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

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James L. Hardy, Ph.D. (1932-1997)

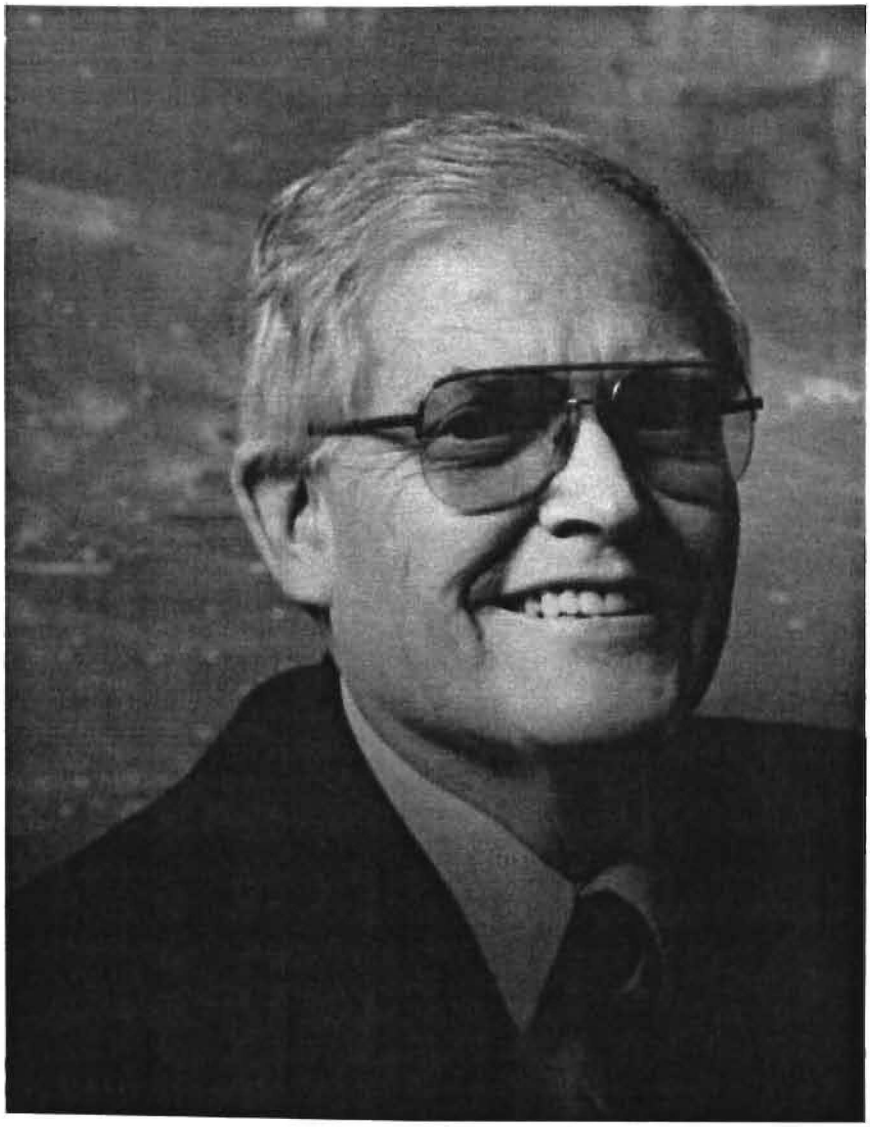
James L. Hardy died peacefully at home in Pleasant Hill, CA, February 15, 1997, after a long battle with cancer. He is survived by his wife, Shirley, and sons James and Jeffrey. Jim was born in Fort Benton, Montana, in 1932, graduated from the University of Montana with a Bachelor's degree in 1954 and a Master's in 1956. His first mentor was Dr. Carl Eklund, an early pioneer in research on encephalitis viruses, poliomyelitis and prions, at the Rocky Mountain Spotted Fever Laboratories, NIH, in Hamilton, Montana, under whose tutelage Jim refined and applied the hemagglutination inhibition test for the diagnosis of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses. Jim then studied Japanese B encephalitis (JE) virus under Drs. Edward L. Buesher and William F. Scherer at the 406th Medical General Laboratory in Tokyo, Japan, and continued with his studies on JE virus at the University of Minnesota under the latter of these two eminent arbovirologists. After he completed his PhD degree in 1962, Jim joined another pioneer in arbovirology, Dr. William C. Reeves, at the School of Public Health at the University of California at Berkeley, with whom he worked closely for the next 35 years.

Jim Hardy was reknowned for his dedication to and enthusiasm for teaching not only in the classroom, but also in the laboratory and the field. This led to his appointment as Assistant Professor of Medical Virology in 1966 and advancement to Professor and Chairman of the Department of Biomedical and Environmental Health Sciences in 1975. As Professor of Medical Virology and head of the Arbovirus Research Program at U. C. Berkeley, Jim influenced many young scientists. He trained a new generation of virologists who now actively work in academia, government, and industry throughout the world.

Jim Hardy had a highly disciplined mind which led to well planned controlled studies to find elusive answers to field questions. The research conducted in his laboratory focused on the interactions of environmental, virologic and entomologic factors that affect the epidemiology of arboviruses. The primary focus of his research was on the natural history of WEE and SLE viruses, although he studied at least 18 other arthropod-borne viruses of California. Research conducted by Jim Hardy and his associates in the Central Valley of CA has increased our understanding of the ecology of *Culex tarsalis*, the primary vector of WEE and SLE viruses. The data generated from these studies continue to provide the basis for the development of alternative and improved approaches to arboviral surveillance and the control of arboviral diseases. Jim's studies on the dynamics of infection and transmission of viruses in mosquitoes laid the foundation for future research on vector competence and vector capacity. His laboratory clearly demonstrated the existence of barriers to infection with and transmisison of virus. His studies on the influence of temperature on the susceptibility of different mosquito populations to viruses was ground-breaking research. In recognition of his research achievements, the American Society of Tropical Medicine and Hygiene awarded Jim Hardy the Bailey K. Ashford Award in 1977 as the outstanding scientist under 45 years of age. In 1990, he received the Richard Moreland Taylor Award from the American Committee on Arthropod-Borne Viruses (ACAV), for outstanding contributions to the field of arbovirology over a significant period of time.

Jim Hardy's research led to over 130 scientific papers of which 32 were published in the American Journal of Tropical Medicine and Hygiene. He served on the editorial board of four journals, including the aforementioned. In addition, Jim Hardy held many consultant appointments with governmental agencies, including the Research Resources Branch of NIH and two study sections of the Division of Research Grants and the National Cancer Institute. He was a program reviewer for the Center for Disease Control and an advisor for the Research Development Command of the U.S. Army. He served on the executive Council of ACAV, of which he was chairman from 1975-1978.

Jim Hardy's interests were not limited to science; he also loved to do fine leatherwork and collect artwork by Remington. But he remained engaged by science, writing and reading manuscripts until the end. He was a mentor and a friend to many of us through the years and enriched our lives. Jim's integrity, quiet sense of humor, intelligence and gentle nature will stay with us forever. We will miss him.



James L. Hardy

William F. Scherer - James L. Hardy Award

The James L. Hardy Award has just been established by the American Committee on Arthropod-Borne Viruses (ACAV) of the American Society of Tropical Medicine and Hygiene to acknowledge and encourage distinguished research by young scientists in the field of arbovirology. It will be combined with the William F. Scherer Award, originally established in 1983. Jim Hardy worked with Bill Scherer in Japan from 1956-1958, and was his doctoral student from 1959 to 1962. Both of these outstanding individuals devoted their lives to research in arbovirology, and to the training of students in the classroom, field and laboratory. The combined award will recognize outstanding graduate students in the final stages of their predoctoral research or initial stage of post-doctoral study in arbovirology. The recipient of the Award will be determined by a committee of three members of ACAV and will provide funds for the recipient to attend the annual meeting of the Society. The first student to receive this combined award will be selected in 1997.

Tax-deductible contributions to this fund are being solicited to make this award possible. Checks should be made out to "ACAV Treasury (Hardy fund)", and mailed to Dr. TomYuill, University of Wisconsin, Institute for Environmental Studies, 1007 WARF Bldg., 610 Walnut St., Madison, WI. 53706. Thank you.

PRESENTATION OF THE RICHARD MORELAND TAYLOR AWARD BY THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

Richard Moreland Taylor was a Founding Father of arbovirology. His pioneering work with the Rockefeller Foundation and his efforts in organizing the American Committee on Arthropod-borne Viruses inspired others to establish the Richard M. Taylor Award, of which Dr. Taylor was the first recipient. Other recipients have included Tommy Aitken, Jordi Casals, Ottis and Calista Causey, Roy Chamberlain, Wil Downs, Pedro Galindo, Bill Hammon, James Hardy, Harry Hoogstraal, Karl Johnson, Bill Reeves, Bob Shope, and Tel Work; quite a group. I now have the honor and pleasure of introducing the most recent winner of this Award, Dr. Thomas Monath.

Actually, the award was announced last year in San Antonio but neither Tom nor many of his admirers were in attendance because of the Government shut-down, so it was decided to present the award at this year's meeting.

Tom was born August 13, 1940 in Hewlett Harbor, NY. He was very well educated at Phillips Exeter Academy in New Hampshire and then at both Harvard College, Cambridge, Massachusetts, where he earned an A.B. degree cum laude, and at Harvard Medical School, Boston, also graduating cum laude. From 1966 to 1968 he was an Intern in Medicine and then an Assistant Resident at Peter Bent Brigham Hospital, Boston.

Having heard Telford Work's tales of arbovirus research in India and elsewhere and having read various papers by Bill Reeves and others, Tom put aside his interests in photography and herpetology and headed for the Centers for Disease Control in Atlanta, where he became the Medical Officer in the Arbovirology Unit. In 1969 he was involved in investigations of Venezuelan equine encephalitis in Ecuador, melding field work and clinical studies. His subsequent studies of this virus in horses and diagnostic techniques provided both insight and laboratory tools for further inquiries.

Assigned as a Medical Epidemiologist to the Federal Ministry of Health of Nigeria, Tom did field and laboratory work with yellow fever and yellow fever virus in the Virus Research Laboratory at the University of Ibadan. He collected rodents, did laboratory and clinical research on Lassa fever in Nigeria and Liberia, work that later was documented in the book and movie "Fever". West Africa is a marvelous place for a person who is intrigued by snakes.

