ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

Number 41 September 1981

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COMMENTS FROM THE EDITOR

When I was approached last November about becoming editor of the Arthropod-borne Virus Information Exchange, I wondered how it would be possible to succeed the two former editors, Dr. Telford H. Work and Dr. Roy Chamberlain. At the time I hoped that Roy would consider postponing his retirement plans and continue editing the "Information Exchange" for at least ten more years. As you may know, he retired from the Centers for Disease Control this past summer and all of his "Information Exchange" files were transferred to my office.

With the assistance of my secretary, Helen Snodgrass, we have put together issue Number 41. Our goal is to have it in the mail by October 15. This should allow enough time for delivery before the meeting of the American Society of Tropical Medicine and Hygiene, November 16-20, in San Juan. The abstracts of arbovirus papers to be presented at this meeting are included in this issue.

I would like to remind all contributors that the deadline for issue Number 42 is March 1, 1982. Please make a note on your calendar to mail articles to arrive in Atlanta by this date. They should be addressed to:

W. Adrian Chappell, Editor
Arthropod-borne Virus Information Exchange
Biological Products Production Branch
Centers for Disease Control
Atlanta, Georgia 30333, U.S.A.

In addition to current information on arbovirus investigations, you are invited to submit news items about investigators of arboviruses, e.g., honors, changes in employment location, retirements, deaths, etc. Announcements of arbovirus meetings will also be made when submitted.

If you have ideas on how the "Information Exchange" can be improved, please let me hear from you.

W. Adrian Chappell

W. Adrian Chappell, Ph.D.

SEAS Report to the ACAV Open Meeting,
5th International Congress of Virology
Strasbourg, France, 1-8 August 1981

The Subcommittee on Evaluation of Arthropod-borne Status (SEAS) has been functioning since November 1970. Its mandate is as follows:

1. Formulate guidelines for deciding if a particular virus is arthropod-borne.

2. Determine the status of each registered virus according to these criteria.

3. Report its decisions to the Arbovirus Catalogue editor.

4. Seek pertinent new information useful in periodic review and updating of decisions.

In addition to the above, the subcommittee assists the Catalogue work with valuable opinions and advice regarding data submitted with each new virus registration.

To date, 434 registered viruses have been evaluated, but only 166 (38%) are acknowledged true or probably true arboviruses. An editorial in the March 1981 issue of the Arbovirus "Info-Exchange" calls attention to the many lacunae in our knowledge, and it is hoped that the future will see an improvement in the characterization of these agents.

Thomas H. G. Aitken
Chairman

TRIBUTE TO DR. TELFORD H. WORK

This issue of the Arthropod-borne Virus Information Exchange is dedicated to

TELFORD H. WORK

who started it in 1960 and who edited it until 1971. The following comments were kindly submitted by Dr. Charles Calisher.

The Arbovirus Information Exchange is 21 years old this year. Coincidentally, Telford H. Work, the first editor of the Infoexchange, was 60 this past July. Both appear to have come of age!

After graduation from Stanford University in 1942 (A.B.), Tel obtained additional degrees from Stanford University Medical School (M.D., 1945), the London School of Hygiene and Tropical Medicine (D.T.M. and H., 1949) and the Johns Hopkins University, School of Hygiene and Public Health (M.P.H., 1952). He took post-graduate studies in the Fiji Islands and joined the Rockefeller Foundation as a staff member, where he was subsequently assigned to Cairo, Egypt, and Poona, India, respectively. In 1960 he became Chief of the Virology Section and Arbovirus Unit, National Communicable Disease Center (now Centers for Disease Control), Atlanta, Georgia, and in 1966 moved back to California to become Vice-Chairman for Public Health Sciences and then Professor and Chairman, Infectious and Tropical Diseases, School of Public Health (U.C.L.A.), Professor of Infectious and Tropical Diseases, Department of Medical Microbiology and Immunology, and Professor of Preventive Medicine, both in the U.C.L.A. School of Medicine.

Those who know Tel will admit that he is persistent and stubborn, voluble and articulate, demanding and giving, intuitive and rigorous, stimulating and exhausting, amusing and annoying. He'll admit to at least half of this.

Now into a fifth decade of field and laboratory research, Tel has published more than 75 scientific articles (he is senior author of more than 40), has served as consultant to numerous scientifically elite committees and panels and W.H.O. and P.A.H.O. study groups, and lectured at numerous universities throughout the world. He is a member of eleven scientific societies and has served on the editorial boards of numerous journals. His lectures on arbovirus disease ecology alone have stimulated medical students to take up careers in the field.

What a breadth of interests and expertise! From Fiji to Novosibirsk, Cairo to Helsinki, Poona to Perth - one would expect that he would take it a bit easy now. But no!, camera in hand he wanders the globe, searching out disease ecosystems, dividing them into component parts, rejoicing with old friends, watching creeks dry, disobeying the doctors' orders and giving everyone the benefit of his experiences and incisive observations. No one has ever said, "Tel who?"

Like the Infoexchange and wine, Tel has mellowed some, but it is hoped that both the informal publication, which grew like Topsy, and Tel have years of usefulness to lend the human communities.

Formation of Bunyavirus Reassortants in Dually-Infected Mosquitoes

Authors: B. J. Beaty and D.H.L. Bishop

Wild-type and temperature-sensitive (ts) bunyaviruses were recovered from Aedes triseriatus mosquitoes after 1-3 weeks extrinsic incubation following intrathoracic inoculation with homologous virus mixtures of Group I and II ts mutants of snowshoe hare (SSH), or LaCrosse (LAC) viruses (e.g. SSH I and SSH II mutants, or LAC I and LAC II mutants). Wild-type and ts viruses were also recovered from mosquitoes dually-infected with heterologous virus mixtures of SSH I and LAC II ts mutants. However, only ts viruses have been obtained from mosquitoes infected with LAC I and SSH II ts mutants. RNA fingerprint analyses of one of the wild-type viruses recovered from a mosquito infected with the SSH I and LAC II ts mutants showed that it had the reassortant virus large/medium/small RNA genotype of SSH/LAC/SSH.

Wild-type and ts viruses were also recovered from suckling mice on which mosquitoes that had been infected with homologous virus Group I and II SSH, or LAC, ts mutants were allowed to feed. Likewise wild-type and ts viruses were recovered from mice on which mosquitoes inoculated with SSH I and LAC II ts mutants were allowed to feed. RNA fingerprint analyses of one of these wild-type viruses indicated that it had the reassortant virus genotype of SSH/LAC/LAC. From mice on which mosquitoes infected with SSH II and LAC I ts mutants had fed, no wild-type viruses were recovered. The inability to detect reassortants having LAC/SSH/LAC and LAC/SSH/SSH genotypes from the in vivo infections expected to yield them (i.e. SSH II plus LAC I) was consistent with previous observations in which such reassortants were only recovered at very low frequencies from in vitro recombination analyses (1).

In these reported studies, mosquitoes were infected by intrathoracic inoculation. Further studies are currently in progress to determine if bunyavirus recombination can occur under normal circumstances of mosquito infection.

IDENTIFICATION OF TWO NEWLY RECOGNIZED VENEZUELAN EQUINE ENCEPHALITIS VIRUSES FROM BRAZIL AND ARGENTINA

Charles H. Calisher, Ph.D., Oscar de Souza Lopes, M.D., Thomas P. Monath, M.D., D. Bruce Francy, Ph.D., Carl J. Mitchell, Ph.D., Richard M. Kinney, M.S., Dennis W. Trent, Ph.D. and Marta Sabbatini, M.D.

During studies of arboviruses in Brazil and Argentina a number of alphaviruses belonging to the Venezuelan equine encephalitis (VEE) complex were isolated. The two isolates from Brazil were from Culex (Melanoconion) species mosquitoes and from a bat (Carollia perspicillata). These isolates were avirulent for 6-to-8 week-old mice and short-haired guinea pigs and were characterized by other biologic and serologic and biochemical means. They were shown serologically to represent a single, newly recognized variety of subtype I. Two dimensional polyacrylamide gel electrophoresis (PAGE) of ribo-nuclease T1 digests of viral ribonucleic acid showed considerable homology between the genomes of the new virus and variety IA. Three structural proteins were discriminated using discontinuous sodium dodecyl sulfate (SDS)-PAGE. Although the smallest protein of both Brazilian isolates migrates with the capsid proteins of other subtype I viruses, the larger structural proteins of the new variety differ in molecular weight from the E_1 and E_2 envelope glycoproteins of the other subtype I varieties. The new isolates produced peptide fragment patterns which were identical to each other, but different from the patterns of other subtype I viruses, following SDS-PAGE of dissociated varions digested with protease. We suggest the classification VEE, IF for these viruses.

Seventeen strains of a VEE complex virus from northern Argentine mosquitoes (mostly Culex (Melanoconion) species) were shown to be serologically identical to one another. The prototype strain, studied by biochemical and serological methods, appears to be yet another new variety within the VEE complex.

The classification of the VEE complex is revised and reviewed.

Molecular studies on tick-borne viruses.

John P.M. Clerx, Hiroshi Ushijima and D.H.L. Bishop, Dept. of Microbiology, The University of Alabama in Birmingham, Birmingham, AL 35294

The bunyavirus family has four defined genera: Bunyavirus, Phlebovirus, Uukuvirus and Nairovirus. At the molecular level the genera can be differentiated on the basis of the sizes of their three RNA species or sizes of virion polypeptides or both.

Bunyavirus genus members have RNA species of 2.7-3.1 x 10^6(L), 1.8-2.3 x 10^6(M) and 0.28-0.50 x 10^6(S) daltons. The major structural polypeptides are 108-120 x 10^3(G1), 29-41 x 10^3(G2) and 19-25 x 10^3(N) daltons. Phlebovirus genus members have RNA species of 2.6-2.8 x 10^6(L), 1.8-2.2 x 10^6(M) and 0.7-0.8 x 10^6(S) daltons. Major structural polypeptides are 55-70 x 10^3(G1), 50-60 x 10^3(G2) and 20-30 x 10^3(N) daltons. Uukuniemi virus (genus Uukuvirus) has RNAs of 2.4 x 10^6(L), 1.1 x 10^6(M) and 0.5 x 10^6(S) daltons. It has major structural polypeptides of 75 x 10^3(G1), 65 x 10^3(G2) and 25 x 10^3(N) daltons. Nairovirus genus members have RNAs of 4.1-4.9 x 10^6(L), 1.5-1.9 x 10^6(M) and 0.6-0.7 x 10^6(S) daltons. Nairoviruses have a nucleoprotein of 48-54 x 10^3 daltons and 2 or 3 glycosylated envelope polypeptides.

We have undertaken molecular analyses of certain tick-borne viruses in order to determine if they are like members of the defined genera of the Bunyaviridae.

Grand Arbaud virus (Uukuvirus genus) has 3 viral RNAs of 2.3, 1.2, and 0.55 x 10^6 daltons and 3 major structural polypeptides (64, 58, and 25 x 10^3 daltons). Thus it closely resembles Uukuniemi virus. Analyses of Silverwater virus (Kaisodi serogroup) indicates that it resembles uukuviruses by having RNAs of 2.4 x 10^6, 1.2 x 10^6 and 0.7 x 10^6 daltons and polypeptides of 60 x 10^3, 50 x 10^3 and 25 x 10^3 daltons.

Sicilian arbovirus 126 (Thogoto serogroup) has RNAs of 2.9 x 10^6, 1.9 x 10^6 and 0.7 x 10^6 daltons. Its polypeptides are 58 x 10^3, 50 x 10^3 and 25 x 10^3 daltons, therefore it resembles the phleboviruses.

Bhanja virus (unassigned, bunyavirus-like) has polypeptides of 68 x 10^3(G1), 58 x 10^3(G2) and 22 x 10^3(N) daltons.

Preliminary studies on Quaranfil virus (Quaranfil serogroup) indicate that this virus is not a bunyavirus, but induces polypeptides in infected cells similar to those induced by arenaviruses.

Prototype Dhori virus (I 611313) has at least 7 virion polypeptides and 7 unique, single-stranded RNA segments with 3' end sequences unlike those of any bunyavirus, phlebovirus/uukuvirus or nairovirus. Our data indicate that Dhori virus which was recovered from ticks collected from camels, is similar to orthomyxoviruses.

MOLECULAR ANALYSIS OF TWO BLUE TONGUE VACCINES AND THEIR RELATIONSHIP WITH RECENT OUTBREAK OF BLUE TONGUE IN WASHINGTON STATE

Ellen Collisson and Polly Roy

Two U.S. BTV vaccines (Colorado and California origin) were studied at the molecular level. Individual RNA's of each vaccine were compared both by gel electrophoresis as well as fingerprint analysis. The individual RNA segments of a recent isolate (1980) of blue tongue virus from Washington State have been analyzed similarly and compared with the corresponding RNA segments of two vaccine viruses.

