June, 1990

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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.

INVITED COMMENTARY

DENGUE

VECTOR COMPETENCE AND VECTOR BIOLOGY

DEVELOPMENT AND APPLICATION OF TECHNIQUES

MOLECULAR BIOLOGY AND MOLECULAR IMMUNOLOGY

EPIDEMICS AND SURVEILLANCE

HANTAVIRUSES

EXPERIMENTAL INFECTIONS

VACCINES

LYME DISEASE

American Committee on Arthropod-borne Viruses

Editor's comments

The next issue is scheduled to be mailed December 1, 1990 (deadline for submissions November 15, 1990). There is nothing that requires you to wait until the last minute. If you have something to communicate in July, August, September, or October, please send it to me. Also, there is nothing that prevents you from submitting a report to every issue. There are no pages charges either, but then again, this is not a publication.

PLEASE !!! Follow the directions for submitting reports. Double-spaced pages take twice as much space as single-spaced pages. Please do not double-space or number pages. Single-space them and leave them unnumbered. Do not staple pages together. One report was on purple paper; thanks, sweetheart.

This has been a remarkable year. It is certainly not appropriate for the editor of a scientific newsletter to comment on political changes, but there is no denying there have been some. To those of you who are struggling with those changes, our best wishes. Try to keep in mind the words of Karl Menninger: "When a trout rising to a fly gets hooked on a line and finds himself unable to swim about freely, he begins with a fight which results in struggles and splashes and sometimes an escape. Often, of course, the situation is too tough for him. In the same way the human being struggles with his environment and with the hooks that catch him. Sometimes he masters his difficulties; sometimes they are too much for him. His struggles are all that the world sees and it naturally misunderstands them. It is hard for a free fish to understand what is happening to a hooked one."

Finally, my apologies to NK Blackburn and P Verani. In the December, 1989 issue I spelled their last names entirely in lower case letters. We all know these are capital people.

Charles H. Calisher, Ph.D.

GUIDELINES FOR SUBMITTING REPORTS

We want to keep this mechanism timely and viable. Therefore, send me only recent news and summaries of your work. **PLEASE** limit the submission to 1 or a very few sheets (21.59 cm x 27.94 cm = 8.5 x 11 inches) plus a table or two; condense as much as you can (**single space** the text); **do not** staple pages together; **do not** number pages. This is essentially a one person operation and I am basically a lazy person; the less work I need to do, the better I like it. The American Committee on Arthropod-borne Viruses, Subcommittee on Information Exchange (Chairman, Dr. Nick Karabatsos), which supervises this effort, has agreed that anyone not submitting a report in any two year period will be dropped from the mailing list. Submission of a brief report seems a small price to pay for all this information.

Needed: Monoclonal antibodies to Semliki Forest virus.

Dr. Christian Mathiot and co-workers have been studying the epidemiology of Semliki Forest virus in the Central African Republic. A recent publication (CC Mathiot, G Grimaud, P Garry, JC Bouquety, A Mada, AM Daguisy, AJ Georges [1990] An outbreak of human Semliki Forest virus infections in Central African Republic. AJTMH 42:386-393) describes many of their findings. However, much more work needs to be done. Not only are the isolates antigenic subtypes of the prototype Semliki Forest virus, but Dr. Mathiot has observed differences in pathogenicities of these isolates. If you have a collection of monoclonal antibodies to Semliki Forest virus or know of such a collection, please write to me and let me know how I can obtain these antibodies or to whom we can write. Thanks.

Charlie Calisher

Needed: *Borrelia burgdorferi* isolates and serum from patients with Lyme disease.

The Division of Vector-Borne Infectious Diseases, CDC is establishing a reference center for Lyme disease (LD). The purpose is to improve and standardize the laboratory diagnosis of LD. We would appreciate receiving any *Borrelia burgdorferi* isolates, serum from patients with LD or experimental animals, or other reagents. We desperately need isolates and large volumes of serum/plasma from the same patients. We can compensate the patient and pay blood bank and shipping costs. Please contact Leonard W. Mayer, Ph.D., Centers for Disease Control, P.O. Box 2087, Fort Collins, Colorado 80522, U.S.A.; telephone: (301) 221-6479, FAX: (301) 221-6476 for more information.

FIFTH AUSTRALIAN ARBOVIRUS SYMPOSIUM, 28 AUGUST - 1 SEPTEMBER 1989, BRISBANE, QLD 4068, AUSTRALIA

A successful Symposium on arboviruses was held in Brisbane, Australia. The five day program brought together scientists of various disciplines. The First Symposium in 1976 in Brisbane was initiated to bring together the separate groups of medical and veterinary researchers into arboviruses, the vectors of arboviruses and the diseases produced by arboviruses. The Third Symposium in 1982 had a very significant international input and this has continued to expand. However, this Fifth Symposium has remained small and very interactive though the character of the content has changed with the international contributions.

The attendance by Australian speakers and delegates was down below the projected number due to a domestic airlines pilots' strike. Very few speakers failed to arrive as alternate transport was widely used by those determined to arrive, even from a vast distance. The total attendance was 171. The Australian attendance suffered more than the international attendance as the strike grounded the domestic airlines but not the international carriers. The other countries represented were New Zealand, USSR, China, USA, United Kingdom, West Germany, Mexico, Papua New Guinea, Taiwan, France, Malaysia, Vietnam, Japan, Sri Lanka, Fiji, Philippines, Switzerland, Indonesia and Singapore. The Pacific Rim was thus well represented by delegates and by subject matter.

The theme of the Symposium was **"Towards 2000 - without prejudice"**. The idea was to encourage speakers to speculate on what the near future holds for arbovirology in an era of massive environmental change. A few speakers accepted the challenge but many did not. Time sorts out hypothesis and theory but it is still worth an attempt to project forward from the hard data, even if you are not always correct.

The Symposium heard single papers or groups of papers on a variety of viruses with disease connotations, their insect vectors and the social effects of arboviruses and their vectors. The content ranged from biotechnology to filed aspects. Subject areas included dengue, Murray Valley Encephalitis, Kunjin, yellow fever, tickborne encephalitis, Ross River, pathogenic Australian bunyaviruses, bluetongue, bovine ephemeral fever, teratogenic arboviruses, vector population monitoring by remote sensing, environmental and direct control of vectors. These subjects were substantially a continuation of lines of investigation of previous symposia of viruses affecting man or animals. A separate stream covering community participation in arbovirus or vector control was added. Over 50% of the papers covered research outside Australia.

The Proceedings is in the editorial stages of preparation. It will contain approximately 105 papers or extended abstracts. The approximate figure is simply a reflection of the fact that not all participants have yet submitted their manuscripts. The Fifth Proceedings will have more pages than the 342 pages of the Fourth Proceedings. The printing date is not yet finalized but will be approximately April 1990. All full registrants at the Symposium will receive a copy. The requirement for additional copies has to be forecast soon. The prepublication price has been set at $70 some time ago. The post-publication price will be higher due to rising costs.

Orders can be placed with:

Editor, Arbovirus Research in Australia
Proceedings 5th Symposium.
CSIRO
Private Bag 3, Indooroopilly,
Queensland 4068, Australia

Price (Australian dollars):

Proceedings prepublication	$70.00
Airmail (outside Australia)	$31.50
Surface/Air (outside Australia)	$19.50
Surface (outside Australia)	$7.80

Cheques or money orders should be made out to CSIRO, Collector of Monies in Australian currency or the equivalent in an internationally exchangeable currency.

(T. D. St.George - Convenor)

Is your subscription to
the Arbovirus Information Exchange
about to expire?

A few years ago I had the honor and opportunity to ask Dr. Jordi Casals, who many of you know, what was the most gratifying feature of his long and remarkable career. He told me it was his personal contacts and working relationships with fellow scientists. If someone would ask me the same question, I would give the same answer. I receive many letters from colleagues around the world thanking me for my efforts in regard to the Arbovirus Information Exchange. This is a labor of love and not any effort at all, but I am grateful that many of you notice.

Unless you are a library, retired, or otherwise unable to submit something, you agreed to send me some information, at least once each two years, for printing in the Arbovirus Information Exchange. Small price to pay for finding out what so many others are doing, right?

Nevertheless, there are many who have not contributed anything since at least the issue before June 1989. That means your name will be removed from the mailing list if I do not have a contribution from you in time for the December 1990 issue.

I know you are out there and I know you are involved in many laboratory or field studies. The intent of the Arbovirus Information Exchange is to let others know what you are doing and find out what others are doing. This is obviously a two-way street.

Keep in mind that the more submissions, the greater is our enthusiasm; the fewer submissions, the less likely the Arbovirus Information Exchange will continue. Let me put it this way: Get off your butt!!!

Your devoted, yet impatient servant.

Charlie Calisher
Charles H. Calisher, Ph. D.

Russian proverb: "If you do not crack the shell, you cannot eat the nut."

Report from the Division of Vector Borne Viral Diseases
Centers for Disease Control
Fort Collins, Colorado
Theodore F. Tsai, M.D.

The epidemiology of Lyme disease in the United States

Although Lyme disease is a bacterial infection, caused by the spirochete Borrelia burgdorferi, arbovirologists should be aware of its features because the disease is an increasingly important domestic and international public health problem. Since its recognition in 1976 as an obscure cause of epidemic arthritis in Connecticut, Lyme disease has developed into the nation's leading vector-borne disease (Figure 1). National incidence has increased steadily with a near doubling of cases each year from 1987 to 1989 (Figure 2). In 1989, approximately 7500 cases were reported nationwide from 44 states. Of the vector-borne infections reported to the Centers for Disease Control from 1983-1988, Lyme disease accounted for almost 60% of the cases. It is pertinent to note that tick-borne infections, Lyme disease, Rocky Mountain spotted fever, tularemia and Colorado tick fever comprised more than 95% of the reported cases of vector-borne infection (Figure 1).

The distribution of Lyme disease in the United States is far from uniform (Figure 3). Cases are concentrated in three regions: in the Northeast; the Upper Midwest, especially in Wisconsin and Minnesota; and on the Pacific Coast. Over 80% of the nation's cases are reported from 6 northeastern states and in 1988 New York alone reported 55% of the nations cases. I. dammini is the principal vector in the East and in the Upper Midwest and I. pacificus is the chief vector species in the West. Although increasing numbers of cases have been reported recently from the Southeast, the identity of the tick vector in this region remains unclear. Bacterial isolates have been made from field collected Amblyomma americanum however experimental infections suggest this species is not likely to be an important vector. Conversely, although few infected I. scapularis have been identified in field collections, experimental data indicate it may be equal to I. dammini in its vector potential. Although numerous ecologic and epidemiologic factors contribute to the risk of transmission of Lyme disease, it is noteworthy that infection rates in the principal vector species roughly parallel disease incidence in their respective regions (Table 1).

Much remains to be learned about the epidemiology of Lyme disease and in particular work is needed to define the circumstances under which infections occur and the ecological and human behavioral factors that contibute to risk. Anecdotal data and limited epidemiologic studies indicate that Lyme disease is acquired in the peridomestic environment. In many areas of the Northeast, secular trends toward preserving forests and the reversion of agricultural lands to uncultivated fields have produced increased habitat for white tailed deer, the principal vertebrate host for adult I. dammini. The juxtaposition of residential housing with these habitats has found people in an environment where all the components of the natural cycle of B. burgdorferi are present: deer, field mice and ticks. Like St. Louis encephalitis, in which peridomestic Cx. pipiens mosquitoes and passerine birds constitute the amplification cycle of SLE virus, the risk for acquiring Lyme disease lies in the backyard.

In other areas of the country, in the North Central states and the Pacific Coast, the epidemiologic circumstances under which infections are acquired are less clear. A comparison of the epidemiologic characteristics of cases from the Pacific region with other areas of the country indicate that there may be differences in the seasonality of Lyme disease and in the age distribution of cases. Lyme disease cases in the Pacific region occur over a broader range of months while the disease exhibits a distinct late spring/summer seasonality in areas where nymphal I. dammini are principally responsible for infecting humans. Regional differences in the epidemiology of Lyme disease should be anticipated because the principal vector species differ in their ecology and human interactions with the vectors also will vary.

It will be important to define more clearly the epidemiologic circumstances under which infections occur in order that the most appropriate prevention and control measures can be designed. In circumstances where exposure occurs sporadically during occupational or avocational activities, personal protection is the logical approach to prevention. These measures include simple alterations in behavior as well as the use of repellents. In the peridomestic environment, where exposure is perennial, environmental modification and the use of acaricides may be appropriate. There may be limits however, to the acceptability of approaches that drastically alter landscaping and that expose humans and pets to potentially toxic acaricides.

Most tick-borne diseases are diseases of place, in which humans put themselves at risk by travelling to the location where infected ticks are present. With the possible exception of tick-borne relapsing fever, Lyme disease is unique because the place of exposure is the peridomestic environment. Innovative approaches are needed to control Lyme disease in this difficult setting.

Table 1.

Comparison of Lyme disease regional incidence and infection rates in vector ticks

Region	Incidence*	Infection rate	
Northeast and North Central	3.7-6.1	I. dammini	40%
Pacific	0.6	I. pacificus	4%
Southeast	0.2	I. scapularis	0.4%
Mountain	0.02	vectors not identified	

* cases/100,000

Figure 1.

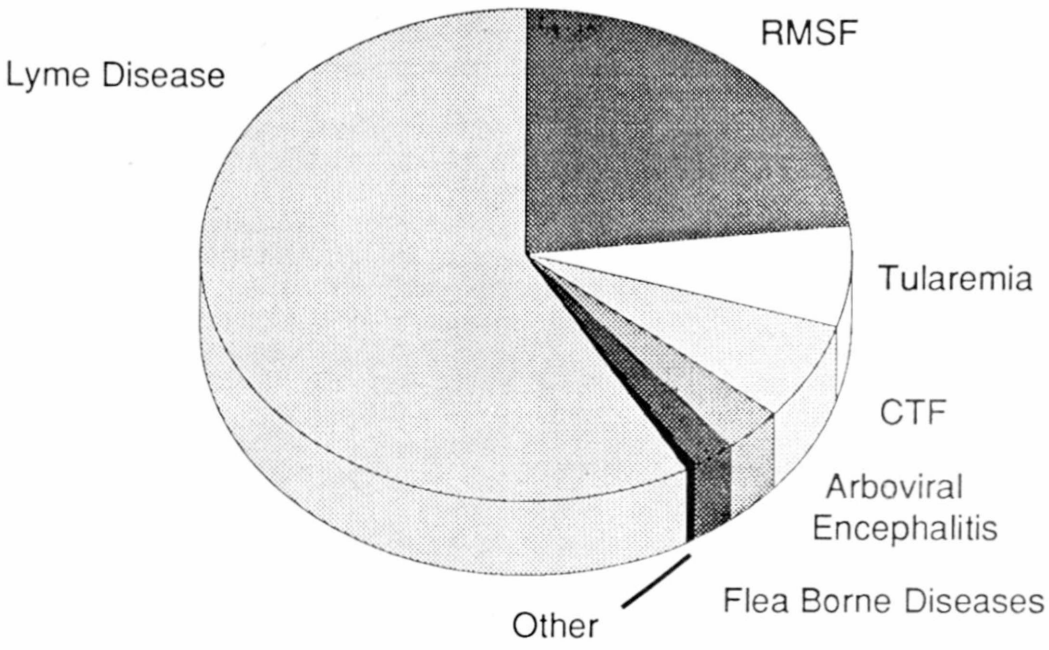


Figure 2.

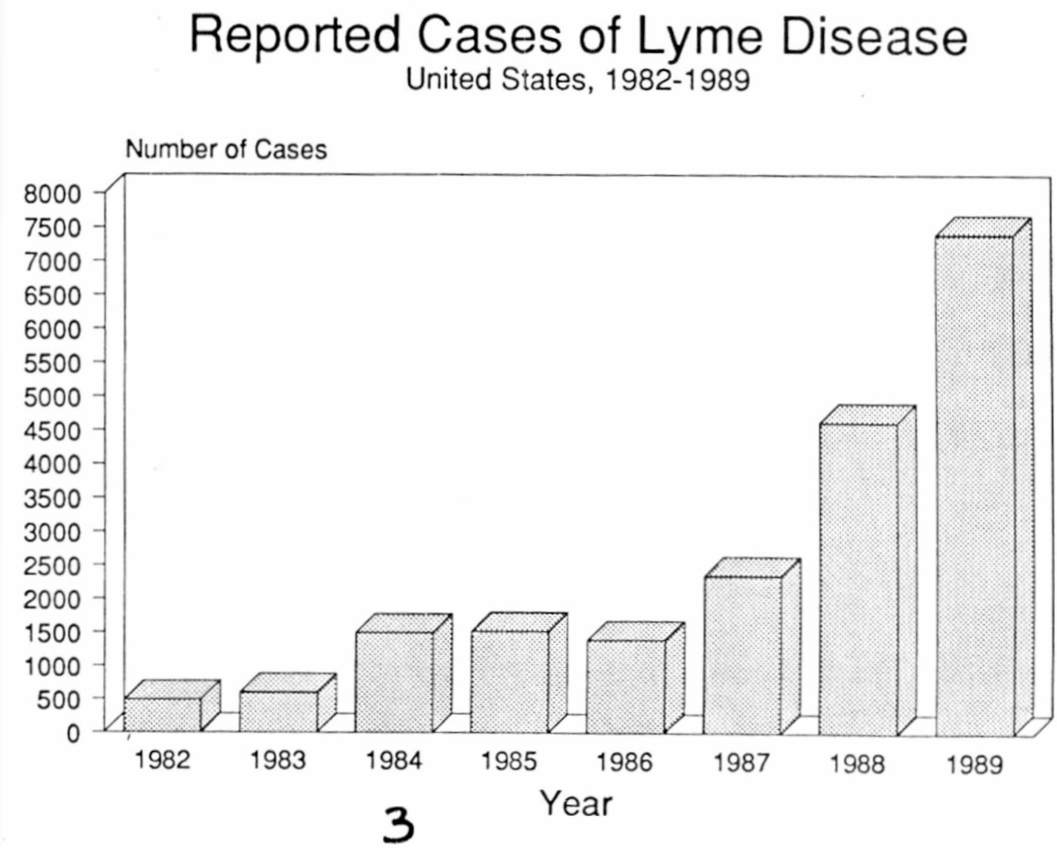
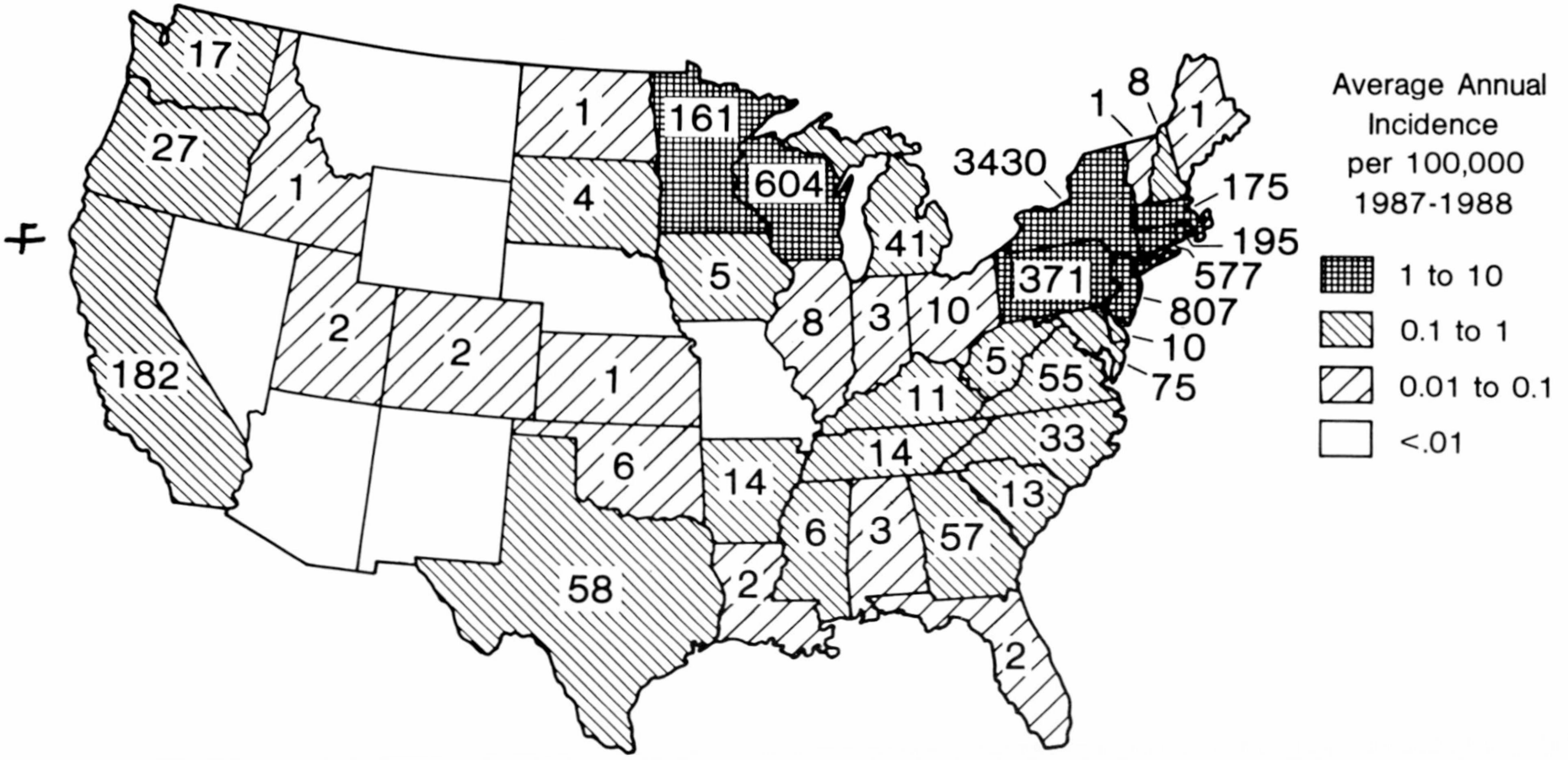