Back in the U.S., Tom became Chief of the Arbovirology Section, CDC/Atlanta, succeeding Tel Work, Brian Henderson, and Roy Chamberlain. He took a year off to serve as a Research Fellow in the Department of Medicine at Harvard Medical School while the rest of us who were transferred to Fort Collins were unpacking, getting the labs organized, and learning how to fish while standing in cold water. He became the Director of the Division of Vector-borne Infectious Diseases, Fort Collins, which housed two W.H.O. Reference Centres

with world-wide responsibilities for arboviruses and plague. During the period 1974-1988 Tom directed work on the epidemiology of equine encephalitis in Argentina, yellow fever in Nigeria, and plague in the western U.S., initiated molecular studies of St. Louis encephalitis, dengue, and yellow fever viruses, designed immunoglobulin assays, applied ELISA to diagnosis of arboviral diseases, organized the development of monoclonal antibodies for research and diagnosis of arboviral infections, conceived and involved himself directly in studies of the pathogenic correlates of yellow fever in monkeys, and supervised field and laboratory studies of vesicular stomatitis, hepatitis, HIV, and many other diseases of tropical and temperate areas. His acuity, logical approach to epidemiologic and other problems, sharp wit, ability to think quickly, and easy going style contributed to his being able to wring the last drop of effort from a large and disparate group of scientists. His articulate and candid approach to problems and even his gullibility were hallmarks of his directorship. I sometimes heard people say they thought he was wrong about this or that but I never heard anyone say they were certain he was wrong. He worked hard, serving as a role model for the rest of us; and he played hard.

I can testify that Tom Monath is a solid friend. During a particularly trying time in my life, and in the lives of my children, Tom stood by with words of encouragement, uplifting ideas, or simply a smile. His charm and intellectual strengths, his abundant generosity, and his willingness to experience all sorts of discomforts to obtain data have made him many, many friends around the world and an ambassador for his employers, for ACAV, and for this Society.

In 1988 Tom left CDC to become a Colonel in the U.S. Army and Chief of the Division of Virology at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland. He conceived and directed programs on genetically engineered vaccines against arbovirus infections and hemorrhagic fever viruses, evaluation of antiviral drugs, relationships of arboviruses and their vectors, and viral pathogenesis.

In 1992 Tom left Ft. Detrick to try his competent hands in the world of commerce, accepting the position of Vice-President for Research and Medical Affairs for OraVax, Inc., in Cambridge, Massachusetts, a venture capital company. Now he is applying his considerable energies to developing vaccines useful in preventing respiratory syncytial virus infections and *Helicobacter pylori* infections of humans, in preventing infections of livestock with bluetongue viruses, and in preparing a *Clostridium difficile* vaccine. I expect that OraVax is thrilled to have made the good decision to have brought him aboard and I have no doubts of his ultimate success with these and other ventures.

Tom has authored well more than 200 scientific papers and book chapters, edited a classical series of books on arboviruses, served in innumerable positions within this Society, including as Program Chairman, and serves the W.H.O. in many capacities, as befits his expertise and practicality. He has organized, advised, and convened, sweet-talked, wheedled, cajoled, and generally charmed funds and support from all directions. He is a lucid and

innovative speaker, researching his subject to great depth. If you missed his Charles Franklin Craig Lecture on yellow fever at the annual meeting in New Orleans, you missed a tour de force. Fortunately, it was published in our journal.

Tom has made numerous and remarkable contributions to ACAV, including a stint as Chair of the Executive Council, to the American Society for Tropical Medicine and Hygiene, including a term as Program Chair, and to arbovirology, which he continues to support. For these and many more reasons, which the constraints of time do not permit me to detail, on behalf of ACAV I have the great personal and professional pleasure to present Thomas Patrick Charles Monath, M.D. the Richard Moreland Taylor Award. Congratulations, Tom, for this well-deserved recognition of your contributions.

(Presented [by Charles H. Calisher, Ph.D., Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Foothills Campus, Colorado State University, Fort Collins, CO 80523] at the American Committee on Arthropod-borne Viruses Open Meeting, Wednesday, December 4, 1996, Baltimore, Maryland)

PRESENTATION OF FIRST DALRYMPLE-YOUNG AWARD BY THE AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

Nathaniel Young was a scientist who had not yet reached his most productive years when he died tragically. At the time of his death he was applying his intelligence and broad skills to virology, pathology and infectious diseases. His father was a Scottish physician who brought his family to this country, eventually working as a TB specialist in Pennsylvania. Nat attended Swarthmore, spending six months of one academic year in Peru and immersing himself in the language and culture of that fascinating country. As an accomplished collegiate golfer he was matched in an NCAA tournament with Jack Nicklaus, which was enough to nudge him toward the study of medicine.

After medical school Nat enlisted in Karl Johnson's small army of brilliant physicians at the N.I.H.'s Middle America Research Unit in Panama. Having been convinced by Telford Work to save everything, Karl had a comprehensive collection of viruses of the Venezuelan equine encephalitis complex and he and Nat worked to understand the antigenic relations and epidemiologic differences of those viruses; the publication is a classic. Moving on to do the first molecular cloning of polioviruses, Nat became involved in other laboratory studies of viruses, of clinical and anatomical pathology, and of other areas necessary to the study of infectious diseases. He surely was headed for a professorship and more when he died. Patricia Webb, Karl Johnson, and others with whom Nat had worked and played, established the Nat Young Award, which was given by ACAV each three years, the recipient required to be <45 years old and to have already made significant contributions to arbovirology.

The equally tragic death of Joel Dalrymple in 1992, at age 53, continues to be one with which many of us have not yet come into equilibrium. Joel was born in Salt Lake City and received his education at the University of Utah. He spent his entire professional career in the Department of the Army, first as a Captain in the Medical Service Corps, then as a civilian, at Walter Reed Army Institute of Research. In 1980 he moved to the U.S. Army Medical Research Institute of Infectious Diseases at Fort Detrick. He generated monoclonal antibodies and applied them to studies of arboviruses, applied gene cloning techniques to the development of vaccines, began studies to express Hantaan, Rift Valley fever, and *Bacillus anthracis* antigens in vaccinia virus, demonstrated the efficacy of these antigens and traveled the world to set up human trials of the resulting vaccines. Joel became a consultant to W.H.O., C.D.C., N.I.H., universities, and other research institutions. His boundless energy and enthusiasm, his remarkable sense of humor, and his personal warmth was legendary. He was a brilliant and funny guy.

This year, with the agreement of the founders of the Nat Young Award, ACAV has established the Dalrymple-Young award to memorialize these two fine scientists and human beings. Each was fun-loving, articulate and creative, and it was anticipated that each would continue to make contributions to our field of interest. Because this is not now possible, this award will be given each third year to a scientist who has already made significant contributions to arbovirology. The Nat Young Award had been conferred only on people who were <45 years of age. However, ACAV has agreed that henceforth this should be a mid-career award to be given independent of age, and so it will be.

Whether strictly true or not, it is usual and proper for the person presenting an award to suggest that all the candidates were of high caliber. Don Burke, Jim LeDuc, Phil Russell and I can attest that this indeed was the case in this inaugural year for the Dalrymple-Young Award. Nonetheless, we have been able to select an awardee, Connie Sue Schmaljohn, nominated by Carol Blair, Head, Department of Microbiology, Colorado State University.

Connie did her undergraduate work at the University of Nebraska in Lincoln and earned a Ph.D. from Colorado State University, staying on for two additional years as a postdoctoral research associate. She moved to Fort Detrick in 1980 and has been there since, currently serving as Chief of the Department of Molecular Virology at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). She is a member of several professional societies, including the American Society for Tropical Medicine and Hygiene, and has been an active player in societal affairs, including the ACAV Executive Council. She serves on numerous societal, national, and international panels and committees, being particularly involved in studies of hantaviruses. Her many publications reflect varied interests but have always focussed on arboviruses and related viruses. Connie's association with Joel Dalrymple was a close one, both personally and professionally. She learned much from Joel but she is now teaching the rest of us much about the molecular biology and biochemistry of hantaviruses. She has published studies of the establishment of the genus *Hantavirus* within the family Bunyaviridae, of biochemical properties of hantaviruses, definition of nucleotide sequences of hantaviruses, delineation of hantavirus gene coding strategy, development of recombinant DNA technology, expression of antigenic subunits of Hantaan virus in baculovirus and vaccinia, identification of Hantaan virus messenger RNA, relationship between antigenic variation and sequences encoding envelope proteins of Hantaan virus, requirement for both G1 and G2 membrane glycoproteins for Hantaan virus targeting of the Golgi complex, epitope mapping of G1 and G2 glycoproteins using monoclonal antibodies, reflections of phylogenetic analyses of hantaviruses, evidence for natural reassortment of hantavirus RNA species, epidemiology of hantaviruses associated with Hantavirus Pulmonary Syndrome in the United States and there is much more to come.

On behalf of the American Committee on Arthropod-borne Viruses, it is with great regard and high honor that I present the first Dalrymple-Young Award to Connie Schmaljohn of USAMRIID.

(Presented [by Charles H. Calisher, Ph.D., Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Foothills Campus, Colorado State University, Fort Collins, CO 80523] at the American Committee on Arthropod-borne Viruses Open Meeting, Wednesday, December 4, 1996, Baltimore, Maryland)

ACAV Treasurer's Report, 1996

ACAV Treasury

	1996 YTD
Net Assets Beginning of Period	$ 10,770
Revenues and Gains:	
Additional Contributions	0
Interest	107
Dividend	109
Total Revenues and Gains	$ 216
Total Expenses	$ 0
Net Assets End of Period	$ 10,986

Scherer Fund

Net Assets Beginning of Period	$ 3,227
Revenues and Gains:	
Additional Contributions	0
Interest	35
Dividend	35
Total Revenues and Gains	$ 70
Total Expenses	0
Net Assets End of Period	$ 3,297

Young Fund

Net Assets Beginning of Period	$ 271
Revenues and Gains:	
Additional Contributions	0
Interest	3
Dividend	3
Total Revenues and Gains	$ 6
Total Expenses	0
Net Assets End of Period	$ 277

Respectfully submitted

Thomas M. Yuill, ACAV Treasurer

12 May 1997
Date

SIRACA Meeting 12/1/96
Baltimore, Maryland

At the Annual Meeting of the American Society for Tropical Medicine and Hygiene in Baltimore, the issue of classification of hantaviruses was discussed during the SIRACA subcommittee meeting (R.E. Shope, Chairman).

