



ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

June, 1991

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PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE:

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

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Editor's comments

The thrust of the Arbovirus Information Exchange has changed because of changes that have taken place in arbovirology. By definition, arbovirology is the study of arthropod-borne viruses. However, only a very few people ever were involved in studies of all arboviruses. More commonly, individual investigators studied viruses that cause diseases in particular countries, or studied the fundamental processes by which these viruses replicate, cause disease, and circulate and spread in nature and in human and animal populations. This continues to be the case. What brings us ("arbovirologists") together is the common fascination with similarities and differences between these viruses, and the ecologic and epidemiologic attributes that characterize them: geography, weather, seasonality, vectors and vertebrate hosts, symptomatology, pathogenesis, customs, economics, religion, history, tradition, etc. The important arboviral diseases have not disappeared. Dengue, Japanese encephalitis, St. Louis encephalitis, yellow fever, Kyasanur Forest disease, the equine encephalitides, LaCrosse encephalitis, Oropouche disease, Rift Valley fever, and others continue to reappear periodically to cause morbidity and mortality in people and agriculturally and economically important animals. Indeed, some of these diseases may be as common, or more common, or more widespread now than they were a decade ago.

If arboviruses and arbovirus diseases are no less common, why is funding less common? No matter the ultimate source of funding, there is a finite amount of wealth that can possibly be devoted to anything. For any project, it is possible to allocate all money, no money, or some amount of money between these extremes. These apportionments are relative to political necessities and public perceptions. No reasonable person would call for a decrease in funding for AIDS research in order to fund bigger and better studies of an obscure, rare virus that is not known to cause disease, causes disease only rarely, or causes severe disease in a few people or animals only once in a great while. Equally obvious, however, is that we must continue to pursue the goal of preventing human misery without destroying the environment. There are aesthetic and economic, as well as altruistic, reasons for this.

Most of those interested in arboviruses are interested in some facet of arbovirology: the virus(es), the vector(s), epidemiology, diagnosis, prevention, or treatment. Today we have sophisticated and sensitive techniques for studying these viruses and the infections they cause, techniques that allow us to peer into the essence of the genetic makeup of the viruses and that allow discrete differentiations in immune responses. Applying these techniques fully, we will be able to determine the genetic basis for infection and replication in the arthropod and vertebrate hosts and the pathogenetic mechanisms by which these viruses cause disease. The next, if distant, step is prevention.

If we continue to follow our instincts, to work in one area of arbovirology while continuing to maintain our fascination with other areas, we will remain innovative, integrate new information, and apply this information to the solution of old problems. Lack of money is the least of our challenges; maintaining a feeling of community is the greatest. (CHC)

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

The next issue is scheduled to be mailed December 1, 1991 (deadline for submissions November 15, 1991). There is nothing that requires you to wait until the last minute. If you have something to communicate in July, August, September, or October, please send it to me. Also, there is nothing that prevents you from submitting a report to every issue. There are no pages charges either but, then again, this is not a publication.

PLEASE !!!

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

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GUIDELINES FOR SUBMITTING REPORTS

We want to keep this mechanism timely and viable. Therefore, submit only recent news and summaries of your work. **PLEASE** limit the submission to 1 or a very few sheets (21.59 cm x 27.94 cm = 8.5 x 11 inches) plus a table or two; condense as much as you can (single space the text); do not staple pages together; do not number pages. This is essentially a one person operation and I am a basically a lazy person; the less work I need to do, the better, I like it.

Letter to the Editor

OBITUARY

Wilbur George Downs (1913 - 1991)

He was one of our most versatile scientists, an intellectual who as a constant searcher of knowledge had the ability to put it to constructive use. Wilbur George Downs was born 7 August 1913 in Perth Amboy, New Jersey. He died in Branford, Connecticut 17 February 1991.

Wil's boyhood was principally spent in Saranac Lake, NY with 3 brothers and a sister. From an early age he showed a keen interest in natural history which remained with him all his life. At Cornell University, he might have pursued a career in medical entomology had the needed faculty stimulation existed. Instead he settled on medicine, but while still an undergraduate he spent 3 summers as a Field Staff Member of the New York Biological Survey which proved a valuable experience in later life. While studying at Cornell Medical College, he and colleague Gustave Dammin utilized the summer of 1937 learning about tropical parasitology with Professor Pedro Kouri at the University of Havana, Cuba.

After obtaining his MD degree, Wil spent the years 1938-40 as intern and resident in medicine at New York Hospital. During this period, he specialized in internal medicine and venereal diseases under Dr. Walsh McDermott. In those days syphilitic paretics were frequently treated with malaria parasites in order to induce a high fever which might destroy the microorganisms in the brain. McDermott's department cooperated with the Rockefeller Foundation's International Health Division (IHD) laboratories across the street where Dr. Lowell Coggeshall was conducting malaria investigations. Wil acted as errand boy, delivering malaria-infected blood as well as interesting himself in Coggeshall's studies. The latter was impressed with Wil's acumen and urged him to apply to Dr. Wilbur Sawyer for an RF appointment. Sawyer replied with a fellowship to study for a Master of Public Health degree at the Johns Hopkins University School of Hygiene & Public Health (1940-41) following which Wil joined the Foundation's IHD field staff. In 1940, Wil married Helen Hartley Geer of New York.

Downs's first assignment was to Trinidad, B.W.I. during the latter half of 1941 where he initiated malaria investigations. The following year he was inducted 1st Lieutenant, MC into the Army of the United States. Wil's malaria studies in Trinidad continued until 1943 when he was transferred to the South Pacific as Malaria Control Officer, first in the New Hebrides, followed by the Russell Islands and New Georgia. In 1944 he was on Bougainville, and in 1945 he became Malaria Control Officer followed by Acting chief of Preventive Medicine on Okinawa. Also during this period and extending into 1945, Downs was associated from time to time with the US NAMRU II on Guam. In 1946, Wil retired from the army and returned to civilian life with the Rockefeller Foundation (RF).

Wil Downs was one of a small group of physicians who delighted

in a prolonged tropical experience resulting in exposure to and familiarity with a great variety of tropical diseases. Throughout the war years, Downs' inherent curiosity brought him into close contact with many other diseases: dengue, venereal diseases of various kinds, leprosy, schistosomiasis, filariasis, scrub typhus, tuberculosis, intestinal parasites, fungal infections, etc. Furthermore, he had to prescribe treatment for many of these afflictions as well as initiate measures for controlling same. The point I am trying to make here is that Wil Downs was a broadly experienced worker in the field of tropical medicine and became one of our most knowledgeable ones.

Getting down to more specific achievements, working as a Rockefeller field staff member, his 1941-42 epidemiological survey of malaria in Trinidad and Tobago ranks as one of the classic studies in the field. Aided by top notch medical entomologist, Raymond Shannon, botanist, Colin Pittendrigh, and Guyanese malariologist, Horace Gillette, these four carried on a complete study of the local malaria problem and made good practical recommendations for its control. They defined the principal vectors (Anopheles aquasalis and Anopheles bellator) and their distribution, identified the breeding places (coastal swamps, aquasalis, and specific epiphytic bromeliads, bellator) and studied their flight and feeding habits (in time and space). Detailed spleen and parasite surveys of school children were conducted to verify or expand on previous findings. Microscopists were given rigorous training and closely supervised. Anophelines of many species were brought in from the wild and examined for natural infections. Other specimens were exposed for feeding on hospitalized malaria patients or malaria-infected paretics whose parasite and gametocyte counts were closely monitored and then the incubated anophelines were examined for oocysts and sporozoites to determine which species were the most susceptible to infection. Detailed studies were made of the bromeliad flora, the species defined, the optimum growing sites recognized and ecological studies undertaken of the relationship of two bromeliad-inhabiting anophelines to the various species of bromeliads and where in the ecosystem they found their preferred niches. These studies eventually led to the control of the "bromeliad malaria" by killing the plants with a dilute copper sulfate spray, thus destroying their anopheline breeding sites. And finally, close liason was maintained with government engineers who were effecting control of many aquasalis larval habitats through the use of concrete tide gates at the sandbar mouths of rivers and streams.

In Mexico, the years 1946-52 were devoted to directing an RF public health and malaria investigation program. Special clinics as well as training centers (for public health nurses, inspectors, malariologists, etc.) were organized within several of the state governments; in addition, cooperative malaria projects were carried out with the Ministry of Health. Together with colleagues Eulogio Bordas and L. Navarro, valuable long term studies with far reaching effect were initiated with DDT, studying its residual action over time as well as when applied to mud (adobe) surfaces. The effect on the incidence of Anopheles pseudopunctipennis-transmitted malaria was carefully related to single annual or biennial DDT

spraying. Likewise the effect of the DDT on the behavior of the house-haunting anophelines was closely examined. These were landmark studies (some of the earliest) of the effect of surface composition on the effectiveness of a sprayed residual insecticide. Malaria parasite and spleen surveys were conducted in various parts of the country and recommendations made to government. Investigations of an entomological nature were varied, including descriptions of new mosquitoes, establishment of insectary-breeding anopheline colonies for malaria transmission experiments and morphological and ecological studies of various species of anophelines.

From Mexico, the RF sent Downs back to Trinidad in 1952 where he founded the Trinidad Regional Virus Laboratory and directed activities until 1961. This laboratory was well conceived and efficiently operated. The staff, both professional and technical, was well trained (most of the latter by Downs) and had a notably fine esprit de corps. There was an excellent, self-contained mouse colony, and tissue culture was soon introduced. Valuable museum collections of mammals, birds, arthropods and plants were created for reference purposes. A well thought out field program operated at all times. This included human serum surveys, hospital visits for acute blood specimens from fever patients, serum collections from chickens and other domestic animals as well as wild birds (by netting or hunting) and mammals (by trapping and releasing, or hunting) and collections of mosquitoes for virus sampling. Studies of mosquito behavior and ecology were strongly encouraged, as were those directed at other medically important hematophagous arthropods. Viruswise, the Trinidad laboratory's investigations were highly successful. Many viruses (35 agents), mostly new to science, were recognized and much information accumulated on their ecology. Laboratory studies were undertaken to determine the susceptibility of various native mammals, birds and mosquitoes to many of the new agents recovered from the field. Valuable new knowledge was acquired for several human virus infections and a yellow fever outbreak in all its ramifications was carefully studied. Wil was a strong advocate of "shoe-leather" epidemiology and much of the success of the field program can be attributed to his persistent and untiring search for follow-up blood specimens of people hidden away in the countryside and often masquerading under an alias or popular name. But those second specimens were "jewels" in the sense that they were frequently the "open sesame" to important knowledge of the virus infection afflicting the unfortunate victim. All of these activities were the result of the cohesive force stimulated by Downs' magnetic personality, wide ranging interests and administrative skills.

From Trinidad Downs was recalled in August 1961 to the Foundation's headquarters in New York City where he was made an Associate Director of the Medical and Natural Sciences Division and given overall responsibility for the RF's world-wide arbovirus research program. Subsequently when the Foundation's New York laboratory was transferred to Yale University, Downs became Professor of Epidemiology and Director of the newly created Yale Arbovirus Research Unit which he headed from 1963 to 1971. That laboratory achieved its own distinction and became WHO's principal

Reference Center for Arboviruses. In 1971 Downs resigned from the RF but continued his teaching and student advising activities at Yale as a Lecturer. From 1973 and thereafter he was appointed Clinical Professor of Epidemiology.

During the course of his New York stewardship of the international arbovirus program, Downs' travels took him to many tropical countries, particularly Africa south of the Sahara where he saw first hand much evidence of the havoc wrought by malaria, onchocerciasis, yellow fever, malnutrition and the newly recognized Lassa Fever and other arena viruses. He repeatedly visited India and S.E. Asia. In more recent years he was engaged in organizing health-and disease-oriented studies associated with the Senegal River impoundment project. He made numerous trips to the area and at one time spent several months with colleagues engaging in parasite and arthropod survey work on the spot.

Wil Downs' technical skills and talents were broad. He was a highly skilled malariologist, virologist, parasitologist, epidemiologist, entomologist and ecologist. He was a keen, critical observer and had a deep and understanding knowledge of natural history. During his years in the tropics, he immersed himself in studies of the local fauna and flora. He was an enthusiastic and knowledgeable ornithologist and competent mammalogist, versed in the ways of wild animals as well as the preparation of museum specimens. He maintained a collection of native orchids and interested himself in hybridization experiments. He was a first class active fisherman of both fresh and saltwaters, ingenious tier of trout flies, versatile sportsman and expert marksman (member of Trinidad's national rifle team at one time). A man of many interests, he was photographer, stamp collector, guitarist and bookbinder. As a bibliophile, he recognized one of man's most precious commodities -- books! His library contained myriads of publications. A voracious reader, his ability introduced him to literature written in Spanish, French, German and Russian. The basement of his home housed a small laboratory with microscope, darkroom and stacks containing a multitude of volumes on entomological and medical subjects. Elsewhere were large collections of books on birds, mammals, fish, plants, geology and other subjects. In recent years Wil had been collaborating with colleague Vernon Nelson compiling a catalogue of the Caddis-flies (Trichoptera) of Connecticut. He was the author/coauthor of over 150 scientific articles as well as a book with Max Theiler, "The Arthropod-Borne Viruses of Vertebrates".

Wil was a first class administrator. He served on numerous committees and boards, both government and other, in many instances chairing same. These included: WHO Comm. on Malaria, WHO Expert Comm. on Arboviruses, Standing Advisory Comm. for Med. Research in the British Caribbean, Comm. on Arbovirus Reagents (USPHS-NIH-NIAD), Malaria Comm. Armed Forces Epidemiol. Board, WHO Comm. for East African Virus Res. Inst. (Uganda), Board of Dir. Hartley House, NYC, etc. Lectureships: several, including (ASTMH's Charles F. Craig (28th) and Fred L. Soper (3rd)). Awards: Military, several, incl. Bronze Star; Civilian, Richard M. Taylor Award (Amer.Comm. Arthropod-Borne Viruses), Johns Hopkins Soc. Scholars Medal, Walter Reed Medal (ASTMH).

Wil Downs' most cherished project was the creation of an overseas research experience for Yale Medical School students. This program, now designated the Downs Student International Health Travel Fellowships, has been in operation since 1965. It has provided stimulating experiences to a legion of young people, permitting them to work in foreign countries, observe health problems first hand and undertake modest research under alien and sometimes frustrating, albeit rewarding, circumstances.

Wil Downs is survived by his wife of a second marriage, the former Dorothy Gardner (Wil's first wife passed away in 1972); a son, William Montague of Kapaa, HI; stepson, Thomas of Bainbridge Is., WA; three daughters, Helen Haller of Ithaca, NY, Anne Carroll of Omaha, NE and Isabel of Santa Barbara, CA; stepdaughter, Nancy Leedy of Bainbridge Is.; a brother, Ray of New Hope, PA; a sister, Jessie of La Conner, WA; and 15 grandchildren.

Thomas H. G. Aitken

ANNOUNCEMENTS

The Regional Oral History Office of the University of California at Berkeley announces the availability of the oral history of Harald Norlin Johnson, M.D., Virologist and Naturalist with the Rockefeller Foundation and the California Department of Public Health.

To order at cost, please contact the Regional Oral History Office, 486 Library, University of California, Berkeley, CA 94720 [415] 642-7395

The California Mosquito and Vector
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**EPIDEMIOLOGY AND CONTROL OF
MOSQUITO-BORNE ARBOVIRUSES
IN CALIFORNIA, 1943-1987**

by

William C. Reeves

in collaboration with

S. Monica Asman, James L. Hardy,
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NOTICE

The National Institutes of Health recently awarded a research grant on tick systematics on a global basis to Dr. James H. Oliver, Jr., Director, the Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, Georgia. As part of the grant award, the Smithsonian Institution has transferred the U.S. National Tick Collection to Georgia Southern University on a long-term enhancement loan. Researchers and students interested in tick systematics or in examining specimens in this collection should contact the Curator:

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Review: Hemorrhagic fever with renal syndrome, tick- and mosquito-borne viruses (Archives of Virology, Supplement 1, Springer-Verlag, Vienna, 1991)

The 347 pages of this well-bound, paper-covered volume contain 35 articles and 7 abstracts. They represent selected presentations from international symposia held in Dubrovnik and Moscow in fall 1989. About one third of the material deals with the epidemiology, diagnosis, and testing of a vaccine for hemorrhagic fever with renal syndrome. A somewhat larger proportion is devoted to tick-borne viruses, and the remainder deals with several other topics, including the geographic distribution of a variety of viruses in the U.S.S.R., Greece, and Canada.

Several contributions on particular themes in addition to hantaviruses were noteworthy. The series of papers on Dugbe virus included additional characterization of viral biochemistry as well as pathogenesis in mice and ticks and represent a substantial contribution to our understanding of nairoviruses, a neglected group of human pathogens. The laboratory studies of Dugbe virus were complemented by excellent field observations on the related nairovirus Crimean-Congo hemorrhagic fever. Distribution of viruses throughout the extensive land mass of the U.S.S.R. was reviewed and the contrast between articles on the findings in northern Eurasia and Canada is interesting. Soviet data on the extensive circulation of California serogroup viruses and their role in human disease also is quite important in extending the potential role of these viruses as human pathogens.

This reviewer estimates that about one third of the information has been published elsewhere. This volume, however, provides a readily accessible English language source for some of the articles. Furthermore, the exposition of the material is unusually excellent, reflecting the nature of the symposia at which the original presentations were made, and the diligent editorial presence. I believe that many readers of the Arbovirus Information Exchange will find this supplement to Archives of Virology of current interest and useful for future reference.

In summary, this reviewer recommends this volume to those interested in the many viruses circulating in nature and posing a disease threat to humans, particularly considering the amount of material from Eastern Europe and the U.S.S.R. The price, unfortunately, will discourage its purchase by many interested scientists.

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DENGUE FEVER AND DENGUE HAEMORRHAGIC FEVER CASES IN THE
CAUCA VALLEY, COLOMBIA, SOUTH AMERICA, 1990.

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Medicine and Health Sciences, University del Valle,
Apartado Aéreo 25360, Cali, Colombia, South America.

An outbreak of classical Dengue Fever (DF), accompanied by several cases of Dengue Haemorrhagic Fever (DHF), were reported in Colombia, from January to April 1990. Although DF is endemic in this country, papers on laboratory confirmations of DHF in Colombia, were not found in several scientific international journals. Due to the presence of DHF in Venezuela, Colombian health authorities were under maximum alert and the amount of blood samples substantially increased at the few virology laboratories with the capability to isolate and identify Dengue virus in this country.

In our laboratory, at the University del Valle, in Cali, we had the opportunity to study 70 patients, 38 of which provided paired sera samples (acute and convalescent). Cali is the largest city in the Cauca Valley province. It is located at 3°27'N and 76°32'W, with an altitude of 995 m above sea level, in the valley of the Cauca river, between two ranges of the Andes, in a tropical dry forest ecological formation (Holdridge's system).

Fourteen of these 38 patients showed virological or serological evidence of recent Dengue virus infection (Table 1). Three of them with a primary response and 11 with a secondary one, including a 3 years old girl, by hemagglutination-inhibition test. Two of the three patients with primary response had specific seroconversion for Dengue virus four (Den-4). However, we only could obtain one Dengue virus isolation using the Aedes albopictus cell line C6/36. It was identified by an indirect fluorescence antibody test (IFA), with specific monoclonal antibodies for each of the four Dengue virus types. The isolated one turned out to be Dengue 1 virus (Den-1). The very low percentage of isolations could be explained because any other cell lines or techniques more appropriate such as mosquito intrathoracic inoculation were not employed.

Only two patients had compatible clinical and clinical pathology criteria for DHF, according to the WHO parameters, but no virus isolation was obtained from them, although both

consistently showed very high serological titers of secondary response including at least two with $\geq 40,960$ (Table 1. patients No. 9 and 11).

One of the two DHF cases was a 22 years old man living in Cali. He had a hemoglobin of 12.7 g/dl and a hematocrit of 38% with normal platelet values, one day after the beginning of the disease. Between days 3 and 8, hemoglobin arose to 15.9 g/dl and the hematocrit got up until 48 %. Thrombocytopenia ($67,000/\text{mm}^3$) occurred between days 3 and 6. Serum transaminases were elevated from the day 5 on. Values at day 7: ALT (=SGPT), 895 U/l ; AST(=SGOT), 765 U/l.

Clinical examination showed fever, chills, headache, retro-ocular pain, myalgia, arthralgia, gastrointestinal symptoms (vomiting and diarrhea), cough, maculopapular rash, petechiae and epistaxis.

The other one was a 40 years old female, from Roldanillo, a city about 150 km north west of Cali in the Cauca Valley. She had thrombocytopenia of $40,000/\text{mm}^3$ at day 10 after the beginning of the symptoms. Prothrombin time of 13 " (control 12 ") and leukocytosis of $17,500/\text{mm}^3$. Other symptoms and signs were similar to those previously mentioned, plus ecchymoses, hematemesis, melaena and metrorrhagia.

These data demonstrated the presence of an outbreak of classical DF, with one isolation of Den-1, as well as the appearance of two cases compatible with DHF, with no viral isolations, and the simultaneous circulation of Den-1 and Den-4 viruses in the Cauca Valley, near the city of Cali.