Figure 3.

REPORTED LYME DISEASE

STATE INCIDENCE - 1987-1988



RAPID DIAGNOSIS OF DENGUE VIRUS SEROTYPES BY GENOMIC AMPLIFICATION.

Polymerase chain reaction (PCR) was developed for the in vitro amplification of dengue virus cDNA genes derived from genomic RNA. A fraction of the N-terminus gene of the envelope protein in the four dengue serotypes was amplified using synthetic oligonucleotide primer pairs. Amplified products of about 260 nucleotides in length (NT 40 to 360 in the E gene) were cloned and used as dengue type-specific probes. Nonradioactive probes were prepared by labeling with N-acetoxy N-2-acetylaminofluorene (AAAF) and used for DNA-DNA or DNA-RNA hybrid detection with anti-AAF monoclonal antibody. Viral RNA in infected cells or amplified cDNA products were doted on nitrocellulose and the dengue serotype was determined by using the four dengue-specific probes. A DNA-DNA hybridization data is shown in figure 1 which confirms the specificity of these dengue probes. We were able to detect and characterize dengue virus serotypes in blood samples by the three-step procedure DNA-PAH consisting in cDNA priming (P), DNA amplification (A) and hybridization (H) using the specific nonradiolabeled probes.
Our findings showed that DNA-PAH was quicker and more sensitive in the identification of the infecting serotype than the mosquito cell cultures (table 1). Moreover, the failure of cultures to detect virus particles in sera containing few copies of viral genome or anti-dengue antibodies justified the DNA-PAH approach to the dengue identification in clinical specimens.

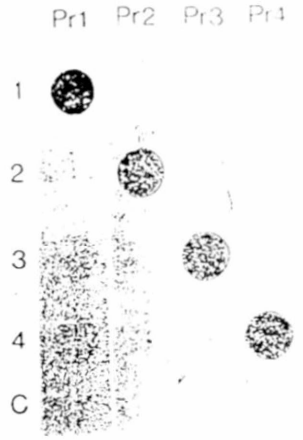


FIGURE 1. Specificity of spot hybridization assays. Rows 1,2,3,4 contained DNA amplified from each dengue serotype. AAF-labelled probes specific to each dengue serotype are indicated Pr1, Pr2, Pr3 and Pr4, respectively. (C): RNA amplified from mock-infected cells. Membrane washing was carried out at 65°C.

Table 1: Comparison of dengue virus PCR to cell culture for dengue virus identification in serum specimens.

	Positive[1]				Negative[1]	
	Virus serotype					
	dengue 1	dengue 2	dengue 3	dengue 4		Negative Control
Cell culture positive[2]	5/32	0/32	5/32	5/32	17/32	0/2
PCR positive[3]	11/32	1/32[4]	7/32	5/32	8/32	0/2

1 All test were done blindly and were duplicated
2 Immunofluorescence for virus identification was performed 10 days after cell infection
3 Serotype determination was performed by dot-hybridization using non radioactive probes
4 Serum was collected 8 days after onset of disease in a laboratory employee accidentally infected

On the other hand, PCR allowed direct sequencing of amplified genes from clinical specimens and provided a powerful tool for molecular epidemiology investigations.
We performed a simple and rapid method (DNA-PAS: cDNA Priming, Amplification, and Sequencing) for sequencing the agarose-eluted DNA using T7 polymerase (Sequanase, USB). Table 2 shows the strain-to-strain comparative data derived from a fraction of the sequence (210 nucleotides) of seven dengue 2 strains (4 were kindly provided by Léon Rosen) belonging to different genotypes (Trent et al., 1983; Kerschner et al., 1986; Rico-Hesse, 1990). Calculation of genetic distances between these strains gave results similar to those previously obtained by sequencing the E/NS1 gene junction of viral RNAs (Rico-Hesse, 1990). Moreover we proved that our method was directly applicable on specimens, thus avoiding the risk of virus selection or mutation during in vitro passages.
Consequently, our DNA-PAS method which is more precise than other technics (Trent et al., 1983; Kerschner et al., 1986) is useful for an easy investigation of relationships among viruses.

Table 2: Pairwise comparison of nucleotide and amino acid sequences in the E gene from dengue 2 geographic variants.

	Jamaica	Porto Rico	Tahiti	Seychelles	Ivory Coast	Burma	Thailand
Jamaica/83	-	14§	20	12	42	9	9
Porto Rico/69	(3)+	-	18	11	42	7	13
Tahiti/71	(3)	(3)	-	19	43	20	19
Seychelles/77	(2)	(1)	(2)	-	36	10	12
Ivory Coast/82	(4)	(5)	(5)	(4)	-	39	41
Burma/76	(1)	(2)	(2)	(1)	(3)	-	4
Thailand/80*	(1)	(2)	(2)	(1)	(3)	(0)	-

* from Gruenberg et al. (1988)
§ number of different nucleotides
+ number of different amino acids

Part of this work will be published in the *Journal of Virological Methods*.

Vincent Deubel and Alain Chippaux
(Laboratory of Arboviruses, National and WHO Collaborating Centers for Arboviruses, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France)

with the participation of Manola Laille
(Laboratory of Arboviruses, Institut Pasteur de Nouvelle Calédonie, BP 61, Nouméa, Nouvelle Calédonie)

REPORT OF THE "PEDRO KOURI" INSTITUTE OF TROPICAL MEDICINE ARBOVIRUS LABORATORY, CURRENT ACTIVITIES

Submitted By: M.G. Guzmán, G. Kouri, S. Vázquez, M. Soler and J.L. Pelegrino

During 1989 and 1990, we continued research and diagnosis activities mainly related with dengue viruses.

RESEARCH:

Studies of the comparison of permissiveness of dengue infection in macrophages of asthmatic and non-asthmatic individuals in immunoenhancement conditions were concluded. We found that viral replication in macrophages of asthmatic patients was significantly higher than in controls. This finding coincides with epidemiological observations done during the 1981 Cuban epidemic in which bronchial asthma was identified as a host's risk factor for DHF/DSS. Currently we are developing the same kind of study in diabetic patients and in individuals of white and black races (from Spain and Angola). In addition, we started studies related to the sequencing of the RNA of various dengue 2 strains.

DIAGNOSIS:

Since 1981 a surveillance system for dengue was established in Cuba in which we study all suspected cases. In 1988, we began using MACELISA and also Inhibition ELISA for the detection of total immunoglobulins to dengue. In 1989, we studies 683 specimens of febrile patients with rash in which the diagnosis of rubella and measles was not confirmed by the laboratory.

We must point out that a very close and systematic entomological surveillance is maintained in the whole country. Aedes aegypti is now eradicated in 13 of 14 provinces in the country and only very rare foci are currently found. Surveillance for the presence of Aedes albopictus is maintained, with no positive reports to now.

Our laboratory studied 284 single sera from suspected dengue cases from Nicaragua and found specific IgM antibodies in 72 (25%). Most of the specimens were from Region III. In January, 1990, two dengue 2 strains were isolated from the sera of two patients with a clinical picture of dengue fever, proving the present circulation in Nicaragua of this serotype during this season.

We also collaborated with Venezuelan authorities during the recent DHF/DSS epidemic that occurred in that country. Our laboratory advised the establishment of diagnostic techniques in the laboratory in Maracay. Additionally, we are studying sera from suspected DHF/DSS cases; the presence in 8 of them of high IgM titers was demonstrated.

In collaboration with the Institute of Hygiene in Guayaquil, Ecuador, we made a seroepidemiological and entomological study in the city. The first phase of this study was concluded and we informed the Ecuadorian health authorities.

TEACHING ACTIVITIES:

Three DENGUE training courses were developed in Dominican Republic (1989 - 1990) and we trained in our laboratories researchers from Argentina, Mexico, Honduras, Dominican Republic, Venezuela and Nicaragua. We advised the National Laboratory in Managua about standardization of diagnostic tools for dengue viruses.

OTHER ACTIVITIES:

We are also doing other studies related to arboviruses, trying to identify the circulation of other arboviruses in the western provinces of Cuba and making clinical correlations. We standardized ELISA for the identification of total Ig for EEE.

In December IPK will develop its THIRD INTERNATIONAL TRAINING COURSE FOR DENGUE AND DHF/DSS. This course includes practical activities for those students interested in laboratory work. For further information, you may write to:

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CUIPK 0512341

Telefax: 215957 IPK Habana Cuba

COMPARATIVE STUDY OF TWO STRAINS OF DENGUE 2 USING ANTIBODY DEPENDENT ENHANCEMENT: PRELIMINARY REPORT

Submitted by Dra. Licel Rodriguez Lay, Dra. Maria G. Guzmán Tirado, Prof. Gustavo Kouri Flores, Lic. Maritza Soler Nodarse and Dr. José R. Bravo

Instituto de Medicina Tropical "Pedro Kouri". Apartado 601, Marianao 13, Habana, Cuba

Clinical and seroepidemiological studies of dengue done in our country showed an increase in the severity of the clinical picture of the disease and the case-fatality rate at the end of the 1981 Dengue Haemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS) Cuban epidemic (Kouri, 1987). Viral virulence apparently increased as the agent was successively passed in the human host and in the mosquito vector.

Two main hypotheses have been proposed to explain the development of DHS/DSS. The first suggests that viral virulence may vary among different strains of the 4 dengue serotypes and that DHF/DSS cases may be caused by infection with exceptionally virulent strains. The second states that sequential dengue infection explains the etiopathogenesis of the severe disease (Halstead, 1981; Rosen, 1986).

Peripheral blood leukocytes were obtained from 10 young adults, whites from both sexes. Cells were prepared on a Ficoll-Visotrast 370 gradient and suspended in medium to a concentration of 10^6 cells/ml in tubes. Virus was added to the cells at a multiplicity of infection of 0.05. One tenth ml of human serum with antibody to serotype 1 at dilutions of 1:640, 1:1280, 1:2560 and 1:5120 was added to each tube. Medium was harvested 3 days postinfection and assayed for virus by plaque formation in BHK-21 cells. Similar cultures without antibodies served as controls. For each case, we determined peak fold-enhancement (the maximum ratio of virus produced by cells infected in the presence of antibodies) and enhancement titer (the highest dilution of antibody-containing preparation at which statistically significant infection enhancement is detected (Halstead, 1984).

Table 1 shows the results obtained. Donors 1 and 10 did not produce any significant antibody-dependent enhancement (ADE). This could be related to the variability of the individual response, as argued by Kouri (1987). Donors 2, 5 and 8 showed significant enhancement, but only with strain A169. The other donors showed significant enhancement with both strains A15 and A169, although the peak fold enhancement was higher in three cases with A169. These results indicate that, at least in vitro, strain A169 reacts in a different way from strain A15.

Whether this finding relates to variations of strain virulence or depends only upon chance needs to be confirmed. Nevertheless, in a comparative study of both strains in P388D1 cells in the presence of 12 monoclonal antibodies, we observed significant ADE with 5 only with A169 and none with strain A15, indicating some difference between viruses.

Table 1: Peak fold enhancement and enhancement titer of the A-15 and A-169 strains in 10 donors.

	Strains	
Donors	A-15	A-169
1	-	-
2	-	3(-3.7)*
3	2(-3.1)	4(-3.1)
4	6(-3.1)	2(-3.1)
5	-	2(-3.1)
6	5(-3.1)	14(-2.8)
7	9.5(-3.4)	15(-2.8)
8	-	12(-2.8)
9	1.4(-3.7)	1.9(-2.8)
10	-	-

*Peak fold-enhancement/log of the reciprocal median ADE titer

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REPORT OF THE INSTITUTE OF TROPICAL MEDICINE " PEDRO KOURI"
DEPARTMENT OF VIROLOGY, HAVANA, CUBA.

Immunopurification of the four Dengue serotypes using a mouse monoclonal antibody

Hybridoma technology for monoclonal antibody generation has become an important tool for bioanalysis in areas as Medicine and Biology. The high purity and large quantities available of these biological reagents have had possible the isolation of proteins from complex mixtures by affinity chromatography (1,2).

In order to get a viral antigen under specificities of purity for diagnostic purposes and for the characterization of viral strains isolated during the 1981 Dengue 2 cuban epidemy, an immunoaffinity column was prepared using a mouse monoclonal antibody generated in our lab (FD2-H3/6) and which recognizes the "Dengue complex" (3). This antibody is able to react with high affinity and specificity against an epitope present on the four Dengue serotype virion as stated by Enzyme Linked Immonosorbent Assay (ELISA) and Indirect Immunofluorescence. We obtained no reaction under the same conditions for some other Flavivirus assayed as San Luis Encephalitis and Yellow Fever.

During the coupling procedure to a matrix of CNBr-Sepharose 4B the amount of antibody bounded was determined as 97%. Then, the four Dengue serotypes, obtained from a mouse brain sucrose/acetone extract, were sequentially applied to the column in a phosphate buffer solution pH 7.2. After a base-line washing, the elution was performed using a buffer containing Glycine 200 mM/L pH 2.8. An affinity peak was collected for each serotype which was dialized and concentrated for further analyses of purity, activity and specificity.

In an "sandwich" indirect ELISA each serotype of Dengue virus was able to be recognized by the FD2-H3/6 antibody. For a wandering about the purity of the affinity fraction for each serotype, a Polyacrylamide Gel Electrophoresis was developed including a sample of non purified virus. A one band pattern was shown for each serotype even when an immunoblotting for each case was performed showing a single band of 59 kd.

On the other hand, a high activity was obtained by the Hemagglutination technique using the purified fractions compared with the crude preparations instead. Also by the immunoelectron microscopy technique was possible to get electromicrographs showing homogeneous viral particles . From these results it is possible that a reasonable degree of purity was achieved and suitable preparations of purified Dengue serotypes are ready for further studies and for diagnostic procedures.

(C. Hermida, M. Pupo*, H. González, S. Vázquez, M. G. Guzmán, M. González, R. Marcet, R. Alemán).

(*) National Bioproducts Center, Havana, Cuba.

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REPORT OF THE INSTITUT OF TROPICAL MEDICINE "PEDRO KOURI", DEPARTMENT OF VIROLOGY, HAVANA, CUBA.

Obtainment of an AP-61 (*Aedes pseudoscutellaris*) cell line clone.

The AP-61 (*Aedes pseudoscutellaris*) cell line obtained by Varma and cols., 1974 was cloned using limiting dilutions in conditioned medium consisting of equal parts of L-15 (Leibovitz) with 10% Tryptose Phosphate Broth (TPB) and 10% fetal calf serum (FCS) and MM/VP-12 supernatant of an AP-61 four day culture 0.45u nitrate cellulose membrane filtered.

The CLA-1 clone grows in L-15 commercial medium supplemented with 10% TPB and 10% FCS while the original line grows in a laboratory prepared media (MM/VP-12) supplemented with 10% FCS. This clone is composed by small epithelial-like cells of rapid and disorganized growth forming a compact monolayer. Its weekly split is 1:15 to 1:20 and it grows at an optimal temperature of 28°C.

A semi-confluent monolayer of CLA-1 and AP-61 is inoculated with Dengue 1 and 2 viruses under the same conditions to check for appearance of a cytophatic effect (CPE) and the detection of viral antigens by indirect immunofluorescence (IFI).

Four to five days post-infection, the AP-61 cells demonstrated syncitial characteristics for both viruses. In the CLA-1 clone no CPE was observed. This may be due to a) the cell's own characteristics, b) the cells are not stressed by a change of medium from MM/VP-12 to L-15 which is common practice in the original line during viral inoculation, and c) the properties of the viral strain used. Nonetheless, the CLA-1 is as efficient as the AP-61 line for viral antigen detection by IFI 72 hours post-infection.

CLA-1 seems to be as sensitive as the AP-61 cell line in relation to D1 and D2 viruses by IFI. Also it improves the culture conditions such as: a high growth velocity and its ability to grow in conventional media. Presently we are testing its capacity for direct viral isolation and its stability for viral sensitivity by increasing the number of passages.