The following guidelines were established regarding the definition of virus species within the genus *Hantavirus* in the Family Bunyaviridae.

Species (viruses) listed are those with ecological, and genetic and/or antigenic characteristics that clearly differentiate them from all other known hantaviruses. Criteria for the classification include:

A. The virus has a distinct econiche.
B. The virus displays a fourfold or greater difference in cross-neutralization with all other hantaviruses in cell culture assays such as a plaque reduction neutralization test or a focus reduction neutralization assay. If NT is not available, other antigenic tests will be used.
C. Comparison of the complete M and S segment gene sequences of the species indicates differences in amino acids equivalent to those observed among antigenically distinct viruses.
D. At least partial gene sequence has been obtained for the L segment, in order to rule out the possibility that the virus is the result of segment reassortment.

Tentative species listed include those which have not been characterized by serological means (not isolated in cell culture) and have not been sufficiently characterized by genetic means. Preliminary sequence analyses, however, suggests that they are unique viruses. Such analyses generally consist of comparisons of nucleotide or deduced amino acid sequences from only one complete genome segment or comparison of partial sequences from one or more segments.

Tentative species:

Juquituba (sequence only; no other information available)
Delgadito (no other information available)
Punchana (no other information available)
Chaco Paraguayo (sequence only; no other information available)

ADVANCES IN THE KNOWLEDGE OF DENGUE AND DENGUE HEMORRHAGIC FEVER IN THE AMERICAS (INTERNATIONAL COURSE)

Considering the importance of Dengue and Dengue Hemorrhagic Fever in our Region and the need of increasing the knowledge of the specialists of our countries in the aspects related to its management, prevention and control, the PAHO/WHO Collaborating Center for Viral Diseases at the Institute of Tropical Medicine Pedro Kourí in Havana, will host from the 18 to the 29 of August of 1997 the International Course on Dengue and Dengue Hemorrhagic Fever, as part of a set of activities that said center since several years carries in the Region to collaborate in the struggle against the dengue.
The course, of two weeks of duration, will consist of masterful conferences offered by Cuban and foreign lecturers (Prof. Vincent Deubel from Pasteur Institute of Paris, Prof. Robert Shope, from the University of Texas, United States, Prof. Hermann Schatzmayr from Institute Oswaldo Cruz, Brazil, Prof. Gustavo Kourí, Prof. M.G.Guzmán, Prof. Juan Bisset from IPK, Prof. Eric Martínez and Prof. Rafael Figueredo from the Cuban Ministery of Health), seminars, group discussions and practical sessions in which will be updated and discussed the following aspects:

Clinical Management of patients.
Molecular Biology of the agent.
Advances in the laboratory diagnosis.
Ethiopathogenesis of the disease. Immunity.
Epidemiological aspects and risk factors for the DHF.
Vector Control.
Community participation in the Control of Dengue
Programs to Eradicate the vector.
Advances in the studies for the obtainment of a vaccine.

Among the practical activities are emphasized those related to:
Laboratory Diagnosis through viral isolation, PCR, immuunohistochemical diagnosis in tissue samples, detection of IgM antibodies in sera and samples of blood dried in filter paper.
Strain characterization through restriction enzyme.
Vector Control
Community participation techniques.

Request for admittance should be sent via FAX before July 1st, 1997 and include: Name and postal address, phone and FAX number, e-mail address and a brief Curriculum Vitae.
Please deliver your request for admittance to:
Prof. María G. Guzmán
Institute "Pedro Kourí"
Autopista Novia del Mediodía Km. 6.
La Lisa, Ciudad de la Habana, Cuba
Phone/FAX 53-7-220633 and 53-7-336051 e-mail ciipk@infomed.sld.cu

Editor's Comments

I would like to announce the establishment of the on-line version of the Arbovirus Information Exchange. With an on-line version, reports can be posted as soon as they are received. This should make information much more rapidly available to people. The eventual goal will be to reduce the number of printed copies of the Arbovirus Information Exchange. This will help us save paper and printing and mailing costs. If you have access to a computer and to the Internet, please try to submit your reports electronically and access the Arbovirus Information Exchange online. We will not be able to reduce the number of printed copies until everyone submits their reports electronically.

The home page for the Arbovirus Information Exchange can be found at the following address:

`http://www.utmb.edu/ctd/arbovirus`

All submissions received electronically (either by e-mail or on a computer disk) will be posted on the website. Reports received only as printed documents (not submitted electronically or on a disk) will **not** be posted on the website. Please feel free to make any suggestions for improvements or changes on the website. If you have interesting hyperlinks, photographs or other materials you would like to see placed on our home page, feel free to let me know (by e-mail please) and we will add them to the site.

Several of you have written to me already with positive remarks about the on-line version. I hope that we will have good success with this. I think we are off to a good start: in this issue of the Arbovirus Information Exchange, all of the preliminary announcements, and nine of the fifteen reports were submitted electronically. These can be viewed on the website immediately. Whether you submit electronically or on paper, please follow the same instructions that are printed below.

Laura J. Chandler
Editor

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

I prefer to receive reports electronically, in WordPerfect or Microsoft Word. Rich Text or ASCII text formats are also acceptable. Either Macintosh or DOS/Windows based documents are acceptable. (Be sure to indicate which format you have used). If you have access to e-mail, your reports may be sent to me at:

`lchandle@marlin.utmb.edu` *or* `laura.chandler@utmb.edu`

If submitting by e-mail, attach the report as a document to your e-mail message. If you like, you may also send your report on a computer disk. Printed reports and reports on computer disks may be mailed to me at the address below.

Laura Chandler, Ph.D.
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Galveston, Texas 77555-0609
FAX 409-747-2437

Previous Editors of the Arbovirus Information Exchange

Telford H. Work 1960-1972

Roy W. Chamberlain 1972-1981

W. Adrian Chappell 1981-1984

Barry R. Miller 1984-1989

Charles H. Calisher 1989-1996

Summary of the activities of the WHO Collaborative Centre of Reference and Research on Arboviruses and Viral Hemorrhagic Fever Viruses

Institute Pasteur of Dakar.

J. Thonnon, A. A. Sall

The CRORA, which is the WHO Collaborative Centre of Reference and Research on Arboviruses and Hemorragic Fever Viruses, carries out the identifications of the strains isolated by the Pasteur Institute of Dakar and by its corresponding members.

In 1996, 39 strains have been identified representing 12 different arboviruses coming from: Institut Pasteur de Côte d'Ivoire (1), Institut Pasteur de Madagascar(3), IMTSSA-Le Pharo- Marseille (2) and Institut Pasteur de Dakar(33). The proportion of identifications coming from Dakar increases each year. This result is probably due to the present excellent collaboration with the ORSTOM entomologist team. The biodiversity studies through different viral ecology programs lead to the isolation of apparently new strains, i.e. recognised by none of the collection of reagents stored at the CRORA.

Since its inauguration, the Centre has identified 5525 virus strains. the reference collection currently includes 201 arboviruses or vertebrate viruses as well as 31 other viruses. For arboviruses the distribution is:: Alphavirus 11, Flavivirus 38, Bunyavirus 35, Phlebovirus 8, Nairovirus 7, Bunyaviruslike 16, Orbivirus 31, Rhabdovirus 32, Arenavirus 1, Poxvirus 2, Orthomyxovirus 1, unclassified viruses 19.

A few outstanding events are to be noted:

- a re-emergence of yellow fever was notifed in Africa during the last decade. In Senegal, two outbreaks emerged: Koungheul in 1995 and Kaffrine(14.5°N, 16.4°W) in 1996. These outbreaks happened after a latent period dating from the Djourbel epidemic of 1965. Incidence of the infection was evaluated by IgM immunocapture. A total of 128 cases of yellow fever were detected: 28 probable cases (clinically defined), 31 confirmed(either by IgM or virus isolations) and 69 asymptomatic patients with IgM. Eight strains of YF were isolated from human cases. The ORSTOM team's carried out the entomological investigations of this outbreak in Kaffrine: larval indices and adult mosquitoes collections. One strain of YF were isolated from *Aedes aegypti.*

The distribution according to the age showed a high rate (91,4%) of cases in children. Thirty six deaths were notified, all of the victims were under 20. The immunity against YF in the area, before the outbreak, was estimated by detecting IgG. Only 25,3% of the children before 10 years had specific antibodies, despite the inclusion of YF vaccine in the EPI.

The CRORA was asked by the WHO to organise a workshop to strengthen the diagnosis of YF for 11 French speaking countries in Africa.

The survey of potential vectors incriminated in different cycles of arboviruses pathogenic for human continued: YF, DEN, RVF, WN. Evidence of selvatic amplification occurred this year in the Kedougou area(South -Eastern Senegal). Evidence of vertical transmission of YF in the selvatic vector was also notified in Kedougou, this confirms the results of the last year.

Interesting results have been obtained through the study of sand flies with the isolation of Tete virus and of an apparent new strain Ar D 111740.

In 1995, serological evidence of the circulation of the RVF virus in sheep flocks was established along the Senegal River. In 1996, the serological survey did not show any increase in the viral activity, either in herds or humans.

The molecular virological laboratory continued its work on molecular epidemiology of RVFV. It showed reassortments between strains isolated in different areas of Africa. For Flaviviruses, sequencing of different parts of the genome is in progress: DEN-2, West African strains, YF strains of the latest outbreaks and from flaviviruses with unknown vectors (Apoi, Saboya, Bukalasa-Bat et Kadam).

DENGUE OUTBREAK IN FRENCH GUIANA, 1996-1997

A. Talarmin[1], M. Senes[2], P. Maurer[2], F. Fouque[1], B. Labeau[1], G. du Fou[1], J. L. Sarthou[1]

[1] Centre national de référence pour la surveillance de la dengue et de la fièvre jaune, Institut Pasteur de la Guyane, BP 6010, 97306, Cayenne, Guyane Française; [2] Réseau sentinelle, 97310, Kourou, Guyane française.