TABLE 1

DATA OF DENGUE OUTBREAK IN CALI, CAUCA VALLEY, COLOMBIA 1990

Patient No.	Sex	Age in Year	Serum	Date	Days of Evolution	Antibody titer by Hemagglutination Inhibition Test					Isolate	Type of Serologic Response
						DEN-1	DEN-2	DEN-3	DEN-4	Yellow fever		
1	F	44	A*	24-01-90	3	< 10	< 10	10	10	20	Den-1	S***
			C	04-02-90	14	≥ 1280	≥ 1280	≥ 1280	≥ 1280	≥ 1280		
2	M	27	A	26-01-90	7	< 10	< 10	< 10	< 10	< 10	None	P
			C	14-02-90	26	40	40	80	80	40		
3	M	25	A	29-01-90	2	< 10	< 10	< 10	< 10	10	None	P
			C	14-02-90	18	< 10	< 10	< 10	40	20		
4	M	53	A	31-01-90	1	< 10	< 10	< 10	< 10	< 10	None	S
			C	14-02-90	14	640	≥ 1280	≥ 1280	≥ 1280	≥ 1280		
5	F	29	A	31-01-90	7	320	640	640	≥ 1280	≥ 1280	None	S
			C	14-02-90	21	320	640	640	≥ 1280	≥ 1280		
6	F	40	A	01-02-90	1	< 10	< 10	< 10	< 10	10	None	S
			C	21-02-90	20	1280	1280	1280	2560	1280		
7	M	18	A	02-02-90	7	≥ 1280	≥ 1280	≥ 1280	≥ 1280	≥ 1280	None	S
			C	16-02-90	21	≥ 1280	≥ 1280	≥ 1280	≥ 1280	≥ 1280		
8	M	45	A	07-02-90	7	< 10	< 10	< 10	< 10	< 10	None	P
			C	01-03-90	29	20	10	20	40	10		
9	M	32	A	07-02-90	8	5120	5120	5120	≥ 40960	5120	None	S
			C	15-02-90	16	2560	5120	5120	20480	5120		
10	M	26	A	13-02-90	8	80	80	80	640	1280	None	S
			C	29-02-90	52	1280	2560	1280	2560	2560		
11	F	40	A	13-02-90	10	20480	20480	10240	≥ 40960	10240	None	S
			C	14-03-90	39	10240	5120	10240	5120	2560		
12	F	3	A	26-01-90	3	40	80	40	80	80	None	S
			C	13-02-90	21	≥ 1280	≥ 1280	≥ 1280	≥ 1280	≥ 1280		
13	F	28	A	30-03-90	10	10	10	10	20	40	ND**	S
			C	18-04-90	27	≥ 1280	≥ 1280	≥ 1280	≥ 1280	≥ 1280		
14	F	30	A	28-09-90	2	10	20	40	40	40	ND	S
			C	16-10-90	20	≥ 1280	≥ 1280	≥ 1280	≥ 1280	≥ 1280		

* A= Acute, C= Convalescent;

**ND= Not done;

*** S= Secondary, P= Primary

Dengue Haemorrhagic Fever outbreak in Sri Lanka

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Upto 1988 Sri Lanka has had endemic transmission of Dengue with many cases of classical Dengue Fever (DF) with an average of four cases (range 0-10 cases) of Dengue Haemorrhagic Fever (DHF). In 1966 and 1967 there was an islandwide outbreak of DF associated with dengue types 1 and 2 and there were 12 and 27 cases of DHF with 10 deaths. As such Sri Lanka along with other countries in South Asia was considered a "silent" area for DHF. The annual dengue infection rate ranged from 10-20% and since 1980 all four serotypes have been endemic in the capital city, Colombo.

The main vector species in the central parts of Colombo and some of the other towns has been Aedes aegypti. However in the peripheral part of Colombo and in some smaller towns there has been a higher density of Aedes albopictus. Breeding of Aedes aegypti was mainly in small containers - tyres, tins, bottles and pots, gutters and flower pots and vases. 90% of breeding sites were located outdoors.

From the beginning of 1989 it was noticed that there was an increase in the incidence of DHF, mainly in Colombo and the suburbs. This peaked during the rainy months (May to August and November to January).

The Virus Department of the Medical Research Institute received specimens from 203 clinically diagnosed cases of DHF, 20 of whom died, and 87 were serologically (HI test) confirmed as cases of dengue.

This increase was sustained at a higher level during 1990 with peaks during the rainy months. The MRI received samples from 1087 clinically diagnosed DHF cases of whom 60 died, and 347 were confirmed as dengue in the laboratory. While most cases continued to come from Colombo and suburbs there was a distinct increase of cases of DHF in the major towns in the South, West, North central and Central parts of the country.

While the majority of cases of DHF were in children with a slight excess of males, about a quarter of cases were in the adolescent and older age groups. The fatalities however were largely in children, though not exclusively so.

While about a third of cases developed shock, the most common presenting feature was haematemesis. The occurrence of skin bleeding was much less than has been described in other countries in South East Asia.

The viruses isolated during the outbreak period were dengue type 3 and dengue type 2 (at Dr. Duane Gubler's laboratory). From limited data on primary DHF cases it would appear that dengue type 3 may be the main virus associated with the outbreak, but this needs further study.

From studies conducted since 1980 at municipal clinics in Colombo the number of DF cases in 1989 and 1990 had not increased significantly.

The Entomology studies which also had been carried out since 1980 in different parts of Colombo did not show a significant increase of Aedes aegypti breeding during the last two years (Breteau index range of 20-30)

These preliminary findings tend to favour the establishment of one or more virulent dengue virus serotypes in Sri Lanka in the last two years. This remains to be confirmed by genetic studies.

DENGUE IN VILLAGES IN INDIA FROM 1981-1990

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Occurrence of dengue fevers in India is known for over 200 years. But virologically confirmed cases were recognised subsequent to World War II. Unto the turn of 1970s epidemics were known only from large cities. This could be attributed to the urban distribution of their principal vector mosquito, Ae. aegypti.

During 1980s a shift in the epidemiological pattern of dengue fever in India was noted. Outbreaks were reported from several villages from different states in India viz., Kerala, Maharashtra and Gujarat.

KERALA: The earliest report of the occurrence of dengue in villages came from Kerala. In Moovathapuzha taluka,* Ernakulum district over 22 villages had proven and/or suspected cases of dengue between 1983-85. In 1983 one dengue virus strain of type-1 was isolated from a patient with dengue like illness and 8 out of 88 serum samples from patients and contacts had the evidence of recent DEN infection. These villages included farmsteads, hamlets amidst agricultural clearing and large villages such as Koothuttukulum and Kolencherry. These villages were visited in 1985 for the study of the distribution of Ae. aegypti breeding. In only one village, Koothattukulum, this species was noted. Therefore, it was felt large towns may have Ae. aegypti breeding and disease was contracted while the people were on errands in these townships. Two townships were visited for this purpose viz., (1) Moovathupuzha municipal area and (2) Ernakulum - Cochin town group. Ae. aegypti was breeding extensively in both residential areas and Kerala State Road Transport Corporation depots, Cochin Ship Yard and Air Port area.

In rural settlements, however, Ae. albopictus mosquitoes were found which bred extensively in the tree holes, fallen palm bracts, discarded coconut shells, rotten cocoa fruits, leaf axils of pineapple, Colocasia sp., Morphos sp., coconut shell cups used for rubber tapping etc. that stand unused during monsoon period. It can be surmised that Ae. albopictus could act as a vector in rural setting in Kerala.

* Taluka = an administrative unit consisting of several villages.

MAHARASHTRA: During this decade only one episode of epidemic of dengue was investigated in an urban area viz. Miraj City. The fever cases reported in March-April 1985 were confirmed to be due to DEN type-3 virus. Peculiarity of the epidemic was that all the fever cases occurred in a hospital campus. Concomittant extensive surveys conducted in rest of the township showed very low prevalence of Ae. aegypti. In the hospital area the vector Ae. aegypti were found to breed in good numbers in defunct toilet flush tanks containing water. Very low populations of Ae. aegypti were noted and no more DEN activity was recorded after their control in April 1985.

Later two strains of DEN type-2 was isolated from wild caught Ae. aegypti in Pune urban agglomeration from 1983 onwards. These were the only evidences of DEN activity in Pune since 1975 episodes. (Gokhale & Kaul : unpublished data from NIV Pune).

During July-August 1986 extensive outbreaks of DEN were reported from the villages covered by Dhaba and Risode primary health centres (PHC) of Akola district and Methikheda PHC of Yavatmal district. While the disease outbreaks continued to occur indifferent rural areas of these districts during subsequent years, the DEN activity was noted in adjacent districts of Amaravati in 1987. Aurangabad and Parbhani districts from 1988 onwards and Buldhana district in 1990. The vigil on nonmalarious fevers by the offices of the state's Directorate of Health Services has facilitated the timely detection of DEN virus infections.

Outbreaks at 3 villages in Parbhani district were investigated in depth. Clinically the illness was typical of dengue fever except for the absence of maculopapular rash. 42 acute, 14 late acute, 73 convalescent and 19 sera from contacts were collected. There were 12 isolations of DEN type-2 and 1 of type-1.

These epidemics also had convincing serologic evidences of wide spread DEN viral activity.

In Aurangabad district, at Ranjangaon-Khuri village, there was an epidemic in 1988. The morbidity rate was 33.64 percent in a population of 3526. The number of cases reported at PHC sub centre at Ranjangaon sharply declined and the epidemic came into control following indoor residual sprays for 50% DDT (WP), with a coverage of 75% occupied households (n=533) and treatment

of the larval habitats of Ae. aegypti with approx. 1 PPM temephos. An antecedent of a breakdown in a pump for drinking water supplies seemed to have augmented the residential water storages and consequently to mosquitoenic conditions which culminated into this outbreak.

GUJARAT STATE: Following the reports of epidemics of febrile illness from several rural and urban areas of Gujarat State in 1988, virological and epidemiological investigations were carried out. Evidence of dengue virus activity was demonstrated in large cities like Surat and Rajkot as well as several small villages in Sabarkantha district. From human sera 4 strains of dengue type-2 virus were isolated of which two were from Surat city and other two were from a village Boria becharjee in Sabarkantha district. Of the 560 sera tested from different areas (including villages and townships), 122 showed the evidence of dengue infection and another 236 showed a broad reaction with flaviviruses. Entomological investigations showed a widespread distribution of Ae. aegypti both in urban and rural areas investigated. Six strains of dengue virus were isolated from Ae. aegypti collected at Chotasan village, Sabarkantha district, four of these were found to be of DEN type-2. In the household conditions this mosquito was found to breed predominantly in containers holding non-potable water. The transportation of cement tanks manufactured in cities seemed to play an important role in the dispersal of the species of to the villages. On the other hand in the non-residential areas, prolific breeding of Ae. aegypti was observed in automobile tyre dumps, and also in varied types of scraps, principally in the urban areas but also some times in rural areas. The patterns of Ae. aegypti distribution and relative prevalence was studied in 36 rural settlements and 10 urban settlements in seven districts in the state, covering the spectrum of settlements in rural-urban continuum. These studies provided cues for the mechanism of DEN dispersion through human migration, transport systems, the process of urbanization, etc. A long term surveillance of the disease and vectors is suggested.

1. INTRODUCTION

L'endémisation de la dengue sous sa forme classique ou sous sa forme hémorragique en Guyane française reste une préoccupation constante de même que la menace d'importation des cas de fièvre jaune des pays limitrophes ou voisins. Surveillance épidémiologique et mise en oeuvre des moyens préventifs (lutte antivectorielle et vaccination anti-amariile) sont les deux mesures à appliquer avec rigueur et constance.

2. METHODES DE SURVEILLANCE

2.1. Surveillance clinique

Un réseau de médecins praticiens hospitaliers ou libéraux concourt au dépistage des cas cliniquement suspects pour lesquels sont demandés les tests de confirmation biologique. En 1990, un total de 475 prélèvements nous ont été adressés : 327 de Guyane, 146 de Guadeloupe, 2 du Surinam.

2.2. Surveillance biologique

Le médecin virologue en charge du laboratoire des arbovirus a quitté l'Institut Pasteur le 30 juin 90. En septembre nous avons engagé un nouveau virologue qui a effectué 1 mois de stage au laboratoire des arbovirus de l'Institut Pasteur à Paris. Il a pris ses fonctions le 1er octobre. L'intérim a été assuré par le médecin biologiste en charge du laboratoire de microbiologie.

2.2.1. Isolement des virus

Les tentatives d'isolement des virus se font à partir du sérum par inoculation de cultures cellulaires MOS61.

L'identification se fait par IFI à l'aide d'anticorps monoclonaux.

2.2.2. Sérologie

Les techniques utilisées sont l'inhibition de l'hémagglutination et la fixation du complément en utilisant les antigènes de 4 flavivirus (Fièvre jaune, Dengue 2, Dengue 3 et Ilheus).

Nous modifions actuellement le panel d'antigènes qui comporte :

- 1 virus du groupe A : virus TONATE (isolé en Guyane) qui sera remplacé prochainement par le VEE (virus de l'encéphalite équine vénézuélienne) dont le potentiel de pathogénicité chez l'homme est beaucoup plus sévère
- 5 virus du groupe B : Dengue 1,2,3, 4 et Fièvre jaune. Intéressant ce groupe nous avons préparé et stocké l'antigène du virus SLE (encéphalite Saint-Louis) pour faire face à toute éventualité.

.../...

Pour les paires de sérums (précoce et tardif) l'ascension du titre des anticorps de 2 dilutions est significative. Pour les sérums isolés (cas le plus fréquent) titre égal ou supérieur en IHA à 640.

3. RESULTATS

3.1. Isolement de souches virales

En Guyane seulement deux souches de virus dengue de type 1 ont été isolées en 1990. Aucune souche de virus amarile n'a été isolée.

3.2. Sérologie

Les résultats suivants ont été enregistrés en 1990

PROVENANCE DES SERUMS	NOMBRE DE SERUMS TESTES	DENGUE RECENTE CONFIRMEE PAR DIAGNOSTIC SEROLOGIQUE
GUYANE	327	29
GUADELOUPE	146	20
SURINAM	2	0

4. COMMENTAIRES

Les situations de la dengue au cours des années précédentes a été la suivante :

1978-79 : Epidémie DEN.1 et DEN.2
1982 : Epidémie DEN.4
1983-85 : Aucun isolement
1986 : Isolement de 7 souches de DEN.2
1987 : " 1 " DEN.2
1988 : " 2 " DEN.2
1989 : " 5 " DEN.2 et 1 DEN.1
1990 : " 2 " DEN.1

Au vu de ces résultats l'hypothèse d'une endémisation du sérotype 2 avait été envisagée en 1989. Toutefois à la fin de l'année dernière 1 souche DEN 1 était isolée. Cette année la DEN 1 semble avoir pris le relais de la DEN 2. Les cas cependant sont restés isolés ; aucun groupement de type épidémique n'a été mis en évidence. Le génie épidémiogène du virus dengue est imprévisible. La substitution de la DEN.2 par la DEN. 1 représente toutefois une situation favorable pour la survenue d'une épidémie. Un paramètre nous manque actuellement celui des études entomologiques. Le principe du financement en 1991 d'une unité d'entomologie médicale au sein de l'Institut Pasteur de Guyane par la Direction Générale de la Santé a été retenu. Cette unité devrait voir le jour au cours de l'année 1991.

.../...

Aucun cas de fièvre jaune ne s'est déclaré en 1990. Un cas suspect toutefois a été observé en avril. Une patiente de 51 ans est décédée dans un tableau d'hépatite fulminante. L'histopathologie d'un fragment nécropsique du foie montrait des lésions de nécrose massive de type amarile. Il nous a été possible de retrouver un sérum de la patiente. Le titre d'Ac antiamarile était les suivant :

IgM par technique MAC ELISA : NEGATIF
IgG par technique IHA : 160

Les registres de vaccinations antiamariles nous ont permis de constater que la patiente avait été vaccinée le 19 octobre 1989. Le titre d'IgG confirme la bonne réponse immunitaire et permet d'écarter le diagnostic de fièvre jaune. Le diagnostic étiologique de cette hépatite fulminante n'a toutefois pu être établi.

La couverture vaccinale antiamarile en Guyane est largement assurée. Le nombre de vaccinations des dernières années sont les suivants :

1987 : 8 741 1989 : 13 330
1988 : 10 735 1990 : 7 576

La situation dans les pays voisins montre une diminution des cas déclarés. Toutefois les variations de ces chiffres sont à interpréter avec prudence (Tableau I)

Tableau I

La fièvre jaune en Amérique du Sud.

PAYS	NOMBRE DE CAS DECLARES			
	1987	1988	1989	1990
BOLIVIE	23	12	97	-
BRESIL	16	20	8	2
COLOMBIE	17	9	-	-
PEROU	179	194	-	7

.../...

CONCLUSION :

La circulation des virus dengue reste permanente en Guyane. Le sérotype 2 a été remplacé en 1990 par le sérotype 1. Les risques d'épidémisation sont une constante qui impose de ne pas relâcher les efforts de surveillance. Pour la fièvre jaune la politique de vaccination de l'ensemble de la population est très efficace.

Le point faible de notre dispositif, la surveillance entomologique, devrait disparaître en 1991 avec la création d'une unité d'entomologie financée par la Direction Générale de la Santé.

THE DENGUE SITUATION IN MALAYSIA IN 1990

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In 1990, 5590 patients suspected to have dengue/dengue hemorrhagic fever (DHF) were referred to the Arbovirus Unit of the Division of Virology, Institute for Medical Research, Malaysia from various government hospitals and clinics throughout the country. Of these 5590 patients, 1880 were confirmed by serological tests as having dengue. Of these, 176 were classified as DHF on the basis of clinical features, leukopenia and thrombocytopenia. Virus isolation was not possible in most cases during the course of the year due to problems associated with transport of samples and the unsuitable nature of specimens received by the laboratory.

Available data show a gradual increase in the number of laboratory-confirmed cases over the period February-May (average: 50 positives per month). A marked increase, however, was noted in the month of June, in which 243 laboratory-confirmed cases were noted. The number of confirmed cases subsequently remained high throughout the second half of 1990, with a peak of 313 confirmed cases in November. Geographically, the most affected states were Sarawak (603 cases), Johore (346 cases), the Federal Territory of Kuala Lumpur (255 cases), Selan gor (160 cases), Penang (124 cases) and Sabah (122 cases). The main age group affected was the 25- to 59-year group (718 cases), followed by the 15- to 24-year group (664 cases) and the 5- to 14-year group (379 cases). There appears to be a shift to the young adults and older age groups for dengue/DHF infections, as compared to the pattern observed a decade earlier, when children <14 years old were mainly affected.

A marked increase in referrals (5590) as well as confirmed cases of dengue/DHF (1880) in 1990 was noted when compared to the previous year, when 3419 patients were referred and 947 were confirmed to have dengue/DHF.

AEDES AEGYPTI, A POTENTIAL DENGUE VECTOR IN SOUTH AFRICA: MORPHOLOGY AND THE TAXONOMIC STATUS OF ANTHROPOPHILIC AND NON-ANTHROPOPHILIC POPULATIONS.

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Dengue fever occurred as an epidemic in Durban in 1926-1927. Since then it has not been reported in South Africa until 1985 when dengue 1 virus was isolated from one person and dengue antibodies detected in two other people, all of whom were Durban residents recently returned from trips to India. Because of this development and the recent outbreaks of dengue in neighbouring Mocambique and the Seychelles, there has been concern that the virus might be reintroduced into South Africa. It was thus decided that a study should be undertaken of *Ae. aegypti* and other mosquitoes, particularly *Aedes (Stegomyia)* species to evaluate their candidature as potential vectors which could participate in epidemic transmission. The overall study has several aspects and this report, our first on the project, deals with a morphological study of *Ae. aegypti* populations to elucidate their taxonomic status.

Previous work by our Unit, and recent observations in the present project have shown that both non-anthropophilic and anthropophilic populations exist in South Africa. Feral populations collected inland have been non-anthropophilic while domestic and peridomestic populations samples along the Natal coastline have been anthropophilic. Families were reared from *Ae. aegypti* collected at 18 localities, 4 representing non-anthropophilic populations and 14 anthropophilic populations. The number of white scales on the first abdominal tergite (T_1) and the number in the basal band on the second tergite (T_2) were counted. We wished to determine whether distinct type (nominata) and *formosus* forms were present in South Africa. If they were present, we wished to know whether they bred true and could be related to geographically and ecologically different populations of *Ae. aegypti*.

The results obtained from the examination of T_1 enabled each mosquito in the sample drawn from each family to be designated as belonging either to the typical form (presence of scales) or the *formosus* form (no scales). In turn this permitted each family to be classified as homogeneous for the typical form of the mosquito, homogeneous for the *formosus* form or heterogenous containing both forms. Out of 196 families, representing 18 localities, 75 (38,3%) were homogeneous typical form, 3 (1,5%) were homogeneous *formosus* form and 118 (60,2%) were heterogenous with both forms.

In a second treatment of the scale counts made on T_1 and T_2 , all the specimens representing a locality, usually belonging to 10-13 families, were regarded as one sample. For each of the 18 such samples, the ranges of values for T_1 and T_2 were recorded and the respective means and standard deviations calculated. All the ranges overlapped for both T_1 and T_2 . These results were used to calculate the $(1-\alpha)$ 100% large sample confidence limits, where $\alpha = 0.05$, for T_1 and T_2 respectively. It was thus shown that the confidence limits for T_1 and T_2 on the various populations differ in length and position, although some populations were very close to one another. When, however, the means of the populations were compared by the Walter-Duncan test in respect of T_1 alone, it was found that none of the 4 non-anthropophilic populations differed consistently from the remaining anthropophilic populations ($P < 0.05$). When both sample means (T_1 and T_2) were compared together by multivariate

analysis, all 18 populations were found to be significantly different from one another ($P < 0.0001$) using 4 statistics (Wilk's Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root).

It is concluded that the basis for separating *Ae. aegypti* into nominate and *formosus* subspecies proposed by Mattingly (1957) should be regarded as invalid. The finding that all 18 populations differed significantly regardless of their biting preference or geographic location supports the view that *Ae. aegypti* is a single polymorphic species in South Africa with a variable preference for man. The presence of some rural sylvan non-anthropophilic populations in South Africa might, however, indicate that speciation has begun.

Mattingly, P.F. 1957. Ann. trop. Med. Parasit. 51: 392-408.

REPORT OF INSTITUTE OF TROPICAL MEDICINE "PEDRO KOURI"
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Comparitive study of viral sensibility of CLA-1 (AP-61 cell line clone)

The obtainment of several mosquito cell lines during the 70s has permitted a considerable development in the studies of the viruses transmitted by arthropods as well as offering a good isolation system. Nevertheless with the increasing number of passages the viral susceptibility of these cells decreased thereby justifying the study of new cell lines and sublines of the same origin which can overcome these difficulties.

The CLA-1 clone was obtained in our laboratory from the AP-61 cell line (*Aedes pseudoscutellaris*) reported by Varma and cols in 1974. The study of its viral sensibility was carried out comparing high (70-75) and low (45-50) subcultivation numbers of the CLA-1 line with both subculture levels of the AP-61 cell line as also with the C6/36 clone used widely in arbovirological isolation studies. We infected the three systems with Dengue viruses D1 (CV-1 strain), D2 (A-35 strain) and D4 (H241 strain) at decreasing multiplicities (m.o.i.) from 0.5 to 0.01 checking 24 to 96 hours post infection. Viral antigens were detected by indirect immunofluorescence.