Dengue Virus Development in *Aedes aegypti* Salivary Glands

by

Robert S. Copeland

Ultrastructural aspects of the replication of dengue type 2 virus in salivary glands of *Aedes aegypti* were studied, following intrathoracic inoculation. Viral particles first appeared in cells of the proximal portion of the lateral lobe at 5 days, and subsequently were found in all regions of the glands except the intermediate portion of the median lobe. Membranous bodies, or vesicles, 30-180 nm. in diameter, always accompanied viral production. Viral matrices, which increased in size over the course of the infection, were found in cells of the proximal and distal portions. Virions were seen in vacuoles, within cisternae of the endoplasmic reticulum, between adjoining cells, and extracellularly. They were also found dispersed and in crystals within salivary secretion in the apical cavities. Vacuoles containing spherical bodies were found in non-secretory cell types, as were precursor particles, suggesting that viral morphogenesis varies with host cell type. Crystalloid inclusions with translucent subunits were seen in the distal portion.

Dengue morphogenesis is intimately associated with cytoplasmic membranes. It is proposed that the vesicles act as a replicative complex, and that viral maturation, at least within secretory cell types, occurs within cisternae of the endoplasmic reticulum, perhaps in viral matrices. Exocytosis is the main mechanism of viral release.

Clinical and laboratory findings in 31 laboratory-confirmed outpatient cases of Dengue in Puerto Rico

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During late 1975, 31 patients who reported to an outpatient facility with dengue-like illnesses (defined as an acute febrile illness with at least one or more of the following symptoms or signs: headache, retro-orbital pain, bodyaches, or rash) were examined from 1-5 times during their first 12 days of illness and again at approximately 30 days after onset. Tourniquet tests were performed at each physical examination during the first 12 days and repeated at the 30 day follow-up upon patients who were positive during their acute illness. Multiple blood samples were obtained for virus isolation, HI and CF serology, complete blood counts (CBC's) and platelet counts (PC's). No patients were judged to require hospitalization. Dengue 2 virus was isolated from 13 (41%) of the patients and all 31 were serologically positive by standard criteria (71% primary responses).

Of the 31 laboratory-confirmed cases, 14 were males, 20% were $\geq$30 yrs. of age, 67% were 15-29 yrs and 13% were $\leq$15 yrs. Of the 31 patients, 100% were febrile and 71% or more complained of headache, retro-orbital pain, body aches, rash, lympadenopathy, and chills. Nausea and conjunctivitis each occurred in 42%, sore throat in 39% and cough and nasal congestion each in 23%. The only hemorrhagic manifestations reported or observed were petechiae (42% of patients) during a tourniquet test in the acute illness only.

No overt bleeding was detected in these patients, but 15 (48%) had an abnormally low HCT which returned to normal or near normal on follow-up examination. Twenty-eight patients (90%) had neutropenia during their acute illness (WBC range 1,700-4,900). A statistically significant increase in WBC occurred between the acute illness and follow-up (McNemar test $p < 0.001$). PC's of less than 150,000 were obtained from blood specimens of 9 patients (29%). A statistically significant increase in PC's occurred at the follow-up visit in both thrombocytopenic and normal patients (McNemar test, $p < 0.01$). The frequency of respiratory and gastrointestinal symptoms, the high frequency of positive tourniquet tests, and the tendency toward absolute or relative pancytopenia was unsuspected in this predominantly adult population with "uncomplicated dengue fever and suggests that dengue may present with a wider variety of clinical and laboratory abnormalities than are classically described.

Evaluation of Mosquito Cell Lines for the Preparation of Dengue Virus Vaccines

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The C6/36 clone (Igarashi) of Aedes albopictus cells and two different cell lines derived by Tesh from Toxorhynchites amboinensis, were evaluated as substrates for dengue virus replication. The C6/36 cells produced 10 to 100 fold more DEN-2 and DEN-3 virus than a vertebrate cell currently in use for vaccine production. Virus yields from the C6/36 cells were also superior by 100 to 1,000 fold to those yields resulting from infection of the T. amboinensis cell lines.

The DEN-2 S-1 vaccine virus was propagated in C6/36 cells, but temperature insensitive virus exhibiting variable plaque sizes was found in the early passages. This was followed within 7 passages by the selection for a stable and homogeneous, temperature sensitive (ts), small, faint plaque producing virus population. Selection for a ts phenotype was also observed after passage of a DEN-3 vaccine candidate virus.

Use of the C6/36 clone as a vaccine substrate cell line requires extensive testing of the cells to rule out contamination with adventitious microbial agents. Standard tests used for the isolation of bacteria, mycoplasma, and viruses as well as electron microscopy have demonstrated that no conventional agents are present in these cells. Additionally, the C6/36 cells were not tumorigenic in newborn hamsters, and karyologic analysis revealed genome stability in terms of both structural and numerical variations of chromosomes.

The C6/36 clone of Aedes albopictus offers several advantages for use as a dengue virus vaccine substrate: high virus yields, selection for ts variants, and the absence of adventitious agents.

An attenuated mutant of Venezuelan encephalitis virus: Biochemical alterations and their genetic association with attenuation. EMILIO A. EMINI and MICHAEL E. WIEBE, Cornell University Medical College, New York, N.Y.

A temperature-sensitive mutant, ts 126, derived from the hamster-virulent 68U201 wild-type (wt) strain of Venezuelan encephalitis virus, an alphavirus, has been shown to be attenuated. Although it is temperature-sensitive, the mutant replicates in infected hamsters and elicits production of protective antibodies (Krieger et al., 1979, Infect. Immun. 25: 873-879).

We have characterized additional phenotypic differences between ts 126 and 68U201 wt in an attempt to localize the genetic basis of the mutant's attenuated virulence. ts 126 was shown to differ from the parent virus with respect to virion structure-dependent, particularly surface structure-dependent, characteristics: temperature-lability, plaque sizes in Vero cells, and binding properties to hydroxylapatite. A surface difference was identified by isoelectric focusing of the virion envelope glycoproteins as an alteration in the E_1 glycoprotein. The common genetic basis of all these phenotypic differences was demonstrated by the isolation of independently-arising, stable genetic revertants of ts 126. These revertants exhibit characteristics identical in every respect to 68U201 wt.

It appears from these studies that the mutation which gave rise to the ts 126 mutant virus occurred in the structural gene coding for the E_1 envelope glycoprotein and that the resultant phenotypic alteration in this glycoprotein is genetically associated with the mutant's lack of virulence.

Experimental studies of SLE virus transmission among mosquitoes of the Culex pipiens complex.

D. Bruce Francy, William A. Rush, and Moises Montoya

Experiments were conducted to establish time related transmission efficiency of SLE virus by Culex p. pipiens and Cx. p. quinquefasciatus. Both subspecies were highly susceptible to infection with SLE virus and no difference in infection rates were noted. For transmission studies, a standard extrinsic incubation period of 11 days was used. Cx. p. pipiens transmission rates were 59.3% and those of Cx. p. quinquefasciatus were 9.0%. Hybrids of both types had transmission rates intermediate between the parental types (28.1 and 29.8%). Results of experiments support the hypothesis that differences in arbovirus transmission efficiency may be genetically controlled.

Additional information on the relationship of virus titers in donor vertebrate hosts, virus titers in mosquitoes, and transmission rates will also be presented.

Experimental Transmission of Rift Valley Fever Virus by Mosquitoes

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Until recently, Rift Valley fever (RVF) was considered to be a disease mainly of domestic animals in sub-Saharan Africa, while in man it was considered serious, but rarely fatal. The virus was first reported north of the Sahara Desert in 1977, when it caused an epidemic in the Nile Delta in Egypt. This epidemic resulted in considerable animal mortality and human involvement with a number of fatalities. Experimental transmission studies with a single colony of Culex pipiens L. implicated the species as a vector of RVF virus in the 1977-78 epizootics in Egypt (Meegan et al, 1980, Am. J. Trop. Med. Hyg. 29:1405-1410). However, this report also mentioned that different transmission rates were obtained with Cx. pipiens colonies originating from different localities. We are interested in the variation in the vector potential of different populations of Egyptian Cx. pipiens in anticipation that such information might clarify the role of the species in the epidemiology of RVF.

This report describes experiments designed to measure the vector competence of certain colonized Egyptian and North American mosquitoes for RVF virus. Five colonies of Egyptian Cx. pipiens originated from adults and larvae collected during 1980-81 from 3 localities: Sharqiya, Qalumbiya and Ismailiya. A North American colony of Cx. pipiens originated from adults collected in 1975 in New Jersey. The history of the Aedes taeniorhynchus colony tested is not available. Golden Syrian hamsters were used as an animal model, because they are uniformly susceptible, circulate virus to high titers and normally die from the infection within 5 days of virus inoculation. Viral replication was monitored in the mosquito strains for 11 days after feeding on infected hamsters. In transmission experiments, groups of mosquitoes were allowed to engorge on infected hamsters, held in the insectary for various periods of time and then allowed to feed individually on susceptible hamsters. Mosquitoes were assayed for virus by a standard plaque assay of triturated suspensions on Vero E-6 monolayers. Hamsters involved in transmission trials were observed for 27 days and liver samples from dead animals were assayed for virus. Sera from survivors were assayed for RVF virus antibody by the IFA technique.

Viral replication experiments showed an eclipse phase consisting of a 2-4 log decrease in virus titer on day 2 post-infectious blood meal. Peak titers in infected mosquitoes occurred on days 6 and 11. Not all mosquitoes within each strain, however, became infected. RVF virus was transmitted to hamsters by 4 of the 5 Egyptian Cx. pipiens strains and also by the American strains of Cx. pipiens and Ae. taeniorhynchus. The shortest extrinsic incubation period for virus transmission was 4 days post-infectious blood meal. Variation in both infection and transmission rates was observed with the Egyptian Cx. pipiens strains. The percent infected and transmitting in the Egyptian strains ranged from 0-78% and 0-32%, respectively. RVF virus transmission was accomplished not only by replete individuals, but also by nonengorged probing mosquitoes. We observed 3 hamsters infected with RVF virus via mosquito bite which did not succumb to the infection, but seroconverted to the virus within 27 days after exposure to the infectious mosquitoes.

Development of Monoclonal Antibodies for Identification of Dengue Virus Serotypes

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Extensive serological cross-reactions occuring among dengue virus serotypes frequently interfere with their identification. We developed hybridoma-derived reagents directed against the prototype dengue virus strains: DEN-1(Hawaiian), DEN-2(New Guinea C), DEN-3(Philippines H-87), and DEN-4(Philippines H-241). Lymphocyte hybridomas were prepared by fusing P3x63Ag8 mouse myeloma cells with spleen cells from mice immunized with dengue virus antigens. Antibodies secreted by hybridomas were detected by solid phase radioimmunoassay (SPRIA) using antigen extracts from dengue-infected C6/36 (Aedes albopictus) cells. Hybrid cells from selected lines were cloned and injected into Pristane-primed mice for preparation of ascitic fluids containing higher concentrations of anti-dengue antibody. Each ascitic fluid was evaluated by plaque reduction neutralization (PRNT), hemagglutination inhibition (HAI) and indirect immunofluorescence (IFA) for type-specificity.

Several anti-DEN-1 monoclonal antibody preparations have been selected for use as serotyping reagents. Two cell lines produced high-titered (1:320;1:1280) type-specific antibody by IFA on virus-infected cells. However, these monoclonal antibodies were not reactive by PRNT and HAI. A third cell line produced an antibody that titered 1:1100 to DEN-1 and 1:10 to DEN-3 by PRNT but was cross-reactive to higher titers by IFA and HAI. Those cell lines type-specific by IFA have recently been used successfully to type isolates from a recent dengue outbreak in Jamaica.

Monoclonal antibodies to DEN-2 virus, reported at the previous annual meeting, include 5 preparations that are type-specific by IFA, one of which reacts specifically to high titer by PRNT, but not by HAI, and four of which react specifically to high titer by HAI but not PRNT. These monoclones also reacted by IFA with other DEN-2 strains from Asia and Caribbean geographical regions.

Monoclonal antibody preparations to DEN-3 included one that was serotype specific to low titer by IFA and HAI, and another that was type-specific to high titer (1:2560) by IFA only. Three monoclonal antibody preparations to DEN-4 were serotype specific by HAI. One of these was type-specific by IFA (1:640) and two were cross-reactive by IFA. There was little, if any, PRNT activity by these DEN-3 or DEN-4 monoclonal antibodies.

Characterization of monoclonal antibodies to the dengue viruses has shown that there are distinct antigenic determinants that react in the PRNT and HAI tests, both of which involve reactions with the major glycoprotein of the virus. Thus, it is unlikely that a single monoclonal antibody preparation would be available for all serological tests that depend on the virion as the test antigen. It is clear, however, that the IFA test can be used with these monoclonal antibody preparations at the present time in field laboratories to identify each serotype of dengue virus, and that it can continue to be used if the antigenic determinants on the intracellular viral specific polypeptides remain stable.