After the DHF outbreak due to dengue 2 in 1991-92, dengue cases were only sporadic in French Guiana from 1993 to 1996, until the outbreak of classical dengue fever (DF) that occured in september 1996. This epidemic first began in Kourou, a town of 15,000 inhabitants located on the atlantic coast, at 60 kms in the North West of Cayenne (figure 1). This epidemic was mainly due to DEN-1 (53 DEN-1 and 7 DEN-2 strains isolated). In January, the outbreak reached Cayenne the capital city (figure 2); but, curiously, DEN-2 was responsible for most cases in this town (49 DEN-2 and 21 DEN-1 strains isolated in January and February 1997).

We are now in a situation where DEN-1 is the only virus isolated in the West of French Guiana, its frequency decreasing from the West to the East of the country. The inverse is observed for DEN-2 which is the only virus isolated in the East.

This virological dissociation is acompanied by clinical differences. Whereas only classical dengue fever cases are observed in the towns where DEN-1 is circulating, some DHF cases are reported in places where DEN-2 is predominant, one DEN-2 being isolated from one of this patient. These observations suggest that only DEN-2 is responsible for DHF in French Guiana.

These epidemics have evidenced the limits of our dengue surveillance system based on biological data (there is no clinically based dengue surveillance system in French Guiana). After the outbreak in Kourou, a new surveillance system has been initiated, based on the number of negative malaria diagnosis. This biological is often prescribed by physicians, in patients with febrile illness, to confirm or more frequently to exclude the diagnosis of malaria. The curve of malaria diagnosis has the same pattern as the curves of suspected and confirmed cases but it may be more reliable since nearly all physicians prescribe this test, which is not the case for dengue diagnosis (figures 1, 2).

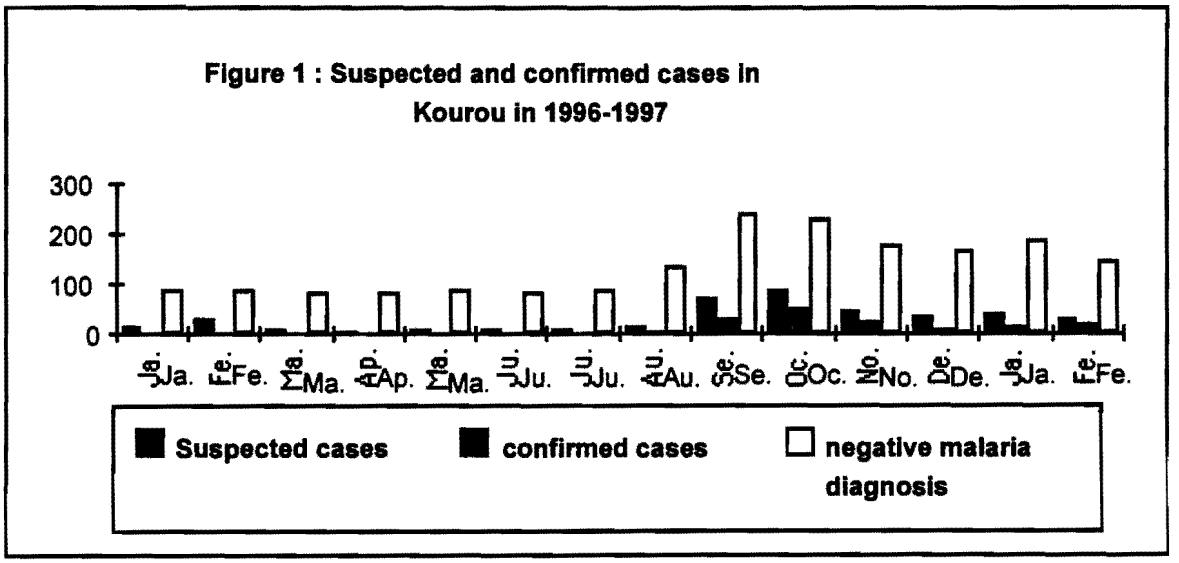
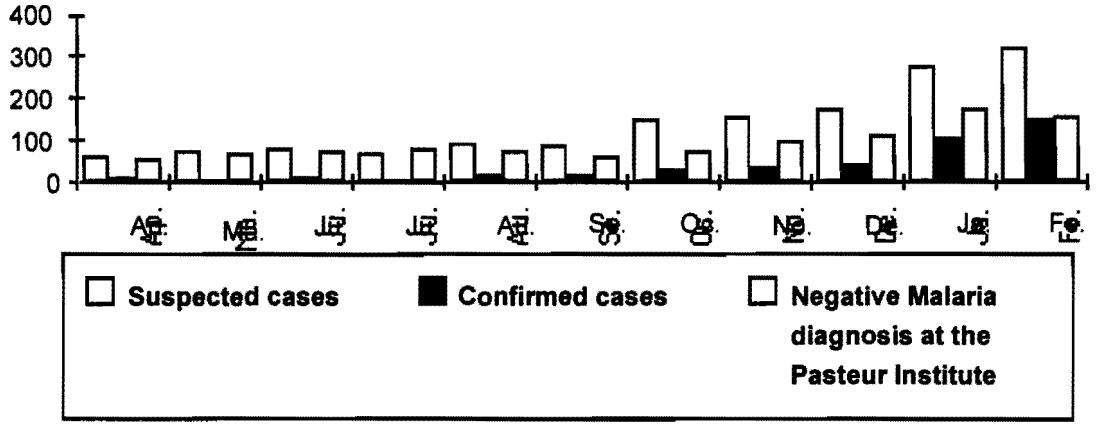


Figure 2 : Suspected and confirmed DF cases in Cayenne in 1996-1997



Microneutralization test for Dengue 2 virus on an Enzyme Immunoassay (NtEIA).

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Hemagglutination Inhibition, Complement Fixation and Enzyme-linked Immunosorbent Assay (ELISA) are of limited value in dengue diagnosis and seroepidemiological studies because they often cannot identify the infecting serotype. Assay of neutralizing antibody is the most specific and great sensitivity. Currently, plaque reduction neutralization technique (PRNT) is used to define serologically the serotype in primary and secondary infection however it's expensive and requires relatively large serum volumes, besides the large number of assays that often must be done simultaneously. Even more, many variables influencing dengue virus plaquing efficiency.

We are developing a microneutralization test in which dengue 2 virus replication is detected by enzyme-linked immunosorbent assay (NtEIA). The results obtained were compared with those previously obtained by PRNT (1).

Dengue virus type 2 (A15 strain) was propagate in sucking mouse brains and from these brains 20% homogenates were prepared in MEM supplemented with 2% FCS.

Virus titration and the proper neutralization assays were performed in 96-well, flat-bottomed tissue culture microtiter plates. About $4x10^4$ BHK21 cells were dispensed into each well of the plates and incubated in a CO_2 incubator for two days at 37°C. Volumes of 25ul of serial dilutions of the virus were inoculated onto the BHK21 monolayers. After adsorption for 2h at 37°C the cells were covered with the maintenance medium. After 5 days incubation at 37°C and 5% CO_2 for dengue 2 and 7 days for the others viruses the plates were freezed and thawed three times at -70°C. Antigen detection was done by ELISA.

Dilutions of human serum or hyperimmune ascitic fluid were made in sterile wells of 96-well flat-bottomed polysterene microtiter plates. 75ul volume of each serum dilution was incubated at 37°C for 1.5 h with 75ul of a working dilution of virus calculated to give 50-100 tissue culture infective doses in the final volume of virus-serum mixture. All dilutions were done in MEM-2% FCS. 50ul of the virus-serum mixtures were inoculated onto BHK21 monolayers. The rest of the assay has been described above. Each dilution of antibody was run in triplicate and each plate included control wells with uninfected cells, a back titration of the virus and a positive and a negative serum control.

Antigen detection by ELISA: The optimal dilutions of the sensitizing antibody (anti-dengue human IgG), the capture antibody (anti-dengue 2 ascitic fluid) and the peroxidase-conjugate (anti-mouse Ig) were determined by checkerboard titrations. The optimal time and temperature for incubations were also determined. ELISA plates were sensitized for 18h at 4°C with anti-dengue human IgG at 7.5ug/ml in coating-buffer pH 9.6. Sheep serum (SS) at 5% in PBS was used as

blocking buffer. 50ul of the antigen samples were added to each well and incubated at 37°C for 1h. The capture antibody diluted 1\4000 in PBS\0.05%Tween 20\2%SS was added to all wells and incubated for 1h. The conjugate was diluted 1\8000 in PBS\0,05%Tween20\ 5%SS and incubated for 1h. The adequate substrate was added and the reaction was stopped after 30min incubation at RT. An absorbance reading of>=2 OD above the mean of 10 control wells was considered to be evidence of viral replication. Titres were calculated using the formula of Reed and Muench.
The mean absorbance of uninfected control cells was 0.08.
Our previous studies demonstrated that NtEIA was able to detect 0.23 PFU of the dengue 2 virus. The titres by ELISA were compared with those obtained by PRNT. Table 1 shows the comparison of antibody neutralizing dengue 2 titer obtained by both methods in some sera.

Table 1. Comparison of neutralizing titers obtained by PRNT and NTEIA*.

No.	EIAWt	PRNT	No.	EIAWt	PRNT
1	4542	640	8	54423	60000
2	10240	4700	9	846	640
3	2024	1500	10	>2560	>2560
4	504	800	11	<40	<40
5	8096	7500	NAF	<40	<40
6	<160	<40	D2AF	>2560	ND**
7	54466	50000	D4AF	<40	ND

* Reciprocal of the Nt titer.
** ND: not done

The NtEIA requires smaller volumes of cells, media and specially sera for neutralization assays (Nt) so that Nt can be made on a larger number of serum specimens in a single experiment and can be apply to seroepidemiological surveys. Our preliminary results suggests the usefulness of NtEIA to dengue diagnosis and seroepidemiologic studies.
Currently a higher number of sera are being studied to dengue 1-4.

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IMMUNOHISTOCHEMISTRY IN DENGUE INFECTED PARAFFIN-EMBEDDED TISSUE

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During the 1980s, the incidence of dengue infections in the Americas increased considerably, and its activity was characterised by a marked geographic spread in the region. The 1981 outbreak of DHF/DSS in Cuba was the most important event in the history of dengue in the Americas. During this epidemic, associated with dengue-2 virus, a total of 158 deaths (of which 101 were children) were reported.

