



# ARTHOPOD-BORNE VIRUS INFORMATION EXCHANGE

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The Arbovirus Information Exchange is a newsletter prepared under the auspices of the Subcommittee on Information Exchange (Nick Karabatsos, Chairman), American Committee on Arthropod-borne Viruses. Printing and mailing costs of the Arbovirus Information Exchange are paid by 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.

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## Editor's Comments

We have a small but interesting issue of the Arbovirus Information Exchange this time. Most of our reports in this issue concern outbreaks and/or arbovirus activity, including yellow fever activity in Brazil, dengue-1 in Brazil and the first report of dengue in Argentina since 1916. The Introductory Notes from Issue 4 (published in October of 1961) are included in this issue of the Arbovirus Information Exchange as the commemoration. I think you will find them enjoyable to read.

I'd like to remind everyone that they are encouraged to submit a report - at least once every two years. Reports can consist of brief summaries or preliminary data.

Thanks to all who submitted reports electronically. In this issue, all but one of the reports were submitted either by e-mail or on a computer diskette. This not only saves postage costs, but also allows us to post the reports on our website. As a reminder, the home page for the Arbovirus Information Exchange can be found at the following address: <http://lablink.utmb.edu/arbovirus>.

Laura Chandler  
University of Texas Medical Branch  
Galveston, Texas

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

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

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

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

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

If sending reports by mail, please use this address:

Laura Chandler, Ph.D.  
Department of Pathology  
Keiller Bldg. Rm. 2.138A  
University of Texas Medical Branch  
Galveston, Texas 77555-0609

You may also send reports by FAX to: 409-747-2437

**ACAV Treasurer's Report**  
1 January through 31 December 1997

**ACAV Treasury**

	<u>1997 YTD</u>
Net Assets Beginning of Period	\$ 10,986
Revenues and Gains:	
Additional Contributions	525
Interest	219
Dividend	<u>111</u>
Total Revenues and Gains	<u>\$ 854</u>
Total Assets End of Period	\$ 11,840
Total Expenses	<u>\$ 2,127</u>
Net Assets End of Period	<u>\$ 9,714</u>

**Scherer Fund**

Net Assets Beginning of Period	\$ 3,296
Revenues and Gains:	
Additional Contributions	0
Interest	68
Dividend	<u>34</u>
Total Revenues and Gains	<u>\$ 102</u>
Total Expenses	<u>0</u>
Net Assets End of Period	<u>\$ 3,397</u>

**Hardy Fund**

Net Assets Beginning of Period	\$ 0
Revenues and Gains:	
Contributions	2,120
Interest	13
Dividend	<u>6</u>
Total Revenues and Gains	<u>2,139</u>
Total Expenses	<u>0</u>
Net Assets End of Period	<u>\$ 2,139</u>

**Dalrymple-Young Fund**

Net Assets Beginning of Period	\$ 277
Revenues and Gains:	
Additional Contributions	0
Interest	6
Dividend	<u>3</u>
Total Revenues and Gains	<u>\$ 9</u>
Total Expenses	<u>0</u>
Net Assets End of Period	<u>\$ 285</u>

Respectfully submitted,

Thomas M. Yuill, ACAV Treasurer

## BOOK AVAILABLE

A new book, entitled "An Overview of Arboviruses in Brazil and Neighbouring Countries" is available from the Instituto Evandro Chagas. This book was edited by Drs. Amelia P.A. Travassos da Rosa, Pedro F.C. Vasconcelos, and Jorge F.S. Travassos da Rosa. The publisher is: Evandro Chagas Institute, P.O. Box 1128, Belem, Para, Brazil.

The book is divided into three main sections: Part 1: Classification, systematics and molecular biology; Part 2: Clinic, laboratorial diagnostic, and epidemiology of arboviruses and certain other viruses of vertebrates; Part 3: Control of vectors and ecology of arboviruses.

The book is available free of charge to people who are interested in arboviruses. You may contact Dr. Amelia P.A. Travassos Da Rosa or Dr. Pedro Fernando Da Costa Vasconcelos. Their addresses and telephone numbers are printed below.

Instituto Evandro Chagas  
Av. Almirante Borroso 492  
66090-000, Belem, Para, Brazil

Tel. 55-91-226-5262/211-4409  
FAX 55-91-226-1284  
email: [Pedrofcv@amazon.com.br](mailto:Pedrofcv@amazon.com.br)

**PAHO/WHO COLLABORATING CENTER FOR VIRAL DISEASES  
OF THE INSTITUTE OF TROPICAL MEDICINE "PEDRO KOURI"  
MINISTRY OF HEALTH, REPUBLIC OF CUBA  
PAN AMERICAN HEALTH ORGANIZATION**

*Dengue Fever, a menace at the doors of the year 2000*  
International Course on Dengue and Dengue Hemorrhagic Fever

La Habana, Cuba, August 1999.

The PAHO/WHO Collaborating Center for Viral Diseases of the Institute of Tropical Medicine "Pedro Kouri" is pleased to announce its traditional bi-annual International Course on Dengue and Dengue Hemorrhagic Fever, in its facilities at the Institute "Pedro Kouri", La Habana, Cuba.

Jointly sponsored by PAHO, the Cuban Ministry of Health and other private institutions, this Course results not only an adequate forum for training and update on activities like vector control and laboratory diagnosis; but also for the discussion of the most relevant problematic the countries in the Region of the Americas are facing through with the Dengue Epidemic.

Topics of the Course will be among others:

- Current Situation of the Dengue Epidemic in the Americas and the rest of the world
- Advances in the Laboratory Research and search of Vaccines
- The eradication or control of *Aedes aegypti*

As well as many other interesting thematics, relative to the Epidemiology of the disease and the participation of the Community in the Control, the Clinical Presentation in the Adult and the Child, the Histopathology and risk factors and some others. The course includes lectures, laboratory and field practices and conferences.

Invited lecturers from PAHO, other PAHO/WHO Collaborating Centers and other foreign institutions are confirmed to participate.

For more information, please contact to:

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Previous Editors of the Arbovirus Information Exchange

Telford H. Work 1960-1972

Roy W. Chamberlain 1972-1981

W. Adrian Chappell 1981-1984

Barry R. Miller 1984-1989

Charles H. Calisher 1989-1996

*CENTRE COLLABORATEUR OMS  
DE REFERENCE ET DE RECHERCHE  
POUR LES ARBOVIRUS ET LES VIRUS  
DE FIEVRES HEMORRAGIQUES  
CRORA  
INSTITUT PASTEUR DE DAKAR*

*INSTITUT FRANCAIS DE  
RECHERCHE SCIENTIFIQUE  
POUR LE DEVELOPPEMENT EN  
COOPERATION  
ORSTOM  
DAKAR ET PARIS*

*INSTITUT PASTEUR  
PARIS*

## **UP TO DATE CRORA REPORT ON THE WEB**

During July 1998, a new version on the interactive report from CRORA, Pasteur Institute of Dakar will be installed on the WEB :

Pasteur Institute of Paris : <http://www.pasteur.fr/Bio/CRORA/>  
ORSTOM Paris : <http://www.orstom.fr/CRORA/>

Pasteur Institute and ORSTOM are associated to collect data and to create this report using HTML language. This new report involves all the data collected by Pasteur Institute and ORSTOM since 1962, about 5,682 isolated strains on 187 arboviruses or mixed arboviruses.

For each virus, all the observed hosts or vectors are given, with the number of collected strains in each country. New bibliographic data are now added, so now 521 strains are connected with their own bibliographical data. A new HTML anchor gives a list of all the reference concerning all the strains of the same viral species. As previously, for each host, or vector, the number of collected strains of each virus in all the countries is given. A special listing is given for the virus isolated from male mosquitoes.

As for the first version for all the 5682 isolated and identified strains, listing give the exact place (latitude and longitude), date of collect and the host (or vector). In this new version, we added a new HTML anchor connecting with technical data, used methods to identify the strain, comments and conclusion. All these data are given for 5493 strains for some cases, tables give all the cross reactions used.

As previously for all the viruses, hosts or vectors, and countries, HTML anchors give the opportunity to have an immediat access to the concerned subject. Actually 1649 indirect immunofluorescence, 966 fluorescences using monoclonal antibodies, 1823 neutralizations (seeckling mice or continuous cells lines) and 4036 complement fixations had been performed to identify the 5.682 strains.

The first CRORA report required 1.413 files, the next one will require more than 7100 files.

Jean-Pierre DIGOUTTE  
INSTITUT PASTEUR

François ADAM  
ORSTOM

**THE DENGUE 1 OUTBREAK IN MANAUS, AMAZONAS, BRAZIL  
BEGINNING IN MARCH, 1998.**

**Betsy Dutary Thatcher, Jose Joaquin Sandoval, Marcus Vinicius Guerra and  
Wilson Alecrim**

**INSTITUTO DE MEDICINA TROPICAL DE AMAZONAS, MANAUS, AMAZONAS, BRAZIL.**

Dengue is endemic in most of Brazil and there are 3 dengue serotypes circulating in some areas of this vast country. There have also been confirmed cases of Dengue Hemorrhagic Fever (DHF) in Rio de Janeiro (RJ) and Fortaleza (Ceara). Amazonas State, nevertheless, had been free of this disease since 1942, when *Aedes aegypti* was eradicated from the area.

