
1984	BOORT	Horse spine	Boort, Victoria	5smb/3v
1972	CH 15139C	*Culex annulirostris*	Charleville, QLD	3smb/3v
1974	CH 16465C	*Cx annulirostris*	Charleville, QLD	3smb/3v
1974	CH 16483E	*Cx annulirostris*	Charleville, QLD	3smb/3v
1974	CH 16514C	*Cx annulirostris*	Charleville, QLD	3smb/3v
1974	CH 16532C	*Cx annulirostris*	Charleville, QLD	3smb/3v
1974	CH 16549E	*Cx annulirostris*	Charleville, QLD	3smb/3v
1982	CX 238	*Cx annulirostris*	Ord river, East Kimberley	1smb/1v
1982	CX 255	*Cx annulirostris*	Ord river, East Kimberley	2smb/3v
1986	FC 15	*Cx annulirostris*	West Kimberley	2smb/1v
1991	HU 6774	Human	Southern NSW	1c6
1991	HU XSMB	Human	Southern NSW	1c6/1smb
1989	K 1738	*Cx annulirostris*	Kimberley	2c6/3v
1984	K 2499	*Cx annulirostris*	Kimberley	2c6/3v
1989	K 5374	*Cx annulirostris*	South-east Kimberley	3c6/3v
1991	K 6547	*Cx annulirostris*	South-east Kimberley	2c6/2v
1991	K 6590	*Cx annulirostris*	Broome, West Kimberley	?
1982	M 695	*Cx annulirostris*	N. Victoria	2smb/3v
1983	M 1465	*Cx annulirostris*	N. Victoria	3smb/3v
1966	MRM 5373	*Oriolus flavocintus*	Mitchell river mission, N. QLD	3smb/3v
1960	MRM 16	*Cx annulirostris*	Mitchell river mission, N. QLD	7smb/3v
1960	MRM 61C	*Cx annulirostris*	Mitchell river mission, N. QLD	?
1973	OR 130	*Cx annulirostris*	Ord river, East Kimberley	5smb/3v
1973	OR 134	*Cx annulirostris*	Ord river, East Kimberley	2smb/3v
1973	OR 166	*Cx annulirostris*	Ord river, East Kimberley	3smb/3v
1973	OR 205	*Aedes tremulus*	Ord river, East Kimberley	6smb/3v
1974	OR 354	*Cx annulirostris*	Ord river, East Kimberley	2smb/3v
1974	OR 393	*Cx annulirostris*	Ord river, East Kimberley	5smb/3v
1972	OR 4	*Cx annulirostris*	Ord river, East Kimberley	5smb/1v
1991	SH 83	Chicken	Victoria	4smb/3v
1979	WK 436	*Cx annulirostris*	Camballin, West Kimberley	2c6/4v
1966	MP502-66	*Cx pseudovishnui*	Sarawak	?

Table 2: Phenotypic Variation Among Kunjin Virus Isolates. Glycosylation was detected using Endo F enzyme digestion (0.5 units); the antigenic type was determined from binding patterns of a panel of six Mabs to KUN viral antigen cultured in C6/36 cell immunoperoxidase assay; and syncytia observed when cultured in C6/36 cells.

Virus Identification	Glycosylation Status.	Antigenic Type C6/36 Cells	Formation of Syncytia
35911(1)	Non-glycosylated	1	NO
BOORT	Glycosylated	2	YES
CH 15139C	Glycosylated ⊕	1	YES
CH 16465C	Non-glycosylated	1	YES
CH 16483E	Non-glycosylated	1	YES
CH 16514C	Non-glycosylated	1	NO
CH 16532C	Non-glycosylated	1	NO
CH 16549E	Glycosylated ⊕	1	YES
CX 238	Glycosylated	1*	YES
CX 255	Non-glycosylated	1*	YES
FC 15	Glycosylated	2	YES
HU 6774 ♥	Glycosylated	2	NO
HU XSMB ♥	Glycosylated ⊕	1*	YES
K 1738	Glycosylated ⊕	2	YES
K 2499	Glycosylated	1*	NO
K 5374	Glycosylated	2	NO
K 6547	Glycosylated	2	NO
K 6590	Glycosylated ⊕	2	NO
M 695	Glycosylated	1	YES
M 1465	Non-glycosylated	1	NO
MRM 5373	Non-glycosylated	1	NO
MRM 16	Non-glycosylated	1	YES
MRM 61C	Glycosylated	1*	YES
OR 130	Non-glycosylated	1	YES
OR 134	Glycosylated ⊕	1	YES
OR 166	Glycosylated	1	YES
OR 205	Non-glycosylated	1*	YES
OR 354	Glycosylated	1*	NO
OR 393	Glycosylated	1	NO
OR 4	Non-glycosylated	1*	NO
SH 83	Non-glycosylated	2	NO
WK 436	Glycosylated ⊕	3*	NO
MP 502-66	Glycosylated	4	NO
MVE OR 1	Glycosylated		NO
WEST NILE	Glycosylated		NO

* Indicates that the antigenic type changed between culture in C6/36 cells and Vero cells.

♥ These isolates are from the same patient.

⊕ Double band on digestion.

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The S RNA genome segments of Batai, Cache Valley, Guaroa, Kairi, Lumbo, Main Drain and Northway bunyaviruses: sequence determination and analysis.

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Bunyaviruses have a genome comprising three segments of negative sense RNA. The smallest RNA segment, S, encodes the nucleocapsid protein, N, and a nonstructural protein, NSs, in overlapping reading frames. The sequences of the S genome RNA segments of seven bunyaviruses (Batai, Cache Valley, Guaroa, Kairi, Main Drain, Northway and Lumbo) were determined from cloned cDNAs obtained using a one-step reverse transcription-polymerase chain reaction protocol. These sequences were compared to those of six viruses previously published; the features of these sequences are summarized in Table 1. Sequence homologies between N proteins correlated with the subdivision of these viruses into three serogroups, Bunyamwera, California and Simbu (Table 2). The encoded N proteins are either 233 or 235 amino acids in length, depending on the serogroup, whereas the NSs proteins are more variable (83-109 amino acids). Guaroa virus has a Bunyamwera-like rather than California-like N protein. A dendrogram showing the relationship of the N proteins is shown in Figure 1. Certain nucleotide sequence motifs are conserved in the S segments of the Bunyamwera and California serogroup viruses, including the spacing of the AUG initiation codons for the N and NSs proteins (except Guaroa virus), and a CA-rich motif in the virion-sense RNA just downstream of the predicted mRNA termination site. A duplicated sequence was observed in the 3' noncoding region of the Lumbo virus S segment, which accounts for the significantly longer S genome segment of this virus. More detailed comparisons of these sequences will appear in the Journal of General Virology in 1994.

Table 1. Summary of bunyavirus S RNA sequences

Serogroup	Virus	Nucleotides				Amino Acids		Accession
		Total	5' nc*	3' nc	A+U%	N	NSs	number
Bunyamwera	BAT	946	68	176	59.5	233	101	X73464
	BUN	961	85	174	58.2	233	101	D00379
	CV	950	74	174	59.8	233	101	X73465
	GER	980	87	191	57.6	233	109	M19420
	GRO	954	26	226	62.4	233	83	X73466
	KRI	992	81	209	60.6	233	109	X73467
	MAG	945	73	170	59.9	233	101	D00380
	MD	954	76	176	60.0	233	101	X73469
	NOR	945	74	169	60.5	233	101	X73470
California	LAC	981	81	192	58.8	235	92	K00108
	LUM	1077	78	291	58.7	235	97	X73468
	SSH	982	79	195	56.3	235	92	J02390
Simbu	AINO	850	34	114	55.9	233	91	M22011

* nc, noncoding region of positive-sense RNA.

Table 2. Amino acid identities between bunyavirus N proteins and between bunyavirus NSs proteins.

% amino acid identity between N proteins

Virus	BAT	BUN	CV	GER	GRO	KRI	MAG	MD	NOR	LAC	LUM	SSH	AINO
BAT		91.8	94.0	77.3	70.4	68.2	93.1	89.3	95.0	43.4	42.5	45.1	41.7
BUN	89.0		90.6	75.1	68.7	70.8	91.0	90.1	92.7	43.4	43.4	45.1	43.0
CV	94.1	88.1		75.1	70.8	69.1	95.7	89.7	96.1	44.6	44.2	45.9	42.2
GER	68.7	71.0	71.7		63.5	62.2	73.8	74.3	76.0	45.5	42.1	46.4	43.2
GRO	43.9	43.8	43.9	43.2		69.5	69.1	68.7	70.0	47.6	45.1	47.6	44.2
KRI	67.3	67.3	69.3	56.5	51.2		67.8	70.4	69.5	48.1	45.5	49.7	43.3
MAG	85.1	89.0	97.0	69.7	42.7	68.3		91.4	94.9	43.4	42.9	44.6	41.7
MD	90.1	86.1	94.1	67.7	47.6	69.3	93.1		91.9	43.4	41.6	44.6	40.4
NOR	94.1	88.1	96.0	68.7	45.1	70.3	95.1	95.1		44.2	42.5	45.9	42.1
LAC	38.2	38.2	38.9	34.1	35.5	32.7	39.3	40.5	39.3		84.7	90.6	45.0
LUM	34.0	30.9	41.1	32.3	33.3	34.0	41.1	44.4	41.1	75.0		87.7	44.6
SSH	31.9	29.7	31.5	31.9	36.4	33.7	31.9	37.1	31.9	85.8	76.1		45.0
AINO	28.9	30.7	28.6	24.2	38.0	31.9	27.8	31.1	30.0	30.7	37.1	34.1	

% amino acid identity between NSs proteins

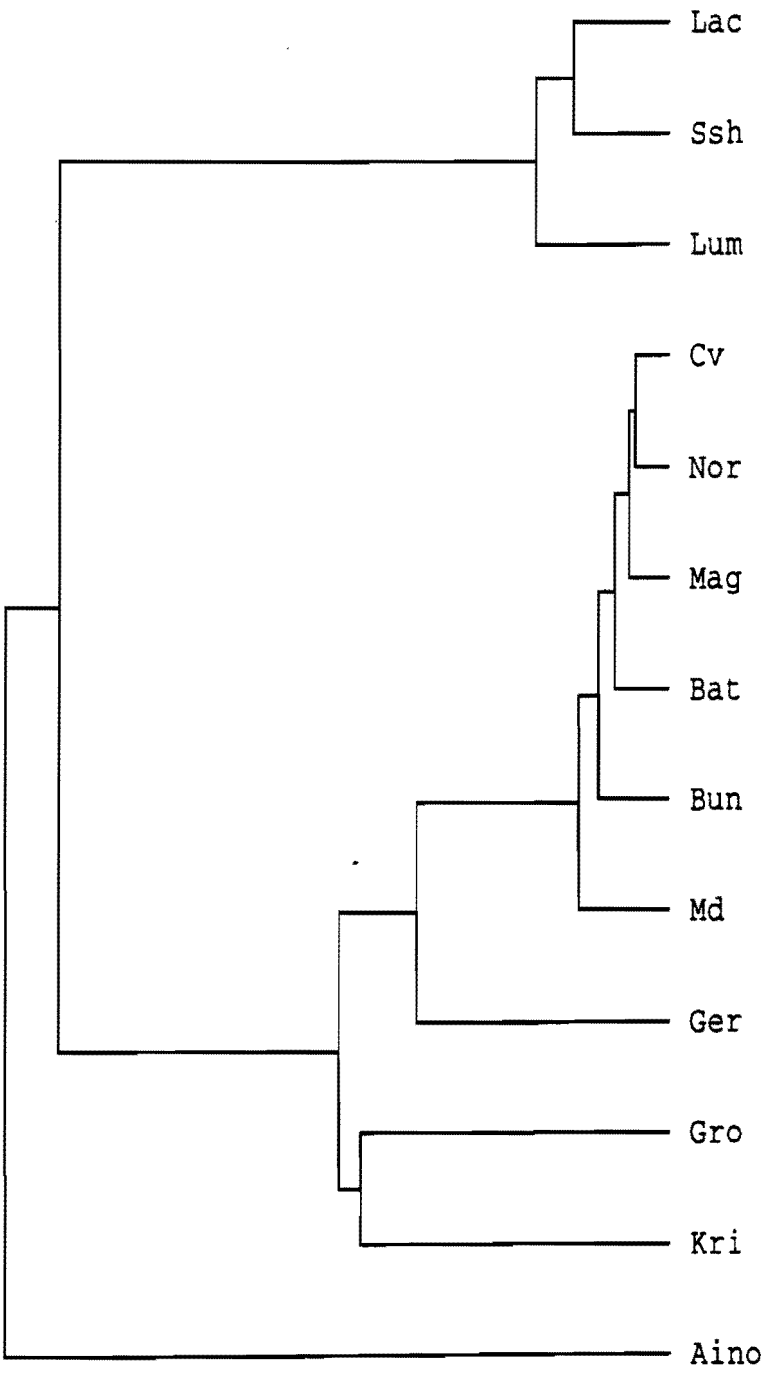

Figure 1. Dendrogram showing relationship between bunyavirus N proteins.

REPORT FROM ARBOVIRUS UNIT
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RVFV monoclonal antibodies - Terry G Besselaar, N K Blackburn

Analysis of the mechanisms involved in neutralization of RVFV had previously revealed that the inhibition of virus infectivity is most likely the result of several different processes, including synergistic neutralization by combinations of different antibodies. Some MAbs were unable to neutralize cell-bound virus, suggesting that they may act by preventing virus from attaching to the cell or by inhibiting cell entry of the adsorbed virus. The present findings showed that none of the MAbs prevented virus attachment, suggesting that the epitopes they define are spatially separate from the site(s) responsible for virus binding to the cellular receptor. Some of the G1-specific MAbs, including the strongly neutralizing and protective MAb mapping to site G1 la, also had no effect on viral internalization and thus must neutralize solely at an intracellular stage.

The remaining neutralizing MAbs directed against the RVFV envelope proteins substantially prevented virus internalization. The observation that both G1 and G2-specific MAbs were capable of inhibiting virus penetration not only reveals a further neutralization strategy employed by immune antibodies but also demonstrates that both envelope glycoproteins have an important function in entry of RVFV into the host cell. This finding is of significance as it is the first time that the involvement of both proteins in RVFV entry into the cell has been definitively shown.

CHARACTERIZATION OF FLANDERS VIRUS cDNA; EVIDENCE FOR SIX GENES

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We have previously identified 8 virus-specific proteins from Flanders virus (FV) infected Vero cells that are also detected in purified virions. One of these is glycosylated and phosphorylated, and two others are phosphorylated [Boyd and Whitaker-Dowling (1988), Virology 163, 349-358]. In vitro translation reactions containing poly A+ RNA from FV-infected BHK cells produced seven FV-specific proteins of which six comigrated with proteins from FV-infected Vero cells and the seventh (mw = 64,000) represents the nonglycosylated form of the glycoprotein (mw = 81,000) [Arbovirus Information Exchange, Dec. 1990].

A cDNA library was constructed with poly A+ RNA from FV-infected BHK cells using the SUPERSCRIPT Plasmid System and dsDNA was directionally cloned into pSPORT 1. DH10B competent cells were transformed, grown in the presence of IPTG, and FV-specific clones were identified with polyclonal FV-immune ascitic fluid. Cross-hybridization screening among 400 clones yielded six groups of clones. To confirm that the representative clone from each group was unique, RNA probes prepared from each insert failed to hybridize with inserts from the other 5 clones. In addition, sequence analysis of the 3′ (mRNA sense) end of each insert confirmed that each was distinct from the others.

Northern blot analysis of RNA prepared from FV-infected cells using negative sense RNA probes prepared from each of the six clones showed that each hybridized to FV-specific RNA. The protein coding assignment for each clone was determined by hybrid selection of RNA from FV-infected cells and in vitro translation. A summary of the data from the two procedures follows.