The Ap-61 at low passage, CLA-1 and C6/36 at both levels of subcultivation were capable of detecting viral antigens by 48 hours post infection at a m.o.i. of 0.5 while the AP-61, at high passages, emits fluorescence at 72 hours with the same m.o.i.. By 96 hours virus was not detected with a m.o.i. of 0.01 in this line at high passage while the CLA-1 and C6/36 detected viral infection at high passage 96 hours at the lowest m.o.i. assayed. The CLA-1 cell line was as sensitive to the viruses studies as the original AP-61 at low subcultivation levels and this sensitivity is maintained when the number of subcultures is increased. This characteristic is similar to the C6/36 clone. Both these clone lines have the same growth rates and both grow in commercial media, making it the line of choice over original lines.

At present we are increasing the viral strains tested and including new cell lines such as the AP-64 (*Aedes pseudoscutellaris*) Pudney and cols, 1975 and CLA-2 (a subline of CLA-1) which have proven their usefulness in the multiplication of D1 and D2.

(Morier.L., A.Castillo and V.Perez.-Cell Culture laboratory)

RETROSPECTIVE SEROEPIDEMIOLOGICAL DENGUE SURVEY IN GUAYAQUIL, 1988.

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Instituto Nacional de Medicina Tropical e Higiene "Leopoldo Izquieta Pérez", Guayaquil, Ecuador.

After more than 30 years without Aedes aegypti mosquitoes, in 1985, this vector was detected in Guayaquil, Ecuador, and early in 1988 a dengue 1 epidemic was reported. Clinically, the illness was mild (classical dengue fever) and reports on the number of cases had ranged from 30000 to over 400000. In september 1988, a seroepidemiological survey was carried on with the aim to determine the prevalence of dengue antibodies (abs) and their relation to age, sex and race, and to know the percentage of the local population that could suffer a secondary infection if another serotype is introduced in the city.

The survey was designed and conducted in Guayaquil city using an equal probabilistic random sampling method. 11 "Parroquias" were selected out of a total of 14. From these, 500 dwellings were choosen. Within each dwelling, a single family was selected; 2101 blood samples were collected by finger prick on 2 Nobuto filter papers and tested to dengue abs by inhibition ELISA test. Positive ELISA samples were tested for neutralizing dengue 1 and dengue 2 abs by PRNT on BHK21 cells.

Total attack rate was 36%, these means that more than 455000 dengue infections occurred in Guayaquil during the outbreak. Table 1 shows the antibody prevalence to dengue by age group. An increase in the prevalence of dengue Abs by age was observed. Attack rate in children (0-14 years) was 23% and in adults 41% (P< 0.01). 33% (297\1883) of males and 37% (443\1213) of females presented dengue abs. Females had a higher abs prevalence (p> 0.05).

According to the reported date of onset of illness in persons with dengue abs, the outbreak apparently began early 1988 (January) and declined in August-September. The epidemic peak occurred in April.

Clinical manifestations corresponded with dengue fever picture although a low number of mild hemorrhagic manifestations were reported in individuals with antibodies.

4% of positive persons had abs titers 1\1280. From these 25 (86%) individuals were 45 years and 2 (7%) from 25 to 44. Two children (5 and 9) showed high abs titers. The antibody base line was 1\80 by ELISA test.

From 300 positives ELISA samples, 87% presented neutralizing abs to dengue 1 virus, 5% to dengue 2 and 8% to both viruses. This preliminary result suggests a possible circulation of dengue 2 virus (during the epidemic some sera presented high IH abs titers indicating a possible secondary infection).

The results obtained suggest that dengue 1 virus circulated widely during the outbreak and produced a critical mass (more than 400000 individuals) of susceptible population to the circulating serotype and at risk of a secondary infection. It is necessary to take effective vector control measures and to establish an efficient active dengue surveillance system.

TABLE 1

Dengue prevalence by age group, Guayaquil 1988 (ELISA Test)

AGE	NO\TOTAL	%
0-4	39\219	18
5-9	70\266	26
10-14	87\249	27
15-24	138\447	31
25-34	132\316	42
35-44	86\214	40
45-54	72\151	48
55-64	74\123	60
>-65	60\104	58
TOTAL	758\2089	36

(Guzmán M., Bravo, J., Alava, A., Kourí, G., Vázquez, S., Soler, M., Rodríguez, L.)

REPORT FROM VIRUS LABORATORY, DIVISION OF TROPICAL MEDICINE,
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Genomic Amplification and Cloning of Dengue Virus cDNA using
Polymerase Chain Reaction

E. ZULKARNAIN, S. HOTTA and T. TAKEGAMI

I. Detection of D1 virus(Mochizuki strain) genome by PCR

We prepared a synthetic oligonucleotide as a primer (P12), which was complementary to a coding region for the amino terminus of envelope protein of D1 virus (Mason et al.,1987). By the reverse transcriptase reaction, D1-cDNA was synthesized and then subjected to PCR using Tth DNA polymerase (TOYOBO) and the above P12 together with P15 which is of the same sequence at the 5' end of D1 genome. Twenty-five cycles of PCR were performed. The product amplified was of ca. 1kb in length, including a noncoding region at 5' end and a core-preM-M coding region. Analyses of this product by restriction enzymes including PstI, BglII and BallI, indicated that Mochizuki strain genome was similar to D1-genome reported by Mason et al.(1987), except XbaI site.

II. Cloning work using PCR product.

The above PCR product was inserted into pUC19 DNA. We obtained a plasmid, pDEN-1 containing D1-core-preM-M. In addition, we constructed an expression vector, pDEN-1(T7) which had T7 promoter in the upstream of D1 coding region. This component has an initiation codon which is important for the regulation of dengue virus protein synthesis. Therefore it is probably useful to analyze the function of noncoding region and role(s) of core-preM-M proteins in dengue virus replication. Experiments along with this line of scheme are being undertaken.

(Reported by S.HOTTA and T.TAKEGAMI)

Direct nucleotide sequencing using total cellular RNA from dengue virus infected mosquito cells.

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In recent years the nucleotide sequences of several flaviviruses have been determined, revealing a unique genome organisation.

Generally, the approach taken involved synthesizing and cloning cDNA using highly purified virus RNA.

In our laboratory several local isolates of dengue viruses have been partially sequenced using a set of specific primers. This direct sequencing was initially accomplished using viral RNA purified through linear sucrose gradients. Since the yield of dengue viruses from cell culture is known to be rather low, between 25 to 50 cell culture flasks (150 cm²) were routinely infected, and virus harvested on three consecutive days.

Recently, a method for direct sequencing of flavivirus RNA isolated from infected mouse brain has been published (1). Mouse brain inoculation was chosen to increase the virus titer, and viral particles had to be purified prior to RNA extraction.

We are currently performing direct RNA sequencing using total RNA isolated from C6/36 cells infected with dengue viruses. The cells are disrupted by incubation with a buffer containing SDS and proteinase K, the proteins are removed by repeated phenol/chloro-form extractions, and the RNA precipitated with ethanol. This approach represents a significant improvement over other methods, since it does not require the rather time consuming purification of viruses. The yield of viral RNA is routinely very high presumably due to the presence of viral particles and free viral RNA in the cytoplasm of infected cells.

There is no need for further purification of this RNA prior to chain termination sequencing using specific primers and reverse transcriptase, and heat treatment alone seems to be sufficient to denature possible secondary structures that could interfere with chain elongation. Our sequencing protocol does not require the end-labelling of the primer molecule and is therefore suitable for large scale sequencing with many different primers.

(1) Gibson et al. (1990) *J. Virol. Methods.* **29**, 167-176

**Report from the
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AUSTRALIA**

Detection of the Dengue Virus Nonstructural Glycoprotein, NS1 in Human Sera by Antigen-Capture ELISA.

An enzyme-linked immunosorbent assay (ELISA) was developed for the detection of the dengue virus nonstructural protein, NS1 in human serum. The assay was in the form of a double sandwich ELISA in which a polyclonal rabbit anti-NS1 serum was immobilised to a microtitre plate as the capture antibody. The captured antigen was detected with mouse monoclonal antibodies (either dengue-2 specific or group-reactive) and labelled with a goat anti-mouse IgG peroxidase conjugate. Using purified NS1 as a standard, the detection sensitivity of the assay was found to be approximately 4 ng/ml (P/N > 3.0) with the dengue-2 specific monoclonal antibodies (1H7.4 and 5H4.4) as probes and approximately 60 ng/ml with the dengue group-reactive monoclonals (3D1.4 and 3A5.4) as probes (Figure 1).

As the sensitivity of the assay was not affected by the dilution of NS1 in pooled normal human sera we used the test to directly measure NS1 levels in serum samples from a 1983 dengue virus outbreak in Thailand in which the major infecting serotype was dengue 2 (based on HI data kindly supplied by Dr C Hoke). In preliminary experiments, we found between 0.5 and as much as 2.6 ug/ml of NS1 in 7 out of 17 acute-phase sera from secondary infections. In general, detection of NS1 correlated with those sera with relatively low anti-NS1 antibody titres. NS1 was not detected in any of six samples from primary cases or in secondary cases with high anti-NS1 levels.

The absence of detectable NS1 in sera with high corresponding antibody titres was possibly due to the presence of immune complexes and so a number of dissociation techniques were used in an attempt to measure any complexed NS1. In each case however, antigenic stability was found to be a problem and we were not able to generate consistent data with any of these approaches. Additional protocols are currently being investigated. Nevertheless, the absence of detectable NS1 in any of the serum samples from primary infections or those taken during the convalescent phase of secondary infections may suggest that this secreted glycoprotein is present in significant quantities only transiently during infection. This interpretation is supported by the much earlier demonstration of NS1-specific antibodies in human sera from individuals undergoing secondary infections but not in serum samples from patients with primary infections (1). Further studies are now in progress to assess the value of this capture assay in diagnostic applications as well as in basic investigations of the role that NS1 may play in dengue virus immunopathogenesis.

(Paige Hilditch, Andrew Falconar and Paul Young)

REFERENCE

1. Falkler, W.A. et.al.(1973) *J.Immunol.* 111 1804-1809.

NS1 ELISA CAPTURE ASSAY

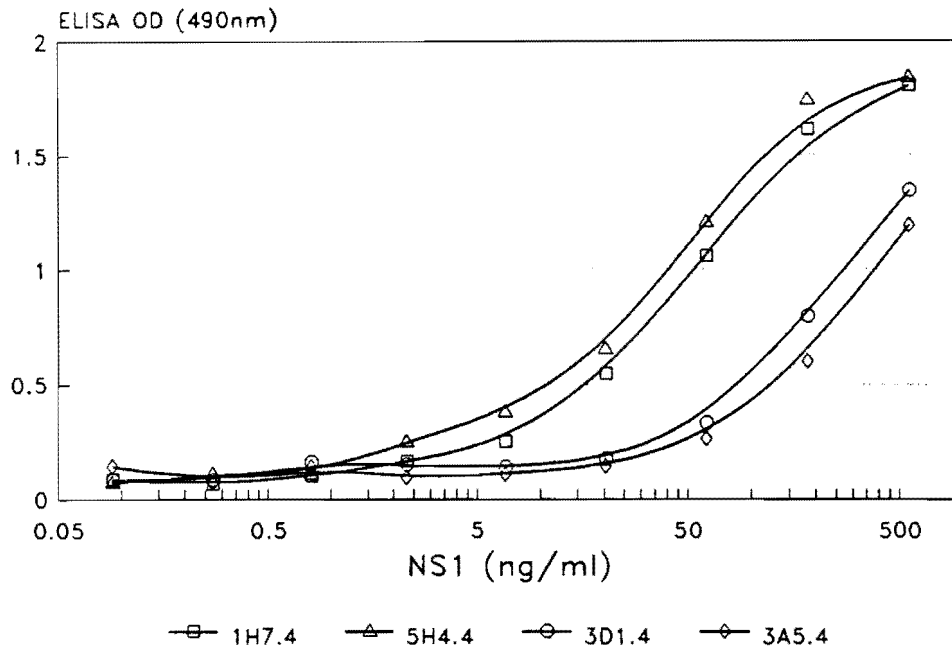


Figure 1. ELISA capture assay for the dengue virus non-structural glycoprotein, NS1. Polyclonal rabbit anti-NS1 serum diluted 1:1500 was coated to a microtitre plate and used to capture a 3-fold dilution series of a purified NS1 preparation (550ng/ml). After incubation, the bound NS1 was detected with a 1:1000 dilution of either DEN 2 type-specific (1H7.4 and 5H4.4) or DEN group-reactive (3D1.4 and 3A5.4) monoclonal antibodies and goat anti-mouse IgG-peroxidase.

THE ASSESSMENT OF GONOTROPHIC AGE IN CULISETA INORNATA WILLISTON. A. Fox and R.A. Brust, Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

Criteria for determining the gonotrophic age of Culiseta inornata were established from the analysis of ovarioles of females of known oviposition history. It is assumed that wild mosquitoes are primarily anautogenous and seek a blood meal within a few days of emergence and oviposition. Ovarioles were considered nulliparous if the pedicel had a least 6 cells and no dilatation; otherwise, the number of dilatations was considered synonymous with the degree of parity. Mosquitoes were reared at $21 \pm 1^\circ\text{C}$ with a 16-h photophase. The ovaries of live females were teased apart in physiological saline and assessed using phase contrast microscopy. All the nulliparous females had an average of 87.9 nulliparous ovarioles, and 50% had an average of 2.6 uniparous ovarioles. All the uniparous females had an average of 72.1 uniparous ovarioles, and 47% had an average 2.0 biparous ovarioles. Ninety-four percent of biparous females had an average of 12.1 biparous ovarioles, and 28% had an average of 1.4 triparous ovarioles. The following criteria were established for assessing the gonotrophic age of Culiseta inornata. A nulliparous female has at least 25 nulliparous ovarioles, and a uniparous or multiparous female has at least 15 uniparous ovarioles. A biparous female has at least 5 biparous ovarioles, and between 1-4 biparous ovarioles if there is a triparous ovariole.

On vector-host relationships in the Central European tick-borne encephalitis foci (West Slovakia)

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On the study territory of Záhorská Lowland, Small Carpathians and Danubian Lowland (West Slovakia) in total 2,057 small terrestrial mammals of 11 species have been trapped and 16,127 larvae and nymphs of *Ixodes ricinus* (12,034 larvae, 437 nymphs), *Dermacentor reticulatus* (2,071 larvae, 867 nymphs) and *Haemaphysalis concinna* (696 larvae, 22 nymphs) have been collected in the three consecutive years study. *I. ricinus* ticks and small rodents are supposed to be the principal partners in Central European encephalitis virus circulation, but the numbers of *I. ricinus* nymphs in comparison with larvae are surprisingly low.

The preference of ticks to the main groups of small terrestrial mammals has been calculated. *I. ricinus* larvae clearly preferred Murinae, *D. reticulatus* larvae preferred Microtinae (index of preference calculated as percentual participation of ticks/percentual participation of mammals rate was higher than one). Nymphs of these two tick species in main features followed the preference of larvae. *H. concinna* larvae and nymphs did not prefer either of the studied mammal groups.

The range of tick infestation on small mammals was from one to 242 ticks and in average, it was 10.7 ticks on an examined animal. In total, 12.4 % of mammals have had more than 20 ticks, among them 24.8 % of *Apodemus flavicollis* and only 8.0 % of *Clethrionomys glareolus*, to mention the most abundant species. In agreement with these observations 20.2 % of *C. glareolus* and only 8.9 % of *A. flavicollis* have been observed with a single tick infestation.

The contacts among the immatures of the three tick species have been, due to the seasonal activity, concentrated into June, July and August. The activity of *I. ricinus* dispersed from early spring till late autumn with the peak in June. The activity of *H. concinna* was from April till August, while the activity of *D. reticulatus* was strictly limited into summer months.

Mosquito spiroplasmas

Mosquito spiroplasmas (msp), although recently discovered (1982), have rapidly created considerable interest among entomologists and microbiologists. These organisms exhibit the same general properties as those of other spiroplasmas and, therefore, may be studied by the same morphological, biological and molecular methods.

Only three species are fully described at this time: Spiroplasma culicicola from the U.S.A., S. sabaudiense from France and S. taiwanense from the Far East. Another species, the Cantharis spiroplasma, although isolated from mosquitoes, has been recovered from other insects, including beetles, a wasp, a firefly and from a plant (Cirsium sp.). The latter isolates may represent a cluster of closely related serovars or species, originating in France and the U.S.A.

Since 1985, the ecology of msp from different areas of France has been studied by our group; only partial conclusions may be drawn at present. The Cantharis spiroplasma seems to be acquired from flowers through nectar-feeding in early spring. It then colonizes mosquito populations during the summer months, only to disappear rapidly in autumn. Inexplicably, only female mosquitoes are infected. A large number of mosquito species (of the genera Aedes, Anopheles, Culex, Coquillettia) have been found infected in both France and the U.S.A. On the contrary, very little is known about the life history of S. sabaudiense or other msp.

One important question about msp concerns their potential pathogenicity for mosquitoes, for other invertebrates, and for warm-blooded vertebrates, including a possible role in Creutzfeldt-Jakob disease. Our group has established good experimental evidence that S. taiwanense significantly reduces the life span of certain mosquito vectors, such as Aedes aegypti and Anopheles stephensi. To date, msp have been shown to be incapable of multiplication at 37°C and there is no epidemiologic proof that they might infect humans or other mammals. This clearly makes msp good candidates for use in biological control against mosquito vectors of human and animal diseases.

Obviously, more studies are needed on these enigmatic organisms, especially in the fields of ecology and pathogenicity mechanisms; however, data presently accumulated have delineated many promising avenues for future investigations.

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(C. Chastel, F. Le Goff, I. Humphery-Smith, M.L. Abalain-Colloc, O. Grulet, M. Odermatt and F. Guyonnet; Virus Laboratory, Faculty of Medicine, B.P. 815, 29285, Brest, France)

TITERS OF VESICULAR STOMATITIS VIRUS NEW JERSEY SEROTYPE IN MALE AND FEMALE SAND FLIES (LUTZOMYIA SHANNONI) COLLECTED ON OSSABAW ISLAND, GEORGIA

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The New Jersey serotype of vesicular stomatitis virus (VSNJ) is enzootic on Ossabaw Island, Georgia, and has been studied intensively at this focus for more than 10 years. Field and laboratory studies have confirmed that Lutzomyia shannoni sand flies (Diptera: Psychodidae) are biological vectors of VSNJ at this site (Am J Trop Med Hyg 42:476-490).

In 1989, approximately 30,000 L. shannoni were collected in light traps and tested for VSNJ infection. Three isolates of VSNJ were recovered: 1 from a pool of nonblooded females and 2 from pools of males. In 1990, 8,000 sand flies were collected in funnel traps placed over openings leading into hollow trees. Four isolates of VSNJ were obtained, including 2 from nonblooded females and 1 from males. Thirteen isolates of VSNJ from sand flies collected at this site have been made during the past 3 years. The 3 isolates from male L. shannoni are the first ever reported from male insects for VSNJ.

A plaque assay was performed with portions of original suspensions which tested positive for VSNJ in 1990. Titers of 3 of the pools, including the pool of male sand flies, were high, and the suspensions contained between 10,000 and 100,000 plaque-forming units of VSNJ per pool. These are the highest titers of VSNJ ever obtained from pools of insects, and suggest that the positive flies within these pools were actively infected with VSNJ and had supported virus replication.

One of the isolates of VSNJ obtained in 1990 was from a pool of female flies that were collected from a hollow tree located 10 meters from a pen-style pigtrap. Feral swine were trapped at this site throughout the 1990 field season, and samples were taken from these animals and processed for virus isolation on a weekly basis. VSNJ was isolated from 5 of these swine during a 5-week period 4 months prior to the collection of the VSNJ-positive sand fly pool. These isolates demonstrate a close spatial association between infected sand flies and feral swine on Ossabaw Island.

The rate of isolation of VSNJ has not varied significantly during the past 3 years. More than 57,000 L. shannoni have been tested for VSNJ virus infection, and the overall infection rate has been 23 infected sand flies per 100,000. Among females, the rate has been slightly higher: 76 infected females per 100,000.

We still do not know how the flies acquire VSNJ in nature or the role that transovarial transmission has in virus maintenance. Field and laboratory studies will continue toward resolving these unknowns.

Mosquito vectors of California and Bunyamwera serogroup
bunyaviruses in California

Report from the Arbovirus Research Program, University of California, Berkeley (L Kramer, J Hardy, W Reeves, S Presser, M Milby, W Reisen) and Dept. of Entomology, University of California, Davis (B Eldridge)

Experiments are being conducted to determine the vectors of California (CAL) and Bunyamwera (BUN) serogroup bunyaviruses in coastal, inland, and alpine habitats of California (Cal). Virus isolations from Aedes melanimon collected in the field as immatures and adults, and vector competence studies performed in the laboratory indicate this mosquito species is the primary vector of California encephalitis virus (CEV) in inland agricultural valleys of California. Since 1989, 10 strains of a virus closely related antigenically to CEV have been isolated from Aedes squamiger collected as immatures in coastal tide water pools. Laboratory vector competence studies confirmed that this species is highly susceptible to peroral infection with CE(like)V and transmits virus efficiently in salivary gland (SG) secretions after 7 days incubation at 20C or 30 days at 10C. Aedes squamiger also transmits CE(like)V vertically following IT inoculation or plegget feeding. Aedes dorsalis collected on the coast was a highly competent horizontal and vertical vector of CEV, but a less efficient horizontal vector of CE(like)V. Coastal Aedes increpitus was highly refractory to peroral infection with both CEV and CE(like)V. Culiseta inornata from coastal and inland habitats were susceptible but inefficient transmitters of CEV and CE(like)V. Snowpool Aedes species became infected with CEV and CE(like)V in the laboratory, but were unable to transmit due to mesenteronal escape, SG infection, and SG escape barriers. This is in keeping with the finding that antibody to CEV has not been found in deer and horses from alpine habitats in California.

On the other hand, antibody to another CAL serogroup virus, Jamestown canyon virus (JCV), has been detected frequently in vertebrates from mountainous habitats in California, and 8 strains of JCV have been isolated from snowpool Aedes since 1988. Studies conducted in the laboratory indicate that each of the snowpool species examined (i.e., Aedes cataphylla, Aedes communis, Aedes hexodontus) is a competent vector of JCV strains isolated from these species. Of the coastal mosquitoes, Aedes squamiger was a better horizontal vector of JCV isolated from snowpool mosquitoes than were Ae. dorsalis and Cs. inornata, whereas coastal Ae. increpitus was highly refractory to peroral infection. Culiseta inornata from inland habitats was susceptible but an inefficient peroral transmitter of an alpine strain of JCV, but females infected by plegget feeding were efficient vertical transmitters of virus.

