December, 1994

TABLE OF CONTENTS

DENGUE

Multiple host contacts and transmission of dengue-2 virus by *Aedes aegypti* (JL Putnam, TW Scott)

Improving the stability of dengue virus hemagglutination antigens (V Vázquez, R Hidaldo, G González, I Gutierrez, L Valdés, JO Castañeda)

An ELISA for the detection of monoclonal antibodies against dengue virus E and NS1 proteins (S Vázquez, M Pupo, JL Pelegrino, L Morier, A Castillo, A Otero, MG Guzmán)

Epidemic of dengue fever with dengue hemorrhagic cases in Fortaleza, Ceará State, Brazil, 1994 (PFC Vasconcelos, DB de Menezes, LP Melo, ETF Paula Pessoa, SG Rodrigues, ES Travassos da Rosa, MJ Timbó, ICB Coelho, F Montenegro, JFS Travassos da Rosa, FMO Andrade, APA Travassos da Rosa)

Circulation of dengue-2 in the northwest of Mexico (C Ramos, D Hernández, P Chen, J Ramos-Casteñada, E Gallardo)

VECTOR COMPETENCE AND VECTOR BIOLOGY

The emergence of *Anopheles albitarsis* in south-eastern Brazil (OP Forattini, I Kakitani, E Massad, D Marucci)

The mosquito vectors (Diptera: Culicidae) of yellow fever in south of Maranhao State, Brazil, 1993-1994 (N Dégallier, B Mondet, APA Travassos da Rosa, PFC Vasconcelos, JFS Travassos da Rosa, ES Travassos da Rosa)

Direct pathological effects and/or vector role of *Ixodes pari* (= *I. frontalis*) for wild birds in France (C Chastel, C Guiguen, JC Beaucournu, O Chastel)

DEVELOPMENT AND APPLICATION OF TECHNIQUES

Retrospective analysis of the ability of antigen capture EIA to detect field isolates of St. Louis encephalitis, western equine encephalitis, and eastern equine encephalitis viruses (RS Nasci, CJ Mitchell)

PCR-based detection of arboviral RNA from mosquitoes homogenized in detergent (MH Vodkin, RJ Novak)

Ultramicroelisa adenovirus: a new tool for seroepidemiological studies (C Savó, J Laferté, A Goyenechea, A Valdivia, L Morier)

BIOLOGY

Infection with Japanese encephalitis virus of cultured mouse brain astrocytes (NK Suri, K Banerjee)

HANTAVIRUSES

Nephropathia epidemica (NE) complicated by adult respiratory distress syndrome (ARDS) and acute renal failure (ARF): a novel pneumo-renal syndrome (P Colson, Ph Lefebvre, C Lescot, J Clement, P McKenna, J Neyts, J Coeck)

OTHER

Phylogenetic relationships of ovine, bovine and porcine pestiviruses (DG Sullivan, R Akkina)

ELISA and ultramicroELISA for Coxsackie A9 and antiviral antibodies in relation with an outbreak of Epidemic Nephropathy in Cuba (A Balmaseda, J Laferte, M Soler, S Vazquez, L Rodriguez, A Otero, V Vazquez, R Falcon, R Hidalgo, JL Pelegrino, R Palomero, MG Guzman, S Resik, P Mas)

ProMED - the Program for Monitoring Emerging Diseases

Numerous recent episodes of emerging and re-emerging infections, including the global AIDS pandemic, the continuing spread of dengue viruses, the now frequent appearance of hitherto unrecognized diseases such as the hemorrhagic fevers, the resurgence of old scourges like tuberculosis and cholera in new, more severe forms, and the economic and environmental dangers of similar occurrences in animals and plants, attest to our continuing vulnerability to infectious diseases throughout the world. Many experts, both within and outside government, have warned of the need to improve capabilities for dealing with emerging infectious diseases, and the development of an effective global infectious disease surveillance system has been the primary recommendation of expert analyses.

A program to identify and quickly respond to unusual outbreaks of infectious diseases in order to provide help to affected areas and to prevent spread is essential, not only to the region of origin but to the entire world. Unfortunately, existing international structures to do this are understaffed and lack coordination. The same is true for animal and plant diseases which could threaten food supplies and, in some cases, infect humans - some of the outbreaks that have attracted attention recently, such as Hantavirus pulmonary syndrome, are zoonoses.

ProMED, the Program for Monitoring Emerging Diseases, was set up specifically to fill this void. It was inaugurated in September 1993 at a conference in Geneva, Switzerland, co-sponsored by the World Health Organization and the Federation of American Scientists. At that conference 60 prominent experts in human, animal and plant health called for a coordinated global program to identify and respond to emerging infectious diseases, and to provide a forum for coordinating plans, with the participation of interested parties at all levels. Members of the Steering Committee of ProMED come from all over the world and include representatives of WHO, CDC, NIH and OIE (the International Office of Epizootics), in addition to other organizations and academic institutions.

ProMED electronic conference

A central goal of ProMED is to establish a direct partnership among scientists concerned with infectious diseases in all parts of the world; building the appropriate networks to encourage communicating and sharing information is a key objective. In cooperation with SatelLife and HealthNet, ProMED has inaugurated an e-mail conference system on the Internet, to encourage timely information sharing and discussion on emerging disease problems worldwide. Through HealthNet, this low-cost system reaches participants in developing countries and remote areas.

ProMED invites and welcomes the participation of all interested colleagues. To sign on to the ProMED electronic conference, send an e-mail message to:

promed-request@usa.healthnet.org

Leave the Subject line blank, and write **subscribe promed** in the text space. You will receive an automatic reply with information on how to access past files. From then on, you will

receive the messages posted to the ProMED conference as they are received. You can cancel this at any time. The only cost is your phone call to your e-mail server.

Dr Stephen S. Morse, Chair, ProMED, The Rockefeller University, New York NY
Dr Jack Woodall, Coordinator, ProMED Communications Task Force, NYS DOH, Albany NY

NEW PERIODICAL ON EMERGING INFECTIONS PLANNED

The Centers for Disease Control and Prevention (CDC) plans to launch a new journal, Emerging Infectious Diseases. The publication, which will debut in January 1995, is part of CDC's plan for combatting emerging infectious diseases; the plan is outlined in a recently published document, "Addressing Emerging Infectious Disease Threats - a Prevention Strategy for the United States." One of the main goals of CDC's plan is to enhance communication of public health information about emerging diseases, so that prevention measures can be implemented without delay.

Emerging Infectious Diseases will be peer reviewed and will provide information on emerging infections in three broad categories:

1) **PERSPECTIVES**: a section addressing factors that underlie disease emergence, including microbial adaptation and change; human demographics and behavior; technology and industry; economic development and land use; international travel and commerce; and breakdown of public health measures.

2) **SYNOPSES**: concise state-of-the-art summaries of specific diseases or syndromes, and related emerging infectious disease issues.

3) **DISPATCHES**: brief laboratory or epidemiologic reports with an international scope.

Editor of Emerging Infectious Diseases will be Joseph E. McDade, Ph.D., National Center for Infectious Diseases, CDC. Section editors will be Stephen S. Morse, Ph.D., Rockefeller University; Phillip J. Baker, Ph.D., National Institute of Allergy and Infectious Diseases, NIH; and Stephen Ostroff, M.D., National Center for Infectious Diseases, CDC.

Emerging Infectious Diseases will be published quarterly, and CDC plans to make it accessible through the Internet (File Transfer Protocol) as well as through ProMED (see NOTICES, page ii). Dispatches may become available separately as soon as they have been cleared for publication.

Emerging Infectious Diseases will be sent to all current subscribers of the Arbovirus Information Exchange.

Emerging Infectious Diseases actively solicits and welcomes contributions.

For additional information about receiving and contributing to this new journal, contact Editor, Emerging Infectious Diseases. E-mail address: eideditor@cidod1.em.cdc.gov

Editor, Emerging Infectious Diseases, Mailstop C12, National center for Infectious Diseases, CDC, 1600 Clifton Rd., Atlanta, GA 30333

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0015 1 303 225 1903
Dr Charles E Calisher

Dear Charlie,
I have just got finalisation on the dates for a regional symposium on bluetongue in China. Is it possible to make the next Arbovirus Information Exchange with an announcement?. The intention is to have a look at these viruses in the somewhat unknown area of the world as far as bluetongue is concerned. A joint research project has begun with ACIAR, the same group who sponsored the Beijing BEF symposium.

South-East Asia Pacific Regional Bluetongue Symposium

A 3 day symposium , 22-24 August 1995, will be held in Kunming , Peoples Republic of China on bluetongue, in this first regional review of this important group of arboviruses and the disease they cause.
The topics will be limited to aspects of pathogenesis, epidemiology and its effects , vaccines and control, and vector entomology. The bulk of invited delegates will be from various regions of China, countries of south-east and east Asia to Australia. Other key speakers are expected from additional countries with an interest in bluetongue.
Further information for those interested in attending can be obtained by contacting

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Editor's comments

The 43rd annual meeting of the American Society for Tropical Medicine and Hygiene was held in Cincinnati, Ohio, 13-17 November, 1994. Attendance at these gatherings allows one to attend Society committee meetings, symposia, scientific sessions, and commercial exhibits, and to have the opportunity to renew acquaintances and make new ones. The general meeting was well attended and the American Committee on Arthropod-borne Viruses (ACAV) was quite active. At least to me, there seems to be a renewed interest in arboviruses and a younger cadre of arbovirologists forming.

Pertinent to the readership of Arbovirus Information Exchange, there were sessions or symposia on hantaviruses and arenaviruses; "Speciation and Implications for Vector Borne Diseases" (sponsored by the American Committee on Medical Entomology); "Spatial Analysis of Infectious Disease Dynamics"; alphaviruses; flaviviruses; and viral epidemiology. The ACAV Open meeting symposium was entitled "Molecular taxonomy and phylogeny of viruses: practical approaches". These sessions were well attended and discussions often were lively.

Through the good offices of the International Committee on Taxonomy of Viruses, a definition of the term "species" (type), as related to viruses, has been published (Van Regenmortel, M.H., J. Maniloff, and C.H. Calisher. The concept of virus species. Arch. Virol. 120:313-314, 1991). However, this definition does not (cannot) clearly spell out the practical details and, therefore, a current problem is the definition of a virus species, a problem caused by the great variety in virus characteristics and the rapid development of molecular methods. It is now possible to identify a virus, partially characterize it, express its proteins, prepare antibodies against those proteins, devise a vaccine, define the epidemiology of the virus, etc. before the virus was isolated; this has been done with the virus causing Hantavirus Pulmonary Syndrome. The problem enters when someone attempts to register a well-characterized virus that has not been isolated. This is not peculiar to arboviruses by any means, c.f. Hepatitis C virus. The ACAV Subcommittee on InterRelationships Among Catalogued Arboviruses (SIRACA) met in Cincinnati and discussed at length many facets of this problem. Aided by the considerable input and expertise of Drs. Connie Schmaljohn (USAMRIID) and Stuart Nichol (CDC), SIRACA was able to come to some conclusions (see FEATURED REPORT, page 1). This report clearly has set a precedent.

On a more personal note, I recently returned from another month in Australia. (My wife was painting the house, so I did not return to Ft. Collins until I thought she was finished.) This trip took me to Sydney (Westmead Hospital), Canberra (Australian National University), Wagga Wagga (Charles Sturt University), Melbourne (Australian Society for Microbiology annual meeting), Geelong (Australian Animal Health Laboratory), and a self-guided tour of Tasmania. The days were excellent and some of the nights were remarkable. An equine outbreak (and a fatal human infection) of an apparently newly recognized morbillivirus was the scientific highlight of the stay; the people at Geelong and elsewhere in Australia did a marvelous job of working out the epidemiology, controlling the outbreak, excluding known agents, and isolating and identifying the virus. Electronic mail systems such as ProMed (see NOTICES, page ii) and on-line periodicals such as Journal of Emerging Diseases (see NOTICES, page iv) should help keep us up-to-date on such happenings and are at no cost to you. I recommend them to the readers of Arbovirus Information Exchange.

Book Review

Positive-Strand RNA Viruses, M.A. Brinton, C.H. Calisher, and R. Rueckert (eds), Springer-Verlag, Vienna, 1994.

This is a supplement to Archives of Virology, based on the plenary lectures from the Third International Symposium on Positive Strand RNA Viruses, held in Clearwater, Florida, in September 1992. It was published in early 1994, and I would have had this review ready earlier, but (among other things) I lent the book to some DNA virologist friends who were working with alphavirus expression vectors. They found it useful.

Like its predecessors, the strength of this meeting was in bringing together researchers who discussed similarities in structure and molecular biology between viruses of plants, insects, and vertebrates.

There are nine major headings, titled "Genome replication and transcription," "RNA recombination," "RNA replication", "RNA-protein interactions and host-virus interactions", "Protein expression and virion maturation", etc. As these suggest, the emphasis is on molecular biology and there is a great deal of overlap between sections, and assignment of each of the short papers to a section is fairly arbitrary. There is no cross-referencing between chapters or sections, and there is no index, so each chapter stands on its own as a concise review. A major underlying theme is application of recent findings on molecular structure and replication to control of virus infections through vaccine, drug, transgenic, and immune modulation strategies.

Most of the papers have a minireview format. Some summarize several years' recent work from a particular laboratory, such as Venezuelan equine encephalitis virus pathogenesis as defined by molecular genetics (Nancy Davis, Bob Johnston, et al) and use of transgenic mice to study poliovirus pathogenesis (Vincent Racaniello); whereas others describe specific experiments done over a short period of time, such as classification by cDNA cloning and sequencing of astro- and caliciviruses (M.J. Carter) and Borna virus (which is determined to be a negative-strand RNA virus; Janice Clements et al.). Most are concise and well-written, and are current (despite the tardiness of this review), due to their nature as plenary lectures rather than research reports.

Only eight or so of the 52 chapters are on arboviruses, and those do not deal with the arthropod-borne aspects of the alpha and flaviviruses which are discussed. Nevertheless, this book is an excellent resource for teaching and for consideration of new approaches to old research problems. Check the price and ask your library to purchase it.

Reviewed by: Carol Blair, Ph.D., Department of Microbiology, Colorado State University, Fort Collins, CO 80523

The International Committee on Taxonomy of Viruses Study Group on Unclassified Viruses is accumulating information about unclassified viruses. If you have any unclassified viruses, from any source (human, arthropod, rodent, elephant, wombat, ostrich--anything), please let me know. At this time, we do not want the viruses, all we want is the available information. I look forward to hearing from you.

Charlie Calisher
Chairman, SGUV

If you are willing to be part of an e-mail network of arbovirologists, please send me your e-mail address. I would like to put together a directory of as many arbovirologists and others as I can.

Also, and if there is sufficient interest, I might be able to provide the Arbovirus Information Exchange on the Internet. Interested?

Charlie Calisher
ccalisher@vines.colostate.edu

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

PLEASE READ CAREFULLY

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

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

c/o DVBID, NCID, CDC, P.O. Box 2087, Ft. Collins, CO 80522 (U.S.A.) or

Arthropod-borne and Infectious Diseases Laboratory, Colorado State University Foothills Campus, Ft. Collins, CO 80523 (U.S.A.) TEL: (303) 491-8604 or (303) 223-8586, FAX: 303-491-1815 or 303-225-1903

You also may send reports to me by **e-mail**: ccalisher@vines.colostate.edu

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

C.H. Calisher

NEXT ISSUE

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

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

At the 1994 meeting of this Subcommittee, held during the 43rd annual meeting of the American Society of Tropical Medicine and Hygiene (Cincinnati, Ohio), SIRACA approved a proposal for the taxonomy of hantaviruses based on molecular characteristics. The proposal was founded on sequence data available from GEN BANK and is compatible with serological classification where serologic data are also recorded.

- A hantavirus type (species) differs by at least 25% of nucleotides in at least one gene segment from all other hantaviruses. Additionally, the fit in the evolutionary tree is consistent with type status.

- A hantavirus subtype (subspecies) differs by 5-24% of nucleotides in at least one gene segment.

- Sequences differing by less than 4% in all three gene segments are the same subtype.

- An isolate ideally should be available. In the absence of an isolate, whether or not sequence data are acceptable as a basis for taxonomic classification and for registration in the International Catalogue of Arboviruses will be the decision of the Subcommittee on Information Exchange and of the Executive Council, American Committee on Arthropod-Borne Viruses.

Current molecular taxonomic status of recognized hantaviruses.

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

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

Robert E. Shope (Chairman), Walter Brandt, Charles H. Calisher, Jordi Casals, Erik Henchal, Nick Karabatsos, Dennis Knudson, Thomas Ksiazek, James LeDuc, Patricia Repik, John Roehrig, Robert B. Tesh

EMERGING AND RE-EMERGING INFECTIOUS DISEASES: WHO RESPONDS TO A GLOBAL THREAT

The World Health Organization called today (26 April 1994) for the establishment of a global network of scientific centres to identify and combat new or re-emerging diseases, including those caused by antibiotic-resistant strains of infectious agents, that threaten the lives of countless millions of people.