Clone Group No.	Size(Kbp)	Hybridization RNA, Size (Kb)	Protein Kd	Tentative Assignment
1	1.4	1.4	54	N
2	1.2	1.2 & 0.7	33	P
3	1.0	1.0 & 0.5	19	M
4	2.2	2.2 & 2.9	64	G
5.	0.7	0.7 & 2.9	27	?
6.	3.1	6.0	180	L

These new data suggest that the FV genome consists of at least 6 genes and that at least 6 proteins are translated directly from viral mRNA. We are proceeding with further analyses to (1) determine why the Group 2 probe and Group 3 probe each hybridized to two species of RNA (2) confirm the gene order and intergenic sequences (3) characterize the "sixth" gene and (4) assess the relatedness of FV to other negative sense RNA viruses including other Hart Park Serogroup viruses by comparisons of nucleotide sequences.

RFLP typing applied to the molecular epidemiology of Euro-African West Nile virus isolates

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The West Nile (WN) flavivirus is a very common mosquito-borne pathogenic agent responsible in human for the WN fever. Although considered as a minor public health problem, the WN virus has received much attention due to its puzzeling epidemiological features. Indeed, the WN virus exhibits an extended geographic distribution (Africa, Europe, Middle East, India) and can replicate in arthropods (several mosquito and tick species) as well as in various vertebrate hosts (rodent, cattle, birds...). Additionally, WN infections are associated with variable clinical manifestations ranging from mild febrile illness to fatal encephalitis and fulminant hepatitis. However, up to now very few molecular data were available concerning the variability and the circulation of WN virus strains in endemic countries.

Recently, we developed a genomic typing assay intended to the grouping of mosquito-borne flaviviruses strains identified at the WHO Collaborating Centre for Reference and Research on Arboviruses (CRORA, Pasteur Institute, Dakar). We used consensus primers for the RT-PCR amplification of RNA segments representative of about 5% of the flavivirus genome. Consequently, the genomic variability was assessed by amplicon RFLP mapping. This new tool was used to investigate the genetic diversity among 44 WN strains isolated from France and 8 African countries. A total of 30 RFLP alleles were identified with 4 frequent cutter restriction enzymes. Natural alleles combination yielded 23 distinct genotypes that were grouped in 8 families on the basis of allele similarities. The temporal and geographic distribution of West Nile Virus genotypes is consistent with highly mobility of WN virus isolates. In addition, two families of closely related genotypes were found to circulate in large and overlapping domains encompassing respectively North/Western/Central and Western/Central/ Eastern Africa. French WN strains were classified in the North/Western/Central African subgroup.

These data strongly support the hypothesis that birds migrational movements are instrumental to land-to-land and continent-to-continent dissemination of the WN virus. Consequently, the definition of topotypes for the WN virus remains hazardous since in our experiments no correlation was found between a specific genotype and a given geographic origin. Results obtained for the WN virus confirm that the region amplified with our consensus primers (end of NS5-3' non coding region) is suitable for studying strain relatedness and phylogeny among mosquito-borne flaviviruses. This project is now actively investigated by our research groups.

Report from the Abteilung für Virologie,
Technische Universität München, Germany

Thermostability of EYACH virus

As previously reported, we succeeded to establish a system to culture EYACH virus (EYA), an European isolate related to Colorado tick fever virus (CTF), in Vero cells. This enables us to study more in detail virus characteristics and virus-cell interactions. Here we report our results on thermosensitivity of EYA virus.

Culture fluids (MEM + 3% FCS) of EYA (8th Vero passage) infected cells were used as inocula. Previous to each experiment the cell cultures were tested for EYA dsRNA by polyacrylamid electrophoresis and EYA antigen by indirect immunofluorescence using CTF hyperimmune serum, to ascertain that virus was present in the cell culture.

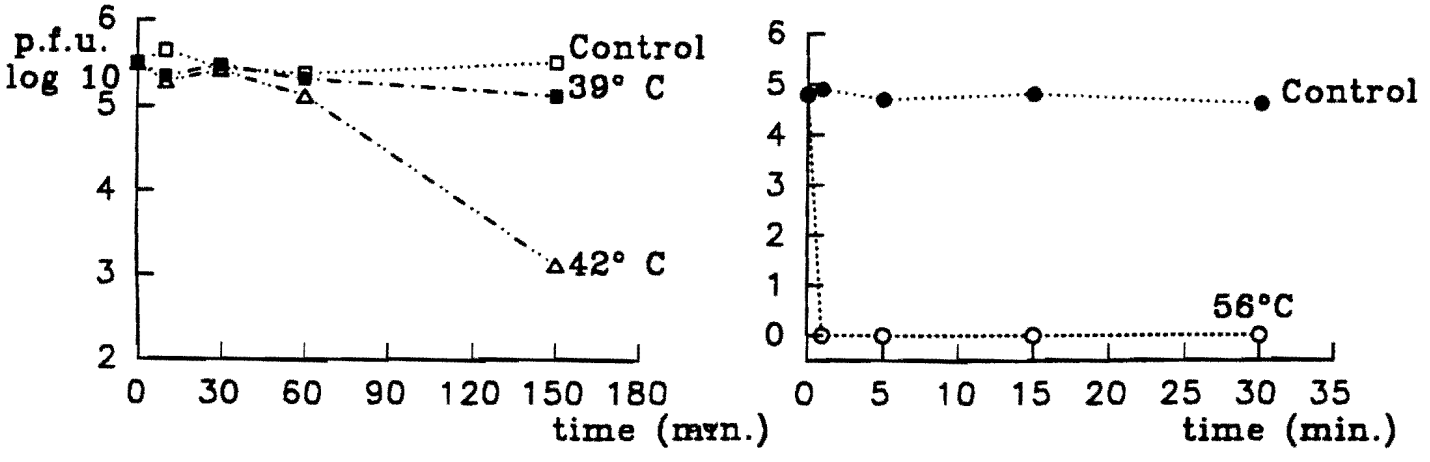
0,5ml of culture fluids were exposed to 39°C, 42°C, and 56°C. Exposure times for 39°C and 42°C were 10, 30, 60, and 150 minutes. Exposure times for 56°C were 1, 5, 15, and 30 minutes. After the respective exposure times the culture fluids were inoculated in triplicate on Vero cells and titrated by a plaque test. As controls the same culture fluids used in the experiments were held for the respective times at 33°C, the temperature commonly used to cultivate EYA.

The results are shown in the figure below. EYA is thermostable at 39°C for the exposure times tested. At 42°C EYA was stable for 60 minutes, while after 150 minutes a decrease of EYA titer of 2 logs was found. At 56°C EYA was completely inactivated after only 1 minute of exposure.

We compared our results to those of thermal inactivation of CTF (Trent et al., J. Bacteriol., 91,1282-1288, 1966). At 39°C EYA was more stable than CTF, while the results at 42°C are similar. At 56°C EYA was found to be much more unstable compared to CTF, which lost only 2 logs after 30 minutes at 56°. The differences can in part be explained by different cell cultures and inocula fluids used in both systems.

(G. Dobler)

Thermostability of Eyach virus



THE ANTIGENIC VARIANTS OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS.

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Crimean-Congo hemorrhagic fever (CCHF) is a severe human infection transmitted by Hyalomma ticks and distributed in Europe, Asia and Africa within the area of these ticks. The CCHF virus belongs to family Bunyaviridae, genus Nairivirus.

The antigenic structure of CCHF virus, as studied by conventional CF, IF, ID and NT tests with immune sera to whole virion, was considered to be gomogenious.

In present investigation the antigenic structure of CCHF virus strains by means of monoclonal antibodies (mAbs) in conjunction with highly sensitive enzimeimmunoassay (EIA) and time-resolved fluoroimmunoassay (TR-FIA) methods (E. Soini and J. Hemmila (1979))were examined. 16 strains of CCHF virus originated from Africa, Asia and Europe were studied (table 1). mAbs GEMA-9, GEMA-10, GEMA-24 and GEMA-12 raised to strain Uz 10145 of CCHF virus, were employed (S. Ya. Gaidamovich et. al. 1989), as well as polyclonal antibodies (pAbs) to Uz 10145 and Ug 3010 strains. The mAbs are probably anti-N specificity. Antibodies were purified from mouse IAF by affinity chromatography on protein A sepharose 4B. In EIA and TR-FIA tests two pAbs and four mAbs were used as a primary (capture) antibodies ; only GEMA-9 and GEMA-12 served as detector antibodies. The results of EIA, presented in table 2, show that GEMA-9 and GEMA-12 were not identical in the reaction with African strain Ug 3010. GEMA-12 distinguished strain Ug 3010 from Uz 10145 if used as a secondary antibodies independently on the type of primary antibodies. GEMA-9 as well as pAbs could not differentiate any strains. In further analysis of 16 strains in EIA and TR-FIA . 3 various type of antigens were used for each strain: crude, SAA and tissue culture antigens. In EIA clear difference of Ug 3010 and IBAR 10200 from the other strainswas detected with cultural antigens. With crude and SAA antigens only Ug 3010 strain could be differentiated.

The results of TR-FIA coincided in general with EIA, but due to more sensitivity of TR-FIA test, it was shown that Astrakhan and IBAR 10200 take intermediate position, being closer to Asian strains.

Conclusion.

Comparative examination of 16 strains of CCHF virus by mAbs in combination with EIA and TR-FIA methods revealed antigenic variation of CCHF virus and showed that there are both conserved and variable epitopes on the N protein of CCHF virus.

Acknowledgements.

We are grateful to Dr. R. Shope, Dr. S. Vasilenko and Dr. V. Gromashevsky for providing us strains of CCHF virus.

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Table 1

Characteristics of CCHF virus strains

	CCHF virus strain	Origin of country	Year	Reservoire	Reciprocul CF titre	Infectious titre in lg LD50/0,02ml
1	Uz 10145	Uzbekistan	1985	Ticks	640	6,5
2	China C	China	1982?	Ticks?	640	n.t.
3	Tad 8966	Tadjikistan	1990	Patien's blood	320	5,8
4	Tad 8973	-"-	-"-	-"-	640	6,1
5	Tad 8975	-"-	-"-	-"-	640	6,2
6	Tad 8976	-"-	-"-	-"-	320	6,0
7	Tad 8978	-"-	-"-	-"-	640	6,3
8	Tad 9102	-"-	1991	-"-	640	5,9
9	Tad 9112	-"-	-"-	-"-	640	6,1
10	Tad 9115	-"-	-"-	-"-	320	5,8
11	Bul 42	Bulgaria	1975?	-"-	640	7,0
12	Bul 517	-"-	1978?	-"-	640	7,0
13	LEIV 22261	Russia	1991	-"-	320	n.t.
14	LEIV 22463	-"-	-"-	Ticks	320	n.t.
15	IbAr 10200	Nigeria	1970	Ticks	n.t.	6,5
16	Ug 3010	Zair	1956	Patient's blood	640	6,4

Table 2

Detection and differentiation of Uz 10145 and Ug 3010 strains (SAA) in EIA with polyclonal and monoclonal antibodies.

Capture antibodies	Detector antibodies					
	Uz 10145		GEMA 9		GEMA 12	
	10145	3010	10145	3010	10145	3010
Poly Uz 10145	320	640	320	160	640	< 10
Poly Ug 3010	640	320	320	320	320	< 10
mAbs GEMA 9	320	320	640	320	160	< 10
mAbs GEMA 10	160	320	640	640	320	< 10
mAbs GEMA 12	640	640	640	320	320	< 10
mAbs GEMA 24	320	320	640	160	160	< 10

Isolation and identification of Coltivirus new members from mosquitoes collected in China

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Up to now ,535 arboviruses have been reported in the world already.But, in China,only Japanese B encephalitis, Dengue,and Crimean-Congo hemorrhagic fever were isolated. In the nearly ten years,some Orbivirus members (ds RNA, 10 segment),some probable Coltivirus members(dsRNA,12segment), Chikungunya and some Alphavirus members were isolated in China. Serological study showed that at least 19 arboviruses activity in China but have not been isolated yet.Therefor arbovirus survey in China is very essential and important.

Mosquitoes were collected in Wudu county and Winxing county,Gansu province,central-western China, situated in the border between Gansu and Sichuan province,105° E,33° N , during July 15 to August 14,1991. During October 24 to November 3,1991,we collected some mosquitoes in Taoranting Park ,located in southern Beijing city and Changping county, approximately 20Km north of Beijing city,116.4° E,40° N.

10 viruses were isolated,they were called WDC_2,WX_1,WX_2, WX_3,ACH,TRT_2,TRT_3,LY_1,LY_2,LY_3,respectively,after the name of the place where the virus was isolated.

The viruses caused CPE on C6/36,BHK-21 and Vero cells, pathogenic for new- born mice, and caused death, not sensitivity to ether and BUDR.Electron micrography showed the viruses were spherical virons without envelope and about 62.7 ± 3.13 nm in diameter.

By a simple,rapid and specific PAGE,the isolates were assigned to four distinct RNA electropherotypes repeatedly. The Gansu province isolates had three RNA patterns,WDC2:2-3-1-3-1-2; WX_3:2-2-2-3-1-2; WX_1,WX_2 and ACH were similar :2-3-2-3-1-2. All of the Beijing isolates were similar:2-2-2-3-1-2. The Yunnan isolate AV which was isolated by Dr. Xu before was:2-2-2-3-1-2. It was easy to find that AV different from both WX_3 and Beijing isolates.

Cross - neutralization test and cross - complement fixation test showed Chinese isolates had antigenic variation. The Gansu province isolate WX_3 is clearly distinct from AV strain of Yunnan province. Chinese isolates have cross reaction with CTF(Florio) , and California strain $S_{3-14-03}$ (titre from 1:4 to 1:8) , but are clearly distinct from them. This result revealed that Chinese isolates are the new members of Coltivirus.

Serological study indicated that at least in Inner Mongolia, Heilongjiang, Liaoning, Beijing, Hebei, Henan, Sichuan and Hunan province,positive results of Coltivirus new member infection were found,the infection rate ranged from 3.2% to 100%,averange rate is 52.3%.

More than fourfold IgG antibody titre increase was demonstrated in the paired serum samples from 3 cases of encephalitis. IgM antibody was detected in the acute sera also .This result suggested that the Coltivirus new member may be a cause of encephalitis in human in the summer and autumn.

Key Words : Coltivirus new member,
Isolation, Identification,
Antigenic variation,
Encephalitis, Pathogen.