The Epidemiologic Significance of An Increased Prevalence of Jamestown Canyon Virus Isolated from Mosquitoes in New York State, 1979-1980.

Margaret A. Grayson, Sunthorn Srihongse, Rudolph Deibel, Edward M. Bosler and Thomas F. Bast

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A total of 5,594 pools of approximately 255,000 adult female mosquitoes collected with CDC light traps from May to October, 1979 and 1980, were inoculated in suckling mice and/or Vero cell cultures for virus isolation attempts. Forty-three California group (CAL) viruses were isolated, 13 in 1979 and 30 in 1980. Thirty-seven of these isolates were compared by complement-fixation technique with 6 CAL reference strains; California encephalitis (CE), La Crosse (LAC), Snowshoe Hare (SSH), Jamestown Canyon (JC), Keystone (KEY) and Trivittatus (TVT). Results of these tests identified 34 JC and 3 SSH viruses. Jamestown Canyon virus infections were widespread, occurring in 11 species of Aedes collected in 9 counties throughout the state, and accounted for 90% (27/30) of the CAL viruses isolated in 1980, a year in which no human cases of California encephalitis were detected.

Fifty-eight percent (16/27) of the JC strains obtained in 1980 were isolated from Aedes communis group mosquitoes which constituted 20% of all Aedes collected that year. Field infection rates were highest in Aedes triseriatus (1:1,091), which yielded 11% (3/27) of the JC viruses. Over half (17/27) of these isolates came from 2 populated counties in northeastern New York and one-fourth (7/27) were from 3 counties in the western region. Jamestown Canyon virus infections were detected each month from May to August peaking in July, the month in which 3 SSH viruses were also obtained.

The 9:1 ratio of JC:SSH viruses obtained from mosquitoes examined during 1980 is in sharp contrast with that encountered in 1978, when these viruses were isolated in equivalent numbers and 23 cases of California encephalitis, including 1 fatality, were diagnosed from New York. The epidemiologic and ecologic significance of these findings will be discussed.

Towards an In-Depth Comparison at the Molecular Level of Prototype La Crosse Virus and New York State Isolates. (Leo J. Grady, Sunthorn Srihongse, Margaret A. Grayson and Rudolf Deibel).

Oligonucleotide fingerprint analyses were used by El Said et al. (Am. J. Trop. Med. Hyg. 28, 364-368, 1979) to show that La Crosse (LAC) virus isolates from various areas of the northern United States were all distinguishable from one another, as well as from prototype LAC virus. Of particular interest to our laboratory was the finding that the oligonucleotide fingerprints of the RNA genomes of LAC isolates from the region around Albany, New York were sufficiently different from prototype LAC that these investigators suggested that they be considered as varieties of LAC virus and not merely variants. As the first step in an investigation of whether or not these genomic differences are biologically meaningful, we have confirmed the observations of El Said et al., and have also analyzed several other LAC isolates from New York State. These data will be presented along with a report on the progress of studies currently being initiated to compare the protein components of prototype LAC with those of the New York isolates.

NUCLEOTIDE SEQUENCE AND CODING ANALYSES OF BUNAYVIRUSES.

Corrie M. Clerx-van Haaster, Hiroomi Akashi, Fred Fuller, John P.M. Clerx, Hiroshi Ushijima, and David H.L. Bishop. Dept. of Microbiology, University of Alabama in Birmingham, Birmingham, AL 35294

Genome RNA sequence analyses have shown that the three RNA segments of snowshoe hare (SSH), La Crosse (LAC), Main Drain, Mermet, Turlock, Pahayokee and Boraceia bunyaviruses share a common 3' terminal sequence of eleven nucleotides: 3' $_{HO}$UCAUCACAUGA. Qalyub, Dugbe, Hughes, Abu Mina, Avalon and Hazara nairoviruses have a common 3' terminal sequence of nine nucleotides: 3'$_{HO}$AGAG$\begin{matrix}A\\U\end{matrix}$UUCU. Punta Toro and Buenaventura phleboviruses have a common 3' terminal nucleotide sequence of nine nucleotides: 3'$_{HO}$UGUGUUUCG. These results show that within a genus member viruses have similar terminal sequences, but that these sequences are different for different Bunyaviridae genera.

Further sequence analyses have been undertaken for SSH, LAC, and an alternate LAC virus isolate (L74) to determine their coding capabilities. The sequences obtained for the S RNA's predict two open, overlapping reading frames for translation of the complementary mRNA's. The M and L RNA sequences so far allow only one open reading frame. Amino acid analyses of tryptic peptides of the viral structural and nonstructural proteins are being undertaken to compare the amino acid sequence of these peptides with the amino acid sequences deduced from the nucleotide sequences.

Vector Competence of Several Species of Mosquitoes from Panama for St. Louis Encephalitis Virus

C. G. Hayes, A. J. Adames and P. Calindo

Gorgas Memorial Laboratory, Republic of Panama

The experimental vector potential of several species of Panamanian mosquitoes for St. Louis encephalitis (SLE) virus has been determined. The mosquitoes used were based upon 1) previous isolations of SLE virus from the species in Panama, 2) feeding patterns associated with suspected vertebrate hosts in Panama, or 3) known involvement as a vector of SLE virus in North America. Species studied to date are Culex pipiens quinquefasciatus, Mansonia dyari, Deinocerites pseudes, Sabethes cyaneus and Haemagogus equinus.

Females of all five species listed above have been fed on baby chickens and fledgling cormorants (Phalacrocorax olivaceus) that were experimentally infected with SLE virus. Cx. p. quinquefasciatus was the most susceptible species followed by H. equinus; whereas, M. dyari, S. cyaneus and D. pseudes appear to be poor vectors based on these experiments.

Oral susceptibility to SLE virus has been determined for H. equinus and 3 strains of Cx. p. quinquefasciatus by feeding females on blood-virus mixtures. The median infective dose (ID_{50}) of SLE virus for H. equinus was $10^{3.5}$ plaque forming units (PFU). The ID_{50} for a colonized strains of Cx. p. quinquefasciatus was $10^{2.8}$ PFU; the other two strains of this species which consisted of field collected material from the towns of Juan Diaz and Panama City had ID_{50}'s of $10^{2.5}$ and $10^{3.1}$ PFU of SLE virus, respectively. Both H. equinus and Cx. p. quinquefasciatus were able to transmit SLE virus to susceptible hosts after becoming infected by feeding on blood-virus mixtures.

The results of the vector competence studies will be discussed in relation to ecological observations on the transmission cycles of SLE virus in Panama.

RADIOIMMUNE PRECIPITATION OF DENGUE VIRUS PROTEINS BY DENGUE HEMORRHAGIC FEVER PATIENT SERA

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Identification of all viral proteins recognized immunologically by dengue hemorrhagic fever (DHF) patients is unknown. Proper assessment of the immune response of these patients is important not only to development of appropriate vaccines but also to understanding the immunopathological steps leading to dengue hemorrhagic fever. Our primary objective in this study was the identification of those viral proteins which can be immunoprecipitated by acute and convalescent sera from DHF patients.

Uninfected or dengue-infected C6/36 (Aedes albopictus) or LLC-MK_2 (monkey kidney) cells were radiolabeled continuously from 48 to 120 hours postinfection using ^{35}S-methionine and ^{14}C-amino acids. Radioimmune precipitation (RIP) antigen was prepared by detergent solubilization of radiolabeled whole cell proteins in Tris buffer, pH 8.0, containing 1% Triton X-100, 1% sodium deoxycholate and 0.1% sodium dodecyl sulfate (SDS). Ten microliter aliquots of acute and convalescent sera from Thai dengue fever patients were reacted overnight with 0.1 ml of RIP antigen ($1x10^6$ cpm) at 4 C. Anti-dengue hyperimmune mouse ascitic fluid (HMAF), normal mouse ascitic fluid (NAF); normal human serum (NHS), and ascitic fluid prepared against host cell (C6/36 or LLC-MK_2) proteins (HCAF) were run as controls. Immune complexes formed were absorbed to formalin-treated Staphlococcus aureus cells and solubilized using an SDS-mercaptoethanol buffer. Samples were characterized using Laemmli 10-20% gradient polyacrylamide slab gels. Protein bands were visualized after exposure of PPO-treated (fluorography), dried gels to x-ray film. Virus-specific proteins were identified by comparison to molecular weight standards, proteins from partially purified virions, and host proteins precipitated by HCAF.

Dengue-specific HMAF precipitated approximately ten virus-specific infected cell proteins ranging in molecular weight from 70,000 daltons to 11,000 daltons. The most predominent bands had approximate molecular weights of 58,000, 43,000, 36,000, 32,000, 17,000, 12,000, 11,000 daltons (ICP 58, ICP 43, ICP 36, ICP 32, ICP-17, ICP 12, and ICP 11, respectively). Convalescent sera from DHF patients reacted principally with the same polypeptides; however, acute sera failed to precipitate or had much diminished reactions to ICP 43, ICP 36, ICP 32, ICP 12 and ICP 11. Since RIP relies primarily on the presence of uncomplexed antibody, these results suggest that certain virus-specific antibodies may be bound to circulating or fixed dengue antigens or are not present in the sera in large quantities.

Title: Detection of LaCrosse virus antigen within mosquito pools by enzymatic immunoassays (EIA).

By: Stephen W. Hildreth and Barry J. Beaty

Enzyme-linked immunoassays, using either a chromatic (EIAC) or a fluorogenic (EIAF) reaction product, were developed to replace common virological isolation and identification assays used in the surveillance of arboviruses. These techniques were demonstrated capable of detection and identification of LAC within 5 hours after specimen procurement.

An indirect sandwich ELISA method was used. Micro-titer plates were coated with anti-virus mouse IgG (or Ig), followed by the sequential addition of mosquito pool sample, anti-virus rabbit IgG (or Ig), goat anti-rabbit IgG conjugated with alkaline phosphatase, and substrate (either, chromatic= p-nitrophenyl phosphate or fluorogenic = 4-methyl umbelliferyl phosphate). The EIAC was evaluated spectrophotometrically on a Titertek Multiskan plate reader (Flow Lab., Inc.) and visually scored on an ordinal scale (maximum +4). The EIAF was also visually scored on a similar ordinal scale during exposure to a C-62 Blak-ray translumminator (Ultra-Violet Products, Inc.).

The minimum number of LAC infected Ae. triseriatus mosquitoes detectable by the EIA systems was determined using pools of known composition. One hundred percent concordance was found between virus isolation with BHK-21 cells and the EIA with mosquito pools containing 1 or 3 infected mosqitoes in addition to various numbers of non-infected mosquitoes (7 to 99). Non-specific reactions (background) associated with non-infected mosquito pools was found to be negligible when evaluated spectrophotometrically and non-existing when evaluated qualitatively. Using virus titrations of mosquito pools, the detection limit of the EIA was estimated to be 2-3 log10 TCID50 units per ml for a 5 hour procedure and 1-2 log10 TCID50 units per ml with an overnight procedure.

Mosquito pools containing individuals infected with LAC, Snowshoe Hare, Jamestown Canyon, Keystone, Trivittatus, Western Equine Encephalitis, Dengue, and Flanders viruses were used to determine the specificity of the LAC-antigen EIA systems. Only certain members of the California virus (LAC, Snowshoe Hare, and Jamestown Canyon) complex cross-reacted in the EIAC. The specificity between the three cross-reacting viruses was improved considerably with the EIAF, allowing for a specific diagnosis of the LAC virus infected mosquito pools.

Qualitative scoring of the final enzymatic product, whether chromatic or fluorogenic, was shown to correlate with the quantitative results of the spectrophotometric plate reader. Absorbance values were significantly associated with the qualitative results of EIAC ($r=0.935$, $p<.001$) and EIAF ($r=0.921$, $p<.001$). Qualitative scoring of EIAC and EIAF were highly correlated ($r=0.933$, $p<.001$), with an agreement of 82.5%, adjusting for chance agreement (kappa=0.825, $p<.0001$). In general, the discordant scoring between the chromatic and fluorogenic systems indicated EIAF was not only superior in sensitivity but also in specificity.

The EIAC was tested to determine if LAC viral antigens could be detected in mosquito pools undergoing multiple freeze-thaw cycles. Pools containing LAC infected mosquitoes were frozen and thawed between 2 to 7 times. All LAC containing pools were diagnosed as positive by the EIAC despite the number of times the sample passed through $0^{o}C$.

For the purpose of surveillance of a known virus within a mosquito population, the ELISA method for detection of viral antigens appeared to be as sensitive and specific as a standard surveillance method. As such, mosquito pools could be tested under limited field conditions, supplied with standard electrical current, using the visually read EIAC or EIAF systems with not only rapid and specific results but also an increase in testable specimen volume. Studies are currently underway to evaluate antigen detecting EIA systems for Eastern Equine Encephalitis, Highland-J, Dengue, and Flanders viruses.