*Aedes aegypti* re-infested Manaus, during 1996. The mosquito was first found in oviposition traps located in the auto parts district of the city. Lack of a mosquito control program, allowed dissemination of the mosquito; the intense river travel between Manaus and dengue infected communities resulted in the introduction of this virus; and the presence of a new arbovirology laboratory facility at the Instituto de Medicina Tropical de Amazonas (IMT-AM), permitted the early detection of imported cases first, and autochthonous cases soon after. On March 28, 1998, dengue cases were detected by IgM ELISA, using dengue antigens from CDC obtained through the courtesy of Dr. Duane Gubler.

Immediately after the announcement of the first cases, a commission was formed including representatives of different sectors of the federal, state and municipal governments. This commission meets at the IMT-AM and it took the following immediate actions: 1. Members of the medical community were given instructions as to how they should attend and clinically diagnose dengue patients at IMT-AM and all other health centers in Manaus; 2. Differential malaria diagnosis in suspected patients, as well as clinical laboratory data (thrombocytopenia e hypoproteinemia) were required; and 3. Tests were conducted to detect the presence of IgM antibodies in the sera of 1 in 10 patients in which 5 or more days had elapsed since initial symptoms appeared. Additionally, official announcements were made through the press, radio and television to inform the population of the signs and symptoms of dengue, what to do, where to go and which immediate mosquito control measures, they could take to reduce the risk of becoming infected.

**Incoming samples per week (01/01/98 - 05/12/98) were as follow:**

<b>Week</b>	<b>N°Sera received</b>	<b>Week</b>	<b>N°Sera received</b>
1		11	
2		12	9
3		13	74
4	1	14	1129
5		15	152
6	1	16	394
7	2	17	1320
8		18	607
9		19	544
10		20	

Up to May 8 1998, 4,233 blood specimens had been received at the Arbovirology Laboratory. Sera from patients with less than 5 days of initial symptoms are stored for virus isolation. One thousand three hundred and seventy five had been processed by the capture IgM ELISA

procedure. Of these samples, 587 (42.7%) were positive and 788 (57.3%) were negative.

**Results by age groups and Sex.**

Age groups	Males		Females		Totals
	Total	Positives	Total	Positives	
		%		%	
0 - 4 yrs	36	2.61	14	2.61	72
5 - 14 yrs	63	4.58	21	4.07	119
15 - 24 yrs	161	11.7	74	9.23	288
25 - 40 yrs	227	16.5	111	21.16	518
41 - 65 yrs	140	10.18	57	13.89	331
> 65 yrs	12	0.87	2	2.4	45
Unknown	2	0.14			2
<b>Totals</b>	<b>641</b>	<b>46.47</b>	<b>279</b>	<b>53.38</b>	<b>1375</b>

The more affected cohorts were males and females between ages of 15 to 65. Dengue infected patients were found in all of the 57 boroughs of the city. Positive samples reacted strongly to Dengue 1 antigens and that is the only serotype that has been found so far. The duration of the symptoms varied between 4 and 6 days and the complete recovery has been taking up to 2 weeks. The sera with dengue negative serology will be examined for other acute febrile illnesses. The symptoms frequency in seropositive patients are still being analyzed at the present time.

**Frequency of Symptoms in the Manaus, Dengue 1 Outbreak**

Symptoms	Seropositive patients	
	Male (279)	Female (306)
Fever	144	171
Headache	140	164
Retro-orbital pain	125	134
Mialgia	137	154
Arthralgia	119	141
Bone pain	86	111
Rash	56	84
Hemorrhagic manifestations	7	4
Under investigation	135	135

The official vector control program is just starting. Sampling the larval population in 1% of the houses of each "bairro" would allow determination of a house index in Manaus in the coming months. A group of newly trained community workers will help educate the local population in methods of eliminating mosquito breeding sites.

This study was funded in part by USAMRU-Br.

## YELLOW FEVER EPIZOOTIE IN ALTAMIRA, PARÁ, BRAZIL

Mondet B.<sup>1</sup>, Travassos da Rosa A.P.A.<sup>2</sup>, Travassos da Rosa E.S.<sup>2</sup>, Rodrigues S.G.<sup>2</sup>, Travassos da Rosa J.F.S.<sup>2</sup>

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<sup>2</sup> Instituto Evandro Chagas, Serviço de Arbovírus, Av. Almirante Barroso, 492  
66090-000 Belém, Pará, Brazil

In the Oriental Amazon Forest, close to the Xingu river, at about 50 kilometers east from Altamira, a city located on the Transamazonian road (3° 09' S; 52° 06' W), about two weeks after the first dead monkeys Alouatta spp. were encountered, collections of mosquitoes revealed a concentration of Haemagogus janthinomys, the main sylvatic vector of yellow fever in South America. A total of 456 females was obtained in 7 days, last week of April of 1998, using 2 to 5 human baits, three hours per day; 63,59 % of them captured up to 10 meters from the ground and 36,41 % on the ground.

The percentage of parous females was 72,87 %. From all the females, about 20 % have carried out three to six trophogonic cycles (1). That means a very good survival, in this period corresponding to the end of the rainy season. The females were divided in 23 pools, inoculated intracerebrally into baby mice. Ten days after, 12 of them are positive for Yellow Fever virus, indicating a minimum infection rate (MIR) of 2,63 %, which represents a very high percentage of infection in the mosquito population. If we consider just the parous females, this percentage rise to 3,61 %. Due to an immediate campaign of vaccination, none human case was to regret.

### Reference :

1. Mondet B., 1993. — Application de la méthode de Polovodova à la détermination de l'âge physiologique des *Aedes* (Diptera, Culicidae) vecteurs de fièvre jaune. — *Ann. Soc. Entomol. Fr. (N.S.)*, 29 (1) : 61-76.

## **JUNGLE YELLOW FEVER ON “MORCEGO” RIVER, AFUÁ, PARÁ, BRAZIL**

Travassos da Rosa A.P.A.<sup>1</sup>, Rodrigues S.G.<sup>1</sup>, Travassos da Rosa E.S.<sup>1</sup>, Cruz A.C.R.<sup>1</sup>,  
Vasconcelos P.F.C.<sup>1</sup>, Mondet B.<sup>2</sup>, Travassos da Rosa J.F.S.<sup>1</sup>

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At the beginning of March 1998, one human case of Yellow Fever was reported by the Pará Health Secretary, from the “rio Morcego” (0° 06' S; 50° 20' W), nearby Afuá, a city of about 20 000 habitants, located in the northern part of the Marajó Island, in the estuary of the Amazon River, in the north of the Pará State, at about 300 Km NW from Belém, the capital. Nine other cases followed, distributed in April and May. All of them have been recently laboratory confirmed as Yellow Fever, one by virus isolation, nine by serology (MAC ELISA) at the Institute Evandro Chagas (IEC). Of these cases, seven were males and three females, between 16 and 44 years old. The first case was a male, aged 23 who died after 11 days in the hospital, the others restored after some days of illness. An intensive campaign of vaccination began at the end of April, and a technical team from the IEC is now doing entomological investigations.

The place of the contamination seems to be the banks of the Morcego River, where people are established in little built-up areas, some of them working in a sawmill. No dead monkey was ever reported, but it seems possible that the virus was circulating as soon as February, because of two dead people from the same place, now suspected of YF, without any confirmation from the laboratory. Yellow fever is reported for the first time from this area, at the oriental limit of the Amazon Region. The last known human cases nearby were notified in 1974, at Gurupá, about 150 km SW, up the Amazon River.

## CHIKUNGUNYA VIRUS ACTIVITY IN SENEGAL

JOCELYN THONNON<sup>1</sup>, MALOUTH DIALLO<sup>1</sup>, DIDIER FONTENILLE<sup>2</sup>

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<sup>2</sup>Laboratoire de Zoologie Médicale à l'Institut Pasteur de Dakar. Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) BP 1386 Dakar Sénégal.

As part of programs aiming at monitoring yellow fever, dengue and Rift valley fever viruses in Senegal, arboviruses which are not specifically targeted at in this precise survey are often isolated from mosquito pools. The Chikungunya (CHIK) virus being one of these entomological surveys were carried out annually during the rainy season in Kedougou (12°11N, 12°33' W; a sudano-guinean region). Mosquitoes were captured using crepuscular human bait and CO<sub>2</sub> light traps, then sorted, pooled and stored in liquid nitrogen until isolation attempts. AP 61 cells were used for virus isolations and viruses were detected by IFI using specific immune ascitic fluids. Since 1972, 178 strains of CHIK have been isolated. The major sylvatic vectors therefore identified were *Aedes furcifer*, *Aedes taylori* and *Aedes luteocephalus* whereas the major domestic vector was *Aedes aegypti*. The amplification of the sylvatic transmission occurred with a 4 year periodicity.