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Antibody to a BUN serogroup bunyavirus, Northway virus (NORV), has been found in deer at both low and high mountainous elevations in California. Since 1970, 6 isolations of NORV have been made from mosquitoes collected in the Central Valley: 1 from Aedes sierrensis, 4 from Anopheles freeborni and 1 from Cs. inornata. Snowpool Aedes and Ae. sierrensis were incompetent horizontal vectors of NORV. Vertical transmission studies with Ae. sierrensis are still in progress. Culiseta inornata and An. freeborni were both susceptible and able to transmit virus perorally. In addition, Cs. inornata was able to transmit NORV vertically following plegget feeding, but at a low rate.

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

ENZYME IMMUNOASSAY FOR DIAGNOSIS OF TICK-BORNE ENCEPHALITIS (CENTRAL EUROPEAN ENCEPHALITIS) VIRUS INFECTION (M. Sekeyová and M. Grešíková)

To prepare an antigen for enzyme-linked immunosorbent assay (ELISA), Vero E6 cells were infected with a fresh isolate of tick-borne encephalitis (TBE) virus. Extracellular virus was concentrated with PEG 6,000. The antigen had hemagglutinating activity (1:80) at pH 6.4. Control antigen was prepared from supernatant fluid of non-infected Vero E6 cells.

For ELISA tests, polystyrene microtiter plates were coated with human IgG to TBE virus and incubated overnight at 4°C. Wells were washed 3 times with phosphate buffered saline (PBS) containing 0.5% Tween 20. To each well we added 100 µL of antigen. After incubation for 1h at 37°C, patient serum was added and incubated for 18h at 4°C. Wells were then washed 3 times with PBS/Tween 20. After washing, 100 µL of anti-human horse radish peroxidase conjugate was added. After 3 washes with PBS, substrate (σ-phenylenediamine) was added and the plate incubated at room temperature. After 30 min, the enzyme reaction was stopped by the addition of 2M H₂SO₄. Absorbance was measured in a Dynatech Minireader. Results are presented in the table below. Three sera reacted by hemagglutination-inhibition but not by ELISA. We suggest using two serological tests for diagnosis of TBE virus infections.

Hemagglutination-inhibition (HI) and enzyme-linked immunosorbent assays (ELISA) for antibody to tick-borne encephalitis virus in sera from patients with clinically diagnosed disease.

Serum no.	HI titer	ELISA titer
1	640	<100
2	1280	<100
3	640	1600
4	640	100
5	1280	800
6	320	800
7	1280	<100
8	1280	1600
9	640	400
10	640	800
11	1280	1600
control	<10	<100

A REPORT FROM THE MOSQUITO CONTROL LABORATORY,
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Research is currently underway at QIMR on the development of a RR-specific ELISA, as part of a project funded jointly by the Australian Water Research Advisory Council and by the Townsville/Thurangowa Water Board. The project as a whole deals with the management of tropical dams in relation to recreation and health. Research efforts are being directed towards the surveillance and control of mosquitoes, arboviruses, avian schistosomes and trombiculate mites around the Ross River Dam area. We envisage our final ELISA will be used in surveillance of the arbovirus infection levels. Such surveillance is felt to be necessary in order to determine the risks of arboviral infection associated with human use of the dam for recreational purposes. Paula Clancy at the Department of Tropical Medical Veterinary Science of James Cook University has raised a panel of monoclonal antibodies against three Australian arboviruses (RR, GET and SIN) but has found none of her monoclonals to be type specific (personal communication). She used traditional immunization protocols. We intend to continue her work but to use two alternative immunization protocols in an attempt to produce a panel of monoclonal antibodies type-specific for RR.

The first protocol involves the use of cyclophosphamide- a cytotoxic agent which acts to suppress proliferating B- and T-cell clones. The drug has successfully been used in directing the specificity of the immune response (Thomas, J. Imm. Meth. 97(1987) 237-243; Matthew and Sandroek 100(1987): 73 -82). In order to produce an immune response to epitopes of Ross River not shared by the other Australian arboviruses, we have innoculated Balb C mice firstly with either SIN, BF or GET antigen. Cyclophosphamide treatment has then been used to suppress the resulting immune response so that immunization with a second antigen - here RR -should result in the production of type-specific RR monoclonal antibodies.

The second protocol involves immunization with synthetic peptides. The complete amino-acid sequences of the structural proteins of the alphaviruses SF, SIN and RR have been published (Garoff et al, Nature (1980) 288: 236-241; Rice and Strauss, Proc. Nat. Acad. Sci. USA (1981) 78: 2062-2066; Dalgarno, L. et al, Virology (1983) 129:170 - 186). We have compared the antigenicity profiles of the E1 and E2 proteins of these viruses and have isolated portions of each protein with both a high antigenicity profile and very little amino acid sequence homology to the other alphaviruses. A number of peptides corresponding to these portions have been made and conjugated to molecules of diptheria toxin. Balb C mice are currently being immunized with these conjugates. It is hoped that some hybridomas produced from these mice will produce anti-peptide antibodies specific for the corresponding native protein of Ross River.

Work on a MVE-specific ELISA and a more general Flavivirus specific ELISA is also underway in collaboration with Dr. Roy Hall at the QE II Medical Centre at the University of Western Australia. Dr Hall is currently raising polyclonal antisera in rabbits immunized with either the purified NS1 or E protein of MVE. At QIMR both anti-NS1 and anti-E ascitic fluids are being produced from Hall's MVE-specific hybridoma cell lines.

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COMPARISON OF THE PREDICTED AMINO ACID SEQUENCE AND CODING ASSIGNMENTS OF BROADHAVEN VIRUS AND BLUETONGUE VIRUS.

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Broadhaven (BRD) virus is a member of the Great Island subgroup of the Kemerovo (KEM) serogroup of orbiviruses. The virus was originally isolated from a pool of *Ixodes uriae* ticks collected from a seabird colony at St Abb's Head, Scotland. The genome comprises 10 segments of double-stranded RNA which migrate during polyacrylamide gel electrophoresis (PAGE) in a characteristic 2:4:3:1 pattern.

Sequence data has been obtained for 9 of the 10 genomic RNA segments of BRD virus, and comparison of this data with that of bluetongue (BTV) has revealed a number of significant differences, that are summarised in Table 1. The major determinants of neutralization for the two viruses are VP5 and VP2 for BRD and BTV, respectively. However, VP5 of BRD shows sequence homology with VP5 of BTV and no relationship with VP2. The product of segment 6, NS1, is highly conserved amongst different serotypes of BTV. Nevertheless, the product of segment 6 BRD only shows 29% homology when compared to NS1 of BTV 10. The two major core proteins of BTV, VP3 and VP7, are encoded by segments 3 and 7, respectively. The product of segment 7 of BRD virus shows homology with VP7 of BTV whereas the product of segment 2 (and not segment 3) shows homology with VP3 of BTV. Analysis of the 5' and 3' terminal sequences of BRD RNA segments reveal that they differ from the end sequences of BTV, and do not appear to be conserved. These data suggest significant evolutionary divergence between tick-borne orbiviruses (KEM serogroup) and insect-borne orbiviruses (BTV and related serogroups).

Table 1. Coding assignments of KEM serogroup viruses compared with BTV

BTV			KEM	
RNA	Protein	Properties	Protein	RNA
L1	VP1	minor core; replicase/transcriptase	(142)	1
L2	VP2	outer capsid; serotype, haemagglutinin	(92)	2
L3	VP3	core; core architecture; serogroup	(75)	3
M4	VP4	minor core; capping enzyme	(69)	4
M5	VP5	outer capsid; influences serotype	(53)	5
M6	NS1	tubules	(62)	6
S7	VP7	major core; serogroup	(43)	7
S8	NS2	phosphoprotein; RNA binding?	(38)	8
S9	VP6	minor core; RNA binding	(28)	9
S10	NS3	?	(21)	10

REPORT FROM ARBOVIRUS UNIT, DEPARTMENT OF VIROLOGY
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Two major foci of Toscana virus (TOS, Bunyaviridae, Phlebovirus) have been identified in Italy: one in the Tuscany region (Central-West) and one in the Marche region (Central-East). During field studies conducted with the aim to ascertain the diffusion of the virus, more than 50 strains were isolated from two species of sand flies (Phlebotomus perniciosus and P.perfiliewi) collected in both foci; a strain was also isolated from the brain of a bat (Pipistrellus khuli) captured in Tuscany region. Moreover, several strains were isolated from cerebrospinal fluid of human cases of acute central nervous system infection in both regions.

A panel of monoclonal antibodies (MAB) against TOS virus N protein was used to initiate a study on the variability of TOS virus isolates. In a previous report we showed that these monoclonals could be classified into three groups. One (MAB group 1) reacted (in IFA test) with TOS virus as well as with Sandfly fever Naples (SFN) and Tehran (TEH) viruses, both belonging to the same serological group as TOS virus. The second group (MAB group 2) recognized TOS and SFN viruses but not TEH virus. The third group (MAB group 3) was specific for TOS virus.

The Table 1 shows the origin of the strains used. The results of IFA test are summarized in Table 2. On the basis of their reactivity, the isolates could be divided into three groups: 1) the P.perniciosus derived isolates (ISS Phl. 3, 19, 32, 40); 2) the P.perfiliewi and the bat isolates (ISS Phl. 99, Pip. 2); 3) the human isolates (ISS M.D., P.I.).

No differences could be evidenced in the proteic pattern expressed in BHK-21 cells infected with the different strains.

Studies are in progress on the capability of the different isolates to multiply in some human cell lines of neurologic origin (glioblastoma, neuroblastoma) as well as on sequence determination of the S segment of the genomes.

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TABLE 1. ORIGIN OF TOS VIRUS STRAINS

Strain	Source	Sex	Site	Year
ISS Phl 3	Phlebotomus perniciosus	F	Tuscany	1971
ISS Phl 19	" "	F	"	1980
ISS Phl 32	" "	F	"	1981
ISS Phl 40	" "	M	"	1981
ISS Phl 99	" <u>perfiliewi</u>	M	"	1982
ISS Pip 2	<u>Pipistrellus khuli</u>	?	"	1984
ISS M.D.	Human	F	"	1983
ISS P.I.	"	M	Marche	1985

TABLE 2. REACTIVITY PATTERNS OF DIFFERENT TOSCANA VIRUS ISOLATES WITH ANTI-TOSCANA N MONOCLONAL ANTIBODIES (IFA TEST).

Strain	Monoclonal antibody:									MIAF
	1/2	1/5	1/6	2/2	2/3	2/5	2/6	2/7	3/6	
ISS Phl 3	≥640	320	320	320	80	160	80	160	40	≥640
ISS Phl 19	≥640	320	320	320	80	160	80	160	40	≥640
ISS Phl 32	160	80	160	160	40	40	40	40	40	320
ISS Phl 40	320	320	320	neg	±10	10	neg	neg	20	≥640
ISS Phl 99	80	40	80	±10	neg	neg	neg	neg	20	≥640
ISS Pip 2	320	160	320	±10	±10	neg	neg	±10	20	320
ISS M.D.	40	neg	20	10	neg	neg	neg	neg	neg	320
ISS P.I.	40	neg	20	neg	neg	neg	neg	neg	neg	80

Isolation of *Ťahyňa* virus from biting midges in Czechoslovakia

Arbovirus *Ťahyňa* (TAH), a well-known virus of the California group (family *Bunyaviridae*) with a nearly world-wide distribution (Europe, Asia, Africa), has been isolated from mosquitoes (*Culicidae*) many times. Several species of these diptera seem to be the only specific vector of TAH virus.

During attempts to clarify the role of various haematophagous insects as potential vectors of viruses in Czechoslovakia, isolation experiments were made from biting midges (*Diptera: Ceratopogonidae*), too. In 1986-1987, females of haematophagous biting midges and their larvae were collected in two different regions of Moravia, CSFR: I, an area around the town Telč, district of Jihlava; II, water reservoirs at Nové Mlýny, district of Břeclav. In total, 14,250 females of the following species were examined: *Culicoides obsoletus*, *C. impunctatus*, *C. achrayi*, *C. pallidicornis*, *C. pulicaris*, *C. heliophilus*, *C. grisescens*, *C. punctatus*, *C. odibilis* and *C. reconditus*. Moreover, 7,500 larvae of the genus *Culicoides* were collected in the region II and examined.

Suspensions were prepared from pools consisting of ca. 250 imagoes, 100 larvae of the 1st to 3rd instar, or 50 larvae of the 4th instar. Virus isolation attempts were carried out by intracerebral inoculation into suckling SPF mice.

Two pathogenic agents (C/T38 and C/T39) were isolated from two pools of female biting midges *Culicoides* spp. (largely *C. obsoletus*), collected in the region I on June 25, 1986. The strains were antigenically identical and identified in the virus-neutralization test as arbovirus *Ťahyňa*. This is the first report of TAH virus in biting midges in Europe and the first isolation of a California group virus from haematophagous insects of the family *Ceratopogonidae* in the world. The evidence of circulation of TAH virus in the area investigated is supported by detecting the TAH virus-neutralizing antibodies in sera of local domestic animals and birds: 8.5% cows (224 examined), 3 of 8 sheep, 2% of ducks (600 examined).

The role of biting midges in the circulation of TAH virus should be studied in detail.

(J. Halouzka, M. Pejčoch, Z. Hubálek, J. Knoz)

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

Arbovirus Surveillance in New Jersey, 1990

During the 1990 surveillance period from June into October, 420 mosquito pools containing up to 100 mosquitoes each were tested for viruses in day old chicks. There were 38 mosquito pools positive for Eastern encephalitis (EE) and Highlands J (HJ) was isolated from 13.

Table 1 summarizes the collection area totals, species of mosquito and time of collection for the EE isolates. Activity began with early July collections and continued into October. All of the 38 isolates were from pools containing Culiseta melanura mosquitoes at 5 sites.

HJ mosquito activity is summarized in Table 2. The late July collections gave the first isolates with continued observation of HJ activity into late September. There were 13 isolates from Culiseta melanura at 3 sites.

EE isolates were also made from September into October in 7 horses in southern coastal and central counties. A single pheasant flock isolate was made in the same coastal area in October.

HJ isolates were made from a chucker partridge flock from birds which exhibited neurologic signs. The flock, however, had been over medicated with a number of drugs which could have been causal.

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

New Jersey, Department of Health
Trenton, N.J. 08625

Table 1
1990
EE MOSQUITO POOL ISOLATES
FOR WEEK ENDING

AREA COLLECTED	MOSQUITO SPECIES	7/6	7/13	7/20	7/27	8/3	8/10	8/17	8/24	8/31	9/7	9/14	9/21	9/28	10/5	AREA TOTALS
Bass River	Cs. melanura					4	3	2		2	2		1		1	15
Centerton	Cs. melanura				1	1	1	2	2	1	1		1			10
Dennisville	Cs. melanura		2			1		1	1	1						6
Hammonton	Cs. melanura			2				1			1					4
Ocean City	Cs. melanura	1	1							1						3
WEEKLY TOTALS		1	3	2	1	6	4	6	3	5	4	0	2	0	1	38

37

Table 2
1990
HJ MOSQUITO POOL ISOLATES
FOR WEEK ENDING

AREA COLLECTED	MOSQUITO SPECIES	7/27	8/3	8/10	8/17	8/24	8/31	9/7	9/14	9/21	AREA TOTALS
Bass River	Cs. melanura	1			3	2	1	1			8
Centerton	Cs. melanura					2				1	3
Hammonton	Cs. melanura				1	1					2
WEEKLY TOTALS		1	0	0	4	5	1	1	0	1	13

SEROLOGICAL EVIDENCE OF ARBOVIRUS INFECTION IN NATIVE AND DOMESTICATED MAMMALS ON THE SOUTH COAST OF NEW SOUTH WALES

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Sera from 12 species of native and 5 species of introduced mammals collected on the south coast of New South Wales between 1982 and 1988 were tested for antibodies to the following arboviruses: Ross River virus (621 animals tested), Barmah Forest virus (371), Gan Gan virus (337) and Trubanaman virus (378). Serum neutralizing antibodies to Ross River virus were found in bandicoots, wallabies, kangaroos, cattle, goats and horses; to Barmah Forest virus in kangaroo, cattle and horses; to Gan Gan virus in kangaroos, wallabies, rat, cows, horses and sheep; and to Trubanaman virus in kangaroos, wallabies, cows and horses. Titres to Ross River virus in seropositive native animal sera ranged from 32-1024 and those in seropositive domesticated animal sera ranged from 8-32,768. Prevalence of serum antibodies in macropodids, cattle and horses was: Ross River virus- 68, 19, 62%; Barmah Forest virus- 4, 26, 9%; Gan Gan virus- 44, 13, 13%; Trubanaman virus- 60, 3, 10% respectively. Evidence suggests that (i) kangaroos and wallabies are major vertebrate hosts for Ross River virus, (ii) the role of bandicoots warrants further investigation and (iii) horses may be important amplifying hosts of the virus, which causes epidemic polyarthrititis in man in Australia.

**ST. LOUIS ENCEPHALITIS VIRUS INFECTION RATES
IN CULEX NIGRIPALPUS DURING THE 1990 FLORIDA EPIDEMIC**

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Indian River County, Florida, was the apparent epicenter of a 1990 epidemic of St. Louis encephalitis (SLE), and provided both the index cases and the highest attack rates seen anywhere in the state.^{1,2} Indian River County was also the only epidemic region in which SLE virus infection rates were monitored in vector mosquitoes collected during the course of the epidemic. Routine, prospective monitoring of mosquito infection rates is not performed in Indian River County, or elsewhere in Florida. The data discussed in this report come from emergency studies conducted in response to the clues provided by ongoing research and surveillance activities in the county.

Virtually all sentinel chickens exposed to biting mosquitoes in Indian River County were infected in late June or early July, regardless of flock location. The near 100% seropositivity (by HI) was first disbelieved, but confirming serological tests available on the first days of August convinced us that risk of epidemic activity was probably extremely high. Intensive sampling of mosquito populations commenced two weeks before any suspect SLE cases were identified. As is typically the case, dates of onset were available much after the fact and the index case of SLE was not reported until August 13.

During the epidemic mosquito populations were sampled almost exclusively by chicken-baited "lard can" traps. These traps are highly effective in trapping large numbers of host-seeking Culex nigripalpus females, but tend to collect relatively low numbers of other species regardless of their true abundance. Trapped mosquitoes were maintained in the lab for 2-4 days before they were killed for virus assay. Mosquitoes from each collection were then identified and pooled, almost always in groups of 100. Each pool was triturated, then centrifuged before use in infectivity assays. A ten-fold dilution of each pool was inoculated into groups of 10 laboratory-reared, uninfected Culex quinquefasciatus mosquitoes used as bioassay hosts. Heads of the bioassay mosquitoes were squashed on slides ten days after inoculation, and were scored for presence of SLE viral antigen by an indirect immunofluorescent assay. Infection rates for each sample were calculated by the method of Le.³ During the epidemic, collections were processed by the principle of "Last in, First out." This was done to provide timely data that would be useful in guiding operational decisions in epidemic control at the local level.

The first week of field sampling occurred on three nights, in three different parts of the county. These six population samples (a total of 9465 Cx. nigripalpus in 96 pools) yielded 41 SLE virus isolates, greatly exceeding the number of isolates made during the remainder of the epidemic. Infection rate of each sample was

impressively high (ranging from 3.4/1000 - 8.5/1000), even though the three sites were several miles apart. This observation was consistent with other evidence that SLE activity during the epidemic was decidedly non-focal within Indian River County. Of nearly 33,000 Culex nigripalpus tested so far from the period of August 5 through November 15, the calculated infection rate overall is 1.4 per 1000 (or 1:698).

Significance testing is problematic for sample infection rates based on testing of pooled specimens, and it is impossible to reliably estimate infection rate of a mosquito population unless very large samples are available. With that limitation in mind, Figure 1 is based on only a subset of the field collections tested for SLE virus infection. Namely, the mosquito infection rates (represented by black dots) only show collections having more than 1000 mosquitoes. While this excludes much data, the sample rates plotted are probably reasonable estimates of true population infection rates. When plotted against date of onset for the Indian River County human cases, it is apparent that the high sample infection rates seen August 5-9 preceded peak dates of onset in the county by 2-3 weeks. This figure leaves the distinct impression that "peak" mosquito infection rates were coincident with the time that most SLE cases were actually bitten.

There are important caveats to consider that may compromise this explanation of the data. First, note that several cases were infected at times when mosquito samples had SLE infection rates that were less than 1 per 1000 - far less than those seen earlier. In fact, the last case with onset November 9 was also the only fatality observed in the county.

There are also no mosquito infection data at all for the critical period between August 9 and August 24, nor for any time before August 5, 1990. There are no clues as to when mosquito infection rates rose to what we now know to be a dangerous level, or what rates were during the period in which sentinel chickens first became infected.