Diagnosis in fatal cases is mainly clinical and supported by laboratory findings like hemoconcentration and thrombocytopenia, but the confirmation is very difficult. Death occur few days after onset and in this moment only the acute sample for serology is available. On the other hand, isolation and molecular techniques are very expensive, and many labs in hospitals and clinics have any possibilities to diagnosis.

The histological studies need a big expertise, because dengue is little pathognomonic, and tissue damage is similar to other haemorrhagic diseases.

One method to employ for the diagnosis of fatal cases are the immunohistochemical techniques (IHQ). Because tissues in many cases are the only available material to confirm clinical diagnosis and the examination techniques are affordable in many places.

IHQ technique using paraffin-embedded tissue from 2 patients of the 1981 Cuban epidemic of DHF/DSS was standardised in our laboratory using an anti-dengue complex Mab (obtained at IPK). Routine haematoxylin-eosine technique was used to obtain coincidence with the preview histological diagnosis made in 1981. Tissues from liver, spleen and linfanodes of both cases were processed to eliminated endogenous peroxidase and then blocked with foetal calf serum. After overnight Mab incubation, a secondary anti-mouse serum (Fab') and streptABComplex (DAKO) were applied.

Positive reaction was observed in both cases. In liver tissues, dengue antigens were demonstrated by a brown precipitate in Kuppfer and endothelial cytoplasm cells. Spleen and lymphatic nodes were positives too. Slides from those organs showed a positive staining in sinusoidal macrophages.

The use of this technique, provided our lab with an useful tool for retrospective studies of our epidemic and also for reference in our region

Rapid detection and typing of Dengue Viruses from Clinical samples using PCR. Evaluation of genomic fragment nt 134 to nt 644 for Dengue virus genetic characterization.

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The Polymerase Chain Reaction (PCR) allows a selective and rapid amplification of short sequences of genome which can further be either identified with type-specific probes or analyzed further as dsDNA molecules for molecular epidemiology investigations. Several methods have been developed for the detection of PCR- amplified dengue virus RNA using either labelled cDNA clones of each of the four dengue serotypes (Deubel et al., J. Virol. Methods., 1990, 30, 41-54) or semi-nested PCR using type-specific oligonucleotides primers for the second series amplification. In this study, we have chosen to amplify a genome fragment in C-prM genes for specific and sensitive dengue virus diagnosis (Lanciotti et al., J. Clin. Microbiol., 1992, 30, 545-551).

27 serum samples from patients with Dengue Fever (DF) or DHF/DSS from epidemics in Nicaragua, Colombia and Panama were directly subjected to reverse transcriptase and PCR for detection of Dengue virus. The resulting double- standed DNA product was typed by second round of PCR amplification (nested PCR) with type specific primers and compared these results with the conventional Indirect Immunofluorescence Assay (Henchal et al., Am. J. Trop. Med. Hyg., 1982, 31, 830-836) for the identification of Dengue viruses currently isolated in insect cell culture from patients' sera and with ELISA for IgM anti- Dengue antibodies (Bundo et al., J. Virol. Methods, 1985, 11, 15-22). The amplified virus genome was detected and typed within 8 hours. The calculated parameters for nested RT-PCR diagnosis with viral isolation / IFA as the " gold standard " were 100 % sensitivity, 78 % specificity, 69 % PPV and 100 % NPV. It is noteworthy that two of the nested RT-PCR positive and isolation negative specimens exhibited specific IgM antibodies. RT-PCR results corresponded closely to results of viral isolation, suggesting that PCR procedure greatly facilitates to rapid diagnosis of Dengue infection.

Information about genetic variation in one virus type or information about epidemiological origins of new dengue isolates has been obtained by mapping the RNase T1- resistant oligonucleotides (Repick et al., Am. J. Trop. Med. Hyg, 1983, 577-589; Trent et al., Virology, 1983, 128, 271-284) by hybridization of RNA using synthetic oligonucleotides as probes (Kenschner et al., 1986, 67, 2645-2661), by comparative analysis of RNA sequences (Deubel et al., J. Virol. Methods, 1990, 30, 41-54; Rico Hesse, Virology, 1990, 174, 479-493) and by restriction fragment length polymorphism (RFLP) (Vorndan et al., J. Virol. Methods, 1994, 48, 237-244) The Dengue gene fragment (nt 134 to nt 644) obtained by RT/PCR using D1 and D2 dengue universal primers (flanked by sequences conserved between dengue viruses) have been exploited for rapid and simple procedure for identify geographic subgroups of dengue virus serotypes 1 and 2 for preliminary epidemiological investigations. The genomic fragment proposed by Lanciotti et al., 1992, for dengue diagnostic was amplified and the products were digested with the endonucleases Hae III, Alu I, Rsa I, and Hinf I. By comparing the electrophoretic patterns of produced, we recognized a pattern for each serotype - prototype strain, 3 groups for 18 serotype 1- dengue virus strain (A1, B1 and C1) (Table) and 4 groups for 15 serotype 2- dengue virus strains (A2, B2, C2, and D2) (Table) corresponding closely to those previously determined by oligonucleotide fingerprinting, and by comparative analysis of RNA sequences. This procedure can be performed in 1 or 2 days, and the results can be interpreted without computer analysis. We aim to accomplish a similar study with Dengue 3 and Dengue 4 strains. So this is a useful tool for rapidly screening multiple virus isolated, without using a great quantity of resources.

	Code	Location	HaeIII	RsaI	AluI	HinfI	Pattern
Dengue 1	SH29177	Senegal,79	1	1	2	1	A1
	TVP3892226	Fiji,75	1	1	2	1	A1
	41374*	Hawaii,44	1	1	2	1	A1
	NIC69	Nicaragua,85	1	1	2	1	A1
	NIC1	Nicaragua,94	1	1	2	1	A1
	TVP1971	Angola,88	1	1	1	1	B1
	TVP1749	Colombia,87	1	1	1	1	B1
	CR271	C. Rica,94	1	1	1	1	B1
	CR1591	C. Rica, 94	1	1	1	1	B1
	R.Pretto	Brazil,91	1	1	1	1	B1
	461466	Panama,94	1	1	1	1	B1
	35291	Brazil,87	1	1	1	1	B1
	38153	Brazil,89	1	1	1	1	B1
	402	Colombia,94	1	1	1	1	B1
	CV1636/77	Jamaica,77	1	1	1	1	B1
	28489	Brazil,86	1	1	1	1	B1
	3 Perú	Peru,90	1	1	1	1	B1
	PRS228686	Burma,76	1	1	3	1	C1
Dengue 2	1251	Tonga,74	1	1	1	1	A2
	TVP-1752	Colombia,88	1	1	1	1	A2
	TVP-961	Indonesia,78	1	1	1	1	A2
	TVP-965	Jamaica,82	1	1	1	1	A2
	453715	Hondura,91	1	1	1	1	A2
	457951	Panama,93	1	1	1	1	A2
	A15	Cuba,81	1	1	1	1	A2
	A35	Cuba,81	1	1	1	1	A2
	NGC *	N. Guinea,44	1	1	1	1	A2
	PM-3974	R.Guinea,81	2	2	1	2	B2
	PL-001	Taiwan,81	2	3	1	3	C2
	818394	Trinidad,80	2	1	1	1	D2
	810827	Trinidad,80	2	1	1	1	D2
	39216	Brazil,90	2	1	1	1	D2
	369	Colombia,94	2	1	1	1	D2

ISOLATION AND TYPING OF DENGUE VIRUS IN THE STATE OF GUERRERO, MEXICO.

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The trend of Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) cases in Mexico is increasing since 1978 when serotype 1 was introduced (1,2,3). In 1982 and 1983, the serotypes 2 and 4, respectively, were detected in Mexico (2). Additionally, in 1995 the serotype 3 was recovered from DF cases occurred in the southeast of the country (3) Therefore, at the present time, all four serotypes are widely circulating in most regions of the country wherever *Aedes aegypti* is present (1). During the period of 1995-1996 more than 16,000 cases of DF were recognized and 2,000 cases of DHF were notified around the country (4).

The State of Guerrero is located in the south of Mexico (See Map) and cases of DF and DHF are annually reported (2,4). So far, serotypes 1, 2 and 4 have been isolated from dengue cases (2,4,5). Surveillance data reported in 1996 by the General Directoriate of Epidemiology (Ministry of Health), showed that the most f DF and DHF cases were registered in the municipality of Acapulco (4). In the summer of 1988 an outbreak of dengue fever was reported in Taxco, Guerrero, located at 1,700 meters above the sea level ; This was the first report in Mexico showing the transmission of dengue virus at a high altitude (6).

Since, many regions of Guerrero State are considered endemic for dengue virus and consequently with a high risk for DHF epidemics, the School of Biological Sciences (Guerrero University) and the Department of Arboviruses, Center for Research in Infectious Diseases (National Institute of Public Health, Cuernavaca, Morelos), initiated in October 1996, a collaborative surveillance activity for the isolation and typing of dengue virus from febrile cases.

A team of graduated students were trained for blood collection from individuals presenting signs and symptoms suggestive of dengue fever (fever, myalgia, arthralgia, headache, retro-ocular pain) and clinical and epidemiological data were registered. During the first stage of this work, the following localities were included: Tres Palos (A), Chilpancingo (B) and Cerrito de Oro ©. (See Map). Blood samples were processed and serum was delivered in dry ice to the Dept. of Arboviruses in Cuernavaca, Morelos.

Dengue viruses were isolated in C6/36 cell cultures according to the procedure described by Gubler et al (7). Briefly, one drop of washed cell suspension was placed in each well of a 12-well slide, dried, fixed with cold acetone and stained with a polyclonal antibody against dengue labelled with fluorescein isothiocyanate. Positive cultures of Dengue-1 (Hawaii strain), Dengue-2, (NGC strain), Dengue-3 (H-87 strain), and Dengue-4 (H-241 strain) kindly donated by Dr. Rebeca Rico- Hesse, Southwest Foundation for Biomedical Research, San Antonio, Texas), were included as controls. Positive serum cultures were

processed for serotype identification by indirect immunofluorescence using specific monoclonal antibodies.

The collection of serum samples will continue during the period of 1997-1998 and localities with reported clinical cases of dengue will be surveyed.