Table 1. Arbovirus serological study in China

	human sera	swine sera	monkey sera
Togaviridae	CHIK EEE MAY SIN VEE SF GET	CHIK EEE RR-CHIK RR-EEE	CHIK GET
Flaviviridae	JE KUN ZIKA MVE WN SEP TMU KFD POW LGT DEN	JE-MVE JE-MVE-WN	KUN DEN1 DEN2 DEN4 LGT
Bunyaviridae	AINO SSH JC TAH		

Table 2. Antibody titres from cross - testing 5 isolates by NT

	Antibody to				
Virus	WX_1	WX_2	WDC_2	AV	TRT_2
WX_1	> 640	⟩ 640	⟩ 640	⟨ 5	⟨ 320
WX_2	80	> 640	⟩ 640	⟨10	⟩ 640
WDC_2	⟩ 640	⟩ 640	> 640	5	⟨ 20
AV	> 640	⟩ 640	⟩ 640	640	320
TRT_2	⟩ 640	⟩ 640	640	⟨10	> 640

Table 3 Antibody titres from cross-testing Coltivirus members and Chinese isolates by CFT

	Antibody to							
Antigen	AV	TRT_2	WDC_2	WX_3	WX_1	CTF	Eyach	$S_{6-14-03}$
AV	16	< 64	< 16	< 8	< 32	< 4	⟨ 4	< 8
TRT_2	< 16	< 32	8	< 16	32	4	⟨ 4	8
WDC_2	⟨ 4	< 8	16	32	32	< 4	< 4	8
WX_3	⟨ 4	16	16	64	64	4	⟨ 4	8
WX_1	⟨ 4	8	16	64	64	⟨ 4	⟨ 4	8
CTF	⟨ 4	⟨ 4	⟨ 4	⟨ 4	⟨ 4	< 64	< 32	< 32
Eyach	⟨ 4	⟨ 4	⟨ 4	⟨ 4	⟨ 4	16	⟩ 256	< 64

Table 4 Detection of Coltivirus new member antibody by indirect ELISA

Location	IgG		IgM	
	No. of positive / No. of samples	rate (%)	No. of positive / No. of sample	rate (%)
Heilongjiang	1/31	3.2	NT	
Hunan	2/9	22.2	NT	
Liaoning	1/4	25	NT	
Hebei and Beijing	18/31	58	6/13	46.2
Inner Mongolia	22/30	73.3	NT	
Henan	9/9	100	0/9	0
Sichuan	14/14	100	4/15	26.7
Total	67/128	52.3	10/37	27

NT: Not tested

Table 5 Titration of Coltivirus new member and JEV antibody in six cases of human encephalitis

Case No.	Name	Sex	Age	Location	Onset date	Coltivirus new member IgG (Indirect ELISA)		JEV (RPHI)			
						First serum	Second serum	First serum		Second serum	
								IgG	IgM	IgG	IgM
1.	Wu	M	30	Beijing	92.9.3	400	1600	–	160	–	320
2.	Song	F	14	Beijing	92.8.24	800	3200	20	80	320	–
3.	Wang	F	23	Beijing	92.9.8	100	800	40	–	20	–
4.	Wu	F	28	Beijing	93.7.26	1600	1600	20	–	20	–
5.	Bao	M	4.5	Beijing	92.8.6	1600 (IgM400)	NT	–	–	NT	
6.	Yang	M	27	Shenyang	92.8.15	6400 (IgM800)	NT	–	–	NT	

NT: Not tested. – : Negative

Acknowledgements

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Preliminary report on an outbreak of human illness in the Northwestern Hawaiian Islands

The Northwestern Hawaiian Islands (NWHI) consist of small atolls and volcanic remnants lying to the northwest of the main inhabited Hawaiian islands. They are remote, and most of the islands are protected as wildlife refuges for seabirds and other birds, seals, and sea turtles. The only humans on these islands are federal wildlife officials and wildlife researchers.

Following an illness in several biologists on Laysan Island during 1991, a survey identified a syndrome of human illness among people beginning in 1989, termed "Laysan fever" for convenience. The symptoms were of a non-specific febrile illness and included low-grade fever, headache, nausea, anorexia, bodyaches, and considerable fatigue. Less frequent symptoms included joint pain, lymphadenopathy, and sleep disturbance. The initial cases were self-limited, with symptoms lasting 1 to 7 days (median 4 days). Subsequent surveillance identified a subset of illness which lasted up to 7 weeks and included the symptoms of abdominal pain and diarrhea, indicating the possibility of two overlapping diseases.

The epidemiology of Laysan fever suggests that avian ticks are involved. The illness is confined to the spring and summer, which is the period of observed tick activity. There is a non-significant association with reported tick bites:

	reported tick bite	no bite reported	total
Laysan fever	5	0	5
no Laysan fever	11	6	17
total	16	6	22

Odds ratio not defined.
Fisher's exact test p = 0.166

Although three species of avian ticks have been identified in the NWHI, Ixodes laysanensis appears to be quite uncommon. Ornithodoros capensis is well known both from the NWHI and from other tropical or subtropical seabird colonies around the world. Of the specimens which have been brought in from the field, all have been Ornithodoros ticks and all which were submitted to the National Tick Collection at Statesboro, Georgia, were identified as O. capensis. O. capensis complex ticks (including the closely related O. denmarki) are known to harbor and transmit a number of avian viruses in various parts of the world. In the central Pacific Ocean area, three such viruses have been identified thus far: Johnston Atoll virus, Midway virus, and Soldado virus[1]. There is one report of human illness circumstantially associated with Soldado virus on an island off Morocco[2], but nothing is known about

the potential of Johnston Atoll or Midway viruses to cause human illness.

Since ticks may harbor and transmit a great variety of microbial pathogens as well as introduce various toxic substances through their bites, there are a variety of considerations for the etiologic agent(s) of Laysan fever. However, one or more arboviruses remain high on the list of candidates. Accordingly, we are attempting to isolate a virus from ticks collected in the NWHI. At the same time, we have collected pre-exposure and post-exposure serum samples from people going to the NWHI during 1993; we have asked the CDC's Division of Vector-Borne Infectious Diseases for assistance in testing for antibody to several of the potential etiologic agents.

We have instituted efforts to prevent tick bites to the biologists. These have consisted of education about ticks and their potential for transmitting disease, permethrin aerosol for use on clothing and footwear, and DEET for use on exposed skin. These personal protection measures have been only partially effective thus far. These and other protection efforts are continuing.

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A BRIEF SUMMARY OF ARBOVIRUS ISOLATION AND IDENTIFICATION IN THE NORTHERN TERRITORY, AUSTRALIA

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Mosquitoes have been collected from population centres around the Northern Territory by the Dept of Medical Entomology since 1981, using carbon dioxide light traps.

Medical Entomology speciates the mosquitoes and stores them in liquid nitrogen. Pools of not more than 50 mosquitoes are then presented to the A.L.Rose Virology Laboratory for virus isolation and identification.

For the period 1982-92 314,712 mosquitoes were processed and more than 350 CPE-causing agents were isolated.
Serological tests were performed on each isolate to viruses routinely identified at the laboratory, these include :

Rhabdoviridae - Adelaide River, Berrimah, Bovine Ephemeral Fever, Kimberley.

Reoviridae - Bluetongue Virus : 1, 3, 9, 15, 16, 20, 21, 23.

Palyam : Bunyip Creek, CSIRO Village, D'Aguilar, Marrakai, DPP 66.

EHDV : 2, 5, 6, 7, 8.

Bunyaviridae- Simbu : Akabane, Aino, Douglas, Peaton, Tinaroo.

Togaviridae - Ross River Virus, Barmah Forest, Sindbis.

Flaviviridae- Murray Valley Encephalitis, Kunjin.

Viral isolates that could not be identified were stored at +4 C and -70 C in 10ml and 2ml aliquots respectively.

Dr C. Calisher visited the laboratory in November 1992 and initiated screening of unidentified virus, using antisera to approximately 250 arboviruses supplied by NIH and CDC.

To date 200 viruses have been tested, representing 12 antigenic groups including: Wongorr 65, Wallal 8, Warrego 7, Eubenangee 3, Corriparta 15, Palyam 1, Mapputta 7, Kowanyama 4, Alphavirus I 14, Alphvirus II 21, Alphavirus III 1, Flavivirus 5, Unidentified 49.

Negative contrast electronmicroscopy and thin section electronmicroscopy has been performed on several unidentified isolates by Dr Alex Hyatt at the Australian Animal Health Laboratory. This examination has revealed members of the Reoviridae, Bunyaviridae and Flaviviridae families.

An Outbreak of Australian Encephalitis in the north of Western Australia in 1993

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Murray valley encephalitis virus (MVE) is an important mosquito-borne flavivirus which is the main cause of the disease Australian Encephalitis (AE), a potentially fatal disease in humans. MVE activity is usually confined to the Australian mainland and to Papua New Guinea. The virus is known to be enzootic in parts of the tropical Kimberley region in the north of Western Australia (WA), particularly around Kununurra but only occurs in epizootics in other areas of Australia. Murray Valley encephalitis virus has been responsible for several large epidemics of AE in the eastern states of Australia, the last being in 1974. However since 1974 the majority of reported cases of AE (28 out of 45) have been from WA with another 13 cases from the Northern Territory. The north of WA has a tropical, monsoonal climate with a wet season usually extending from November to May and varying in intensity from year to year. In years with heavy wet season rainfall there is an associated increase in mosquito breeding which leads to an increased health risk to the residents in the north of the State. This occurred in 1993 when widespread rain, thunderstorms and flooding were recorded in February in both the east and west Kimberley regions in the far north of Western Australia. Flood damage was extensive and many cattle stations and Aboriginal communities in the area were isolated for several weeks. Widespread, heavy rain was also recorded in areas of the Pilbara. The large areas of water that were left behind after the floods receded provided excellent breeding grounds for mosquitoes, particularly *Culex annulirostris* mosquitoes, the major vector for MVE virus.

Flavivirus activity in the north of WA is monitored year-round by the Arbovirus Laboratory at the University of WA using sentinel chicken flocks. This programme is carried out in conjunction with the Health Department of WA. At present there are 24 chicken flocks maintained at most towns and some minesites in the Kimberley and Pilbara regions. The chickens are bled fortnightly during the wet season and monthly at other times to provide an early warning of an increase in MVE activity. The first evidence of an increase in MVE activity in 1993 was seen in the second week of March when one chicken in the Kununurra flock seroconverted. By the end of March all of the Kununurra flock were positive to MVE and a large number of seroconversions had also been recorded in other flocks throughout the Kimberley. By the end of the wet season a total of 60/60 (100%) of the chickens in the East Kimberley and 52/60 (87%) of the chickens in the West Kimberley were positive for antibodies to MVE. During April, MVE activity was also detected further south, in flocks located in the Pilbara. The spread of virus to this region was probably due to the movement of viraemic waterbirds from endemic areas of the Kimberley.

During the 1993 wet season 8 cases of AE were reported from WA and a further 6 from adjacent areas of the Northern Territory (Map 1). This is the most cases ever confirmed from the north of WA in a single season. The first case was reported from Fitzroy Crossing with an approximate date of the onset of symptoms on 20/03/93, only 9 days after the first seroconversions in sentinel chickens. Further cases were

reported from Billiluna, Warmun, Kununurra and Halls Creek in the East Kimberley and Derby in the West Kimberley. Details of the cases in WA are presented in the following table. All the cases shown were confirmed by a rising titre in MVE specific antibodies in paired sera and/or the presence of MVE specific IgM in the CSF.

Table showing details of the AE cases from WA in 1993

Case	Location	Age	Sex	Race	Onset Date
1	Fitzroy Crossing, WK	1 y	M	Aboriginal	20/03/93
2	Billiluna, EK	1 y	M	Aboriginal	28/03/93
3	Warmun, EK	2 y	F	Aboriginal	13/04/93
4	Halls Creek, EK	24 y	M	non -Aboriginal	15/04/93
5	Kununurra, EK	39 y	F	non -Aboriginal	24/04/93
6	Kununurra, EK	9 m	M	non- Aboriginal	02/05/93
7	Derby, EK	3 m	M	non- Aboriginal	20/05/93
8	Halls Creek, EK	14 m	M	Aboriginal	11/06/93

Abbreviations : WK = West Kimberley, EK = East Kimberley, y = years, m = month, M = male, F = female

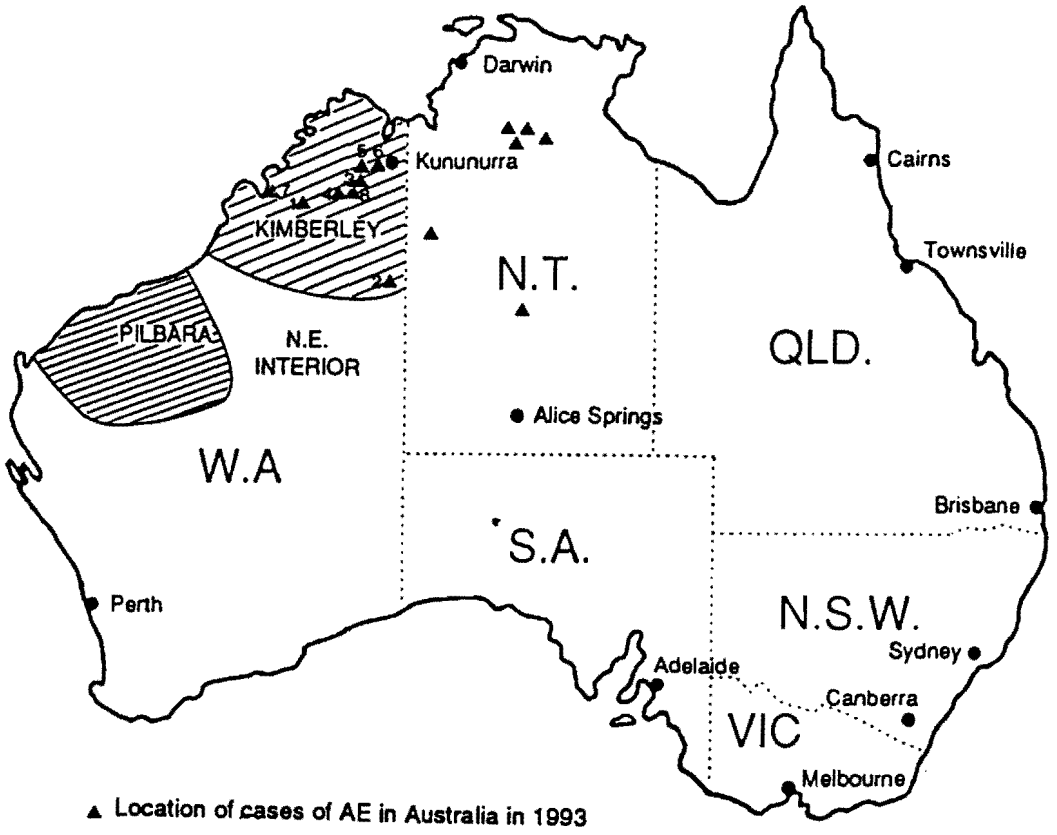
Of the 8 cases, 6 were children aged 2 years or less and 2 were adults. Four of the six children were Aboriginals while both adults were non-Aboriginal. All three deaths occurred in children, two Aboriginal and one non-Aboriginal. This pattern of infection and mortality is usual for the Kimberley region of WA (Mackenzie et al., 1993). These cases also showed the same strong male predominance in young children. The 3 month-old child is the youngest case of MVE infection documented in WA. Interestingly the mother did not have flavivirus antibody detectable in her serum. The lack of passively transferred maternal antibody may have made this child more susceptible than most young infants in endemic regions.

The cases of AE in 1993 occurred as a result of extremely heavy wet season rainfall and flooding in the north of Australia which in turn allowed increased mosquito breeding. This led to a subsequent increase in MVE activity and this high level of activity was maintained for several months in the Kimberley region. Previously, major outbreaks in the south east of Australia have been preceded by an increased level of MVE activity in the Kimberley region and therefore rainfall and other environmental factors which predispose to outbreaks of AE should be closely monitored in 1994.

Reference: Mackenzie, J.S., Smith, D.W., Broom, A.K. and Bucens, M.R. Australian encephalitis in Western Australia, 1978-1991. Med. J. Aust. 1993 ; 158 : 591-595.

The authors would like to thank the Health Department of Western Australia who fund this project, all the people involved in the sentinel chicken programme and Maria D'Ercole, Dora Li and Rosa Egerton-Warburton for their technical assistance

Map 1. Location of the AE cases in northern Australia in 1993



Report from the Virus Laboratory

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Sero-epidemiological survey for "Flavivirus" infections on Reunion Island, Indian Ocean

The frequency of Flavivirus infections was studied on Reunion Island, Mascaraignes Archipelago, Indian Ocean. Two thousand five hundred and seven human sera were sampled in 1987 from a randomised group of adults. They were tested against five viral strains (yellow fever, dengue type 1 and 2, West Nile and Wesselsbron) using the haemagglutination inhibition test (HIT). One hundred and thirteen animal sera from various species were added in 1989 and tested against the same antigens according to the same technique.