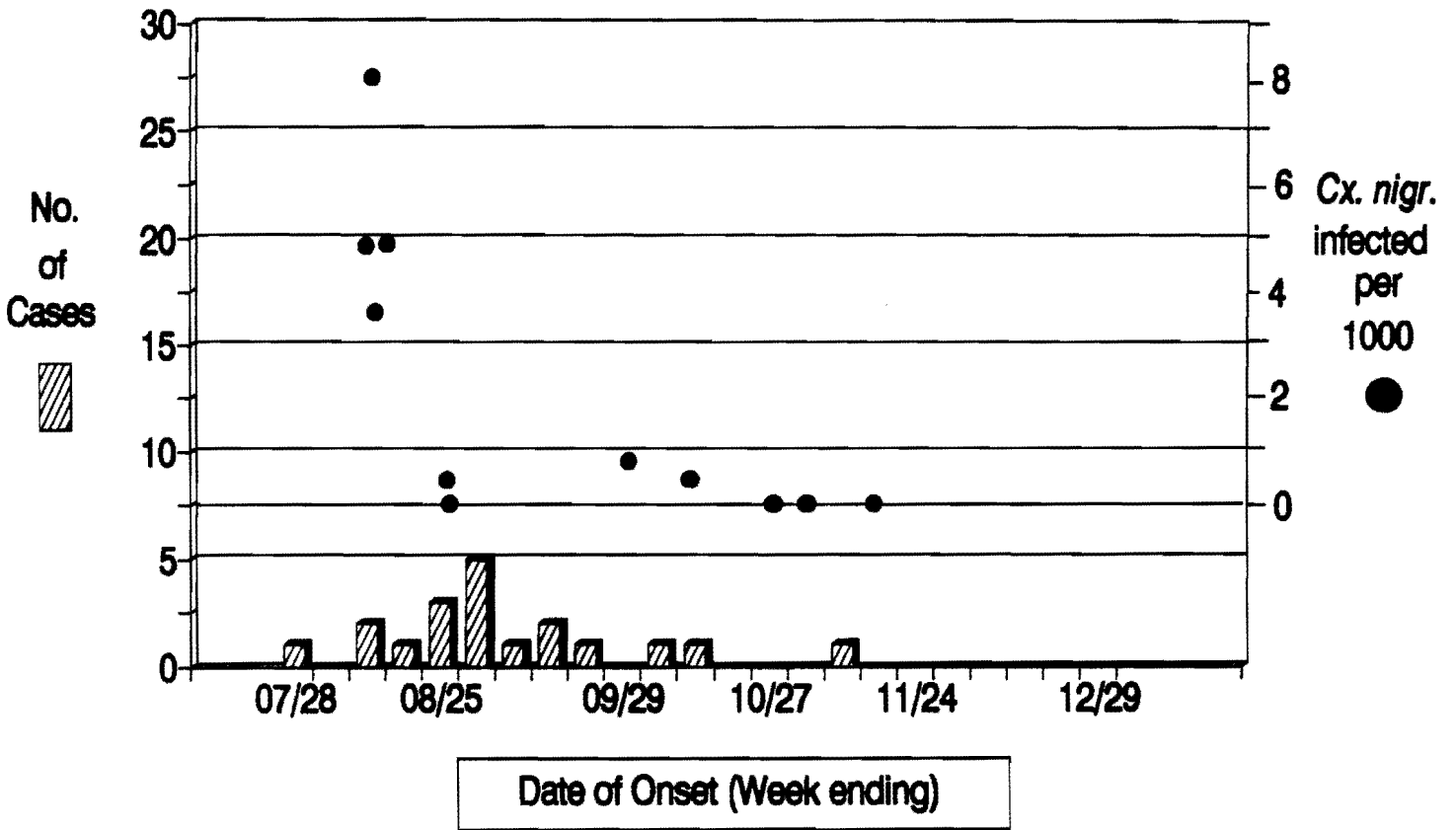
Although the lard can traps used preferentially trap Culex nigripalpus, other mosquitoes were nonetheless captured in small numbers. While many pools of these miscellaneous species remain to be tested, those tested to date have all been SLE-negative. Culex quinquefasciatus was conspicuously absent from the collections.

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FIGURE 1

Indian River County, Florida - 1990



**THE USE OF AVIAN SEROSURVEYS TO TRACK ST. LOUIS
ENCEPHALITIS VIRUS TRANSMISSION IN NATURE**

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The 1990 epidemic of St. Louis encephalitis (SLE) virus in southern Florida provided us with a unique opportunity to simultaneously compare the sensitivity of two avian monitoring systems for tracking SLE transmission in the field.

Sentinel chickens made up our first avian monitoring system and were maintained in Indian River County by personnel from Indian River Mosquito Control District since 1978. Six flocks of six birds each were placed in the field in June and bled once a week through December. During years of high arbovirus transmission, this bleeding schedule was usually continued from January through May of the following year.

The 12 year (1978 to 1989) sentinel chicken data set showed two important trends prior to 1990. First, on average, seven percent of the sentinel chickens in Indian River County seroconverted to SLE virus annually. The range was zero to 28 percent. Second, sentinel chicken SLE seroconversions never occurred before August.

Our second avian monitoring system consisted of a year round serosurvey of resident and migratory wild birds in Indian River County. This study began in January 1989.

By the end of June 1990, we had yet to see any SLE positive wild birds, but three of our sentinel chickens had tested positive for hemagglutination inhibition antibody to SLE virus. By the middle of July, all of our sentinel chickens had tested positive for SLE antibody, and we had still not seen any seropositive wild birds. By the end of July, seropositive wild birds had begun to appear and in August and September virtually 100 percent of the wild birds showed positive SLE antibody titers.

The epicenter of the 1990 Florida SLE epidemic was in the Indian River County area. Sentinel chickens maintained there seroconverted to SLE virus in significant numbers four weeks prior to the first human case. Wild birds, on the other hand, also seroconverted in large numbers, but after human cases began to appear.

DISCOVERY OF THE NATURAL FOCUS OF CHIKUNGUNYA
IN YUNNAN PROVINCE, CHINA AND STUDY OF BIOLO-
GICAL PROPERTIES OF CHIKUNGUNYA VIRUS

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In 1986-1988, two strains of Chikungunya(CHIK) virus were isolated from the brain tissues of bats(Rousettus leschenaulti), three strains from Aedes albopictus and Culex tritaeniorhynchus, and a strain from serum sample of unknown fever case collected from Xishuangbanna area, Yunnan province, China. These six virus strains could cause illness death in suckling mice and produced evident CPE in the C6/36, BHK21, Vero and primary hamster kidney cells. These virus strains contain RNA, are sensitive to acid and ether but resistant to 5-FUdR. The strains were also found capable of agglutinating red blood cells of dove, goose, chick, duckling, and sheep at pH5.75-6.4. By electron microscopic examination of ultra-thin sections of brain tissue of infected suckling mice, spherical enveloped virus particles were found, approximately 66.8nm in external diameter and 47.7nm in internal diameter. These viruses have been identified serologically by hemagglutination inhibition(HI), immunofluorescent and neutralization tests. Each showed specific reactivity with high titres to CHIK virus only. Therefore these six virus strains have been proved as CHIK virus. This is the first report to isolation of CHIK virus in China.

Sera from health human population and animals in Yunnan were tested for antibodies against CHIK virus by HI test with positive results of 10.07%(275/2731) for persons, 49.30%(140/284) for Rousettus leschenaulti, 55.56%(45/81) for Openpopelia tranquebarica, 18.18%(2/11) for Suncus murinus, 4.06%(8/197) for pigs, 2.59%(9/347) for Rattus flavipectus, 2.45%(5/204) for Macaca mullata and 1.80%

(2/111) for dogs. These results demonstrate that CHIK virus infection occurs in humans and animals in Yunnan Province. Evidence from these investigations show that the west and south parts of Yunnan Province are natural foci of CHIK virus, that bats, birds, pigs, and monkeys might be important hosts, and that *Aedes albopictus* and *Culex tritaeniorhynchus* might be the vectors of CHIK virus.



STUDY OF NATURAL INFECTION OF BATS WITH JAPANESE ENCEPHALITIS VIRUS

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In July-August 1988, three strains of viruses were isolated from 453 samples of brain tissues of bats (*Rousettus leschenaulti*) collected from Jinghong county, Yunnan Province, China. These viruses have been identified by hemagglutination-inhibition (HI), complement fixation, immunofluorescence, and neutralization tests. The isolates have been identified as strains of Japanese encephalitis (JE) virus. Sera from *Rousettus leschenaulti* were tested for antibodies against JE virus by the HI tests, positive results were obtained for 150/291 (51.55%). The results demonstrate that natural infection with JE virus exists among bats in Yunnan Province. This probably indicates that bats might be a persistent host of JE virus.

USA/USSR Joint Program on Arboviruses

During the summer of 1990 scientists from the USA and the Soviet Union participated in the first joint USA/USSR expedition to Western Siberia to collect mosquitoes for arbovirus investigations and taxonomic studies. A primary objective was to clarify the vector relationships of California serogroup viruses. A portion of the collection has been processed at the D.I. Ivanovsky Institute of Virology, Moscow, under the direction of Dr. Dimitry Lvov. A portion of the collection also was brought to the Division of Vector-Borne Infectious Diseases, CDC, Fort Collins, where more precise entomological assessments have been made.

Thus far, approximately one-half of the collection brought to Fort Collins has been processed and tested for virus (Table 1). Two virus isolations have been made from mosquitoes collected in the South Taiga zone from sites south of Tyumen City and Tobolsk, respectively. The virus isolates will be identified when the remainder of the collection has been processed and tested for virus. In addition to the mosquitoes shown in Table 1, we have tested 9,650 simuliids in 70 pools and 37 ceratopogonids in 2 pools with negative results.

Following completion of processing of the collection for virus isolation, pinned voucher specimens of female mosquitoes from the species groups will be studied further. This should result in more specific identification of some specimens currently assigned to species groups.

Report submitted by Carl J. Mitchell, Harry M. Savage, and Gordon C. Smith, Medical Entomology-Ecology Branch, Division of Vector-Borne Infectious Diseases, Center for Infectious Diseases, Centers for Disease Control, Fort Collins, and, Sergei Lvov, Gamaleya Institute for Epidemiology and Microbiology, Moscow.

Table 1. Summary of mosquitoes collected in Tyumen Region, Western Siberia, U.S.S.R., during the summer of 1990 and tested for virus.

Taxa	No. Specimens	No. Pools	No. Positive Pools
<u>An. (Ano.) maculipennis</u> complex	64	11	
<u>Anopheles</u> spp.	5	1	
<u>Ae. (Aed.) cinereus</u>	5987	141	
<u>Ae. (Aed.)</u> sp. "A"	1971	62	
<u>Ae. (Adm.) vexans</u>	66	7	
<u>Ae. (Och.) annulipes</u>	335	28	
<u>Ae. (Och.) behningi</u>	115	11	
<u>Ae. (Och.) behningi</u> subgroup	43	4	
<u>Ae. (Och.) cyprius</u>	7	3	
<u>Ae. (Och.) cantans</u> group	669	33	
<u>Ae. (Och.) communis</u> group	186	9	
<u>Ae. (Och.) communis</u> subgroup	762	34	1
<u>Ae. (Och.) communis</u> complex	601	28	
<u>Ae. (Och.) cataphylla-punctor</u> subgroup	333	22	1
<u>Ae. (Och.) decticus</u> subgroup	23	6	
<u>Ae. (Och.) decticus-communis</u> subgroup	70	5	
<u>Ae. (Och.) eudes</u>	88	4	
<u>Ae. (Och.) eudes</u> subgroup	107	14	
<u>Ae. (Och.) excrucians</u>	4264	105	
<u>Ae. (Och.) flavescens</u>	26	9	
<u>Ae. (Och.) intrudens</u>	166	20	
<u>Ae. (Och.) intrudens</u> subgroup	17	3	
<u>Ae. (Och.) punctor</u> subgroup	1371	63	
<u>Ae. (Och.) sticticus-communis</u> complex	18	5	
<u>Ae. (Och.)</u> sp. "B"	10	4	
<u>Ae. (Och.)</u> sp. "C"	10	6	
<u>Ae. (Och.)</u> spp.	24	2	
<u>Aedes</u> spp.	1102	36	
<u>Cx. (Bar.) modestus</u>	897	23	
<u>Cx. (Bar.)</u> spp.	30	3	
<u>Culex</u> spp.	43	3	
<u>Cs. (Cuc.) morsitans</u>	255	9	
<u>Cs. (Cuc.) ochroptera</u>	57	2	
<u>Cs. (Cus.) alaskaensis</u>	4	1	
<u>Cq. (Coq.) richiardii</u>	80	18	
Totals	19,806	735	2

Report from the W.H.O. Collaborating Center of Arbovirus Reference and Research (CRORA, Institute Pasteur BP 220, Dakar, Senegal)

Activity of the WHO Collaborating Center of Reference and Research on Arboviruses (CRORA) remained at a high level. In addition to the virological results, the year was marked by two important facts. First of all, establishment of a data base allowing management of stocks of all biological products of the Reference Center. Then the production of a very important number of reagents prepared from reference strains. These reagents have all been tested for specificity using homologous and heterologous strains. Marie-Armande Calvo-Wilson is thanked for this important work. All this has been achieved in spite of the need to maintain a high level of other activities.

In 1990, the Center identified 217 strains coming from Pasteur institutes in Abidjan (67), Bangui (6), Tananarive (1), Dakar (142) and from USAMRIID Fort Detrick (1). Since its opening, the Center has identified 4233 virus strains. This year, viruses came from different African regions and were from mosquitoes, ticks and humans. They are: chikungunya (58), Igbo-Ora (1), Bagaza (3), ArD 65239 near Bagaza (39), Wesselsbron (5), Yaoundé (3), West Nile (3), yellow fever (3), Zika (15), Kedougou (6), dengue-2 (22), Bwamba (1), Pongola (4), M'Poko (1), Tanga (1), CHF-Congo (1), Bhanja (3), UGZ 52969 Kasokero (1), Tataguine (2), Bangui (1), Jos (1), variant Wad Medani (63), Orungo (1), Ndelle (1), and a virus considered new, for the time being (ArD 66707).

The reference collection presently is composed of 192 arboviruses of vertebrates. Some modifications have been made to the classification of these viruses.

This year, many significant facts are to be noted:

- in Senegal, the isolation of strains of yellow fever virus in the Kedougou region. This confirms the virus maintenance in a same ecological niche.

- the maintenance of dengue-2 virus in the same ecological niche of Kedougou, with isolations this year from humans living or who have lived in this region.

- the isolation of a strain of Bangui virus from a pool of *Anopheles pharoensis* captured by a USAMRIID team supervised by Scott Gordon.

- the display of a new virus of the Corriparta group from a pool of *Culex poicilipes*. This virus is antigenically related to but different from Acado virus.

- the confirmation of the human pathogenicity of UGZ 52969 (Kasokero virus). It is a laboratory contamination.

The reference Center participates actively in epidemiologic surveys by looking for the presence of virus in all mosquito pools provided by ORSTOM epidemiologists. Within the context of this program, 3071 pools of mosquitoes have been tested. 70 virus strains have been isolated.

During 1989, the number of associations of dengue-2 and Zika viruses (isolated in *Aedes pseudoscutellaris* [AP-61] mosquito cells) from pools of unusual vectors has been particularly convincing. A simple calculation of probability showed that this phenomenon was presenting a probability less than 0.001 to be uncertain. An experimental study revealed that dengue-2 and Zika viruses, when isolated separately in these cells, have an action which is different than the one of which it normally loses its neurovirulence for the suckling mice after passage in AP-61, preserves it when it is associated with dengue-2 and Zika.

J.P. Digoutte, M.A. Calvo-Wilson, M. Mondo

REPORT FROM DEPARTMENT OF VIROLOGY, COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN¹

A SEROEPIDEMIOLOGICAL STUDY OF PREVIOUSLY UNREPORTED ALPHA AND BUNYAVIRUSES
IN NIGERIAN HUMAN AND ANIMAL POPULATIONS

1.0. We report preliminary findings of a seroepidemiological study on sera from Nigerian human and animal populations for antibodies to alpha- and bunyaviruses previously undescribed in Nigeria. The following viruses were used in the study, ALPHAVIRUSES: Eastern Equine encephalitis - (EEE), Western Equine encephalitis - (WEE), Mayaro - (MAY), and Mucambo - (MUC); BUNYAVIRUSES: Group C, Caraparu - (CAR); California group, Guaroa - (GRO) Simbu group, Oropouche - (ORO), Anopheles A group, Tacaiuma - (TCM) Bunyamwera group, Maguari - (MAG) and Guama group, Catu - (CATU). Antibodies were evaluated by the haemagglutination-inhibition (HI) and mouse neutralisation (N) tests.

2.0. RESULTS

The results are summarised in Tables 1 - 4.

- 2.1. Alphaviruses: Some of the interesting findings include the detection of HI antibody (titre 1:20 or greater) to WEE in 11 or 16.7% of 66 horse sera (Table 1). Four of the HI antibody positive sera were also found positive in N tests for WEE virus antibody with LNI ranging from 2.0 dex to 3.7 dex. These four sera were negative either in HI or N test for antibodies to the other viruses MAY & MUC. (Table 2). Preliminary investigations reveal that two of the four horses positive for N antibodies to WEE virus were imported in 1988 to Nigeria from Argentina for the annual Polo festival, while the other two are local horses.
- 2.2. Serum from one cow reacted monospecifically in HI and N tests for antibodies to WEE. The HI titer was 1:80, while LNI was 2.0 dex. Another cow serum also gave monospecific reactions in both HI and N tests for MUC virus antibody. The HI titer was 1:40, while the LNI was 3.0 dex. (Table 1 and 2).
- 2.3. We detected HI antibodies in 26 (4.4%) of 588 human sera to WEE virus, while 31 (5.3%) were positive for MAY virus antibody. Only 9 (1.5%) of the human sera were positive for MUC virus antibody. However, all the sera positive for WEE virus HI antibody were negative in N test with the same virus, (LNI less than 2.0 dex). On the other hand, 22 or 71% of 31 sera positive in HI test for MAY virus antibody were confirmed positive for N antibody to the same virus. The LNI ranged from 2.5 dex to 4.7 dex.
- 2.4. In Nigeria, we have reported the isolation of only 4 members of the alphaviruses including Chikungunya (CHK), Semiliki Forest (SF), Sindbis (SIN), and Igbo-Ora. Further investigations are in progress to

determine the implications of these findings in the ecology of previously unreported alphaviruses in Nigeria.

3.0. Bunyaviruses

The highlight of the results was the detection by HI test and the confirmation in N test of antibodies to CAR, ORO, TCM and MAG viruses (human sera); CAR, ORO, TCM, MAG and CATU viruses (cattle sera); and CAR and MAG viruses (horse sera). (Tables 3 & 4). These positive results may have resulted from the presence in Nigeria of some bunyaviruses (ILE, BWA, PGA, SAN, SAT, SHA, SIM, SHU, TETE). However, we detected in the sera of rural Nigerians and indigenous cattle and horse N antibody titers with LNI ranging from 3.0 dex to 4.4 dex to viruses such as CAR, TCM & CATU. These viruses have only been isolated in either South or North America.

Again these findings call for further and more detailed field and laboratory investigations in the arboviruses in Nigeria.

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¹ These studies were conducted at Belem while OT was on a Fellowship of the third World Academy of Science, Tresle, Italy from September - December, 1990.

TABLE 1

Prevalence of HI antibodies to alphaviruses in
Nigerian sera

SAMPLE	TOTAL NO. TESTED	EEE*	WEE	MAY	MUC
Human	588	17(2.9)**	26(4.4)	31(5.3)	9(1.5)
Cattle	80	1(1.3)	1(1.3)	0 (0)	1(1.3)
Horse	66	3(4.5)	11(16.7)	0 (0)	0 (0)

* See text for virus names

** Number (%) positive

Table 2

Prevalence of N antibodies to alphaviruses in
Nigerian sera

SAMPLE	WEE	MAY	MUC
Human	0/26(0)*	22/31(71)	3/9(33)
Cattle	1/3(33)**	0/10(0)	1/1(100)**
Horse	4/19(21.1)	0/19(0)	0/19(0)
Range of LNI (dex)	(2.0 - 3.7)	(2.5 - 4.7)	(1.6 - 3.0)

* No. positive/No. tested (% positive)

** Monospecific reactions in both the
HI & N tests.

TABLE 3

Prevalence of HI antibodies to Bunyamwera supergroup
viruses in Nigerian sera

SAMPLE	TOTAL NO. TESTED	CAR*	GRO	ORO	TCM	MAG	CATU
Human	588	11(1.7)**	0(0)	6(1.0)	6(1.0)	16(2.7)	8(1.4)
Cattle	80	6(5.0)	0(0)	3(3.8)	13(16.3)	8(10)	4(5.0)
Horse	66	2(3.0)	0(0)	3(4.5)	0(0)	11(16.7)	4(6.1)

* See text for virus names

** Number (%) positive

TABLE 4

Prevalence of N antibody to Bunyamwera supergroup viruses
in Nigerian sera

SAMPLE	CAR*	GRO	ORO	TCM	MAG	CATU
Human	11/11(100)**	0/10(0)	1/6(16.6)	1/6(16.6)	5/16(31.3)	0/8(0)
Cattle	6/6(100)	0/10(0)	1/3(33.3)	11/13(84.6)	3/8(37.5)	4/4(100)
Horse	1/2(50)	0/10(0)	0/3(0)	0/2(0)	5/11(45.4)	0/4(0)
Range of LNI (dex)	2.4 - 4.4	-	1.5	1.4 - 2.9	1.6 - 2.4	1.4 - 3.6

* See text for virus names

** No. positive/No. tested (%) positive

A Brief Summary of Surveillance for Mosquito-borne Encephalitis Virus Activity in California, 1990.

As usual, an extensive surveillance program was conducted, involving cooperative efforts by many groups and individuals from local mosquito control agencies; the Arbovirus Research Program at the University of California at Berkeley; the California Mosquito and Vector Control Association and the CMVCA Research Foundation; county and local public health departments; the California Department of Food and Agriculture; physicians and veterinarians throughout California; and three branches of the California Department of Health Services - the Infectious Disease Branch, the Environmental Management Branch, and the Viral and Rickettsial Disease Laboratory of the Division of Laboratories. A complete summary will be published in the proceedings of the California Mosquito and Vector Control Association annual conference.

Announcements about the program were disseminated from February to May, and 29 weekly bulletins (April 9 - December 21) were distributed widely during the season to give data about the statewide surveillance results. Many participants now receive these via electronic mail. In addition to the weekly summary of all results, positive findings are telephoned immediately to the agency which submitted the mosquito pools or sentinel chicken sera.

Clinical and laboratory surveillance for human and equine cases of encephalitis, meningoencephalitis and meningitis detected only two human cases of St Louis encephalitis (SLE), in contrast to the 1989 season when 29 confirmed or presumptive-positive human cases of SLE were found. The first case detected was in a 19 year old man from Porterville, Tulare County, with onset 9/14/90 who most probably acquired infection from mosquitoes at a canal (Porter Slough) just east of his home. The second case was a 28 year old man with onset approximately 9/1/90, who had recently traveled in Texas, left there 8/31, stopped in the Colorado River area briefly, then returned home 9/2-3/90 and was hospitalized in Los Angeles. Place of contraction was most likely near the Colorado River site but this could not be exactly determined. Only a single blood sample obtained 9/19/90 was available for testing, but high SLE-IgM antibody, and both SLE-IgM and IgG antibodies in a cerebrospinal fluid sample substantiated the diagnosis.

There were no confirmed cases of WEE in humans or horses during 1990. There were 5,262 mosquito pools tested during the year, yielding 47 viral isolates in the VRDL: 18 SLE, and 29 California serogroup viruses. Additional pools from Southern California (935 pools) and Kern County (640 pools) were tested by the U.C. Berkeley Arbovirus Laboratory, yielding Hart Park, Turlock and other isolates but no additional SLE or WEE.