Vector-Competence Studies with Bluetongue and Epizootic Hemorrhagic Disease Viruses and Culicoides variipennis in 1978-1980

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Analysis (6169 specimens) of 1978-80 data for 4 states of the United States gave two facts about Culicoides variipennis, the primary vector of bluetongue virus (BTV) in the U.S.A., that were important to the U.S. export market for bovine genetic stock. Populations collected in the northeast were resistant to oral infection with 4 strains of BTV. This was indicated by the low infection rate (IR) of 2.7% (43/1586) for 8 New York populations of which 4 were tested 2-3 times in 1980. A population from Virginia was also resistant with an IR of 0.8% (5/617). Populations from BT enzootic areas were susceptible as shown by the data for 8 Colorado populations with an 8.5% IR (89/1041) and. for 18 California populations with a 19.1% IR (440/2306). The resistance of populations appeared to be due to environmental suppression of the IR as laboratory reared F_1 populations were significantly ($P<0.01$) more susceptible than their parent field populations for Colorado and California (IRs: P 18.0% [121/673]; F_1 39.7% [223/562]). Although the IRs for the P and F_1 New York populations were not significantly different, a colony derived from a New York population was susceptible with a mean IR of 45% for the F_{6-8} (48/106), and a cross mating of the New York colony with a standard colony that has a maximum IR of ca 30% produced a colony with a mean IR of 77% for the $F_{11-13,\ 17}$ (183/236).

The 1978-80 data of 9193 specimens from 8 states were for both BTV and epizootic hemorrhagic disease virus (EHDV) and indicated additional facts, some of which will require additional data in 1981 for confirmation of present concepts. The IRs for the 2 strains of EHDV were similar to each other and to the mean IRs for BTV for each state except for Colorado where 4 populations had a greater mean IR for EHDV-NJ than for EHDV-KY. The data for California, where IRs ranged from 0% to 59% for 2306 specimens, suggested other facts for BTV: 1) the pre-selection of a strain to represent a serotype may not be relevant as the difference between IRs for 2 strains of a serotype was almost as great as among serotypes, 2) northern populations appeared more resistant to oral infection in support of the concept that BT is not enzootic above about the 43rd parallel, and 3) there may be differences among IRs over time (seasonally and among years), in support of the concept that IRs would be higher in areas where BT is in outbreak due to favorable climatic conditions.

Some Aspects of Guaroa Virus in the Isthmus of Panama: Virus Isolation and Neutralizing Antibody Found in Residents of Selected Areas of Panama

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Guaroa virus was originally isolated from blood of apparently healthy settlers of Guaroa Village, Colombia, in 1956. Later the virus was recovered from pools of Anopheles neivai collected in the forest near Buenaventura, Colombia, and from blood of sick forest workers near Belem, Brazil. Guaroa virus was isolated in 1961 in Panama from a pool of Anopheles neivai collected in Almirante, Bocas del Toro Province.

Recently, Guaroa virus was isolated from the blood of a spider monkey collected during a yellow fever surveillance program in Darien, Panama in 1979. Antibodies were detected also in howler (10/10) and spider (9/35) monkeys collected in this area. During these studies a laboratory technician suffered a febrile illness and Guaroa virus was recovered from his blood on days 2 and 4 after onset of illness.

As part of environmental impact studies for hydroelectric projects in the areas of Bayano in Panama Province (east of the Canal); in the Teribe, Changuinola and Oeste Rivers in Bocas del Toro (on the Atlantic, west of the Canal) and in the Tabasará River in Chiriquí Province (on the Pacific, west of the Canal), a serological survey was undertaken to determine the prevalence of antibody to selected arboviruses. Antibodies to Guaroa virus were found in a high percentage of residents (232/405) in the Bayano area, while a lower percentage of positives (31/410) were found in the Bocas del Toro. Results from the Tabasara area are pending. These results will be presented and discussed.

EPIDEMIOLOGIC INVESTIGATIONS OF DENGUE INFECTION IN MEXICO, 1980

Jonathan E. Kaplan, D. Eliason, M. Moore, G. Sather, L. Cabrera-Coello, and J. Fernandez de Castro

Dengue 1 infection was documented in the Caribbean in 1977, and outbreaks of dengue-like illness were reported in Central America in 1978 and in southern Mexico in 1979. In order to document the existence of dengue 1 in Mexico and to study epidemiologic characteristics of dengue, a binational investigation was conducted in 2 Mexican cities in 1980--Merida and Tampico. Two study areas were selected in each of the cities; in each area, public health nurses obtained blood specimens and clinical information from residents in February and in September. Dengue 1 activity was documented in both cities by virus isolation and serologic testing.

One hundred seven individuals (26% of the study population) showed serologic evidence of recent dengue 1 infection. Infection rates in the 4 study areas (10%-55%) increased with age in 3 of the 4 areas and were higher in females in all 4 areas. Dengue-like illness (fever and eye pain OR fever and headache, with myalgias, arthralgias, or back pain) was reported by 67% of infected individuals (range 36%-78%); this figure increased significantly with the age of the patient ($p<.05$). Dengue-like illness was also reported by 34% of individuals, regardless of age, who were not infected; these illnesses were similar to those reported by infected individuals in date of onset and duration of symptoms, but rash was reported significantly less frequently ($p<.001$). A positive correlation between dengue-like illness and dengue infection was found in only 1 area in the study, where the infection rate was 55%.

Dengue-like illness is a nonspecific indicator of dengue infection in a community, and a correlation with infection can be expected only when infection rates are high. The higher infection rates observed among females and older individuals may be related to greater exposure to infectious mosquitoes at home; Aedes aegypti bite most actively during daylight hours, and both females ($p<.05$) and individuals 30 years of age or older ($p<.001$) were more likely than males or persons <30 to be in the home when the study was conducted.

A Survey of Potential Bluetongue Vectors in Florida

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In the U.S., the vector most commonly associated with Bluetongue (BT) virus is Culicoides variipennis. There are areas within the state of Florida where the vector transmission of BT virus appears to occur in the absence of C. variipennis. Serological surveys conducted by our laboratory have indicated that in these areas over 80% of the cattle have antibodies to BT virus. Six study sites were established for collection of Culicoides midges as well as virological and serological examination of resident cattle herds. For the past 18 months we have been conducting an extensive survey of the Culicoides species associated with cattle in Florida and attempting to isolate BT virus from field populations of these species. In 1980 over 60,000 midges were trapped and identified. These collections indicate that the major cattle-associated species in southern Florida is Culicoides insignis and this midge certainly appears to be a potential vector of BT virus. C. variipennis and C. insignis exhibit a sympatric distribution in central Florida while C. variipennis is the major cattle-associated species in north Florida. Other species found to be associated with cattle but collected in variable numbers at the study sites are C. edeni, C. pusillus, and C. stellifer. Thus far, all attempts to isolate virus from field-collected C. insignis have been unsuccessful but these efforts are continuing in 1981. Additionally, a sentinel system of cattle herds has been established in Florida to provide information on seasonality of BT virus transmission.

PERSISTENT INFECTION OF A NONVECTOR MOSQUITO CELL LINE WITH DENGUE VIRUSES

Kuno, Goro[1]

All studies of persistent infection of arboviruses in mosquito cell cultures have been conducted with the use of cell lines derived from vector mosquitoes and laboratory-adapted viruses. Before a generalization of persistent infection in mosquito cell cultures is attempted, it is important to study the persistent infection of arboviruses in cell lines from nonvector mosquitoes.

In this study, 4 serotypes of dengue viruses with different passage histories were inoculated into cell cultures of a nonvector mosquito cell line, TRA-171, derived from Toxorhynchites amboinensis. Both laboratory-adapted and unadapted wild strains could establish and sustain persistent infections that lasted over a year. Adapted strains produced apparent infection throughout, while unadapted strains, after initially producing apparent infection for several weeks, produced mostly inapparent infection.

Alterations of the viruses in the persistently-infected cultures were demonstrated by (i) reduced homologous and increased heterologous neutralizing titers, (ii) increased ability to induce syncytia in normal cell cultures, (iii) increased temperature sensitivity, and (iv) reduced neurovirulence in suckling mice.

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IN VITRO INHIBITION OF DENGUE VIRUS TYPE-3 REPLICATION BY DIDEMNIN A

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The in vitro effect of a new class of depsipeptide, hidroxyisolvaleryl-propionate(didemnin A) (Rinehart K.L., et al., Science 212, 935-937, 1981) on dengue virus Type-3 (D-3) replication was examined. Didemnin A reduced the plaque forming ability of D-3 virus(strain PR-38) on LLC-MK2 cell monolayers. Drug concentrations of 2.1 ug/ml and 0.82 ug/ml reduced the number of D-3 virus plaques by 90 and 50 percent, respectively. No evidence of cytopathic effect was observed at these drug concentrations. However, didemnin A concentrations of 5 ug/ml or higher resulted in marked cell toxicity (See table below).

Preliminary results indicate that antiviral effect of didemnin A was maximal when the drug was added to the infected cultures immediately after virus adsorption. Exposure of the cells to the drug 24 hours before infection did not have any effect on viral replication.

Effect of didemnin A[a,b] on the Plaque forming ability of D-3 Virus

Concentration of Didemnin A(ug/ml)[c]	No. of D-3(PR-38) Plaques	% of Plaques
0 (Control 1% DMSO)	190 ± 4.2	100
5.0[d]	0	0
2.5	10.5 ± 2.1	5.5 ± .8
1.25	53.0 ± 8.4	27.9 ± 3.1
0.62	141.0 ± 2.8	74.2 ± 1.6
0.31	171.5 ± 12.0	90.2 ± 2.4

a. Didemnin A was added to infected cultures immediately after virus adsorption.
b. Values are the mean ± S.E. of the mean from two replica point determinations.
c. Stock solution of didemnin A (10 mg/ml) was made in 100% dimethylsulfoxide (DMSO). Final concentration of DMSO in the agar overlay media was 1% for experimental conditions.
d. This concentration of didemnin A was found to be toxic to cells.

It is concluded that didemnin A significantly inhibits the in vitro replication of dengue-3 virus when used at concentrations that do not produce cellular toxicity.

* Author presenting the paper.

KAWASAKI DISEASE--SEARCH FOR THE SMOKING GUN. N.J.Marchette, M.E. Melish, R.M. Hicks, E.Sam and S. Kihara. Dept. of Tropical Medicine and Dept. of Pediatrics, University of Hawaii, School of Medicine, Honolulu, Hi. 96816.

Kawasaki Disease is an acute febrile illness of infants and young children--peak age 6 months to 2 years (range 6 weeks to 8 years). It occurs sporadically throughout the world and is endemic in Japan and Hawaii. Cases in Hawaii occur in every month of the year without apparent time clustering except for outbreaks of increased incidence during winter and spring months of some years. Three sharply defined epidemics of 32, 25 and 45 cases respectively occurred in February-June, 1978, October-May, 1979-80 and January-May, 1981. There is a statistically significant overrepresentation of Japanese: Caucasians and Filipinos are underrepresented. The etiology of the disease is not known and attempts to implicate an infectious agent have not been successful to date. No bacterial agent has been isolated consistently from cases and there is no serological evidence for infection with leptospires, legionella or chlamydiae associated with the disease. Serological conversions to adenovirus group antigen were recorded in 4/52 and to respiratory syncytial virus (RSV) in 4/40 patients. Antibody patterns to mumps, measles, rubella, parainfluenza, herpes simplex, cytomegalovirus (CMV), varicella, influenza A, coronavirus, hepatitis A and Mycoplasma pneumoniae did not differ significantly from that in a normal pediatric population of this age group. Rotavirus was detected in the stool of only 1/29 patients tested. Of unknown significance is the detection of low level antibody reacting with a strain of Korean hemorrhagic fever virus in 5/23 patients. These and additional sera are currently being tested against several additional strains of KHF. Attempts have been made to isolate viruses from plasma, buffy coat, stool, urine, nasal-pharyngeal and conjunctival secretions and CSF from 71 patients using standard tissue culture methods. To date, 33 strains of virus have been isolated from 23 (32%) patients: 6 CMV, 4 RSV or RSV-like viruses, 2 adenoviruses, 16 enteroviruses, 1 parainfluenza virus and 4 viruses not yet grouped. Definitive etiological relationship to the disease has not been established for any of these isolates although work is still proceeding on some. The variety of viruses isolated and the lack of any consistent pattern suggests that this syndrome may be indirectly related to an infectious agent (or agents) which triggers an abnormal immunological response in certain individuals. The detection of circulating immune complexes (by the Clq binding assay) in 48/81 (60%) patients tested is supporting evidence.