We are also studying a cell line obtained by Pudney and cols. in 1975 from *Aedes pseudoscutellaris* (AP-64) and we determined that the best growth and inoculation media are MM/VP-12 + 10% FCS and L-15 + 10% TPB + 2% FCS respectively. Their split per week is 1:10 to 1:15 and the viral sensitivity for Dengue 1, 2 and 4 viruses is similar to AP-61 but AP-64 is capable to detect Dengue 2 virus by indirect immunofluorecence 24 hours earlier at the same multiplicity of infection.

(L. Morier, R. Alemán, A. Castillo, V. Pérez. Cell Culture Laboratory).

REPORT OF THE INSTITUTE OF TROPICAL MEDICINE "PEDRO KOURI", DEPARTMENT OF ARBOVIROLOGY, HAVANA, CUBA.

Effect of various factors during cryopreservation on the morfology and viability of several poikilothermic cell lines used in arbovirology.

Cell cultures have gained in importance due to their usefulness in Virology, Pharmacology, Bacteriology, etc. For this reason, cell lines and strains must be maintained in cryopreservation in cell culture laboratories to carry out comparative studies of different stages of development. It also reduces laboratory work, contamination risks and the appearance of undesirable traits.

Although there are several discrepancies concerning cell damage during cryopreservation, most investigators prefer a slow process of freezing, maintenance in liquid nitrogen, quick thawing and the use of Dimethyl sulfoxide (DMSO) for cell protection.

Of the main factors that affect cryopreserved cells we have placed special interest on the following points: 1) if the cryopreservation methods described in the texts for mammalian cells applies to poikilothermic cells and 2) if the time kept in cryopreservation affects the cells in some way (morphology or viability).

The cell lines used were the AP-61 (*Aedes pseudoscutellaris*) in subcultures 36-38 maintained in MM/VP-12 medium supplemented with 10% heat inactivated fetal calf serum (FCS) made up of a heterogenous fibroblastic-like, epithelial-like and round cell population; clone C6/36 in subcultures 38 to 40 maintained in Eagle-MEM with glutamine and non-essential aminoacids supplemented with 10% FCS and made up of small epithelial-like cell population; and the XL-2 line (*Xenopus laevis*) in subcultures 61-63 grown in L-15 medium with 10% Triptose Phosphate Broth and supplemented with 10% FCS also made up of epithelial-like cells. The three cell lines grow at 28°C and were dispersed mechanically.

The three cell systems were frozen according to the methods described for mammalian cells and maintained in liquid nitrogen. Lots of each were thawed 2,4 and 8 years later and the Tripan Blue Exclusion test used to determine viability inmediately after thawing. They were also seeded into Leighton tubes to study their morphology staining them with Haematoxilin-Eosin.

No morphologic alterations were observed for the C6/36 and XL-2 cell lines at the times tested and their viabilities were adequate. At two years of cryopreservation the indicators

measured for the AP-61 were natural. With this, we can concluded that the freezing method described for mammalian cell lines can be applied succesfully for at least these three poikilothermic cells studied. Nonetheless, increasing the cryopreservation period for the AP-61 cells alterations were observed proportionally and their viability affected seriously (Table I). This may be due to the nature of the cells frozen, as reported by other authors since the XL-2 were obtained from an amphibian while the AP-61 is derived from a mosquito. For the C6/36 cell line, a subpopulation of the Singh´s original line resistant to freezing may have been cloned by chance.

There are cell lines that are affected by long periods of cryopreservation and the AP-61 appears to be one of them and its is therefore advisable that the lots in liquid nitrogen be renewed periodically. This, of course is not exclusive for the AP-61 line, and, consequently, opens interogations for those who work with these cells widely used in Arbovirology.

(A. Castillo, L. Morier, V. Pérez, I. Lezcano. Cell Culture Laboratory).

Table I. Effect of cryopreservation period on the viability and morphology of three poikilothermic cell lines.

Line	Cryopreservation (years)	Viability (%)	Morphology
AP-61	2	95-98	N
	4	65-70	A
	8	< 50	SA
C6/36	2	95	N
	4	95	N
	8	95	N
XL-2	2	95-98	N
	4	95-98	N
	8	95-98	N

N= Normal; A= Affected; SA= Seriously affected

MULTIPLE BLOOD FEEDING BY AEDES AEGYPTI COLLECTED IN SAN JUAN, PUERTO RICO

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We are using a histological technique to examine engorged Aedes aegypti collected from a dengue virus endemic area in Puerto Rico for signs of multiple blood feeding during a single gonotrophic cycle. An increase in the rate that Ae. aegypti bite humans might increase the probability of virus transmission. This would help explain sustained transmission during interepidemic periods, provide an explanation for clusters of DEN infections within one household, and possibly improve our understanding of the explosive spread of disease during epidemics.

During March 1988, we collected 88 engorged Ae. aegypti from houses in San Juan, Puerto Rico. Fixed specimens were sent to the University of Maryland where they were sectioned, stained, and examined by light microscopy. Twenty-four percent (20/88) of those mosquitoes had clearly imbibed two meals during a single gonotrophic cycle.

Laboratory studies are currently underway to standardize the histological technique with this species. Preliminary results show that multiple feeding can be detected 85% of the time (34/40) when separated by approximately 1.5-24 hr.

During the fall of 1990 we will begin more detailed studies in Puerto Rico designed to define the frequency and under what conditions Ae. aegypti engages in multiple feeding and what effect multiple feeding has on virus transmission. Our preliminary results suggest that multiple feeding by mosquito vectors may be an important factor in the dynamics of dengue virus transmission.

PERORAL SUSCEPTIBILITY OF AEDES ALBIFASCIATUS AND CULEX PIPIENS COMPLEX MOSQUITOES (DIPTERA:CULICIDAE) FROM ARGENTINA TO WESTERN EQUINE ENCEPHALITIS VIRUS.

The transmission cycle of western equine encephalitis (WEE) virus in South America is unknown. A WEE virus strain was isolated from Aedes albifasciatus in Argentina during the WEE epizootic of 1982-83. Also, Culex pipiens from Argentina was reported to be able to transmit WEE virus experimentally, but other results indicate that Cx. pipiens from the USA is refractory to this virus. We determined the susceptibility of Argentina strains of Ae. albifasciatus and Culex pipiens complex mosquitoes to infection by WEE virus by the oral route. Adult females were fed on chicks infected with a WEE virus strain isolated in Córdoba Province, Argentina, or were fed on a blood/virus suspension. Each mosquito ingested between $10^{1.6}$ to $10^{6.4}$ vero cell plaque-forming units of virus. Each of 28 Ae. albifasciatus was positive for virus from the fourth day postfeeding, and there was evidence for virus replication (Table 1). In contrast, 0/44 Cx. p. quinquefasciatus and only 1/15 Cx. p. pipiens was positive. Aedes albifasciatus is susceptible to infection by WEE virus and should be considered a potential vector of this virus in Argentina. Both subspecies of Cx. pipiens are refractory to peroral infection by WEE virus and probably do not play a role in the WEE virus cycle in Argentina.

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TABLE 1. Cba CIV 180 WEE virus titers in the infective meals and in individual *Aedes albifasciatus* mosquitoes infected by peroral route.

Infective meal virus pre-post exposure period (log PFU /5 µl)	Virus titers (log PFU/mosquito) of individual mosquitoes per day of extrinsic incubation								
	1	2	4	5	6	8	9	11	13
1.6 - 2.7	4.4		5.3			6.7			
			6.7			5.8			
			6.0						
			4.6						
3.9 - 3.9	3.9		5.5						
			5.7						
			6.6						
4.1 - 4.4				6.3					4.5
				6.5					4.5
									5.6
4.7 - 4.9				4.5					
				5.5					
5.0 - 5.4	5.3			3.5					
				5.9					
4.6 - 5.7	3.9							5.6	
	4.8								
5.4 - 5.7	5.8		6.3				6.0		
	5.8		6.5						
			6.6						
5.7*	5.8		5.3		5.0				
5.0 - 5.8		4.9				3.3			
5.2 - 6.0	4.8						5.0		
5.5 - 6.3	6.6								
	5.9								
3.7 - 6.4	6.5						5.3		
n	12	1	11	6	1	3	3	1	3
$\bar{x}$	5.3		5.9	5.3		5.2	5.4		4.8

* viral suspension

VECTOR COMPETENCE OF *AEDES ALBOPICTUS* FOR A NEWLY RECOGNIZED *BUNYAVIRUS* FROM MOSQUITOES COLLECTED IN POTOSI, MISSOURI

The vector competence of a Lexington, Kentucky, strain of *Aedes albopictus* was assessed for a newly recognized *Bunyavirus*, related to Tensaw virus, isolated from *Ae. albopictus* collected in Potosi, Missouri, during August and September, 1989. Females are susceptible to peroral infection; 11.1% became infected after ingesting about 2 Vero cell plaque-forming units (PFU) of virus and 44.7% did so after ingesting about 15 PFU (Table 1). Irrespective of infective dose, virus replicated rapidly in infected mosquitoes and reached average titers of $10^{5.4}$ to $10^{6.0}$ PFU/mosquito by day 7 postfeeding (Table 2). Forty percent of 35 females tested in an *in vitro* virus transmission experiment were subsequently shown to be infected, and 3 (21.4%) of the 14 infected females transmitted virus. There was no evidence of vertical transmission among 1,196 progeny of a group of mothers exposed to infection perorally and having a 44.7% infection rate. Also, 6,635 progeny of mothers uniformly infected by parenteral inoculation of virus were negative. The apparent absence or infrequency of vertical transmission of the newly recognized *Bunyavirus* by *Ae. albopictus*, suggests that the virus was not introduced into Potosi, Missouri, via infected *Ae. albopictus* eggs. Although vertical transmission occurs rarely if at all, *Ae. albopictus* is a competent vector of this virus and we provide the first experimental evidence for incriminating *Ae. albopictus* as a vector in a natural arbovirus transmission cycle in the United States.

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Table 1. Peroral infection rates of Lexington, KY, Ae. albopictus for a newly recognized Bunyavirus.

Titer of Infectious Meal*	No. Tested	% Infected Day-14 Incubation
2 PFU	54	11.1
8 PFU	48	22.9
15 PFU	38	44.7

*Number of Vero cell plaque-forming units (PFU) per 5 µl, i.e., estimated bloodmeal volume.

Table 2. Replication of a newly recognized Bunyavirus in perorally infected Aedes albopictus.

Day Post-feeding	Titers[a] of Infectious Meals: 2 PFU/female No. Positive/ No. Tested	2 PFU/female Titer $\bar{X}$ (S.E.)	8 PFU/female No. Postive/ No. Tested	8 PFU/female Titer $\bar{X}$ (S.E.)	15 PFU/female No. Positive/ No. Tested	15 PFU/female Titer $\bar{X}$ (S.E.)
0	3/3	0.3[a]	2/3	0.9[a]	3/3	1.2[a]
2	0/3		1/3	3.6[b]	1/3	3.5[b]
4	0/3		0/2		0/3	
7	1/10	6.0[b]	2/10	5.5[b] (0.2)	6/10	5.4[b] (0.8)
9	0/3		0/3		1/3	5.5[b]
12	Not Done		Not Done		1/3	5.7[b]
14	6/54	5.5[b] (0.3)	11/48	5.5[b] (0.1)	17/38	5.5[b] (0.3)

[a]Based on $\log_{10}$ Vero cell PFU titer of infectious bloodmeals and estimated bloodmeal volume of 5 µl per mosquito.

[b]Log_{10} Vero cell PFU/female

COLLECTION AND ESTIMATION OF INFECTION RATES OF POST-LARVAL *AMBLYOMMA* TICKS IN ZIMBABWE.

In order to estimate rates of infection with pathogens in ixodid tick populations of endemic areas, post-larval forms (which are usually randomly dispersed) must be collected. This is relatively easy with many 3-host species because of their predilection to quest on vegetation while awaiting chance contact with a host, and their ready attraction to CO_2 vapors. However, nymphs and adults of two African species of *Amblyomma*, *A. variegatum* and *A. hebraeum* (both important vectors of viral, bacterial, fungal or protozoan diseases of domestic livestock or man) are difficult to collect in the unfed state. Rather than questing on vegetation, they secrete themselves beneath ground litter, emerging and actively seeking out their host (usually a large domestic or wild ruminant) only when chemical emissions signal its presence nearby. Hence, they cannot be collected on drags. In addition, although stimulated by CO_2 vapors, they do not readily migrate to the source of the gas.

Recently we determined that directional movement, over distances of up to 5 meters, of post-larval *A. hebraeum* was mediated by an aggregation-attachment pheromone (AAP) produced by fed male ticks (Norval, Andrew and Yunker, 1989. **Science**. 243: 364-365). Thus, unfed forms can be lured to CO_2 traps baited with fed males or extracts of AAP from fed males. The wild ticks may then be tested for infectivity. By this means we collected post-larval *A. hebraeum* and subsequently tested them for infection with *Cowdria ruminantium*, the causal agent of heartwater disease of domestic ruminants (Andrew et al., **Vet. Parasitol.**, in press). Collections were made in the lowveld of southern Zimbabwe, where the disease is enzootic. Because ruminants are the hosts of choice for demonstration of this infection, ticks, upon collection, were pooled according to stage or sex and each pool was fed on a heartwater-susceptible sheep. Pools fed on sheep that later developed heartwater were considered to contain one or more infected individuals and the infection rate for the population of the area was determined by the probability estimate of Chiang and Reeves (1962. **Amer. J. Hyg.** 75: 377-391). Of 39 pools of ticks tested over a 2-year period, 18 were positive for heartwater. The estimated infection rates were 0.0 - 44.9% (males), 20.0 - 36.1% (females) and 0.0 - 13.4% (nymphs). Most of these rates are considerably higher than those reported previously in areas enzootic for heartwater, and have implications for epidemiology and control of the disease. They suggest that ticks are an important reservoir, as well as vector, of the infection and that enzootic stability may be maintained despite relatively low numbers of vectors. Further, eradication of the disease in southern Africa may be impossible without first eradicating the vector.

In subsequent tests (Yunker et al., **J. Insect Behavior**, in press), we found that extracts of AAP from *A. variegatum* or *A. hebraeum* were equally attractive to adults of either species (Tables I,II). Within the past decade, virologists working in the Central African Republic have accumulated evidence that *A. variegatum* may play a role in the dispersal or survival of yellow fever virus, especially during times of unfavourable climatic events. Our findings would indicate that unfed *A. variegatum* may now be easily collected for assay of infection with yellow fever virus or other pathogens.
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(C.E. Yunker, R.A.I. Norval and H.R. Andrew. University of Florida/U.S. Agency for International Development/Zimbabwe Heartwater Research Project, P.O. Box 8101, Causeway, Zimbabwe.)

TABLE I. ATTRACTION OF RELEASED AMBLYOMMA HEBRAEUM ADULTS TO SOURCES OF MALE AGGREGATION-ATTACHMENT PHEROMONE (AAP) AND CO_2.[1,2]

	Conspecific attraction *A. hebraeum* AAP + CO_2				Interspecific attraction *A. variegatum* AAP + CO_2				Control CO_2			
Trial No.	Females		Males		Females		Males		Females		Males	
	n/N	%n	n/N	%n	n/N	%n	n/N	%n	n/N	%n	n/N	%n
1	44/94	47.8	32/98	32.7	NT	-	NT	-	0/93	0.0	0/93	0.0
2	17/38	44.7	25/44	56.8	20/44	45.5	21/40	52.5	2/42	4.8	1/42	2.4
3	32/40	80.0	24/45	53.3	36/46	78.3	35/43	81.4	2/41	4.9	6/48	12.5
Total ($\bar{x}$)	93/172[3]	(54.1)	81/187[3]	(43.3)	56/90[4]	(62.2)	56/83[4]	(67.5)	4/176	(2.3)	7/183	(3.8)

1. Ticks moving from 4 m away to within 50 cm of test substance in 1 hr were counted and removed from experiment.

2. N = No. of ticks released; n = No. of ticks recovered; NT = not rested.

3. Adjusted X^2 value for difference between females and males = 3.73; $P > 0.05$ (df=1).

4. Adjusted X^2 value for difference between females and males = 0.78; $P > 0.25$ (df=1).

TABLE II. ATTRACTION OF RELEASED AMBLYOMMA VARIEGATUM ADULTS TO SOURCES OF MALE AGGREGATION-ATTACHMENT PHEROMONES (AAP) AND CO_2[1,2]

Trial No.	Conspecific attraction								Interspecific attraction							
	A. variegatum AAP + CO_2				Control (CO_2)				A. variegatum AAP + CO_2				Control (CO_2)			
	Females		Males		Females		Males		Females		Males		Females		Males	
	n/N	%n	n/N	%n	n/N	%n	n/N	%n	n/N	%n	n/N	%n	n/N	%n	n/N	%n
1	24/49	49.0	10/48	20.8	0/32	0.0	0/39	0.0	25/94	26.6	18/88	20.5	0/95	0.0	2/93	2.2
2	32/45	71.1	37/50	74.0	2/46	4.3	1/44	2.3	31/43	72.1	27/39	69.2	4/48	8.3	5/44	11.4
3	NT	-	NT	-	NT	-	NT	-	17/25	68.0	36/75	48.0				
Total ($\bar{x}$)	56/94[3]	(59.6)	47/98[3]	(48.0)	2/78	(2.6)	1/83	(1.2)	73/162[4]	(45.1)	81/202[4]	(40.3)	4/143	(2.8)	7/137	(5.1)

1,2. See footnotes for Table I.

3. Adjusted X^2 value for difference between females and males = 2.16; $P > 0.05$ (df=1).

4. Adjusted X^2 value for difference between females and males = 0.72; $P > 0.25$ (df=1).

Genetics of Culicoides variipennis oral susceptibility to infection with bluetongue virus. **Walter J. Tabachnick, Arthropod-borne Animal Diseases Research Laboratory, USDA-ARS, P.O. Box 3965, University Station, Laramie, Wyoming 82071**

A family selection scheme and an enzyme linked immunoabsorbant assay were used to select lines of Culicoides variipennis which were either resistant, or susceptible, to oral infection with bluetongue virus (BTV). Several lines were tested for oral infection with BTV for 8 - 10 generations and four lines did not show significant differences in infectivity between generations. Two independently derived resistant lines showed an average of 5 - 10% susceptibility while two independently derived susceptible lines showed an average of 80 - 90% susceptibility.

Crosses between the resistant and susceptible lines showed results consistent with the hypothesis that oral susceptibility for BTV is controlled by a single genetic locus. The F_1 progeny also demonstrated a maternal effect. Crosses between resistant males and susceptible females resulted in susceptible offspring, while reciprocal crosses between susceptible males and resistant females resulted in resistant offspring.

The family selection scheme provided resistant or susceptible lines within 1 or 2 generations. This rapid progress in selection is similar to that previously reported by Jones and Foster (J. Med. Ent. 11:316 - 323, 1974). for C. variipennis oral susceptibility to BTV. They also observed rapid progress in selection, and as a result they suggested the likelihood of control by a single genetic locus.