Interestingly, human CHIK outbreaks have rarely been reported in Senegal except in 1966 and 1982 but an elevated seroprevalence of CHIK antibodies was found in rural populations, increasing with age. We recently reported two outbreaks, diagnosed by IgM detection using a standard MAC-ELISA. The first one occurred in Kaffrine, in an area where an outbreak of YF was investigated at the end of the 1996 rainy season. The recognition of the CHIK occurrence was the result of a systematic detection of IgM antibodies directed to the most frequently encountered arboviruses in Senegal. The population tested comprised 447 individuals, and the incidence rates were 21% for YF virus and 35,3 % for CHIK virus. The second outbreak occurred in Niakhar, during the 1997 dry season with a more limited incidence rate: 8,5% (among 576 tested individuals).

The diagnosis of severe and febrile infectious diseases is not easy in Africa for several socio-economic and cultural reasons. The challenge is even harder for milder (non life-threatening or disabling) infections. For example, in Kaffrine, the YF outbreak was recognised on behalf of a health structure sentinel, but it is very likely that the most active circulation of CHIK virus happened one or two months before it was identified. In Niakhar, the first diagnosed cases affected French expatriates. Clinical features of CHIK disease (fever, joint pains, rash) are non-specific and always rapidly resolutive without fatality, though the occurrence of chronic rheumatismal syndrome has been reported. This clinical feature explains why an epidemic of CHIK virus can occur without being notified to the health care systems in the absence of further independent investigation. For Kaffrine it was interesting to note that the same mosquito populations can transmit concomitantly two (or more) arboviruses, one being clearly identified (YF) and the other one (CHIK) requiring a specific, laboratory supported, investigation.

RECENT ACTIVITY OF JAPANESE ENCEPHALITIS VIRUS  
IN PAPUA NEW GUINEA AND A POSSIBLE LINK TO ARBOVIRAL DISEASE IN AUSTRALIA

C. Johansen<sup>1</sup>, S. Flew<sup>2</sup>, J. Oakley<sup>3</sup>, S. Ritchie<sup>4</sup>, A. van den Hurk<sup>4</sup>, R. Paru<sup>5</sup>, D. Phillips<sup>6</sup>,  
R. Hall<sup>1</sup> and J. Mackenzie<sup>1</sup>.

<sup>1</sup>Department of Microbiology, The University of Queensland, St. Lucia QLD 4072. <sup>2</sup>Ok Tedi Mining Ltd, Health Services Department, Tabubil, Western Province PNG. <sup>3</sup>Rumginae Health Clinic, via Kiunga, Western Province PNG. <sup>4</sup>Tropical Public Health Unit, Cairns QLD 4870. <sup>5</sup>Institute of Medical Research, Madang, Western Highlands Province PNG. <sup>6</sup>Centre of Public Health Science, Queensland Health, Brisbane QLD 4000.

The first appearance of Japanese encephalitis (JE) virus in Australia occurred in March/April 1995, when three cases of encephalitis were diagnosed on Badu Island, in the Torres Strait. Two of the cases were fatal. Fifty five subclinical infections were detected in humans residing on outer Torres Strait islands. In addition to the human infections, seroconversions were detected in 90 pigs on nine Torres Strait islands (Hanna *et al.*, 1996). Two isolates were obtained from subclinical human infections and eight from pools of *Culex annulirostris* collected during the Badu Island outbreak (Hanna *et al.*, 1996). This outbreak was unprecedented, as the nearest known focus of JE virus was Bali, 3000 km west of the Torres Strait, and the nearest site of isolation of JE virus was Flores, 2200 km west of the Torres Strait (Mackenzie 1997).

Sequencing of the 1995 isolates of JE virus revealed they had a common source, but the source could not be determined (Mackenzie 1997). It was thought that the most likely source was Papua New Guinea (PNG) or Irian Jaya, given their proximity to islands of the Torres Strait. As a result of the outbreak in the Torres Strait in 1995, a study was undertaken to investigate whether JE virus did exist in PNG and, if so, determine its prevalence.

Field trips to collect adult mosquitoes in different localities in the Western Province of PNG (that being closest to the Torres Strait islands) were carried out during the late wet season in 1996, 1997 and 1998. Mosquitoes were sorted to species and processed for virus isolation (mosquitoes collected in 1998 are yet to be processed) using C6/36, BHK-21 and PSEK cells (Johansen *et al.*, 1997). To date, one isolate of JE virus has been obtained from a pool of *Cx. annulirostris* collected at Lake Murray (near the PNG border with Irian Jaya) in the late wet season of 1997 (C. Johansen, S. Ritchie, A. van den Hurk, R. Paru and J. Mackenzie, unpublished results). Sequencing revealed the JE virus isolate from PNG had the same deletion in the 3' UTR (unpublished results) which was unique to all JE virus isolates obtained during the Badu Island outbreak in 1995 (Hanna *et al.*, 1996; Ritchie *et al.*, 1997), as well as an isolate obtained from the serum of a sentinel pig on Badu Island in 1998 (D. Phillips, unpublished results).

Serological surveys of humans and pigs in the Western Province of PNG have shown JE virus infections to be widespread. Forty percent of pig sera collected from various sites in the Western Province in 1995 had antibodies to JE virus (J. Shield, unpublished results). Almost 22% of human sera collected from the Daru area of the Western Province in 1989 had antibodies to JE virus (Johansen *et al.*, 1997). In the Upper Fly River area of the Western Province, the seroprevalence of antibodies to JE virus appears to have increased from 8% in 1990-91 to over 40% in 1997 (S. Flew, C. Johansen, R. Hall, J. Mackenzie, unpublished results). Serological evidence of JE virus infections were also detected



in the Gulf and Southern Highlands Provinces in 1993 and 1994 respectively (Johansen *et al.*, 1997). Thus, it appears JE virus has been enzootic in certain areas of PNG since at least 1989.

Three cases of JE have recently been confirmed in patients residing in the Upper Fly River area. Two cases were acquired in late 1997 (one of which was fatal), whilst one was acquired in February/March 1998 (J. Oakley, S. Flew, C. Johansen, R. Hall, D. Phillips and J. Mackenzie, unpublished results). It is highly likely that other cases of JE have occurred, but have gone undetected due to the lack of health care and laboratory diagnostic facilities in many remote parts of PNG (Mackenzie *et al.*, 1997). Thus, the three human cases of JE confirmed in the region so far are likely to be an underestimate of the true situation.

JE virus activity has been detected in northern Australia each year since the 1995 outbreak. During the wet seasons of 1996 and 1997 seroconversions were detected in sentinel pigs located on Saibai Island in the northern Torres Strait (J. Lee and D. Phillips, unpublished results) and more recently, another two cases of JE were diagnosed in early 1998. The first case occurred in an unvaccinated child on Badu Island and the second in a fisherman working in the Mitchell River area of Cape York, northern Queensland. The latter case of JE virus infection is the first record of JE virus activity on mainland Australia (J. Hanna, S. Ritchie, D. Phillips, personal communication).

The virus isolation and serosurveys in PNG show JE virus is clearly enzootic in certain areas, particularly the Western Province. Genetic analysis of the virus isolate obtained from Lake Murray suggests JE virus is being introduced into northern Australia from PNG. Efforts are being made to further clarify the prevalence of JE virus in PNG and determine the likely mechanism by which JE is being introduced into northern Australia from PNG.

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## **Murray Valley encephalitis virus activity in the north of Western Australia during the 1997 wet season**

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Murray Valley encephalitis (MVE) virus is a flavivirus found in the tropical north of Western Australia that circulates in complex transmission cycles between vertebrate hosts and mosquito vectors. This virus is an important human pathogen and causes rare but potentially fatal encephalitis in humans. The major vertebrate hosts of MVE virus are birds, particularly herons and other waterbirds and *Culex annulirostris* are the major mosquito vectors. MVE virus activity increases during the late wet season (February to May) and outbreaks of disease occur in years with above average monsoonal rainfall. MVE virus is known to be enzootic in the Kimberley region of Western Australia, particularly in the Ord River region near Kununurra.

The University of Western Australia carries out surveillance in the north of Western Australia to monitor MVE activity. This program is funded by the Health Department of Western Australia. Ten sentinel chicken flocks are maintained in major towns and some Aboriginal communities in the Kimberley region. Figure 1 shows the location of these sites. Blood samples are taken regularly throughout the year and sera are tested for antibodies to MVE virus using a competitive ELISA with monoclonal antibodies. This gives an early warning of increases in virus transmission and health warnings can then be issued to residents in the region who are at risk of serious disease. Annual mosquito collecting trips are also carried out to the Kimberley region and these are usually timed to coincide with the end of the wet season when virus activity is at its' peak. A maximum of 500 mosquitoes are identified from each trap and processed for virus isolation. Virus isolates are identified using specific monoclonal antibodies. By analysing these results we hope to be able to predict more accurately which environmental conditions are likely to give rise to significant increases in MVE virus activity.

During the 1997 wet season there was average rainfall in the East Kimberley with record rainfall and extensive localised flooding recorded in February in Broome in the West Kimberley. The first seroconversions to MVE virus in the sentinel chicken flocks were recorded from Derby (West Kimberley) in early March 1997. Further seroconversions occurred in the sentinel flocks throughout the Kimberley from March to June and the results are presented in Table 1. Health warnings were issued to the Kimberley residents by the Health Department of Western Australia after the heavy rain and again after the first sentinel chicken seroconversions. A mild case of encephalitis caused by MVE virus was reported from a 32-year-old female from Broome in May 1997.