International experts are meeting at WHO headquarters in Geneva today because of growing concern about such diseases, and the related problems of their spread by international travel, the international exchange of foodstuffs and live animals, and resistance to antibiotics. Under the chairmanship of Nobel prize-winner, Professor Joshua Lederberg, of Rockefeller University, New York, the experts are discussing how the planned network of surveillance and monitoring centres can be set up.

New and re-emerging infectious diseases are those that have been recently discovered, have increased in humans during the last 20 years, or threaten to grow in the near future. The most dramatic example is AIDS, due to the human immunodeficiency virus (HIV) whose existence was unknown barely 10 years ago. The resurgence of tuberculosis, including antibiotic-resistant strains, and the spread of other diseases regarded until recently as under control - such as cholera, yellow fever, and dengue - are further examples.

In addition, there have been recent epidemics of infections due to contaminated food and public water supplies, and the emergence of new diseases, such as Hantavirus Pulmonary Syndrome. This hitherto unrecognized disease was recently discovered in the southwestern United States. It is caused by a previously unknown hantavirus, transmitted to humans by field mice, and has been fatal in more than half the 60 cases now identified.

The last few years have also seen the emergence of a completely new strain of cholera in Asia; and, in South America, Venezuelan haemorrhagic fever and Brazilian haemorrhagic fever - both fatal and both caused by newly-recognized arenaviruses, related to that which causes Lassa fever in Africa.

"These examples vividly illustrate that the world remains extremely vulnerable to new and re-emerging infections, and emphasizes the need for increased vigilance", said Dr James Le Duc, of WHO's Division of Communicable Diseases, and organizer of today's meeting. "They also show the urgent need for a better global system to identify these infectious agents and permit a timely response."

In 1993, the biggest outbreak of water-borne illness ever recorded in the United States occurred - more than 400 000 people in Milwaukee, Wisconsin, suffered prolonged diarrhoea, and about 4400 of them needed hospital treatment. The cause: water supplies contaminated by human or animal faeces containing the intestinal parasite *Cryptosporidium*.

Other examples of resurgence of infectious diseases in 1993 include cholera in Latin America, yellow fever in Kenya - where outbreaks of the disease had never been recorded before - outbreaks of illness caused by the food-borne bacterium, *Escherichia coli (E. coli)* in South Africa and Swaziland, dengue in Costa Rica, and diphtheria in Russia.

MULTIPLE HOST CONTACTS AND TRANSMISSION OF DENGUE-2 VIRUS BY AEDES AEGYPTI

John L. Putnam and Thomas W. Scott
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Relative to a mosquito contacting only one host per gonotrophic cycle, multiple host contacts could increase mosquito-borne disease by increasing the opportunities for susceptible mosquitoes to become infected or for infected ones to transmit a disease (Klowden and Lea 1979, Scott et al. 1993a). An important untested assumption of the hypothesis that multiple host contacts by *Ae. aegypti* is an important component of dengue virus transmission (Scott et al. 1993a & Scott et al. 1993b) is that virus transmission is unaltered when mosquitoes repeatedly search for a blood meal--probing their mouthparts into host tissues--or imbibes multiple blood meals. If mosquito infectivity is reduced following an attempt to locate blood, subsequent contacts may not result in transmission of an infectious virus dose. Studies with malaria parasites support a rigorous challenge of the assumption that transmission is unaltered. Some of those studies suggested that multiple host contacts may reduce the infectivity of anopheline vectors (Burkot 1988, Rosenberg et al. 1990), but others concluded that one mosquito can transmit malaria parasites to a series of hosts during one gonotrophic cycle (Kelly & Edman 1992).

In this study we tested the assumption that multiple contacts do not alter virus transmission by examining the efficiency of dengue-2 virus transmission by infected *Ae. aegypti* that probed their mouthparts into the tissues and attempt to imbibe blood more than once from a vertebrate host during a single gonotrophic cycle. Transmission experiments were carried out with F_2 generation *Ae. aegypti* from San Juan, Puerto Rico and a dengue-2 virus strain isolated in 1986 from a 5 month old infant who became ill and died in San Juan. We used the Rexville strain of *Ae. aegypti*, also from San Juan, for amplifying dengue-2 viruses and as recipient mosquitoes during the assay for virus by mosquito inoculation. We infected female *Ae. aegypti* by intrathoracic inoculation (Gubler & Rosen 1974) or by allowing them to engorge from "hanging drops" of articially infected blood (Rosen & Gubler 1976, Miller et al. 1982). The mosquito inoculation technique (Rosen & Gubler 1974) in combination with the direct fluorescent antibody technique (DFAT) (Kuberski and Rosen, 1977) was used to assay for dengue-2 viruses. To determine if a mosquito was infective after probing, we used a transmission model with two components: 1) a guinea pig for the mosquitoes to probe into; and 2) Aitken's (1977) *in vitro* transmission assay to determine if the mosquitoes were infective. To evaluate the effect of probing on infectivity of *Ae. aegypti*, we compared transmission rates of mosquitoes that had just completed a probing bout to mosquitoes that had not probed. Probing bouts consisted of 5, 10 or 20 consecutive 45 second probes. Infection status of all mosquitoes was confirmed with the DFAT.

Probing 5, 10 and even 20 times consecutively did not significantly alter the infectivity of parenterally infected *Ae. aegypti* ($P > 0.05$, $df = 2$ or 3; Table 1). Orally

infected *Ae. aegypti*, that probed 20 consecutive times, transmitted dengue viruses at a significantly higher rate than did mosquitoes that had not probed prior to the transmission assay ($P < 0.05$, df = 2; Table 2).

We draw two conclusions from our study. First, our findings are consistent with the hypothesis that multiple host contacts contribute to dengue virus transmission. Second, our data suggests that *Ae. aegypti* remain infective with dengue virus regardless of their probing history. This information, combined with reports on the tendency for *Ae. aegypti* to take multiple blood meals (reviewed by Scott et al. 1993a & Scott et al. 1993b), implies that once infective *Ae. aegypti* are extremely efficient disseminators of virus.

ACKNOWLEDGEMENTS

We thank Drs. Gary Clark and Paul Reiter from the Dengue Branch, San Juan Laboratories, Centers for Disease Control for supplying us with mosquito eggs, conjugated antibodies, and dengue-2 virus.

LITERATURE CITED

Aitken, T. 1977. Mosq. News 37: 130-133.
Burkot, T.R. 1988. Parasit. Today 4: 156-162.
Gubler, D.J. and L. Rosen. 1976. Am. J. Trop. Med. Hyg. 25: 146-150.
Kelly, R. and J.D. Edman. 1992. J. Med. Entomol. 29: 329-331.
Klowden, M.J. and A.O. Lea. 1979. J. Med. Ent. 15: 514-517.
Kuberski, T. and L. Rosen. 1977. Am. J. Trop. Med. Hyg. 26: 533-537.
Miller, B.R., B.J. Beaty, T.H.G. Aitken, K.H. Eckles and P.K. Russell. 1982. Am. J. Trop. Med. Hyg. 31: 1232-1237.
Rosen, L. and D. Gubler. 1974. Am. J. Trop. Med. Hyg. 23: 1153-1160.
Rosenberg, R., R.A. Wirtz, I. Schneider and R. Burge. 1990. Trans. Roy. Soc. Trop. Med. Hyg. 84: 209-212.
Scott, T.W., G.G. Clark, L.H. Lorenz, P.H. Amerasinghe, P. Reiter and J.D. Edman. 1993a. J. Med. Ent. 30: 94-99.
Scott, T.W., E. Chow, D. Strickman, P. Kittayapong, R.A. Wirtz and J.D. Edman. 1993b. J. Med. Ent. 30: 922-927.

Table 1. *In vitro* transmission rates of dengue-2 virus by parenterally infected *Aedes aegypti* ... guinea pig 5, 10, or 20 times consecutively compared with mosquitoes that had not probed before the transmission attempt.

	% Transmission (transmission detected/total infected mosquitoes assayed)					
	Experiment 1		Experiment 2		Experiment 3	
Replicate	Following 5 probes	Control[a]	Following 10 probes	Control	Following 20 probes	Control
1	100 (5/5)	100 (5/5)	80 (4/5)	100 (5/5)	100 (3/3)	100 (4/4)
2	100 (5/5)	100 (5/5)	80 (4/5)	100 (5/5)	100 (4/4)	75 (3/4)
3	100 (5/5)	80 (4/5)	100 (5/5)	60 (3/5)	100 (4/4)	100 (1/1)
4	-	-	-	-	75 (3/4)	100 (4/4)
Mean	100 (15/15)	93 (14/15)	87 (13/15)	87 (13/15)	94 (14/15)	94 (12/13)
SE	0	6.6	7.4	7.4	4.4	4.4
Mean probe duration (sec)	33.7	-	28.4	-	28.4	-

[a] - Cohort mosquitoes that had not probed prior to the *in vitro* transmission assay.
... experiment no treatments were significantly different (paired t-test, $P > 0.05$).

Table 2. *In vitro* transmission rates of dengue-2 virus by orally infected *Aedes aegypti* after having probed a guinea pig 20 times consecutively compared with mosquitoes that did not probe prior to the transmission attempt.

Replicate	% Transmission (transmission detected/total infected mosquitoes assayed)	
	Following 20 probes	No probes prior to transmission
1	100 (4/4)	50 (2/4)
2	100 (3/3)	60 (3/5)
3	100 (6/6)	83 (5/6)
Mean	100[a] (13/13)	64 (10/15)
SE	0	9.8
Mean probe duration	32 sec	-

[a] - Significantly greater than mosquitoes that did not probe (paired t-test, $P < 0.05$).

Title:

IMPROVING THE STABILITY OF DENGUE VIRUS HEMAGGLUTINANTION ANTIGENS

Authors:

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The hemaglutination antigens from saccharose-acetone extracted brain of infected mice (Clarke and Casals, 1958) are highly thermo-labile, and moderate temperatures cause rapid loss of their activity (Monath, 1990). Preservation of them, therefore, requires low temperatue storage.
The hemagglutination activity of Dengue virus serotype 2 strain New Guinea was studied after freeze-drying (F/D) (9 hours, 6.2 mBares, -70 C to room temperature) with or without additives at intervals over a three month storage period at various temperatures (Table 1).
A mathemathical model based upon the Arrhenius equation was applied to calculate the relative degradation rate so as to describe its relation with temperature and time of storage in oder to predict stability under various conditions (Kirkwood, 1977).
The appearance of the final freeze-dried product was also evaluated by visual inspection.
The results indicate that gelatin (1%) and dextran (5%) improve the esthetic quality of freeze-dried Dengue virus type 2 hemagglutination antigens (D2HA) and saccharose protects its activity. It is also noticed that skimmed milk, despite the fact taht it confers a good appearance to the lyophilized cake, present a background hemagglutination activity which could be undesirable.
The validity of the model applied was assessed and the parameters estimated showed that shipment and storage of Dengue virus serotype 2 strain New Guinea hemagglutinins is possible without the need of very low temperatures under the proposed conditions, thereby reducing costs.

TABLE 1. Appearance and antigenicity of freeze dried D2HA immediately and after subsequent storage at either 37, 4 and -20 Celsius (T).

Additives[a]		(1)	(2)	(3)	(4)	(5)
Appearance[b]		001	111	110	000	010
Post F/D		8.317	8.317	NA[c]	8.317	8.317
T	days					
37	10	5.545	7.624	NA	7.624	6.238
	21	4.158	6.238	NA	6.931	4.852
	30	2.079	4.852	NA	6.238	3.465
4	10	6.931	7.624	NA	8.030	6.931
	30	5.545	6.643	NA	7.624	6.238
	45	4.158	5.545	NA	6.931	4.852
-20	60	6.931	7.624	NA	8.030	7.336
	91	6.643	7.336	NA	7.624	6.931

[a]. (1) 1.5 M NaCl, 0.5 M H_3BO_3, 1 M NaOH; (2) a mixture of 5% dextran, 1% gelatin and 5% saccharose; (3) a mixture of 4% peptone, 1% gelatin and 10% skimmed milk; (4) 30% saccharose and (5) a mixture of 5% peptone and 1% gelatin. Additives from (2) to (5) were prepared in (1).

[b]. Binary code for appereance evaluation. First digit 0, collapsed; 1, amorfous shape; second digit 0, dark; 1, solid clear color; last digit 0, formation of aggregates after reconstitution (turbid solution); 1, clear solution.

[c]. Not attempted.

Numbers represent the natural logarithm of hemagglutination activity.

REFERENCES

Clarke, D.H. and Casals, J. (1958) Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. J. Trop. Med. Hyg. 7(5), 561-573.

Kirkwood, T.B.L. (1977) Predicting the stability of biological standards and products. Biometrics. 33, 736-742.

Monath, T.P. (1990) Flaviviruses. In: B.N. Fields, D.M. Knipe et. al. (Eds), Virology. Raven Press Ltd, New York.

An ELISA for the detection of Monoclonal Antibodies against Dengue virus E and NS1 proteins.

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Dengue virus is composed of three proteins: the envelope (E), t membrane (M), and the capside (C) . The last one is complex with the single-stranded genomic RNA. The RNA also codifi for seven non structural proteins: NS1. NS2a, NS2b,NS NS4a, NS4b, and NS5.

The protective immune response of some of these proteins h been previously studied, E protein confers the most effecti protection and the NS1 protein seems to be also involved in t immune protection.

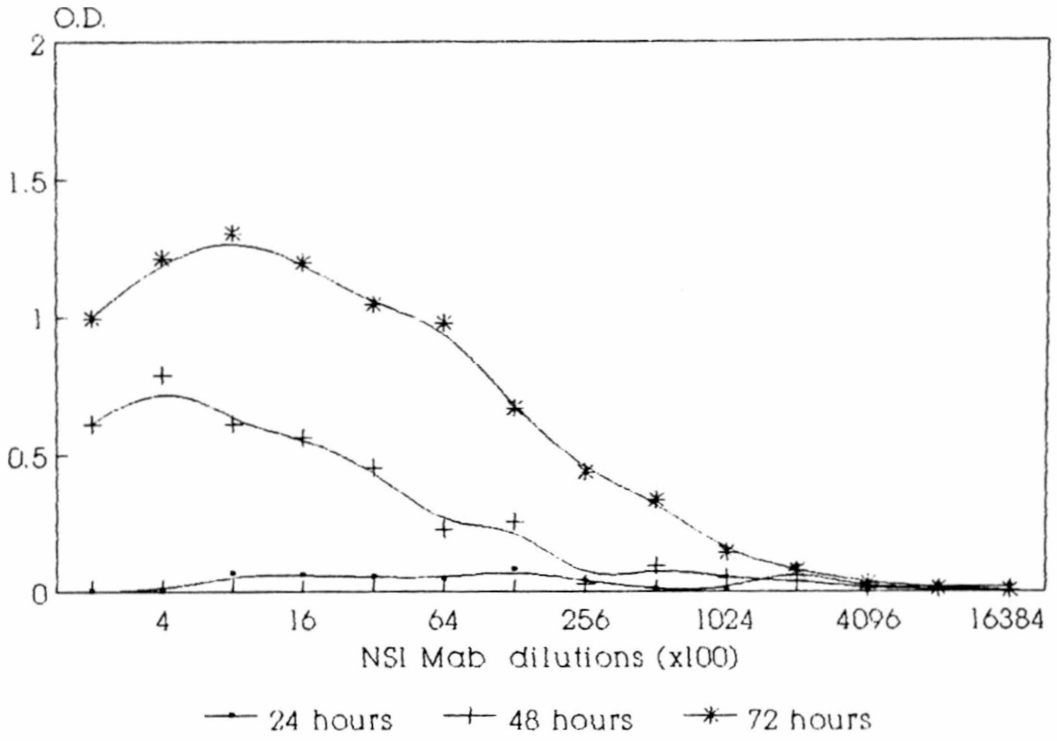
Monoclonal antibodies against dengue virus provide sensiti means for studying these proteins and they have be valuable tools in different studies.

A method is described for the detection of monoclon antibodies against NS1 non structural and E structural deng virus proteins. In this cellular ELISA, dengue 2 infect C6/36 mosquito cells were attached to flat bottomed microtit plates using glutaraldehyde as fixative. Anti-NS1 and anti Monoclonal antibodies were used for setting up the ass as positive controls. Normal ascitic fluid was used as negati control. Anti- mouse IgG peroxidase conjugate with appropria substrate solution were used for revealing the reaction.

A maximal response between controls were obtained with cel after 72 hours post inoculation for both proteins (Fig.1 and 2 The sensitivity was of 14.7 ng/ml for anti-NS1 and 1.43 ng/ml f anti-E Mabs.