Experimental Infection of Vertebrates with SLE Virus

R. G. McLean and K. Holcomb

Species of birds and mammals found naturally exposed to SLE virus in Memphis, Tennessee, were experimentally inoculated with strains of SLE virus to determine their potential as natural hosts. Culex sp. mosquitoes were allowed to feed on some of the inoculated vertebrate species and were held for 14 days and tested for SLE infection. Also, the feeding success of vector mosquitoes on these vertebrate species was measured under laboratory conditions.

Significant differences in susceptibility and viremic response among the various vertebrate species were noted. The Cardinal, Robin, and Bobwhite Quail were uniformly susceptible; whereas, the Coturnix Quail, Raccoon, and Cotton Rat were resistant to SLE virus challenge. The Robins had the highest titer of circulating virus in their blood but were viremic for the shortest period of time. The Bobwhites had lower peak titers but had a longer duration of viremia.

Cx. pipiens complex mosquitoes readily became infected after feeding on Cardinals. Culex sp. mosquitoes feeding on Raccoons did not become infected, but SLE virus was isolated from a single Cx. nigripalpus 14 days after it fed on an inoculated opossum. No viremia could be detected in any of the sera from Opossums, and the remaining mosquitoes of two species which fed on the Opossums were negative for virus.

Differences in the feeding success of several species of Culex mosquitoes on the various vertebrate species were observed. The feeding success of Cx. pipiens complex mosquitoes on Bobwhites was significantly less than for other bird species like the Cardinal which was tolerant of mosquito feeding. This intolerance was due to anti-mosquito feeding behavior by the Bobwhite.

Comparisons of the experimental data with data obtained from field investigations provided a better understanding of the contributions of the various vertebrate species to the transmission and maintenance of SLE virus in nature.

A Retrospective Study of the Enzyme-Linked Immunosorbent Assay for Rapid Antigen and Antibody Detection in Diagnostic Specimens Collected During the Egyptian Rift Valley Fever Epizootics

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An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to Rift Valley fever (RVF) virus was developed using as antigen a beta-propiolactone inactivated infected mouse liver. Studies showed that purifying the antigen, including negative antigen preparations, and adding agents to reduce non-specific binding, eliminated false positive ELISA readings. The following groups of sera representing a variety of field collection situations and antibody levels have been evaluated in the ELISA: (1) acute and serially collected convalescent human sera; (2) sera collected during the epizootic from humans and animals residing in both heavily infected and marginally infected areas; (3) human sera from Sudan collected three or more years after an epizootic; and (4) pre and post-vaccination human sera. In greater than 180 sera tested to date, there has been complete agreement between the ELISA, hemagglutination-inhibition, and plaque-reduction neutralization tests. The enzyme-linked fluorescence assay (ELFA) was developed and tested in parallel with the ELISA. The ELFA was 4 to 10-fold more sensitive than the visually read ELISA.

An indirect antigen detection ELISA was developed using antibodies purified by affinity chromatography. Preliminary positive results with laboratory prepared antigens are currently being extended to acute human and animal sera collected during the 1977 and 1978 Egyptian epizootics.

Variation in infectivity of St. Louis encephalitis viral strains for Culex pipiens quinquefasciatus and correlation with virulence markers in vertebrates.

Carl J. Mitchell and Duane J. Gubler

Abstract: Only six isolations of St. Louis encephalitis (SLE) virus have been made from Argentina: two from patients with undifferentiated fever, two from rodents (CorAn-9124 and CorAn-9275), and two from mosquitoes (78V-6507 and 79V-2533). The strains from rodents are notable because of their lack of virulence for weanling mice and the weak viremic response they produce in house sparrows. We compared the infectivity of these four SLE viral strains for a colony strain of Argentina Culex pipiens quinquefasciatus following parenteral inoculation of approximately $10^{1.2}$ Vero cell plaque-forming units (PFU) per mosquito, and following ingestion of a range of virus doses (approximately 10^3 to 10^6 PFU/ml) contained in blood/virus and sugar (2%) mixtures. In addition, virus growth and dissemination in mosquitoes were determined in individuals fed on suspensions containing approximately 10^6 PFU/ml of CorAn-9275 or 79V-2533. Mosquitoes were dissected on days 1, 3, 5, 10 and 13 of extrinsic incubation. Head squashes were examined for the presence of SLE viral antigen using the direct fluorescent antibody test (DFAT), and mesenterons and body remnants were assayed for virus in Vero cell culture.

Each viral strain grew to high titer following parenteral inoculation into Cx. p. quinquefasciatus; however, day-20 infection rates never exceeded 7% in groups of mosquitoes fed on suspensions of CorAn-9124 and CorAn-9275. In contrast, infection rates were 90.9% and 87.5% in mosquitoes fed on suspensions of 78V-6507 and 79V-2533, and LD_{50}'s were $10^{5.2}$ and $10^{5.8}$ PFU/ml respectively. Further, CorAn-9275 did not grow well in Cx p. quinquefasciatus mesenterons (avg. titers 10^2 and $10^{2.1}$ PFU/ml on days 10 and 13) and rarely passed the mesenteronal barrier. On the otherhand, 79V-2533 readily infected mosquitoes by the oral route and grew to high titer (avg. $10^{4.4}$ PFU/ml) in the mesenteron and began disseminating by day 5 (9 of 9 mesenterons and 2 of 9 remnants positive). Widespread dissemination of virus occurred by day 10 (7 of 9 remnants and 3 of 9 head squashes positive) but virus spread and amplification continued at least through day 13 by which time viral antigen was detectable in head squashes of practically all (8 of 9) mosquitoes tested.

The low infectivity of CorAn-9124 and CorAn-9275 and the high infectivity of 78V-6507 and 79V-2533 for Cx. p. quinquefasciatus correlates well with published reports on the virulence characteristics of these viral strains. With the exception of certain attenuated vaccine strains, we believe this is the first demonstration that strains of the same arbovirus may vary in their ability to infect a competent arthropod vector, and that such infectivity assays may be useful in discriminating between virulent and attenuated viral strains from nature.

ONTOGENY OF YELLOW FEVER (YF) 17D VACCINE: RNA OLIGONUCLEOTIDE FINGERPRINT ANALYSIS OF VACCINES PRODUCED WORLDWIDE

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Concern on the part of PAHO/WHO about current YF vaccine production practices and capabilities has spurred an initiative to investigate means of modernization, including use of cell culture substrates for vaccine production. In this context, there is a need for better virological characterization of YF 17D virus. Vaccines produced in different countries have disparate origins and passage histories. Although all vaccines have a common ancestor (original 17D), two vaccines (made in Brazil and Senegal) were derived from the 17DD substrain, whereas those produced elsewhere were derived from the 17D-204 substrain. The total number of subcultures of the world's vaccines range from 233 to 287, and various manipulations have been done to free certain vaccines from avian leukosis virus (ALV). The neurovirulence of YF 17D virus is known to be a relatively unstable marker, and safety testing of seed stocks by ic inoculation of monkeys is a mandatory (if crude and increasingly expensive) procedure. We thus investigated the heterogeneity of YF 17D vaccine preparations at the molecular level and studied the possibility that changes in neurovirulence are reflected in RNA oligonucleotide maps.

YF 17D vaccines and secondary and primary seed stocks obtained from the 12 producers worldwide were grown, purified, and subjected to RNA extraction. Viral RNA was digested with T_1 RNase and the oligonucleotide fragments labeled in vitro at the 5' end by use of polynucleotide kinase - ^{32}P - gamma ATP reaction. Oligonucleotide fingerprints were prepared by two-dimensional gel electrophoresis. One lot (2654LE) of 17D vaccine produced by Connaught Laboratories, Inc. (USA) was used as a reference strain for comparison with other strains. In analysis of other strains, a total of 42 large oligonucleotides were enumerated and compared.

A high level of homogeneity among 11 vaccine preparations studied to date was demonstrated: 40 or more of 42 oligonucleotides were shared with reference strain 2654 LE (95-100% homology). The 2 vaccines (Brazil and Senegal) derived from the 17DD substrain both were found to lack reference oligonucleotide #37. Only one of the 9 vaccines (South Africa) derived from substrain 17D 204 differed from the reference strain (missing oligonucleotide #11). Genome differences thus appeared to correlate with substrain origin. The results indicate a high degree of stability of YF 17D despite the different pedigrees. No genetic differences were found between vaccines from the same producer which were contaminated with or had been freed of ALV.

A vaccine seed lot (Connaught 6766) found to be unsatisfatory on neurovirulence testing in monkeys did not differ from the reference strain.

Work in progress on parent (Asibi) virus and other neurovirulent 17D preparations will be presented.

TEMPERATURE EFFECTS ON DENGUE TRANSMISSION IN PUERTO RICO

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Recent reports have shown that environmental temperatures affect the extrinsic incubation period of dengue virus. We report evidence of apparent temperature effects on cool-season dengue transmission in three climatologically distinct zones in Puerto Rico.

Over 90% of the Puerto Rican population resides in only three of the five recognized ecological zones (Holdridge system) that make up the island. The three zones, Subtropical wet (January-February temperature = 22°C, rainfall = 92 mm/month), Subtropical Moist (24°C, 79 mm/month), and Subtropical Dry (25°C, 25 mm/month), are referred to here as cool/wet, mild/moist, and hot/dry, respectively.

Of a group of 7,664 patients with dengue-like illness with suitable samples and that were serologically or virologically tested between 1975 and 1980, a total of 4,122 had documented dengue infections. Of those, 4,106 reported an onset date. Patients were assigned to the ecological zones described above based on stated place of residence.

Fewer cases occurred in all zones during the cool season. Only 418 (10%) cases occurred in the 4 months from December to March. Of those 418, only 15 (4%) were from the cool/wet zone. During weeks 4 through 8, there was only one case (0.3% of all cases in the zone), while 64 (2.3% of cases in the zone) and 27 (2.6% of cases in the zone) cases were reported from mild/moist and hot/dry zones respectively. Reduced rainfall lowered vector indexes in the hot/dry zone during the cool season, but transmission continued at about the same rate as in the mild/moist zone, where vector densities were more stable.

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Lassa fever in an endemic area of West Africa. Preliminary report of prospective epidemiologic serosurveillance in a sentinel village.

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In conjunction with a comprehensive study of rodent surveillance and control in and around an area highly endemic for Lassa fever, the farming village of Konia was placed under prospective surveillance from October, 1978, until March, 1980. Eighty-two households were randomly selected and information was sought and serum specimens obtained from 748 residents. A field team remained in Konia for the entire 17-month period examining all villagers with febrile illnesses and conducting follow-up serosurveys at 9 and 17 months. The prevalence of Lassa IF antibody in Konia (titer $\geq$ 1:16) was 40.8%, with a trend towards prevalence varying with age up to the 15-19 year age group. Antibody was also found in toddlers and older children. The incidence of documented Lassa fever in Konia was 3.3 cases per 100 susceptible person-years. Of 51 initially seronegative persons with one or more documented febrile illness from whom follow-up serum specimens were obtained 10 or more days after onset, 2 (3.9%) had seroconversions. The high Lassa inapparent to apparent infection ratio of 4.0 may have been biased by incomplete ascertainment of illness. Overall, 1 of 2 persons with documented febrile illness associated with Lassa seroconversion died, 0 of 8 with reputedly asymptomatic Lassa seroconversion died, and 2 of 49 persons with febrile illnesses not associated with Lassa infection died. Lassa may be a common cause of febrile illness and asymptomatic infection in an endemic area of West Africa, occurring with similar incidence in a farming village and a previously reported diamond mining village.

An Attenuated Junin Virus Vaccine Candidate for Argentine Hemorrhagic Fever

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We have attempted to develop a cloned derivative of Junin virus, the etiologic virus of Argentine hemorrhagic fever (AHF), more attenuated than the XJ Clone 3 strain of Junin virus, inoculated experimentally into approximately 600 volunteers without significant adverse reactions in Argentina ten years ago. We obtained a related Junin parent virus strain which was more acceptable than XJ Clone 3 with respect to passage history and was approximately as attenuated as the mouse brain-origin product used in man in the above trial.

Cloning efforts of the parent virus, XJ passage 44, were carried out by plaque isolation, terminal dilution or the pseudo-single burst method of Walen et al. All passages and cloning procedures were done in diploid cell lines of certified vaccine substrate quality. Virulence assays were carried out in IC inoculated, 12 day old suckling mice and in adult and baby guinea pigs.

Only the pseudo-single burst method yielded viral clones significantly more attenuated than either the parent or the XJ Clone 3 virus. One of these clones, designated Candidate #1, was more attenuated than all other clones isolated and measured. When compared to XJ Clone 3 and parent virus for virulence in the suckling mice system, 15,000 plaque forming units (pfu) of Candidate #1 were required for one suckling mouse ICLD-50 whereas only 100 pfu and 26 pfu constituted an LD-50 for XJ Clone 3 and parent virus respectively. This comparison was repeated in several tests. Virulence comparisons of Candidate #1, XJ Clone 3 and parent virus in guinea pigs showed a respective percent mortality over a range of doses of 8%, 66% and 62%. Preliminary monkey neurovirulence and genetic stability studies are also favorable for Candidate #1 virus.