**Table 1.** Number of MVE seroconversions in the Kimberley sentinel chicken flocks during the 1997 wet season

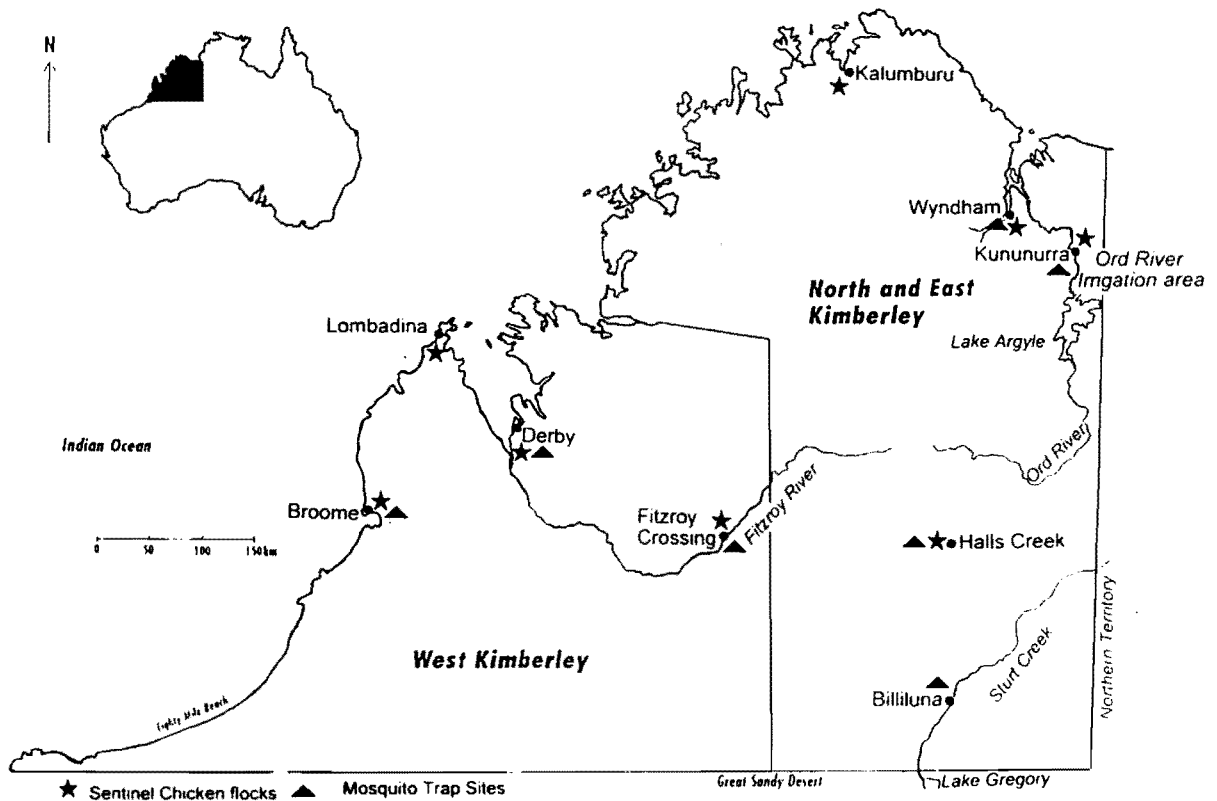
Location	Number of seroconversions in sentinel chicken flocks					
	January	February	March	April	May	June
West Kimberley	0/72	0/72	5/72	19/72	2/72	5/72
East Kimberley	0/48	0/48	3/48	11/48	2/48	4/48

Mosquitoes were collected from the six major towns in the Kimberley and from Billiluna, a small Aboriginal community in the southeast Kimberley (Figure 1), during March and April 1997. A total of 228 EVS/CO<sub>2</sub> traps were set, with an average of 2907 mosquitoes obtained per trap. Record numbers of mosquitoes were trapped in and around Broome in the West Kimberley in March 1998, with up to 40,000 mosquitoes caught in a single trap in one night. The majority of these mosquitoes were *Cx. annulirostris*, the major vector species of MVE virus. MVE virus isolates were obtained from mosquitoes trapped in all towns in the Kimberley except Fitzroy Crossing and from Billiluna. The majority of the isolates (19/24) were from *Cx. annulirostris* mosquitoes. MVE isolates were also obtained from pools of *Cx. pullus* and *An. annulipes*. Total numbers of MVE isolates and infection rates in *Cx. annulirostris* are shown in Table 2. The highest number of MVE isolates were obtained from mosquitoes trapped at Billiluna, but infection rates in 1997 were low compared to previous years when infection rates as high as 1:104 were recorded (Broom *et al*, 1997). Mosquito infection rates throughout the Kimberley were low during the 1997 wet season which was surprising given the number of seroconversions in sentinel chickens and the amount of rain that was recorded from the West Kimberley. These results suggest that above average rain and flooding must also occur in the East Kimberley region for high levels of MVE activity to be maintained.

**Table 2.** MVE isolates and infection rates in *Cx. annulirostris* from the Kimberley region, 1997

District	Location	Number of MVE isolates	Infection rates in <i>Cx. annulirostris</i>
West Kimberley	Broome	3	1:8477
	Derby	4	1:3206
East Kimberley	Kununurra	6	1:4030
	Wyndham	3	1:5841
	Halls Creek	1	1:509
	Billiluna	7	1:1214

**Figure 1.** Map of the Kimberley region showing the location of sentinel chicken flocks and mosquito trapping sites.



Map courtesy of Michael Lindsay, Department of Microbiology, The University of Western Australia

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**ACTIVITIES RELATIVE TO THE DENGUE VIRUS CARRIED AT THE PAHO/WHO COLLABORATING CENTER ON VIRAL DISEASES, INSTITUTE OF TROPICAL MEDICINE "PEDRO KOURÍ", LA HABANA (1994-1997).**

The Center was nominated in January 1994, as a PAHO/WHO Collaborating Center for the Study of Viral Diseases and Subregional Reference Center for Measles. Since then, intense diagnostic and referential functions in Cuba for the main viral diseases of human importance have been carried, as well as for other countries in the Region, characterized by the Advisory, training of human resources and the evaluation of Epidemiological Surveillance Programs. Collaborative research with other Latin American institutions has been also part of the activities undertaken during this period. The Center also rules the training of Virologists at the pre and postgraduate levels and conducts both Master Degree and Specialization courses on Virology (the latter for M.D. microbiologists) During these four years important activities were carried in coordination with PAHO:

- *Diagnosis of the re-introduction and circulation of Den-3 virus in the American region, after an absence of 15 years and mainly through an extensive study of the Epidemic of Nicaragua in 1994.*
- *Participation in the evaluation of the Control and Prevention Programs in Guatemala (1994), Colombia (1995) and Venezuela (1996), which has been organized jointly by PAHO and the respective countries.*
- *On-request supply of biological laboratory reagents to several institutions, either through direct request to the Center or according to a Project sponsored by PAHO for the provision of IgM detection kits for Dengue.*
- *A Serological Proficiency Study for Dengue in the Region (1996-1997) in close coordination with PAHO.*
- *Two International Courses: International Course on Dengue and Hemorrhagic Dengue in 1995 and Advances to the Knowledge on Dengue in 1997. Both co-sponsored by PAHO and with the participation of invited lecturers from other Collaborating Centers.*
- *Advisory on Dengue to different countries in the area.*
- *Training of staff from several laboratories of the Region.*
- *Joint research projects on Dengue with Costa Rica, Panama, Colombia, Bolivia and Brazil.*
- *Other activities linked to the functions inherent to the Subregional Reference Laboratory for Measles.*

Research at the Center is essentially directed towards the transfer or the original design of new technologies for the laboratory study of disease causing microorganisms, including the most recent advances on Molecular Biology and Recombinant DNA, Monoclonal Antibodies and Cell Culture production as well as to ensure the specialized diagnostic services to the Units of the National Ministry of Health and Quality Assurance of their laboratories all over the country.

Among the most important research projects conducted with other institutions from the area can be signaled:

- Virological and Serological Studies in relation to the Epidemic of Dengue 3 in Nicaragua, 1994, carried in collaboration with the National Institute of Health (NIH) and the Ministry of Health of Nicaragua.
- Serological Dengue Survey in Panama, in collaboration with the Gorgas Memorial Laboratory, 1994.
- Serological Dengue Survey in Medellín, in collaboration with the Departmental Laboratory, 1995.
- Role of the Vaccination Against Yellow Fever as a Risk Factor to the Development of Hemorrhagic Dengue (ongoing), Bucaramanga, Colombia.
- Study on the Epidemic of Achuapa, Nicaragua, 1995. In collaboration with the NIH and the Ministry of Health of Nicaragua.
- Usefulness of the Filter Paper for the Collection of Blood Samples for the Detection of IgM against Dengue, 1995-1996, In collaboration with the NIH of Nicaragua and INCIENSA in Costa Rica.
- Serological Survey of Dengue and Yellow Fever in the city of Santa Cruz, Bolivia, 1997-1998, in collaboration with CENETROP (ongoing).
- Serological Studies on Dengue in the City of Salta, Argentina, 1997 in collaboration with the University.
- Genomic characterization of Dengue strains from the Central American Region, in collaboration with the Departmental Laboratory, Medellín (Colombia) and the Gorgas Memorial Laboratory, Panama.