We obtained a sensitivity less than 1 ug/ml for both kind antibodies, which is enough low for detecting monoclonal antibo producing hybridomas in culture supernatants.

We recommend this ELISA as a rapid and easy method for detecti relevant dengue monoclonal antibodies.



Figl. Titration of NSI Mab at differents times

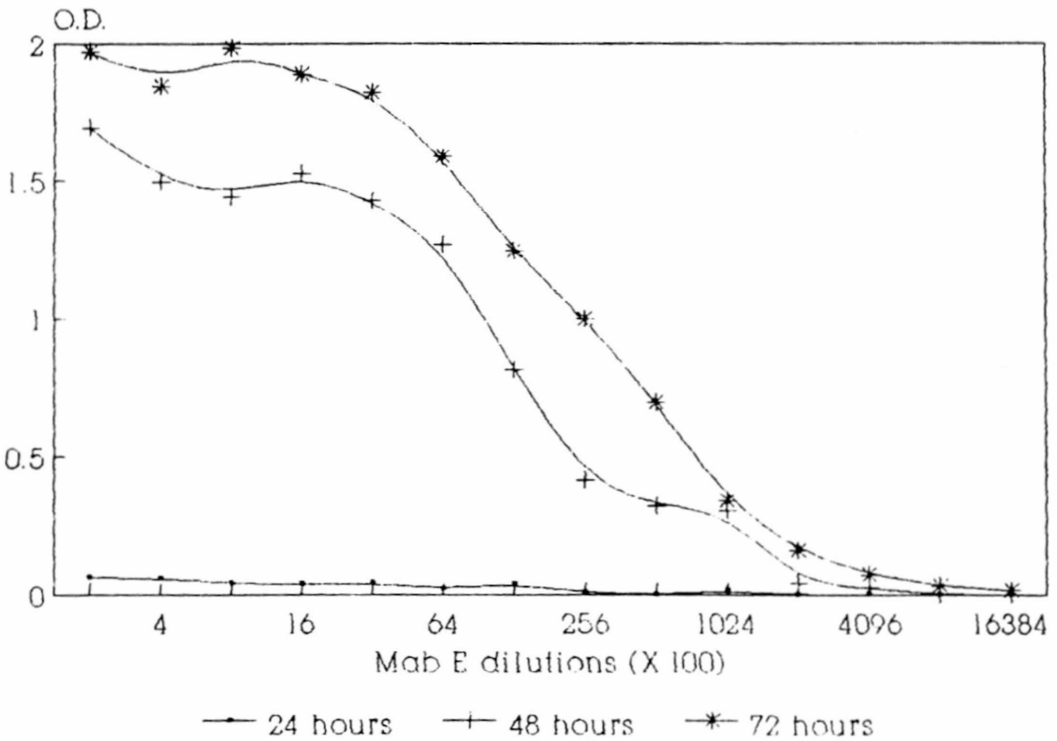


Fig2. Titration of Mab E at differets times

EPIDEMIC OF DENGUE FEVER WITH DENGUE HEMORRHAGIC CASES IN FORTALEZA, CEARÁ STATE, BRAZIL, 1994.

During the last eight years, the Ceará State have been experienced several outbreaks of dengue fever (DF). From 1986 to 1993, only serotype 1 of dengue virus was responsible for the epidemics. In 1994 dengue virus serotypes 1 (DEN 1) and 2 (DEN 2) were isolated from blood of sick persons. Several counties were affected, although Fortaleza city was the principal target area in this outbreak.

The first cases were reported in the 10th epidemiological week and the last ones in 32nd. In this period a total of 19,304 cases were reported in Fortaleza (Figure 1). Other counties have also notified cases to the Department of Epidemiology of Health Secretary of Ceará State (Figure 2), of which 178 were suspected of DHF. After an exhaustive revision according of WHO and Ministry of Health criteria's for DHF, 26 were confirmed (Figure 3) , age ranging from 13 to 93 years old with mean value of 42 y.o., (11 male and 15 female), 134 were discarded and 18 are being analyzed. Of the confirmed cases, 14 (54%) had fatal outcome (5 male and 9 female). Preliminary analysis of the temporal distribution of cases suggest the outbreak peak occurred towards the 21st and 23rd weeks.

Data collected from patients autopsied showed that the most common alterations at macroscopically level were gastrointestinal hemorrhages (especially gum bleeding and melena), ascites, bilateral pleural effusion and CNS edema. At microscopy level were found diffuse cappilarity and alveolar infiltrate. It is noteworthy that once more in Brazil, DHF have been associated with high severity in age groups between 19 to 49 y.o. The severe gastrointestinal bleeding was the main mechanism responsible to the fatal outcome. Notwithstanding, several patients have been manifested encephalitic signs as have been reported in Asian patients with DHF, in such cases, virus or signs of viral encephalitis were not found in the microscopy.

Through May-July 1994, 12 dengue virus isolates were obtained at IEC. Most isolates belonged to DEN 2 (11) and one was DEN 1. The strains were isolated from 68 samples inoculated. Two (one of each serotype) of these isolates came from fatal cases. Antidengue antibodies were demonstrated in 59 patients, among them, 9 DHF cases. With the exception of a girl of 13 y.o., whose immune response was primary type, all DHF patients examined showed secondary type response and have been confirmed by dengue IgM capture test (MAC ELISA).

Aedes aegypti control measures including ULV application and focal treatment, carried out by National Health Foundation, have broken virus transmission.

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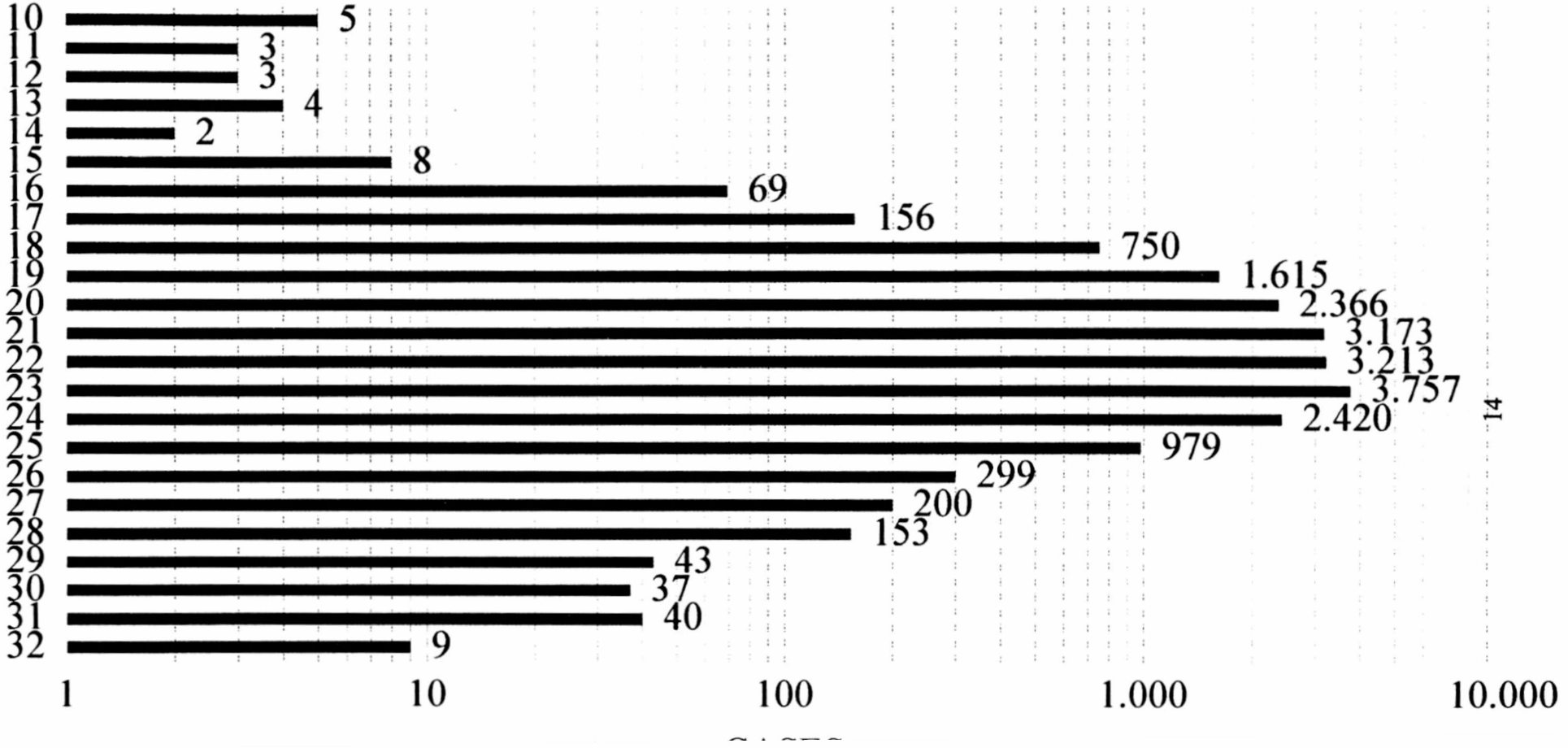
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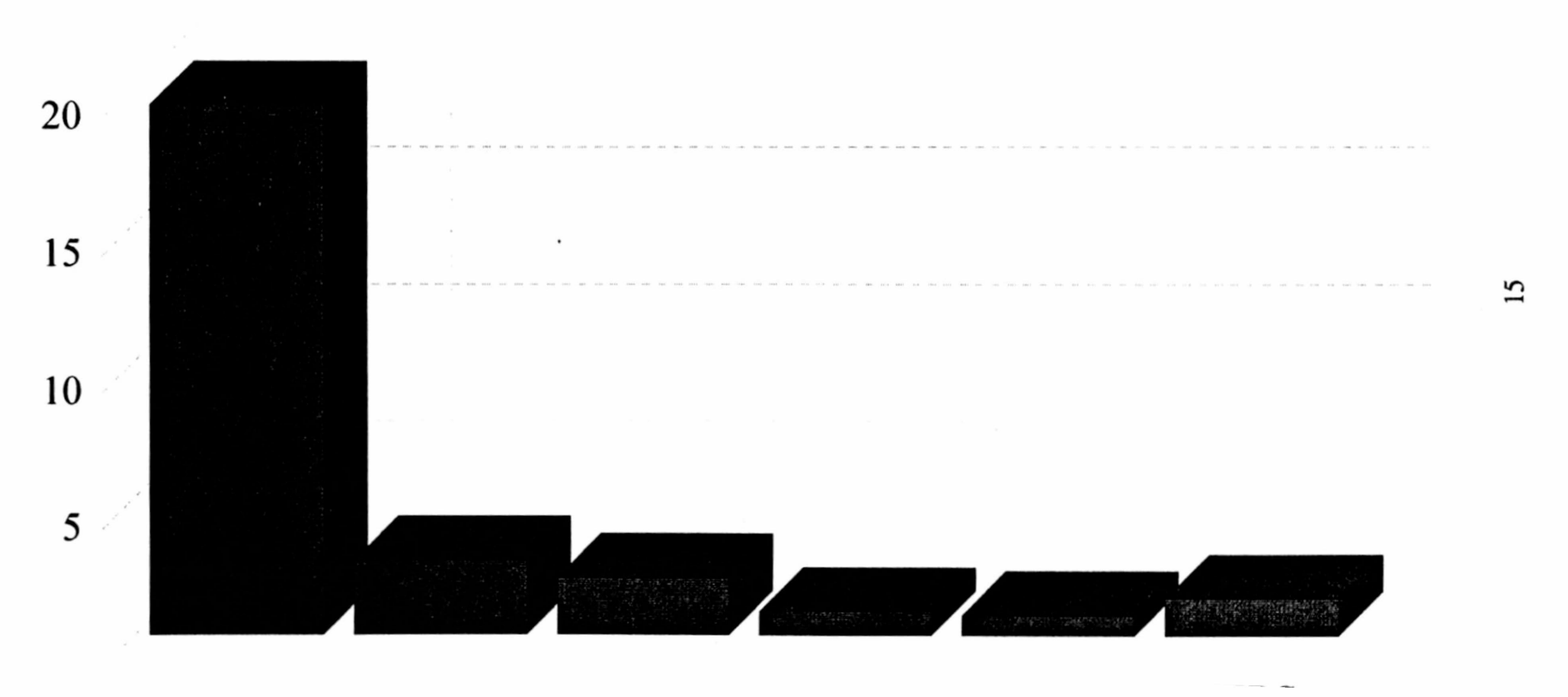
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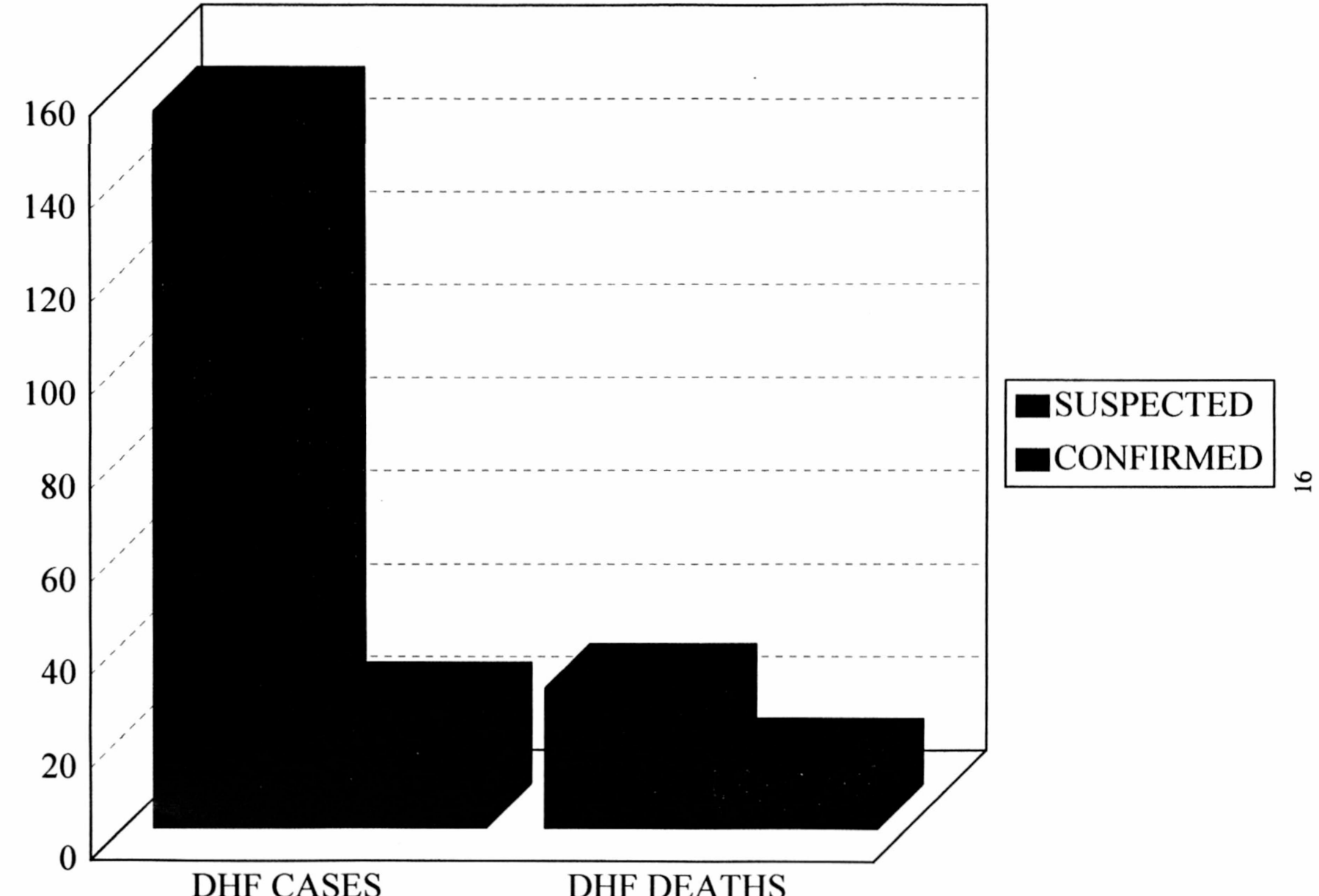
Figure 3: DENGUE/DHF OUTBREAK IN CLARA, 1994



CIRCULATION OF DENGUE-2 IN THE NORTHWEST OF MEXICO.