These results offer encouragement for genetic studies to elucidate genetic and environmental factors controlling variation in vector competence for arboviruses. We are in the process of identifying DNA restriction fragment length polymorphisms (RFLPs) as genetic markers in C. variipennis for mapping studies to locate vector competence loci. Additional resistant or susceptible lines are needed to identify other controlling loci, and for use in studies to determine environmental effects on changing the vector competence phenotype.

REPORT FROM MEDICAL ENTOMOLOGY AND ECOLOGY BRANCH, DIVISION OF VECTOR-BORNE INFECTIOUS DISEASES, CID, CDC, FORT COLLINS, COLORADO 80522

Effectiveness of ULV Aerial Applications of Malathion for the Control of *Aedes albopictus* in New Orleans, Louisiana

This study was designed to evaluate the use of aerial ultralow volume (ULV) spraying as a means of rapidly suppressing *Ae. albopictus* mosquito populations. Between May and October, 1989, eight aerial ULV applications of malathion were made. Two treatments were applied in single doses and 3 treatments were made in pairs separated by 2-3 days. A 2040 acre area was treated using the maximum malathion label rate of 3 ounces per acre. Applications were made at sunrise, at an altitude of 200 ft., and at an airspeed of 150 mph.

The treatment site was a suburban residential area located in north central New Orleans that has well-established populations of *Ae. albopictus* and *Ae. aegypti*. For experimental comparison, *Ae. albopictus* populations were monitored in another suburban residential area of similar size and habitat type which was located about 8 miles east of the treatment area. Within the treatment and nontreatment sites, mosquito populations were monitored daily using ovitraps, landing-biting collections, and adult resting collections.

Each insecticide application was monitored in relation to droplet size, droplet density, and the effect on bioassay specimens. Bioassays of target species employed larvae, pupae, females, and males of *Ae. albopictus* and *Ae. aegypti*. Non-target organisms tested in bioassays were *Toxorhynchites amboinensis*, several species of larvivorous cyclops, and a species of fish, *Cyprinodon variegatus*. Bioassay specimens were also placed in open and sheltered areas. In some experiments, specimens were also situated inside tires which were placed in either open or sheltered sites.

Since space will not permit a discussion of each treatment, only the results from applications made during August will be presented. Malathion ULV aerial treatments were made on August 15 and 18. The first application was applied with light surface winds (<4 mph), while the second had no measurable wind. The average length median diameters (LMD) of droplets were 34.6 and 32.3 uM in the first and second tests, respectively. In the first treatment, the mean density of droplets was 1.9 and 0.7 per cm^2 for open and sheltered areas, respectively. Droplet densities in the second treatment were 5.4 and 1.9 per cm^2 for open and sheltered areas.

Adult bioassay results showed that 90% of caged female and male *Ae. albopictus* mosquitoes in exposed locations and 45% of both sexes in sheltered sites were killed by the malathion treatment. Nearly identical results were also obtained for *Ae. aegypti*. When caged adults were placed in the upper inside of tires, mortality was low, ranging between 10 and 20%.

Larval bioassay results showed that nearly all larvae in exposed sites and about a third of those in sequestered locations were killed within 24 hours of treatment. Similar results were obtained when bioassay larvae were placed inside tires.

Samples of the natural mosquito population showed that after treatment there was a sharp decline in the number of eggs and adults collected. A comparison of ovitrap collections 7 days before and after treatment showed an 82% reduction in the number of eggs oviposited. No reduction in egg laying was observed in the untreated area.

During the 7 posttreatment days, *Aedes albopictus* human biting collections were reduced an average 76% reduction over the 7 day pretreatment level. In addition, the *Ae. aegypti* population was almost completely suppressed for nearly 7 days. The population of *Ae. albopictus* mosquitoes caught in resting collections was reduced an average of 75% during the 7 days following treatment. The population remained below pretreatment levels for the remainder of August.

The data suggests that, at least for New Orleans, aerial ULV applications of malathion would be likely to suppress a population of *Ae. albopictus* rapidly and to a level reducing the risk of disease transmission.

(Freier, J.E., Francy, D.B., and Eliason, D.A.)

Line Immuno Assay (LIA) in Diagnosis of Hantavirus Infection

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LIA is a recently developed serological test in which multiple antigens can be applied in parallel lines onto nitrocellulose strips.

This technique has been successfully applied to the serological detection of Malarial (2), Syphilitic (3) and HIV (4,5) infections in man.

LIA could be of particular importance in the field of Hantavirus serology, since it allows in a single experiment the presence of antibodies against different Hantavirus serotypes to be determined.

Preliminary tests have been carried out in our laboratories using the more commonly applied Indirect Immunofluorescent Assay (IFA) and Western blotting (WB) methods to detect the presence of serum antibodies to various Hantavirus strains. Comparisons were then made with results obtained by LIA testing of these same serum samples. Seven Hantaviral antigens from different geographical regions and representative of 4 different serotypes were employed in our tests, namely : Hantaan type (Hantaan 76-118, Korea); Seoul type (TCH, U.S.A.); Rattus type (R22 VP30, China); Puumala type (Hällnas, Sweden, CG 18-20, U.S.S.R., CG 14604, Belgium); Prospect Hill type (PH, U.S.A.).

The antigens were prepared from the supernatant of Vero E6 Hantaviral infected cells using a freeze-thawing, centrifugal technique - control antigen being derived from the supernatant of non infected Vero E6 cells.

Sera analysed via LIA were considered positive if a coloured reaction was obtained with the Hantaviral antigen and the subsequent intensity of the band was stronger than that of the control antigen. Sera were considered positive by Western blotting when a band corresponding to 45 - 55 Kdalton was recorded with the Hantaviral antigen and where the control antigen did not produce such a reaction.

Our initial results showed that the overall correlation between IFA/LIA and IFA/WB was 85.3 and 77.6 percent respectively and the correlation between WB/LIA was 90.6 percent (Table 1). Therefore it would appear that LIA is more sensitive than WB when compared to IFA, but that both LIA and WB are less sensitive than IFA in detection of antibodies to Hantaviruses.

Among the LIA false negatives, 75 % of the sera had a low IFA titre in the range 16 - 32 and of the WB false negatives, 60 % of the sera had a low IFA titre in this same range.

The extent however to which these low IFA titres represent false negative results from WB and LIA testing would require verification via another specific test such as RIPA.

Upon comparing LIA with WB, it was observed that 88 % of discrepant results were LIA positive and WB negative and that such sera had IFA titres higher than or equal to 64.

It could therefore be inferred from our preliminary findings that LIA does compare favourably with other frequently used diagnostic tests.

In the future, we propose to use this self same technique in the testing of recombinant Hantaviral peptides with batteries of documented sera.

Table 1 : Overall correlation between different diagnostic tests for the determination of antibodies against seven Hantavirus strains

	WB	LIA		LIA
IFA			WB	
HTN 76-118	99	94	HTN 76-118	100
TCH	83	90	TCH	95
R22	50	84	R22	71
Hällnas	77	75	Hällnas	91
CG 18-20	83	88	CG 18-20	96
CG 14604	79	75	CG 14604	98
PH	72	91	PH	83
Average	77.6 %	85.3 %	Average	90.6 %

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USING SATELLITE DATA TO FORECAST THE OCCURRENCE OF THE COMMON TICK IXODES RICINUS – MAIN VECTOR OF TICK-BORNE ENCEPHALITIS IN CENTRAL EUROPE

To forecast the incidence of the common tick, Ixodes ricinus, the authors used the finding that some type of vegetation may indicate the presence of the ticks. To obtain the necessary information in a scope that would enable its practical application in medicine to prevent human exposure to ticks, remote sensing data were utilized as obtained from the Multispectral Scanner operating aboard LANDSAT 5. A file was selected from the full scene representing a territory measuring 41 by 41 kilometers in the center of which there was an area known to have consistently high tick numbers, as revealed in a 25-year continuous study of I. ricinus, and which was virologically proved to be a natural focus of tick-borne encephalitis. This area was taken as a model and was compared with its surroundings. Six landscape classes were examined /1 – coniferous forest, 2 – leaved forest, 3 – mixed forest, 4 – water basins, 5 – glades, 6 – housing developments/, the former three being of crucial importance to evaluate the probability of tick presence. The 6th category is significant in assessing human exposure to ticks.

Data processing was carried out by supervised classification using Bayes decision rule of maximum likelihood. The findings were obtained both in a graphic form /colour copy made by computer printer on scale corresponding to conventional map 1: 100 000/ and in the form of statistical reviews as regards the presence of appropriate landscape categories.

The presence of I. ricinus is primarily associated with areas covered my mixed and leaved forests, but also with coniferous forest scattered in numerous small islands in a complex with the two former categories. In area under consideration, this mosaic pattern of different vegetation types suggests very favorable conditions for the emergence and existence of ecotones which is extremely important for the presence of I. ricinus and availability of a natural TBE focus.

The reference area is situated only 5 km to the west and the number of the ticks there reach levels which are not epidemiologically significant. This area is visually characterized by a markedly greater and homogenous distribution of coniferous forests.

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DELTA : A SIMPLE, VERSATILE AND POWERFUL SOFTWARE SYSTEM FOR MANAGING DATA ON VIRUS STRAINS*[1].

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& VASCONCELOS (P.F.C.)***

(*This work is part of ORSTOM, CNPq & Fond. SESP, MS. Research Program on Yellow Fever and other arboviruses in Brazil, and was supported by funds from all three organizations;
**ORSTOM, C.P. 75 Belém 66000 PA Brazil & Instituto Evandro Chagas;
***Instituto Evandro Chagas C.P. 1128 Belém 66000 PA;
***ENSP/ FIOCRUZ, rua Leopoldo Bulhões 1480 Manguinhos, Rio de Janeiro 21040 RJ)

As consequences of both its location in Brazilian Amazonia and its historical concern in Amazonian and Brazilian arbovirology, the Arbovirus Laboratory of the Evandro Chagas Institute/ Fond. SESP was designated by WHO as a Collaborating Centre for Arbovirus Reference and Research since 1982, and by the Brazilian Ministry of Health as Reference Center for Brazil since 1989.

Thereafter, it became clear that the techniques used since the creation of the Laboratory (in 1954), perfectly suited for virological research and strain conservation, were not adapted for easy information stockage, retrieval and diffusion. The cardboard filing and paper archiving system did not allow an easy actualization of the list and description of a rapidly evolving strain collection. In order to computerize these data, a cheap, simple and powerful system was selected, called DELTA (for DEscription Language for TAxonomy), distributed freely by Dr. M.J. Dallwitz (CSIRO Div. of Entomology, G.P.O. Box 1700, Canberra, A.C.T. 2601 Australia).

The set of programs, recorded on two 360 Kb discs, together with a short but clear and complete documentation, was operationalized in two days on an IBM - compatible microcomputer. An ASCII file gets all basic informations about the characters (62 in the present case) and their respective states, each one being coded. Another file using the latter data gets the descriptions of the viral types ("items"). The format of the descriptions is very simple: the code number of each character is followed by a comma and then by the code of its state in the item. Commands are then available to check the correctness of the data, to reorder the characters, to print lists, identification keys, or natural-language descriptions, to do online identifications, & s. o.

Contrary to other software, the updating of the data is straightforward and the system is open to improvements. As a first objective, it is planned to enter the descriptions of all arbovirus' prototypes (at least 163) in the Reference Center.

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[1] Summary of a work presented as a poster demonstration under the title "DELTA: um sistema informático simples,versatil e potente para manejamento de dados sobre amostras virológicas" during the "I Encontro Regional Sul de Virologia - Virológica 89", Florianópolis SC, 09 - 13 October, 1989.

A NEW METHOD TO CHARACTERIZE SEQUENCE VARIATION AMONG ISOLATES OF RNA VIRUSES: THE DETECTION OF MISMATCHED CYTOSINE AND THYMINE IN HETERODUPLEXES BY CHEMICAL CLEAVAGE AT SINGLE BASE PAIR MISMATCHES

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We are developing a chemical cleavage method of general application to the study of variation in the nucleotide sequences of RNA viruses. The technique enables the detection of single base changes. Provided appropriate cDNA probes are available, significant regions of a genome can be specific targets for analysis. In some instances, such as epidemiological surveys, a "fingerprint" of difference for a region using a probe of one sense only may be sufficient. Alternatively, more detailed information can be obtained using DNA probes of both senses against positive and negative strand RNA.

In our initial experiments, an end-labelled cDNA probe of known sequence (negative sense) was prepared from the PUO-218 isolate of dengue virus type 2 (DEN-2), and used to form RNA.DNA heteroduplexes with viral RNA of two other DEN-2 isolates, namely New Guinea C and D80-100 (Cotton and Wright, 1989). The viral RNA was prepared from purified virions, or was a total RNA preparation from infected cells. The end-labelled probe was specifically cleaved at mismatched cytosine and thymine bases following treatment with hydroxylamine and osmium tetroxide respectively (Cotton et al., 1988). The points of cleavage in the end-labelled DNA probe were located by electrophoresis of the heteroduplex samples through denaturing gels in parallel with standard Maxam and Gilbert reactions of the same probe. The probe used in this study contained the coding region for the carboxy terminus of prM and the amino terminus of E. The mismatches detected in the heteroduplexes with New Guinea C virus were consistent with the published sequence for that virus. The nucleotide sequence of D80-100 has not been determined, but the results indicated a close similarity to PUO-218 in this region of the genome.

Some potential uses of the chemical cleavage at mismatch (CCM) method are in epidemiological surveys and diagnosis, ascertaining genetic variability in selected genomic regions and identifying new isolates. Because the method can locate single base changes, it can be used to map genetic changes in isolates with altered phenotypes in tropism, pathogenicity, and resistance to neutralizing monoclonal antibodies. It can also be used to map cross-over points in recombinant viruses.

Cotton, R. G. H., Rodrigues, N. R. and Campbell, R. D. (1988) Proc. Natl. Acad. Sci. USA 85, 4397-4401.

Cotton, R. G. H., and Wright, P. J. (1989) J. Virol. Methods. 26, 67-76.

Protein synthesis in **L-A9** and **Aedes albopictus** cells infected with Mayaro virus in hypertonic medium.

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The modification of intracellular concentration of Na^+ and K^+ was found in cells infected with a number of viruses. Curiously the mRNA specified by a number of DNA and RNA viruses are efficiently translated under altered ionic conditions wich block cellular protein synthesis. Exposure of infected cells to hypertonic medium provides a useful procedure to study early events in the infectious cycle by permitting the efficient traslation of virus-specific polypeptides.

We found that elevation of the NaCl concentration in the growth medium of **L-A9** and **A. albopictus** cells caused an inhibition of the protein synthesis. **Aedes albpictus** cells (clone C6/36) and **L-A9** cells (mouse fibroblast) were infected or mock infected with Mayaro virus (10 PFU/cell) and maintained in growth medium during 24 hours. After this period the cells were treated with isotonic medium (116mm NaCl) or hypertonic medium (220mM NaCl) for 1 hour and further labelled with ^{35}S methionine (10 uCi/ml) for 1 hour. Cellular extracts were subjected to polyacrylamide gel electrophoresis and autoradiography. Under hypertonic conditions cellular protein synthesis was selectively suppressed and an enhancement of virus proteins was observed. In **Aedes albopictus** cells infected with Mayaro cells no inhibition of host protein synthesis was observed, however the cells respond to changes in intracellular lever of Na^+ and K^+ ions (in hypertonic medium) wich stimulated the synthesis of virus proteins.

(Marcia Christina T. Vasconcelos; Izabel Christina P.P. Frugulhetti and Moacyr A. Rebello)

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June 1, 1990

Charles H. Calisher, Ph.D.
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Dear Charlie,

The Recombinant DNA Laboratory (RDL) is a recent addition (four years) to the USDA-Agricultural Research Service's Arthropod-borne Animal Diseases Research Laboratory (ABADRL). Our interest is in the use of modern technology to study arboviruses. Our present emphasis is on diagnosis and vaccine development for bluetongue (BT) and epizootic hemorrhagic disease (EHD) viruses. In these studies we have cloned the genes that encode VP2 and VP3 of EHDV serotype 1. The first clone is serotype-specific and the latter is serogroup reactive. The RDL has developed and maximized the sensitivity of an RNA/cDNA hybridization test. This test was applied to the detection of BT and EHD virus in infected Culicoides variipennis, the principal insect vector of these viruses in North America. The RDL has developed a one step polymerase chain reaction (PCR) test to detect all US serotypes of BT virus. Optimized sample preparation and serotype specificity is currently being investigated. The paper concerning the EHDV-cDNAs will be printed in the June issue of Amer. J. Vet. Res.

In addition, the RDL and Dr. J. Mecham's Molecular Biology Unit, is investigating the antigenicity of various viral peptides to aid design of subunit vaccines. Collaboratively, with Dr. J. Ellis (Department of Veterinary Sciences, University of Wyoming), the RDL is investigating the effect of host immune responses on BT susceptibility.

This brief report was intended to give your readers an indication of where the RDL is headed. I hope that we will be able to provide a few more details of our work in an upcoming issue.

Sincerely,

William C. Wilson

William C. Wilson, Ph.D.
Research Chemist

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

Arbovirus Surveillance in New Jersey, 1989

During the 1989 surveillance period from June into October, 2158 mosquito pools containing up to 100 mosquitoes each were tested for viruses in day old chicks. There were 103 mosquito pools positive for Eastern encephalitis (EE) and Highlands J (HJ) was isolated from 37.

Table 1 summarizes the collection area totals, species of mosquito and time of collection for the EE isolates. Activity began with late July collections and continued into October. All of the 103 isolates were from pools containing Culiseta melanura mosquitoes at 10 sites.

HJ mosquito activity is summarized in Table 2. The early August collections gave the first isolates with continued observation of HJ activity into late October. There were 37 isolates from Culiseta melanura at 7 sites.

EE isolates were also made from August into October in 10 horses in southern coastal counties. A single pheasant flock isolate was made in the same area in October.