Table I shows the positive serum samples for virus isolation (16/21 samples, 76%) and 81% of them corresponded to serotype 2. The negative serum samples for virus isolation will be processed by Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) for virus detection. In spite of few analyzed samples, it is interesting to note that serotypes 1, 2 and 4 were isolated from residents of Cerrito de Oro, a village near to Acapulco City. Since multiple circulation of dengue serotypes could be associated with a risk for hemorrhagic cases, it is important to strengthen the epidemiological surveillance activities.

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LOCATION OF THE STATE OF GUERRERO AND VILLAGES (A,B,C) SELECTED FOR BLOOD COLLECTION

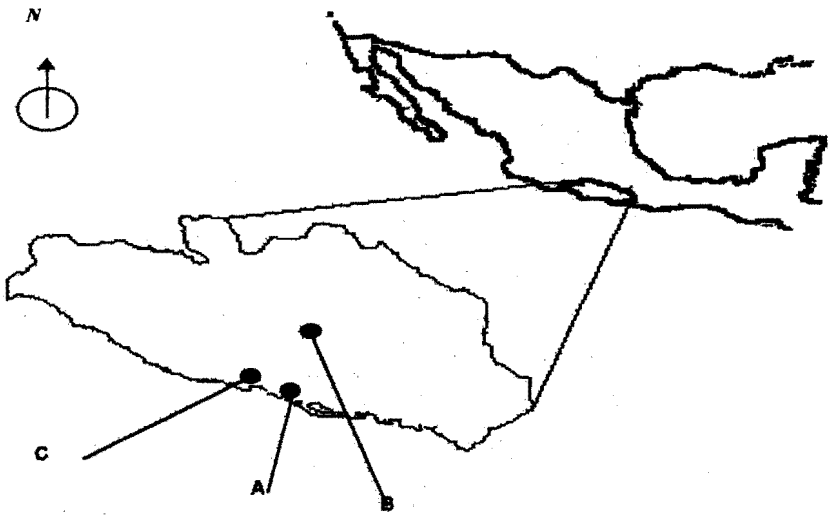

TABLE 1
ISOLATION AND TYPING OF DENGUE VIRUS IN THE STATE OF GUERRERO, MEXICO.

SERUM CODE	ORIGIN	SEROTYPE	SERUM CODE	ORIGIN	SEROTYPE
827	TRES PALOS (A)	DENGUE-2	840	CERRITO DE ORO (C)	DENGUE-4
828	TRES PALOS (A)	DENGUE-2	849	CERRITO DE ORO (C)	DENGUE-2
832	CHILPANCINGO (B)	DENGUE-2	850	CERRITO DE ORO (C)	DENGUE-2
833	CHILPANCINGO (B)	DENGUE-2	852	CERRITO DE ORO (C)	DENGUE-1
834	CHILPANCINGO (B)	DENGUE-2	853	CERRITO DE ORO (C)	DENGUE-2
835	CHILPANCINGO (B)	DENGUE-2	855	CERRITO DE ORO (C)	DENGUE-2
836	CHILPANCINGO (B)	DENGUE-2			
837	CHILPANCINGO (B)	DENGUE-2			
838	CHILPANCINGO (B)	DENGUE-4			
839	CHILPANCINGO (B)	DENGUE-2			

Decrease in rodent seroprevalence to hantavirus at 1993-94 hantavirus pulmonary syndrome case sites.

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Peromyscus mice trapped from 1993-1994 hantavirus pulmonary syndrome (HPS) case sites, in Arizona and Colorado, had seroprevalence rates to hantavirus of up to 63% (testing provided by Centers for Disease Control, Atlanta GA). Seven of these sites were re-trapped three years later, using the same trapping protocol, in order to get a point-in-time longitudinal estimate of changes in rodent density and seroprevalence. Four of these locations are from Colorado and three are from Arizona.

In 1993-94, a total of 233 rodents were trapped at these seven case sites, of which 145 (62.2%) were *Peromyscus* mice. Fifty-seven (40.4%) of the *Peromyscus* were seropositive for antibodies to hantavirus (range = 0% to 63% per site). In 1996-97, a total of 146 rodents were trapped at these same locations, at the same time of year, using the same trapping protocol. Ninety (61.6%) of the rodents were *Peromyscus*, one (1.1%) of which was positive for Sin Nombre virus antibodies. These data show a significant decrease in *Peromyscus* seroprevalence from the 1993-94 outbreak trapping period to the 1996-97 re-trapping period ($X^2 = 43.59$, $p < 0.00001$).

The total number of rodents, the number of *Peromyscus* mice, and the seroprevalence rate all decreased between the outbreak period of 1993-94 and three years later in 1996-97. The decreases in the rodent numbers are probably related to a variety of things, including overall population density changes and natural and man-made changes around the case sites. Several man-made environmental manipulations, including homesite reconstruction, brush and clutter reduction and previous rodent elimination attempts were seen at several of the sites. Two other sites were retrapped, but lacked comparable data and were not included in the analysis. One of the these sites was a vacant homesite in eastern Arizona. The site was abandoned after one of the inhabitants became ill with HPS in 1993. No environmental manipulations occurred at the homesite between 1993 and 1996. The overall number of captured rodents decreased from 44 to 32, but the number of cricetid rodents increased from 22 to 25, of which two were positive in 1993 and one was positive in 1996 (all cricetid rodents, including *Peromyscus* and *Reithrodontomys*, were lumped together as a few rodent identifications at this site may have been inaccurate in 1993). The other site without comparable data was in southwest Colorado, where one rodent was captured in 1993. The rodent was not identified or tested. Two negative *Peromyscus* were trapped at this place three years later.

Even if man-made manipulation was the cause for the decrease in rodent numbers, it is doubtful that these manipulations were the cause of the significant decrease in *Peromyscus* seroprevalence to hantavirus. It is possible that, like rodent densities, seroprevalence rates fluctuate naturally, and that the overall seroprevalence of *Peromyscus* rodents in the Four-Corners states is much lower now than during the 1993 HPS outbreak. Ongoing longitudinal rodent trapping and testing studies at selected locations in Arizona, Colorado, and New Mexico will produce data that should further clarify this phenomenon.

LONGITUDINAL STUDIES OF HANTAVIRUSES IN DIVERSE ECOSYSTEMS IN COLORADO, 1994-97.

Longitudinal capture-release studies of transseasonal transmission of the hantavirus Sin Nombre virus (SNV; the etiologic agent of hantavirus pulmonary syndrome) are being done at three ecologically diverse sites in Colorado. In southwest Colorado antibody prevalence has ranged from 15.6% in 1994 to 0% in 1996 in deer mice (*Peromyscus maniculatus*, the ostensible principal vertebrate host of SNV). In west central Colorado antibody prevalence has ranged from 0% in 1994 to 33% in 1995 and 24% in spring-summer 1996 and to 0% in August, September, and November 1996. In southeastern Colorado, Western harvest mice (*Reithrodontomys megalotis*), piñon mice (*P. truei*), and deer mice have been the most common species and the only ones with antibody; 10% of western harvest mice (antibody in western harvest mice likely due to infection with El Moro Canyon virus), 1.6% of piñon mice, and 1.2% of deer mice (antibody in deer mice and piñon mice likely due to infection with SNV).

Increased moisture (rainfall, snow), a condition ostensibly conducive to increased rates of transmission and prevalence of hantavirus infections had not occurred through early 1997. Fluctuations in populations of some rodent species have been observed but because of the absence of increases in populations of deer mice, Western harvest mice, and piñon mice, no increase in virus prevalence has been noted.

Differences in antibody prevalence rates by gender are clear (Table 1). Antibody prevalence is more than twice as high in male than in female deer mice, although males represent only about 55% of the population. This pattern is even more well-defined among western harvest mice, of which 90.5% of seropositive animals have been males.

Data collected thus far suggest that hantavirus infections can occur at essentially any time of year but more frequently occur in the summer-autumn and winter-spring periods. Peak population densities and cold weather "huddling" of *Peromyscus* sp. rodents may constitute risk factors for acquiring infection with the hantaviruses at these sites. Seroconversions in females are more likely to occur in the winter-spring period than in the summer-fall period; males appear to seroconvert as often in winter-spring as in summer-fall periods. This pattern may not hold for western harvest mice at PCMS.

Until recently, cotton rats (*Sigmodon hispidus*) were not known to occur at PCMS. Since we began our studies the cotton rat population has abruptly increased, such that in August of this year 83% of the rodents at one site, 24% at another, and 20% at a third site were cotton rats. With this increase we have seen a concomitant decrease in deer mice and western harvest mice, which are competitors of cotton rats and are the hosts of hantaviruses at Piñon Canyon. Cotton rats are predators of the young of these mice. Piñon mouse populations have not been impacted because they are not found in the low, moist areas which cotton rats, deer mice, and other rodents inhabit. Cotton rats do not store food and they disperse, rather than fight, when their populations increase. During the winter of 1996-1997 the cotton rat population at PCMS "crashed", a phenomenon known to occur in this species in winter.

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Table 1. Antibody prevalence by gender at three sites (data combined) in Colorado.

Peromyscus sp.

Gender	Total with antibody (%)	Total animals (%)	Percent with antibody
Male	34 (68)	455 (55.4)	7.5
Female	16 (32)	366 (44.6)	4.4
Total	50	821	6.1

Reithrodontomys megalotis

Gender	Total with antibody (%)	Total animals (%)	Percent with antibody
Male	19 (90.5)	101 (51.8)	18.8
Female	2 (9.5)	94 (48.2)	2.1
Total	21	195	10.8

Interpreting St. Louis encephalitis virus transmission activity by concurrent evaluation of "sentinel" mosquito populations.

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Surveillance of sporadically occurring indicators of arboviral activity is inherently complicated by the difficulty of realistically interpreting any data that are gathered. Integrated surveillance strategies that employ several different and complementary surveillance tools are less likely to fail than strategies that rely upon a single surveillance tool.

St. Louis encephalitis virus (SLE) is endemic to south Florida, and is detectable most years in Indian River County. The principal SLE vector in Indian River County is the tropical floodwater mosquito, *Culex nigripalpus*. This mosquito is not only the most common and locally abundant species, but the adults are widely dispersing. Furthermore, *Cx. nigripalpus* is largely untouched by extant mosquito control activities within IRMCD, and substantial numbers are present year-round. The extensive larval habitat includes not only natural freshwater wetlands, but inter-row furrows in approximately 80,000 acres of citrus groves.