The results of animal sero-survey were inconclusive.

For human beings, our results were interpreted according to the individual registers of responses obtained in the 1987 versus the results of a previous investigation on the Reunion island carried out in 1971 by F. RODHAIN (Institut Pasteur, Paris). The total prevalence found in 1987 (42,68 %) is significantly higher than that observed in 1971 (16,5%). The multivalent reactions represent practically three fourths of the positive reactions. No precise focus was reported. Two phenomena seem to account for these positivities : a severe dengue outbreak (dengue type 2) on the island in 1977-1978 and, on the other hand, the possible activity of one (or perhaps several) flavivirus(es) in the region. Epidemiological data lead to the hypothesis that it would be either a dengue or a West Nile virus.

Among the risk factors examined, neither sex, nor profession, nor contact with the recorded animal species seem to have any effect on the positivity ratio. On the contrary, a precise study of the age of the infected subjects and their living surroundings (countryside or town) versus the geographical distribution of the increase in seroprevalence between 1971 and 1987 confirm the part of the last recorded outbreak of dengue on the level of positivity, as well as the possible circulation of one (or several) flavivirus(es) among the Reunion population.

A new entomological survey concerning the whole Reunion island is urgently requested, since previous data have been collected some forty years ago (Hamon, 1953) and taking in account the potential risk of reintroduction of dengue fever from the surrounding infected areas such as Madagascar, Comores and Seychelles islands. Another potential risk for the Reunion island is represented by the possible dissemintion of Japanese encephalitis from Sri Lanka, India or another Asian focus to this region of Indian Ocean.

Reference : KLES Virginie. "Contribution à l'étude des arboviroses sur l'île de la Réunion : enquête séro-épidémiologique". Thèse, Université de Bretagne Occidentale, Brest (1993), 149 pages.

Key words : Flaviviridae ; Sero-epidemiology ; Reunion island ; Indian Ocean.

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VIRUS ISOLATION FROM FIELD CAUGHT MOSQUITOES AND TICKS IN SRI LANKA

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As part of a study of arbovirus epidemiology in Sri Lanka (see references [1,2,3,4]), virus isolation was attempted on 178,181 female mosquitoes and 229 Haemaphysalis intermedia ticks collected between 1984 and 1989 from different ecological areas of Sri Lanka. Innoculation of suckling mice and AP61 (Aedes pseudoscutellaris) cells were the methods used for virus isolation. Thirty two virus isolates obtained (Table) were identified as Japanese encephalitis (JE) virus (n=17), Getah virus (n=9), a Batai related bunyavirus (n=3), Arkonam virus (n=2) and a nairovirus (n=1).

JE virus was isolated from Cx. tritaeniorhynchus, Cx. gelidus, Cx. fuscocephala and Cx. whitmorei during a human epidemic that occurred in 1987 in the dry zone of the country. Correlation of viral carriage in mosquitoes with vector abundence, porcine seroconversion and human infection has been reported previously [2]. Culex tritaeniorhynchus and Mansonia uniformis mosquitoes were found to carry JE virus in a dry zone non-epidemic area, and Cx. pseudovishnui in a wet zone non-epidemic area. Getah virus was isolated from Cx. tritaeniorhynchus, Cx. gelidus and Cx. fuscocephala collected in the vicinity of swine. A nairovirus related to Crimean-Congo haemorrhagic fever (CCHF) and Nairobi sheep disease viruses was isolated from Haemaphysalis intermedia ticks. Isolation of Getah virus, Arkonam virus, Batai related viruses and a CCHF / Nairobi sheep disease related virus from Sri Lanka are reported for the first time.

References:

1) Arunagiri et al. A serologic study of California serogroup bunyaviruses in Sri Lanka. Am J Trop Med Hyg. 1991; 45: 377-382.

2) Peiris et al. Japanese encephalitis in Sri Lanka - the study of an epidemic: vector incrimination, porcine infection and human disease. Trans. Roy Soc Trop Med Hyg. 1992; 86: 307-313.

3) Peiris et al. Japanese encephalitis in Sri Lanka: comparison of vector and virus ecology in different agro-climatological areas. Trans Roy Soc Trop Med Hyg. 1993 - in press.

4) Peiris et al. Seroepidemiology of dengue and other arboviruses in a natural population of toque macaques (Macaca sinica) at Polonnaruwa, Sri Lanka. J Med Primatol. 1993; 22: 1-12.

TABLE : VIRUS ISOLATES FROM MOSQUITOES

Virus Isolate Code	Date of Mosquito Collection	MOSQUITO SPECIES	+Mosquito Pool Size	Method of Mosquito Collection	Identification of Virus
ANURADHAPURA (dry zone/low elevation, JE epidemic area):					
An 380	28 Nov 86	Culex gelidus	200	CDC/LT	Getah (provisional)[1]
An 432	20 Dec 86	Cx. fuscocephal	200	CDC/LT	Getah
An 457	28 Nov 86	Cx.tritaeniorhynchus	240	CDC/LT	Getah (provisional)[1]
An 514	28 Nov 86	Cx.tritaeniorhynchus	200	CDC/LT	Getah
A 817	27 Oct 87	Cx.gelidus	100	CDC/LT	JE
A 756	2 Nov 87	Cx.tritaeniorhynchus	100	CDC/LT	JE
A 798	27 Oct 87	Cx.gelidus	100	CDC/LT	JE
A 794	27 Oct 87	Cx.gelidus	100	CDC/LT	JE
A 795	27 Oct 87	Cx.gelidus	100	CDC/LT	JE
A 815	27 Oct 87	Cx.tritaeniorhynchus	100	CDC/LT	JE
A 833	27 Oct 87	Cx.tritaeniorhynchus	100	CDC/LT	Getah related[2]
A 836	27 Oct 87	Cx.tritaeniorhynchus	100	CDC/LT	Getah
A 890	16 Nov 87	Cx.tritaeniorhynchus	100	CDC/LT	Getah related[2]
A 949	26 Nov 87	Cx.gelidus	100	CDC/LT	Arkonam
A 966	4 Dec 87	Cx.tritaeniorhynchus	16	CDC/LT	JE
A 995	4 Dec 87	Cx.tritaeniorhynchus	100	CDC/LT	JE
A 989	4 Dec 87	Cx.tritaeniorhynchus	100	CDC/LT	Getah related[2]
A 998	17 Dec 87	Cx.whitmorei	100	CDC/LT	JE
A 1420	11 Jan 89	Cx.fuscocephala	100	CDC/LT	JE
A 1463	8 Dec 88	Cx.gelidus	100	CDC/LT	JE
DEHIYATTAKANDIYA (dry zone/low elevation)					
M 178	6 Oct 85	Cx.tritaeniorhynchus	25	HBC	JE
M 441	22 Dec 86	Cx.fuscocephala	295	HBC/CBC	Arkonam
M 1181	22 Nov 86	Cx.tritaeniorhynchus	187	CDC/LT	JE
M 1315	22 Sep 86	Ma.uniformis	18	CDC/LT	JE
M 1318	20 Oct 87	Ma.uniformis	70	CDC/LT	JE
M 1113	24 Jan 88	Cx.triraeniorhynchus	100	CDC/LT	JE[3]
KANDY (wet zone/mid elevation)					
CBC 79	29 Jan 85	Anopheles vagus	76	CBC	Batai
CBC 80	23 Jan 85	An.peditaeniatus	131	CBC	Batai
CBC 190	20 Nov 85	Cx.fuscocephala	100	CBC	Batai
K 307	17 Jul 86	Cx.pseudovishnui	53	CDC/LT	JE
K 348	15 Oct 86	Cx.tritaeniorhynchus	100	CBC	Getah (provisional)[1]

+ Methods of Mosquito collection: CDC/LT=CDC Light traps; HBC = human bait catch; CBC = cattle bait catch.

[1] antiserum to prototype Getah virus neutralizes isolate to "homologous titre".

[2] identification based on immunofluorescence tests with Getah hyperimmune ascitic fluid.

[3] identification based only on JE ELISA reactivity of virus isolate

An Otbreak of Japanese Encephalitis in Dali Region, Yunnan Province, China in 1991

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During July-November 1991, an outbreak of Japanese encephalitis(JE) occurred in the Dali region. The first case of the epidemic had reported in Eryuan county, on 21st July, the number of cases had peaked in September, and by November JE patients had dwindled to negligible numbers. 582 patients of JE were reported during the period, 106 deaths, the case fatality rate of 18.21 per cent. The morbidity rate and mortality rate were 19.21 and 3.50 per 100000 population respectively. Cases of JE were distributed in twelve counties of the region, of these, 118 patients were occurred in Eryuan with the morbidity rate of 41.43 per 100000 population and the case-fatality rate of 24.58 per cent. There were 59.24 per cent males and 40.76 per cent females in the cases. Patients of JE distributed in all age-groups, and the highest frequency of JE cases was 0-6 year-old (40.72 per cent), followed by 7-14 year-old(20.27per cent), 15-34 year-old(27.66per cent), and above 34 years (11.34 per cent). The number of children(below 15 years) JE cases was higher than that for adult(above 15 years).

It was confirmed by virological investigations that the epidemic was caused by JE virus. Serological tests were carried out in 76 actute sera or paired sera with 65 showing IgM positive or antibody four-fold rises to JE virus. Four strains of JE virus were isolated from acute period blood of patients. In mid-September 1991, 925 Culex tritaeniorhychus mosquitoes collected from Eryuan were pooled into 18 pools and isolated for JE virus using C6/36 cell method and suckling mouse method. The positive isolation rate was 11.11 per cent(2/18), and the mosquitoes body virus carrier rate was 1:462.5. Isolation of JE virus was negative to other species of mosquitoes including Anophees sinensis(2080 mosquitoes), C. theleri(289), Aedes vexans(180), C. fatigans(159), C. pseudovishnui(15), C. fuscocephala(2) and Armigeres subalbatus(1). These results indicated that C. tritaeniorhyncus was the main vector of JE virus in the epidemic.

ARBOVIRAL DISEASE SURVEILLANCE IN CALIFORNIA, 1993

A busy surveillance effort during 1993 has again documented extensive activity by western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses in California, but fortunately little human disease has been noted:

Following the recognition of early WEE activity in Sacramento County in mid to late June, a major effort was made to alert the medical community to watch for suspect cases of aseptic meningitis and encephalitis. Over 200 suspect cases were tested at the State's Viral and Rickettsial Disease Laboratory, and an unknown further number by other laboratories, thus far revealing only two cases: (1) a 64 year old male resident of San Bernardino City, San Bernardino County, who became ill 8/12/93, was hospitalized from 8/15-8/23, and recovered completely. His only known recent travel had been within 20-30 miles of his home; (2) a 19 year old male resident of San Diego County, who became ill 9/11/93 with fever, headache, nausea, stiff neck and transient diplopia, but recovered completely. On 8/31, he had travelled to Imperial County and fished in the West Main Canal, the most likely source of mosquito exposure.

In addition to the SLE cases, extensive WEE activity detected in sentinel chicken flocks and mosquito pools, especially in the Sacramento Valley northward, was reflected in one fatal case of WEE in a four month old quarter horse filly from Red Bluff, Tehama County, 8/21/93; and extensive involvement of a relatively new and exotic species in California, the emu, which is being raised commercially and which has proven to be quite susceptible to the WEE virus.

The routine surveillance program utilizing sentinel chickens and mosquito collections for virus isolation has again proven to be a useful and successful means of providing early alerts to virus activity in various regions of the State and to help focus mosquito control efforts in the most critical areas. The 117 flocks of sentinel chickens were bled every two weeks, using a new filter paper blood collection technique, yielding nearly 17,000 blood samples for testing. Of these, 276 were positive for WEE antibodies and 86 for SLE antibodies. The dried blood samples are mailed at ambient temperature, saving significantly on postage. Of over 3,500 mosquito pools tested, at least 166 were positive for WEE virus, and only five were found positive for SLE virus. The filter paper collection method and streamlined laboratory methods for virus isolation and identification have helped to reduce the costs and extend the surveillance network for this program. The surveillance program involves cooperative efforts by many groups, including: local Mosquito Abatement Agencies; the Arbovirus Research Program at the University of California, Berkeley; the California Mosquito and Vector Control Association; the CMVCA Research Foundation; county and local public health departments; the California Department of Food and Agriculture; physicians and veterinarians throughout the State; and four units of the California Department of Health Services' Division of Communicable Disease Control (Vector Borne Disease Section, Veterinary Public Health Section, Disease Investigation Section, and the Viral and Rickettsial Disease Laboratory).

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Viral and Rickettsial Disease Laboratory

REPORT FROM THE QUEENSLAND HEALTH - QUEENSLAND UNIVERSITY OF TECHNOLOGY W.H.O COLLABORATIVE CENTRE FOR ARBOVIRUS REFERENCE AND RESEARCH, BRISBANE AUSTRALIA

Outbreak of dengue in North Queensland

Dengue fever reemerged in north Queensland in May 1990 after an absence of almost ten years. The first clinical infection was diagnosed in a resident of Townsville. Later that month, dengue patients from Cairns were diagnosed with the outbreak spreading to Thursday Island by July 1990. Sporadic, laboratory confirmed, dengue infections were detected until February 1991. In each centre, the total number of cases diagnosed remained small (Cairns 6, Townsville 8, Thursday Island 19). Dengue 1 was the only serotype isolated from acute phase sera from patients.

Concern was raised in March 1992 when a different serotype, dengue 2 was isolated from a resident of Townsville who had not recently travelled outside Australia.

A total of 466 dengue infections were confirmed during 1992 with all except two occurring in residents of or visitors to Townsville. The majority of diagnoses were made in May and June (356 or 76%). Haemagglutination inhibition antibody assays of 135 randomly selected patients revealed that 25%, based on WHO criteria, were experiencing presumptive secondary dengue infections. Cases continued to occur sporadically throughout the summer months into 1993.

This year a similar pattern emerged with a dramatic increase in confirmed infections in April and May and 805 laboratory confirmed dengue infections to October 1993. Dengue 2 remains the only serotype isolated.

Cases have been diagnosed in centres in north Queensland other than Townsville (Charters Towers 203, Cairns 17, Hughenden 5, Mackay 5, Ingham 2). The ratio of female to male patients has been 1:3:1 with females of the 20-40 age group most commonly affected.

With the introduction of a second dengue serotype the potential exists for a more severe form of dengue infection, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Spontaneous minor haemorrhagic manifestations such as gingival bleeding, epistaxis and hypermenorrhoea have been observed. One patient from Charters Towers, a 42 year old female, matched the WHO criteria for grade II dengue haemorrhagic fever. This was the first confirmed case of haemorrhagic fever in the current epidemic.

Candidate Ross River Virus Vaccine

With the advent of comprehensive serological testing, the number of diagnosed cases of epidemic polyarthritis (Ross River virus infection) exceeded 5,000 in 1992. The continuing increase in the number of diagnosed epidemic polyarthritis patients has prompted a effort to develop a killed Ross

River virus vaccine. We have not proceeded with evaluation of a live attenuated vaccine because of the cost of licencing it.

Virus was isolated from a clinically typical epidemic polyarthritis patient and limit diluted to produce a relatively homogeneous virus population. After inactivation with binary ethylene-imine (BEI), adjuvanted and non adjuvanted vaccine was injected intramuscularly into mice. The serological response is shown in Figure 1.

The BEI treatment had no effect on the binding of IgG or IgM antibodies to the virions. This contrasts with the damage done to sulphur containing amino acids by β-propiolactone.