Sentinel chicken flocks at 68 sites showed only low-level SLE activity in a few San Joaquin Valley (5% seroconversion) and Southern California (13% seroconversion) sites in late summer and fall, and no evidence of WEE activity (over 7,600 chicken sera tested). This was the largest surveillance effort in the past 20 years, and the first year since 1943 with no evidence of WEE activity, by any parameter.

An even greater and well-focused effort is needed during 1991 to attempt to anticipate the build up of virus activity and take steps to suppress it before the threat of epidemic encephalitis in humans or equines becomes a reality.

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

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

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

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

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

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

Table 2

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

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

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

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INFECTIVITY OF JAMESTOWN CANYON TOPOYPES ISOLATED IN CANADA
FOR NEW ZEALAND WHITE RABBITS

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Seawright et al (1974) previously reported that New Zealand white rabbits inoculated intravenously (iv) with a Wisconsin toptype of Jamestown Canyon (JC) virus did not become viremic and that nine of ten rabbits inoculated also did not produce antibodies to JC virus. They concluded that the rabbit is not a reliable indicator for JC virus. Since sentinel rabbits have been used in numerous California serogroup studies undertaken in Canada, an experiment was undertaken to determine the infectivity of several JC toptypes isolated in Canada for rabbits.

Seven New Zealand white rabbits were inoculated iv with 1×10^5 tissue culture infective dose₅₀ of one of the following vero-propagated viruses: JC prototype 61V-2235 and JC isolates from Newfoundland - toptype 78-80, Quebec - toptypes 137A17 and 140A8, Ontario - toptypes 9-178-79 and 9-237-79, Manitoba - toptype Mn256-260 and Saskatchewan - toptype YGMC 216-82. Rabbits were bled at days 7, 14, 21 and 28 postinoculation (pi) and antibody responses measured by hemagglutination inhibition using a hemagglutinin prepared from toptype Mn256-260 (Grimstad et al 1987).

All seven toptypes of JC virus infected New Zealand white rabbits with antibodies detected by day 7 pi (Table 1). However, no antibody response was observed in the rabbit inoculated with prototype strain 61V-2235. This latter observation is consistent with results obtained by Seawright et al (1974) with Wisconsin toptype 686-67.

A previous study of 13 neutralization identified JC toptypes isolated from five Canadian provinces revealed serological heterogeneity among these toptypes (Artsob et al 1984). Further studies using monoclonal antibodies produced to prototype JC virus have also indicated serological differences between naturally occurring toptypes (unpublished data). More extensive experiments are required to confirm and extend the observations reported here but these preliminary results give rise to the possibility that differences also exist in virulence properties between naturally occurring JC toptypes.

TABLE 1. Hemagglutination inhibition serology of New Zealand white rabbits inoculated intravenously with different Jamestown Canyon virus strains.

Jamestown Canyon virus strain inoculated	Place of Isolation	Days Postinoculation			
		7	14	21	28
prototype 61V-2235	Colorado	- ¹	-	-	-
topotype 78-80	Newfoundland	20 ²	40	40	40
137A17	Quebec	N.T. ³	N.T.	N.T.	160
140A8	Quebec	20	20	40	40
9-178-79	Ontario	10	20	20	40
9-237-79	Ontario	N.T.	N.T.	N.T.	40
Mn256-260	Manitoba	20	80	80	80
YGMC216-82	Saskatchewan	10	40	40	20

¹ - = hemagglutination inhibition titre <1:10

² results are expressed as reciprocal of antibody titre

³ N.T. = not tested

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**REPORT FROM CSIRO, DIVISION OF TROPICAL ANIMAL PRODUCTION,
PRIVATE BAG NO.3 INDOOROPILLY 4068 AUSTRALIA.**

Bovine Ephemeral Fever Glycoprotein (BEFgp) Vaccine

BEFgp was extracted from purified BEFV by Triton X-100 solubilisation. In a series of experiments, BEFgp has been shown to produce immunity against BEF infection in cattle. Using the saponin Quil A as an adjuvant, the following parameters have been investigated:- dose rate, storage & vaccination schedules & the duration of immunity. A final vaccination schedule of 2 vaccinations at a 21 day interval with BEFgp at 1.0 ug/animal is now routinely used in our continuing experimental investigations. A blocking ELISA test utilising BEFgp specific MAB's has been used to monitor antibody response to the vaccine. High levels of both ELISA & neutralising antibody are produced by vaccinated cattle. The immunity conferred by BEFgp vaccination using the above protocol is not long-lasting, with protection lasting approx. 10-12 months.

CMI Response to BEFgp Vaccine

Using a mouse model, it has been demonstrated that there is a reduced CMI response to immunisation with BEFgp when compared with purified whole virus. The following table presents the stimulation index response of murine splenocytes to in-vitro stimulation with purified BEFV after immunisation with BEFgp & BEFV.

Immunised with	Day Post Stimulation						
	1	2	3	4	5	6	7
BEFV	103	110	155	139	181	77	52
BEFgp	100	88	127	109	142	107	52

Future Work

Present and future studies will further investigate the CMI response to different viral components and attempt to increase the time period for effective immunity to BEF.

(Michael Uren, Peter Walker, Toby St.George, Daisy Cybinski and Helen Zakrzewski)

**Proceedings 5th Australian Arbovirus Symposium
Brisbane, Australia. Aug 28 - Sept 1 1989.**

This symposium brought together many prominent investigators in the field of arbovirology. The theme of the symposium was "Towards 2000" with special emphasis on virus-vector interaction. The conference also included a substantial input by workers from countries involved in community participation in tropical health programmes. The proceedings contains each speakers presentation in full and represents a valuable summary of the current thinking on many aspects of arbovirus research. The cost is Aust\$70.00 plus \$30.00 postage and handling. Orders for copies should be sent to The Librarian at the above address. Cheques and money order should be made payable to : CSIRO Collector of Monies.

CURRENT STATUS OF THE MUTAGEN-ATTENUATED RIFT VALLEY FEVER
VACCINE (RVF MP-12)

A mutagen-attenuated strain of Rift Valley fever (RVF) virus, developed as a potential vaccine, has been shown to be immunogenic, avirulent, nonabortigenic, and protective for dam and fetus against challenge inoculation with 5×10^5 pfu of virulent RVF virus in sheep and cattle.^(1,2,3,4) The genome of this virus has at least one attenuating mutation on each of the three segments suggesting that reversion to virulence is unlikely and that reassortment with wild-type virus would produce attenuated progeny.^(5,6) In neurovirulence tests done in juvenile rhesus monkeys, this virus strain was highly attenuated and minimally neuroinvasive.

Further studies have shown that rhesus monkeys inoculated intramuscularly with 3000 pfu of RVF MP-12 develop neutralizing antibody titers of 1:320 to 1:5120 within 10 days of inoculation and those antibodies protected against intravenous or aerosol challenge with 10^5 pfu of virulent ZH-501 strain of RVF virus. Vaccine virus was isolated from oropharyngeal swabs from two of nine vaccinated monkeys at four or seven days post vaccination. Post vaccination virus titers of $\leq 1.8 \log_{10}$ pfu/ml of serum were detected in three of nine vaccinated monkeys at one to three days after vaccination. No detectable elevations in serum ALT and GGT values were seen after vaccination. Following intravenous or aerosol challenge, no viremia was detected in any of the vaccinees and serum enzyme values were not affected. Unvaccinated control monkeys challenged intravenously with an identical dose of virus developed viremia titers of 5.4 to 6.6 \log_{10} pfu/ml of serum and had three- to four-fold increases in serum ALT values. Unvaccinated control monkeys challenged by the aerosol route had viremia titers of 4.5 to 5.7 \log_{10} pfu/ml of serum and two- to three-fold elevations in serum ALT.

The MP-12 vaccine candidate may have advantages over the Smithburn strain for veterinary use. MP-12 does not induce fetal damage in sheep or cattle and appears to be fully protective in both species so that timing of inoculation during the breeding season is not necessary. It has not undergone serial passage in nervous tissue and appears less neurovirulent for rhesus monkeys than the Smithburn strain, perhaps providing additional safety to humans involved in the vaccination procedure. Additional field trials in African animals are needed to provide information on breed differences and the effects of prior infection with other phleboviruses. There is also a need for developmental work on

items such as inexpensive means of production, freeze-drying, and field stability.

The current vaccine lot is undergoing review prior to human testing at USAMRIID in September 1991. If MP-12 proves to be safe and efficacious it may be useful in protecting those in high-risk professions (veterinarians, slaughterhouse employees, laboratory workers in endemic regions) or residents in areas of uncontrolled epizootics. Since MP-12 is a live-attenuated vaccine it would be expected to provide several advantages over the currently used inactivated vaccine, including single injection immunization, rapid onset of protection, long duration of immunity, and lower cost per dose.

Recent studies have also shown that rhesus monkeys exposed to 10^4 pfu of RVF MP-12 by small particle aerosol or nasal drops have no untoward effects and develop neutralizing antibodies. Studies are now in progress to determine the efficacy of aerosol immunization.

(Morrill, J.C. and Peters, C.J.)

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SURVEY FOR LYME BORRELIOSIS VECTORS IN MANITOBA, CANADA

Ixodes dammini was reported in the province of Manitoba for the first time in 1989. Because of the presence of *Borrelia* antibodies in humans, it was imperative that we learn more about the distribution and prevalence of *I. dammini*. A joint project initiated by T. Galloway (Dept. of Entomology, University of Manitoba) and L. Sekla and W. Stackiw (Cadhams Provincial Health Laboratory, Winnipeg) was funded by the Manitoba Health Research Council to determine the status of *I. dammini* in Manitoba, and to try to isolate *Borrelia burgdorferi* from wild rodents.

Field work began in May (D. Mitchell - until August) and continued into early December (J. Christie - July-December). Small mammals were live trapped at over 100 locations in southeastern Manitoba, involving nearly 4000 trap nights. Over 1000 mammals (34 species) were examined for ectoparasites. Over 300 rodents (5 species, predominantly the deer mouse, *Peromyscus maniculatus*) were sacrificed and tissues harvested in the laboratory for isolation of *B. burgdorferi*. Dragging for ticks was carried out at most locations, as time permitted, using a 1 m² flannel drag. Cumulative distance dragged exceeded 200 km. The assistance of the public was solicited via the media to submit ticks to the Dept. of Entomology for identification.

Seven species of ticks (three genera) were collected from examined mammals and dragging efforts. No *I. dammini* were collected. A single unfed *I. dammini* nymph was submitted by a man in the Charleswood area of Winnipeg. The tick had been removed from his dog. The site was revisited several times thereafter, but no additional specimens could be found. One adult female *I. dammini* was submitted by a man who had just returned from a camping trip in the Minneapolis area. Hundreds of *Dermacentor variabilis* and a small series of *Rhipicephalus sanguineus* were also submitted by the general public for identification. No *B. burgdorferi* were isolated from the collected rodents.

We were unable to establish the presence of reproducing populations of *I. dammini* in Manitoba in 1990. The occurrence of *I. dammini* here has been confirmed, but at the present time we must conclude that these specimens are adventive, or that if *I. dammini* persists in southeastern Manitoba, it does so in low numbers and scattered populations.

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January 1991

INDIGENOUS LYME DISEASE IN ONTARIO

INTRODUCTION

Lyme disease (LD) (Lyme borreliosis) was first described as a separate clinical entity during 1977 due to a temporal and geographic clustering of juvenile rheumatoid arthritis in Lyme, Connecticut¹. That year, Ixodid ticks were epidemiologically linked to those patients who also exhibited erythema migrans (EM)². Later in 1982, Burgdorfer isolated the previously unrecognized spirochete, *Borrelia burgdorferi* from *Ixodes dammini* ticks³ and from patients with erythema migrans.⁴ A causative agent and an efficient vector for the transmission of LD had been discovered. Since then, LD has been recognized in 43 U.S. states as well as Europe, China, Japan and Australia. In Canada, 7 provinces have documented cases of LD but it is estimated that only 65 of the 112 reported cases are likely to be indigenous. At the present time, the disease is reportable only in Ontario.

ONTARIO EXPERIENCE (1984-1989)

This report reviews the Ontario experience for LD from 1984-1989. As the case definition for LD used in Ontario for surveillance purposes continues to be revised, all individuals who had been reported as cases were retrospectively assessed according to the current case definition. The surveillance case definition used is:

The occurrence of clinically compatible signs and symptoms and possible exposure to ticks with:

- A. Endemic
- 1) isolation of *B. burgdorferi* from a clinical specimen;
 - 2) erythema migrans;
 - 3) positive serology (optical density of .40 or greater with the ELISA test when compared to a known positive standard) and involvement of at least one of the three following organ systems: neurologic, cardiac, or musculo-skeletal.
- B. Non-endemic
- 1) isolation of *B. burgdorferi* from a clinical specimen;
 - 2) positive serology and erythema migrans;
 - 3) positive serology and involvement of at least two of the above mentioned organ systems.

The only endemic area currently identified in Ontario is Long Point (Norfolk County) on the north shore of Lake Erie.

A total of 76 cases of LD were reported in Ontario from 1984 to 1989, of which 44 had no history of travel to an endemic area outside of Ontario during the incubation period and are therefore considered indigenous. Of those Ontario residents who acquired the infection out of the province, 72% (23/32) had recently visited the north and southeastern United States. Of the 44 indigenous cases, all exhibited positive serology and 82% had evidence of EM (36/44) (Figure 1). Eight cases were epidemiologically linked to occupational or recreational activities in the endemic area of the National Wildlife Area or the Provincial Park at Long Point. Temporal distribution of indigenous cases (Figure 2) suggests a marked annual increase, particularly from 1988 to 1989. However, some of the increase may be attributed to the disease being made reportable in 1988 and to the increasing public and medical awareness. Lyme disease appears to have a seasonal distribution, with 77% (34/44) of the indigenous cases being acquired from May to August with a peak in June (Figure 3). This coincides with the time of the year when the nymph stage of the tick is feeding on both small and large hosts, including humans.

The distribution of indigenous cases by sex and age is illustrated in Figure 4. The mean age of the cases was 38 years with a range of two to 69 years. Fifty-five percent (24/44) of the indigenous cases occurred in males, most of whom were aged 30 to 59 years, and in females from 10 to 49 years of age. This may be attributable to a greater likelihood of occupational and recreational exposure for those individuals. The activity most commonly described with the occurrence of LD was camping followed by hiking in wooded areas, peridomestic exposure (e.g. cutting grass, deer in backyard, etc.), occupational exposure (one biologist, one conservationist and three bush cutters) and recreation (one golfer, two deer hunters, and one birder). Of interest, only 45% (24/44) of these cases could recollect a previous insect (9) or tick bite (15) during the incubation period. However, no ticks were saved and subsequently identified.

The incidence rate of LD per 100,000 individuals by site of acquisition was highest in northwestern Ontario. This may be explained by the increase of the domestic deer population in that area, although evidence of the *I. dammini* tick has not been demonstrated. Also of concern is the close proximity of northwestern Ontario to the endemic areas of north central USA (states of Minnesota and Wisconsin). Figure 5A and 5B are graphic depictions of indigenous cases of LD by health unit where exposure is believed to have taken place. Eight cases occurred in Haldimand-Norfolk, all of

which are associated with visiting Long Point. Seven cases originated from Thunder Bay District Health Unit with three from both Windsor-Essex County and the Niagara Regional Area Health Units. Dr. I.K. Barker, Associate Professor, Wildlife Diseases, University of Guelph has conducted tick surveillance and animal serology studies in 22 different localities in Ontario from Dryden in the northwest to Point Pelee National Park in the southwest to Presqu'île Provincial Park (Prince Edward County) in the east from 1987 to 1989. Both the *I. dammini* tick and the causative organism, *B. burgdorferi*, have been recovered from small mammals in the National Wildlife Area and Provincial Park at Long Point on the north shore of Lake Erie. Serologic surveys also support this focus of cyclical infection in Long Point deer and mice. Sixty-five percent of *Peromyscus* mice and 50% white-tailed deer examined at those sites were seroreactive to the indirect fluorescent antibody test. *I. dammini* ticks have not been recovered from the mainland at 3 sites 6-20 km from the base of Long Point. The distribution of reported cases other than in Long Point cannot be explained in terms of a known mouse-tick-deer cycle.

It is possible that the current case definition is too non-specific for surveillance purposes. False-positive laboratory tests using ELISA have been attributed to cross-reactions with *T. pallidum*, *Leptospira* sp, other *Borellia* sp, and to the occurrence of infectious mononucleosis and certain auto-immune diseases. False-negative results can occur due to early submission of serum samples (within three to four weeks after exposure) or post-antibiotic therapy. Laboratory confirmation therefore requires a more specific test such as the western blot. To ensure uniform reporting of LD nationally, a case definition for national surveillance is being developed by the Advisory Committee on Epidemiology. This is expected to be published early in 1991.

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Lyme Disease by Method of Diagnosis Indigenous Cases, Ontario 1984 to 1989

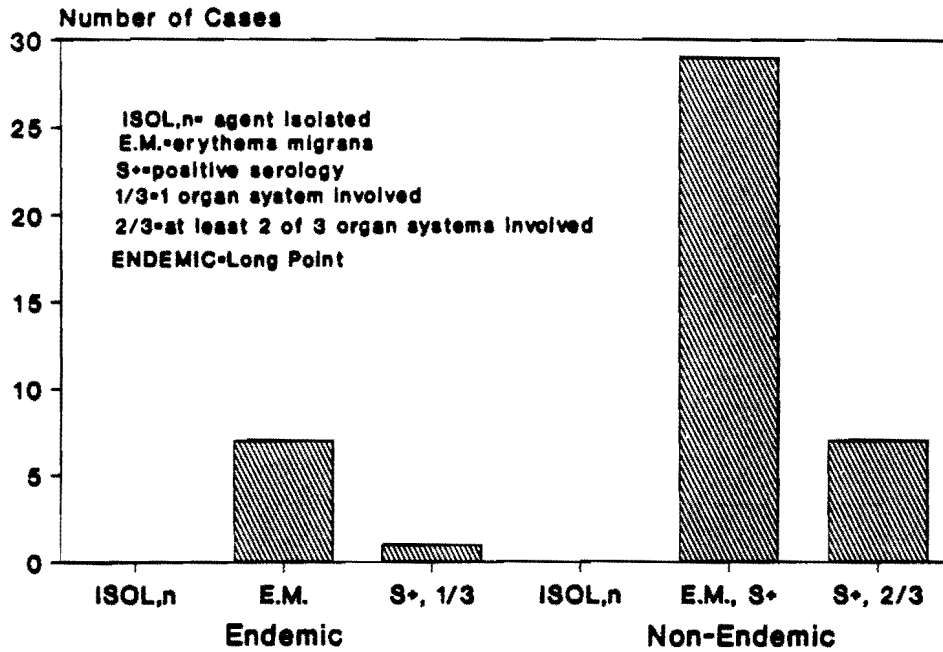


Figure 1

Lyme Disease by Annual Distribution Indigenous Cases, Ontario 1984 to 1989

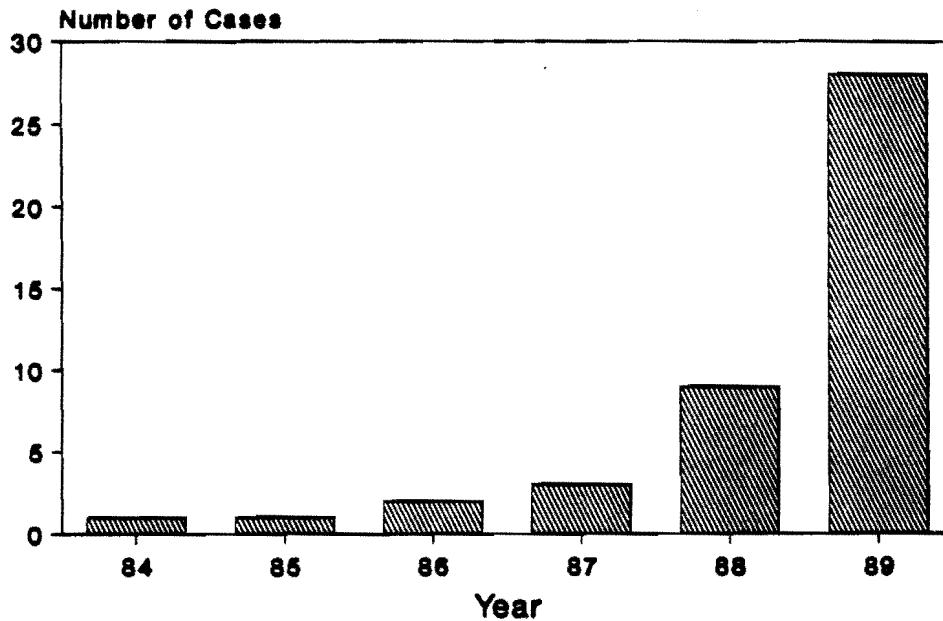


Figure 2

Lyme Disease by Seasonal Distribution Indigenous Cases, Ontario 1984 to 1989

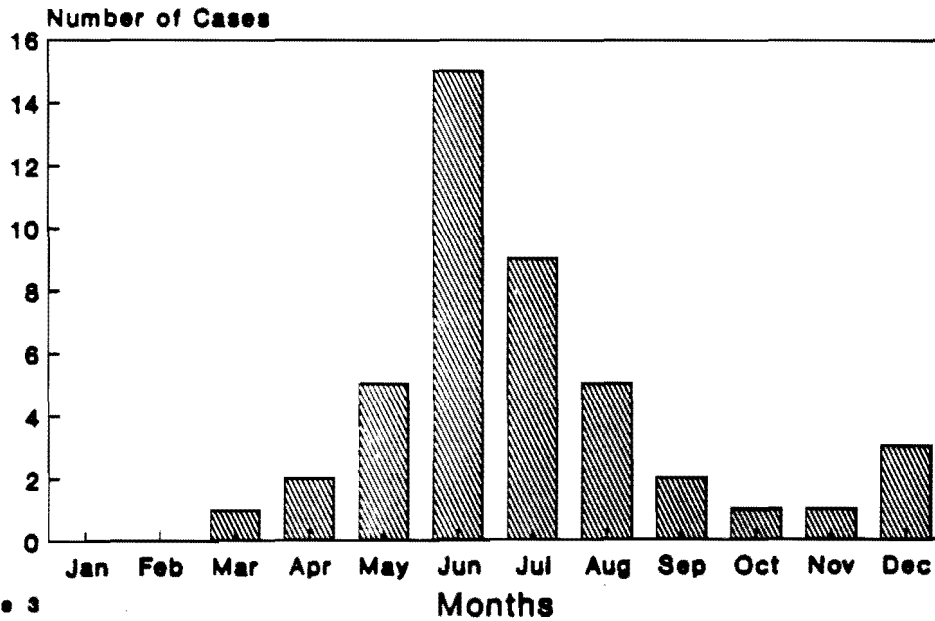


Figure 3

Lyme Disease by Age and Sex Indigenous Cases, Ontario 1984 to 1989

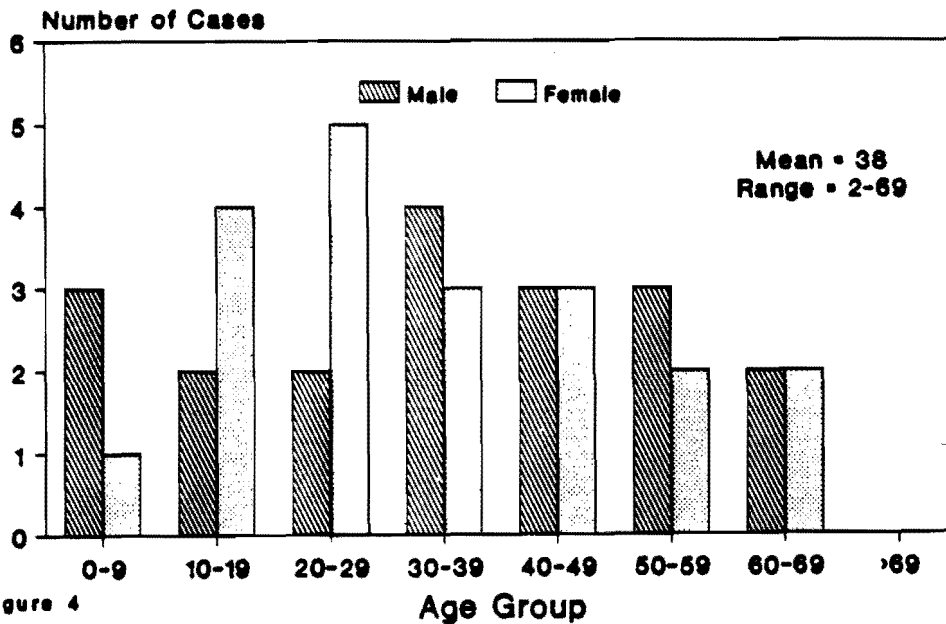


Figure 4

Lyme Disease by Health Unit of Exposure Indigenous Cases, Northern Ontario 1984 - 1989