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

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Table 1
1989
EE MOSQUITO POOL ISOLATES
FOR WEEK ENDING

AREA COLLECTED	MOSQUITO SPECIES	7/21	7/28	8/4	8/11	8/18	8/25	9/1	9/8	9/15	9/22	9/29	10/6	10/13	AREA TOTALS
Bass River	Cs. melanura		1	1	2	1	3	4		1	1				14
Centerton	Cs. melanura						2	3	1	9	6	3	2	1	27
Dennisville	Cs. melanura		1	3	2	2	2	13	1		2	1	1		28
Green Bank	Cs. melanura	2	2	2	1		1	1	2	1					12
Hammonton	Cs. melanura				1	1	1		1						4
Indian Mills	Cs. melanura						1	1	1						3
Ocean City	Cs. melanura		1		1	2	1	1	2	1					9
South Dennis	Cs. melanura						4								4
Tuckahoe	Cs. melanura							1							1
Woodbine	Cs. melanura						1								1
WEEKLY TOTALS		2	5	6	7	6	16	24	8	12	9	4	3	2	103

Table 2
1989
HJ MOSQUITO POOL ISOLATES
FOR WEEK ENDING

AREA COLLECTED	MOSQUITO SPECIES	8/4	8/11	8/18	8/25	9/1	9/8	9/15	9/22	9/29	10/6	10/13	10/20	10/27	AREA TOTALS
Bass River	Cs. melanura				1										1
Centerton	Cs. melanura					1	1							2	4
Dennisville	Cs. melanura	7	2	4	3	4	1								21
Green Bank	Cs. melanura				1		2	1			1				5
Indian Mills	Cs. melanura										1				1
Ocean City	Cs. melanura				1										1
South Dennis	Cs. melanura				3										3
Woodbine	Cs. melanura				1										1
WEEKLY TOTALS		7	2	4	10	5	4	1	0	0	2	0	0	2	37

ARBOVIRUS STUDIES IN LUANDA, POPULAR REPUBLIC OF ANGOLA

In cooperation with the Ministry of Health of the Popular Republic of Angola, we have made several attempts to isolate arboviruses from mosquitoes collected in the city of Luanda. From a pool of 21 *Aedes aegypti* females, we isolated a virus pathogenic for laboratory mice. The virus was later identified by personnel of the Yale Arbovirus Research Unit as a strain of chikungunya virus. This is the second strain of chikungunya virus isolated in Luanda, the first being isolated in 1971. Serological surveys have shown that 13% of humans in Luanda had antibody to chikungunya virus in 1971 but 20% had antibody to this virus in 1988. Chikungunya virus has been endemic in Luanda and neighboring areas since at least 1970.

(AR Filipe, J Dupret, Center for Zoonoses Research, National Institute of Health, 2965 Aguas de Moura, Portugal)

RISK EVALUATION FOR EASTERN EQUINE ENCEPHALITIS TRANSMISSION FOLLOWING A HURRICANE IN SOUTH CAROLINA, 1989. Medical Entomology-Ecology Branch, Division of Vector-Borne Infectious Diseases, CDC, Fort Collins, Colorado, USA.

On September 28, 1989, hurricane Hugo struck the coast of South Carolina, causing widespread damage due to winds and flooding. At the request of the South Carolina Department of Health and Environmental Control and the Federal Emergency Management Agency, CDC's Division of Vector-Borne Infectious Diseases collected mosquitoes and conducted active surveillance for eastern equine encephalitis (EEE) in the area affected by the hurricane, and in several surrounding counties.

CO_2-baited CDC light traps operated at 67 sites in 13 counties (Fig. 1) collected an estimated 199,000 mosquitoes in 164 trap nights (1,211/trap night). Subsamples of up to about 1,600 specimens from each site were identified to species, pooled, and inoculated into Vero cell cultures for virus isolation. Table 1 summarizes results of the survey. A total of 76,990 specimens were processed in 1,336 pools. One isolate of EEE was obtained from a pool of Culex salinarius collected in Williamsburg Co. Another 6 isolates of viruses other than EEE were isolated from Aedes spp. collected in Charleston Co., and 1 non-EEE isolate came from a pool of Ae. atlanticus/tormentor from Florence Co. These latter viruses all appear similar, and 2 have been shown to be California serogroup viruses. Further identification is pending.

(D. Beard, E. Campos, M. Crabtree, B. Cropp, D. Eliason, B. Francy, J. Freier, C. Happ, N. Karabatsos, L. Kirk, R. McLean, B. Miller, C. Mitchell, C. Moore, J. Piesman, P. Schneider, R. Shriner, G. Smith, T. Tsai, and G. Wiggett)

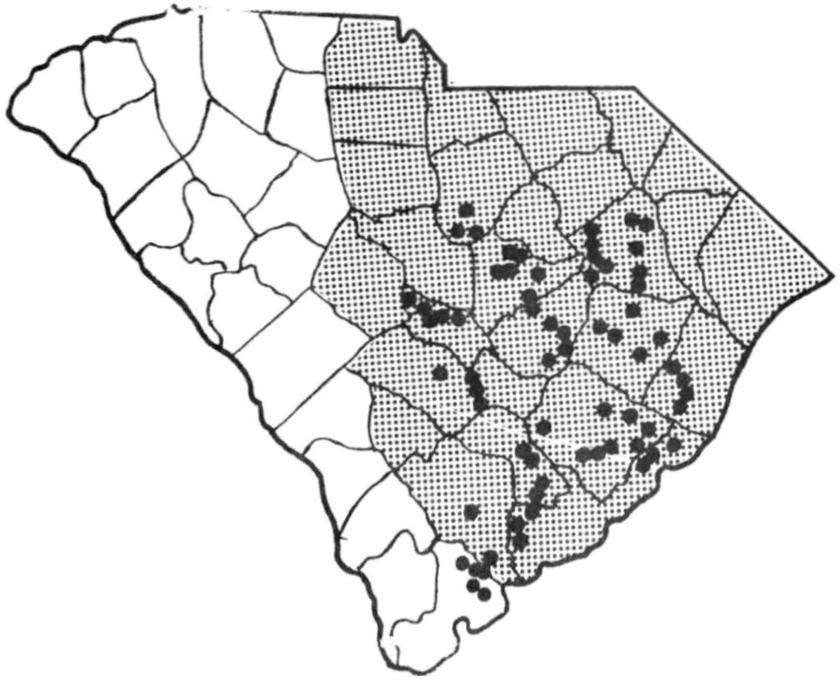

Table 1. Mosquito collections and virus isolations from South Carolina counties following hurricane Hugo, October, 1989.

Species	Total pools	Total mosquitoes	Viruses isolated	
			EEE	Unidentified
Aedes spp.	297	26,594		6
aegypti	2	5		
atlanticus/tormentor	41	2,791		1
canadensis	34	879		
fulvus pallens	7	90		
infirmatus	4	178		
sollicitans	11	45		
taeniorhynchus	17	417		
triseriatus/hendersoni	2	2		
vexans	98	6,201		
Coquilettidia perturbans	18	97		
Culex spp.	114	7,502		
nigripalpus	20	1,000		
p. quinquefasciatus	18	350		
restuans	13	78		
salinarius	169	13,670	1	
territans	3	7		
Culiseta inornata	1	1		
melanura	55	1,114		
Psorophora spp.	18	478		
ciliata	82	1,038		
columbiae	58	1,732		
cyanescens	2	4		
ferox	213	12,257		
howardii	39	460		
Totals	1,336	76,990	1	7

Mosquito-Borne Encephalitis Virus Activity in California, 1989

This brief report updates the preliminary report given in the December, 1989, issue. The full report will be published in the Proceedings and Papers of the 58th Annual Conference of the California Mosquito and Vector Control Association, Inc. The surveillance program is a cooperative effort by local mosquito control agencies; the Arbovirus Research Program at the University of California, Berkeley; county and local health departments; the California Department of Food and Agriculture; the Viral and Rickettsial Disease Laboratory (VRDL), the Infectious Disease Branch, and the Environmental Management Branch of the California Department of Health Services; and physicians and veterinarians in California.

Surveillance for human and equine cases of encephalitis/meningitis yielded 29 laboratory-confirmed human cases of St. Louis encephalitis (SLE), but no proven human or equine cases of western equine encephalomyelitis (WEE). The initial case of SLE occurred in the Antelope Valley area of northern Los Angeles County on 8/11/89, an area not previously surveyed or known to be endemic for virus activity. The additional 28 cases of SLE occurred from 8/17/89 to 10/8/89 in Kings, Tulare, and Kern Counties which had not had much virus activity detected in the previous few years. One non-fatal encephalitis-like illness occurred in a horse in Kern County 9/16/89 and high, stationary SLE antibody titers in paired sera suggested, though did not prove, an etiologic association. SLE antibody has been found in 35 of 633 equines tested in the VRDL from 1971 through 1988, but rising antibody titers and serologic proof of SLE clinical disease in equines have been very rare.

There were 3,845 mosquito pools tested by the VRDL, 75% collected in Imperial, Los Angeles, Riverside, San Bernardino and Kern Counties. Pools were selected to include mostly the major transmitting species: Culex tarsalis 69%; Culex pipiens complex 24%; and Culex stigmatosoma, Aedes melanimon and miscellaneous others the rest. There were 133 viral isolates: 118 SLE, 13 WEE and 2 California serogroup (CE) viruses. All isolates of SLE and WEE were from Cx.tarsalis except 1 WEE and 15 SLE from Cx. quinquefasciatus and 2 SLE from Cx.stigmatosoma, and the 2 CE isolates were from Ae.melanimon. An additional 2,748 pools from southern California and Kern County were tested by the U.C. Berkeley Arbovirus Laboratory, yielding 8 isolates of SLE virus from Cx.tarsalis and 1 from Cx. quinquefasciatus, 2 WEE (Cx.tarsalis and Cx. erythrothorax), 4 Hart Park and 1 Turlock from Cx.tarsalis, and 6 not yet identified viruses from Cx.tarsalis.

Sentinel chicken flocks at 68 sites throughout endemic areas of the state were bled monthly and showed SLE seroconversions from June, 1989 through January, 1990 in 57 chickens from Southern California flocks, and from July through November in 210 chickens from the Southern San Joaquin Valley. SLE seroconversions in Kern, Tulare and Kings counties preceded or coincided with the occurrence of human cases in these counties. WEE seroconversions were found in 35 chickens in flocks from the Imperial Valley and Needles. Thus the surveillance program detected virus activity in 9 counties, and the 3 counties where significant numbers of SLE cases occurred had early evidence of virus activity, which helped define the region where special search for human cases should be focussed.

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California Department of Health Services

REPORT FROM THE STATE OF NEW YORK, DEPARTMENT OF HEALTH WADSWORTH CENTER FOR LABORATORIES & RESEARCH, ALBANY, NEW YORK

Surveillance for Arbovirus Activity: 1989

In 1989, a total of 3,859 samples consisting of 2,329 pools of 159,135 mosquitoes, 767 vertebrate tissues and 763 sera from non-human vertebrates was submitted to the Arbovirus Laboratory for testing. Adult female specimens, representing at least 22 species in 6 genera of mosquitoes, were collected between 5/31 and 9/14/89 from 11 counties throughout New York State; 5 mosquito pools comprising 463 specimens yielded Bunyaviruses. On Long Island, Jamestown Canyon (JC) virus, a member of the California serogroup, was isolated from 1 pool each of Aedes canadensis and Aedes cantator captured in Suffolk County. This virus was also obtained from Aedes canadensis and Aedes stimulans group mosquitoes taken in Oswego and Seneca Counties, located in upstate and western New York, respectively. In addition, a pool of 100 Coquilletidia perturbans collected in Oswego county yielded 1 isolation of CV virus. No isolations of EEE or Highlands J (HJ), a western encephalitis complex (WEC) virus, were obtained from mosquitoes. Of 767 blood and tissue specimens tested for Alphavirus, 3 were from equines, 118 from sentinel pheasants and 646 from 23 species of wild avians. No arboviruses were isolated from these samples.

Hemagglutination-inhibition (HI) tests for antibody to 3 Alphaviruses, EEE, WEE, and HJ, were completed on 763 vertebrate sera representing 3 equines, 117 pheasants, and 643 wild avians (22 species) from 2 counties. No seropositive results indicative of current infection were found in equines or pheasants. However 96 wild avians in 14 species, all from Oswego County, were seropositive for 1 or more Alphaviruses as follows: EEE (43), HJ (21), Western Complex (7) and Alphavirus (25). The 3 most prevalent avian species sampled in the area, ranked in descending order of abundance, were: Gray Catbird, Song Sparrow, and Veery. Serum specimens from these 3 species made up 68% of the total; they yielded 63% and 67% of the EEE and HJ seropositive reactors, respectively. It should be noted that in 1988 EEE seropositive prevalence rates and antibody titers were significantly higher in resident birds sampled during the first 3 weeks of September than in those bled during the previous 3 months, supporting the contention that an outbreak of EEE in wild birds was initiated towards the end of August that year. However, in 1989 the reverse occurred; 69% of all the seropositive specimens were from adult birds sampled between 6/12 and 6/27, suggesting that these reactions were due to persisting antibody from infections acquired the previous year.

(Margaret A. Grayson, Ph.D., Robert D. Boromisa, Ph.D. and Leo J. Grady, Ph.D.)

Report from the W.H.O. Collaborating Centre for Arbovirus Reference and Research, Institute of Virology, Bratislava, Czechoslovakia.

Hemagglutination-inhibiting antibodies to some arboviruses in sera of Vietnamese inhabitants.

Altogether, 665 human sera collected in Vietnamese Democratic Republic (VDR) were examined for the presence of hemagglutination-inhibiting (HI) antibodies against the following arboviruses: chikungunya (Togaviridae), dengue types 1, 2, 3, and 4 and Japanese encephalitis (Flaviviridae). Two localities were selected:

Locality I: Ho Chi Minh City with more than 3 million inhabitants, mostly Vietnamese nationality, living in 9 urban districts and 4 suburban districts. From these, 11 sampling points were selected for survey.

Locality II: Daklak area, represented by two villages in Ajumpa District, Province Gialai-Kontum, and by two villages in Krong Pach District, Province Buon Ma Thuot. This area is situated 200-600 m above sea level in the southwest highlands of Vietnam. It is inhabited mostly by the Giarai and Ede nationalities as well as by minorities Viet and Muong. In all, 383 sera were from people in Locality I and 282 from people in Locality II.

HI tests with sera from people in Locality I revealed that 52.2% had antibody to chikungunya (CHIK) virus (Table 1). Antibody titers ranged from 20 to 10,240. Tests for antibody to flaviviruses are presented in Table 2: nearly all had antibody to these viruses and cross-reactions were common. Antibody titers to dengue viruses were from 20 to 5120 (Table 3).

HI tests with sera from people in Locality II revealed that 24.2% had antibody to CHIK virus (Table 1). Tests for antibody to flaviviruses are presented in Table 2; many had antibody to these viruses and cross-reactions were common. Antibody titers to dengue viruses were from 20 to 5120 (Table 3).

As follows from the results we obtained, the proportion of positive reactions depended on the locality in which the sera were collected. Antibody to all viruses studies were most frequently detected in sera from Locality I.

(Grešíková, M., Sekeyová, M., Elečková, E.)

Table 1. HI antibodies to chikungunya virus in sera of inhabitants of VDR, Localities I and II.

Titer	Number of positive sera	
	Locality I	Locality II
10	0	0
20	3	1
40	8	1
80	17	1
160	10	10
320	29	13
640	45	12
1280	53	16
2560	27	8
5120	5	1
10240	3	0
TOTAL (%)	200 (52.2)	63 (22.3)

Table 2. HI antibodies to five flaviviruses in sera of inhabitants of VDR, Localities I and II.

Virus	Percent positive	
	Locality I	Locality II
dengue-1	97.3	77.3
dengue-2	89.2	61.4
dengue-3	96.3	73.4
dengue-4	96.6	74.2
Japanese enc.	96.3	89.6

Table 3. HI antibody titers to five flaviviruses in sera of inhabitants of VDR, Localities I and II.

	Number of sera									
	Locality I					Locality II				
Titer	DEN-1	DEN-2	DEN-3	DEN-4	JE	DEN-1	DEN-2	DEN-3	DEN-4	JE
10	0	0	0	0	0	0	0	0	0	0
20	5	5	6	2	7	15	22	17	16	6
40	14	21	21	14	13	28	35	34	30	16
80	35	51	62	44	35	58	47	55	51	37
160	91	101	104	96	73	36	33	44	51	53
320	106	109	116	106	76	44	12	34	24	54
640	80	43	40	74	79	14	9	5	17	52
1280	33	10	15	34	41	5	0	2	3	7
2560	5	2	5	0	10	1	0	0	1	8
5120	4	0	0	0	1	0	0	0	0	0
TOTAL	373	342	369	370	335	201	158	191	193	233

SEROLOGIC PREVALENCE OF ARBOVIRUSES IN WHITE-TAILED DEER IN SOUTH FLORIDA, 1984-1988.

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White-tailed deer (Odocoileus virginianus) were collected at numerous sites throughout south Florida during 1984-1988 to evaluate the health status of the deer population. A total of 227 serum samples were obtained from hunter-killed and trapped deer. The serum samples were tested by the plaque-reduction neutralization (Nt) test against 11 arboviruses. Serologic positive serum samples were found for 9 of the arboviruses tested. The highest prevalence of Nt antibody occurred for Tensaw virus (95 %) followed by the California group viruses (70 %), Everglades (EVE) virus (19 %), vesicular stomatitis-New Jersey virus (12 %), St. Louis encephalitis (SLE) virus (5 %), eastern equine encephalitis (EEE) virus (5 %), vesicular stomatitis-Indiana virus (3 %) and Highlands J virus (1 %). Of the 149 serum specimens positive for the California group viruses, 54% were identified by single reactor or highest titer (greater than two-fold difference in titer). Of those identified, 60% were seropositive against Keystone virus, 39% against Jamestown Canyon virus, and 1% against Trivittatus virus. A geographic pattern in the distribution of some of the viruses was evident. A north-south gradient in the prevalence of antibody against EVE, SLE and EEE viruses was observed with higher prevalences of EVE antibody in the south toward the Everglades National Park and higher prevalences of SLE and EEE antibody north of the Big Cypress National Preserve.

REPORT FROM THE VIRUS LABORATORY
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AN UUKUVIRUS FROM TUNISIA

Tunisia is a small north-African country located in the eastern part of the Maghreb. A number of serosurveys have been performed in man (Porterfield, 1983; Nabli et al, 1970; Tesh, 1976) and in animals (Chastel et al, 1977, 1983; Haddad, 1980) and have shown a number of arboviruses to be present. Here we report upon a virus not previously isolated from this country.

In October 1989, Professeur C. Vermeil, Nantes, collected ticks from rodent burrows and other biotopes in Tunisia. A total of 214 ticks (mainly Ornithodoros erraticus and O. normandi) was studied virologically. A strain of a virus (Brest Ar/T 2756) was isolated and reisolated in suckling mice (not in VERO cells) from a pool of 24 adults of Argas reflexus group recently fed on domestic pigeons inhabiting dwellings of the Faculty of Medicine, Tunis.

This virus is pathogenic for SM by both ic and ip routes, inducing paralysis and death by 8 days, p.i. It possesses a lipid envelope and it passes a 200 nm filter. No hemagglutinin was demonstrated but potent CF antigen was obtained by sucrose acetone extraction of infected SM brain.

When this virus was studied by CF test using a number of "group" or "type" immune fluids, weak antigenic relationships were demonstrated with two members of the Uukuniemi group: RML 105 355 and Brest Ar/T 260, both isolated from Ixodes uriae ticks, in Alaska and Brittany, respectively (Table). No serological relationship was found with Grand Arbaud virus (Argas reflexus, Camargue, France). Comparison with Ponteves virus, another uukuvirus from the same source, was not possible because no reference antibody nor antigen was available for this "lost" virus. Cross-microprecipitation tests confirmed the relationship of T 2756 to uukuviruses.