The data demonstrate a significantly lower virulence for Candidate #1 than for XJ Clone 3 or parent virus. Inasmuch as XJ Clone 3 has already been used in 600 persons, we believe the more attenuated Candidate #1 warrants consideration for use as a live-attenuated AHF vaccine.

Pathogenesis of Phlebovirus Infections

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The newly defined Phlebovirus genus of the family Bunyaviradae contains more than 30 serologically distinct members including Rift Valley Fever (RVF), Punta Toro (PT), Sicilian, Gordil, St. Floris, Gabeck Forest, Arumowot, Karimbad, and Rio Grande viruses. Several strains of RVF virus were pathogenic for adult ICR mice (AM) and adult hamsters (AH) after subcutaneous (SC) inoculation of 10^3 to 10^5 pfu. Prototype strains of the other viruses failed to kill AM and only Arumowot was lethal for AH when 10^5 pfu were given SC. The resistance of AM to Phleboviruses was not T-cell dependent since nude athymic mice survived SC challenge with 8 prototype strains. Conventional and athymic mice died after intracranial inoculation of all the viruses except St. Floris, indicating that many of these viruses have some degree of mouse neurovirulence if they reach the central nervous system.

When we tested several strains of PT in AH, three isolates from eastern Panama were lethal in contrast to 3 strains from western Panama which behaved like prototype PT. This increased pathogenicity was not seen when AM were inoculated with these 6 PT strains or when Wistar-Furth rats were given one of the eastern strains (Adames). The LD_{50} of the Adames strain for AH was 100 pfu but 5×10^6 pfu prototype virus failed to kill AH. AH dying 3 to 5 days after Adames strain infection had high viremia (10^8-10^9) and extensive necrosis of the liver and spleen. These findings are similar to those reported for RVF: Egyptian RVF strains, in contrast to Ugandan or South African isolates, are highly pathogenic for Wistar-Furth rats but not other inbred rat strains. They produce fulminant hepatitis with rampant viral replication. This exquisite "lock and key" relationship in which a specific viral isolate is highly pathogenic for a single species or genotype may be a general property of Phlebotomus fever and possibly even other viruses.

Rift Valley Fever Virus - RNA and Protein Characterization

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Rift Valley Fever Virus (RVFV) is a major human and animal pathogen belonging to the Phlebovirus genus within the family Bunyaviridae. Molecular characterization of this agent has been difficult due in part to problems associated with virus purification and the restrictions of containment. However, such characterization is essential for precise identification of different isolates and definition of the major antigens of the virus.

RVFV (Zagazig Hospital 501) was propagated in Vero cells, concentrated by pelleting, and purified by rate zonal centrifugation on Renografin® gradients. Purified virions approaching 2 x 10^{10} PFU/ml were disrupted with Triton X-100, releasing ribonucleoprotein (RNP) which were analyzed on sucrose gradients. Three RNP complexes were clearly distinguished sedimenting at 140 S, 126 S, and 88 S. Subsequent RNA analysis of these complexes revealed that each contained only one species of virion RNA - the large (L), medium (M), or small (S) RNA, respectively. The fact that approximately 90% of the RNA within the RNP complexes were sensitive to RNase digestion suggests that the protein is not tightly associated with the RNA. The apparent molecular weights of the L, M, and S RNA species were determined to be 2.9 X 10^6, 2.0 X 10^6, and 0.74 X 10^6 daltons, respectively, by electrophoresis in non-denaturing polyacrylamide gels. These molecular weights are essentially identical to those of Punta Toro, a serologically related Phlebovirus. The three RNA species were unique as shown by oligonucleotide fingerprint analysis. Few similarities, if any, exist between the fingerprint patterns of RVFV and those previously reported for other Phleboviruses.

Virus structural proteins were isolated and identified by electrophoresis in 12% polyacrylamide gels (G1 = 65,000, G2 = 56,000, and NC = 25,000 daltons MW). The two envelope glycoproteins were readily distinguished by electrofocusing. The G1 glycoprotein focused at pH 4.8 which was clearly separate from the G2 glycoprotein at pH 9.6. The NC protein was detected in the pH 8 region under denaturing conditions necessary to remove RNA.

These data provide a basis for comparison of RVFV with other Phleboviruses and establish parameters for separation and identification of purified virion components.

BIOCHEMICAL AND BIOLOGICAL ANALYSIS OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS AND ITS DISEASE PROCESS USING MONOCLONAL ANTIBODIES

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Monoclonal antibodies directed against the TC-83 vaccine strain of Venezuelan equine encephalomyelitis virus (VEE) were isolated by fusion of spleenic lymphocytes from VEE immunized BALB/c mice, and Sp 2/0-Ag14 non-secreting mouse myeloma cells. These antibodies were used to characterize the biochemical and biological functions of the envelope glycoproteins of TC-83. Four antigenic epitopes were identified on the E2 glycoprotein, and five antigenic epitopes were identified on the E1 glycoprotein by cross-reactivity analysis and competitive binding assay. The vaccine parent, Trinidad Donkey virus, differed from the TC-83 strain in 3 of the 9 total antigenic epitopes. Comparisons of members of the remaining VEE subtypes indicates variation in 8 of the 9 epitopes. Relatedness between the subtypes reflect the relatedness demonstrated by standard serology, with the exception of a closer relationship of subtype 2 (4 of 9 epitopes shared), and a more distant relationship of subtype IE (2 of 9 epitopes shared). The hemagglutinin and neutralization sites we define to be the same on TC-83 virus. This site is shared with IB, IC, and ID subtypes. The neutralization function only is shared with subtype II.

Protection studies with the VEE monoclones indicate that 3 week old mice can be protected from intraperitoneal challenge with 100 LD_{50} of Trinidad Donkey virus by passive immunization of as little as 20 ug antibody per mouse. Other biological activities will be discussed.

An indigenous hereditary virus of *Culex quinquefasciatus* mosquitoes which induces carbon dioxide sensitivity

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Naturally occurring CO_2 sensitivity in mosquitoes was discovered when *Culex quinquefasciatus* mosquitoes from a laboratory colony were anesthetized by exposure to low temperature and CO_2 in order to inject them with a virus. Surprisingly, all the mosquitoes from this colony failed to recover from the anesthesia though *Cx. quinquefasciatus* from another colony anesthetized in the same way and injected with the same virus recovered as expected. This finding that a particular mosquito colony (originally started from specimens from Matsu Island near the coast of mainland China) was lethally sensitive to CO_2 is similar to the observation which led to the discovery in *Drosophila melanogaster* of the hereditary virus called sigma. As is the case with *D. melanogaster* and sigma virus, the CO_2 sensitivity observed in *Cx. quinquefasciatus* is temperature dependent (i.e. CO_2 is lethal only at low temperatures and not at 25°C).

Inheritance of CO_2 sensitivity in *Cx. quinquefasciatus* appears to follow the same unusual rules as inheritance of CO_2 sensitivity in *D. melanogaster*. For example, a *Cx. quinquefasciatus* colony has remained consistently CO_2 sensitive despite the fact that the females of each generation are mated to nonsensitive males from another source. Thus, the CO_2 sensitivity can be maintained exclusively by maternal inheritance. On the other hand, as with *D. melanogaster*, CO_2 sensitive male mosquitoes which inherit their sensitivity from their mothers can transmit the sensitivity to a portion, but not all, of their progeny when mated with a nonsensitive female. Sensitive male progeny from such a cross, however, cannot transmit the sensitivity to the next generation.

Another important finding is that CO_2 sensitivity in *Cx. quinquefasciatus* appears to be common in natural populations. The study of field populations has just begun but sensitive individuals already have been found among the first few families of this mosquito species collected on Oahu, Hawaii.

CO_2 sensitivity can be transmitted to nonsensitive *Cx. quinquefasciatus* and *Toxorhynchites amboinensis* mosquitoes by inoculation of filtered (450 nm Millipore) extracts of sensitive mosquitoes. Inoculated mosquitoes become sensitive to CO_2 only after a latent period of more than 7 days, suggesting replication of an infectious agent.

Since sigma virus is now known to be a rhabdovirus, "Matsu" virus may also belong to that group. If so, it will be of interest to determine its relationship to rhabdoviruses, such as Flanders, which are commonly isolated from *Cx. quinquefasciatus* mosquitoes by inoculation of mice.

ANTIBODIES TO VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN DIABETIC PATIENTS

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Several reports in the literature refer to the relationship between a viral infection and the subsequent development of diabetes. The most implicated agents are Coxsackie B and Mumps virus. Experiments in hamsters and mice, innoculated with Venezuelan Equine Encephalitis virus (VEE), demonstrated complete destruction of pancreatic beta cells or some abnormalities in the glucose tolerance test.

Being diabetes a relevant public health problem and since the northern region of the Zulia State, Venezuela, is frequently affected by VEE epidemies, we tried to study the possible correlation between these two nosologic entities.

The presence of antibodies against VEE was determined by the hemagglutination inhibition test in the sera of 90 diabetic outpatients at a local hospital. The results were compared with those from individuals with normal glycemia that attended the same hospital for causes unrelated to diabetes.

No significative difference was found between the two groups. A 14.4% of the diabetics presented antibodies against VEE, compared to 9% of controls. The majority of the diabetics with antibodies (70%) were individuals over 60 years old, whose diabetes could be catalogued as insulin-independent. In a more selective study, of 13 children with diagnosis of juvenile diabetes, ages ranging fron 3 to 13 years, no antibodies against VEE were detected.

These results plus previous reports fron our laboratory, in which no important alterations were found in the glucose tolerance test from individuals who suffered encephalitis during a VEE epidemy, seem to indicate low susceptibility of the studied population to pancreatic involvement caused by this virus.

Longitudinal Epidemiologic Study on Dengue Hemorrhagic Fever in Thailand: First-Year Report

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A WHO-sponsored five-year longitudinal study on dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) was established in Thailand in 1980. Its purpose is to measure transmission rates of specific dengue virus types in a defined population and relate these to hospitalization rates of DHF/DSS. Similar studies are being done elsewhere in South and Southeast Asia in DHF/DSS endemic and "silent" areas.

In January-April 1980, blood was collected from 3171 children, ages <1-14 years, resident in Rayong municipality and 4 adjacent villages (200 km southeast of Bangkok, population - 52,935, area - 73 sq. km). Finger-tip blood was collected. Of children ages <1-4, 60-70% had dengue HI antibody; antibody prevalence rose progressively from 70-100% in children ages 5-14 years. Sera with HI antibodies <1:10 were also without dengue 1-4 plaque reduction neutralizing antibody (PRNT).

During August-October, 50 Rayong children had serologically and clinically confirmed DHF/DSS (48 secondary infections; 2 primary infections). Fifteen of these children, 8 with shock, had been bled in January-April. Two strains of dengue 2 and one dengue 1 were isolated from the 50 DHF/DSS cases.

In December 1980-January 1981, one hundred sixty-nine children who were without HI antibody in January-April 1980, were bled. Monospecific neutralizing antibodies were as follows: dengue 1 - 18.9%; dengue 2 - 9.5%; dengue 3 - 2.4%; dengue 4 - 1.8%; two infections - 13.6%; no infection - 53.8%.

Of eight shock cases bled before DHF/DSS, seven had seroconversions in the neutralization test. One of these had no antibody in pre-illness serum (dengue 2 recovered during illness); three had previous dengue 1, two had dengue 3 and one had dengue 4 infections. In all instances where pre-illness antibody was documented, post-illness (4-6 months) sera exhibited evidence of an original antigenic sin antibody response. The child without pre-illness antibody experienced a classical secondary antibody response with shock syndrome (two infections within 8 months).

Although definitive evidence is lacking, the data are compatible with the possibility that dengue 2 virus was always the secondary infecting virus in shock cases. It should be noted that dengue 2 virus was not the dominant dengue virus transmitted in Rayong in 1980.

EFFECT OF PREVIOUS YELLOW FEVER IMMUNIZATION ON CLINICAL AND SEROLOGICAL RESPONSES TO A DENGUE TYPE 2 (DEN-2) VACCINE

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Initial studies of a small plaque, temperature-sensitive, dengue-2 vaccine (PR-159/S-1) showed acceptable safety and immunogenicity in volunteers, but raised the possibility of increased frequency and severity of vaccine-related symptoms in subjects with previous yellow fever immunization. Further, subjects having short intervals between yellow fever and Den-2 immunization appeared to have more severe symptoms. To further examine these questions, a placebo-controlled, double-blind study was performed in 148 soldiers, who were randomized so that 99 received vaccine (V) and 49 received placebo (P). The vaccine was inoculated at a mean dose of 8.1×10^4 PFU. Following inoculation all subjects were followed for vaccine-related symptoms for 30 days and serum was obtained for serology at 1, 2 and 6 months.