When challenged intravenously with 10^5 TCID of the prototype (T48) strain of Ross River virus, all mice given two doeses of vaccine 90 days apart failed to produce any detectable viraemia. Interestingly, 0.2 and 2.0μg adjuvanted vaccine afforded less protection than did vaccine alone.

Histological examination of mouse muscles injected with vaccine revealed a transient weak inflammatory response to vaccine alone and a strong response resulting in granulomata formation when adjuvant (alhydrogel) was used.

Preliminary experiments with serum from the vaccine immunised mice suggest that, *in vitro*, they neutralise isolates of Ross River virus made over the last fifteen years from widely dispersed regions and countries.

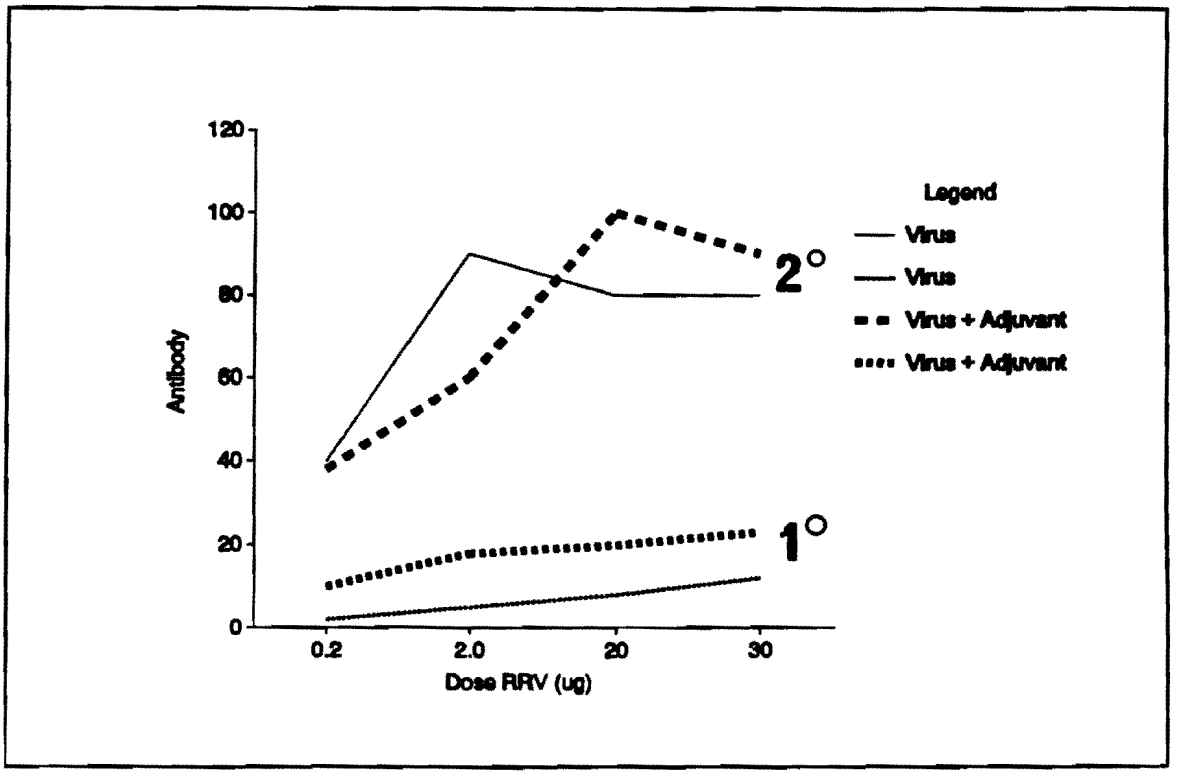

Figure 1: Levels of anti-Ross River virus antibodies (detected by ELISA) in serum from BALB/c mice 28 days after a primary or secondary immunisation with the Ross River virus vaccine.

Table 1. Viraemia in mice challenged intravenously with 10^5 TCID prototype strain of Ross River virus 90 days after primary or secondary immunisation with inactivated virus.

Immunisation	Viraemia (log $TCID_{10}$)				
	Dose of virus	Adjuvant	4 hr	48 hr	72 hr
Primary	0.2	-	1.4(2/5)*	<1.0(1/5)	<1.0(0/5)
	0.2	+	4.2(5/5)	2.4(2/5)	1.1(1/5)
	2.0	-	<1.0	<1.0	<1.0
	2.0	+	3.3(4/5)	1.0(2/5)	<1.0(0/5)
	20.0	-	<1.0	<1.0	<1.0
	20.0	+	<1.0	<1.0	<1.0
	30.0	-	<1.0	<1.0	<1.0
	30.0	+	<1.0	<1.0	<1.0
	-	-	4.3(5/5)	1.8(5/5)	1.3(4/5)
Secondary	0.2	-	<1.0	<1.0	<1.0
	0.2	+	<1.0	<1.0	<1.0
	2.0	-	<1.0	<1.0	<1.0
	2.0	+	<1.0	<1.0	<1.0
	20.0	-	<1.0	<1.0	<1.0
	20.0	+	<1.0	<1.0	<1.0
	30.0	-	n.d.	n.d.	n.d.
	30.0	+	n.d.	n.d.	n.d.
	-	-	3.5(4/4)	2.2(4/4)	<1.0(0/4)

*figures in parenthesis indicate the number of viraemic mice.

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Bluetongue research in Indonesia

Sentinel herds of cattle, buffaloes, goats and sheep were established in different sites in Indonesia, including West Java, Bali, Timor and Irian Jaya, which is some 4000 km from our laboratory. Heparinized blood, serum and insects are collected from at these sentinel sites.

The seasonality of bluetongue infection was figured and indicated that seroconversions were mostly occurred at the end of wet season. A number of bluetongue virus isolations have been made from blood of sentinel animals and insects. Some isolates have been serotyped. Serotypes isolated to date are Bluetongue serotypes 1, 7, 9, 12, 21 and 23. Other arboviruses such as epizootic haemorrhagic disease of deer was also isolated. Bluetongue serotype 21 was also detected from a mixed pool of Culicoides fulvus and Culicoides orientalis.

Trials to determine the pathogenicity of Indonesian bluetongue viruses are currently being conducted using imported merinos sheep and local Indonesian sheep. Topotyping of Indonesian isolates is also planned.

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REPORT FROM THE LABORATORY FOR ARBOVIRUS RESEARCH and SURVEILLANCE
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Studies of the ecology and epidemiology of select California serogroup viruses, especially Jamestown Canyon (JC) virus continue to focus on vector competence, virus variation and human infection. In recently completed studies (M. Zhang, doctoral dissertation) 38 North American isolates and reference strains of JC virus, including 23 isolates from Aedes mosquitoes and white-tailed deer, were characterized by plaque assays, temperature sensitivity tests, neutralization tests, and ELISAs. The isolates separated into four different antigenic groups. Analyses of representative JC isolates revealed marked differences in viral growth in cell culture, virulence for suckling mice, and infectivity for an experimental vector, Aedes epactius.

The results indicated a high rate of variability among JC isolates/strains; the geographic variation among strains (Midwestern, East Coast, Canadian and prototype) was no greater than that seen among 20 JC strains isolated from mosquitoes (14/20 from Aedes provocans) at our north central Michigan study site. Use of monoclonal antibodies in ELISAs and Nt tests indicated that the majority of the Michigan mosquito isolates (all taken from one site from 1984-1989) in a given year were antigenically very similar, but that the subsequent year's isolates predominantly produced a different ELISA reaction pattern. The similarity of deer isolates from one year and mosquito isolates in the following year suggests that vertebrate passage has a major influence on strain variation at this site. In instances where mosquito isolates from two or more successive years were identical, transovarial transmission is presumed to have occurred.

Molecular techniques were used to characterize JC viral protein components. A partially denatured gel electrophoresis protocol was developed, which enabled the viral proteins to be identified using stains and immunoblots without radio-labeling. Four structural proteins were identified: the large (L) protein, the nucleocapsid (N) protein and two envelope glycoproteins (G1 and G2). The G1 protein is responsible for infection of vertebrate cells and induction of neutralizing antibodies. That protein bears a California serogroup-specific neutralizable epitope and two JC virus-specific epitopes, one with and one without neutralizing activity. The antibody binding activity of these epitopes appeared to be dependent on tertiary structure. Jamestown Canyon virus is easily distinguished from La Crosse (LAC) and trivittatus (TVT) viruses based on G1 protein reaction patterns in Western blots. One notable exception to the typical patterns was isolate JC-89-0056 (from Michigan Aedes provocans) which cross-reacted strongly with LAC and TVT viruses. That isolate also elicited an immune response in rabbits which other tested United States JC strains do not do. The G2 protein left a very weak band with Coomassie blue staining and produced no signal in immunoblots. Results suggest that the G2 protein apparently is not involved in vertebrate cell infection or neutralizing activity. The N protein represented another common antigen within the California serogroup, a shared non-neutralizable epitope within the Melao virus complex. This protein retained its antigenicity following SDS and 2-mercaptoethanol treatment.

A modified ELISA was developed for large-scale serosurveys of deer populations to monitor herd seroconversions and aid human epidemiologic studies.

Our overall work has increasingly included Cache Valley (CV) virus, sparked by its growing veterinary importance. During investigations of eastern equine encephalitis outbreaks in south western Michigan during August-October 1980-82, CV was isolated from 89 pools of mainly Anopheles and Coquillettidia perturbans (Calisher et al. 1986). The seroprevalence for CV among horses in the region was 66% with an estimated yearly seroconversion rate of 10-20% (McLean et al. 1987). We are further evaluating the spread and virulence of the CV virus strains circulating in northern Indiana. Preliminary results from a serosurvey of large domestic animals using routine blood samples from a veterinary clinic in the area indicated a seroprevalence of 44% (n=66) in cattle, 4% (n=232) in swine, 73% (n=73) in horses and 25% (n=4) in goats. Some animals (4% n=125) also had antibodies against Potosi virus.

A statewide antibody survey of 10,200 Indiana residents in 1978-79, on the heels of the large St. Louis encephalitis epidemic of 1975, revealed California group virus transmission throughout the state (Grimstad et al. 1984). Serologies were based on neutralizing (N) antibodies, with the screening antigen being LAC virus. Differences in regional distributions were noted: LAC was most prevalent in the southern half of Indiana, JC was most prevalent in the north, and TVT was most prevalent in the west-central region. Subsequent studies based on screening tests with JC virus revealed a much higher JC antibody prevalence (P.R. Grimstad, unpublished).

Based on those results, a second study nearing completion was initiated in 1989 (as a 10-season follow up) to look at the occurrence of other bunyaviruses and register any marked changes in LAC and JC seroprevalence from the previous estimates. Between September 1989 and January 1990, sera and demographic data were collected from 3447 hospital patients. The samples were intended to reflect cross-sections of the populations in 28 counties; 3 of the state's 4 quadrants were represented. Tests for N antibody were conducted with Vero cells and 96-well plates. All samples were screened for N antibody against JC virus; approximately a third were screened against 3 additional California group viruses, namely LAC, TVT, and Keystone (KEY) viruses. A subset of 2784 samples was screened against CV virus; smaller proportions were also screened against Tensaw (TEN), Potosi, and Northway (NOR) viruses. Screen-positive samples were then titered to endpoint against a battery of 4-8 viruses (4 from each serogroup). Homologous viruses were named for samples with a 4-fold or greater difference in N titers and a maximum titer of at least 8.

Table 1 incorporates all seropositive samples regardless of the antigen(s) which initially detected them in the screening step. The regional distributions of CAL group viruses mirrored the results of 1978-79. JC and TVT were more prevalent than LAC; CV was widely distributed, with county-based prevalence rates highest in the north. Antibodies to KEY, TEN, and Potosi occurred too infrequently for these viruses to be called endemic. The 3 human sera containing Potosi-specific antibody were collected in 2 counties in the far northwest part of the state. The 8 samples containing TEN-specific antibody were relatively evenly distributed, as were the 3 sera with KEY-specific antibody. The large number of "indeterminate" sera was comprised of samples with either (a) equal titers to 2 or more viruses, (b) titers differing by no more than a factor of 2, or (c) maximum titers below 8.

Samples from 8 counties were screened with LAC virus, and their results were compared with the corresponding data from 1978-79. There was no difference in LAC seroprevalence between the 2 study periods. Analyses of JC seroprevalence (16 counties) yielded similar results. A positive correlation between deer density and JC seroprevalence, first noted in Michigan (Grimstad et al. 1986), was calculated from the

Indiana data (n= 28 counties, r=0.586, p=.001).

From these data we conclude that JC, LAC, and TVT are endemic in Indiana at roughly equal levels, that their ranges overlap, and the levels of transmission of JC and LAC are not appreciably different in the 2 periods studied. CV is presumably endemic, overlaps in distribution with the California group viruses, and occurs at similar levels. Furthermore, Potosi virus can infect humans. The effort to interpret the large number of indeterminate sera brings to light several unresolved issues. Among these are (a) the likelihood of second exposures, (b) variation in the breadth or cross-reactivity of primary and anamnestic antibody responses in humans, (c) the rates of decline of homologous and heterologous antibody, and (d) the influence of strain variation on the strength and specificity of the antibody response.

Table 1. Percentage of samples containing N antibody to California or Bunyamwera serogroup viruses, Indiana, 1989.

Homologous virus*	All counties combined	Range for individual counties
JC	2.7	0-16.7
LAC	1.5	0-11.4
TVT	2.7	0-9.5
KEY	0.1	0-0.8
Indeterminate	3.3	0-8.2
Combined CAL group	10.3	1.5-22.2
CV	1.4	0-12.0
TEN	0.3	0-2.6
Potosi	0.1	0-1.1
NOR	0	0
Indeterminate	5.8	0-14.0
Combined BUN group	7.5	0-26.0

* Prototype viral strains were used, with the exceptions of JC and LAC, which were isolated from mosquitoes in Indiana, and Potosi, which was collected concurrently with the prototype strain from Aedes albopictus in Missouri.

A new investigation is focusing on human exposure to these agents in one northern Indiana county not part of the 1989-1990 sampling. Of approximately 200 sera tested to date (collected since September, 1993) 24.5% are positive for antibodies against one or more California group member (16% specifically against JC virus) and an additional 10% positive for antibodies against one or more Bunyamwera group member (4% to CV virus). Results of this study, and others involving mosquito vector competence, strain variation with sequencing of JC strains and animal pathogenicity studies with JC and CV will be presented in later exchanges.

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MISSOURI FLOOD EMERGENCY SURVEILLANCE - 1993

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Mosquitoes were collected in areas of Missouri effected by flooding of the Mississippi and Missouri Rivers and assayed for St. Louis Encephalitis antigen by an ELISA. The state was divided into five regions (Figure I) and mosquitoes were collected from the 5th of August to the 18th of September using light traps baited with CO_2. Navy DVECC teams collected in regions 1, 2 and 4 while individuals from the Missouri Department of Health trapped in region three and personnel from St. Louis County Department of Health collected in region five. Culex pipiens complex, Culex tarsalis, and Culex salinarius mosquitoes were sent to Southeast Missouri State University in pools of 100 or less. Tables I and II summarize the pools and mosquitos received and tested at Southeast. During the first two weeks of the project, 38,734 mosquitoes in 525 pools were sent from regions 1, 2 (week 1 only), 3, and 4 to the National Center for Infectious Diseases, Centers for Disease Control laboratories in Fort Collins, CO (Table III). An additional 37 pools containing 2,223 mosquitos collected in region 5 prior to the start of this project were also tested at Southeast. In all 1,796 pools and 126,086 mosquitoes were assayed for SLE antigen.