The Indian River County Public Health Unit (a county government agency) is responsible for responding to arbovirus threats, but is entirely dependent on the Indian River Mosquito Control District (IRMCD) for local surveillance data. IRMCD is a relatively small, independent government agency, with only 20 employees (most dedicated to control of coastal salt marsh mosquitoes). Only 1.5 personnel are dedicated to arbovirus surveillance. Since 1978, IRMCD has relied primarily on the detection of SLE virus ***transmission*** to sentinel chickens as an indicator of virus activity. In 1990 it was possible to detect a period of imminent risk of SLE epidemic activity, using not only transmission data but concurrent data pertaining to the population dynamics of *Cx. nigripalpus*. This period would later prove to be the beginning of the most widespread SLE epidemic experienced in Florida.

Occasional sentinel seroconversions are common in "normal" years. In other years, temporal clumping of seroconversions and the simultaneous transmission of virus to sentinels at multiple sites suggests greater risk of transmission to man. At such times reliable monitoring of the vector population's reproductive status is instrumental in determining how IRMCD advises local health officials, and in directing any emergency adulticiding efforts. Based on the experiences of the 1990 epidemic year, SLE transmission data are now routinely interpreted in the context of real-time monitoring of two "sentinel" *Cx. nigripalpus* populations. Resting adult

mosquitoes in these populations are regularly collected, and categorized by sex and age class (in the case of females). The proportions and absolute numbers of freshly bloodfed and gravid females (i.e., females previously bloodfed, with fully developed egg batches ready for oviposition) are particularly important to track in "real-time." Dispersal, oviposition and subsequent re-feeding of gravid females is highly dependent on rainfall. Large rainfall events serve to synchronize bloodfeeding by these physiologically "older" females, and are often temporally associated with the transmission of SLE virus to sentinel chickens in the field. Thus, an accumulation of gravid females during periods of short-term drought becomes a cause for concern when this is coincident with any significant SLE transmission to sentinels. In such circumstances, the next major rainfall event is likely to create a period of elevated transmission risk to both birds and man.

Conserved antigenic regions on the G2 protein of California encephalitis serogroup viruses

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The G2 protein of La Crosse encephalitis virus (LACV) has been implicated in the ability of the virus to infect mosquito midgut cells (1,2). We have produced and characterized a panel of monoclonal antibodies (Mabs) directed against LACV G2 (3). In this report, we describe our use of these antibodies to identify the antigenic regions of the G2 protein and to determine if these regions are conserved in other California encephalitis group viruses.

Reciprocal competetive ELISA's were performed on LACV- infected Vero cells, using 5 monoclonal antibodies directed against G2, and 2 monoclonal antibodies that recognize an antigenic area present on both G1 and G2. Our data suggest the presence of 4 antigenic regions on the G2 protein (Table 1). It is possible that area A and B may overlap. None of the antibodies tested competed with an antibody which was directed against the LACV G1 protein, nor with an irrelevant monoclonal antibody.

Table 1. A summary of reciprocal competitive ELISA

Mab	Specificity	Subtype	Epitope
3D9.4	G2	IgG2a	A
9E7.2	G2	IgG2a	A
4A5.5	G2	IgG2a	A
9B7.5	G2	IgG2a	A′ or B
7H12.1	G2	IgG1	A′ or B
10G5.4	G1+G2	IgG2a	C
807-22	G1+G2	IgG2a	D

To determine if these antigenic areas on LACV were present on other CE serogroup viruses, we initially examined six CE serogroup viruses, and one Bunyamwera group virus by indirect fluorescent assay on virus- infected Vero cells, using a pool of four anti -LACV G2 Mabs (Table 2). The Mabs tested were 3D9.4, 4A5.5, 7H12.1, and 9B7.5.

Table 2. Indirect Fluorescent Assay with Pooled Anti LACV G2 Mabs

Virus	Results
La Crosse	Positive
Jamestown Canyon	Positive
California Encephalitis	Positive
Snowshoe Hare	Positive
Trivatatus	Negative
Jerry Slough	Positive
Bunyamwera	Negative
Bluetongue-17 (neg control)	Negative

We then extended these studies to include additional viruses tested against individual Mabs. These results (Table 3) indicate that two antigenic areas are conserved on all four CE subgroup viruses examined, as well as on the three Melao subgroup viruses examined. The antigenic regions were not conserved on three Bunyamwera serogroup viruses nor on Trivattatus virus.

Table 3. Indirect fluorescent assay of bunyaviruses with anti-LACV G2 Mabs

		Mabs			
Serogroup/subgroup	**Virus**	**3D9.4**	**4A5.5**	**7H12.1**	**9B7.5**
CAL / CE	LAC	+	+	+	+
	SSH	+	+	+	+
	CE	+	+	+	+
	SA	+	+	+	+
CAL / Melao	JC	+	+	+	+
	KEY	-	+	+	+
	JS	+	+	+	+
CAL / TVT	TVT	-	-	-	-
BUN	BUN	-	-	-	-
	CV	-	-	-	-
	TEN	-	-	-	-

We are currently using *in vitro* mutagenesis combined with sequencing data to localize the antigenic regions on the G2 protein. Conserved regions may be important in determining mosquito infectivity and specificity.

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Isolation of Coltivirus from mosquitoes collected in Beijing and northeast part of China

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Several virus strains were isolated from mosquitoes collected in Beijing in 1994 and northeast part of China in 1996, respectively,. The biological characteristics showed that the new isolates caused cytopathogenic effects on C6/36 cells, but not on BHK and Vero cells , and not lethal for new born mice and three week old mice. The new isolates were resistant to 5'-IDU and ether,but sensitive to acid pH 3.0. Primary identification of isolates showed that they were nonenvelop 12 segment RNA by polyacrylamid Gel Electrophoresis (PAGE) and can be neutralized by immune sera of Coltivirus TRT2 , which was isolated from mosquitoes collected in Beijing in 1991. The results indicated that the new isolates can be identified as Coltivirus.

The PAGE profiles of Beijing new isolates were 6-6,which was similar to TRT2 strain,but the new isolates from northeast part of China were 6-5-1, which was different from that of TRT2 strain. The PAGE profiles of Coltivirus from both Beijing and northeast part of China was significant different from Colorado Tick Fever virus from USA.

CERULENIN BLOCKS LIPID SYNTHESIS AND INHIBITS MAYARO VIRUS REPLICATION IN *Aedes albopictus* CELLS. **H.S. Pereira and M.A. Rebello. Instituto de Biofísica Carlos Chagas Filho and Departamento de Virologia do Instituto de Microbiologia Prof. Paulo de Góes. Universidade Federal do Rio de Janeiro, 21941-590, Rio de Janeiro-RJ, Brasil.**

Cerulenin is an antibiotic synthesized by *Cephalosporium caerulens* that inhibits de novo fatty acid and cholesterol biosynthesis. The presence of this compound to growing cultures of diferent fungi, bacteria and yeast was found to result in the inhibition of growth of these organisms. The biological implications of phospholipid synthesis in the replication cycle of animal viruses remain largely unknow. The glycoproteins that form spikes on the surface of many enveloped animal viruses undergo a number of modifications as they move from their site of polypeptide synthesis on the endoplasmic reticulum through the golgi apparatus to the plasma membrane of the host cell. Among these modifications are the processing of protein-bound oligosaccharides and fatty acid acylation. In confluent monolayers of *Aedes albopictus* cells infected with Mayaro virus and treated with different concentrations of cerulenin, we found that this compound (20 ug/ml) inhibits in 95% the virus yield. Analysis of [^{35}S] methionine labeled virus proteins by SDS-polyacrylamide gel electrophoresis revealed that addition of cerulenin after virus entry, drastically inhibits virus specific proteins, whereas if the antibiotic is added later, 2, 3, 4 and 5 hours after infection, there is no effect on virus protein synthesis. We investigated the effect of cerulenin on the [^{3}H] glycerol uptake in chloroform soluble substances and in separate phospholipids by thin layer chromatography bidimentional and PI (Inorganic phosphate) dosage. At the earlier stages of infection we observed an inhibition of about 10% (1 ug/ml) in [^{3}H] glycerol incorporation reaching levels of 50% (20ug/ml). In separate phospholipids, we detected an inhibition of 40% in phosphatidilcholine (PC), 20% in phosphatidilethanolamine (PE) and 60% in phosphatidilinositol. At late stages of infection, the pattern of inhibition of [^{3}H] glycerol incorporation and separate phospholipids remained constant, indicating that the effect of cerulenin on replication of Mayaro virus is preferentially in early stages of infection.

Genetic analysis of *Aedes polynesiensis* using isoenzymes and Random Amplified Polymorphic DNA markers.

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Introduction

Aedes polynesiensis (Marks), a mosquito endemic from the Polynesian islands transmits the human lymphatic filariasis caused by the nematode *Wuchereria bancrofti* var. *pacifica* (Kessel, 1957). Our investigations in French Polynesia based on isoenzyme analysis of *Ae. polynesiensis* strains have demonstrated variations in allelic frequencies at some neutral loci. Mosquito spreadings using human unintentionnal transports among islands are source of intensive gene flow (Failloux *et al.*, 1997). Compared to the classical isozyme approach which investigates a limited number of polymorphic loci (Tabachnick and Powell, 1978; Tabachnick *et al.*, 1979), a recent method based at DNA sequences resolution level known as R.A.P.D. (Random Amplified Polymorphism DNA) (Saiki *et al.*, 1988; Williams *et al.*, 1990) was considered to be more powerful in the detection of polymorphic genetic markers. The present report tends to investigate the contribution of RAPD-PCR in mosquito genetic differentiation compared to the classical method of isozyme polymorphism.

Materials and methods

Three *Ae. polynesiensis* strains were used: Tahiti (Society archipelago), Nuku-Hiva (Marquesas archipelago), and Tubuai (Australs archipelago). They were originally collected in 1992 in the field (F0) and were established in colonies for 30 generations (F30) under insectarium conditions (T° = 25±1°C, Relative Humidity = 80±10%).