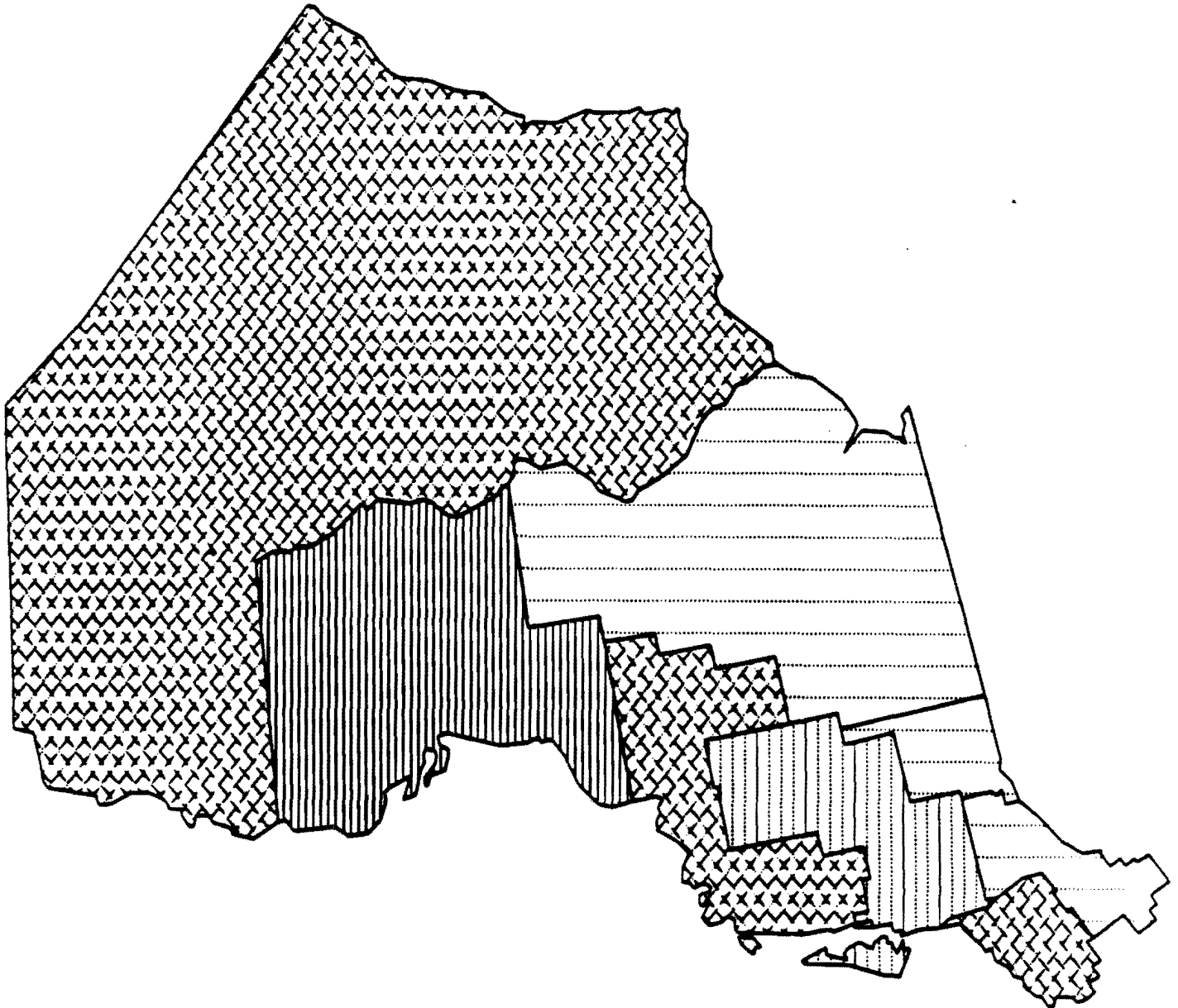


Figure 5A



Thunder Bay: 7 cases

Lyme Disease by Health Unit of Exposure Indigenous Cases, Southern Ontario 1984 - 1989

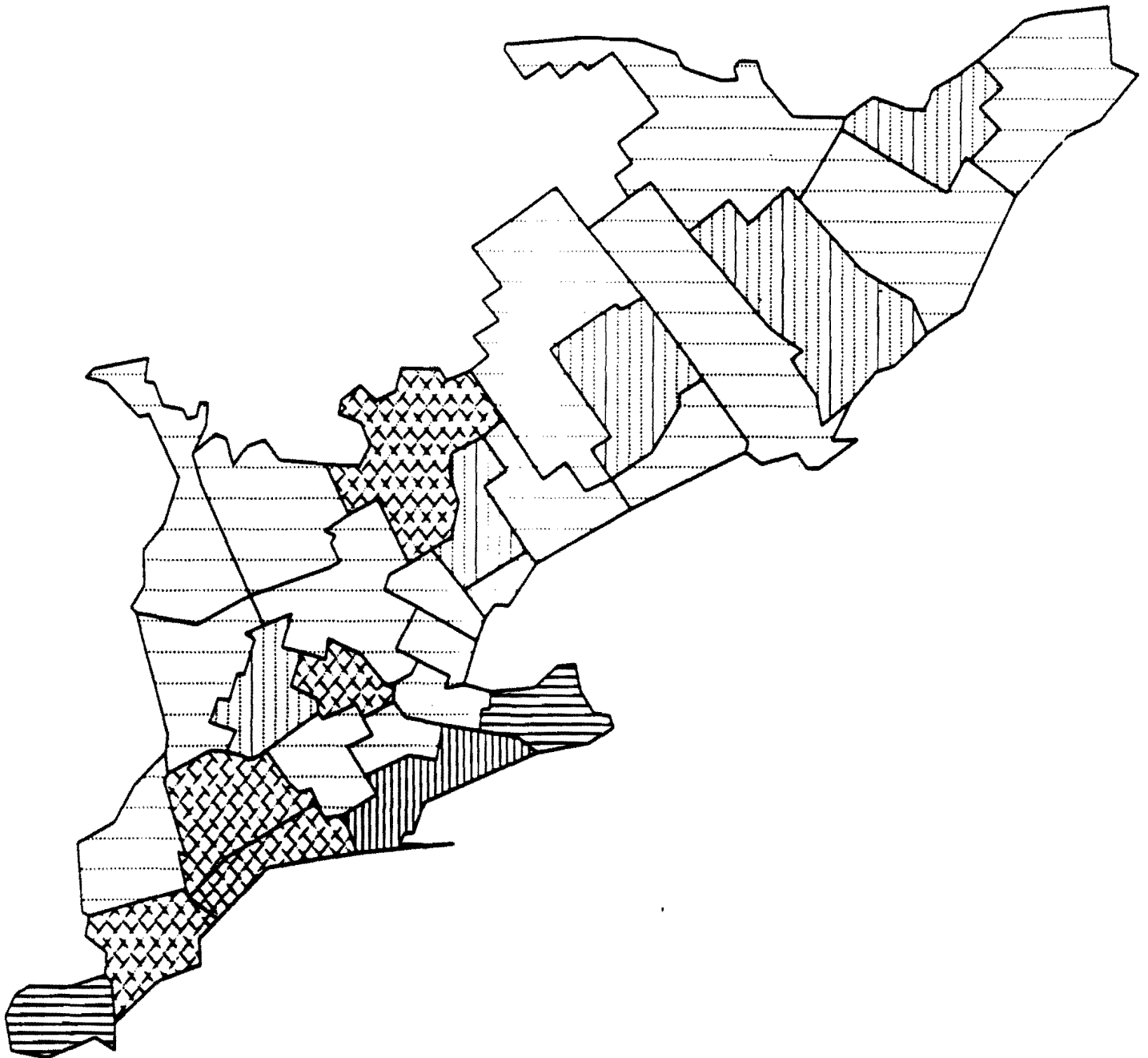
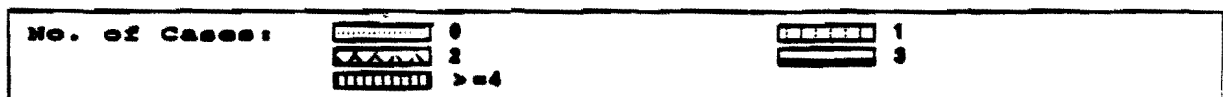


Figure 5B



Highland - Norfolk: 8 cases

PROVISIONAL DATA FOR REPORTED LYME DISEASE CASES - 1990

Cases of Lyme disease (LD) reported by States to CDC for 1990 were collated during early April. As in previous years, these data are published provisionally with the expectation that updated reports will continue to arrive over the next few months. The provisional total of 7,995 cases for 1990 is 557 cases (6.5%) less than the final total of 8,552 reported in 1989 (Table 1). In previous years, over 500 late reports have been received after publication of the first provisional figure.

From 1986 through 1989, nationally reported cases of Lyme disease doubled or nearly doubled each year (Fig. 1). The provisional 1990 data mark a halt in this trend. This may reflect a plateau in case detection, decreased reporting by physicians, or the use of more stringent criteria in defining cases. The new CDC Lyme disease case definition was adopted by the Council of State and Territorial Epidemiologists at its national meeting in April 1990 and became the standard for national reporting in January 1991. Information on the case definition used by each state during 1990 is not yet available.

The geographic distribution of Lyme disease cases among the 50 states (Fig. 2), and the provisional number of reported cases by federal geographic region (Table 2) show increased reporting from the mid-Atlantic, Pacific, west north-central and east south-central regions. Decreases in reported cases are noted in the northeast, south-Atlantic, west south-central and mountain states. Crude incidence rates by region ranged from a high of 12.9 cases per 100,000 population in the mid-Atlantic states to a low of 0.10/100,000 in the mountain states (Fig. 3).

Data on secular trends by region from 1982 through 1990 are presented graphically in Figs. 4-12. In the mid-Atlantic region, New Jersey and New York reported increases in cases, and Pennsylvania reported a modest decrease (Fig. 4). The decrease in cases in the northeast region reflects decreased numbers reported from Connecticut, Massachusetts, and Rhode Island. The incidence in Rhode Island fell from being the highest in the nation in 1989 to fourth highest in 1990 (Table 3).

The south-Atlantic region showed a decrease of 350 cases in spite of substantial increases in cases reported by Delaware, Maryland, and Virginia. This reflects a marked decrease in cases reported by Georgia (Fig. 6).

In the east north-central region, modest increases were reported from Indiana and Ohio, while decreased numbers were reported from Illinois, Michigan, and Wisconsin.

Lyme disease activity in the Pacific region is greatest in California. The 95 cases reported from California account for nearly all of the regional increase in 1990.

In the west north-central region, reports in 1990 increased by 20%. The increase occurred as a result of a near doubling of reported cases from Missouri (from 107 cases to 205 cases).

The remaining three regions (west south-central, east south-central and mountain) each reported under 100 cases for 1990 (Figs. 10, 11, 12). Of these regions, only the west south-central region has ever reported more than 100 cases in a single year.

**TABLE 1
REPORTED LYME DISEASE CASES - UNITED STATES**

STATE	CASES (FINAL) 1989	CASES (PROVISIONAL) 1990	↑, ↓, or --
ALABAMA	25	33	↑
ALASKA	0	0	--
ARIZONA	0	0	--
ARKANSAS	10	22	↑
CALIFORNIA	250	345	↑
COLORADO	1	0	↓
CONNECTICUT	774	704	↓
DELAWARE	25	54	↑
DC	0	5	↑
FLORIDA	6	6	--
GEORGIA	715	161	↓
HAWAII	1	2	↑
IDAHO	42	5	↓
ILLINOIS	79	30	↓
INDIANA	8	15	↑
IOWA	27	16	↓
KANSAS	15	14	↓
KENTUCKY	21	18	↓
LOUISIANA	2	3	↑
MAINE	3	6	↑
MARYLAND	138	243	↑
MASSACHUSETTS	129	117	↓
MICHIGAN	165	122	↓
MINNESOTA	92	70	↓
MISSISSIPPI	7	6	↓
MISSOURI	107	205	↑

**TABLE 1 (CONTINUED)
REPORTED LYME DISEASE CASES - UNITED STATES**

STATE	CASES (FINAL) 1989	CASES (PROVISIONAL) 1990	↑, ↓, or --
MONTANA	0	0	--
NEBRASKA	0	0	--
NEVADA	7	3	↓
NEW HAMPSHIRE	3	5	↑
NEW JERSEY	680	1,074	↑
NEW MEXICO	5	0	↓
NEW YORK	3,224	3,244	↑
NORTH CAROLINA	61	67	↑
NORTH DAKOTA	12	3	↓
OHIO	99	113	↑
OKLAHOMA	16	13	↓
OREGON	5	11	↑
PENNSYLVANIA	681	553	↓
RHODE ISLAND	415	101	↓
SOUTH CAROLINA	18	7	↓
SOUTH DAKOTA	3	2	↓
TENNESSEE	30	28	↓
TEXAS	82	44	↓
UTAH	3	1	↓
VERMONT	1	11	↑
VIRGINIA	54	129	↑
WASHINGTON	33	31	↓
WEST VIRGINIA	15	11	↓
WISCONSIN	456	337	↓
WYOMING	6	5	↓
UNITED STATES TOTAL	8,551	7,995	6.5%↓

TABLE 2 REGIONAL LYME DISEASE CASES—UNITED STATES
PROVISIONAL DATA—1990;FINAL DATA—1989

<u>REGIONS</u>	<u>CASES-1989</u>	<u>CASES-1990</u>
MIDATLANTIC	4585	4871
NORTHEAST	1325	944
SOUTH ATLANTIC	1033	683
EAST NORTH CENTRAL	807	617
PACIFIC	289	389
WEST NORTH CENTRAL	256	310
WEST SOUTH CENTRAL	110	82
EAST SOUTH CENTRAL	83	85
MOUNTAIN	64	14
UNITED STATES TOTALS	8552	7995

BACTERIAL ZONNOSES BR.,DVBID,CID,CDC.

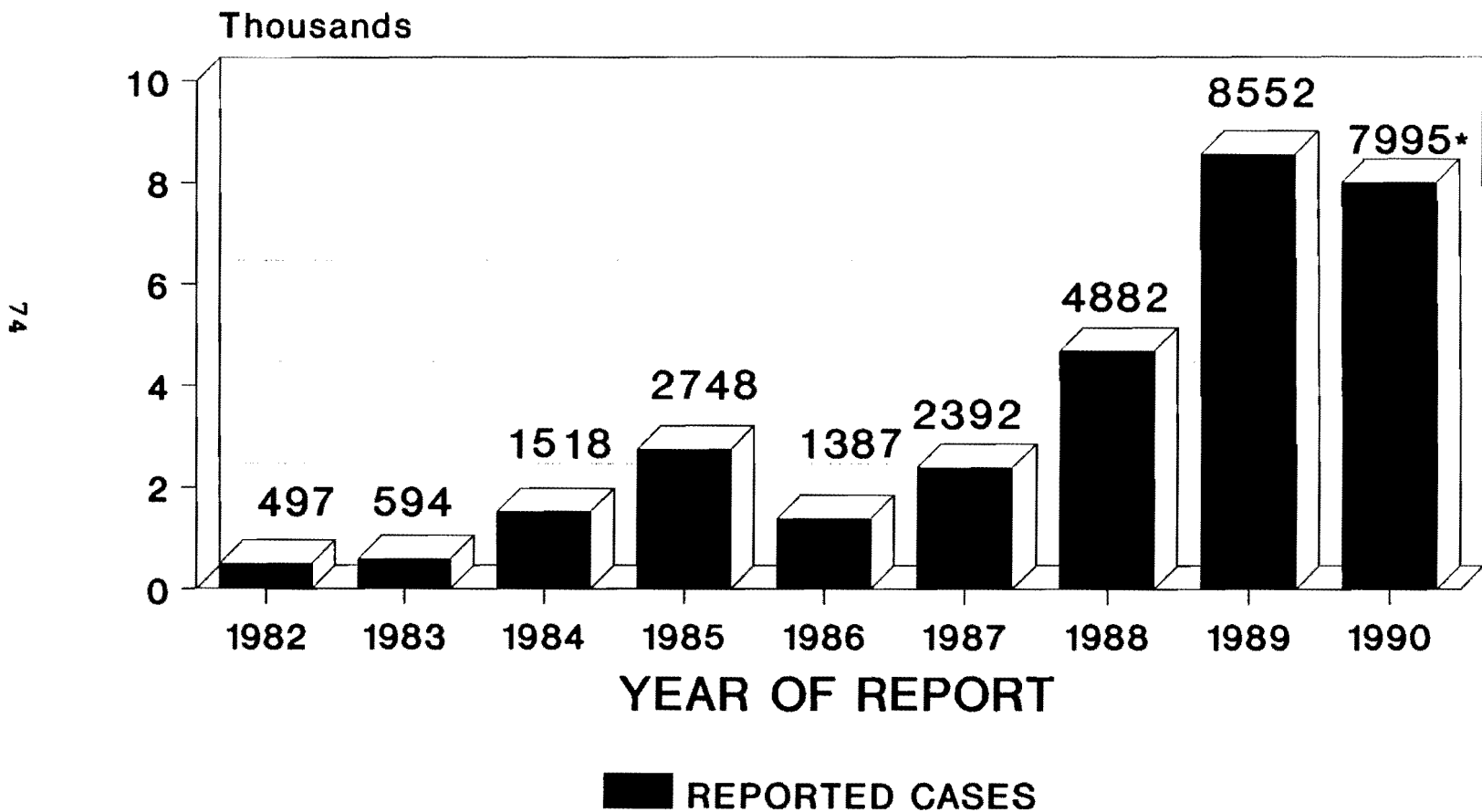
TABLE 3 TEN STATES WITH HIGHEST INCIDENCE OF LYME DISEASE
REPORTED CASES PER 100,000 POPULATION—1990

<u>STATE</u>	<u>INCIDENCE-1990*</u>
CONNECTICUT	21.55
NEW YORK	18.15
NEW JERSEY	13.75
RHODE ISLAND	10.12
DELAWARE	7.92
WISCONSIN	6.88
MARYLAND	5.09
PENNSYLVANIA	4.59
MISSOURI	3.94
GEORGIA	2.44

*INCIDENCE CALCULATED WITH PROVISIONAL REPORTED DATA.

BACTERIAL ZONNOSES BR.,DVBID,CID,CDC.