FIGURE I MISSOURI MOSQUITO SURVEILLANCE AREAS

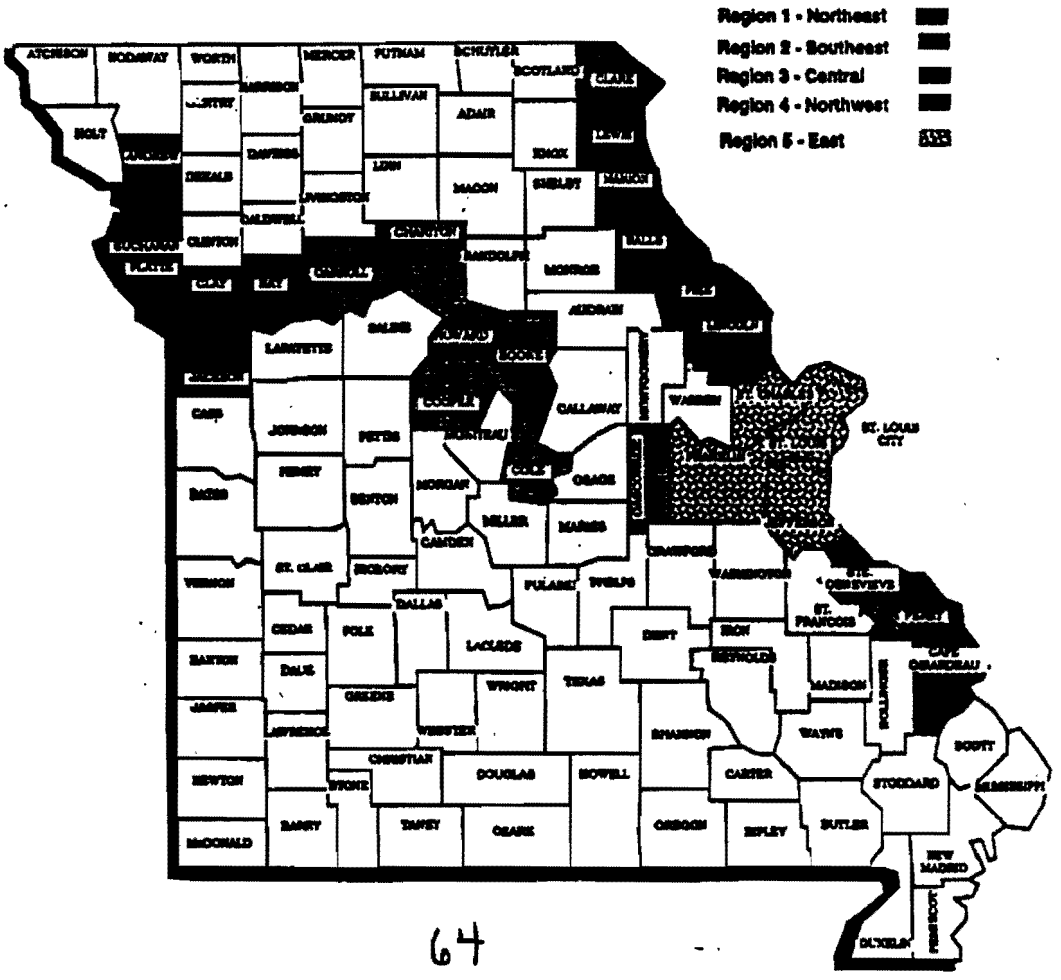

TABLE I SUMMARY OF MOSQUITOES AND POOLS RECEIVED AT SOUTHEAST BY REGION

REGION	SHIPMENTS	DATES	LOCATIONS	SITES	POOLS	MOSQUITOES
1	9	8/25-9/21	8	19	215	15288
2	6	8/16-9/20	19	37	221	11355
3	2	8/25-8/31	9	23	40	1382
4	8	8/25-9/21		21	229	17556
5	7	8/11-9/23	5	91	529	39548
	32	**8/11-9/23**	**41**	**191**	**1234**	**85129**

TABLE II SUMMARY OF MOSQUITOES AND POOLS RECEIVED AT SOUTHEAST BY WEEK

WEEK	REGION 1	REGION 2	REGION 3	REGION 4	REGION 5	TOTAL
8/9-15					2621 (39)	2621 (39)
8/16-22		1593 (22)			928 (14)	2521 (36)
8/23-29	7301 (81)	453 (34)	759 (27)	2381 (29)	13583 (168)	24477 (339)
8/30-9/5	2806 (37)	1944 (29)	623 (13)	3139 (33)	3720 (57)	12232 (169)
9/6-12	3505 (57)	3038 (56)		6589 (95)	4017 (64)	17149 (272)
9/13-19	1292 (32)	2182 (38)		4522 (55)	8030 (106)	16026 (231)
9/20-26	384 (8)	2145 (42)		925 (17)	6649 (81)	10103 (148)
	15288 (215)	**11355 (221)**	**1382 (40)**	**17556 (229)**	**39548 (529)**	**85129(1234)**

TABLE III SUMMARY OF MOSQUITOES AND POOLS SENT TO CDC

DATES	REGION 1	REGION 2	REGION 3	REGION 4	TOTAL
8/9-15	11435 (137)	3505 (53)	2327 (81)	8416 (92)	25683 (363)
8/16-24	7461 (82)		764 (27)	4826 (53)	13051 (162)
	18896(219)	**3505 (53)**	**3091 (108)**	**13242 (145)**	**38734 (525)**

The ELISA was performed using reagents provided by CDC following the protocol outlined by Tsai and colleagues (1). After the antigen was added, the test was incubated overnight at 4 ° C and ABTS was chosen as the enzyme substrate. Pools giving a preliminary positive in the ELISA were tested in an inhibition assay as described by Tsai(1). On average, the assay was completed within three days (range 2-5 days) of receipt of the mosquitoes.

Seventeen pools obtained from collections made between 7/2/93 and 9/18/93 in regions 1, 2, 4, and 5 tested weakly positive in the ELISA. However, none of these demonstrated specificity for St. Louis Encephalitis in the inhibition assay.

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REPORT FROM THE VECTOR-BORNE AND SPECIAL PATHOGENS UNIT

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1. **Surveillance of Vector-Borne Viral and Rickettsial Infections**

During a one year surveillance program ending in April/93 we investigated 2081 samples from 1467 suspected cases of vector-borne disease.

The frequency of patients with travel history continued to increase, 449/1467 (30.6%) since this program was established.

Samples were examined for evidence of vector-borne viral infections by one or more of the following techniques: hemagglutination inhibition, complement fixation, microneutralization and immunofluorescence assay (HAI, CF, MNT, IFA). Such tests involved 6-8 antigens or viruses, depending on the suspect diagnosis and circumstances, these are:

Flaviviruses:
St. Louis encephalitis, Powassan, Dengue and Yellow Fever.

Alphaviruses:
Eastern, Western and Venezuelan equine encephalitis.

California Group Viruses:
Snowshoe Hare (SSH) and Jamestown Canyon (JC).

Viral Hemorrhagic Fevers (VHF):
Congo-Crimean, Rift Valley, Ebola Zaire, Ebola Sudan, Lassa, Marburg and Hantaan (hemorrhagic fever with renal syndrome).

Tests for rickettsial infections were done by IFA and included: (a) Phase 1 and Phase 2 of Q Fever for the three classes of immunoglobulins, IgG, IgM, IgA; the last class is of particular significance in cases of endocarditis; (b) Rocky Mountain spotted fever (RMSF) which cross-reacts with R. conorii, the causative agent of Mediterranean spotted and South African tick bite fevers and murine typhus. Tests were done for IgG and IgM classes only in the latter group.

There were 252/1467 (17.2%) seropositive patients to one or more of the above killed antigens or live viruses. Thirty six of the 252 seropositive patients (14%) had diagnostic findings. The rest had antibodies not necessarily related to the then current or recent diseases. Of these 36 cases, 14 were diagnostic of arboviral infections (39%) and 22 (61%) of rickettsial diseases.

The seropositive patients to above antigens or viruses included 104/252 (41.3%) to flavivirus, 102/252 (40.5%) to the California Group, 12/252 (4.7%) to Alphavirus(es) and 33 (13.1%) to Rickettsia.

This is the highest number of seroreactive findings to alphavirus(es) in one year, six alpha seropositive patients had known histories of travel and none of the 12 patients had acute neurological diseases. The specificity of this reactivity is being ascertained. This is the second year seroreactivity to alphaviruses is markedly observed. Examination of 81 bird sera, from an area reported to us to have one fatal horse case of EEE and one seropositive horse, revealed no detectable antibodies to EEE.

1a. Arthropod-Borne Viral Infections:

Twelve patients had diagnostic findings of flavivirus disease, dengue in the majority of cases. The serological response was usually associated with history of travel to flavi or dengue active area and clinical diagnosis of this disease.

102 patients were seropositive to the California Group Virus, 32 of them had histories of travel mainly to the USA. There were 2 cases with presumptive diagnosis of Jamestown Canyon virus. Antibody presence in the remaining cases, can be categorized as likely not related to the patients' disease. The microneutralization test, which is both sensitive and specific, has been used both as a screening and typing test. Data on 100 of these seropositive cases showed Jamestown Canyon in 57%, snowshoe hare in 11% and showed 32.1% as indeterminate. Those indeterminate reactors are being tested for LaCrosse virus antibodies by microneutralization. It is noteworthy that 19 seroreactive patients had associated history of travel to Florida. Sixteen were typed as Jamestown Canyon and three were indeterminate type. None of these, however, had diagnostic titres to either SSH or JC viruses.

1b. Rickettsial Infections: This year, 359 cases were investigated for rickettsial infections 33 (9.2%) of those were seropositive with 22/33 (67%) having diagnostic significance, all Q fever.

2. **Histories of Travel and Suspect Cases of Exotic Diseases:**

During this period, 449 (30.6%) patients had histories of travel, the highest percentage we have had. Twelve patients had diagnostic findings consistent with flavivirus infections. Five of them had histories of travel to dengue affected area, the details of travel of the remaining seven patients were not available.

3. **Review of Vector-Borne Viral and Rickettsial Infections Associated with Foreign Travels:**

Travels to various disease-endemic areas involve risks of exotic infections; some are hazardous to the laboratory worker, community or both. We reviewed our data on cases with foreign travels over a 12 year period to emphasize the possible occurrence of such risks.

Samples received at the Virus Laboratory are monitored for records of travel. Suspect infections and appropriate containment levels for tests are thus identified. Tests included haemagglutination inhibition, complement fixation, microneutralization and immunofluorescence. Test antigens were selected to be compatible with area of travel and clinical findings.

Over a period of 12 years (1980 - 1992), we investigated 2003 cases with histories of travel. 443 patients or 22% were seropositive: 336 to Flavi, 79 to Bunya and 17 to Alphaviruses; 17 to Rickettsia; and 10 to Arena, Filo and Phlebovirus antigens. These included 95 confirmed (C) and presumptive (P) cases: 3 Lassa (C); 84 Flavivirus: 35 (C), 49 (P) and 8 Rickettsia, 7(C) and 1(P). To our knowledge, there were no secondary cases.

Common infections, e.g. genital herpes, hepatitis A, B, and measles caused disease in some cases. The identification of cases with exotic infections in patients with histories of travels demonstrate that the possibility well exists to import these and other hazardous infections from endemic areas. Identification of such infections allows prompt response, patient management and other public health measures when warranted. Inclusion of recent travels in case histories aids the clinician in the differential diagnosis of disease and the diagnostic or surveillance laboratory in selecting appropriate tests.

4. <u>The Maximum Containment Laboratory (MCL)</u>:

The steam-ethlyne oxide sterilizer releasing decontaminated laboratory supplies, instruments or biowaste is being replaced. A biodecontamination system utilizing steam, vapourized hydrogen peroxide or formaldehyde is being installed. This system has a double sliding door - sterilizer with contamination seals on both ends, the contained room and the clean corridor end. These double contamination seals provide means to service the sterilizer from the clean side, if needed, without having service personnel enter into containment rooms. The sterilizer chamber has been modified, furthermore, to allow using it as a pass-through, for treating material with either vapourized hydrogen peroxide or formaldehyde vapours as required. Vapourized hydrogen peroxide or formaldehyde are provided by generators which have been modified to couple with the sterilizer chamber through quick disconnect fittings guarded by ball valves. To our knowledge the steam-vapourized hydrogen peroxide or formaldehyde system with coupling capability is the first such unit for steam-gaseous decontamination.

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Antibodies in indigenous populations to undescribed viruses of the Anopheles A, C, and Guama bunyavirus serogroups in Nigeria.

Using hemagglutination-inhibition (HI) and neutralization (N) tests, we examined human and animal sera from Nigeria for antibodies to representatives of Anopheles A, C, Guama, and California serogroups (family Bunyaviridae, genus *Bunyavirus*). The following viruses were used: Tacaiuma (Anopheles A), Caraparu (C), Catu (Guama), and Guaroa (California). A total of 734 (588 human, 80 cattle, 66 horse) sera were tested.

The prevalence of HI antibodies to Tacaiuma virus was 4.8%. The prevalences for HI antibodies to Caraparu and Catu viruses were 2.9% and 2.5%, respectively. None of the 734 sera was positive for HI antibody to Guaroa virus.

Thirty-two of 35 sera with HI antibody to Tacaiuma virus were also positive in N tests. HI antibody to Caraparu virus were confirmed by N tests in all 21 sera and to Catu virus in 12/18 sera. Results are summarized in Tables 1 and 2. Further studies are in progress. We are attempting to isolate these or related viruses in Nigeria.

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Table 1. Prevalences of HI antibodies to Tacaiuma, Caraparu, and Catu viruses in human and animal sera from Nigeria.

Source	No. tested	Tacaiuma No. (%) positive	Caraparu No. (%) positive	Catu No. (%) positive
Human	588	9 (1.5)	10 (1.7)	6 (1.0)
Bovine	80	26 (32.5)	9 (11.3)	8 (10.0)
Equine	66	0	2 (3.0)	4 (6.0)
TOTAL	734	35 (4.8)	21 (2.9)	18 (2.5)
Range of titers		20-80	20-80	20-80

Table 2. Prevalences of N antibodies to Tacaiuma, Caraparu, and Catu viruses in HI positive human and animal sera from Nigeria.

Source	Tacaiuma No. (%) positive	Caraparu No. (%) positive	Catu No. (%) positive
Human	6 (67)	10 (100)	4 (67)
Bovine	26 (100)	9 (100)	4 (50)
Equine	-	2 (100)	4 (100)
TOTAL	32 (91.4)	21 (100)	12 (67)
Range of LNI (DEX)	1.5-3.0	2.5-4.5	1.5-4.0

HANTAVIRUS ACTIVITY IN PATIENTS WITH HAEMORRHAGIC FEVER IN ARGENTINA

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In Argentina, anti-Hantaan antibodies have been detected in rats from the port cities of Buenos Aires and Mar del Plata (1) as well as in healthy humans living in several areas of Argentina (2). Asymptomatic hantavirus infections have been documented among laboratory personnel and experimental rodents in Argentina as well (2,3).

Recently, we tested sera from 591 patients suspected clinically of having Argentine Haemorrhagic Fever (AHF) between 1984-1991, in whom Junin antibodies could not be detected, using an ELISA specific for Hantaaan (HTN, strain 76-188) antibodies.

One serum from each patient was tested for the presence of IgG antibodies by ELISA in 96 well plates coated with lysates of E6 VERO cells infected with HTN virus or normal E6 cells (as control). Sera were added in twofold dilutions, beginning 1:100 and anti-Hantaan IgG antibodies were detected by peroxidase labeled anti-human IgG.

Acute and early convalescent sera from 11 positive patients detected by this screening, were then examined using both IgG and an Ab-capture IgM ELISA. According to the temporal appearance and evolution of IgM and IgG antibodies, 7 cases were determined to be recent infections (table 1). Three cases were considered past infections (table 2) and the status of one was not interpretable.