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Dengue virus was introduced in Mexico in 1978 as a consequence of transmission of the virus (serotype 1) in the caribbean and Central America[1]. Further virus spread occurred in 1979 and many cases were reported in Chiapas, Quintana Roo, Yucatan, Veracruz, Tabasco, Tamaulipas, Oaxaca and San Luis Potosi[2]. The rapid spread of serotype 1 throughout the Gulf of Mexico was responsible of dengue fever cases in the northeast border of Mexico and, in 1980, the Centers for Disease Control reported cases of dengue fever caused by dengue-1 in Texas[1,3-5]. In 1982 and 1983 the serotypes 2 and 4, respectively, were detected in Mexico. Kaplan et al (1983) showed serologic evidence of recent dengue-1 infection in two mexican cities: Merida, Yucatan and Tampico, Tamaulipas[6]. The transmission of dengue virus (serotypes 1 and 2) was detected by Gomez et al (1988) in the pacific coast of Mexico in epidemics occurred in Manzanillo, Colima, Puerto Vallarta, Jalisco and Santiago Ixcuintla, Nayarit[7]. In 1984 an outbreak of dengue 4 occurred in Yucatan and dengue fever was confirmed by serologic and/or virologic procedures; several hemorrhagic cases were recognized including 4 fatal cases (Loroño et al, 1993)[8]. In the summer of 1988 an outbreak of dengue fever was reported in Taxco, State of Guerrero; this is a town located at 1,700 meters above sea level and this is the first report in Mexico where dengue virus transmission occurs at a high altitude[9]. More recently, Ramos et al (manuscript in preparation) shown the circulation of dengue-4 in the State of Guerrero, dengue-1,2 and 4 in the State of Morelos and dengue-1 and 4 in the State of Puebla. At the present time, the majority of reported cases in Mexico are dengue fever, however cases of dengue hemorrhagic fever and/or dengue shock syndrome are sporadically reported.

In this work we report the circulation of dengue-2 in the states of Sinaloa, Sonora and Chihuahua, located in the northwest of Mexico.

In 1992, the General Directorate of Preventive Medicine (Mexico) submitted to our laboratory 119 human sera collected from individuals with suspected dengue fever who presented fever, headache, myalgia, arthralgia, rash and retro-orbital pain. The serum samples were analyzed for virus isolation and serotype identification in the mosquito cell line TRA-284. The cells were grown in glass tubes at 28°C in medium Leibowitz (L-15) supplemented with 50% of Tryptose phosphate broth (vol/vol) and 1% of heat inactivated fetal calf serum. In order to increase the detection of dengue virus, Actinomycin D (an inhibitor of cellular DNA transcription) was added to the cell cultures as described by Ramos et al (Trans. Roy. Soc. Trop. Med. & Hyg., accepted for publication). Briefly, Actinomycin D (Sigma Chemical) was added at a final concentration of 0.001 µg/ml to the cell cultures 5 days after infection and 2 days later (7 days postinfection), the cells were harvested for virus detection by direct immunofluorescence. Virus was identified by indirect immunofluorescence using type-specific monoclonal antibodies according to Gubler et al[10]. The prototypes dengue-1 (Hawaii), dengue-2 (NGC), dengue-3 (H-87) and dengue-4 (H-241) were included as controls.

Additional serological analysis were performed by the detection of IgG anti-dengue by an ELISA technique. Flat-bottom microplates were coated with 100 µl of goat antibody against human IgG diluted 1:200 in carbonate buffer (0.1 M, pH 9.0). After 4 hr of incubation at 37°C, the plates were washed 5 times with phosphate-buffered saline (PBS) 0.02 M, pH 7.4 containing 0.5% Tween 20 (PBS-T). Thereafter the plates were blocked with 200 µl of Casein 1% in PBS during 1 hr at 37°C. After washing with PBS-T, 50 µl of diluted sera in 0.5% BSA-PBS were added and the plates were incubate for 2 hr at 37°C. After washing, prototype dengue suspensions prepared in suckling mouse brain as antigens diluted in 0.5% BSA-PBS were added. The plates were incubated overnight at 4°C. Fifty µl of anti-flavivirus-peroxidase conjugate diluted 1:600 in 0.5% BSA-PBS were added and the plates were incubated during 1 hr at 37°C and washed. The peroxidase reaction was developed by the addition of 100µl of a substrate solution containig o-phenilendiamine and H_2O_2 in citrate buffer (0.1 M, pH 5.0). After 30 min of incubation at room temperature, the reaction was stopped by adding 50µl of 2.5 M sulphuric acid. The optical density (OD) values at 490 nm were measured with an

automated ELISA plate reader. A reaction was considered positive when the OD of the test serum was greater than the mean OD plus 3 standard devations of negative serum samples.

Our virologic and serologic results evidence the circulation of dengue virus in the northwest of Mexico (Table 1). In this region of the country, there is not information on recent transmission of dengue virus, however outbreaks of dengue fever have been recently reported in the near states of Nayarit ant Jalisco.

TABLE 1

PREVALENCE OF ANTIBODIES AGAINST DENGUE VIRUS (IgG-ELISA) AND VIRUS ISOLATION IN SERUM FROM RESIDENTS OF SINALOA, SONORA Y CHIHUAHUA (MEXICO), 1992.

LOCALITIES	TESTED SERA	IgG-ELISA POSITIVE/TOTAL (%)	VIRUS ISOLATION (SEROTYPE 2) POSITIVE/TOTAL (%)
Los Mochis, Sinaloa.	10	5/10 (50)	3/10 (30)
Guasave, Sinaloa	13	5/13 (38)	3/13 (23)
Navojoa, Sonora	68	45/68 (66)	17/68 (25)
Chinipas, Chihuahua	28	8/28 (29)	4/28 (14)
Total	119	63/119 (53)	27/119 (23)

REFERENCES

1.Pinhero, F.P. Dengue in the Americas 1980-1987. Epidemiol. Bull. (1989) 10:1-8

2. Paludismo y dengue. De la erradicacion a las zonas de riesgo. Cuadernos de Salud. Sepulveda A.J. (Coordinador). Secretaria de Salud, México. (1994) pp.55-91

3. Centers for Disease Control. Dengue -Mexico. Morbidity Mortality Weekly Rep. (1979) 28: 402-404

4. Centers for Disease Control. Follow-up on dengue-Mexico. Mexico. Morbidity Mortality Weekly Rep. (1980) 29: 169-170

5. Kappus, K.D., Sather, G.E., Kaplan, J.E., Schonberger, L.B., Monath, T.P. & Calisher, C.H. Human arboviral infecctions in the United States in 1980. J. Infect. Dis. (1982) 145:283-286.

6. Kaplan, J.E., Eliason, D.A., Moore, M., Sather G.E., Schonberger, L.B., Cabrera-Coello, L. & Fernandez de Castro, J. Epidemiologic investigations of dengue infection in Mexico. Am. J. Epidemiol. (1983) 117: 335-343

7. Gomez, D. H., Koopman, J.S., Addy, C.L., Zarate, M.L., Vaca-Marin, M.A.,Longini, I.M., Sarti, E., Alcantara, V., Garcia, L. & Rivas, E. Dengue epidemics on the pacific coast of Mexico. Int. J. Epidemiol. (1988) 17: 178-186

8. Loroño, P.M.A., Farfan, A.J.A., Rosado, P.E.P., Kuno, G., & Gubler, D.J. Epidemic of dengue in the Yucatan, Mexico. Rev. Inst. Med. Trop. Sao Paulo. (1993) 35: 449-455

9. Herrera-Basto, E., Prevots, D.R., Zarate, M.L., Silva, L. & Sepulveda-Amor J. First reported outbreak of classical dengue fever at 1,700 meters above sea level in Guerrero state, Mexico, june 1988. Am. J. Trop. Med. Hyg. (1992) 46: 649-653

10. Gubler, D.J., Kuno, G., Sather, G.E., Velez, M. & Oliver, A. Mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. Am. J. Trop. Med. Hyg. (1984) 33: 158-165

THE EMERGENCE OF *ANOPHELES ALBITARSIS* IN SOUTH-EASTERN BRAZIL.

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The Ribeira Valley region of São Paulo State (South-Eastern Brazil) actually presents malaria and encephalitis prevalences at a hypoendemic level. However, some factors may contribute to their ressurgence. One of them it may be the impact of agricultural irrigation on the mosquito population. The regional implantation of rice cultivation employing the artificial irrigation technique provided the opportunity to carry out studies on that matter. Previous observations on adult culicid activities in the same region have been published putting in evidence the *Anopheles albitarsis* emergence (Forattini et al.[1993] Rev.Saúde Pública, 27:313-25). Continuing these observations, the 1993-1994 rice cultivation cycle was followed. Breeding of *An. albitarsis* occurred in the early stage 3 of permanent paddies flooding, till five weeks after transplantation when rice plant height was small. So an inverse correlation between anopheline breeding and rice growth was observed. At the rice fields empty situation, corresponding to the first two stages of the cultivation cycle (follow uncultivated and ploughing), *Aedes scapularis*, *Culex nigripalpus* and *Cx. mollis* were the predominant species found. So, it would be considered that, depending of the rice field extent, the densities of these mosquitoes may will be of epidemiological significance.

The global data obtained during the rice cultivation cycle were as showed in the next Table.

Larval densities(*) in paddies during the stages of rice cultivation cycle 1993-1994.

stages (rice cultivation cycle)	Condition	*Ae. scapularis*	*An. albitarsis*	*Cx. nigripalpus*	*Cx. mollis*
1- Fallow uncultivated	empty	17.2	-	39.0	6.1
2-Ploughed	empty	5.5	-	5.0	-
Graded	transient flooded	-	-	-	-
3-Transplantation	permanent flooded	-	5.6	-	-
4-Young	permanent flooded	-	0.2	-	-
5-Middle	permanent flooded	-	0.1	-	-
6-Mature	permanent flooded	-	-	-	-
7-Harvested	empty	-	-	-	-

* Number of larvae per ten dips.

THE MOSQUITO VECTORS (DIPTERA: CULICIDAE) OF YELLOW FEVER IN SOUTH OF MARANHÃO STATE, BRAZIL, 1993 - 1994[1].

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Yellow Fever (YF) virus has been active from April to June, 1993 in three counties of south- eastern Maranhão State: Barra do Corda, Esperantinópolis and Mirador. The latter county was the most affected, with 62 confirmed human cases. Three localities in Mirador county have been surveyed for infected mosquitoes: Caiçarinha, Cana Brava and Araponga. Only one strain of YF virus has been isolated from *Haemagogus janthinomys* mosquitoes (real infection rate: 0.35 %) collected in Araponga. Based upon the importance of the epidemic and the fact that high densities of "capuchin" monkeys (*Cebus apella*) were noticed in the area, it was interesting to verify the hypothesis that YF would circulate again in 1994. Human YF has been diagnosticated in April, 1994 in a county (Pastos Bons) situated more or less 100 km South-East of Mirador. Thus, collecting of mosquitoes has been done both at the place "positive" in 1993 and in Pastos Bons county.

1196 (48 pools) and 1245 (55 pools) *Hg. janthinomys* have been collected in Araponga and Pastos Bons, respectively, and inoculated in new-born mice. Other potential vectors of YF were *Sabethes chloropterus* (267 or 13 pools and 73 or 3 pools from Araponga and Pastos Bons, respectively) and *Hg. leucocelaenus* (only 1 pool of 23 individuals from Araponga).

Sixteen strains have been isolated from *Hg. janthinomys* collected in Pastos Bons. The estimated real infection rate was 1.34 %, *i. e.* a much higher value than that obtained for the former year, indicating a high level transmission rate. In this context, as was also the case during Campo Grande, MS epizootics in 1992, one strain has been isolated from *Sa. chloropterus* mosquitoes (real infection rate = 1.67 %).

The relative abundance of these vectors (nr. of landings on one human bait / hour [collecting period: 10:00 - 15:00 h]) is varying according to the places and periods:

- *Hg. janthinomys* : Araponga, 1993: 299/194 = 1.5; Araponga, 1994: 1196/480 = 2.4; Pastos Bons, 1994:1245/350 = 3.5. It is noteworthy that the densities of *Hg. janthinomys* were much higher in 1994 than in 1993.

Another important factor for estimating the vectorial capacity of a mosquito population is the proportion of parous females. Preliminary results show that in Pastos Bons, 49.3 % (68/138) of the *Hg. janthinomys* females were parous. The only suitable data for comparisons are the parous rates of the populations of *Hg. janthinomys* estimated during the 1992 epizootics in Campo Grande area - MS, which were higher, varying from 57.5 to 61.8 %.

It is concluded from these data that, contrary to what occurred in Southern Mato Grosso in 1992, it was the high relative density of vectors which accounted for the high transmission rate.

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[1]Work presented as a poster at the "VII Encontro Nacional de Virologia, 20 / 23 nov 94, São Lourenço - MG Centro de Convenções do hotel Primus", Sociedade Brasileira de Virologia, Brazil.

Report from the Virus Laboratory

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Direct Pathological effects and/or vector role of Ixodes pari (= I. frontalis) for wild birds in France

Ixodes (Scaphixodes) pari Leach 1815 (= Ixodes (S.) frontalis Panzer 1795) is a strictly ornithophilic tick rarely found free in the wild, parasitizing mainly Passeriformes eating on the ground and living in hedges, bramble bushes, etc... Its biology is poorly documented and its role in the transmission and the dissemination of viruses or other pathogens remains to be clarified.

In a previous work (Chastel et al., 1991) we have studied the pathological effects of the infestation of twenty wild birds by I. pari and the eventual vector role of this tick for arboviruses. It has been concluded that I. pari was actually responsible per se for severe pathological disorders associated with hemorrhages and thrombosis leading to the death of the bird. Some years ago we have also observed paralysis, convulsions and tremors in an infested collared Dove (Streptopelia decaocto) followed by rapid death of the bird (Chastel et al., 1981).

However, such pathological events apparently occur only under peculiar conditions : unusual bird host and/or the attachment of one or more ovipositing female ticks.

During this previous work no virus isolation attempt has succeeded from 78 specimens of I. pari collected from the twenty wild birds studied and it has been concluded that I. pari probably did not play an important role in arbovirus transmission and dissemination (Chastel et al., 1991).

Since this first survey, ten more wild birds were collected and 23 more specimens of I.pari were virologically studied. This leads to the isolation of an arbovirus provisionally named "Chizé virus" from one nymph of I. pari parasitizing a Wren (Troglodytes troglodyte) found dead on the road near Chizé, south western France. Preliminary serological screening of the isolate (Brest/Ar/T 2913) performed in Brest Virus Laboratory suggests this agent is a new tick-borne Phlebovirus of the Uukuniemi group, Bunyaviridae family. We are unaware of the possible pathogenic power of "Chizé virus" for wild birds or other vertebrates. This virus is at present under study at C.D.C., Fort Collins, Co (Doctor Nick Karabatsos).

Russian workers have previously established that I. pari harboured Coxiella burnetii (Basova et al., 1960) and the eventual role of this ornithophilic tick in concerving/disseminating Borrelia burgdorferi deserves further attention since it was established that migrating birds may carry ticks infected by the agent of Lyme disease in the U.S.A. (Weisbrod and Johnson, 1989).

References :

- CHASTEL C., MONNAT J.Y., LE LAY G., BEAUCOURNU J.C. - Syndrome neurologique mortel chez une tourterelle turque (Streptopelia decaocto) et fixation de la tique Ixodes pari (= I. frontalis). Ann. Parasitol. Hum. Comp., 1981, 56, 349-351.

- CHASTEL C., GUIGUEN C., CHASTEL O., BEAUCOURNU J.C. - Pouvoir pathogène, rôle vecteur et hôtes nouveaux d'Ixodes pari (= I. frontalis) (Acari ; Ixodoidea ; Ixodidae). Ann. Parasitol. Hum. Comp., 1991, 66, 27-32.

- BASOVA N.N., CHERNIKOVA T.M., SUCHKOV I.G., RUDNEV M.M. - Q fever and Ornithosis in wild birds. Vopr. Virusol., 1960, 5, 586-591.

- WEISBROD A.R. and JOHNSON R.C. - Lyme disease and migrating birds in the Saint Croix River Valley. Appl. Environ. Microbiol., 1989, 55, 1921-1924.

- C. CHASTEL Virus Laboratory, Brest - C. GUIGUEN and J.C. BEAUCOURNU, Parasitology, Rennes - O. CHASTEL, CEBC / CNRS, Chizé, France.

RETROSPECTIVE ANALYSIS OF THE ABILITY OF ANTIGEN CAPTURE EIA TO DETECT FIELD ISOLATES OF ST. LOUIS ENCEPHALITIS, WESTERN EQUINE ENCEPHALITIS, AND EASTERN EQUINE ENCEPHALITIS VIRUSES.

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Antigen capture enzyme immunoassay (EIA) methods for detecting arboviral antigens were developed in response to the need for economical and rapid diagnostic tests in mosquito-based surveillance systems. In many surveillance programs, EIA is replacing tissue culture plaque assay as the standard virus detection technique. While EIA offers speed and economy not possible with tissue culture, it is not as sensitive as tissue culture and may not detect virus in mosquito pools with low virus titers. Mosquito infection rates calculated from EIA results may differ significantly from those calculated from tissue culture plaque assay results. Therefore, it is essential to know the proportion of mosquito pools positive by plaque assay that are detectable with EIA techniques.