Using electron microscopy, virus particles with a mean diameter of 92 nm were found in both SM brain sections and negatively stained brain suspension. They exhibit an envelope and large spikes. This morphology is compatible with that described for other Bunyaviridae within the Uukuvirus genus. Thus, the inclusion of T 2756 in this genus is warranted.

T 2756, tentatively named "Tunis" virus, appears to be either a new Uukuvirus or a strain of Ponteves virus, but we have no means to confirm this.

Ticks of Argas reflexus group, i.e. Argas r. reflexus (Europe) and A. r. hermanni (North Africa) readily attack birds and mammals, including man (Hoogstraal and Kohls, 1960); Hoogstraal et al, 1979). In Tunisia, antibody to Uukuniemi virus (S 23) has been found in small mammals (Chastel et al, 1977). Thus "Tunis" virus (T 2756) may represent a potential pathogen for humans in this country, in particular children living in the vicinity of domestic pigeons or swallows, the principal hosts of these ticks.

C. CHASTEL, G. LE LAV, O. GRULET, F. LE GOFF, M. ODERMATT (Brest), A. BOUATTOUR, D. BACH-HAMBA (Tunis) and C. VERMEIL (Nantes).

TABLE: T 2756 identification using CF test and UUK antibodies*

Immune fluid	CF titers against T 2756	Homologous titers
Oceanside	<4	1 024
RML 105 355	16	256
Uukuniemi (S23)	<4	128
Grand Arbaud	<4	256
Manawa	<4	256
Zaliv Terpeniya	<4	256
Brest Ar/T 260	8	512
Sumakh	<4	256

* Ponteves antibody not available

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUT PASTEUR DE COTE D'IVOIRE
BP 490 - ABIDJAN 01

Submitted by D. J. Vincent

In a collaborative work with a team of ORSTOM[1] Medical Entomologists, 465 strains of arboviruses were isolated from 1973 to the end of 1989 by inoculation of new-born mice, C6/36 and Vero cells. ELISA derived (in case of mouse brain suspensions) and indirect IF techniques (in case of cell cultures) were used for strain identification. The identified strains were transmitted to the regional WHO Reference Center (Institut Pasteur, Dakar) to confirm our identification.

In three cases, mixed infections of mosquitoes could be detected (CHIK + WSL; CHIK + BOUBOUI; ORUNGO + YF). The isolates were distributed among 34 main serotypes[2]:

Flaviviruses: yellow Fever (120); dengue 1 (1); dengue 2 (39); Zika (71); Bagaza (20); Wesselsbron (21); West Nile (2); Bouboui (15), Spondweni (4); Uganda S (3).

Alphaviruses: Chikungunya (74); Semliki forest (2); Sindbis (1); Y 251 [Sindbis subtype] (3); Middelburg (2); Ndumu (2); Igbo-Ora (20).

Bunyaviridae: Shokwe (2); Simbu (2); Bwamba (2); Odrenisrou [new serotype] (1); Pongola (5); M'Poko (6); Tanga (1).

Bunyavirus-like, other groups and ungrouped: Tai [new serotype] (2); Dugbe (1); Bangui (1); Jos (1); Orungo (11); Oubangui (1); Nyando (2); Eretmapodites 147 (2); Nkolbisson (4); Oubi [new serotype] (1); Okola (1).

The situation during the last 17 years can be summarized and commented as follows:

Yellow Fever: Outbreaks in Ivory Coast (1982-85) and one outbreak in Burkina-Faso (1983) yielded 120 strains from humans and from vectors. In 1987, an epidemic in Mali resulted in the isolation of 9 strains. From that time, no YF strain was isolated in our laboratory from Ivory Coast or from neighbouring countries.

Dengue 2: Principally isolated in 1980. Despite the number of strains from vectors, only one human case was recorded. A non-human transmission cycle is possible.

Zika: A peak in isolations of Zika was noted in 1984. The other flaviviruses were more or less regularly distributed during the period considered.

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[1] Office de la Recherche Scientifique et Technique Outre-Mer.

[2] Numbers in parentheses correspond to the number of strains.

Chikungunya : From 1973 to 1987, only 4 strains were isolated in suckling mice, but from the time we had the facilities to inoculate cultured cells in parallel, many CHIK were isolated from mosquitoes, often only in cell cultures, which resulted in 70 strains during the last 3 years.

Many of the isolates react serologically in a one-way pattern with the reference strain (WHO Reference Laboratory comments). A strain of CHIK was isolated from a pool of Aedes furcifer males. Transovarian transmission of CHIK can be considered. Despite the number of strains isolated in suckling mice and/or in cell cultures, the virus does not seem for the present time to have a great importance in human pathology here.

Igbo-Ora: Serologically related to CHIK, this virus is antigenically more distant from CHIK than the viruses mentioned above. For the first time an epidemic due to Igbo-Ora was reported and documented in Ivory Coast (1984 - 1985, in the vicinity of Yamoussoukro)[3]. The causal agent was isolated from patients and vectors. The main symptoms were, sequentially: fever, painful arthralgias lasting 4 - 5 days, and rash at the 3rd day of evolution lasting for approximately 3 - 4 days. All symptoms disappeared after one week without sequelae. Children under ten were more frequently affected than adults, while clinical signs were more severe in the latter. At the beginning of this year, an epidemic clinically similar to the one of 1984 - 1985 was detected approximately 100 km south of Yamoussoukro. Only one strain was isolated from 20 blood samples and none from mosquitoes. This virus was easily isolated in C6/36 and Vero cell cultures. Weakly pathogenic for newborn mice even after passage, it is under more precise identification at the WHO Regional Reference Center; serological investigations will be attempted.

Of more than 200 human sera collected in rural areas, antibodies to Rift Valley fever virus (or other phleboviruses) were detected at low titer (160) in one serum (region of Man: western part of the country). No antibodies to Congo-CHF or Hantaan were demonstrated in the same sera and till now no cases of hemorrhagic fevers have been reported in Ivory Coast (apart from YF), though strains of Zinga and one strain of Congo-CHF were isolated at the WHO Reference Center from human sera of a neighbouring country BURKINA-FASO (previously named Upper Volta).

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[3] Bull. Soc. Path. 81 1988.000.000

CALIFORNIA AND BUNYAMWERA SEROGROUP VIRUS STUDIES IN CALIFORNIA

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Serological studies. Tests of paired blood samples from cattle in northeastern California drawn before and after moving cattle from low elevation ranges to high elevation ranges in Modoc County showed high rates of seroconversion to Jamestown Canyon (JC) virus.

Virus studies. Eight partially identified or unidentified Bunyamwera serogroup viruses isolated from mosquitoes collected in California and Oregon between 1969 and 1985 were studied by cross-neutralization tests and subjected to taxonomic analysis. Two viruses isolated from *Culiseta inornata* collected in 1969 in Umatilla County, Oregon, may represent a novel virus serotype in the Bunyamwera serocomplex of the Bunyamwera serogroup. Four viruses isolated from *Anopheles freeborni* and a strain from *Aedes sierrensis* collected in 1970-1971 in Butte County in the northern central Valley of California are closely related to the prototype strain of Northway virus. The isolation from *Ae. sierrensis* is the first isolation of a virus from this species.

Mosquito studies. More than 31,000 mosquitoes in 4 genera were collected from high elevation (>1000 m) areas of California and tested for virus by plaque assay in Vero cells. Mosquitoes in the *Aedes communis* group, including *Ae. communis*, *Aedes cataphylla*, and *Aedes hexodontus* made up more than 80% of the collections. A total of 6 viruses were isolated, all from snow pool *Aedes* collected in Alpine County, California at approximately 2,300 m elevation. One virus was isolated from a pool of *Ae. cataphylla* collected as larvae, which is evidence of vertical transmission. Four viruses were isolated from *Ae. communis*, and one virus was isolated from *Ae. hexodontus*, all from mosquitoes collected as adult females. Cross-neutralization tests showed all 6 viral strains to related very closely to both the Jerry Slough and prototype strains of JC virus. These isolations represent the first viral isolates from boreal *Aedes* mosquitoes in California, and the first isolates of JC virus from *Ae. cataphylla* and *Ae. hexodontus* anywhere.

More than 16,000 mosquitoes in 393 pools were collected from low elevation (<1,000 m) areas of California and Oregon and assayed for virus. Samples were from 13 California and 1 Oregon county, and included 8 species in 4 genera. Five strains of a California (CAL) serogroup virus closely related to California encephalitis (CE) virus were isolated from *Ae. squamiger* collected as larvae and pupae at sea

level in coastal salt marsh habitat at Morro Bay, San Luis Obispo County, California. The collection site specific minimum infection ratio for these isolates was 4.8 per 1,000. *Ae. squamiger* is considered to be an evolutionary descendant of snow pool *Aedes* mosquitoes, and this association and the possibility that viruses in the CAL serogroup may have co-evolved with their mosquito hosts formed the basis for our sampling of this mosquito species. These isolates represent the first viruses known to be associated with *Ae. squamiger*. The public health significance of the isolates remains to be determined, but this species of mosquito is an important human pest in coastal urban California, and studies to establish the public health significance should be done.

Vector competence studies in mosquitoes. Studies were begun to determine the vector competence (horizontal and vertical transmission) of several snow pool *Aedes* species for JC and CE virus. In the summer of 1989 several large collections of adult female *Ae. communis* were made in Alpine County, California and shipped to the Berkeley laboratory. The females were permitted to feed on a JC virus-blood mixture, then tested for various factors related to transmission (transmission to cell culture, infected salivary glands, infected midguts, infected body remnants). Virus was isolated from Vero cells in 41% and 29% of females tested 14 and 21 days after the infectious blood meal, respectively. In another series of experiments, female mosquitoes were infected by intrathoracic inoculation. Virus isolations were similar to those from pledget-fed females, but with a higher proportion of infected females as measured by the other parameters. Experiments on vector competence of *Ae. squamiger* and *Ae. increpitus* for CE and JC virus are currently underway. Preliminary results demonstrate marked differences in vector competence between these two species.

Population studies of mosquitoes. We have completed a preliminary study of the population genetics of mosquitoes in the *Aedes stimulans* group in the western United States, including a comprehensive study of *Ae. increpitus* from California, Oregon, and Utah. Based on these studies, we have concluded that the *Ae. stimulans* group is a natural (phylogenetic) grouping, and that *Ae. increpitus* is the closest western North American relative of *Ae. squamiger*. These studies are related to our studies of the evolutionary relationships between CAL serogroup viruses and their mosquito hosts. Our studies of *Ae. increpitus*, based on starch gel electrophoresis, demonstrated very large genetic distances among western U.S. populations, especially between those from high Sierra habitats in south central California, and northern California or coastal California populations. These differences are consistent with those found between different species of mosquitoes. We have also found differences in larval morphology among these populations.

Vector ecology studies. We studied survival in an *Ae. communis* population in a high mountain forested site in Alpine County using the mark-release-recapture technique. With 3 separate releases of marked mosquitoes, we estimated (by regression analysis) survival rates of 0.90, 0.91, and 0.88 per day. These rates are very high and

indicative of a relatively long adult life span. Such a high daily survivorship rate also is consistent with a high degree of vector capacity.

Evolution studies. We have concluded an analysis of over 100 published records of isolations of CAL serogroup viruses from mosquitoes and other arthropods, followed by a comparison of a contemporary classification of mosquitoes of the genus *Aedes* with a contemporary classification of CAL serogroup viruses. Using criteria of 5 or more total isolations of a given virus from a given species of mosquito, and an MIR of 1:10,000 or greater, it was found that *Aedes* and *Culiseta* mosquito species were the arthropods most frequently involved as vectors. It was concluded that such a comparison could not explain all virus-vector relationships on the basis of coevolution of viruses in *Aedes* mosquitoes because of crossing over of viruses to other mosquito vector species through horizontal transmission mechanisms, and because of deficiencies in presently available classifications. Based on this study, we offered a hypothetical reconstruction of virus-mosquito relationships over time and possible routes of dispersal of CAL serogroup viruses to their present geographical distribution. This study also formed the theoretical backdrop to our studies of *Ae. squamiger*, a coastal California mosquito species from which we isolated 5 strains of CAL serogroup virus not yet identified (see above).

These studies were supported by NIH grant No. AI 26154.

AUSTRALIA'S NATIONAL BLUETONGUE STRATEGY

Animal health personnel and the livestock industries are currently concerned about the bluetongue situation in Australia. There was a flurry of activity for several years after the virus was first detected in 1977. However initial concerns diminished when early investigations indicated that the virus had been circulating for at least 20 years and was mostly confined to northern tropical areas where few sheep are farmed and experimental infections suggested that the three first virus types recovered (1, 20 and 21) were not very pathogenic.

There are several reasons for the current upsurge of interest in bluetongue. Eight serotypes (1,3,9,15,16,20,21 and 23) have now been isolated in northern Australia and some at least seem to be recent introductions. A vector (Culicoides wadai), first found in northern Australia in 1971 has yet to reach its southern distribution limits and transmission trials with several serotypes, using unadapted field inoculum, have caused severe disease in sheep. There is a realisation that bluetongue is still evolving in Australia, that further virus types and vectors may still enter northern Australia from Asia and that eventually, infection of commercial sheep flocks may occur.

To address the national concerns over bluetongue, an expert panel was appointed in 1988 and comprised representatives from Commonwealth, State, industry and research bodies. The panel recommended

1. That a comprehensive national stratgegy be implemented over the three years 1989/90 to 1991/92 that will

(a) minimise disruption to trade domestically and internationally, caused by bluetongue virus infections
(b) define and monitor bluetongue-free areas consistent with the international regionalisation concept for export purposes, and
(c) ensure a response capability at field level should outbreaks of clinical bluetongue disease occur.

2. That, to provide a scientific basis for this strategy

(a) existing investigations associated with surveillance, pathogenesis and recombinant vaccine development be maintained, and
(b) additional work be undertaken in the following four major areas:
   - attenuated vaccine development and evaluation,
   - insect vector studies
   - field epidemiological studies based on virological and serological surveillance
   - rapid virus identification, alternative bluetongue control techniques, effects on germ plasm, and bluetongue ecology.

The research strategy will be managed by a coordinating committee, comprising Graham Alexander, Toby St George and Geoff Gard. It is anticipated that substantial information on bluetongue, and other arboviruses, will be obtained in Australia during this 3 year period.

(Contributed by Geoff Gard, Bureau of Rural Resources, GPO Box 858, Canberra, 2600, Australia.)

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Diagnostic of arbovirus infections in humans

Active foci of Tahyna virus (TAH) are found in inundation forests in the Upper Rhine area. 5 virus strains had been isolated from mosquitoes (predominant species Aedes vexans) during September 1981 in an inundation forest near Germersheim. A total of 1,563 human sera had been collected between October 1985 and June 1986 from patients in 9 hospitals in the Upper Rhine area between Boppard in the north and Breisach in the south. TAH virus antibody titers were estimated by employing the indirect immunofluorescence assay and the plaque reduction neutralization test. TAH antibody prevalence was highest in Germersheim with 22.2 % and Ingelheim with 17.3 %, indicating that there are at least two natural foci in this area: the inundation forests "Insel Grün" and "Kühkopf" [2,3,4,5].

For the diagnosis of Dengue virus (DEN) infections the hemagglutination inhibition and the plaque reduction neutralization test were employed. We found DEN antibodies in 21 out of 23 (91 %) sera from natives in the Maldive Islands and 32 out of 55 (58 %) sera from tourists or foreigners working in this country. Antibodies against DEN virus types 1 and 2 were predominant. Although clinical symptoms typical for Dengue had been observed in tourists and foreigners, no case of Dengue hemorrhagic fever or Dengue shock syndrome was found in this group [6,7].

A German tourist who had visited a mangrove swamp at the coast of Sierra Leone (West Africa) for two days fell ill 16 days later with clinical symptoms like fever (38-39°C), severe pain in muscles and joints, and swelling of lymph glands. An antibody titer of 1 : 40 against Chikungunya virus (CHIK) could be demonstrated in his serum by hemagglutination inhibition test. A similar titer was found in the serum of one out of two companions who had visited the same swamp and fell ill during the same time.

CHIK antibodies were found in 3 out of 130 (2.3 %) sera from German tourists and in 6 out of 105 (5.7 %) sera from natives living in Uganda (East Africa). In 9 out of 105 (8.6 %) sera from Uganda also antibodies against Sindbis virus (SIN) could be demonstrated, whereas all 130 tourist sera were SIN negative. The human sera from Uganda had been kindly supplied to us by Dr. Kalunda, Uganda Virus Research Institute at Entebbe [1].

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(J. PILASKI, H. MACKENSTEIN, A. GREWE, H. NIEHAUS, V. ULLNER, and R. JANSEN-ROSSECK)

Report from Department of Veterinary Public Health
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Epidemiological studies of Japanese encephalitis virus in swine in Hokkaido, Japan.

Epidemiological studies of Japanese encephalitis (JE) were conducted in swine population in Hokkaido which is located at northern limit of JE virus distribution in Japan. Main aim of the studies was to reveal the overwintering mechanism of JE virus at northern template zone.

During 1984 to 1986, JE epidemic was observed among swine population in Hokkaido. Three virus strains were isolated from aborted fetuses of swine at different localities. Monoclonal antibodies analysis of these isolates showed that Hokkaido strains had unique antigenic characteristics distinct from other strains isolated in southern part of Japan.

Interestingly, one of the outbreaks of JE abortion in swine was recorded during winter time. Abortion started on February 15th and ended on May 1st. The JE virus was isolated from one of the aborted fetuses which were delivered on March 6th.

Antibody survey was conducted on the pig farms located near Sapporo city during 1984 to 1986 period. The results showed that antibody-positive farms were always located at the same areas among many negative farms.

Collectively, the results suggested that indigenous virus overwintered locally at distinct foci and that JE virus were not introduced every years from southern part of Eastasia where JE activities were recorded during whole year period.

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(Reported by I. Takashima)

SEROLOGICAL COMPARISON OF HANTAVIRUS ISOLATES USING PLAQUE REDUCTION NEUTRALIZATION TESTING

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Following the development of virus isolation and identification techniques and the distribution of diagnostic reagents, *Hantavirus* isolates in great number have been made from around the world. Many laboratories have reported viruses exhibiting antigenic differences based upon a variety of different serological techniques and batteries of polyclonal and monoclonal immune reagents. In order to initiate comparative serological comparisons of some of these viruses, a number of *Hantavirus* isolates were obtained from the WHO Collaborating Centre for Hemorrhagic Fever with Renal Syndrome (HFRS) in Seoul Korea and added to a collection of viruses maintained at USAMRIID. Viruses were propagated in Vero (E6) cells and examined for contaminating bacteria, mycoplasma and/or extraneous viruses. Stock suspensions of each of these virus isolates were used in plaque reduction neutralization tests employing the Vero (E6) cell line and plaque doses of 50-100 plaque forming units of each of the virus isolates. Convalescent antisera were obtained from both laboratory rats and New Zealand white rabbits which were infected by a single intramuscular injection of cell culture seed virus preparations of each of the isolates. Antiserum dilutions were mixed with the appropriate virus dose and incubated overnight at 4° C together with guinea pig complement prior to the plaque assay.