The most common clinical findings among presuntive cases were fever, headache, palatal petechiae, oliguria or polyuria, and pulmonary edema. Laboratory findings in the seven patients with recent infection are presented in table 3. Thrombocytopenia, a characteristic of Junin Virus infection (AHF), also was present in all patients. In contrast to finding in patients with AHF, leukopenia and round cells in urine(4) were absent in all seven patients.

The 591 sera screened for Hantavirus antibodies were obtained from patients living throghout much of the endemic area for AHF. The 11 individuals whose sera were reactive by ELISA to Hantaan virus all lived in communities bordering the Parana river.

We present here the first cases with a temporal association between a haemorrhagic fever syndrome and hantavirus infection in Argentina.

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TABLE1

PATIENTS WITH RECENT HANTAVIRUS INFECTION:
RESULTS OF ANTIBODY DETECTION BY ELISA

Patient	Sex/ Age	Early Sera			Convalescent Sera								
		Days from onset	IgG	IgM	Days from onset	IgG	IgM	Days from onset	IgG	IgM	Days from onset	IgG	IgM
R.M.	M 27	6	0	NT	39	200	0	69	800	0			
J.M.	M 39	6	200	1600	40	400	1600	69	3200	1600			
J.C.	M 16	5	1600	800	38	3200	0	66	1600	0	95	1600	0
J.P.	M 64	9	1600	3200	40	800	800	71	1600	800	106	6400	800
A.B.	F 22	8	0	≥12800	39	800	3200						
M.D.	M 46	11	3200	1600									
E.D.V.	M 37	19	≥12800	≥12800	50	≥12800	3200	80	≥12800	800	110	3200	800

Titers are expressed as the reciprocal of the dilution
NT= Not tested
M= Male
F= Female

TABLE 2

PATIENTS WITH PAST HANTAVIRUS INFECTION:
RESULTS OF ANTIBODY DETECTION BY ELISA

Patient	Sex/ Age	Early Sera			Convalescent Sera		
		Days from onset	IgG	IgM	Days from onset	IgG	IgM
E.Z.	M 22	8	1600	0	34	1600	0
R.B.	M 40	5	6400	0			
M.G.	F 23	5	12800	0			

Titers are expressed as the reciprocal of the dilution
M= Male
F= Female

TABLE 3

LABORATORY FINDINGS IN THE RECENT
HANTAVIRUS INFECTED PATIENTS

ASSAY	NUMBER STUDIED	ABNORMAL	
		NUMBER	MEAN (RANGE)
MINIMAL PLATELET COUNT (/MM^3)	7	7	43,571* (20,000-85,000)
PEAK LEVELS OF WHITE BLOOD COUNT (/MM^3)	7	2	14,325 (12,800-15,850)
PEAK OF PROTEINURIA (g/l)	7	7	2.2 (0.3-6.9)
PEAK LEVELS OF BLOOD CREATININE (mg/l)	6	5	33.3 (12.5-87.0)

* Between 8th and 11th days from the onset

NEUTRALIZING HUMAN MONOCLONAL ANTIBODIES AGAINST PUUMALA VIRUS

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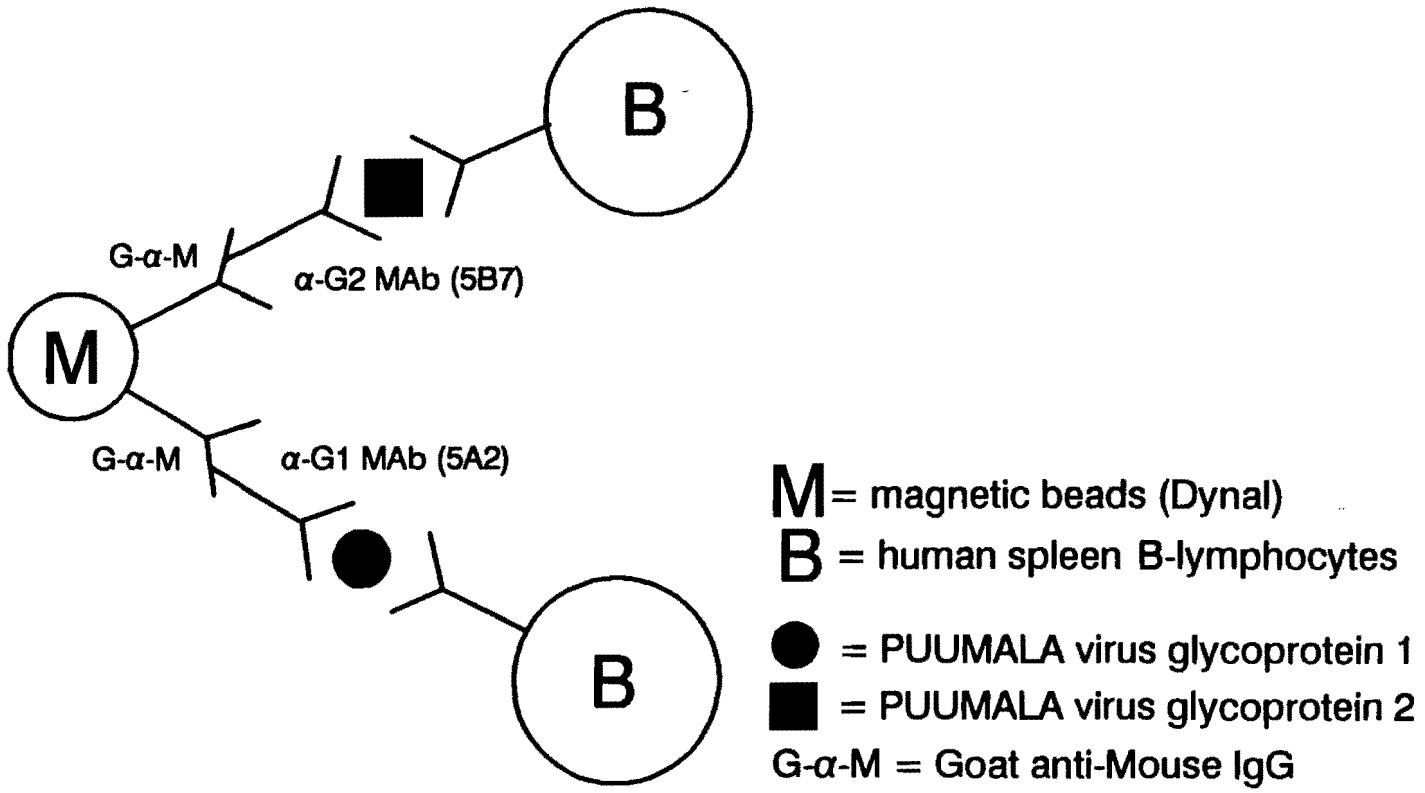
Development of human monoclonal antibodies opens new possibilities for both treatment and prophylaxis to several arthropod borne and hemorrhagic fever viruses. We used Puumala virus as a model system to generate and characterize human monoclonal antibodies (MAbs). Human spleen cells were obtained from a 60-year old PUU virus immune patient splenectomized because of idiopathic trombocytopenic purpura. B-lymphocytes were pre-selected for specific surface immunoglobulin (Ig) by magnetic beads coated with the viral glycoproteins, and subsequently immortalized by Epstein-Barr virus transformation (see figure). Four IgG positive monoclonal lymphoblastoid cell lines (LCLs) were established and have remained stable MAb secretors for over 12 months. Analyses of the antigen and epitope specificities recognized by the MAbs showed overlapping binding patterns of four anti-glycoprotein 2-specific clones. Identical isotypes (IgG1λ) and isoelectric points (9.2) of the four MAbs suggested that they were derived from the same original clone. The MAbs reacted with eight PUU virus-like strains, but were negative for Hantaan, Seoul, and Prospect Hill viruses in an immunofluorescence assay, indicating binding to a conserved epitope unique for strains associated to the European form of hemorrhagic fever with renal syndrome, nephropathia epidemica. The MAbs neutralized all investigated PUU virus-like strains in a focus reduction neutralization test. The MAb neutralizing activity was significantly enhanced in the presence of human or guinea pig complement. To stabilize and increase the antibody secretion and to reduce the demand of culture medium supplements (e.g fetal calf serum), three of the monoclonal LCLs were fused with the non-secreting human x mouse partner SPAM-8. Several of the established human x [human x mouse] monoclonal triomas grew faster and produced larger amounts of MAbs as compared to the original LCLs.


B
G-α-M
α-G2 MAb (5B7)
M
G-α-M
α-G1 MAb (5A2)
B
M = magnetic beads (Dynal)
B = human spleen B-lymphocytes
= PUUMALA virus glycoprotein 1
= PUUMALA virus glycoprotein 2
G-α-M = Goat anti-Mouse IgG

Problems of Isolating the Lyme Disease Spirochaete from *Ixodes ricinus* Ticks Collected in the United Kingdom

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Comparatively little is known of the ecology of *Borrelia burgdorferi* (the aetiological agent of Lyme borreliosis) in the UK. Although ecological similarities with continental Europe are apparent, the physical isolation of the UK (as an island), and the different host preferences of *Ixodes ricinus* (the principal European vector of *B. burgdorferi*) suggest likely differences. Indeed, *I. ricinus* is known as the sheep tick in the UK whereas on the Continent it is called the wood tick.

An essential step in understanding the ecology of Lyme borreliosis is defining the properties of the aetiological agent. This is important for selecting suitable diagnostic reagents and in understanding the infection characteristics that influence transmission dynamics. Isolation of UK *B. burgdorferi* has proved difficult and to date only one isolate obtained by Dr S. Cutler (Charing Cross Hospital, London) is generally available for study.

We succeeded in obtaining only one isolate from 89 tick pools (representing 504 questing *I. ricinus* nymphs and adults) collected in the UK. The isolate was not maintained beyond the sixth *in vitro* passage. In contrast, using identical conditions, *B. burgdorferi* was isolated from one of 7 tick pools (87 ticks) from Switzerland, and from a single pool of 10 ticks from Slovakia, and both isolates grew readily in the culture medium.

Examination of 108 questing *I. ricinus* nymphs and 32 questing adults from a UK Lyme disease focus, using direct immunofluorescence, revealed 8 nymphs and 6 adults positive for *B. burgdorferi* spirochaetes. Of these, all the adults and 6 nymphs contained 1 to 10 spirochaetes and only one nymph had more than 100 spirochaetes. The low numbers of spirochaetes compared with those recorded in ticks collected on the Continent (5000 to 500,000 spirochaetes per infected tick) partly explain the poor success rate in isolating UK *B. burgdorferi*. However, even if the one successful isolation was due to the presence of a highly infected tick, we would expect to have obtained a greater number of UK *B. burgdorferi* isolates.

To examine the problem further, the isolation procedures were monitored by the polymerase chain reaction (PCR using a nested set of primers specific for the OspA gene, and a single primer pair specific for the chromosome of *B. burgdorferi*). Eleven of 12 tick samples were PCR positive after 2 weeks in culture but only one sample was positive after 4 weeks and motile spirochaetes were not detected by dark field microscopy. The results indicate that UK B. *burgdorferi* did not adapt to the culture conditions.

The difficulties of isolating and culturing the Lyme disease spirochaete from ticks collected in the UK, together with the comparative ease with which Swiss and Slovakian *B. burgdorferi* were isolated using identical conditions and culture medium, suggest that the growth requirements of UK *B. burgdorferi* differ significantly from those of other *B. burgdorferi* strains.

CANINE LYME DISEASE IN BELGIUM

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Lyme borreliosis is a multisystem disease caused by the tick-borne spirochete Borrelia burgdorferi (Bb) (Steere et al, 1983), (Steere, 1989). Human Lyme disease has been recognised as a distinct entity since 1975 when the geographic clustering of cases of oligoarticular arthritis in the American town Lyme (Connecticut) was established. In addition to causing human illness, borreliosis has also been recorded in many species of mammals including domestic animals which come in contact with the infected tick vector. The first case of Lyme borreliosis in a dog was described in 1984 (Lissman et al). However, unlike human Lyme disease, the canine version of the illness is not so well documented in Europe. We describe here, the first two cases to our knowledge of Lyme borreliosis in dogs from Belgium.

Serum from a 6 year old, male, pedigree Dobermannpinscher (Dog 1) from a forested region north of the city of Antwerp (Province of Antwerp), Belgium, and a 7 year old, female, non thoroughbred cross between a Maline Shepherd and a Greyhound (Dog 2) from the Province of Brabant, Belgium, both showing symptoms of Lyme disease, were screened via indirect immunofluorescent asssay (IFA) (Steere et al, 1983) for borrelia specific IgG antibodies. A titer of >1/64 was considered as evidence of a previous Bb infection (Magnarelli et al, 1985 & 1987), (Kornblatt et al, 1985). In addition, an ELISA assay with the American B31 borrelia strain was used to detect IgM specific Bb serum antibodies. Both animals were found to possess IgG antibodies to borrelia. This positive serology was confirmed with an equally positive Lyme passive haemagglutination test (LYMAG, Diagast, Lille Cedex, France) (See Table I). Only dog 1 was found to possess IgM Bb specific antibodies, however, this is not necessarily a prerequisite for a diagnosis of canine Lyme disease (Magnarelli et al, 1985). A differential diagnosis was also made for other rheumatoid or systemic diseases and furthermore the renal functions were tested by mearuring the serum creatinine and ureum values (See Table II).

The unique clinical symptomatology exhibited by these two dogs in combination with the serological findings, persuades us to affirm a diagnosis of canine Lyme borreliosis. Both animals presented with the typical and much described affliction of recurrent lameness and general prostration. In addition, dog 1 displayed recurrent paralysis of the N., V, VII, IX, and X, leading to an outspoken degree of dysphagia associated more typically with human cases of Lyme (Lacau St. Guily et al, 1993), as well as total bilateral paralysis of the mandibular muscles. A positive response to antibiotic treatment was also recorded in both cases.
We would therefore conclude that it is important to consider Lyme borreliosis in the diagnosis of dogs presenting with illnesses marked by fever, anorexia, fatigue, non-traumatic lameness and / or certain forms of paralysis.

TABLE I : Screening of canine sera via IFA, ELISA and PCR and Passive Hemagglutination for the presence of Borrelia specific antibodies.

Serum	**Reciprocal IFA IgG Bb Titer**	**ELISA IgM Test**	**LYMAG Hemagglutinat^{n} Test**	**PCR**
Dog 1 (24/04/93')	512	+	+1	-
(31/05/93')	128	+	NT	-
Dog 2 (10/03/93')	64	-	+1	-
(23/04/93')	64	-	NT	-

Key:

IFA cut-off value : 1/64
IFA screening antigen : Heat inactivated European Strain (Medor, Germany)
LYMAG Haemagglutination test : A scale of positivity is recorded ranging from +1 to +4
NT : Not Tested

TABLE II : Differential diagnostic testing of canine sera.

Serum	Ureum mg %	Creat mg %	RF IU/ml	ANA	DNP	LSP
Dog 1 24/4/93	20	0.71	22.8	-	-	-
Dog 2 10/3/93	17	0.67	<18.8	-	-	-

Key : Creat : Creatinine, (normal values: 0.5-1.5 mg%)
Ureum : normal values: (20-50 mg%)
ANA : Antinuclear Antibody present in Indirect IFA
DNP : Macroscopic agglutination in deoxyribonucleoprotein Latex test
LSP : Macroscopic Passive agglutination in the Leptospirosis screening test

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SECOND LYME DISEASE NEWSLETTER FOR THE U. K., AUTUMN 1993

From Dot Carey and Pat Nuttall

We initiated a Lyme Disease Newsletter in 1991 in an attempt to record the distribution of *Borrelia burgdorferi* infected ticks in the UK. This report gives details of the progress made over the last two years. The second edition has been sent out to >150 individuals who have expressed an interest in our research and in many cases contributed ticks.
Responses to our call for ticks have come from a wide selection of the community ranging from veterinary, medical or environmental health practitioners, academics, forest rangers, ecologists, gamekeepers and members of the public.