The laboratory has also provided services of Virological and Serological Diagnosis for samples sent from diverse countries of the region.

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**Studies about *Aedes albopictus* in south-eastern Brazil.**

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Project FAPESP 95/0381-4

Larval habitat productivity of *Ae. albopictus* was measured following methodology used for *Ae. aegypti*. A water reservoir situated at the Cananéia City periphery was chosen as breeding place of that mosquito. Observations were made during November 1996 until May 1997. Results showed that a mean of 14.7/day females were produced. Aspiration and human bait combined with that results showed 22.8 females a day available as man baiting. Besides these observations, *Ae. albopictus* breeding in bromeliads was found at the locality of Pedrinhas in the same region.

DISTRIBUTION OF TICK-BORNE ENCEPHALITIS VIRUS IN JAPAN  
AND EFFICACY OF AN EXISTING VACCINE  
TO THE PREVALENT VIRUS

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The distribution of tick-borne encephalitis (TBE) foci in Hokkaido and the efficacy of commercially available vaccine for Western subtype virus against the Hokkaido prevalent virus are studied. TBE virus was isolated from one rodent (*Apodemus speciosus*) out of 39 rodents captured in 1995. Two strains of TBE virus were isolated from 600 *Ixodes ovatus* ticks collected by flagging method in 1996, giving a minimum field infection rate of 0.33%(2/600). Sequencing analysis of the envelope gene indicated that virus isolates from the ticks and rodents were also the RSSE type and almost identical with an isolate recovered from dogs. Seroepidemiological survey using rodent sera showed that TBE foci are scattered at several survey points in K Town other than this case study site. Further survey using dog sera in wide areas in Hokkaido revealed several antibody positive areas in different districts. Efficacy of commercial vaccine was examined by measuring neutralizing antibody with the prevalent virus strain in the sera of vaccinated humans. The results demonstrated that Western type TBE vaccine afforded good neutralization antibody responses to the prevalent TBE virus. The results clearly indicated circulation of RSSE type virus among mammals and ticks in the case study site and the endemic foci are distributed over a wide range in Hokkaido. Western type vaccine appears to be effective against the prevalent virus.



## DUAL CAPTURES OF RODENTS IN COLORADO SUPPORT A THEORY OF SOCIAL TRAVELING

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In 1993 Sin Nombre virus was identified as the etiologic agent of a severe human respiratory disease, and the rodent host of this zoonotic hantavirus was identified as the deer mouse, *Peromyscus maniculatus*. We established longitudinal studies at three sites in Colorado in order to monitor transmission and persistence of Sin Nombre virus in rodent populations. As a complement to surveillance for hantaviral infection in rodents at these communities, we examined demographic and ecologic characteristics of these populations to evaluate factors that may influence transmission of virus. Occasionally during these studies, we captured two rodents in a single trap, presenting an opportunity to compare our dual capture results with those previously reported by others. Our multiple capture data indicate that dual captures of rodents is unusual but not rare and occur among individuals of certain species. Because most often the pair was comprised of rodents of the same species, that males more often were captured as pairs than were females, that adult females were never captured together, that juvenile males were never captured with adults of either gender, that pairs of rodents of the same species could be recaptured as pairs, and that pairs of certain species were not captured, the data support a hypothesis of social traveling, rather than random encounters.

If dual trap success is an index of social traveling or cohesiveness, a reasonable hypothesis could be that species with higher degrees of social contact have higher prevalences of infection with hantaviruses transmitted through close contact. Although data are fragmentary at this junction and alternative interpretations and caveats can be offered, it is still interesting to note that in several studies on hantaviruses circulating in western rodent populations during non-epidemic periods, the prevalence of antibody to Sin Nombre virus was highest in western harvest mice (23%) and lowest in pinon mice (3%), while deer mice maintained a middle position (11%).

## Characterization of the Conserved Antigenic Epitopes on the G2 Glycoproteins among California Serogroup Bunyaviruses

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California (CAL) serogroup viruses in the family of Bunyaviridae are significant agents of arboviral encephalitis in humans, and are maintained and transmitted in nature by mosquito vectors to the preferred vertebrate amplifying hosts. G2 glycoproteins of La Crosse (LAC) virus, a member of the CAL serogroup, were proposed by Ludwig *et al.* to be important determinants for attachment to mosquito midgut cells, whereas G1 proteins were responsible for attachment to mammalian cell receptors (1,2). G2 proteins showed an overall highly conserved amino acid sequence among the four available M segment sequences of bunyaviruses suggesting the evolutionary relatedness of the G2 glycoproteins of viruses from two serogroups of the Bunyavirus genus (3). The mechanism(s) or the selection pressure exerted by mosquito vectors on the virus is obscure, it is probably evolutionary beneficial and economic for viruses from the same lineage to share a conserved viral attachment protein region(s) for the mosquito midgut cells. The specific objectives of this study were to investigate the antigenic sites of G2 glycoproteins of LAC virus that are involved in neutralization and to determine the relatedness of G2 proteins among California serogroup viruses.

We have previously characterized the topological mapping of antigenic sites on G2 of LAC virus by a competitive ELISA. We identified four partially overlapping G2 antigenic epitopes (A to D) which were conserved among CE and Melao subgroup viruses in the CAL serogroup, but not in the TVT subgroup, nor in BUN serogroup (4). Comparison of the extracellular deduced amino acid sequences with IFA data of total 11 bunyaviruses allowed us to predict the possible locations of the sequences involved in the formation of the G2 epitopes. Sequences for LAC, SSH, BUN, and GER were obtained from Gen Bank. Additional G2 protein partial exodomain sequences were generated by RT-PCR using universal primers (G06 and G05) to amplify sequences of CAL serogroup viruses (5) followed by direct sequencing or cloning into Bluescript plasmid (Stratagene) vectors, sequencing analysis and the GCG alignment program. These G2 amino acid sequences were further analyzed by GCG program and PredictProtein Help program (Burkhard Rost). Computer predictions of the secondary structure of G2 protein were conducted. The amino acid sequence alignment shows a striking conservation of secondary structure and antigenic domains among CAL viruses (Fig. 1).

The eight CAL group viruses include a) La Crosse (LAC), snowshoe hare (SSH), California encephalitis (CE), and San Angelo (SAN) viruses from California encephalitis complex; b) Jamestown Canyon (JC), Keystone (KEY), and Jerry Slough (JS) viruses from Melao (MEL) complex; and c) trivittatus (TVT), the only virus in the TVT complex. Three viruses from Bunyamwera serogroup are Bunyamwera (BUN), the prototype virus of the genus, Cache Valley (CV), and Tensaw (TEN) viruses. The \* indicates the positions of two predicted N-linked glycosylation sites. Computer analyses of the sequences by the PredictProtein Help program (B. Rost) reveal that regions I, III, IV, V and VI are loop domains in the external regions of the G2 protein. Region II is most likely a helix, but also in the external protein of the glycoprotein. These sequence regions, highlighted in bold type (I. to VI.), have higher hydrophilic properties and greater antibody binding capacity (antigenicity index). In addition, the seven CAL group viruses which crossreact with our LACV G2 Mabs share conserved G2 amino acid sequences.