Complaints of illness were made by 54% of Vs and 47% of Ps (p=N.S.) and medical consultation was sought by 31% of Vs and 20% of Ps (p=N.S.). Analysis of vaccine-related morbidity showed the following symptoms to occur more frequently in vaccine recipients: chills ($p<0.005$), abdominal pain ($p<0.001$), headache ($p<0.05$), fever ($p<0.05$), night sweats ($p<0.05$) and nausea ($p<0.05$). A combination of one or more of these symptoms was experienced by 52% of Vs and 26% of Ps ($p<0.01$), led to medical consultation in 29% of Vs and 6% of Ps ($p<0.002$) and resulted in placement on quarters in 15% of Vs and 2% of Ps ($p<0.02$). Symptoms began 9.1±5.3 days after immunization and lasted 4.1±2.4 days. The average duration of assignment to quarters was 1.9 days with a range of 1 to 4 days. Previous yellow fever immunization was documented in 76% of Vs. The frequency and severity of vaccine-related symptoms was no greater in the recipients who were yellow fever immune than those who were not. Also, no relationship was found between the interval from yellow fever to DEN-2 immunization and the severity of symptoms.

By one month, 58% of Vs had seroconverted to DEN-2 by hemagglutination inhibition to a titer of 1:40. As predicted by earlier studies, DEN-2 seroconversions occurred more frequently in yellow fever immunes (73%) than in the yellow fever nonimmunes (32%). Geometric mean titers in yellow fever immunes and nonimmunes were 176.3 and 7.6, respectively. These results clearly showed that previous yellow fever immunization augmented the antibody response to the DEN-2.

As the candidate DEN-2 vaccine was more immunogenic in subjects previously immunized to yellow fever and as the frequency and severity of symptoms was similar to that observed in yellow fever nonimmunes, plans for future use of this vaccine should consider preliminary immunization with yellow fever vaccine.

Factors influencing transovarial transmission of Japanese encephalitis virus in Culex tritaeniorhynchus

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We have demonstrated that Japanese encephalitis (JE) virus can be transovarially transmitted to progeny of 12 species of mosquito, including the important vectors to man, Culex tritaeniorhynchus and Culex annulus. Vertically transmitted virus was found in larvae and adult progeny of these two species, but filial infection rates varied markedly from experiment to experiment. Progeny from the same set of infected mothers were pooled, randomized, and split into two portions. One portion was assayed for virus in the 4th larval stage, while the other portion was assayed as adults. In these experiments, infected larvae could always be demonstrated, but adults were often not infected. For Cx. tritaeniorhynchus minimum infection rates (MIR) ranged from 1:100-1:900 for larvae, but when adults were infected their MIR's ranged from 1:400-1:3000. Similarly, MIR's for Cx. annulus larvae ranged from 1:100-1:319, and in the sole demonstration of adult infection the MIR was about 1:500. This apparent reduction in transtadial transmission to adults is the subject of continuing study, but temperature may have an important effect. In comparison to insects reared entirely at 25°C, mosquitoes exposed to 20°C during the 4th larval and pupal stages had lower adult infection rates.

We have no evidence that route of maternal infection (parenteral vs. oral) influences MIR's for JE virus. The two virus strains employed to date have not yielded different filial infection rates, nor have we detected significant differences in MIR's with three different strains of Cx. tritaeniorhynchus. However, recent preliminary observations with several other strains of Cx. tritaeniorhynchus have encouraged us to further investigate the possibility that efficiency of transovarial transmission is related to genetic differences in different populations of the same mosquito species. The results of these new studies will be presented.

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Cytoplasmic Inheritance of California Encephalitis Virus in *Aedes* Mosquitoes

There is evidence that California encephalitis (CE) virus may be a mosquito virus which is primarily maintained in nature in subpopulations of its vectors, *Aedes dorsalis* and *Aedes melanimon*. Three subpopulations of *Ae. dorsalis* were selected which transmitted CE virus vertically to over 90% of their progeny. Infected progeny in these subpopulations transmitted virus at similar rates through 5 generations and females from the last generation transmitted virus by bite to suckling mice. The high rates of vertical transmission appeared to be due to cytoplasmic inheritance rather than to genetic selection for a more efficient transmitter. Evidence for this was that females transmitted CE virus vertically to over 90% of their progeny even when females from the high transmitting subpopulation were mated for 2 consecutive generations with males from an uninfected colony. Females infected either orally, or by intrathoracic inoculation, transmitted virus transovarially to approximately 20% of their progeny, and developed 10-fold higher viral titers than did females from the high transmitting subpopulation. The pattern of high viral titer and low vertical transmission rates is typical of a nonstabilized viral infection, while the pattern of lower viral titers and high (nearly 100%) vertical transmission rates observed in the high transmission subpopulation is typical of a stabilized viral infection.

The demonstration of stabilized infections with CE virus in *Aedes* mosquitoes requires modification of the model for viral maintenance developed by Fine and LeDuc (1978). The model is modified by using separate maternal vertical transmission rates for parental females with stabilized and nonstabilized infections. These rates were applied to the prevalence rates of stabilized and nonstabilized infections in adult female *Ae. melanimon* during the breeding season to determine the prevalence of viral infections in the next generation.

Transovarially infected females can infect vertebrates by bite. However, the occurrence of subpopulations with highly efficient vertical transmission, the lack of increasing prevalence rates of viral infection in naturally infected *Ae. melanimon* as the summer progresses, and the relatively low viremia titers developed by most vertebrates indicates that there may be little need for an amplification cycle between vertebrates and mosquitoes to assure the maintenance of viral infections in the vector populations.

Intestinal infection and transmission thresholds of Middle American enzootic and epizootic strains of Venezuelan encephalitis virus in an enzootic vector mosquito, Culex (Melanoconion) taeniopus from Guatemala

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Intestinal thresholds of Venezuelan encephalitis virus (minimal intestinal doses of virus) for four Middle American HI subtype I-E enzootic strains have been compared with three Middle American HI subtype I-AB epizootic strains in an enzootic vector mosquito, Cu. (Mel.) taeniopus from Guatemala. This mosquito species is only the third proven enzootic vector of VE virus; the other species are Cu. (Mel.) portesi in Trinidad West Indies and aikenii (ocossa and panocossa) in Panama.

Mosquitoes were allowed to bite hamsters at selected times after subcutaneous inoculation of virus to obtain different oral input quantities of virus. Samples of individual mosquitoes were frozen immediately after engorgement of viremic blood. Remaining engorged mosquitoes were kept at 27°C, about 80% relative humidity with 15 hours of light, including artifical dusk and dawn, for extrinsic incubation periods of 12-28 days. Suspensions of mosquitoes were assayed for virus content by plaque formation in chicken embryonic cell cultures. Mosquitoes for many experiments were wild-caught in Guatemala and others were from F_2, F_4 or F_8 generations of a colony derived from Guatemalan Cu. (Mel.) taeniopus in 1979.

Overall an average of 84% of 228 mosquitoes was infected after ingesting <5-4000 plaque-forming units (pfu) of one Middle American enzootic VE strain, 92% of 31 mosquitoes for a second strain, 60% of 64 for a third strain and 100% of 7 mosquitoes for a fourth enzootic strain. No striking differences in virus susceptibility at different mosquito generation levels were evident.

In contrast, only 2% of 166, 3% of 159 and 6% of 34 mosquitoes became infected after oral inputs of <5-250,000 pfu of three Middle American epizootic VE strains.

Mean quantities of virus per infected mosquito after extrinsic incubation were $10^{4.1-5.4}$ pfu of enzootic VE strains and $10^{2.6-3.5}$ of epizootic strains.

Mosquitoes transmitted all four enzootic strains of VE virus to hamsters, usually in 50-100% frequencies. No transmissions of epizootic virus occurred among the three infected mosquitoes that bit hamsters after oral infection and extrinsic incubation.

In other experiments, two Middle American epizootic VE strains replicated after intrathoracic inoculation in all of 40 and 8 Cu. (Mel.) taeniopus, with mean inputs as low as 10 pfu.

These data support the following concepts and conjectures. 1) There is a fundamental difference between Middle American enzootic HI subtype I-E and epizootic HI subtype I-AB virions with respect to ability to infect enzootic vector Cu. (Mel.) taeniopus mosquitoes orally. 2) An intestinal barrier exists for epizootic but not enzootic VE virus in Cu. (Mel.) taeniopus. 3) This intestinal barrier helps to explain the apparent failure of epizootic VE virus to persist in Middle America after the 1969-1971 epidemic and equine-epizootic.

Reduction of Lassa Virus Transmission in a Village by Intensive Rodent Trapping

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The population (862) of a small village in eastern Sierra Leone was known to have endemic Lassa fever and as a consequence was chosen for a prospective study of the control of Lassa virus transmission by rodent trapping. Surveillance was established on a daily basis and a cohort of people chosen for initial bleeding and follow-up. After 9 months of surveillance, an intensive trap out was accomplished of the rodents in the village. Surveillance was continued for 9 more months after the trap out to test the efficacy of the interim trapping as a method of local control of Lassa virus transmission. Four hundred sixty-three rodents were trapped over a 5-week period, 37% of which were Mastomys natalensis, the reservoir of Lassa virus. The largest number of Mastomys caught in any one house was 14. Many houses had no Mastomys. Fifteen of 56 Mastomys tested had antibody to Lassa virus. Thirteen of 101 had Lassa virus isolated from a blood specimen. Forty-five percent of 85 houses contained 1 or more Mastomys. Only 10 houses contained virus-positive Mastomys. The rate of human seroconversion during the pre trap out surveillance period was 4.1/100 persons/year. The rate during the post surveillance period was 1.9/100 persons/year. The relative risk for seroconverting to Lassa virus was 2.2 in the pre compared to the post trap out period. There was no correlation between seroconversion and the presence or absence of Mastomys in a given house, nor was there correlation between the presence of virus or antibody-positive Mastomys and seroconversion. Intensive village rodent trapping in houses offers some hope in reducing Lassa virus transmission rates in an endemic setting.

REPORT FROM WELLCOME VIRUS LABORATORY, TAMAVUA HOSPITAL, SUVA, FIJI ISLANDS

STUDIES ON THE VECTOR COMPETENCE ON FIJI MOSQUITOES FOR ROSS RIVER VIRUS

In a preliminary experiment in which Aedes aegypti, Ae.polynesiensis and Ae.pseudoscutellaris had been allowed to feed on different suckling mice from the same litter with RRV viraemia, Ae.polynesiensis had been infected and had transmitted the virus by bites to further suckling mice, while the other species despite satisfactory engorgement had failed to become infected.

We have normally collected adult mosquitoes, allowed them to oviposit in the laboratory and used the progeny of this generation for experiments. Sometimes field collected larvae had been reared and used. In the absence of current evidence of Ross River virus circulation this appears satisfactory. We have not used any colonised mosquito lines.

Following a suggestion by Dr.D.Gubler, the experiments summarised in the tables were undertaken. Groups of 10 females of the three Stegomyias and of Culex annulirostris were placed in plastic tubes with the end covered with nylon mesh and a suckling mouse was placed between each pair. Normally they were allowed to feed for 6 hours, but since Cx.annulirostris was reluctant to feed during the day in such experiments the mice were exposed overnight for 15 hours. At the end of the periods the mouse blood was titrated.

The mosquitoes were allowed to feed on suckling mice in groups on days 7 & 14(occasionally day 8) and the survivors to day 21 or occasionally day 16 were encouraged to engorge individually. The mosquitoes were then pooled in their original groups. The contained virus was titrated and the titre expressed as S M L D $_{50}$ per mosquito.

The survival of Ae.polynesiensis and, to a lesser extent Ae.pseudoscutellaris at 29°C was poor under conditions which allowed 90% of Ae.aegypti and over 40% of Cx.annulirostris to survive for 21 days. However the results confirmed the sensitivity of Ae.polynesiensis which was the only species infected by blood from a mouse with viraemia below 7.7 log 10 S M L D 50/ml. The mouse with a blood titre of 7.0 log $_{10}$ SMLD$_{50}$/ml which infected Ae.polynesiensis failed to infect Ae.aegypti. From this experiment, only two of five Ae.polynesiensis surviving to day 21 engorged. Both transmitted infection. The titre of virus per mosquito in the group of 5 on day 21 was 4.3 log $_{10}$ SMLD 50 per mosquito.

Ae.pseudoscutellaris gave lower virus titres per mosquito. Presumably in the case where transmission was obtained on day 14, but no virus was detected on day 21 the infected mosquito was one which died between days 14 & 21.