To obtain background on the distribution of titers in mosquito pools, we examined historical virus isolation records of the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention from 1974 through 1993. Virus titers in the plaque-positive pools were estimated and the pools were classified according to the published limits of sensitivity of the EIA. If the estimated titer was ≥ 3.5 $\log_{10}$ PFU/ml, we assumed that there would be sufficient viral antigen in the pool to be detectable with EIA. If the titer was 3.0-3.5 $\log_{10}$ PFU/ml, the pool was coded as equivocal with EIA, and if the titer was <3.0 $\log_{10}$ PFU/ml we assumed that it was undetectable with EIA. The number of theoretically detectable, equivocal, and undetectable pools was tabulated and analyzed for each major arbovirus, for individual arbovirus outbreak investigations, and for arbovirus-vector combinations.

Only 39% of SLE pools, 74% of EEE pools, and 64% of WEE pools positive with plaque assay would likely be detected by EIA. Also, the proportion of high-titered, theoretically detectable pools varied considerably among years and locations (examples shown in Table 1) and among mosquito species (examples shown in Table 2). The results suggest that the interpretation of data from surveillance systems using EIA as the only virus detection system should be modified to adjust to the decreased sensitivity of EIA and differences in virus susceptibility among mosquito species.

Table 1. Virus isolates from selected SLE, EEE and WEE investigations during 1974-1993 and the theoretical ability of EIA antigen capture to detect the isolates.

Year	State	Virus	Cells*	N	Proportion of isolates		
					Undetectable	Equivocal	Detectable
75	TN	SLE	Vero	136	0.62	0.18	0.21
76	TN	SLE	Vero	84	0.49	0.14	0.37
80	TX	SLE	Vero	54	0.17	0.07	0.76
83	AZ	SLE	Vero	29	0.24	0.03	0.72
91	AR	SLE	Vero	12	0.67	0.17	0.17
75	ND	WEE	DE	266	0.35	0.17	0.47
77	CO	WEE	DE	257	0.21	0.06	0.73
83	MN	WEE	DE	110	0.22	0.03	0.75
83	ND	WEE	DE	231	0.25	0.02	0.73
87	CO	WEE	Vero	38	0.29	0.05	0.66
81	MI	EEE	DE	36	0.17	0.03	0.81
82	FL	EEE	DE	13	0.08	0.08	0.85
83	MI	EEE	Vero	33	0.15	0.00	0.85
83	RI	EEE	DE	27	0.19	0.04	0.78
91	FL	EEE	Vero	14	0.36	0.00	0.64

* DE = duck embryo.

Table 2. Virus isolates from SLE, WEE and EEE investigations during 1974-1993 separated by mosquito species, and the theoretical ability of EIA antigen capture to detect the isolates.

Mosquito species	Cells*	N	Proportion of isolates		
			Undetectable	Equivocal	Detectable
Cx. nigripalpus	Vero	4	0.25	0.50	0.25
Cx. pipiens	Vero	147	0.54	0.16	0.31
Cx. quinquefasciatus	Vero	82	0.28	0.10	0.56
Cx. tarsalis	Vero	36	0.25	0.08	0.67
Ae. vexans	DE	28	0.68	0.04	0.29
Cx. tarsalis	DE	1133	0.26	0.08	0.66
Ae. albopictus	Vero	14	0.36	0.00	0.64
Ae. canadensis	DE	2	1.00	0.00	0.00
Ae. vexans	DE	4	1.00	0.00	0.00
Cq. perturbans	DE	20	0.35	0.00	0.65
Cs. melanura	DE	113	0.11	0.02	0.87

* DE = duck embryo.

PCR-Based Detection of Arboviral RNA from Mosquitoes Homogenized in Detergent

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An improved method for the extraction of viral RNAs was developed to facilitate the reverse transcription (RT)-polymerase chain reaction (PCR) detection of mosquitoes infected with western equine encephalitis virus (WEEV) or LaCrosse virus (LACV). The solubilization method, which involves boiling mosquito homogenates in a buffered SDS-EDTA solution (final concentrations 1.0% and 5mM, respectively) followed by dilution (10-2) of sample, allows accurate viral detection through the use of random hexamers for the RT followed by specific primers for the PCR. Three primers were selected from a 223 base pair (bp) region that encompasses the 3'region of the capsid and the 5' region of the contiguous envelope gene of WEEV. Two primers were selected from a 158 bp region of the RNA polymerase gene of LACV. Our level of detection is one infected mosquito in a group of 100. Identities of the reaction products were confirmed either by sequencing or restriction endonuclease digestion. Previous methods for the extraction of RNA for the coupled RT-PCR depended on combinations of guanidinium isothiocyanate, acid phenol, detergents, and multiple centrifugations. Ideally, routine detection of viral RNAs for diagnostic purposes should bypass many of the above steps, while still providing a sensitive assay. Preliminary evidence suggests that this detergent extraction method may be applicable to other arboviral RNAs.

Further information is available in the July, 1994 issue of BioTechniques.

ULTRAMICROELISA ADENOVIRUS. A NEW TOOL FOR SEROEPIDEMIOLOGICAL STUDIES.

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A 10 µl ultramicroELISA assays (UMELISA) have been recently introduced for the diagnosis of different viral agents, including Herpes, Cytomegalovirus, Rubella, Dengue and others, showing several advantages over the currently used conventional technique (1,2,3,4).
A 10 µl indirect ultramicroELISA assay for the detection of IgG antibodies to Adenovirus was standardized. The method uses a crude antigen obtained from infected vero cells with Adenovirus type 3 strain. The sera were tested at 1:40 dilution, and sheep antibodies to human IgG, conjugated to alkaline phosphatase, were used. The sensitivity of the assay was increased by using a fluorescent substrate. The method was easy and rapid to perform requiring only 2 hours to be completed.
The UMELISA was compared with the Complement Fixation (CF) and Indirect Immunofluorescence tests (IFA) using 44 serum samples from children showing no symptoms of respiratory diseases , age range from 0 to 14 years old, in order to estimate the virus circulation. The Umelisa regarding the two other methods showed a 100% sensitivity, the agreement with CF was 65.9% and with IFA 88.6%.
The results obtained support the used of UMELISA for the detection of IgG antibodies against Adenovirus as a tool in the epidemiological surveillance programs related to this agent.

REFERENCES

Laferté, J. et al. (1992). UMELISA for the detection of IgG antibodies to Citomegalovirus. Rev. do Instituto de Medicina Tropical de sao Paulo. 34(1), 43-47.

Rivas, M.A. et al. (1992) UMELISA for the detection of IgG antibodies to Herpes Virus. Rev. Cub. Med. Trop. 44(2), 20-27.

Laferté J. et al (1992) Rapid diagnosis of Dengue virus infections by using a capture IgM ultramicroELISA assay. Advances in Biotechnology. 1(19), 4-6.

Guzmán M.G. et al (1993) Umelisa Rubella. A new tool for Rubella serology. Biotecnlogia Aplicada. 10(3), 15-18.

After primary and secondary immunization with yellow fever virus, lymphocytic 2′,5′-oligoadenylate synthetase activity increases prior to neutralizing, immunoglobulin M, and immunoglobulin G antibodies.

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The immune defense against a virus is dependent on production of several lymphokines, such as interferons (IFN) and interleukins (Il), released from antigen-presenting cells and T-helper (Th) lymphocytes. In the primary recognition and elimination of viruses, the activation of IFN-responsive genes is an early event. Within a few hours after infection the mRNA activity related to these genes is increased. One of the transcribed and translated gene products is the IFN-dependent enzyme 2′,5′-oligoadenylate synthetase (2′,5′AS). From cellular ATP, this enzyme generates 2′,5′-oligoadenylates (2′,5′A), which are diester condensation products of different chain lengths (oligomers). The produced oligomers (dimers, trimers, etc.) activate an endogenous latent ribonuclease (RNaseL) with mRNA and virus degrading activity. The end results of these processes are destruction of viral mRNA and shutdown of cellular protein synthesis.

Primary and secondary immunizations of human volunteers were done with commercial live, attenuated yellow fever vaccine (strain 17D) in order to study the course of appearance of virus neutralizing antibodies, IgM and IgG antibodies directed against the virus, and the IFN-dependent enzyme 2′,5′AS (determined in homogenates of peripheral B- and T-lymphocytes separated by immune rosetting). From cellular ATP, this enzyme generates 2′,5′A that mediate degradation of viral mRNA by stimulating RNaseL. By day 4 after the first immunization, the earliest and highest 2′,5′AS activity was present in the T-lymphocytes fraction. By day 7, the enzyme activity was highest in the B-lymphocytes fraction. Virus neutralizing antibodies appeared 7 days after vaccination and IgM antibodies were detected in serum collected 5 days later.

After a second immunization, done 2 years $\pm$ 2 months later, the only significant increase in 2′,5′AS activity was observed in the T-lymphocyte fraction. Virus neutralizing antibodies were present from day 1, whereas no IgM antibodies were detected. By 12 days after revaccination 80% of the vaccinees had detectable IgG antibodies.

In the primary and secondary (memory) immune response, 2′,5′AS activity is expressed in the T-lymphocyte fraction prior to the appearance of antibodies directed against the virus and may serve as an early and sensitive marker of an on-going virus infection, which otherwise would be difficult to detect. After primary or secondary immunization, no changes in conventional laboratory analyses, such as differential blood cell counts or total immunoglobulins, disclosed immune activity.

Cloning and Functional Expession Antibody VH-Domain in E.coli

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The mouse hybridoma cell line, 1G11, secrects anti-Japanese Encelphalitis B Virus (anti-JE) monoclonal antibody.This line was used as a source of mRNA for the synthesis of cDNA. Subsequently, the cDNA, encoding the variable region of antibody heavy chain (VH)was amplificated by PCR. The expression vector containing the VH gene was constructed in the phagemid pHEN- 1. After induced by help phage M13K07, the VH- domain was secrected into the culture supernatant as a fusion protein with the phage fd gene 3 protein on the surface of phage. The expressed VH-domain fragments have the same specificity compared to the native mAb, and affinity is about 1:32 detected by RPHI.In the animal treatment trial, the purified VH-domain, as a treatment agent, showed a sigificant therapeutic effect to the JE infected mouse.The genetic engineering antibody fragments, as described here, may become an important elment to human for immunotherapy against infectious diseases.

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CCGTCGACGTCAAGGAGCTCCAGTCAGGAGGAGGCTTGGTGCAACCTGGAGGATCCATGAAACTCTCTTGTGCTACTTC