Signal peptide	-> NH <sub>2</sub>	loop I.	helix II.	*	76	
MIRMLVLIVVT...AASPVYQRCFQDGAIVK	Q	NPSKEAVTEVCLKDDVSMIK	TEARYVK	NATGVFSNNVAIRKWL	VS	DW LAC
MICILILFAVT...AASPVYQRCFQDGAIV	J	QNP	SKEAVTEVCLKDDVSMIK	TEARYIK	NATGVKSNNAIRKWL	VS
		RCFQDGAIVK	QNP	SKEAVIEVCLKDDVSMIK	TEARYVKN	SSGVKSNVALRKWL
		RCFQDGAIVK	QNP	SKEAVTEVCLKDDVSMIK	TEARYVRN	SSGVFSNNVALRKWL
		RCFQDGAIVK	QNP	SKEAVTEVCLKDDVSMIK	TEAKYVKN	TGTGFANNIAMRKWL
		RCFQDGAIVK	QNP	SKEAVTEVCLKDDVSMIK	TEAKYVKN	LTGTFSNNVAMRKWL
		QDGAIVK	QNP	SKEAVTEVCLKDDVSMIK	TEAKYVKN	TGTGFSNNIAMRKWL
		RCFQDGAIVR	Q	EYSKNAVTEVCLKDDVSMIK	TEAEYFK	NSTGVFSKNIA
MRILILLAVTQLAVSSPVITRCFHGGQLIA	E	ERKSQTSISEFCIKDDVSM	LKSEIVYTK	NDTGI	FGH	SKVFRHWTITDW BUN
MLFLLLAFLGFSTSNPIAT.RCFHGGQLIA	E	ESKSAKTSISEFCLKDDVSM	LKSEVVYIK	NDTGL	FGHN	KVFRSWTIKDW CV
		HGGQLIA	E	ERKSQTSISEFCLKDDVSM	LKTEVVYIK	NDTGLFGH
		loop III.	loop IV.	loop V.	loop VI.	159
HDCRPFKIVGGHINVIEVGDDLSLHTE	S	YVCSADCTIGVDKETAQVRLQ	DTTHHFEIAGTTVKSQWFK	STTYITLDQT	CEH LAC	
HDCRPFKITGGHINVIEVGDDLSLHTE	S	YVCSADCTIGVDKETAQVRLQ	DTTHHFEIAGITVKSQWFK	STTYITLDQT	CEH SSH	
HDCRPFKITGGHINVIEVGDDLSLHTE	S	YVCSADCTIGIDKETAQIRLQ	DTTNHFEVAGTTVKSQWFK	STTYITLDQT	CEH CE	
HDCRPFKIVGGHINVIEVGDDLSLHTE	S	YVCSADCTIGIDKETAQIRLQ	DTTNHFEVAGTTVKSQWFK	STTYITLDQT	CEH SAN	
HDCRPFQKSSGGHINVIEVGDDLSLHTE	S	YICSADCVITVDKETAQIRLQ	DTTNHFEVAGTTVKSQWFK	STTYITLDQT	CEH JC	
HDCRPFQKRAGGHINVIEVGDDLSLHTE	S	YICSADCVITVDKETAQIRLQ	DTTNHFEVAGTTVKSQWFK	STTYITLDQT	CER KEY	
HDCRPFQKSAGGHINVIEVGDDLSLHTE	S	YICSADCVITVDKETAQIRLQ	DTTNHFEVAGTTVKSQ		JS	
NECRPFQKSANGHINVIEVQPDLSLQ	TNSYVCAATCVISIDKETAQVRLQ	DTTNHFEISGTTVKSQWFK	STTYITLDQT	CEH TVT		
KACNPFVVTAGGSINVIEVDKLN	LNLVTRNYVCTGDCITIVDRKNAQII	FQTDKLNHFEVGT	TISTQWFKSKASVTL	LDRTCEH BUN		
KLCNPIPTAGGTINVIEVNK	DLMLTYICYVCSRDCXITIDKEEAQII	FQTDNPNHFEVSGT	TLLSSGWFKSKASVTL	LDRTCEH CV		
KACNPIVVTAGGTINVIEVNK	DLMLSYVCYVC			TEN		
	->		Hydrophobic Region		235	
LKVS	CAPKSVQFHACFNQHMSCVRFLHRTILPGSIANSICQNI	IIILVTLTLLIFILLSILSKTYICYLLM	PIFIPIAYMY LAC			
LKVS	CGPKSIQFHACFNQHMSCVRFLHRTILPGSIANSICQNI	IIILVTLTLLIFILLSILSKTYICYLLMPVFIPIAYAY SSH				
IKVS	CGKTLQFHACFKQHMSCVRFLHRSILPGSMASICQNI	ELIIITLALCIFIFIIMILTKTYICYVLI	PVVFMPPIAFA BUN			
VKVT	CGKKSL		CV			
	<- *	Zinc binding Site	COOH			
GVI	YNKCKKCKLGLVYHPFTECGTHCVCGARYDTS	SDRMKLHRASGLCPGYKSLRAAR LAC				
GII	YNKCKKCKLGLVYHPFTECGTHCVCGARYDTS	SDRMKLHRASGLCPGYKSLRAAR SSH				
GWAY	NRSCKKCCGLAYHPFTNCGSYCVCGSKFETS	SDRMHRRESGLCQGFKSLRVAR BUN				

Figure 1. Alignment of G2 glycoprotein amino acid sequences of eight California (CAL) serogroup viruses and three Bunyamwera group viruses.

To confirm the location of antigenic sites of G2 proteins, various length segments of the G2 exodomain sequence of LACV were systematically replaced using Morph<sup>TM</sup> mutagenesis kit (5 prime -> 3 prime, Inc.) with analogous sequences from bunyamwera virus that have no reactivity with our LAC G2 Mabs. These segments are separated either by disulfide bonds, by borders of secondary structure, or by regions of sequence identity among the eleven bunyaviruses. The mutated genes, cloned in pBluescript under the control of T7 promoter, were transfected by Transit<sup>TM</sup> polyamine (PanVera Co.) into vaccinia-T7 virus infected HeLa cells and were transiently expressed. HeLa cells were fixed and used in an IFA with the panel of LACV G2 Mabs to determine which mutations would affect Mab binding.

Four discontinuous regions I, II, III, and IV are involved in the formation of conformational epitopes A and B of G2 protein (Table 1). The replacement mutation in region III completely obliterated the binding of G2 Mabs directed against epitopes A and B, whereas mutation in regions I and II partially blocked the binding of Mabs. The mutation in region IV, however, abolished the binding of Mabs to epitope A and partially blocked the binding of Mabs to epitope B. The partial blockage of binding by these Mabs in IFA corresponds to approximately 81 to 87 % loss of binding in ELISA analyses (data not shown). No mutations altered the binding of Mab 10G5.4, the G1/G2 Mab that recognizes epitope C.

Interestingly, Mab 807-22, another G1/G2 Mab, which recognizes epitope D in the competitive ELISA studies, fails to bind to G2 glycoprotein when G2 alone is expressed on the surface of HeLa cells.

Table 5. Detection by IFA of Mab Binding to Native or Mutated LACV G2 proteins.

Epitopes Mabs	A		B		C	D
	3D9.4 (G2)	4A5.5 (G2)	9B7.5 (G2)	7H12.1 (G2)	10G5.4 (G1/G2)	807-22 (G1/G2)
G1/G2 w.t.	+++	+++	+++	++	+++	+++
G2 w.t.	+++	+++	+++	++	+++	+/-
Mut I	+/-	+/-	+/-	+/-	+++	+/-
Mut II	+/-	+/-	+/-	+/-	+++	+/-
Mut III	-	-	-	-	+++	+/-
Mut IV	-	-	+/-	+/-	+++	+/-
Mut V	+++	+++	+++	++	+++	+/-
Mut VI	+++	+++	+++	++	+++	+/-

Our study confirms the importance of using several assays in an epitope analysis. The ELISA assay measures the virus-antibody interaction and provides a sensitive method to determine the interrelationships between epitopes. Since the Mabs used in our studies could neutralize LAC virus infection of mosquito cells (1,6), these observations are probably biologically highly relevant. The sequences identified as Mab binding sites are likely to participate in the virus-mosquito interactions. Furthermore, *in vitro* mutagenic analysis confirmed the location of the shared G2 epitopes. We chose to replace LACV sequences with analogous sequences from BUN, rather than to delete or randomly mutate G2 sequences. Our method proved to be efficient in maintaining the native secondary structure and we were successful in mapping the conformational epitopes. While the sequences in regions V and VI do not appear to be in the antigenic areas recognized by our neutralizing Mabs, they may, however, be important in the binding of virus to mosquito midgut, or in the determination of vector specificity.

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# COMPARATIVE STRUCTURAL DESCRIPTION OF *WUCHERERIA BANCROFTI* DEVELOPMENT IN SUSCEPTIBLE AND REFRACTORY *AEDES* MOSQUITOES.

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**ABSTRACT.** We examined *Wuchereria bancrofti* development in susceptible and refractory species of *Aedes*. In two strains of *Aedes polynesiensis* (Tahiti and Mangareva), the parasite was capable of completing development, to the detriment of the host. In *Aedes aegypti*, which is refractory to *W. bancrofti*, larval development in the thoracic muscles was blocked at the L1 stage.

## INTRODUCTION

*Wuchereria bancrofti* has the broadest geographic distribution of filaria that infect humans. The parasite is commonly found in subtropical and tropical regions of Asia (India, Indonesia), Africa, northern South America, and several Caribbean and Pacific islands (Laurence 1989). In islands of the South Pacific, the parasite was successfully established because of the presence of suitable intermediate hosts. For example, several strains of mosquitoes belonging to the *Aedes (Stegomyia) scutellaris* species are involved in filaria transmission (Belkin 1962). Particular to the South Pacific, *Aedes polynesiensis* Marks is the main vector of *W. bancrofti* (Kessel 1957).

We have previously identified variations of *Ae. polynesiensis* vectorial efficiency (Failloux et al. 1995). In laboratory experiments with mosquitoes infected by using artificial feeders, it was observed that transmission efficiency was related to mosquito geographic origin. Mosquito strains from the Society archipelago supported the highest proportion of infective stage larvae and exhibited the lowest mortality rate when infected with a sympatric Tahitian *W. bancrofti*. In these experiments, no encapsulation or melanization was observed. Except for parasite reduction at the midgut, a phenomenon described as limitation (Bain and Brengues 1972), little is known about the biochemical mechanisms used by *Ae. polynesiensis* to modulate infection.

## MATERIALS AND METHODS

Two laboratory established colonies of *Ae. polynesiensis* described in Failloux et al. (1995) were infected with a Tahitian strain of filaria. Mangareva strain (Gambiers archipelago) supports development of *W. bancrofti* with a low efficiency. Only 46% of ingested microfilariae (mf) successfully completed larval development in the thoracic muscles by 15 days at 25-30°C (Hamon 1981). In contrast, 78%-90% of the *W. bancrofti* reached the mature larvae stage in the Tahiti colony (Society archipelago). As a control, *Aedes aegypti* which originate from Tahiti and are resistant to *Wuchereria*, were infected simultaneously.