Plaque reduction neutralization data are presented in the accompanying two tables for rabbit serum only. Virus isolates closely related to Hantaan 76-118 prototype virus are shown in the first table together with cross-reactions to representatives of other virus serogroups. The second table emphasizes the relationships of Seoul or rat associated viruses. Results have been duplicated on each of the separate tables so that all neutralization cross-reactions can be observed. These data obtained using rabbit antisera do not differ appreciably from comparable testing of convalescent rat antiserum pools except that the Puumala and Prospect Hill related virus antisera exhibit higher neutralizing antibody titers in the rabbit.

Based upon comparative plaque reduction neutralization testing of a collection of different Hantavirus isolates, eight different patterns of serological cross reactions were evident. Virus isolates in either of the two largest groups, Hantaan and Seoul, were difficult to differentiate using this method.

HANTAVIRUS PLAQUE REDUCTION NEUTRALIZATION TEST

VIRUSES

ANTISERUM	HTN	Lee	HoJo	Jin 502	Jin 494	Fojnica	Greek	Chen	A-9	Maagi	CG	SriLan	Thai	Seoul	ProsH.	Puuma	Leaky	Thotta
Hantaan 76-118	2560	640	640	640	640	2560	2560	640	640	2560	2560	2560	40	10	10	10	2560	10
Lee	640	10240	640	2560	2560	640	640	160	640	2560	2560	2560	40	10	10	10	2560	-
HoJo	2560	2560	640	640	2560	2560	2560	2560	2560	2560	640	10240	40	10	10	10	2560	-
Jinhae 502	2560	2560	640	2560	640	2560	2560	640	160	2560	640	2560	40	40	10	40	640	-
Jinhae 494	160	640	160	2560	640	640	640	160	160	2560	640	2560	40	10	-	10	640	-
Fojnica	640	640	160	160	640	2560	640	640	2560	2560	640	2560	10	10	10	10	2560	-
Porogia	640	640	640	2560	640	640	2560	640	40	2560	2560	2560	40	10	10	10	2560	-
Chen	640	640	160	640	640	160	640	160	160	640	640	2560	10	160	40	40	640	-
A-9	640	640	640	640	640	640	640	640	160	640	640	640	40	10	-	10	640	-
Maagi	40	40	10	40	160	160	40	10	10	640	160	160	-	-	-	-	40	-
CG 3883	160	160	40	160	160	160	160	10	10	160	160	160	-	-	-	-	160	-
Sri Lanka	640	160	640	640	160	640	640	160	160	640	640	2560	160	640	-	10	640	-
Thailand 605	640	640	160	640	640	640	640	160	40	640	640	2560	2560	640	40	40	640	10
Seoul	10	40	40	160	10	40	160	40	10	40	40	40	160	2560	-	10	40	-
Prospect Hill	640	640	640	640	640	2560	640	640	160	640	640	2560	40	10	2560	40	640	-
Puumala	10	40	-	-	10	10	40	-	40	40	10	40	10	-	40	2560	40	-
Leaky	160	160	40	160	160	640	640	160	10	640	160	640	10	10	10	10	160	-
Thottapalayam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40

HANTAVIRUS PLAQUE REDUCTION NEUTRALIZATION TESTS

VIRUSES

ANTISERUM	HTN	Sri Lan	Singa	H K	Th 605	Th 749	Seoul	Brazil	Balt	SR-11	Houston	KI262	R-22
Hantaan 76-118	2560	2560	2560	640	40	40	10	10	10	10	40	10	10
Sri Lanka	640	2560	2560	640	160	160	640	640	640	640	640	640	640
Singapore	40	640	640	640	40	160	640	640	640	640	640	2560	2560
Hong Kong	40	640	160	640	-	-	-	-	-	-	-	-	-
Thailand 605	640	2560	2560	2560	2560	2560	640	640	640	640	160	160	2560
Thailand 749	160	640	2560	2560	2560	10240	640	640	640	160	160	40	160
Seoul	10	40	160	160	160	160	2560	2560	2560	640	2560	2560	2560
Brazil	10	40	10	40	160	160	640	640	160	640	640	160	640
Baltimore Rat	10	10	40	640	160	160	640	640	10240	640	10240	10240	10240
SR-11	10	40	40	40	160	640	2560	2560	2560	2560	10240	2560	2560
Houston	40	160	40	640	160	160	2560	2560	2560	640	2560	2560	2560
KI 262	10	10	40	40	40	40	640	640	2560	640	640	2560	2560
R-22	10	160	10	-	640	640	2560	2560	2560	2560	10240	2560	2560
Girard Point	10	40	160	40	640	160	2560	2560	10240	2560	10240	2560	2560
Egypt	-	160	10	-	160	640	2560	2560	2560	2560	640	2560	2560
B-1	160	40	640	640	160	640	2560	2560	2560	2560	640	2560	2560
Tchopitoulas	40	160	40	160	160	160	2560	2560	2560	2560	2560	2560	2560
Hubei	160	640	640	640	40	160	2560	2560	640	2560	640	640	2560
Prospect Hill	640	2560	2560	2560	40	40	10	10	10	10	10	-	10
Wisconsin	-	-	-	-	-	-	-	-	-	10	-	-	-
Puumala	10	40	40	160	10	-	-	-	-	-	-	-	-
USSR 18-20	-	-	-	-	-	-	-	-	-	-	-	-	-
83-223	10	40	40	40	10	40	10	10	10	10	10	-	10
Leaky	160	640	2560	640	10	10	10	10	-	40	-	-	-
Thottapalayam	-	-	-	-	-	-	-	-	-	-	-	-	-

Girard	Egypt	B-1	Tchop	Hubei	PHV	Wiscon	Puuma	USSR	83-223	Leaky	Thotta
10	-	40	10	40	10	-	10	-	10	2560	10
640	160	160	40	640	-	-	10	-	10	640	-
640	640	640	40	640	10	10	10	10	10	160	-
-	-	-	-	-	-	-	-	-	-	160	-
640	40	160	640	2560	40	10	40	10	40	640	10
160	40	640	160	640	40	10	40	10	10	160	10
2560	2560	2560	160	2560	-	-	10	-	10	40	
640	640	640	640	640	-	-	-	-	10	-	-
2560	2560	2560	160	2560	-	10	10	-	10	40	-
10240	2560	2560	640	2560	-	-	10	-	10	10	-
2560	640	2560	160	2560	10	10	10	-	40	10	-
640	2560	2560	2560	2560	-	-	-	-	-	10	-
2560	2560	2560	2560	2560	-	10	40	10	10	40	-
10240	2560	2560	2560	10240	-	-	10	-	-	10	-
10240	2560	640	2560	2560	-	-	10	10	10	-	-
10240	2560	2560	2560	2560	-	-	-	-	10	40	-
10240	2560	2560	2560	2560	10	-	10	-	10	40	-
640	2560	2560	2560	2560	-	-	10	10	-	160	-
10	10	160	10	40	2560	2560	10	160	160	640	-
-	-	-	-	-	160	640	10	10	40	-	-
-	-	-	-	-	40	160	2560	2560	640	40	-
-	-	-	-	-	-	-	40	10	10	-	-
10	10	10	10	10	40	160	2560	2560	640	40	-
-	-	-	10	10	10	-	10	10	40	160	-
-	-	-	-	-	-	-	-	-	-	-	40

Report from Virus Research Institute
Department of Medical Sciences, Ministry of Public Health,
Bangkok, Thailand.

Studies on Antibody to Haemorrhagic Fever with Renal Syndrome (HFRS)

The study was conducted in 1989 to determine the antibody to HFRS both in man and rat. Human sera were collected from Bangkok Metropolis, northern and northeastern part of Thailand. Rat sera were collected from Bangkok Metropolis and northern part of Thailand. The results are as follow:-

Table 1 Prevalence of antibody to HFRS related B-1 virus and Hantaan virus among patients and healthy persons in Thailand, 1985, 1988.

Type of human sera	No examined	No of positive (%)	
		B-1 virus	Hantaan virus
I Patient group			
Jaundice or Hepatitis	70	3 (4.3%)	0
Renal failure	10	0	0
Fever of unknown origin	10	0	0
sub-total	90	3 (3.3%)	0
II Healthy group from			
Nongkhai	12	1 (8.3%)	0
Nonkorn Ratchasima	45	0	0
Phitsanulok	27	0	0
sub-total	84	1 (1.2%)	0
Total	174	4 (2.3%)	0

From Table 1, the result showed that 4 sera from 174 human sera were positive for antibody to HFRS related B-1 virus but none to Hantaan virus. Among the 4 positive sera, one was from the healthy person, and three were from jaundice or hepatitis patients and none from the renal failure or the fever with unknown origin. For the history of these three patients, they were admitted to the hospitals with suspected hepatitis. One was confirmed by laboratory test as hepatitis A but the other two without any antigen or antibody to hepatitis virus. However, these three patients had positive antibody to HFRS. Among these three patients, one patient with hepatitis A virus infection who lived in Nongkhai, had antibody titer of 1:80 and the other two with non hepatitis virus infection, who lived in Bangkok, had antibody titer of 1:80 and 1:20. From the healthy group, only one out of 84 sera from Nongkhai was positive antibody against HFRS related B-1 virus.

Table 2 Prevalence of antibody to HFRS related B-1 virus and Hantaan virus among rats trapped in Bangkok and Northern Thailand, 1988.

Rat	Province	No examined	No of positive (%)		
			B-1 virus	Hantaan virus	Total
Rattus norvegicus	Bangkok	26	1 (3.8%)	0	1 (3.8%)
Rattus exulans	Bangkok	50	1 (2.0%)	4 (8.0%)	5 (10.0%)
	Tak	29	1 (3.5%)	4 (13.8%)	5 (17.2%)
Rattus rattus	Chiengmai	7	0	0	0
Suncus Murinus	Bangkok	13	0	0	0
Total		125	3 (2.4%)	8 (5.6%)	11 (8.8%)

From Table 2, 11 out of 125 rat sera were positive antibody to HFRS related B-1 virus and Hantaan virus. Its positive rate was 8.8% which were 2.4% and 5.6% of positive rate against HFRS related B-1 virus and Hantaan virus, respectively. The titers of antibody to HFRS in these infected rats were low, ranging from 1:20 to 1:80 and having 1:80 in only one rat. For the species of rat, Rattus norvegicus from Bangkok and Rattus exulans from Bangkok and Tak were found antibody. The positive rate was 12.6% in R. exulans which was higher than that (3.8%) in R. norvegicus. Moreover, R. exulans was the only one species that had the prevalence of antibody (10.1%) against Hantaan virus. However, these two species of rat are house rats which widely distributed in urban area.

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Virus Research Institute,
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PERSISTENCE AND SUPERINFECTION RESISTANCE OF LOUPING ILL VIRUS IN PORCINE KIDNEY (PS) CELL LINES

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The ability to infect tissue culture cell lines persistently is a well known characteristic of many flaviviruses. We have found that Louping ill (LI) virus, a member of the family Flaviviridae, readily establishes persistent infections in PS (porcine kidney) cell lines. PS cell monolayers infected with LI virus initially show moderate cytopathic changes but these soon disappear and the monolayers recover to become healthy and persistently infected with LI virus. The infected cells can be subcultured indefinitely. We have studied the persistently infected PS cells by immunofluorescence tests using monoclonal antibodies with defined antigenic and molecular specificities for LI virus. By indirect immunofluorescence using polyclonal antiserum against LI virus at least 80 per cent of the persistently infected cells showed virus specific fluorescence but the quantity of antigen in each cell appeared smaller than that seen when LI virus produced a cytolytic infection.

Normal uninfected (control) PS cells and PI cells were compared for their capacity to support plaque production by LI virus. Only the control PS cells produced plaques.

Subsequently, the susceptibility of PI cells to become superinfected with either mosquito or tick-borne flaviviruses was tested and the fate of each of these superinfecting viruses was monitored using MAb specific for the different viruses. The PI cell cultures were grown on coverslips and superinfected with the viruses shown in Table 1. Normal uninfected PS control cells were also infected at the same time.

Table 1. Comparison of susceptibility of LI persistently infected cells to be superinfected by other flaviviruses.

Superinfecting virus		Control PS cells*	LI-PI cells
1.	CEE	+	-
2.	NEG	+	-
3.	POW	+	-
4.	LGT	+	-
5.	WN	+	-
6.	JE	+	-
7.	YF FNV	+	+

*Indirect immunofluorescence tests were performed on each sample using MAb known to react with the appropriate virus under test.

The superinfected PI cells were then tested for virus-specific fluorescence using a MAb (3.8) that did not react with LI virus but did react with the other viruses used. All the viruses actively infected the normal control PS cells but in the PI cells, with the exception of YF FNV, superinfection with all flaviviruses was

blocked. YF FNV produced a progressively developing infection which eventually killed the PI cells.

The course of infection of YF FNV on the PI cells was further investigated using a time course immunofluorescence test employing a double labelling technique. YF FNV antigen was detected using a specific MAb conjugated with FITC while LI antigen was detected by indirect immunofluorescence using a LI specific MAb and a Texas red label. The LI specific (red) fluorescence was present in nearly 80 per cent of the cells throughout the period of study, whereas the percentage of YF FNV infected cells (with green fluorescence) increased steadily. At 24 hours post infection, a few isolated cells showed YF FNV-specific fluorescence and by six days post-infection almost 100 per cent of the PI cells contained YF FNV antigen. Thus, YF FNV replicated in cells which were infected with LI virus.

As yet, there is no definitive explanation for the exclusive existence of LI virus in the United Kingdom while other closely related tick-borne viruses are present in other parts of western and far eastern Europe. This work is still at an early stage, but it represents a potentially useful model of virus persistence to study superinfection and interference amongst flaviviruses.

REPORT FROM THE MAX-VON-PETTENKOFER-INSTITUT FOR HYGIENE AND MEDICAL MICROBIOLOGY, UNIVERSITY OF MUNICH, FEDERAL REPUBLIC OF GERMANY

CULTIVATION OF *EYACH VIRUS* IN CELL CULTURE

EYACH virus was isolated in 1972 from Ixodes (Ix.) ricinus ticks in South-West Germany and again in 1981 from Ix. ricinus and Ix. ventalloi in Mayenne, France.

EYACH virus is of ecological, epidemiological and molecular interest since it is the only known virus outside North America related to Colorado tick fever virus. Studies at the moment are hampered by the difficult cultivation of the isolated virus strains with titers not high enough for studies with the virus.

We succeeded in adapting a strain of EYACH virus to Vero cells with titers reaching 10^5, so that we can isolate the virus genome, purify the dsRNA and separate it in polyacrylamid gel electrophoresis (PAGE).

EYACH virus (37th suckling mouse brain passage) was a kind gift of Dr. Jan Kopecky, Department of Natural Focality of Diseases, Institute of Parasitology, Cechoslovak Academy of Sciences. Virus was passaged one time in 2-day-old suckling mice and 20% mouse brain suspension of these mice was inoculted in Vero, BHK21, Hela, and MA104 permanent cell lines.

Supernatants were passaged every six days in new cell culture tubes and were tested for typical dsRNA segments in PAGE. Little c.p.e. was seen in the 3rd passage of Vero cells 5 days p.i. In the supernatant of the fourth passage of Vero cells 12 dsRNA segments could be detected in silver stained polyacrylamid gels. No c.p.e. could be detected in one of the other cell lines inoculated, nor could dsRNA be detected in supernatants of other cell lines.

Titers were determined daily by inoculation of ten fold dilutions of supernatants 1-6 days p.i. in Vero cells and by plaque assay of same supernatants in Vero cells. Maximum of titers was reached 3 days p.i. exhibiting $10^{5,4}$ infectious particles, and $10^{5,1}$ pfu per ml respectively. On day 6 p.i., when c.p.e. was evident virus titer was $10^{3,1}$.

It is known from other orbiviruses that 90% of released virus is bound to cell membranes. Therefore we also tested daily titers 1-6 days p.i. after 3 freezing and thawing cycles, but no infectious virus could be found after this procedure.

The adaption of a strain of EYACH virus to Vero cells enormously will facilitate working with this virus. Molecular biologic studies will become possible, as well as reassortment studies and virus evolution studies with its North-American relative Colorado tick fever virus. Hopefully also new diagnostic tests now can be established to examine the pathogenic potential of EYACH virus for humans as mentioned in a Czechoslovak study.

Gerhard G. Dobler, [illegible]

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

Antibody response following indigenous mouse brain JE vaccine in West Bengal.

Since the first epidemic outbreak of Japanese encephalitis (JE) in some of the districts of West Bengal in 1973, the disease has established itself not only in West Bengal but in a number of states of India. Owing to its high morbidity and case fatality, JE has posed a major threat to the already existing health problem in India. Among the possible preventive measures, mosquito control and vaccination of the people, specially the susceptible age group, seem to be a rational approach. Although various anti-mosquito measures are being adopted in the JE affected areas, control of JE with only this operation seems to be quite difficult, considering the requirements of huge quantities of insecticides for coverage of vast JE affected areas along with equipments and staff and continued monitoring of the whole operation. In addition to anti-mosquito measures, vaccination of the susceptible population in the JE affected areas seems to be a practical approach. With this view, the Govt. of India has started manufacturing of formalin inactivated mouse brain JE vaccine at the Central Research Institute, Kasauli, Himachal Pradesh, with the Nakayama-NIH strain of JE virus from Japan. The present communication deals with the field study carried out in 1988 with the above vaccine at Memari Block II of Burdwan district, West Bengal.

Materials & Methods:

Being one of the recognized JE endemic areas of Burdwan district, Memari Block II, with a population of 1,24,419, has been selected for the field study of JE vaccine made at CRI. During the months of May & June, 1988, (inter epidemic period), 24,699 persons belonging to 5-25 years age group were inoculated with 2 doses of vaccine, at an interval of 7-14 days followed by another booster dose after 1 month of the 2nd dose. Each individual received 1 ml. of the vaccine subcutaneously.

A total of 1203 representative pre-vaccinated and 436 post-vaccinated blood samples could be collected from the vaccinees. To ascertain the antibody response, haemagglutination inhibition (HAI) tests were carried out according to the methods of Clarke & Casals, using microtitre techniques. The results of 336 paired (pre and post vaccinated) serum samples have been presented here. Sixty four post vaccinated (24 HAI positive and 40 HAI negative) samples were subjected to neutralization (N) test in infant mice

using intraperitoneal route. Serum samples showing HAI antibodies at a dilution of 1 in 20 or more and N antibody at a dilution of 1 in 10 or more, were considered positive. Positive vaccine effect was considered with the post vaccinated serum samples showing HAI antibody response (conversion or rise) and or detection of N antibody.

Results:

Testing of 336 prevaccinated sera revealed already existing HAI antibody in 184 samples (54.7%). Following 3 doses of JE vaccination significant rise of antibody titre was detected in 155 (84.2%) and conversion in 92 out of 152 seronegatives (60.5%), Table I. Total HAI response e.g. rise along with seroconversion was found in 73.5% of the vaccinees (247 out of 336).