TGGATTCACTTTTAGTGATGCCTGGATGGACTGGGTCCGCCAGTCTCCAGAGAAGGGGCTTGAGTGGGTTGCTGAAATT
                ----------
                     CDR1
AGAAACAAAGCTAATAATCATGCAACATACTATGCTGAGTCTGTGAAAGGGAGGTTCACCATCTCAAGAGATGATTCCAA
------------------------------------------------
                     CDR2
AAGGAGAGTGTACCTGCAAATCAACACCTTAAGAGCTCAAGACTCTGGCATTTATTACTGTACCGGGATCTACTATCATT
                                                  ---------------
                                                        CDR3
ACCCCTGGTTTGCTTACTGGGGCCAAGGGACCAGTCTCACAGTCTCCTCAGAATTCGG
---------------
```

Fig. 1 Nucleotide Sequence of the Anti-JE Monoclonal Antibody VH Gene.
The Complementary Determining Regions Are Underlined.

Fig.2 Results Of JE Treatment Test With VH-Domain Ab

		Total No.	Day of Death	No. of Death	Death Ratio
First	Test	10	9 13 14	3	3/10
	Control	10	6 7 7 8 8 9 10 10 12	9	9/10
Second	Test	10	10 19	2	2/10
	Control	10	7 8 9 10 10 11 12 12	8	8/10

Emergency Arbovirus Surveillance in the Southeastern United States

Tropical storm Alberto struck the Florida panhandle on July 3, 1993. Over the next 10 days, rainfall associated with the tropical storm produced widespread flooding in western and southern Georgia, western portions of the Florida panhandle and the southeastern corner of Alabama. In response to a federal disaster declaration and requests from Georgia and Alabama, the CDC/DVBID provided technical assistance in assessing the potential for increased arbovirus transmission activity. Though historical data indicate that the risk of increased arbovirus disease immediately following flooding is low, there were indications of EEE activity in the region prior to the flood. Therefore, surveillance programs were established to detect changes in arbovirus activity levels in the region.

A sentinel chicken surveillance system was developed in Georgia, where 52 of the state's 159 counties were included in the federal disaster declaration. Twenty-one existing flocks of 10 or more chickens were identified in communities extending from Macon to Bainbridge. Blood samples are being drawn from 10 birds in each flock each week, and are being tested for IgM antibodies to EEE and SLE by the Veterinary Diagnostic and Investigational Laboratory in Tifton, GA. In addition, a notice was sent to all physicians in the disaster area to increase awareness of the potential for EEE and SLE, and a similar notice was sent to all veterinarians in the state to request information on equine and avian cases of EEE. As of August 24, there is no evidence of increased arbovirus activity in Georgia.

In Alabama, ten counties were included in the disaster declaration. Active surveillance was initiated in 5 of the 10 counties. Veterinarians and physicians in these counties were alerted to the possibility of increased disease in equines, ratites, and humans. Wild and domestic birds were bled to be tested for antibodies to EEE and SLE. Mosquitoes were trapped in CO_2-baited CDC light traps for virus isolation. As of August 24, there had been 11 cases of EEE in horses and 2 in emus. Four of the horses were from counties outside the disaster area. All wild birds sampled to date have been negative. However, three sentinel flocks in Mobile county (not in the disaster area) have had seroconversions to EEE in one or more birds. A single virus isolate has been isolated from an initial shipment of mosquitoes. Identification of the virus is pending. Limited perifocal application of resmethrin is being carried out in areas where multiple equine cases have been reported.

A sentinel chicken surveillance system was put in place in Florida, where 13 counties have been included in the disaster declaration. Florida Department of Agriculture reports arbovirus activity in sentinel flocks has been minimal to date. In areas along the Florida-Alabama border, several cases of EEE in Florida animals were diagnosed by Alabama veterinarians. These include four horses and one emu. Adulticiding was carried out by aerial ULV application of naled over approximately 225,000 acres.

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Serological investigations of suspected viral encephalitis cases carried out at NIV, Pune.

Serum and CSF samples from patients with suspected viral encephalitis and febrile illness were received from various hospitals, medical institutes and primary health care centres from different parts of the country. These samples were tested in MAC-ELISA, HI and CF tests.

This is to highlight that besides Dengue and JE, WN viruses were prevalent in Maharashtra and few other places in India.

Serological data are summarised in the Table.

(K Banerjee)

Table : Serological data 1993 (Total no. of samples 1399, Total no. of patients 1089)

State/ District	Neg.	Past flavi	Recent JE	Recent WN	Recent DEN	Recent JE/WN	Recent JE/DEN	Recent WN/DEN	Recent flavi	Total # pts. (# Samples)
ANDHRA PRADESH										
Hyderabad	3	1	4	-	-	-	-	-	-	8 (13)
Tirupati	5	-	1	-	-	-	-	-	-	6 (6)
GOA										
Panaji	96	24	38	3	-	1	-	1	2	165 (259)
GUJARAT										
Ahmedabad	5	11	-	-	1	-	-	-	1	18 (18)
Amroli	4	4	-	-	2	-	-	-	1	11 (11)
Rajkot	13	19	-	-	5	-	1	-	3	41 (42)
HARYANA										
Rohtak	1	1	-	-	2	-	-	-	-	4 (4)
J & K										
Jammu	2	8	-	-	8	-	-	1	-	19 (19)
KARNATAKA										
Gulbarga	-	1	-	-	-	-	-	-	-	1 (3)
Manipal	2	-	-	-	-	-	-	-	-	2 (4)
Sirsi	2	-	-	-	-	-	-	-	-	2 (2)
MADHYA PRADESH										
Bhopal	1	1	-	-	-	-	-	-	-	2 (2)
Kurasia	20	37	-	-	6	-	-	2	-	65 (70)
MAHARASHTRA										
Ahmednagar	1	2	-	1	2	-	-	-	-	6 (6)
Akola	3	25	-	-	4	-	1	-	-	33 (39)
Aurangabad	10	30	-	1	9	-	-	-	1	51 (54)
Beed	38	42	-	3	10	1	-	-	3	97 (130)
Belgaum	-	1	-	-	-	-	-	-	-	1 (1)
Bombay	6	4	-	-	4	-	-	1	-	15 (24)
Buldhana	22	14	-	4	13	-	-	2	1	56 (90)
Chandrapur	1	-	-	-	-	-	-	-	-	1 (1)
Jalagaon	20	16	1	5	7	4	-	3	1	57 (78)
Jalna	9	9	-	2	6	-	-	-	-	26 (34)
Karad	-	1	-	-	-	-	-	-	-	1 (1)
Nasik	7	30	-	3	24	-	-	2	-	66 (97)
Parbhani	4	12	-	-	-	-	-	-	-	16 (16)
Pune	21	19	1	2	5	1	-	-	2	51 (68)
Raigarh	5	-	-	-	-	-	-	-	-	5 (5)
Solapur	7	10	-	-	-	-	-	-	-	17 (17)
Thane	2	2	-	-	-	-	-	-	-	4 (4)
Wardha	5	8	-	-	-	-	-	-	-	13 (18)
Yavatmal	3	14	-	-	4	1	-	-	-	22 (27)
NEW DELHI										
New Delhi	1	5	-	-	1	1	-	-	-	8 (8)
ORISSA										
Rourkela	53	34	11	2	11	-	-	-	2	113 (126)
PUNJAB										
Ludhiana	9	18	-	-	-	-	-	-	-	27 (27)
RAJASTHAN										
Udaipur	11	3	-	1	-	-	-	-	-	15 (17)
UTTAR PRADESH										
Gorakhpur	11	13	20	-	-	-	-	-	-	44 (47)
Total	403	419	76	27	124	9	2	12	17	1089 (1399)

Prevalence of Arbovirus Antibodies in Long-time Travellers to South-East Asia

72 sera of long-time travellers to South-East Asia were tested against DEN-2, CHIK, WN, and SIN antibodies by indirect immunofluorescence (IIF). Slides were prepared from infected Vero cells. Virus strains were provided by Dr. Karabatsos, CDC Vector-borne Diseases Laboratories, Ft. Collins, U.S.A.

Sera were taken from 42 female and 30 male travellers. Average duration of stay was 5.8 years (range: 6 months to 23 years). Travellers with history of travel to other continents (Africa, America) and persons with anamnestic history of a flavivirus vaccination were excluded. Travelling were mainly undertaken for professional reasons.

4 of 72 (6%) sera were found positive against Den-2 and 2 of 72 sera (3%) were found positive against WN with titers 1:20 or greater. One of these serum reacted against DEN-2 and WN. No sera reacted neither against CHIK nor against SIN. The duration of stay of the DEN-2 positives were 7, 4, and 18 years in Indonesia, Thailand, and India, respectively. The person with the serum reacting against DEN-2 and WN stayed for 4 years in Taiwan. The person with the serum reacting positive against WN alone stayed in China for one year.

Our study shows that infections with Dengue viruses and West Nile virus or a related virus (e.g. Japanese encephalitis) pose a danger on long-time travellers to South-East Asia. No antibodies against the other two arboviruses, Chikungunya and Sindbis, were found. In the case of SIN this could be explained by the natural cycle of this virus which only exceptionally involves humans. CHIK and WN (or JE) appear epidemically in South-East Asia while DEN is endemic in the whole region and most often involves humans.

One serum was reactive against DEN and WN. Several possibilities can explain this cross-reaction. There could be infections with the two flaviviruses DEN and WN. Another reason could be the infection with two Dengue virus types, often resulting in a cross-reaction against different flaviviruses. A third possibility is an anamnestically non-reported flavivirus vaccination (YF, JE, TBE). We found this serum positive against YF by IIF (titer 1:80) while the other sera which were positive for DEN-2 or WN were found non-reactive against YF by IIF. All sera were negative against TBE by ELISA.

G. Dobler

REPORT FROM ARBOVIRUS UNIT
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Toscana virus activity in Central Italy (1990-1993)

The sand fly-transmitted Toscana virus (TOS) has been associated with acute symptoms of the human Central Nervous System (CNS) disease, mainly aseptic meningitis or meningoencephalitis (1). During the summer clinical cases of TOS virus meningitis occur in restricted areas of of Central Italy. Simultaneously, the isolation of TOS virus from its vector (*P. perniciosus* and *P. perfiliewi*) in the same areas (natural foci) reaches its highest level (2). The report describes the periodic outbreaks, occurred in the years between 1990-1993 in two regions of Central Italy: Toscana e Marche, where two major foci of this virus have been identified.

Serologic studies on patients with neurologic disease were carried out. All patients examined were residents of rural or suburban areas in Tuscany Region (provinces of Firenze, Siena, Grosseto and Livorno) and in Marche Region (provinces of Fermo, Iesi, Macerata and Pesaro). A total of 189 patients were examined: 87 from Tuscany Region and 102 from Marche Region. Diagnosis was performed by specific enzyme-linked immunosorbent assay (ELISA) for IgG and IgM antibodies (capture method). The presence of IgM antibodies was assumed to indicate a recent infection by TOS virus. As shown in Table 1, of 189 cases of meningitis or meningoencephalitis, 112 (62%) were serologically diagnosed as TOS virus infection: 52 (60%) from Toscana Region and 60 (58%) from Marche Region. All but two patients observed were adults. Two cases were children of 2 and 4 years old.

Table 1. Serologic Diagnosis of TOS Virus Infection in Cases of CNS Disease by ELISA Test (1990-1993)

	Toscana*	Marche*	Total* (%)
1990	18/32	23/34	41/66 (61.1)
1991	7/16	-	7/16 (43.7)
1992	9/13	19/32	28/45 (62.2)
1993	18/26	18/36	36/62 (58.1)
TOT	52/87	60/102	112/189 (59.2)

* Values are N. positive/N. tested

As summarized in Table 2 a total of 87 isolation attempts have been made from cerebrospinal fluid (CSF): 13 viral isolates were obtained. All isolates were identified as TOS virus. All were from the seropositive patients. A strains of TOS virus was isolated from CSF of a 4 years old child from Tuscany Region.

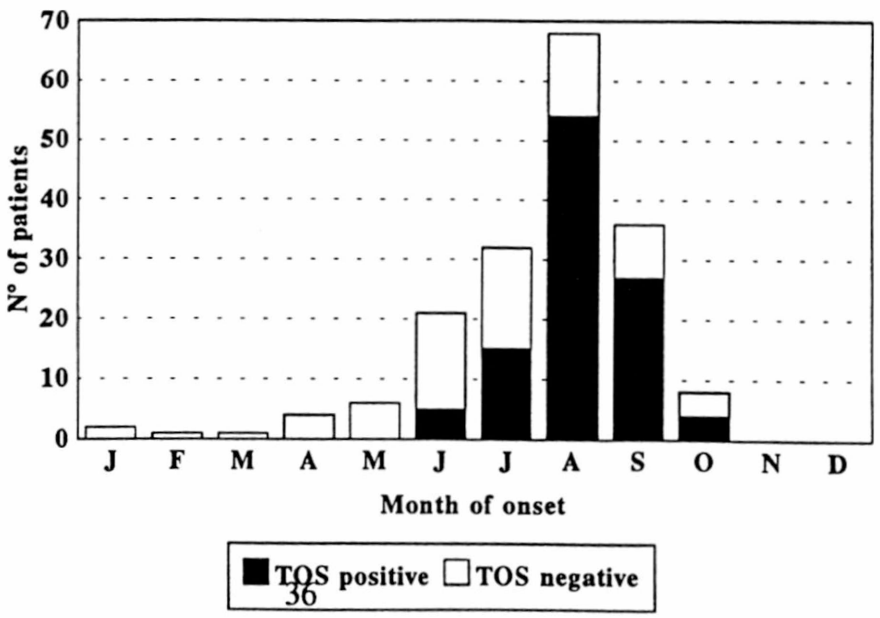
Table 2. TOS Virus Isolation from CSF* of Patients with CNS Disease (1990-1993)

Year of collection	Toscana[@]	Marche[@]	Tot[@] (%)
1990	0/2	3/22	3/24 (12.5)
1991	2/6	-	2/6 (33.3)
1992	1/5	4/21	5/26 (19.2)
1993	2/12	1/19	3/31 (9.7)
TOT	5/25	8/62	13/87 (14.9)

*CSF = cerebrospinal fluid
@Values are N. positive/N. tested

Figure 1 shows the cumulative distribution of examined cases. TOS CNS disease occurred during the summer, reaching the peach in August, when the maximum activity of the sand fly vector occur and virus isolates are obtained in their natural foci.

Figure 1. Cumulative Seasonal Distribution of TOS Virus Infections (1990-1993)



In 1993 healthy people living with patients with meningitis were examined for TOS antibodies: 21% (10/48) had IgG antibodies to TOS virus. Three of them had also IgM antibodies, indicating a recent contact with TOS virus without any symptoms of disease.

M.G. Ciufolini, C. Fiorentini, L. Nicoletti, A. Marchi.

REFERENCES

1) Nicoletti L., Verani P., Caciolli S., Ciufolini M.G., Renzi A., Bartolozzi D., Paci P., Leoncini F., Padovani P., Traini E., Baldereschi M. and Balducci M.
Central nervous system involvement during infection by Phlebovirus Toscana of residents in natural foci in Central Italy (1977-1988).
Am. J. Trop. Med. Hyg. 45: 429-434, 1991

2) Verani P., Ciufolini M.G., Caciolli S., Renzi A., Nicoletti L., Sabatinelli G., Bartolozzi D., Volpi G., Amaducci L., Coluzzi M., Paci P. & Balducci M.
Ecology of viruses isolated from sand flies in Italy and characterization of a new *Phlebovirus* (Arbia virus).
Am. J. Trop. Med. Hyg. 38:433, 1988

Field Studies of California Serogroup Encephalitis in Mississippi and North Carolina

Program plans in the Arbovirus Diseases Branch (ADB) for this past fiscal year included an effort to evaluate California CE) serogroup encephalitis in the US. A fatal case in a 5 year old male resident of Mississippi during 1993 as well as serologic specimens from his family members and neighbors positive for antibodies to the CE serogroup virus, Jamestown Canyon suggested that our 1994 studies could be profitably focused in southern states. The Mississippi State Department of Health was also interested in the possible implications of this outbreak and offered assistance and support for a study in southeastern Mississippi where the fatal case occurred. We also wished to revisit and evaluate the known endemic focus in western North Carolina and secured the support and cooperation of the NC Department of Environment, Health, and Natural Resources to conduct a study there.

Study design was identical in the two states except that a prospective surveillance component was initiated in Mississippi. Physicians in the southeastern part of that state were alerted to the possibility of CE serogroup cases in their area and arrangements were made to collect and test diagnostic specimens. On site studies conducted by ADB in cooperation with personnel from the respective State and Local Health Departments included retrospective audits of hospital records from 1989 - 1993 to identify compatible clinical cases, and entomologic surveys to capture mosquitoes for identification and virus isolation attempts.

A preliminary survey of patterns of medical care utilization in southeastern Mississippi revealed that the proximity of this area to Mobile, AL led to frequent referrals to hospitals there. The Alabama Department of Public Health was very helpful in arranging for hospital records reviews in the Mobile referral hospitals. The definition for a CE serogroup compatible encephalitis case was a discharge diagnosis of viral or aseptic meningitis; or a diagnosis of viral encephalitis; lymphocytic pleocytosis of the cerebrospinal fluid (CSF); and negative bacteriologic cultures of CSF.

Laboratory results from entomologic collections will be completed after resources now being utilized in support of surveillance and diagnostic activities become available later in the fall. Epidemiologic results collected have now been analyzed and summarized results are reported in this issue. Only three clinically compatible cases including the fatal 1993 case were found after reviewing >700 charts in Pascagoula and Hattiesburg, MS and Mobile, AL. Of the two remaining case-patients, one was a 28 year old female with onset in 1993 and the other was a 10 year old female with onset in 1991. Both survived without neurologic sequellae.

In western North Carolina, 36 clinically compatible cases were identified from six counties: Buncombe, Cherokee, Graham, Jackson, Macon and Swain. Twenty nine of the total 36 cases occurred in children aged <15 years (Table 1). Onsets of compatible cases by month and year are shown in Fig. 1. The largest number of cases (9) occurred in 1991. Cases occurred in each of the five study years in Swain County; in four of the five study years in Jackson county; and in three or less of the five years in the remaining counties. Nearly half (17/36) of the total cases occurred in Swain Co., location of the Cherokee Indian Reservation. Twelve of the 36 total cases occurred in the Native American population, all of them children <15 years. Table 2 shows rates per 10,000 for all cases in 5 year age intervals by year and county of onset. Incidence rates vary from 0/10,000 in some age groups to 40/10,000 in the younger age intervals.

While reviewing records in Mobile for cases referred from Mississippi, five compatible cases residing in southwestern Alabama were identified and an additional three cases from that state have been serologically confirmed in 1994, suggesting that Alabama, as well as Mississippi, may be a newly recognized focus of CE serogroup activity.

Submitted by Epidemiology Section, Arbovirus Diseases Branch, Division of Vector-borne Infectious Diseases, National Center for Infectious Diseases, National Centers for Disease Control and Prevention.

Table 1. Age Distribution: CE Serogroup Compatible Cases,North Carolina,'89-'93

Age Interval	Cases
00-04	11
05-09	9
10-14	9
15-19	1
20-24	1
25-29	1
>=30	4
Total	36

	1989		1990	
County	AI	Rate	AI	Rate
Buncombe	05-09	01.0	---	---
Cherokee	---	---	---	---
Graham	10-14	19.0	---	---
Graham	20-24	19.6	---	---
Jackson	---	---	00-04	07.5
Jackson	---	---	35-59	05.6