About 50-100, seven day-old females previously starved for at least 12 hours, were exposed for 1 hour to infected blood through a parafilm membrane (Failloux et al. 1991). Venous blood was diluted with a 10% sucrose solution supplemented with  $5 \cdot 10^{-3}$ M ATP, to give a final concentration of 2500 mf/ml. Fully engorged females were maintained until day 15 at 25°C and 75% relative humidity, and supplied with a 10% sugar solution. At days 0, 4, 6, 11, 13 and 15 post-infection, 2-5 females were killed and fixed in Carnoy solution (3 vol. chloroform, 1 vol. absolute ethanol, 1 vol. acetic acid). The samples were then dehydrated as follows: 8 h in absolute ethanol, 17h in solution 1 (55% n-butanol/40,5% absolute ethanol in H<sub>2</sub>O), 8h in solution 2 (75% n-butanol/22.5% absolute ethanol in H<sub>2</sub>O) and finally 2-3 days in n-butanol. Mosquitoes were embedded in resin (Historesin, Leica instruments). Sections (5 µm) were stained with Hemalun-Eosine and were examined by light microscopy (Leiz).

## RESULTS

We observed mf development in *Ae. polynesiensis* during 15 days post-infection, whereas in *Ae. aegypti*, *W. bancrofti* development was blocked at the L1 stage.

### *W. bancrofti* development in *Ae. polynesiensis*

By one hour after ingestion, mf penetrated the midgut epithelium and migrated to the flight muscles in the thorax. At 4 days post-infection, the larvae were still found in the thoracic muscles of both *Ae. polynesiensis* strains. The larvae had changed shape, however, as they were thicker and shorter than larvae at 1h post-infection. The first

stage larvae (L1, approximately 200  $\mu\text{m}$ ) were bracketed with myofibrils that were lysed in the proximity of the larvae. The rectal cells of the larvae (Bain 1970) were gathered at the posterior end close to the anal pore. At this stage of development, the digestive apparatus was not well defined, as it had many undifferentiated cells at the distal end. At 6 days post-infection, after the first moult, the larvae were longer, but maintained the same width as earlier stages. All larvae displayed a similar development in either Tahiti or Mangareva strains. At 11 days post-infection, the second moult occurred which leads to the third stage larvae that now measure approximately 1500  $\mu\text{m}$  in length. At this size, the larvae occupy a considerable part of the thorax and perturb the structure of the sarcoplasm. The cuticle was thicker than in larvae of previous stages. In addition, the cuticle was associated with the muscles that were present at the onset of the infective mobile stage. These larvae also exhibited many structural modifications at the anterior end which harbours labial papillae, which were not easily seen. At 15 days post-infection, upon reaching the infective stage, the larvae were mobile in both *Ae. polynesiensis* strains, and they emerged from the tissues and reached the mouthparts.

#### *W. bancrofti* development in *Ae. aegypti*

In the refractory species, *Ae. aegypti*, the larvae also reached L1 stage. The larval cavity zone was devoid of organs and invaded by dense granulations clearly observed in the longitudinal sections. No distinct structure separating the larvae from the flight muscle tissue was observed. At 11 days post-infection, the thoracic muscles remained unchanged and the L1 stage larvae were not isolated from the host by any surrounding dense material. At 15 days post-infection, larval development remained blocked at the L1 stage. Although it was not readily distinguishable in our sections, it was likely that larval degeneration may have occurred.

### DISCUSSION

In this study, we examined the development of the parasite *W. bancrofti* in susceptible and refractory mosquitoes. Two strains are of the *Ae. polynesiensis* species which are known to be variably susceptible to *W. bancrofti*, whereas the third strain, *Ae. aegypti*, is naturally resistant. Studies on the variability of competency toward *Wuchereria bancrofti* (Failloux et al. 1995) and of susceptibility toward various insecticides (Failloux et al. 1994) have suggested that *Ae. polynesiensis* populations from different islands exhibit substantial genetic differentiation (Failloux et al. 1997). Our results on the variability of competency toward *W. bancrofti* (Failloux et al. 1995) have suggested that the vectorial efficiency of *Ae. polynesiensis* was related to the geographic origin of the mosquito strain. Experimental infection of various strains of *Ae. polynesiensis* have showed that the mosquitoes from Society archipelago were more efficient intermediate hosts than geographically distant strains when infected with the sympatric Tahitian *W. bancrofti* strain. In the present study, we have achieved structural examinations to compare the responses of susceptible and refractory mosquitoes. In both cases, the microfilariae passed through the midgut and penetrated the thoracic cells without host resistance. At the later stages, host response varied dramatically between the two mosquito species and larvae developed only in *Ae. polynesiensis* strains. The completion of larval development in *Ae. polynesiensis* confirms the role of this species of mosquito in filaria transmission (Rosen 1955). In contrast, in *Ae. aegypti*, larvae did not develop beyond L1 stage. In this latter species, refractoriness could be manifested by encapsulation and melanization of the pathogen in the flight muscle tissue (Ashida et al. 1990). Melanization is catalyzed by the enzyme, phenoloxidase which participates to the synthesis of melanin deposited around parasites (Brey et al. 1988). In our study, examination of degenerative larvae in *Ae. aegypti* suggested that larval death occurred without melanization, whereas a phenoloxidase activity was detected (unpublished data). This indicates that inhibition of the *W. bancrofti* growth and differentiation in *Ae. aegypti* is not related to melanization although the species is capable of melanizing and encapsulating a related microfilariae, *Brugia malayi* (Nayar and Bradley 1994).

Refractoriness to *Wuchereria* species which is under the control of a simple sex-linked allele (MacDonald 1976; Meek and MacDonald 1982) is expressed in the thoracic muscles where physiological and/or biochemical conditions hinder the growth and differentiation of filaria parasites. Therefore, the susceptibility phenotype expressed in thoracic muscles is probably controlled by additional factors. The absence of melanization in *Ae. aegypti* in our study would suggest that inhibition of larval growth is a result of biochemical factors related to the expression of the refractory gene. In this case, intracellular melanization would be a secondary response. Other molecules such as antibacterial peptides were also reported to be induced in the haemolymph of *Ae. aegypti* following the inoculation with *B. malayi* (Chalk et al. 1994).

The fate of *W. bancrofti* in *Aedes* mosquitoes was decided in the thoracic muscles. Our examinations have shown that refractoriness was not related to melanization in *Ae. aegypti* and no additional information on the

biochemical and/or physiological events have been provide with the regard to *Ae. polynesiensis* variability of competency toward *W. bancrofti*.

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## INTRODUCTORY NOTES FROM THE SUBCOMMITTEE ON INFORMATION EXCHANGE

At the last meeting of the so-called Gould House Group in Atlanta in April, several decisions were made which affected the Subcommittee on Information Exchange. For administrative effectiveness, the former Gould House Group was succeeded by a smaller committee designated as the American Committee on Arthropod-borne Viruses. This latter committee now serves as the parent committee for the Subcommittee on Information Exchange (see Summary of Actions of the April 1961 meeting of the Gould House Group). Another decision that affected this subcommittee was that its activities, including both the Catalogue and the Newsletter, be expanded to international coverage. Also the modified form of the questionnaire for registering a virus in the Catalogue was approved, which cleared the way to proceed with the further assembling of the Catalogue.

Since the April meeting, this subcommittee has been engaged in carrying out these directives. This has involved the selection of and sending out of invitations to persons in foreign countries to participate in the Newsletter and to invite them to register any new viruses that should qualify for inclusion in the Catalogue. The conditions for participation in the Newsletter and instructions for registering new viruses in the Catalogue were covered in form letters.

The selection of foreign participants and the method of communication with them are being developed in collaboration with the WHO. For areas that are covered by Regional Reference Laboratories designated by the WHO, the director or some person he may designate in the laboratory, is being utilized as "correspondent" through whom the exchange of information is channeled. So far, Regional Reference Laboratories have been designated in the Soviet Union, Czechoslovakia, England (for Europe and parts of Africa), and Australia to cover both Australia and New Zealand. In areas for which no Regional Laboratories have been designated, correspondence is being carried on directly by the subcommittee with foreign participants. A selection of foreign participants is still proceeding but at the time of this writing, 74 investigators operating in 37 different nations have been



invited to participate. Among these are 13 Americans stationed abroad. The number of recipients of the Newsletter in the United States now stands at 64, making a total of 138.

After the approval of the new form for registering viruses in the Catalogue, special emphasis was directed toward obtaining full registration of all the known arthropod-borne viruses, and invitations were circulated to register "new" and unpublished viruses that should qualify for registration. The objective of securing full registration of the well-known viruses has not yet been reached, but great progress has been realized and a total of 93 viruses have now been registered. In the registration of the published, well-known viruses, an attempt is made to have the person or his collaborators who were responsible for the discovery of the virus make the registration. Failing in this, an investigator who has been devoting major attention to the virus and who is familiar with recent literature is asked to make the registration. This procedure has required time and has accounted for the delay in registration of some of these viruses.