FIGURE 1 REPORTED LYME DISEASE CASES UNITED STATES 1982-1990

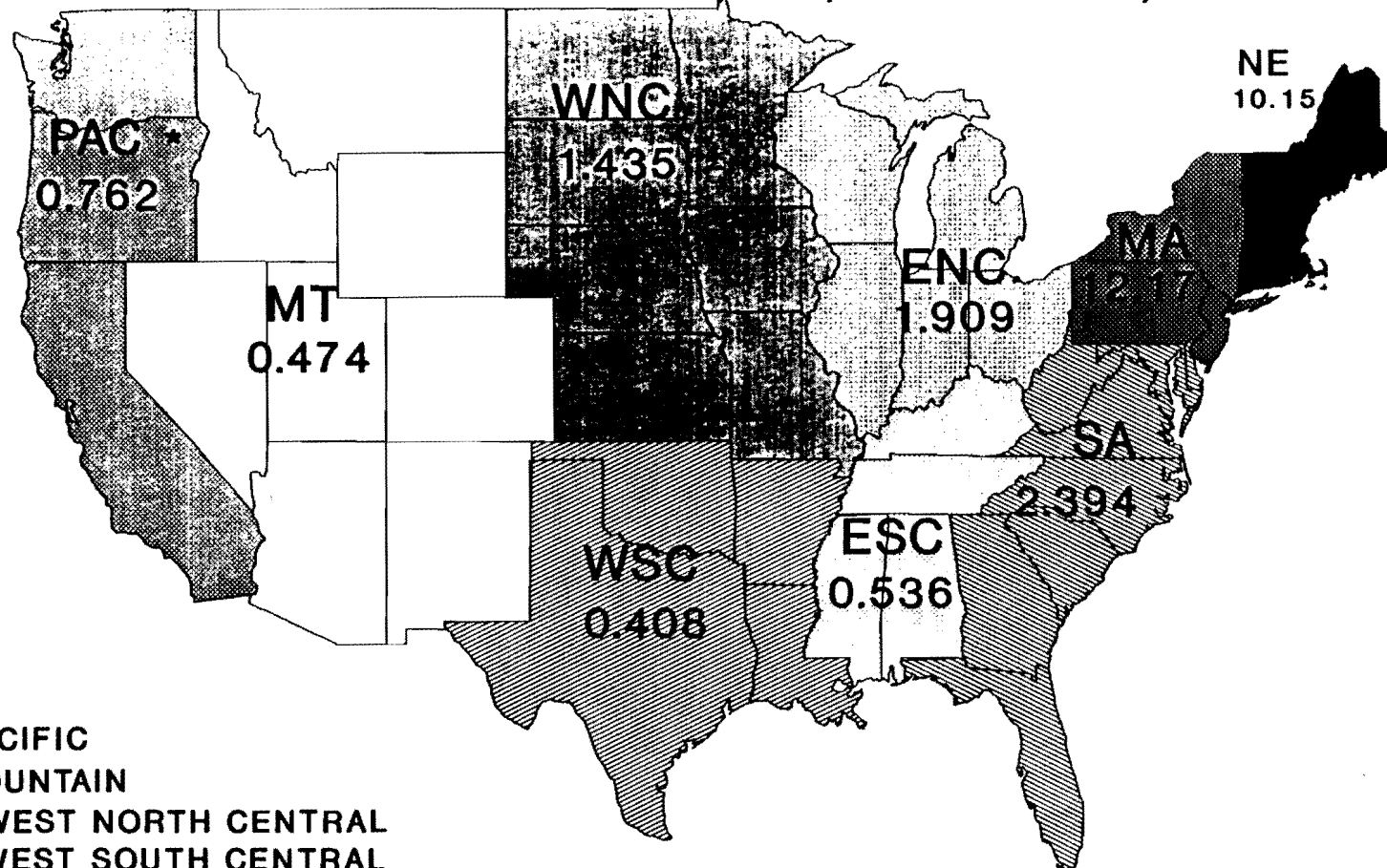


CENTERS FOR DISEASE CONTROL
DIV. OF VECTOR-BORNE INFECTIOUS DISEASES

*PROVISIONAL DATA

FIGURE 2.

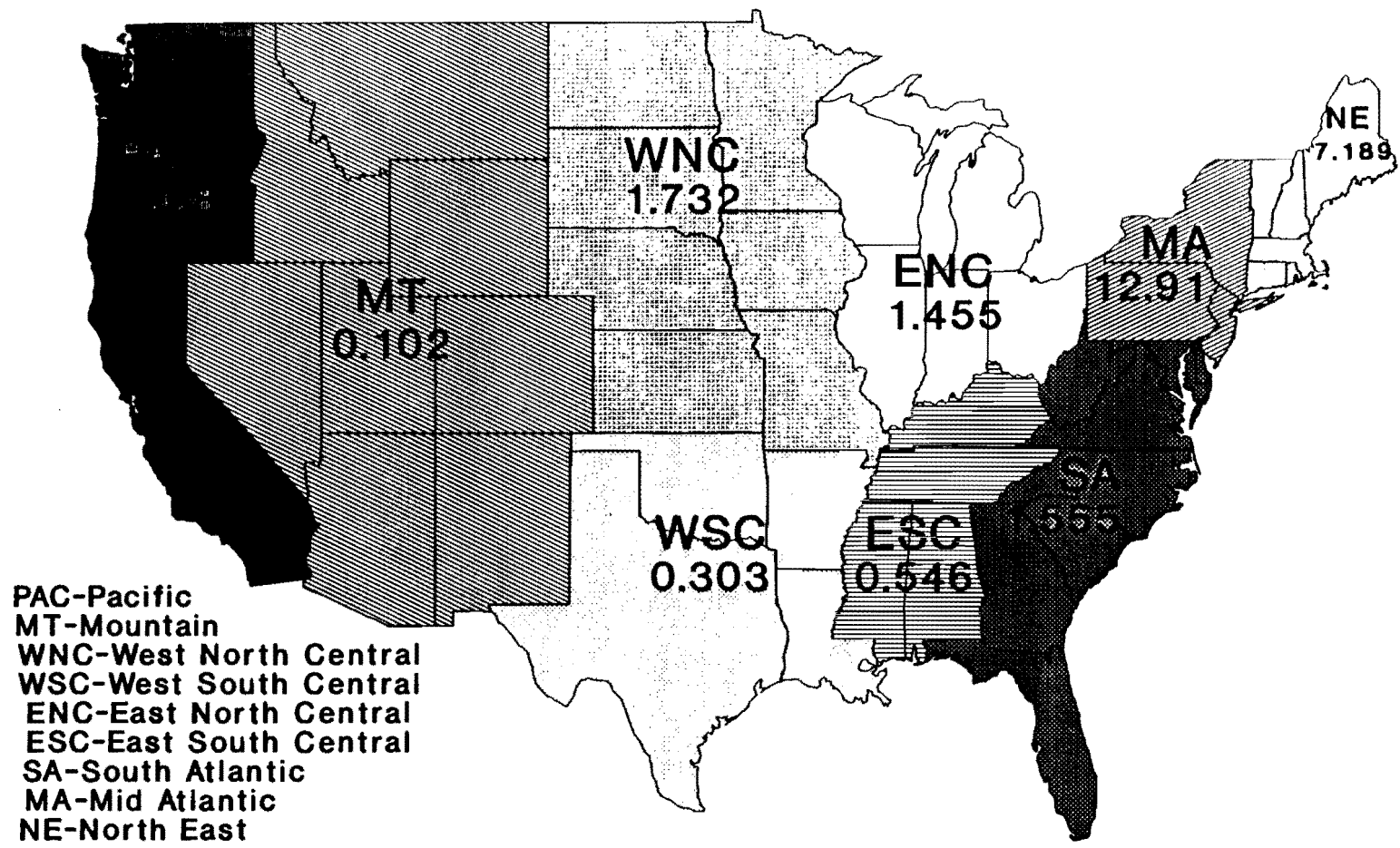
LYME DISEASE INCIDENCE/100,000, BY REGION, 1989



PAC-PACIFIC
MT-MOUNTAIN
WNC-WEST NORTH CENTRAL
WSC-WEST SOUTH CENTRAL
ENC-EAST NORTH CENTRAL
ESC-EAST SOUTH CENTRAL
SA-SOUTH ATLANTIC
MA-MID ATLANTIC
NE-NORTH EAST

Centers for Disease Control
Center for Infectious Diseases
• PAC INCLUDES ALASKA AND HAWAII

FIGURE 3 LYME DISEASE CASE REPORT RATES BY REGION, 1990

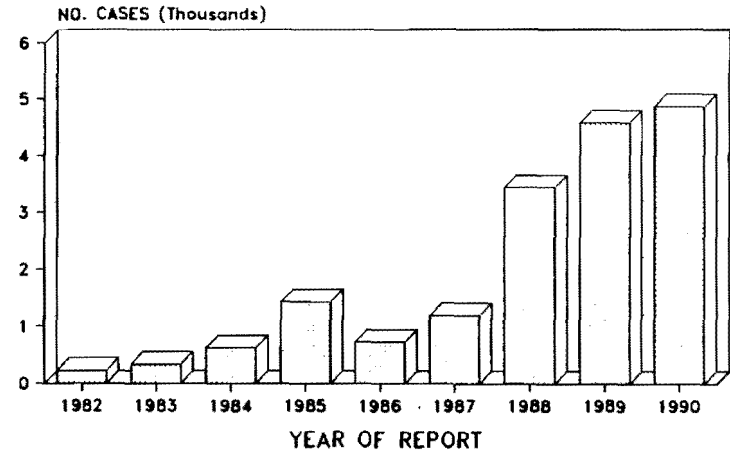


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Bacterial Zoonoses Branch, DVBD
Center for Infectious Diseases
Centers for Disease Control

* Includes Alaska and Hawaii
+ Provisional Rates/100,000 population as of 4/18/91

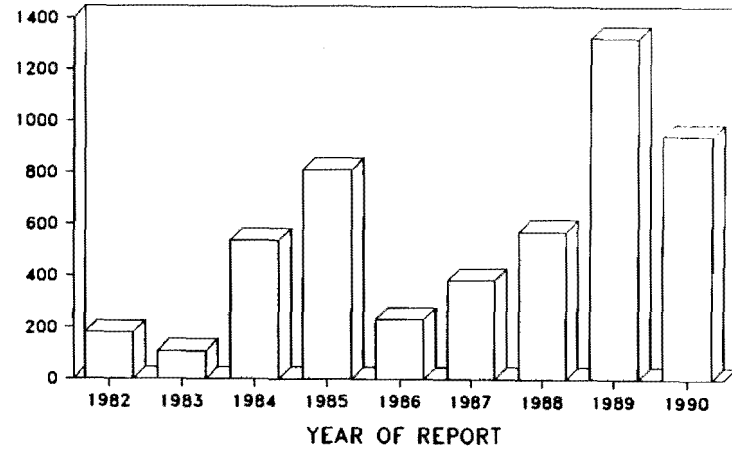
FIGURE 4 MIDATLANTIC REGION REPORTED LYME DISEASE REGIONALLY REPORTED CASES 1982-'90



BACTERIAL ZOOSES BRANCH-DVBID.
CENTER FOR INFECTIOUS DISEASES
CENTERS FOR DISEASE CONTROL

REGION-NJ,NY & PA.

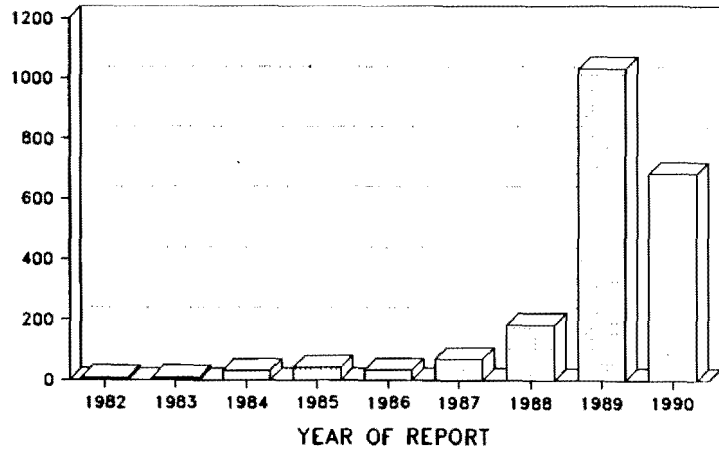
FIGURE 5 NORTHEAST REGION REPORTED LYME DISEASE REGIONALLY REPORTED CASES 1982-'90



BACTERIAL ZOOSES BRANCH-DVBID.
CENTER FOR INFECTIOUS DISEASES
CENTERS FOR DISEASE CONTROL

REGION-CT,ME,MA,RI & VT.

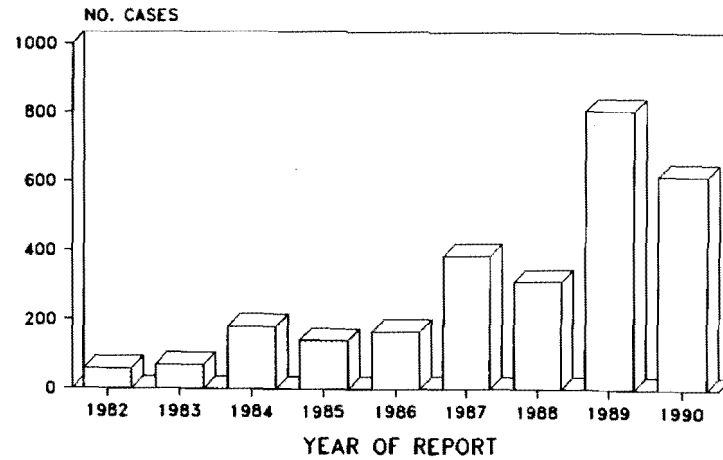
FIGURE 6 SOUTH ATLANTIC ZONE LYME DISEASE CASES REGIONALLY REPORTED CASES 1982-'90



BACTERIAL ZOOSES BRANCH-DVBID.
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CENTERS FOR DISEASE CONTROL

REGION-DE,DC,FL,GA,MD,NC,SC,VA & WV.

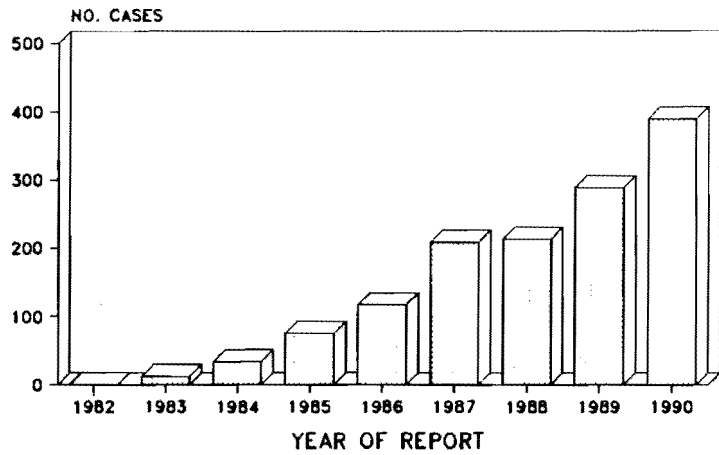
FIGURE 7 EAST NORTH CENTRAL REPORTED LYME DISEASE REGIONALLY REPORTED CASES 1982-'90



BACTERIAL ZOOSES BRANCH-DVBID.
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REGION-IL,IN,MI,OH & WI.

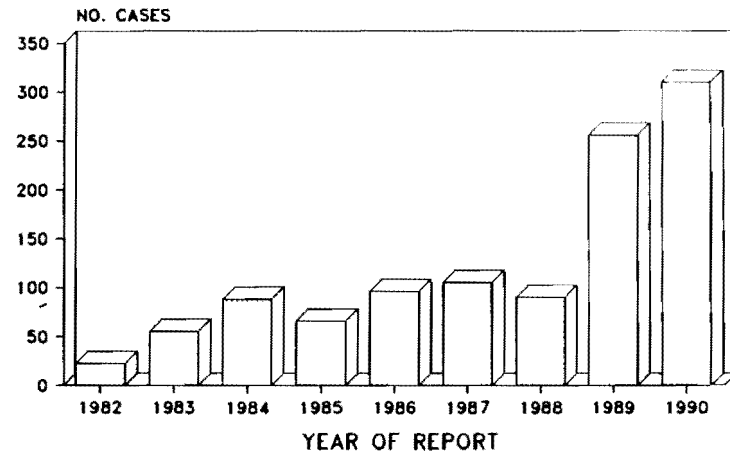
FIGURE 8 PACIFIC REGION REPORTED LYME DISEASE REGIONALLY REPORTED CASES 1982-'90



BACTERIAL ZOOSES BRANCH-DVBID.
CENTER FOR INFECTIOUS DISEASES
CENTERS FOR DISEASE CONTROL

REGION-AK,CA,HI,OR & WA.

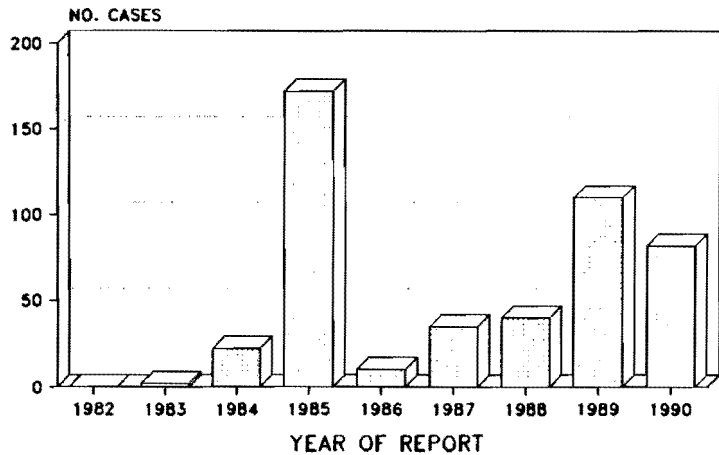
FIGURE 9 WEST NORTH CENTRAL REPORTED LYME DISEASE REGIONALLY REPORTED CASES 1982-'90



BACTERIAL ZOOSES BRANCH-DVBID.
CENTER FOR INFECTIOUS DISEASES
CENTERS FOR DISEASE CONTROL

REGION-IA,KS,MN,MO,NE,ND & SD.

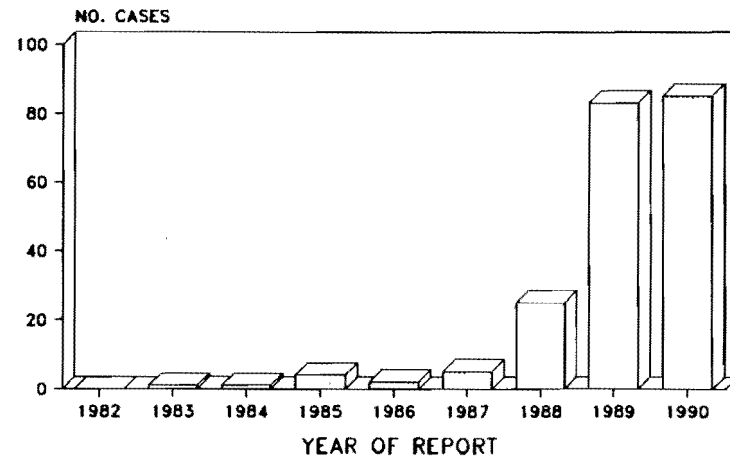
FIGURE 10 WEST SOUTH CENTRAL REPORTED LYME DISEASE REGIONALLY REPORTED CASES 1982-'90



BACTERIAL ZOOSES BRANCH-DVBID.
CENTER FOR INFECTIOUS DISEASES
CENTERS FOR DISEASE CONTROL

REGION-AR,LA,OK & TX.

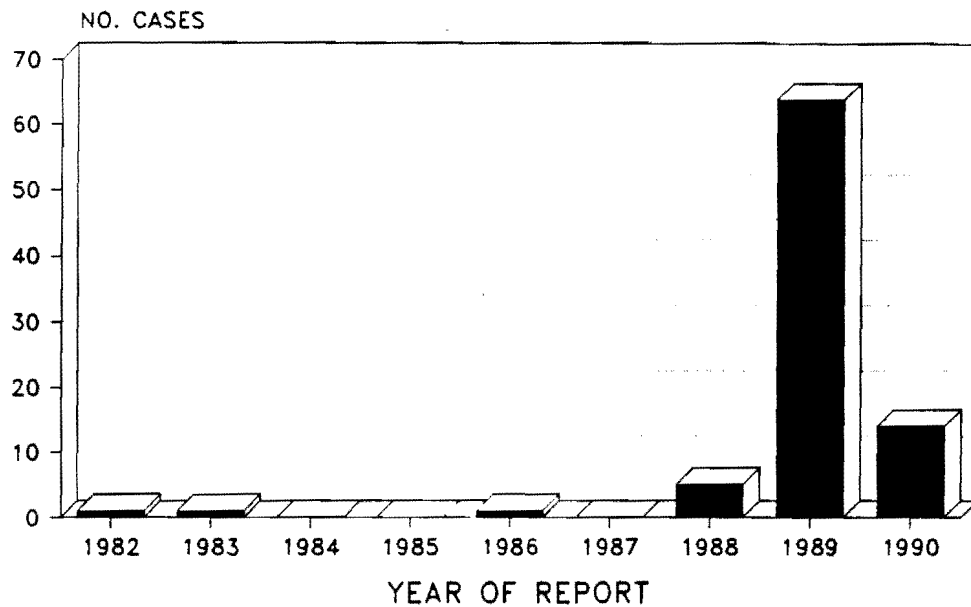
FIGURE 11 EAST SOUTH CENTRAL REPORTED LYME DISEASE REGIONALLY REPORTED CASES 1982-'90



BACTERIAL ZOOSES BRANCH-DVBID.
CENTER FOR INFECTIOUS DISEASES
CENTERS FOR DISEASE CONTROL

REGION-AL,KY,MS & TN

FIGURE 12 MOUNTAIN REGION REPORTED LYME DISEASE
REGIONALLY REPORTED CASES 1982-'90



BACTERIAL ZOOSES BRANCH-DVBID.
CENTER FOR INFECTIOUS DISEASES
CENTERS FOR DISEASE CONTROL

REGION-AZ,CO,ID,MT,NV,NM, & UT.

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BY POPULAR DEMAND (really!), MORE IRRELEVANT QUOTES

Will Rogers: "There's no trick to being a humorist when you have the whole government working for you."

Will Rogers: "Diplomacy is the art of saying "Nice doggie!" until you can find a rock."

Russian proverb: "Since when does the fiddle pick the tune?"

Woody Allen: "I'm not afraid to die. I just don't want to be there when it happens."

Angelo Bartlett Giamatti: "To the Members of the University Community: In order to repair what Milton called the ruin of our grand parents, I wish to announce that henceforth, as a matter of University policy, evil is abolished and paradise restored. I trust all of you will do whatever possible to achieve this policy objective."

Joe Garagiola: "Nolan Ryan is pitching much better now that he has his curve ball straightened out."

Victor Lowmes: "A promiscuous person is someone who is getting more sex than you are."

Karl Kraus: "The relation between psychiatrists and other kinds of lunatic is more or less the relation of a convex to a concave one."

Mahatma Ghandi: "There is more to life than increasing its speed."

Abraham Lincoln: "He can compress the most words into the smallest idea of any man I ever met."

Plato: "Of all the animals, the boy is the most unmanageable."

Bertrand Russell: "One of the symptoms of an approaching nervous breakdown is the belief that one's work is terribly important."

Tom Lehrer: "I wish people who have trouble communicating would just shut up."

Angelo Bartlett Giamatti: "The tale of leaving and seeking home is told in as many ways as one can imagine, and there still occur every season plays on the field that even the most experienced baseball people say they have never seen before. The random events, the variety of incidents, the differing ways various personalities react to pressure, the passion poured into the quest to win, are organized by the rhythms of the innings, by the metric of the count and the pitcher's rhythm and by the cool geometry that is underfoot and overarching."

Duncan J. McGeoch: "Viruses may be mistletoe on the Tree of Life."