Results of neutralization test (Table II) with 64 post vaccinated serum samples revealed N antibody in 80% of HAI negatives (32 out of 40) and 100% among 24 HAI positive samples. Thus, the total vaccine effect (considering both HAI and N antibodies) was found to be 87.5%, which is quite encouraging.

The present preliminary field study in Memari block II of Burdwan district, using indigenous mouse brain JE vaccine is considered quite encouraging, since N antibody response ranged between 75% to 100% among HAI negative and HAI positive subjects respectively.

No severe side effects like gross skin allergy or any neurological manifestations were reported in the present study. A large scale field study with this indigenous JE vaccine with wide coverage of both endemic & non-endemic areas is being contemplated, which may provide more informations enabling to adopt correct strategy in this respect.

Table - I

Post vaccination JE antibody (HAI) response

Age group (in years)	Pre-Vac. AB status		Post vac AB response		Total HAI vaccine effect
	Sero Negatives	Sero Positives	Conversion	Rise	
5 - 10	24	24	16/24	16/24	32/48(66.6*)
11- 15	52	64	40/52	52/64	92/116(79.3)
16- 20	40	48	16/40	44/48	60/88(88.1)
21- 25	36	48	20/36	43/48	63/84(75)
T o t a l	152	184	92/152 (60.5)	155/184 (84.2%)	247/336 (73.5)

* Figures in parenthesis denotes %

Table - II

Results of neutralization (N) test:
A profile of 64 post vaccinated sera

HAI antibody status	Number	N - antibody
Positive	24	24 (100%)
Negative	40	32 (80%)
T o t a l	64	56 (87.5%)

(M.S. Chakraborty, S.K. Chakravarti, K.K. Mukherjee, M.K. Mukherjee, P.N. De, S. Chatterjee & B. Mukherjee)

Report from Virus Research Institute,
Department of Medical Sciences, Ministry of Public Health,
Bangkok, Thailand.

Preliminary Study on Vaccinations of JE Simultaneously with EPI Vaccines in Infants

A total of 172 children in the well baby clinic of the Children's Hospital during April to December 1989 was conducted to determine the possibility of immunizing JE vaccine with the routine vaccinations in infants (EPI). These children were devided into 4 groups: Group I : first dose of JE vaccine was given simultaneously with second dose of DPT and OPV at 4th month, followed by second JE dose at 6th month (along with third DPT and OPV); Group II : JE vaccine at 6th month (together with third DPT and OPV) followed by second JE at 9th month (plus measle vaccine); Group III : JE vaccine at 6th month (with third DPT) and second dose of JE one week later; Group IV : Control group was received only DPT and measles vaccines. JE neutralizing antibodies were determined one month after the last JE dose. Results revealed that almost 100% of seronegative children in all experimental groups showed seroconversions. Only mild side-effects were observed; 2 out of 42 children in group I had low grade fever with a few rash which lasted only a few days. No JE antibodies were detected in the control group at the end of one-month observation period (Table 1).

Our results so far demonstrated that JE could be administered simultaneously with EPI in infants by stimulating antibody responses without severe side-effects. However, antibody determination at 1 year after vaccination should be carried on.

Table 1 Distribution of children with neutralizing antibody titres after 2 doses of JE vaccination with EPI, 1989.

Children age		No Tested	No with antibody ≥1:10	Reciprocol N-antibody titre (%)			
I dose	II dose			≥10	≥40	≥160	≥640
4 m.	6 m.	42	41	1(2.4)	9(22.0)	21(51.2)	10(24.4)
6 m.	9 m.	23	23	0	1(4.3)	13(56.6)	13(56.6)
6 m.	6 m.+1 wk.	11	11	11(9.0)	8(72.8)	0	0
4 m.	6 m. JE unvac.	20	0	0	0	0	0
6 m.	9 m. JE unvac.	11	1	1(100.0)	0	0	0

Suntharee Rojanasuphot
Virus Research Institute,
Bangkok, Thailand.

THE FIRST RECOGNIZED HUMAN LYME DISEASE IN PORTUGAL

A clinical case of Lyme disease in Portugal was identified late in 1989 in the south (Alentejo) of the country. We believe this is the first human infection with *Borrelia burgdorferi* identified in Portugal. We analyzed certain aspects of the ecology and epidemiology of this case. We concluded that in the Alentejo region the vector of *Borrelia burgdorferi* is certainly not the same species of ixodid ticks as has been recognized in other countries in Europe.

(Armindo R. Filipe, Center for Zoonoses Research, National Institute of Health, 2965 Aguas de Moura, Portugal)

ECOLOGICAL RESEARCH ON LYME BORRELIOSIS IN SWEDEN

In 1987 we started to investigate the ecology of Lyme disease in Sweden. This work has proceeded in collaboration with other Swedish scientists, i.a., Sven Bergström (Umeå University), and Anders Hovmark and Eva Åsbrink (Southern Hospital/Karolinska Institute, Stockholm). We have studied (i) the availability of *Ixodes ricinus* and the prevalence of this tick infected with *Borrelia burgdorferi* in relation to geographic locality, time of the year and developmental stage of the tick (ii) if small rodents serve as hosts for *B. burgdorferi* in Sweden, and (iii) the geographic distribution and host relations in Sweden of all tick species occurring here.

The most abundant species, *Ixodes ricinus*, were thought to be restricted to the central and southern parts of Sweden. We have shown that *Borrelia*-infected *I. ricinus* are present also along the coastal areas of northern Sweden (Jaenson, Bergström, Burman et al. Läkartidningen **86**:2584).

Spirochete-infected *I. ricinus* have been demonstrated, by phase-contrast microscopy, in nearly all localities of southern and central Sweden where large samples of nymphs or adults have been investigated.

During 1988-1989 the mean percentages of spirochete-infected nymphal ticks was significantly ($P < 0.02$) greater (12%, N=718) at Torö (coastal locality), south of Stockholm, than at an inland locality near Uppsala (7%, N=314) north of Stockholm. Out of more than 300 larvae dissected none has been found to be infected.

B. burgdorferi was cultivated from *I. ricinus* nymphs and small rodents (*Apodemus flavicollis* and *Clethrionomys glareolus*) (Hovmark, Jaenson, Åsbrink et al. APMIS **96**:917-920)

These studies suggest that in central Sweden small rodents are important reservoirs, while *I. ricinus*, particularly the nymphs, are the main vector of *B. burgdorferi*. Data on the seasonal host feeding activity pattern of the *I. ricinus* nymphs suggest that the risk for humans to acquire Lyme borreliosis in central Sweden is greatest during April-June and August-October.

Thomas G.T. Jaenson & Hans A. Mejlon
Section of Entomology, Department of Zoology, Uppsala University, Box 561, S-751 22 Uppsala, Sweden.

The Medical Entomology-Ecology Branch of the Division of Vector-borne Infectious Diseases, CDC, has modified the ear punch biopsy procedure for the detection of Borrelia burgdorferi in rodents originally described by Sinsky & Piesman 1989 (Journal of Clinical Microbiology 27:1723-27). In order to diminish problems with contamination, wedges of skin taken from the rodent's ear are rinsed in 70% ethanol and then soaked for 15 min in 10% chlorox or wescodyne (3 oz per 5 gal distilled water) solution. The skin is then rinsed again in 70% alcohol, cut into small pieces and placed in 4 ml of modified BSK in snap cap tubes (12X75 mm roundbottom). We have been successful in recovering spirochetes from freshly collected specimens as well as those frozen at -70C. Those interested in submitting specimens from field collected rodents should contact Dr. Joseph Piesman, (303)221-6408, or Mrs. Christine Happ 221-6470.

Another high priority effort of the DVBID is to detect Lyme disease spirochetes in pools of ticks submitted from areas where the tick infection rate is low. We are currently developing a polymerase chain reaction test that will be sensitive and specific for the detection of B. burgdorferi in ticks. While this test is still in the development phase, we have high hopes for the future.

Submitted by: Dr. Joseph Piesman, Lyme Disease Vector Section Chief, Medical Entomology-Ecology Branch, Division of Vector-borne Infectious Diseases, Center for Infectious Diseases, Centers for Disease Control, P.O. Box 2087, Ft. Collins, CO 80522.

The AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

April 19, 1990

EXECUTIVE COUNCIL

Dear Colleagues:

An outstanding slate of candidates has again been nominated to fill the vacancies to be created when two Executive Council members rotate off at the end of this year's annual meeting. Francisco Pinheiro and I will be rotating off, and Drs. Gary Clark (CDC, Puerto Rico), Joel Dalrymple (USAMRIID, Fort Detrick), Tom Ksiazek (USAMRIID, Fort Detrick), and Rebeca Rico-Hesse (Yale University) have agreed to stand for election. Please vote for <u>two</u> candidates and return your ballot to me at the address listed below by 1 August 1990. Results will be announced at the annual meeting.

Let me take this opportunity to also mention several other issues of some importance. First, the Richard M. Taylor Award will be given at the 1990 annual meeting. Formal announcement appeared in the April issue of Tropical Medicine News. The nominating committee is chaired by Dr. Tommy Aitken, with Karl Johnson and Bob Shope as committee members. The Taylor Award has evolved as the most prestigious recognition by ACAV, recognizing lifetime achievement in the field. Please take the time to nominate deserving persons. Nominations should be sent to Dr. Thomas Aitken, Department of Epidemiology and Public Health, Yale Arbovirus Research Unit, Yale University School of Medicine, Box 3333, 60 College Street, New Haven, Connecticut 06510, as soon as possible.

Additional ACAV neckties are in and available for purchase. The price is $15 for one, $25 for two, $35 for three and $10 each for four or more. Colors are blue and burgundy as in the previous set, and two additional colors which are really quite nice, brown and green. Each has the distinctive ACAV pattern in red and white. Please pay by check to "ACAV general fund". Ties are available from me at the address listed below, or from Charlie Calisher at CDC Fort Collins. This is the only "fund raiser" activity of the ACAV, and we have a lot of money invested in these ties. We have yet to reach the break even point, so please participate. Besides, they're nice ties, and great gifts! Buy 4 or more and save!

I have asked all ACAV subcommittee chairmen to consider new members to their subcommittees in an effort to insure that all interested persons are given the opportunity to participate in this important aspect of our group. If you are interested in serving on one or more of the subcommittees, please notify the appropriate subcommittee chairman, or me. Enclosed is a list of each subcommittee, with the names and addresses of the chairmen.

The International Congress of Virology will be held in Berlin, 26-31 August 1990, and the ACAV will hold a business and scientific session in conjunction with the Congress. We are fortunate to have a meeting room provided for us, and it has been reserved for Sunday, 26 August 1990, 12:00 to 13:45. Please plan to attend. I am just now organizing the session, and if you would like to formally present, please let me know as soon as possible, preferably by FAX at 301-663-2492. First priority will be given to the International Advisors; however, I will make every effort to insure that anyone wishing to present will have an opportunity.

For the past few years we have discussed, both at the annual open meeting and in several executive council sessions, creation of formal guidelines for the ACAV. As a result, a revised draft guidelines was prepared and circulated among members of the executive council, and minor modifications were suggested. The council is now in general agreement with the draft which is enclosed. Please take this opportunity to review the enclosed draft guidelines, and return any comments to me prior to the annual meeting. I anticipate a brief discussion of the guidelines during the annual meeting, followed by a show of hands vote. Please come prepared to vote on this important issue.

Dr. Tom Scott was the primary author of the guidelines, and we all owe him a vote of thanks for his efforts.

Dr. Tom Yuill is in the process of organizing the ACAV scientific session for this year's annual meeting. The topic will be "Emerging Viruses", and it promises to be an exceptional session. Please plan to attend.

Sincerely,

James W. LeDuc
Chairman
ACAV Executive Council

Enclosures

GUIDELINES FOR THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

15 November 1989

During the 1987 executive council meeting of the American Committee on Arthropod-borne Viruses (ACAV) in Los Angeles it was agreed that guidelines for the committee need to be established and recorded in a single document. The ACAV currently operates under procedures that are stated in various reports. A summary statement of the ACAV organization would assist new executive council members and chairpersons in understanding how the committee operates. In addition, modifications in procedures could easily be incorporated into a centralized body of information.

The information that follows is intended only as a guide for the ACAV organization; goals of the ACAV are stated in other publications. During the formation of ACAV it was agreed that the group would not be a formal organization with by-laws. Rather, it would provide a forum for exchange of information among people interested in arbovirus research.

The ACAV meets annually in association with the American Society of Tropical Medicine and Hygiene (ASTMH). An executive council is the governing body of the ACAV. The council is chaired by a person who directs council and committee meetings and retains the ACAV files. The chairperson of the executive council serves a 1-year renewable term and is elected by the executive council at the annual council meeting. The council consists of six people who each serve a 4-year term. New council members are elected by members of the ACAV. Council members who are rotating off form a search committee that nominates council candidates; the slate of candidates must be approved by the existing council. The chairperson of the council sends ballots to the ACAV membership. The candidates who receive the most votes are notified and take office at the next annual meeting of the ACAV. There is no quorum for the election of officers or for other council and committee votes. Should a member of the executive council resign or leave the ACAV, a new council member is appointed by the executive council to complete the original member's term.

Other ACAV positions include a secretary, treasurer, historian, and international advisers. The secretary is the newest member of the council and records minutes of the council meeting. The treasurer is appointed to a 4-year renewable term by the executive council and maintains ACAV funds in a subaccount of the ASTMH. Withdrawals and deposits from the ACAV account are arranged with the treasurer. The council chairperson must approve all withdrawals, and the council must be consulted for withdrawals of $100 or more. The historian is designated by the council and provides historical information on the operation of

the ACAV. The council selects international advisers, determines the number of advisers, and defines the length of adviser's terms.

All people interested in joining the ACAV may do so by attending a meeting and signing their name to the attendance sheet. Membership in the ASTMH is not a requirement for membership in the ACAV. People missing three consecutive meetings will lose their membership in the ACAV. Exceptions to this attendance rule are made for people with emeritus status and for some overseas participants. Emeritus status is automatic upon retirement. The executive council must approve an attendance exemption for people living overseas.

Subcommittees are an important part of the ACAV and are formed when sufficient interest is presented to the executive council by ACAV members. The council reviews proposed subcommittees and decides whether a particular subcommittee is needed. When council approves the formation of a subcommittee, it appoints a chairperson. The subcommittee chairperson, in consultation with the ACAV chairperson, appoints subcommittee members. New subcommittees formalize their objectives and structure. All subcommittees report progress on objectives to the annual meeting of the ACAV.

The ACAV presents three awards, when appropriate, in staggered three-year intervals. The chair of the executive council appoints a nominating committee for each award; recipients of awards are selected by the executive council. An announcement requesting nomination for each pending award is published in the Tropical Medicine and Hygiene News at least six months prior to the ASTMH annual meeting. This procedure provides the nominating committee with nominations and documentation from ACAV members. The William F. Scherer Award is given to an outstanding graduate student either before graduation or up to 3 years post-graduation. Accrued interest from the account for this award is used to defray costs associated with the student's participation at the annual ASTMH meeting. The council chairperson may use general ACAV funds to assist in this effort. The Nathaniel Young Award is given to a young investigator, under the age of 45, who has made an outstanding contribution to arbovirology. The Richard M. Taylor Award is given to a person who has made outstanding contributions to arbovirology throughout his or her career. Medals for the Young and Taylor Awards are retained by the treasurer until they are requested by the chairperson for presentation at the annual committee meeting.

In addition to award presentations, the agenda for each annual ACAV meeting includes old and new business, epidemic reports, and a symposium on an arbovirus-related topic. Prospective symposium topics are discussed by the executive council and solicited from the committee membership, but the final decision on what will be presented is determined by the chairperson.

ACAV SUBCOMMITTEES

Subcommittee for the Collection of Low-Passage Arbovirus Strains (SCLAS)

Chairman: R. B. Tesh
YARU, Yale University
School of Medicine
P.O. Box 3333
60 College Street
New Haven, CT 06510-8034

Subcommittee on Arbovirus Laboratory Safety (SALS)

Chairman: J. M. Dalrymple
Chief, Department of Viral Biology
Virology Division, Bldg 1425
USAMRIID, Ft. Detrick
Frederick, MD 21701-5011

Subcommittee on Evaluation of Arthropod-Borne Status (SEAS)

Chairman: M. J. Turell
Department of Arboviral
Entomology
Virology Division, Bldg 1425
USAMRIID, Ft. Detrick
Frederick, MD 21701-5011

Subcommittee on Veterinary Arbovirology (SVA)

Chairman: T. M. Yuill
University of Wisconsin
School of Veterinary Medicine
2015 Linden Drive
Room 2170D
Madison, Wisconsin 53706

Subcommittee on Interrelationships Among Catalogued Arboviruses (SIRACA)

Chairman: R. E. Shope
YARU, Yale University School
of Medicine
Box 3333
60 College Street
New Haven, CT 06510

Subcommittee on Information Exchange (SIE)

Chairman: N. Karabatsos
DVBID/CID/CDC
P.O. Box 2087
Ft. Collins, CO 80522-2087

BY POPULAR DEMAND, AN ENTIRE PAGE OF IRRELEVANT QUOTES

A. Bartlett Giamatti: "Genteel in its American origins, proletarian in its development, egalitarian in its demands and appeal, effortless in its adaptation to nature, raucous, hard-nosed, and glamorous as a profession, expanding with the country like fingers unfolding from a fist, image of a lost past, evergreen reminder of America's best promises, baseball fitted and still fits America. It fits so well because it embodies the interplay of individual and group that we so love and because it conserves our longing for the rule of law while licensing our resentment of lawgivers."

Roseanne Barr (I think): "Beauty is only skin deep but ugly goes clear to the bone."

Tu Fu: "Each single petal swirling down
Diminishes the spring."

Hans Zinsser: "Not everyone realizes that typhus has at least as just a reason to claim that it 'won the war' as any of the contending nations. Many a French barroom fight might have been avoided if this had been clearly understood."

Damon Runyon: "If they ever rematch them, the smart money will still be on Goliath."

Woody Allen: "I hope there is no such thing as reincarnation. I might have to see the Ice Capades again."

Frank Layden: "My father always wanted to be a garbage man. He thought they only worked on Tuesdays."

Frank Layden: "I don't jog. I want to be sick when I die."

George Bernard Shaw: "The power of accurate observation is commonly called cynicism by those who have not got it."

Sydney Smith: "In composing, as a general rule, run your pen through every other word you have written; you have no idea what vigor it will give your style."

David Russell: "The hardest thing to learn in life is which bridge to cross and which to burn."

Gabriel García Márquez (Chronicle of a Death Foretold): "Everything we know about his character has been learned from the brief, which several people helped me look for 20 years later in the Palace of Justice in Riohacha. There was no classification of files whatever, and more than a century of cases were piled up on the floor of the decrepit colonial building that had been Sir Francis Drake's headquarters for two days. The ground floor would be flooded by high tides and the unbound volumes floated about the deserted offices. I searched many times with the water up to my ankles in that lagoon of lost causes, and after five years rummaging around only chance let me rescue some 322 pages filched from the more than 500 that the brief must have contained."