The demand for our work can be measured by the number of receipts:

Date	Code Number	Total Per Annum
1990	1 / 11	11
1991	12 / 64	52
1992	65 / 210	155
1993 to 30/9/93	210 / 310	100

We have received 310 parcels containing ticks. The sample size has varied from single ticks from pet dogs or cats to a collection from a culled 27 year-old deer hind that consisted of a mixture of >350 engorged females and male adult ticks that came in a jam jar to a depth of 1.5 inches! We have succeeded in reaching tick collectors (>120) from Scotland, England and Wales. The 'Emerald Isle' is ably covered by colleagues resident in Dublin.
The samples of ticks are stored at -70° prior to screening using the polymerase chain reaction (PCR). The procedure used is a modification of the method described by Guy and Stanek (J. Clin Pathol., 1991, 44: 610-611.) and uses guanidinium thiocyanate extraction and amplification using a nested set of primers.
Most counties in the UK have been found to have ticks supporting infections of *Borrelia burgdorferi* as defined by the PCR method (see figure). A notable exception is the Midlands area although this region may have isolated pockets of suitable biotopes separated by arable farmland, conurbation or major transport routes.

WHERE HAVE THE TICKS COME FROM?

Ticks have been collected from a wide range of vertebrates. Although randomly sent, a pattern can be found although there is a heavy bias to interested parties. There are basically three types of environments sampled;
- from farms, rural: sheep, cattle, pigs, horse, and working dogs.
- from within the home, urban: dogs, cats, humans.
- from the countryside, wild: deer including red deer, roe deer, muntjac deer, fallow deer, sika deer; fox, weasel, bank vole, hedgehog, field mouse, red squirrel, grey squirrel, shearwater, guillemot, common grouse, pheasant, black grouse.

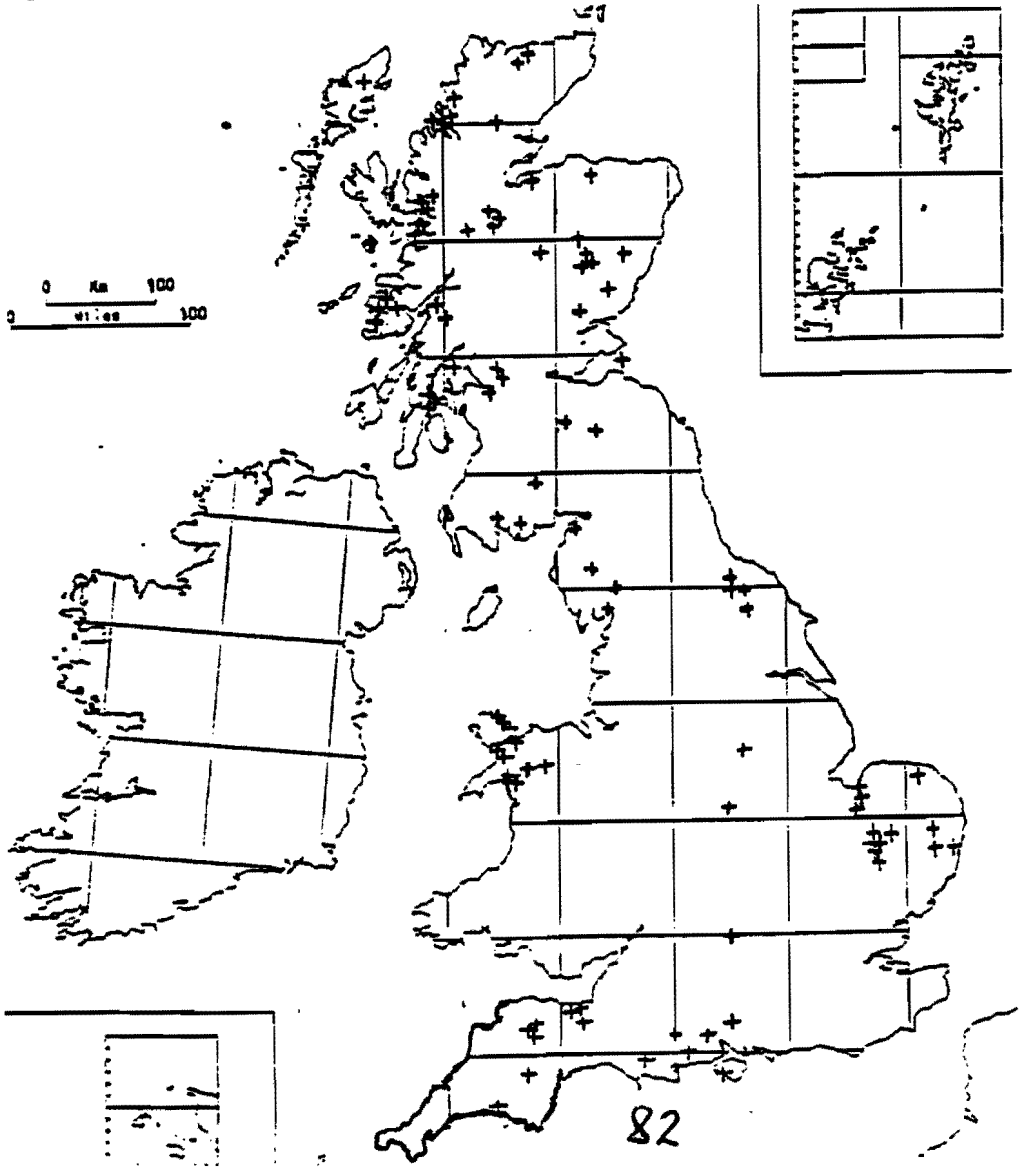
SUMMARY OF RESULTS

Of the 468 ticks examined by PCR, 35% were positive. The ticks were collected from a total of 178 animals and 53% of these individuals were carrying PCR+ ticks.

RESULTS OF PCR ANALYSIS OF TICKS

HOSTS OF TICKS				TICKS		
Source	No.sampled	No.+	% +	No.tested	No.PCR+	% +
deer	18	9	50%	61	13	22%
red deer	13	5	38%	41	7	17%
roe deer	10	2	20%	26	3	11%
muntjac	1	1	100%	14	2	14%
grey squirrel	1	1	100%	13	3	23%
pheasant	4	2	50%	5	3	60%
dog	58	28	48%	133	42	31%
cat	10	5	50%	16	6	37%
horse	6	3	50%	8	3	37%
pig	1	1	100%	1	1	100%
cattle	1	0	0%	6	0	0%
black grouse	1	0	0%	2	0	0%
human	16	6	37%	24	7	29%
others	38	32	84%	118	73	61%
TOTALS	178	95	53%	468	163	35%

MAP OF THE UK SHOWING LOCATIONS OF POSITIVE TICKS



DETECTION OF TRANSFUSION-ASSOCIATED HEPATITIS CAUSED BY NON-A, NON-B, NON-C FLAVIVIRUS

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The existence of the non-A, non-B hepatitis has been recognized almost twenty years ago. However, the discovery of the hepatitis C virus did not clarify the etiology of all transfusion-associated hepatitis cases. There is a series of evidences suggesting the existence of other parenteral non-A, non-B, non-C agents (hepatitis F and beyond).

The serological data indicating the existence of an as yet unidentified flavivirus present in the Hungarian population are described in this paper.

Sera of patients suffering from acute hepatitis, and different forms of chronic hepatitis were found to be reactive to reagents prepared from the yellow fever virus (YF) vaccine strain. Serum samples of 1974 patients were tested, and 133 of them were found to be positive. Hepatitis C virus specific antibodies were absent from the majority of them. The frequency of antibodies to other flaviviruses (tick-borne encephalitis, West Nile) and hepatitis B virus markers proved to be similar to that measured among the population in Hungary positive for any of the surrogate markers of hepatitis infections. Results of both immunofluorescence tests, and Western blots suggest, that there is a non-A, non-B, non-C hepatitis virus circulating among the Hungarian population, which possesses antigenic crossreactivity with the yellow fever virus, but the identity to any of the known flaviviruses could not be verified yet. No history of yellow fever vaccination could be revealed in any of the patients included into this study. The anamnestic data on previous transfusions or surgical operations can be verified only in the case of the half of YFV-positive patients, nevertheless, the sexual transmission seems to be very infrequent. Attempts are continued in order to detect the viral RNA using polymerase chain reaction, and clone cDNA sequences for sequence analysis.

Virus Encefalitis de San Luis

Dra. Norma Evangelina Mettler

Parece nombre de Santo
pero Santo nunca fuí
es que solo porto el nombre
de un pueblo de Misurí

Donde produje epidemia
de encefalitis viral
y terminé siendo aislado
de un caso que fué fatal

Y fué justo de un cerebro
de niño, para peor mal
que vine de prototipo
para iniciar mi historial.

Y fué en este siglo veinte
en la década del treinta
que me encontraron actuando
como causal de epidemias

Que de caracter rural
urbanas y hasta intermedias
fuí dejando mis secuelas
en esta faz de la tierra.

Dicen que soy habitante
de este país argentino
y es cierto, si en las Américas
siempre he vivido y surgido.

Soy amante de mosquitos
de ratones y de humanos
y cuento entre mis hermanos
a Dengue y Fiebre Amarilla

Y fué por esa razón
que me agruparon con ellos
y con otros similares
para constituir un género

Y bueno, en Flavivirus
ya me puedes ubicar
y en familia de los Flavi
me deberás colocar.

soy un virus ARN
que tiene simple cadena
que es positiva y lineal
y además se encuentra entera.

Pero lo más importante
que debes considerar
es que soy un arbovirus
de importancia sin igual

Pues yo causo encefalitis
cuadros gripales y al fin
en esta tierra argentina
enfermo como el Junín.

Uso como reservorios
esos ratones del campo
causo cuadros hemorragicos
al igual que mis hermanos

Pero no olvido señores
de hacer honor a mi nombre
y cuando ataco a los hombres
genéricamente hablando

su cerebro voy mirando
y donde quiero hago foco
y así cuadros yo provoco
que resultan proteiformes

y que a veces tienen nombres
de los tejidos que inflaman
guardandote bien en cama
por tiempos considerables

Y aquí es bueno que les hable
del lugar que voy tomando
pues mientras polio los deja
su espacio yo voy llenando

Y al igual que poliovirus
los puedo paralizar
aunque ni toque las astas
de la médula espinal.

Siempre fuí menos piadoso
con la gente que es mayor
y a muchos de estos ancianos
los pasé a vida mejor.

Sinembargo cambio mucho
para confundir peritos
en el Norte vivo en pájaros
y multiplico en mosquitos

que de variadas especies
los elijo en actuaciones
y esto depende del molde
que imponga a las poblaciones

No te olvides que mi género
hasta infecta por la leche
de vacas,cabras u ovejas
como en Europa acontece

Si mis hermanos del este
despliegan estas virtudes
no por ser americano
tendré menos aptitudes.

Creo que tienes en mí
a un enemigo en acción
y en toda América yo estoy
esperando una reacción.

Soy virus americano
y me encuentro en tu país
infectando a quien alcanzo
y enfermando aquí y allí.

Y creo estar alertando
de mi actividad creciente
quien te habla no te miente
no lo olvides, sed consciente.

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QUOTES

Will Rogers: "Everybody is preaching economy, but the only folks practicing it are the ones that ain't got nothing."

Frank Layden: "I was driving down the freeway and this cop pulls me over. I pull out my driver's license and ask what's the matter. The cop says he thought I ought to know my wife fell out of the car three miles back down the road. I tell him thanks because I was getting worried that I was going deaf."

Francesco Caracciolo: "In England there are sixty different religions and only one sauce."

Cato the Elder: "After I'm dead I'd rather have people ask why I have no monument than why I have one."

Peter B. Medawar: "The human mind treats a new idea the way the body treats a strange protein: it rejects it."

Lewis Thomas ("The Lives of a Cell"): "The viruses, instead of being single-minded agents of disease and death, now begin to look more like mobile genes. Evolution is still an infinitely long and tedious biologic game, with only the winners staying at the table, but the rules are beginning to look more flexible. We live in a dancing matrix of viruses; they dart, rather like bees, from organism to organism, from plant to insect to mammal to me and back again, and into the sea, tugging along pieces of this genome, strings of genes from that, transplanting grafts of DNA, passing around heredity as though at a great party. They may be the mechanism for keeping new, mutant kinds of DNA in widest circulation among us. If this is true, the odd virus disease, on which we must focus so much of our attention in medicine, may be looked on as an accident, something dropped."

The Mishnah: "Where there is no Law, there is no bread."

Lawrence J. Peter: "Cleaning anything involves making something else dirty, but anything can get dirty without something else getting clean."

Nicolas Chanfort: "Rank without merit earns deference without respect."

Edward Abbey: "Growth for the sake of growth is the ideology of the cancer cell."

Casey Stengel: "When a fielder gets the pitcher into trouble, the pitcher has to pitch himself out of a slump he isn't in."

Bernard DeVoto (on the martini): "The rat stops gnawing in the wood, the dungeon walls withdraw, the weight is lifted. Your pulse steadies and the sun has found your heart. The day was not bad, the season has not been bad, and there is a sense and even promise in going on." (Courtesy of Connie Yunker)

From the Great Law of the Iroquois Confederacy: "In our deliberation, we must consider the impact of our decisions on the next seven generations."

Jean Kerr: "I'm tired of all this nonsense about beauty being only skin deep. That's deep enough. What do you want, an adorable pancreas?"

John Maynard Keynes: "It is better than a man should tyrannize over his bank balance than over his fellow citizens."

Groucho Marx: "Remember, we are fighting for this woman's honor, which is probably more than she ever did."

Kermit the Frog: "Calling us Amphibian Americans is going a little too far. I could see how, if you were a salamander, Amphibian American would be a step up, but it seems to me you should call a toad a toad."

Judge Edwin Torres (to a particularly offensive criminal): "Your parole officer has not yet been born."

George Bernard Shaw: "Patriotism is the conviction that your country is superior to all other countries because you were born there."

Ashleigh Brilliant: "There has been an alarming increase in the number of things that I know nothing about."

Harald Johnson (quoting someone else): "It's not what I don't know that bothers me, it's what I know that isn't so."

Antoine de Saint-Exupéry: "Les grandes personnes ne comprennent jamais rien toutes seules, et c'est fatigant, pour les enfants, de toujours et toujours leur donner des explications."

Grantland Rice: "Outlined against a blue-gray October sky, the Four Horsemen rode again. In dramatic lore they were known as Famine, Pestilence, Destruction, and Death. These are only aliases. Their real names are Stuhldreher, Miller, Crowley, and Layden. They formed the crest of the South Bend cyclone before which another fighting Army football team was swept over the precipice at the Polo Grounds yesterday afternoon as 55,000 spectators peered down on the bewildering panorama spread on the green below." (Not much has changed-- Editor)

Will Rogers: "You can't say civilization don't advance, however, for in every war they kill you in a new way."

John D. Rockefeller: "The growth of a large business is merely a survival of the fittest.... The American Beauty rose can be produced in the splendour and fragrance which bring cheer to its beholder only by sacrificing the early buds which grow up around it." (For those of you whose primary language is not English, it should be recognized that this is a statement from the past and should not be taken seriously.)

Arlene Heath: "Classical music is music written by famous dead foreigners."

Napoleon Bonaparte: "Glory is fleeting, but obscurity is forever."

Jack Benny: "I don't deserve this award, but I have arthritis and I don't deserve that either."