Jackson	---	---	---	---
Macon	---	---	25-29	07.5
Swain	00-04	40.0	05-09	39.0
Swain	10-14	13.0	10-14	13.0
Swain	---	---	---	---

1991		1992		1993	
AI	Rate	AI	Rate	AI	Rate
---	---	---	---	---	---
---	---	00-04	09.0	---	---
00-04	24.0	---	---	10-14	19.0
---	---	---	---	---	---
00-04	30.0	00-04	07.5	05-09	07.0
05-09	07.0	---	---	10-14	06.5
30-34	05.9	---	---	15-19	05.0
---	---	30-34	06.5	---	---
00-04	13.0	05-09	26.0	05-09	13.0
10-14	12.6	10-14	25.0	---	---
---	---	55-59	17.4	---	---

THE ISOLATION OF SINDBIS-LIKE VIRUSES IN LANDSCAPE BELTS OF LEAF-BEARING FORESTS AND TAIGA FROM MOSQUITOES AND TICKS.

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Sindbis virus was isolated before only in the south of the European part, in the Caucasus and in Central Asia of the former Soviet Union. The studies carried out in 1990-1991 resulted in the isolation of Sindbis-like strains in the belts of leaf-bearing forests in central part of Russian Plain: of the northern taiga in western Siberia and middle-mountainous taiga in Altai. In Altai the virus was isolated from Dermacentor nuttalli ticks (Table 1).

All the strain were isolated from newborn white mice by intracerebral infection with positive reisolation 1-2 months after of keeping the initial material in t^{0} -70°C. Incubation period was 24-48 hours in subcultivated culture BHK-21 the cytopathic action was observed 24-36 hours later. According to the data of electronic microscopy the viruses are related to Togaviridae family. All the strains had the hemagglutinating activity (HA) with pH 5.4-5.8. IAF was obtained in white mice to the isolated strains.

Serological identification was carried out in CF, HI (Table 2).

Identification of two strains isolated in Tatarstan and in Altai was carried out and in NT on tissues culture BHK-21 (Table 3).

From the mentioned data it follows that all the isolated strains are the parts of antigenic complex of WEE and are the most similar to Sindbis virus. Antigenic features of the strains are in the process of the study. Thus, the obtained data on Sindbis virus circulation in different climatic belts were obtained and the strain LEIV 22939 Tyum is the most northern isolate from known isolates in Russia. Only the Karelian fever - Ockelbö virus was isolated before in the analogous belt. The fact of its isolation from Dermacenter nuttalli ticks in mountains of Altai indicates the high ecologic plasticity of Sindbis virus.

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Table 1. ISOLATION OF THE STRAINS OF SINDBIS-LIKE VIRUSES IN RUSSIA

Strains	The date of collection	Source of isolation	The place of collection of the material			Data of reisolation
			Physico-geographical region	Geographical coordinates	Climatic Landscape belt	
LEIV 2594 Tat	4.07.90.	Aedes cantans	Russian plain	In latitude 55°42' North longitude 52°10' East	Leaf-bearing forests	+
LEIV 2939 Tyum.	11.07.90.	p. Aedes in sp.	Western Sibiria	latitude 60°40' North longitude 68°10' East	Northern taiga	+
LEIV 4817 Alt.	17.04.91.	Dermacentor nuttaill	Altai mountains	latitude 50°20' North longitude 85°30' East	Middle-mountain taiga	+
LEIV 6010 St.	20.07.91.	p. Aedes in sp.	Russian plain	latitude 46°42' North longitude 44°16' East	Leaf-bearing forests	+

Table 2. IDENTIFICATION OF ISOLATED STRAINS IN CF AND HI

Strains	LEIV22594 Tat.		LEIV22939 Tyum.		LEIV24817 Alt.		LEIV26010 St.
IAF	CF	HI	CF	HI	CF	HI	CF
LEIV 22594 Tat	128	n.t.	32/128*)	n.t.	64/128	n.t.	64/128
LEIV 22939 Tyum.	n.t.	n.t.	128	n.t.	n.t.	n.t.	64/128
LEIV 24817 Alt.	256/256	n.t.	n.t.	n.t.	256	n.t.	128/256
LEIV 26010 St.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	64
Sindbis	64/64	640	32/64	1280	64/64	1280	64/64
Karelian fever	32/128	80	8/128	1280	32/128	160	32/128
WEE	8/128	20	8	n.t.	8	10	8
Wataroa	8/64	20	n.t.	n.t.	16/128	10	8/128
Kyl-Agach	8	10	n.t.	n.t.	8	10	8
U-62-33	8	10	8	n.t.	8	10	8
Getah	8/128	10	8	10	n.t.	n.t.	n.t.
O'Nyong-Nyong	8	10	n.t.	n.t.	8/64	10	n.t.
EEE	8	10	n.t.	n.t.	8	10	n.t.
Ndumu	8	10	n.t.	n.t.	8	10	n.t.
Pixuna	8	10	n.t.	n.t.	8	10	n.t.
Mucambo	8	10	8	40	8	10	n.t.

n.t. - not tested

*) numerator - quantity of inverse titre with the used strain
denominator - homologic titre

Table 3. IDENTIFICATION OF SINDBIS-LIKE VIRUSES IN NEUTRALIZATION TEST (ACCORDING TO THE INDEX OF NEUTRALIZATION)

Strains	IAF	Sindbis	Karelian fever	WEE	U-68-88	Getah	Sem
LEIV 22594 Tat		6.0	4.0	4.0	0	0	
LEIV 24817 Alt.		6.0	4.0	0	0	0	

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For the next issue of Arbovirus Information Exchange

ISOLATIONS OF CHANDIPURA VIRUS FROM PHLEBOTOMINE SAND FLIES IN SENEGAL.

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As part of our programme of African Rift Valley Fever (RVF) in Senegal, since RVF virus is a Phlebovirus(Bunyavirideae) and most of the Phleboviruses known in the world were isolated from sand flies, we collected these vectors to investigate the possible natural transmission of RVF virus and other arboviruses.

Sand flies were caught around temporary ground pools in the district of Barkedji, in north Sénégal (15°17 N,14°53W), in Sahelian shrubby savannah, since May 1992. From titurated pools, Arbovirus isolations were made into newborn mice and two continuous cell lines : Vero and AP 61. Identification of the virus isolated was made using the complement fixation technique and confirmation was done using seroneutralisation test.

Up to now we have isolated 3 strains of Chandipura virus: Ar D 89384- Ar D 104134- Ar D 105137 (Rhabdoviridae, Vesiculovirus genus), described by Bhatt, Rodrigues 1967, in India, from patients. In Africa Chandipura has been isolated from hedgehogs.in Nigeria.

The Senegalese strains are the first isolations of Chandipura virus from arthropods in Africa. These need a extensive study, because they may induce an emergent disease in the future.

D Fontenille, M Traore Lamizana, J Trouillet, A Leclerc, M Mondo, Y Ba, JP Digoutte, H Zeller 1994. First isolations of arboviruses from phlebotomine sand flies in west Africa Am J. Med. Hyg.:50(5): 570-574

Serologic survey for Cache Valley and antigenically related viruses (Bunyamwera viruses).

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Bovine serums (50 each) from northeastern states (Maine, Vermont, Connecticut, Massachusetts, New York, Pennsylvania, Maryland, Virginia, and West Virginia), north central states (Ohio, Indiana, Illinois, Iowa, Kentucky, Michigan, Wisconsin, Minnesota, South Dakota, and North Dakota), Alaska, and Hawaii were evaluated for the presence of antibodies to Bunyamwera serogroup viruses Cache Valley, Northway, Tensaw, Lockern, and Main Drain. The virus microneutralization test using Vero cells was used, and an 80 percent inhibition of the virus at 1:10 serum dilution was considered positive for the presence of specific antibody.

Table 1 shows the distribution of antibodies to Cache Valley, Northway, Tensaw, Lockern, and Main Drain viruses. Other than Main Drain, antibodies to the other four viruses appear to be widespread. Antibodies to Bunyamwera virus were not observed in serums from Hawaii.

Serums with antibody to Cache Valley show cross-reaction with Northway, Lockern, and Tensaw viruses (Table 2).[2] These Cache Valley positive serums seem to cross-react with Northway virus to a greater extent than Tensaw and Lockern. There appears to be very low or no cross-reaction with Main Drain virus. Table 3 presents noncross-reacting serums. Other than in Alaska and Maryland-Delaware, Cache Valley virus appears to be the most common virus of Bunyamwera serogroup present in the northeast and north central regions of the United States.[1,3] Noncross-reacting antibodies to other viruses were also present in low levels in many states indicating the presence of these Bunyamwera viruses along with Cache Valley virus.[1]

References:

1. Calisher C, Francy DB, Smith GC, et al. 1986. Distribution of Bunyamwera serogroup viruses in North America. Am J Trop Med Hyg. 35:429-443.

2. McConnells S, Livingston CW, Calisher CH, et al. 1987. Isolation of Cache Valley virus in Texas, 1981. Vet Microbiol. 13:11-18.

3. Yuill TM, Gochenour WS, Lucas FR, et al. 1970. Cache Valley virus in the Del Mar Va peninsula III. Serological evidence for natural infection of dairy cattle. Am J Trop Med Hyg. 19:506-512.

Table 1. Distribution of antibodies to Bunyamwera viruses in serums of cattle (50 samples for each state).

States	Percent serums with antibodies to				
	CVV*	NWV†	TSV‡	LOV§	MDV‖
Alaska	6	10	4	4	0
Hawaii	0	0	0	0	0
Illinois	56	34	10	24	2
Indiana	50	24	8	16	0
Iowa	42	28	10	20	0
Kentucky	36	12	12	10	0
Massachusetts-Connecticut	32	22	8	14	0
Maryland-Delaware	40	22	24	34	4
Maine-Vermont	8	4	2	2	0
Michigan	34	26	16	16	0
Minnesota	48	44	12	22	0
New York	30	6	6	12	2
North Dakota	52	32	4	34	0
Ohio	44	18	6	14	4
Pennsylvania	34	26	6	18	0
South Dakota	54	40	10	16	0
Virginia	16	6	12	22	0
Wisconsin	32	28	14	14	0
West Virginia	50	22	16	6	0

*CVV-Cache Valley virus
†NWV-Northway virus
‡TSV-Tensaw virus
§LOV-Lockern virus
‖MDV-Main Drain virus

Table 2. Samples with antibody against Cache Valley virus (CVV) that had similar antibody titers to other viruses of the Bunyamwera serogroup.

States	Percent serums with antibodies to CVV showing cross-reaction with							
	NWV*, TSV†, LOV‡	NWV, TSV	NWV, LOV	TSV, LOV	NWV	TSV	LOV	MDV§
Alaska	2	2	2	0	0	0	0	0
Hawaii	0	0	0	0	0	0	0	0
Illinois	6	2	10	2	12	0	2	0
Indiana	2	0	6	4	14	2	0	0
Iowa	6	0	6	2	14	0	4	0
Kentucky	6	0	0	2	6	2	2	0
Massachusetts-Connecticut	6	2	6	0	8	0	0	0
Maryland-Delaware	14	4	0	6	2	0	16	0
Maine-Vermont	2	0	0	0	2	0	0	0
Michigan	10	6	4	0	4	0	0	0
Minnesota	10	2	12	0	18	0	0	0
New York	2	0	0	2	4	2	6	0
North Dakota	4	0	16	0	12	0	6	0
Ohio	6	0	4	0	8	0	2	4
Pennsylvania	6	0	8	0	12	0	0	0
South Dakota	2	6	10	2	22	0	2	0
Virginia	2	0	2	4	0	0	2	0
Wisconsin	6	2	8	0	2	0	0	0
West Virginia	6	2	0	0	8	6	0	0

*NWV-Northway virus
†TSV-Tensaw virus
‡LOV-Lockern virus
§MDV-Main Drain virus

Table 3. Presence of noncross-reacting antibodies to viruses of Bunyamwera serogroup in cattle serums.

States	Percent noncross-reacting serums				
	CVV*	NWV†	TSV‡	LOV§	MDV‖
Alaska	0	4	0	0	0
Hawaii	0	0	0	0	0
Illinois	22	4	0	4	2
Indiana	20	2	0	2	0
Iowa	8	0	0	2	0
Kentucky	18	0	2	0	0
Massachusetts-Connecticut	10	0	0	2	0
Maryland-Delaware	0	2	0	0	2
Maine-Vermont	4	0	0	0	0
Michigan	10	2	0	2	0
Minnesota	6	2	0	0	0
New York	14	0	0	2	2
North Dakota	12	0	0	6	0
Ohio	20	0	0	2	0
Pennsylvania	4	0	0	0	0
South Dakota	10	0	0	0	0
Virginia	8	0	2	10	0
Wisconsin	10	6	0	0	0
West Virginia	28	6	2	0	0

*CVV-Cache Valley virus
†NWV-Northway virus
‡TSV-Tensaw virus
§LOV-Lockern virus
‖MDV-Main Drain virus

REPORT FROM THE DIVISION OF ARBOVIROLOGY
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Bwamba virus infection in suspected haemorrhagic fever

In late May of this year, the Institute was informed by MSF (Holland) of a suspected outbreak of haemorrhagic fever in Rwandese refugees in Burigi Camp, Ngara, Tanzania. Clotted blood samples from 5/14 suspect cases, mostly adults were received. Clinical presentation was fever, bleeding from nose and gums, jaundice, an enlarged liver and spleen and very dark urine and faeces. Initial treatment was for suspected malaria but none responded to chloroquine or quinine, nor to penicillin. All 14 cases died within 2 weeks.

Serum from the 5 blood samples was inoculated i/c into suckling baby mice which were observed daily. The first signs of sickness were observed on day 7, post-inoculation and sick mice were collected and frozen at -80C. Only 1/5 of the Rwandese serum specimens caused sickness in the mice over 3 weeks of observation. Brains were harvested, and a 10% suspension, filtered and used for the first passage. Sickness was again noted on day 7 at the first passage and day 4 at the fifth passage and was titred at $10^{-3.5}$.

Whole carcasses from the fifth passage were sent to Fort Collins, together with the original 5 Rwandese sera for further identification. A virus was isolated from the same serum specimen and other sera failed to yield virus. The results of one-way neutralization assays for the Ugandan isolate were as follows:

Test Viruses	90% PRNT titre of antibody to;	
	Bwamba	Pongola
Uganda isolate (SG.372)	1280	40
Bwamba (M459)	1280	<20
Pongola (SAAr 1)	20	1280

PRNT: plaque-reduction neutralization test
Titre: reciprocal of highest dilution giving >90% neutralization

These suspect cases of haemorrhagic fever had high levels of malarial parasites (Dr E Sanders, WHO) and in this context, concurrent Bwamba infection might be coincidental.

(Report from S Rwaguma, RG Downing, SDK Sempala, N Karabatsos, DJ Gubler)

Antibodies to St. Louis encephalitis virus in armadillos from southern Florida: 1990-1991

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From January 1990 through March 1991, 189 nine-banded armadillos (*Dasypus novemcinctus* L.) were collected from Brevard and Glades Counties in southern Florida (USA). The sera were analyzed for hemagglutination-inhibition (HAI) antibodies against St. Louis encephalitis (SLE) and eastern equine encephalomyelitis (EEE) viruses. None of the armadillos had detectable HAI antibody titers to EEE virus, but 59 (31 %) had HAI antibodies against SLE virus (Table 1). Sera from 31 of the HAI-positive armadillos contained significant levels of neutralizing (NT) antibody to SLE virus. Blood samples from 129 of the armadillos were tested for the presence of viruses. No viruses were isolated.

Between July and December 1990, a widespread human SLE epidemic struck south Florida. A total of 226 human SLE infections (including 11 deaths) was reported from 28 Florida Counties, mainly in the central and southern peninsular region of the state. Armadillos captured during the epidemic had a greater prevalence of HAI and NT antibody to SLE virus than did animals captured before the start of the epidemic. This provides evidence that armadillos were fed on by mosquitoes infected with SLE virus.

The nine-banded armadillo was introduced into Florida in Brevard County in 1922 through escape from a local zoo and again by migration from Texas in 1972. The large-scale involvement of mammalian species in the maintenance and amplification of SLE has not been documented. However, SLE virus, SLE hemagglutination-inhibition (HAI) antibody, and SLE neutralizing (NT) antibodies have been detected in a number of wild mammal species suggesting that the SLE amplification and maintenance cycles may involve more than just wild birds and mosquitoes.

There has been little evidence for the involvement of armadillos in SLE maintenance and amplification. Extensive wild vertebrate surveillance during an inter-epidemic period (1965-1974) in Florida yielded positive HAI and NT titers to SLE virus from one of 11 armadillos captured in Orange County in 1965.

Armadillos for our study were captured with long-handled, heavy duty bird nets in Brevard and Glades Counties, Florida between 1 January 1990 and 10 March 1991. After capture, armadillos were housed individually in large Doskocil Vari-kennel cages and maintained on a high quality, commercial cat chow softened with tap water. Once in the laboratory, the sex of each armadillo was determined and they were separated by weight into three age categories: young (< 2.5 kg), young adult (2.6 - 3.0 kg), and adult (> 3.1 kg). To obtain blood, armadillos were anesthetized with a mixture of Ketaset (ketamine hydrochloride equivalent to 100 mg/ml) and PromAce (acepromazine maleate equivalent to 10 mg/ml). The dosage used was 33 mg/kg of Detaset and 1.1 mg/kg of PromAce administered by

intramuscular injection. Three ml of blood were collected from the right rear saphenous vein and the resulting serum was used for SLE and EEE, HAI and SLE, NT antibody analysis.

Fifty-nine (31 %) of the 189 field-collected armadillos tested positive for HAI antibody to SLE virus (Table 1). A significantly ($P < 0.05$) larger number of armadillos collected between September 1990 and March 1991, during and immediately following the 1990 SLE epidemic, had SLE antibodies when compared with those captured in January and February 1990, prior to the start of epidemic transmission. Of the 31, HAI-positive armadillos tested, all were positive for NT antibody to SLE virus.

Table 1. Antibody and viral isolation attempts from field-collected armadillos captured between January 1990 and March 1991 in Brevard and Glades Counties, Florida.

Number sampled	Number of SLE, HAI-positive armadillos (%)	Number of SLE, NT-positive armadillos	Number of viral isolations
189	59 (31 %)	31/31	0/129

The majority (140 of 189) of armadillos were collected in Glades County. Of these, 47 (34 %) were HAI-positive for SLE antibody. Twelve of the 49 (24 %) armadillos collected in Brevard County contained HAI antibody to SLE virus. There was no significant difference between the number of HAI-positive armadillos collected in Brevard and Glades Counties. Seventeen of 70 (24 %) male, and 42 of 119 (35 %) female armadillos were HAI-positive for SLE antibody. This difference was not statistically significant when analyzed by the G-statistic. The young, young adult, and adult age groups were represented by 12, 15, and 162 animals respectively. Twenty-five percent (3 of 12) of the young armadillos were positive for SLE antibody, while 20 % (3 of 15) of the young adults, and 33 % (53 of 162) of the adults were HAI-positive. None of these differences were statistically significant when tested by the G-statistic.