As for the unpublished viruses, the responsibility for registration lies with the discoverer. The duty of the committee goes no further than to extend the invitation to register. The main problem in the registration of new viruses, besides obtaining adequate data on their characteristics, is to decide whether the virus differs sufficiently from previously registered viruses to be regarded as a new species or type. This subcommittee has avoided acting as a judge in this respect and has limited its function to exchange of views among investigators who have had an opportunity to work with the virus in question. Since at present the most generally accepted criteria for classification of viruses relate to their antigenic characteristics, the opinion of Dr. Jordi Casals, who has been the outstanding leader in this field, is frequently sought, and it is a pleasure to acknowledge his collaboration and very helpful advice.

It may be anticipated that the exact bases of classification and the magnitude of divergence from other related viruses that are required for regarding a virus as a distinct entity worthy of separate designation will continue to be a matter of discussion for some time to come. Certainly it is not the function of this subcommittee to lay down rules or make decisions on the acceptance or rejection of a virus for registration; and in order to be guided in this respect, it is hoped a special committee will eventually be

appointed and assume the responsibility of rendering decisions on acceptance or rejection of a virus for registration in the Catalogue. This reference committee should also have the responsibility of reviewing from time to time the Catalogue and make recommendations on viruses that should be deleted.

Clearly, a virus catalogue, to be of value, must be kept current, not only for the registration of new viruses as they are discovered, but also new information concerning the viruses registered should be currently supplied. It was and still is hoped that this responsibility will be assumed by the person registering the virus, but it is questioned whether this source alone is dependable, especially as many of the investigators reporting viruses may be in field laboratories where library facilities are inadequate for keeping abreast with the literature. As an accessory source of information, arrangements have been made to reproduce and distribute abstracts of articles on arthropod-borne viruses from Biological Abstracts. Reproduction of abstracts is now in progress and it is the intention to send out with the Catalogue at the end of October abstracts going back to at least January 1960, and shortly thereafter for the years 1959 and 1958, and possibly 1957. The Director of Biological Abstracts, G. Miles Conrad, has not only been kind enough to grant us this permission, but also generously supplied us without charge back numbers of Section C for the past four years and will send us bimonthly issues as they appear.

These abstracts will be reproduced in the same size print as the original on 3 x 5 inch heavy paper slips. With the thought that an index system for filing would make them more readily useful, each abstract will carry an index code at the top left hand corner and will be accompanied by a supply of index cards. Thus, with the code number and the flag cards, filing is a simple clerical procedure. It will here suffice to say that the index system uses as a framework the main rubrics in the questionnaire for registering viruses in the Catalogue in order that cross reference or inter-transfer of information may be facilitated.

A similar arrangement has been made through Dr. Gordon Smith and Dr. Charles Wilcocks for the utilization of abstracts in future issues of the Bulletin of Hygiene and of Tropical Diseases if it seems desirable, but the initial issue will include only abstracts from Biological Abstracts.

For more current and unpublished information, recourse may be had to the Newsletter.

This fourth issue of the Newsletter is the first that was opened to international participation. The response has certainly vindicated the unanimous decision to share this facility with arbovirus investigators of all nations. This issue marks the achievement in the short period of two years of a truly international sharing of scientific knowledge of timely interest and importance.

Members of the Subcommittee on Information Exchange have recently had the opportunity of visiting a number of laboratories and investigators which previously have been somewhat removed in time and geography from sources of information about one of the most rapidly developing fields of medical and biological science. It is worthy of note to mention here the sincere appreciation expressed by these investigators individually and collectively to the members of the original Gould House Group who freely and generously approved the extension of this mechanism of communication and information exchange to all serious investigators of arthropod-borne viruses.

The increase in number of contributing countries to eighteen and contributing investigators to thirty-nine attests to the international coverage of the newsletter.

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## QUOTES

Elbert Hubbard: "A miracle: an event described by those to whom it was told by men who did not see it."

Jim Backus: "Many a man owes his success to his first wife and his second wife to his success."

Woody Allen: "Of all the wonders of nature, a tree in summer is perhaps the most remarkable, with the possible exception of a moose singing 'Embraceable You' in spats."

Sir Thomas Browne: "We all labour against our own cure, for death is the cure of all diseases."

Sydney J. Harris: "When I hear somebody sigh that 'Life is hard,' I am always tempted to ask, Compared to what?"

Menachem Begin: "The devil himself has not yet created a suitable vengeance for the blood of a slain infant."

George Bernard Shaw: "Progress is impossible without change; and those who cannot change their minds cannot change anything."

Rita Mae Brown: "This (The Gay Olympics) is a celebration of individual freedom, not of homosexuality. No government has the right to tell its citizens when or whom to love. The only queer people are those who don't love anybody."

George Bernard Shaw: "Life does not cease to be funny when people die any more than it ceases to be serious when people laugh."

Bob Uecker: "Let's face it. Umpiring is not an easy or happy way to make a living. In the abuse they suffer, and the pay they get for it, you see an imbalance that can only be explained by their need to stay close to a game they can't resist."

John Brown (American abolitionist on the day of his execution): "I, John Brown, am now quite certain that the crimes of this guilty land will never be purged away but with Blood."

H.L. Mencken: "There is always an easy solution to every human problem...neat, plausible and wrong."

The graduate with a Science degree asks, "Why does it work?" The graduate with an Engineering degree asks, "How does it work?" The graduate with an Accounting degree asks, "How much will it cost?" The graduate with a Liberal Arts degree asks, "You want fries with that?"

Oscar Wilde: "Bigamy is having one wife too many. Monogamy is the same."

Leonard Cohen: "There is a crack in everything. That's how the light gets in."

Henry Beston: "We need another and a wiser and perhaps more mystical concept of animals. In a world older and more complete than ours they move finished and complete, gifted with extensions of the senses we have lost or never attained, living by voices we shall never hear. They are not brethren, they are not underlings; they are not other nations, caught with ourselves in the net of life and time, fellow prisoners of the splendour and travail of the earth."

Slovak aphorism: "A thousand times nothing has killed the donkey."

Deng Xiaoping: "It doesn't matter whether a cat is black or white, as long as it catches mice."

John Glenn: "There is still no cure for the common birthday."

Henny Youngman: "When I read about the evils of drinking, I gave up reading."

Dave Barry: "Without question, the greatest invention in the history of mankind is beer. Oh, I grant you that the wheel was also a fine invention, but the wheel does not go nearly as well with pizza."

Capital Brewery: "People who drink light 'beer' don't like the taste of beer, they just like to pee a lot."

George Will: "Football combines the two worst things about America: it is violence punctuated by committee meetings."

Anonymous: "May those that love us, love us. For those that don't love us, May the Lord turn their hearts. If he won't turn their hearts, May he turn their ankles, So that by their limp we shall know them."

Bruce Baum: "I don't know what's wrong with my television set. I was getting C-SPAN and the Home Shopping Network on the same station. I bought a congressman by accident."

Lily Tomlin: "I always wanted to be somebody, but I should have been more specific."

Jeff Stilson: "I had a linguistics professor who said that it's man's ability to use language that makes him the dominant species on the planet. That may be, but I think there's one other thing that separates us from the animals. We aren't afraid of vacuum cleaners."

Toni Morrison: "Race is the least reliable information you can have about someone. It's real information, but it tells you next to nothing."

Elayne Boosler: "When women are depressed they either eat or go shopping. Men invade another country."

Albert Einstein: "If we knew what we were doing, it wouldn't be called research."

## FIRST DENGUE OUTBREAK IN ARGENTINA AFTER EIGHTY TWO YEARS OF SILENCE

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The last Dengue (DEN) outbreak in Argentina occurred in 1916, in Entre Rios Province, in eastern Argentina. In 1955, Aedes aegypti was distributed from Buenos Aires city to the northern border of the country. In 1963, it was considered eradicated from Argentina. Recently, the National Ministry of Health reported reinfestation by Ae. aegypti in some of the same areas where it previously occurred. In 1997, laboratory surveillance for DEN was carried out in Salta Province and 16 cases due to DEN 2 virus were detected by IgM-Capture-ELISA, neutralization (NT) or PCR tests. In 1998, an outbreak of DEN began in the same localities where cases had been detected the previous year. More than 700 suspected clinical cases occurred between January 3 and May 12; 510 sera from patients were tested by IgM-Capture-ELISA resulting in 346 (68 %) positives. The distribution of the positive cases by locality follows: Tartagal (228/285, 80 %), Salvador Mazza (15/55, 27 %), Salta Capital (6/9, 66 %), Orán (4/13, 30 %), General Mosconi (36/48, 75 %), Embarcación (13/19, 68 %), Aguaray (25/43, 58 %), Colonia Santa Rosa (5/7, 71 %), Santa Victoria Este (2/3, 66 %), El Galpón (1/5, 20 %) and location undetermined (11/23, 47 %). First and second samples, collected 15-30 days after onset, were tested by NT test against the four DEN viruses, resulting in 49/49 positives. Most sera neutralized each of the 4 DEN viruses, but highest titers were obtained with DEN 2. The majority of cases occurred in February. Intensive mosquito control was carried out to reduce vector mosquito population densities and temperature decreased due to seasonal variation; this was accompanied by a decrease in the number of cases. These data are preliminary because, in May, a few cases still were being detected. There have been no reports of dengue hemorrhagic fever. This is the first report of a DEN outbreak in Argentina with laboratory confirmation.