Electrophoresis of single adult homogenates were carried out on starch gel in TME 7.4 buffer systems (Pasteur *et al.*, 1988). Three enzymatic systems were investigated: esterases (Est, EC 3.1.1.1.), glutamate-oxaloacetate transaminases (Got, EC 2.6.1.1.), and phosphoglucomutase (Pgm, EC 2.7.5.1.). Genetic variability of each strain was assessed from the frequency of alleles at each locus. Genetic differentiation between strains was tested using Fisher's exact test on RxC contingency tables for each locus (Raymond and Rousset, 1995).

Batches of adult mosquitoes were ground to powder in liquid nitrogen. The genomic DNA extracts were used as template to PCR reactions performed at low stringency conditions (for method see Failloux, 1994). The four primers used were random 10-base oligomers: 10TS1 5' ACG GTA CAC T 3', 10TS3 5' TGG TCA CTG T 3', 10TS5 5' ATT GCG TCC A 3', and 10TS10 5' ACG GCA CCC T 3'. Amplification DNA products from each mosquito strain were analyzed on agarose gels. Only reproducible strongly amplified fragments between two assays using the same primer and the same mosquito strain were considered. The presence or the absence of fragments within these size ranges for each strain was scored.

Results

Genetic differentiation among samples was highly significant ($P<10^{-3}$) except for the 3 laboratory strains at the locus Est1 (P=0.103) (Table 1). When comparing the genotypic differentiation at each locus for all pairs of samples, combinations between colonies, Tahiti *versus* Nuku-Hiva, and Tahiti *versus* Tubuai, led to a significant differentiation at the loci Pgm and Got2. Nuku-Hiva and Tubuai colonies were differentiated at the locus Got2. Comparatively, allelic comparisons of field populations were differentiated except at the locus Est1 (Tahiti x Nuku-Hiva, Tahiti x Tubuai) and Pgm (Nuku-Hiva x Tubuai).

When using the RAPD technique, a group of 3 fragments was obtained with the primer 10TS1 (800, 1000, and 1400 base pairs), 12 with 10TS3 (3900 - 600 bp), 4 with 10TS5 (4800, 4400, 4200, 3700 bp), and 9 with 10TS10 (1000 - 3900 bp). The calculated percentage of homogeneity ranged from 0.4 (Nuku-Hiva/Tubuai with 10TS10) to 1.00 (Tahiti/Tubuai with 10TS5). Tahiti strain has less diverged from Tubuai and Nuku-Hiva than did Nuku-Hiva from Tubuai.

Conclusion

Based on the enzyme polymorphism, the genetic differentiation estimated among populations was proved to be more important than among established colonies. The lower level of polymorphism inside each colony may enhance the genetic divergence among them and should be considered when making inferences concerning field populations. Comparatively, RAPD markers revealed from DNA isolated from pools of mosquitoes do not permit the detection of any additionnal genetic variation.

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Table 1. Test of genotypic differentiation among *Aedes polynesiensis* samples from F0 and F30. All refers to the combined probability (Fisher exact test) over all loci. Bold characters indicate significant ($P < 0.05$) values.

	(N)	Pgm	Est1	Got2
F0	3	**0.0009**	**$<10^{-5}$**	**$<10^{-5}$**
TahitiXNuku-Hiva	2	**0.004**	**0.031**	0.383
TahitiXTubuai	2	1.00	**0.002**	**0.0001**
Nuku-HivaXTubuai	2	**0.004**	**<10-5**	**0.0007**
F30	3	**$<10^{-5}$**	0.103	**$<10^{-5}$**
TahitiXNuku-Hiva	2	**0.0002**	0.247	**$<10^{-5}$**
TahitiXTubuai	2	**$<10^{-5}$**	0.299	**$<10^{-5}$**
Nuku-HivaXTubuai	2	0.162	**0.04**	**0.0005**

Remote sensing recognition of *Ixodes ricinus* habitats presenting a high epidemiological risk

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Attention was directed to application of remote sensing (RS) methods to the landscape epidemiology of tick-borne diseases with the target to prepare a predictive map of *Ixodes ricinus* high occurrence habitats and of tick-borne diseases risk assessment for the prevention measures. The vegetation types have been used as the indicators of an ecosystem suitable for tick occurrence, for pathogens circulation, and, accordingly, for the existence of natural foci of these infections.

RS methods have been used to determine the indicative plant cover. Satellite data covering an area over 11,500 km^2 in Central Bohemia, the Czech Republic, was acquired by the LANDSAT 5 TM scanner (spatial resolution of 30 meters). The territory under study contains a large diversity of landscape and vegetation types and it contains also very active tick-borne encephalitis (TBE) and Lyme borreliosis (LB) natural foci. Data processing has been accomplished on the IBM RISC 6000 workstation using professional image processing software EASI/PACE of PCI Inc., Canada. Nine forest classes have been recognized in the scene by successive supervised, unsupervised, and once again supervised classifications, and identified in a field checking botanical survey.

Beside the conclusions dealing with the importance of different plant types for high *I. ricinus* occurrence, also the epidemiological TBE and LB maps based on human cases contracted in the territory under study were exploited for the evaluation of particular forest classes significance. These maps are based on routine reports on human cases excerpted from a National Epidemiological Register which has been widely contributed by clinicians for years. The reported places of infection (i. e. tick bites loci in anamneses) were located in a geographical information system (GIS) by matching the places names with those in a database of reference coordinates. Altogether 469 TBE and 866 LB cases have been validated and located.

Short characteristics of studied nine forest classes are as follows:

1. Spruce wood cultures with homogeneous structure.
2. Coniferous (mostly spruce) wood cultures with heterogeneous structure.
3. Mixed woods mostly with tall stems, and with prevalence of conifers.
4. Deciduous shrubs with fine grain size of the stand mosaic.
5. Mixed woods stands mostly young low, and dominated by deciduous trees.
6. Young deciduous stands with highly heterogeneous structure (mostly ecotones).
7. Young deciduous wood stands with homogeneous structure.

8. Tall deciduous woods with homogeneous structure.
9. Tall deciduous woods with heterogeneous structure.

This specification of the forest types has been accepted for the description of forest types having been correlated with tick presence, and TBE or LB risk.

Prior to questing correlation between the maps of TBE, LB, and remotely classified woods, the coordinate systems, projections, and spacing of the grids were unified, and continuous TBE- and LB-risk values were ranked into 8 levels (zones). Being aware of a lack of normality and disparate spatial resolution of the data-layers, a nonparametric robust approach was applied. It is based on an assumption that if there is a forest class linked with the disease, its density would increase from zone to zone along with increasing risk disregarding an absolute abundance of the class in the scene. First, an observed frequency matrix of all 9 x 8 class-risk combinations was computed across the scene; second, an expected one was calculated under the assumption of complete spatial randomness; third, a matrix of relative excesses (i. e. no. of observed/no. of expected pixels per combination) was estimated; fourth, Spearman rank correlation coefficient (SCC) was determined within each row of the excess matrix. Eventually, the resulted SCC's were tested against the null hypothesis: no positive or negative tendency in an aggregation of a class with increasing risk is apparent. An alternative is, of course, either positive or negative class-to-risk association, the absolute magnitude of SCC indicates a 'firmness' of such an association.

Correlation of the classified satellite image with the TBE map: As could be expected, negative association evinces the "non-forest" class "0" (encompassing all agricultural and build-up landscape structures, etc.) Both "coniferous" classes "1" and "2" exhibit neutral relationship with TBE risk. The rest of forest classes shows positive association with the disease in a different level. The class "6" (young broadleaved stands with highly heterogeneous structure, including also ecotones) reveals the closest association with elevated risk zones.

Correlation of the classified wood types with LB map: By analogy with TBE, negative association could be, again, documented in the "non-forest" class "0", while the "coniferous" classes "1" and "2" exhibit neutral position. From the class "3" through the class "9" the positive association increases, reaching a local maximum in the class "6" again. The class "9" exhibits the closest association with LB risk.

The earlier results, received with TBE model and with a smaller shifted satellite scene (model area 70 x 70 km) have been corroborated with more representative data. It might be indicative of relative stability of the method, of course, within a similar landscape. Apart from a general pattern of risk gradation from coniferous to deciduous wood types, both TBE and LB date suggest specific position of the heterogeneous deciduous forest class "6" which presents a local maximum in either risk gradient. Epidemiological significance of the other particular forest classes was assessed by the degrees of positive class-to-risk association.

Predictive maps are realized both in digital, and in printed forms in the scale of 1 : 300 000 for an overall risk evaluation, and in the scale 1 : 25 000 for a detailed

local orientation.

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THE ROLE OF SOME PROINFLAMATORY Th1 AND Th2 CYTOKINES IN THE PATHOGENESIS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS)

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Introduction: In spite of numerous genetic and serologic analyses of different hantaviral isolates world-wide, especially since the hantaviral pulmonary syndrome (HPS) has been recognised in the USA[1], little is known about the pathogenesis of HFRS. Scientists make some strategies to fight against hantaviruses, but our body has its own strategy for defence as well[2,3,4,5]. The aim of our study, was to measure some proinflammatory cytokines that may have role in the pathogenesis of HFRS.

Materials and Methods: We use EIA-test (Quantikine, R&D Systems Inc., UK) for the detection of IL-2, IL-2sRα, IL-6 and IL-6sR in sera of 41 seropositive HFRS patients. Samples were collected among the Croatian soldiers during the greatest HFRS outbreak in Croatia in 1995[6]. During the outbreak, we were looking for immunophenotypic changes in the main lymphocyte populations and activation markers in 22 HFRS patients.

Results: We found elevated levels of IL-2sRα, IL-6 and IL-6sR in patients with HFRS in comparison to healthy controls. IL-2sRα and IL-6 showed negative correlation with the day after the HFRS onset. In 14 patients IL-2sRα positively correlated with the CD25, an early activation marker (detected during the study in 1995). In the same patients we also found positive correlation among the IL-6 and $CD20^+CD21^+$ double-positive B-lymphocytes, CD23, B-cell activation marker and $CD4^+$ and $CD8^+$ - lymphocytes simultaneously expressing both CD45RA and CD45RO markers.

Conclusion: We found an increase of tested proinflammatory cytokines in the early phase of HFRS. Also, we could consider that measured cytokines act as an autocrine and paracrine factors, driving the expansion of antigen-specific lymphocytes and influencing the activity of the other cells within the immune system. Our next aim would be to correlate clinical and biochemical data with the immune response, and to look for their role in the prognosis of HFRS.

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