Brevard and Glades Counties both were involved in the 1990 south Florida SLE epidemic. The SLE seropositive rate for armadillos collected in these counties increased during the epidemic indicating that armadillos were bitten by SLE-infected mosquitoes in the field and that they mounted an immune response to the viral challenge. While it is unlikely that armadillos are more important than passerine birds as amplifying hosts for SLE virus, it is possible, given their unusual immune system and their low body temperature, that armadillos contribute to the maintenance of SLE virus during inter-epidemic periods and that they are involved in viral amplification during pre-epidemic and epidemic periods.

ARBOVIRUS ISOLATIONS IN NEW YORK STATE, 1994

More than 275,000 mosquitoes representing 31 species in seven genera, collected in New York State from the end of May to the end of September, were processed in pools of up to 100 in tubes of Vero and dolphin kidney cell (flavivirus susceptible) tissue cultures for CPE. To date 91 isolations of arboviruses have been made: 68 EEE, 20 Highlands J (HJ), 2 Cache Valley (CV), and 1 California (CAL) group (probably trivittatus virus). Identification was by IFA directly on the cells of the cultures that showed CPE.

The geographical distribution of the isolates was: central New York (Syracuse area) 19 EEE and 20 HJ; eastern Long Island (Town of Brookhaven, Suffolk County) 49 EEE and 2 CV; and western New York (Buffalo area) 1 CAL (TVT).

The mosquitoes involved were: *Culiseta melanura* for all the EEE and HJ isolates from central New York and 43 of the Long Island EEE isolates; *Culex* spp. (3 EEE), *Anopheles quadrimaculatus* (2 EEE), *Coquillettidia perturbans* (1 EEE), *Aedes taeniorhynchus* and *Ae. sollicitans* (1 CV each), all from Long Island; and *Ae. trivittatus* for the CAL (TVT) isolate (western NY state).

The first EEE-positive pool on Long Island was collected on 7 July and the last on 6 September. The 2 CV-positive pools were collected on 8 September. Fourteen pools of *Cs. melanura* collected during one week in mid-August produced isolates of EEE virus.

The dates of collection of virus-positive pools in central New York State were 26 July to 27 September. Through August all isolates were HJ (n=16), with the first EEE isolate coming from mosquitoes collected on 1 September. Isolations of both viruses were made through September. There were no double-infected pools. Collections were suspended at the end of September.

In addition to mosquito pools, sera and clots from sentinel pheasant flocks in the Syracuse area, blood and tissue specimens from suspect equine cases of encephalitis (after excluding rabies), and human clinical specimens were tested for evidence of arbovirus infection. Two pheasants seroconverted to HJ virus, but there was no evidence of EEE in the sentinel flocks. None of the specimens submitted from the 20 horse cases was positive, and there was no evidence of human arboviral infection in New York State in 1994.

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INFECTION WITH JAPANESE ENCEPHALITIS VIRUS OF CULTURED MOUSE BRAIN ASTROCYTES

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Japanese encephalitis virus (JEV) transmitted by the bite of infected culicine group of mosquitoes in rural and semiurban areas of many Asian countries often causes a fatal encephalitis in children. Through many postmortem histopathology reports of brains of JE patients or of experimentally infected laboratory rodent brains and through in vitro infection of cultured rodent brain cells with JEV it has been shown that thevirus infects selectively and specifically neurons only and damages them. Infection of glial cells with JEV has not been reported in these studies. Fatal outcome from the infection has been considered due to coma and respiratory failure caused by loss or damage of brain neurons by unabated growt of virus. In vitro where as infection with JEV of neurons in the cultured mouse brain cells has been shown by the undersigned and others, infection of glial cells, specially the astrocytes have been shown by us. Recently when astrocyte enriched cultured from 3 day old mouse brains were infected with JEV, they not only got infected productively with the virus but also produced a marked cytopathic effect after 11 days of infection. The cells became round giving grape like appearance on the confluent monolayer and got disloged on mild agitation. The thinned monolayer got shrunk and rolled back forming a 3 dimensional trunk like structure. The infected cultures kept shedding free virus in the culture fluid which produced a biphasic growth curve on titration by plaques. The peak titre in the 2nd phase coinsided with appearance of CPE and detachment of cells (Figure). Virus specific immunofluorescence was seen in primary and passaged GFAP positive astrocytes. The probable reason of non detection of infection in astrocytes or other glial cells in the JEV infected brain tissue could be the death of the subject through fast neuronal depletion before the virus could infect the astrocytes and destroy them. In the case of survival of the subject from encephalitis after partial loss of neurons the person may develop palsy or other neurological sequelae. Infection of astrocyte in vivo may lead to prolonged shedding of the virus with production of IgG and IgM for extended length of time with invasion of the CNS by inflammatory cells and T and B cells. Infection of oligodendrocytes may result in mild demyelination with increased amounts of myelin basic protein being shed in the serum

References:

(1) Johnson et al (1985) Ann. Nuerol. 18 : 567
(2) Suri N.K. and Banerjee K. (1987) Indian J. Med. Res. 86 : 148
(3) Hase et al (1990) Int. J. Expt. Med. 7 : 493
(4) Thakare et al (1991) Indian J. Med. Res. 93 : 271
(5) Kimura-Kuroda et al (1992) Arch. Virol. 130-477.

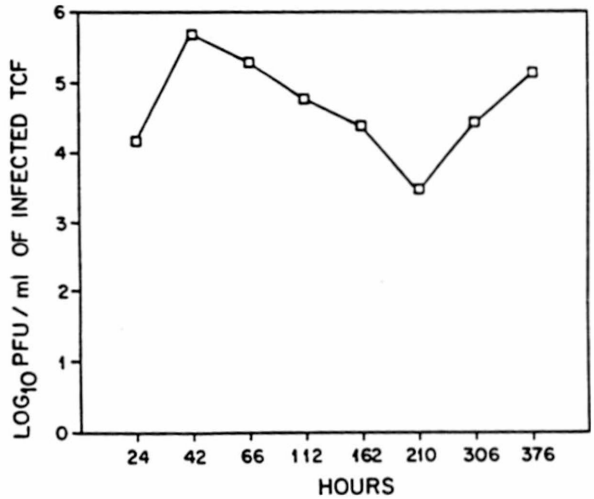

Fig. GROWTH OF JEV IN THE MOUSE BRAIN ASTROCYTE CULTURE.

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NEPHROPATHIA EPIDEMICA (NE) COMPLICATED BY ADULT RESPIRATORY DISTRESS SYNDROME (ARDS) AND ACUTE RENAL FAILURE (ARF): A NOVEL PNEUMO-RENAL SYNDROME.

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NE, the mild European variant of Hantavirus (HV) disease, is induced by the *Puumala* (PUU) HV-serotype and typically presents with thrombocytopenia and ARF. Pulmonary involvement in NE has been scarcely documented so far, and to our knowledge, a full-blown picture of ARDS has never been reported. We report here 2 proven cases of NE with typical kidney biopsies. PUU-infection was confirmed by high IgG IFA *and* IgM EIA PUU-specific titers. Pat. 1 was a 69 years old male hospitalized with a picture of "septic" shock (BP 65/40 mmHg), lactic acidosis, and rapidly progressive ARF (peak S.Creat. 8.9 mg%) prompting 10 consecutive days of peritoneal dialysis. Moreover, he developed diffuse intravascular coagulation (DIC) with purpura and internal hemorrhages, together with severe ARDS (Pa O_2 39 mmHg, Pa CO_2 30 mmHg with R/3 lit/min of O_2) . Fluid overload and / or heart failure (HF) was excluded by hemodynamic monitoring. This patient recovered eventually without sequelae after 20 days of mechanical ventilation. Pat. 2 was a 26 years old male, hospitalized with fever, low BP. (115/60 mm Hg), ARF (peak S. Creat. 9.7 mg %). transient DIC with external hemorrhages, and bilateral acute glaucoma. He also developed dyspnoea with cough, desaturation (Pa O_2 70 mm Hg, Pa Co_2 30 mm Hg with R/5 lit/min of O_2), and diffuse interstitial pulmonary infiltrates on RX, without clinical or radiological signs of fluid overload or HF. He recovered fully without dialysis or ventilation. *In Conclusion* The recently described, so-called "HV-pulmonary syndrome" due to a new American HV-serotype, is not unique to the current HV-epidemic in the USA, but can also exceptionally be seen in PUU-induced cases of NE.

Phylogenetic Relationships of Ovine, Bovine and Porcine Pestiviruses

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Pestiviruses of the *Flaviviridae* family are pathogens of major importance in cattle, pigs and sheep. They are classified according to their host of origin as bovine viral diarrhea virus (BVDV), hog cholera virus (HoCV), and border disease virus (BDV) of sheep. Although the degree of diversity between BVDV and HoCV has been well characterized by protein and genomic analysis, the relationship of these viruses to BDV is not well understood. To gain a better understanding of the degree of diversity between pestiviruses we have cloned and sequenced the entire structural gene coding region of a viral isolate (BD78) from a BDV-affected lamb and the glycoprotein coding region of three additional BDV isolates. cDNA clones generated by reverse transcription and polymerase chain reactions (RT-PCR) were cloned into the pCRII vector. The nucleotide sequence of the cloned fragments were determined using the dideoxy chain termination method.

The structural gene coding region of 3,648 nucleotides contained one open reading frame encoding 1100 amino acids. Analysis and comparisons of the nucleotide and amino acid sequences have been made with those of other pestiviruses. Although the genomic organization is well conserved, significant diversity exists throughout the structural coding region. The most conserved regions are the 5' untranslated region (UTR) and the capsid protein coding region (p14) and the most divergent sequences are in the E2 coding region. Our data suggest that BDV is a distinct virus in the *Pestivirus* genus.

Furthermore, to gain a better understanding of the degree of viral variation among ovine pestiviruses, we also compared the sequence of the glycoprotein coding regions of three additional BDV isolates (BD31 BD97, BDSC) to different strains of BDV, BVDV and HoCV. Approximately 1200 nucleotides have been sequenced for each of the three viral isolates. Interestingly, phylogenetic analysis of these sequences revealed that two sub-types of BDV exists. One of these sub-types is more closely related to HoCV, while the other is more closely related to BVDV. This data supports the premise that the two sub-types of BDV have evolved separately; one originating from HoCV, and the other originating from BVDV. These findings provide insight into virus strain variation that is essential for pestiviral diagnosis, epidemiology and vaccine design.

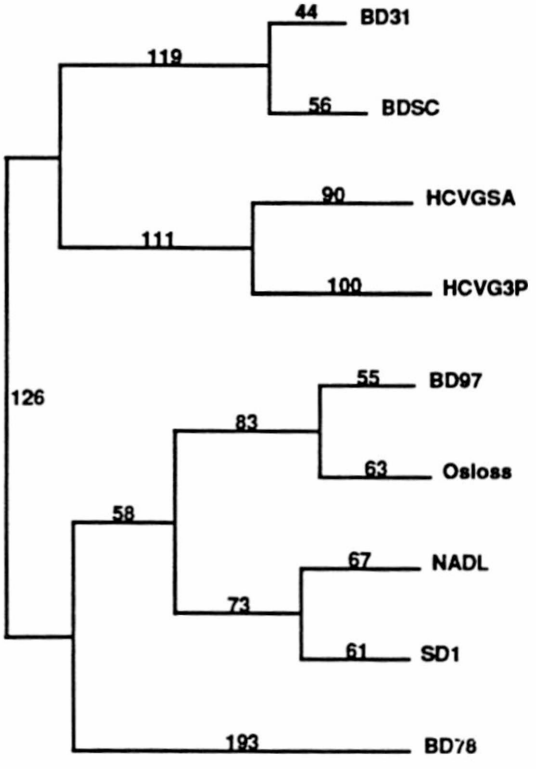

Figure 1. Phylogram depicting genetic relationships among bovine (Osloss, NADL and SD-1), porcine (HCVGSA and HCVG3P) and ovine (BDSC,BD31, BD97 and BD78) pestivirus isolates

ELISA and ultramicroELISA for Coxsackie A9 and antiviral antibodies in relation with an outbreak of Epidemic Neuropathy in Cuba.

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Recently an Epidemic Neuropathy outbreak showing both optic and peripheral manifestations affected our country . The disease is currently considered to have a multi-factorial etiology (infectious and neurotoxic agents, nutritional deficiency, etc). An Enterovirus (47/93 strain) was isolated from patient's cerebrospinal fluid and was later classified as Coxsackie A9 (Cox A9) using the Neutralization test with LBM pools of antisera. We standardized two sandwich immunoenzymatic procedures (100 µL standard ELISA and 10 µL fluorigenic ultramicroELISA) for the rapid identification (three hours) of this agent from culture supernatants. Both sandwich methods were shown to be type specific, as they could identify the isolated agent and other strains of Cox A9 virus including a reference one, and their did not react with 20 other enterovirus strains (Table I). Two aditional methods (Competitive ELISA and 10 µL inhibition fluorigenic ultramicroELISA) were developed for detecting total human antibodies in sera from populations showing a high and low prevalence of the disease. A significant relation was found between the high incidence of the disease and the serological response to this agent (Table II), which could be considered as possibly related to the etiology of the disease.

Table I. Identification of different strains of Co
before and during the Epidemic outbreak.

Strain	ELISA (OD)	UMELISA (
47 (*)	1.200	110
Cox B1	0.070	0
Cox B2	0.064	3
Cox B3	0.081	1
Cox B4	0.092	0
Cox B5	0.073	2
Cox B6	0.053	0
Cox A7	0.080	0
476 (Cox B2)	0.092	0
273 (Cox B2)	0.091	0
152 (Echo 7)	0.085	0
602 (***)	0.063	0
589 (***)	0.075	0
595 (Echo 21)	0.062	0
Cox A9 (**)	0.985	100
35 (*)	0.753	95
453 (*)	1.425	120
231 (*)	1.138	102
135 (*)	1.350	117
590 (***)	0.001	0
57 (*)	1.520	145
Cox A7	0.080	3
Echo 2	0.094	0

Strain	ELISA (OD)	UMELISA (Fluorescence)
Polio 1	0.096	9
Polio 2	0.117	7
Polio 3	0.112	10
HAV	0.065	3

* Coxsackies A9 isolated before and during the Epidemic outbreak
** Standard Coxsackie A9 strain
*** Unidentified Enterovirus
Cut off: ELISA OD > 0. 20 UMELISA Fluorescence > 20

Table II. Serological screening of populations showing high and low incidence of the disease using the Competitive ELISA test.

Population	Samples	Seropositive	%
High incidence	1007	735	72.98
Low incidence	1345	900	66.91

* The chi-square test was used to determine the statistically significant differences among the areas ($p < 0.05$).

Henry David Thoreau: "Some circumstantial evidence is very strong, as when you find a trout in the milk."

Frank Layden (but really Henny Youngman): "I went to a psychiatrist and he said I was crazy. I told him I wanted a second opinion. He said, "You are ugly, too."

John Bright: "Force is not a remedy."

W.E. Gladstone: "We are part of the community of Europe, and we must do our duty as such."

Heinrich Heine: "God will pardon me. It is his trade."

Hiram Johnson: "The first casualty when war comes is truth."

Anonymous (18th Century suicide note): "All this buttoning and unbuttoning."

Sir Arnold Bax: "You should make a point of trying every experience once, except incest and folk-dancing."

Willie Tyler: "The reason lightening doesn't strike twice in the same place is that the same place isn't there a second time."

Unknown: "He swims at the shallow end of the gene pool."

Oliver Wendell Holmes: "State interference is an evil, where it cannot be shown to be a good."

Albert Einstein: "I love the higher values of science, yet I find it humiliating to have to struggle to make a scientific career."

Lyndon Johnson: "While you're saving your face, you are losing your ass."

Cluff Hopla: "The hens are always willing to cackle, but to produce an egg requires more effort."

Mary Wollstonecraft: "The *divine right* of husbands, like the divine right of kings, may, it is hoped, in this enlightened age, be contested without danger."

Oscar Wilde: "A thing is not necessarily true because a man dies for it."

Groucho Marx: "Either that horse is dead or my watch has stopped."

Austin O'Malley: "Revenge is often like biting a dog because the dog bit you."

Havelock Ellis: "The whole religious complexion of the modern world is due to the absence from Jerusalem of a lunatic asylum."

Irving Layton: "In Pierre Elliot Trudeau, Canada has at last produced a political leader worthy of assassination."

Oliver Wendell Holmes: "Long illness is the real vampirism: think of living a year or two after one is dead, by sucking the life-blood out of a frail young creature at one's bedside!"

Anonymous: "If he only knew a little of law, he would know a little of everything."

W.I.E. Gates: "Then there is the man who drowned crossing a stream with an average depth of six inches."

Hans Christian Andersen: "But the Emperor has nothing on at all!"

Matthew Prior: "Cured yesterday of my disease, I died last night of my physician."

Mother Teresa: "The biggest disease today is not leprosy or tuberculosis, but rather the feeling of being unwanted."

Irving Berlin: "The world would not be in such a snarl, had Marx been Groucho instead of Karl."

S.J. Perelman: "I have Bright's disease and he has mine."

Harding's Homily: "It is difficult to be nostalgic when you can't remember anything."

Hylton's Rule: "No job is too small to botch."

Erma Bombeck: "The only concession we had made to automation was a smoke alarm, so we could know when dinner was ready."

Confucius: "There are three degrees of filial piety. The highest is being a credit to our parents, the second is not disgracing them; the lowest is being able simply to support them."

Voltaire: "I advise you to go on living solely to enrage those who are paying your annuities."

Francis Bacon: "There is no comparison between that which is lost by not succeeding and that which is lost by not trying."

Duc de la Rochefoucauld: "If we resist our passions, it is more because of their weakness than because of our strength."

Duc de la Rochefoucauld: "Absence diminishes commonplace passions and increases great ones, as the wind extinguishes candles and kindles fires."

Mark Twain: "Courage is resistance to fear, mastery of fear, not absence of fear."

Frank B. Medo: "The only people who brag about having been poor are the rich."

Judge John L. Kane, Jr.: "An extremist is one who believes you can't have too much of a good value. But too much love of one's country produces fascism- and in the absence of competing values, too much freedom is chaos and too much law is despotism."