Although Ae.aegypti and Cx.annulirostris were not infected by the smallest doses of virus, two of three infected groups of Cx.annulirostris transmitted infection on day 8 and four of seven infected groups of Ae.aegypti on day 7. None of five groups of Ae.polynesiensis and only one of six probably infected groups of Ae.pseudoscutellaris transmitted virus on the day 7. However, one of two groups of Ae.polynesiensis tested on day 8 did transmit virus.

The relative importance of the vectors must remain in doubt. Despite the sensitivity of the local strains of Aedes polynesiensis, the poor survival in the laboratory combined with rather late development of infectivity, suggest that, if field survival is also poor, this mosquito species may be a less important vector than its sensitivity indicates.

J. A. R. Miles and J. U. Mataika

TABLE I

ROSS RIVER VIRUS

Infection of mosquitoes fed on mice with varying levels of viraemia (10 mosquitoes in each group)

Aedes aegypti

Viraemia Log 10 SMLD 50/ ml	7.0	7.7	7.7	7.8	7.9	8.0	8.3	8.3	≫8.7	≫8.7	≫8.7
Virus per mosq. day 21	<2.0	<2.0	<2.0*	4.7	4.3*	<2.0	3.9*	3.9	4.6	3.0	4.6
Transmission day 7	0	0+	0+	+	0+	0	0+	+	+	0	+
" any day	0	0	0	+	+	0	+	+	+	0	+

Ae.polynesiensis

Viraemia	7.0	7.7	≫8.7	≫8.7	≫8.7	≫8.7	≫8.7
Virus per mosq.	4.3	4.9	4.0	✱	-	-	-
Transmission day 7	0	++	0	0	0	0	
" any ady	+	+	+	+	-	-	

Ae.pseudoscutellaris

Viraemia	7.7	7.8	8.0	≫8.7	≫8.7	≫8.7	≫8.7	≫8.7	≫8.7
Virus per mosq.	-	<2.0	-	3.3	3.4	-	-	-	
Transmission day 7	0	0	0	+	0	0	0	0	
" any day	-	+	-	+	+	-	-	-	

Culex annulirostris

Viraemia	7.3	7.7	7.9	8.3
Virus per mosq. day 16	<2.0	3.0	2.8	4.3
Transmission day 8	0	+	0	+
Transmission any day	0	+	0	+

* day 16

\+ day 8

✱ no survivors to end of experiment.

TABLE 2

Pairs of mosquito spp. fed on the same RRV viraemic mouse.
Transmission by bite and titre of virus in mosquitoes.

Viraemia Titre	Pair of Mosquito spp.								
	Species	Transmission any day	Transmission day 21	Titre	Species	Transmission any dy	Transmission dy 21	Titre
7.0+	Ae.aegypti	-	0/10	<2.0	Ae.polyne.	+	2/5	4.3*
7.7	"	-	0/6*	<2.0	"	+	2/4*	4.9
7.7	"	-	0/10*	<2.0	Cx.annuli.	+	1/5*	3.0
7.8	"	+	2/7	4.7	Ae.pseudos.	+	0/4	<2.0
7.9	"	+	1/10*	4.3	Cx.annuli.	+	0/9*	2.8
8.3	"	+	4/10*	3.9	Cx.annuli.	+	5/5*	4.3
8.7	"	+	2/8	4.6	Ae.polyn.	+	2/2	4.0
8.7	"	-	0/10	3.0	Ae.pseudos.	+	1/4	3.3
8.7	"	+	3/9	4.6	"	+	1/5	3.4

\+ Log 10 suckling mouse LD 50/ ml blood.

* Log 10 suckling mouse LD 50/ mosquito

* Day 16, remainder day 21.

REPORT FROM THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH, BRISBANE, AUSTRALIA

COMPARISON OF THE RNA GENOMES OF CERTAIN BLUETONGUE VIRUSES

(B.M. Gorman, J. Taylor, P.M. Finnimore, J.A. Bryant)

The study of bluetongue viruses in Australia is limited by the restrictions on importation of reference viruses.

Small amounts of double-stranded RNA from bluetongue types 1, 4, 6, 10, 15 and 17 were prepared at the Animal Virus Research Institute, Pirbright, England and sent back to Australia. Strict precautions were taken to eliminate the possibility of importing exotic viruses. Double stranded RNA from bluetongue serotypes 1, 4, 10, 15 and 17 was compared with RNA isolated from CSIRO19 (bluetongue type 20) CSIRO154 and CSIRO156.

The electrophoretic separation of the ten genome segments is distinct for each bluetongue serotype. None of the Australian viruses produced a pattern of identity with the other viruses examined. From our work with other orbiviruses we know that although identical viruses produce identical eletrophoretic patterns, electrophoretically equivalent segments of two viruses can contain distinct genetic information. Comparative electrophoretic patterns of RNA can not therefore be used to assess the degree of relationship between viruses.

Method of establishing the genetic relationship between bluetongue viruses

In infected cells enzymes associated with bluetongue virus transcribe each gene into a messenger RNA which is an exact copy of one strand of the double-stranded RNA gene. Each of these single-stranded messenger RNA molecules associates with cellular ribosomes and is translated into a protein. The sequence of molecules in the RNA determines the amino acid sequence and hence the structure of the protein. The comparison of the genetic material of bluetongue viruses involves the isolation of messenger RNA from cells infected with one virus and comparing each directly with the corresponding double-stranded gene of another bluetongue virus. Single-stranded RNA isolated from virus CSIRO156 was compared in reassociation tests with RNA isolated from CSIRO19 and CSIRO154. After reassociation of radiolabelled single-stranded RNA from CSIRO156 with unlabelled double-stranded RNA from each of the viruses, the homologous reaction produced 10 radiolabelled molecules identical in migration with the parent unlabelled double-stranded RNA. Radiolabelled single-stranded RNA from CSIRO156 reassociated with 8 genome segments of the other two viruses. The Australian isolates are closely related genetically. The major differences between them can be interpreted as due to the differences in genome segment 2 (the genetic determinant of one of the virus surface proteins) and possibly in genome segment 6 (the determinant of the other surface protein on the viruses). The relationship between CSIRO19 and CSIRO156 appears closer than that between CSIRO154 and CSIRO156 since more mismatching occurs between equivalent segments of CSIRO156 and 154 leading to abnormal electrophoretic migration than occurs between the viruses CSIRO19 and CSIRO156.

The RNA-RNA reassociation test appears suitable for detailed analysis of closely related viruses. Our attempts to analyse duplex molecules formed between RNA of Australian viruses and viruses exotic to Australia have had limited success. Initial attempts using single-stranded RNA isolated from bluetongue type 20 (CSIRO19) and double-stranded RNA from bluetongue types 1, 4, 10 and 17 produced no duplex molecules and suggested that bluetongue virus type 20 was distantly related genetically from those serotypes compared with it. Further analysis comparing all three Australian isolates with bluetongue types 1, 4, 10, 15 and 17 had confirmed the distant genetic relationship between the exotic viruses and those isolated in Australia.

The results suggest sequence divergence between geographically isolated viruses and not the recent introduction of a bluetongue virus into Australia.

The isolation of bluetongue virus recombinants

Bluetongue viruses like influenza (and other viruses with segmented genomes) present a problem in that in cells infected with two or more viruses the possibility exists for exchange of genetic information between parent viruses and the emergence of new virus genotypes.

Two bluetongue viruses with 10 distinct genes are theoretically able to reassort those genes and produce 210 or 1024 progeny viruses with genes derived from each parent. We have shown such recombination does occur between serotypes of the Wallal group of orbiviruses and that new viruses with genes derived from each serotype can be isolated from cultures infected with both viruses. We have recently conducted a similar experiment to mimic in the laboratory the inoculation of an animal with a polyvalent vaccine. Twenty distinct bluetongue viruses have been described and antiserum to one serotype does not neutralize the infectivity of another serotype and an animal immunised against one serotype is not necessarily protected against further infection with another serotype. The vaccines used consist of live attenuated virus strains containing a single virus strain (used in the United States) or polyvalent vaccines (in South Africa). Currently in South Africa animals receive 3 injections with vaccines containing 5 live attenuated viruses. They are exposed to 15 of the 20 known serotypes of bluetongue virus. We have questioned that policy and have suggested that it be reviewed to take into consideration the information gained over the last 10 years on the structure of orbiviruses and the genetic interactions between them.

In a system designed to mimic the inoculation of an animal with a polyvalent vaccine we have isolated recombinant bluetongue virus. A culture of pig kidney cells was infected simultaneously with 3 bluetongue virus serotypes. The virus stocks were plaque cloned three times to ensure genetic homogeneity for each virus and the electrophoretic pattern of separation of RNA of the viruses was sufficiently distinct to allow us to recognise each parent virus. Cells were inoculated with 1 plaque-forming-unit of each virus and the culture incubated at 37° until cytopathic effect was complete. The composition of the culture was then determined by titrating for plaques under agar. Individual plaques were selected from the plates and the isolated RNA from these plaques compared with that of the parent viruses. We have isolated three recombinant viruses which contain genome segments derived from each of the parent bluetongue viruses. The detailed analysis of the genetic composition and the proteins incorporated into recombinant virus particles require further investigation.

The isolation of these recombinants must be considered in the formulation of vaccination strategy against bluetongue viruses. The problems associated with diversity within the bluetongue virus group will not be overcome by use of vaccines which themselves may generate novel bluetongue virus genotypes.

EPIDEMIOLOGY OF ARBOVIRUSES

(B.H. Kay, I.D. Fanning, R.A. Hall, P. Mottram)

We are continuing our studies of potential amplifiers of Murray Valley encephalitis (MVE) virus and to date have infected, using orally fed *Culex annulirostris*, 121 individuals of the following species: piglets (9), lambs (23), dogs (11), domestic rabbits (5) and wild rabbits (16), calves (6), grey kangaroos (5), agile wallabies (9), chickens (8), galahs (10), sulphur crested cockatoos (15) and black ducks (4).

Results to date suggest that a wide range of animals are likely to be effective amplifiers of MVE virus in nature although the calves, sheep and wallabies were either poorly or insusceptible. Rabbits in particular developed viraemias (average 2.8 $\pm$ 2.1 logs on an infant suckling mouse scale for 5.2 $\pm$ 1.9 days) sufficient enough to suggest that they could have played some role, because of their extreme abundance, during the 1951 epidemic. Grey kangaroos have also proved to be highly susceptible with comparable viraemias. From our vector competence data on *Culex annulirostris*, we believe that a viraemia of 3.2 logs per 0.015 ml is sufficient to infect 50% of *Cx annulirostris* feeding.

We have also been developing a rather simplistic model of MVE in the Murray Valley area. From data on the subclinical infection rate in man, the incubation period in both man, mosquito and amplifier, host-feeding patterns, longevity and vector competence, we believe that rural amplification was initiated at least two months prior to onset of cases. In the case of the 1951 and 1974 epidemics, this means amplification during November at the latest and transport by infected waterbirds, if in fact this is true, commencing as early as August - September. During this time, the inland 1800 km corridor between north and southern Australia would not seem amenable to shifting foci of infection, involving both mosquitoes and vertebrates.

Another interesting point that would seem to be worthy of pursuit is that during October - November when rural amplification occurs in the Murray Valley, *Culex annulirostris* populations are usually low. These points may suggest that (1) the virus is present during inter-epidemic periods and (2) that mosquitoes other than *Cx annulirostris*, e.g., Spring *Aedes* with transovarial infections, *Cx pipiens australicus* may be involved.

We are currently processing mosquitoes from Tonga. Dr Kay spent 4 weeks there collecting mosquitoes for Ross River virus vector competence testing. The following species were fed dilutions of virus orally and held for different extrinsic incubation periods before storing in liquid nitrogen: *Aedes tabu*, *Ae vexans*, *Ae vigilax*, *Ae oceanicus*, *Culex quinquefasciatus*, *Cx sitiens* and *Cx annulirostris*. Because of the time limits, further work is necessary to complete all profiles fully.

Australian encephalitis: Although Murray Valley encephalitis virus is active in south-east Australia in large, serious, but infrequent epidemics, the virus is endemic in the Kimberley region of Western Australia. Since 1972 virus activity has been recorded each year as virus isolations from mosquitoes, serological evidence, or human infections. There is a marked seasonality recorded with peak virus activity occurring in February to May at the height of the wet season. This pattern is consistent throughout the Kimberley. In Western Australia the annual dissemination of MVE has been well documented (see earlier reports), but widespread human infection was not seen until 1978. Further cases were diagnosed in 1979 and 1981 (Figure 1). It is clear that many undiagnosed cases had occurred prior to 1978. Studies in the Pilbara region since 1978 have shown that the dissemination of MVE in 1978 was intense and widespread with all centres as far south as Tom Price and Newman being highly positive for MVE antibodies (see Arbovirus Info. Ech. 38 : 108). No activity was detected in either 1979 or 1980. However, in 1981, cases were again seen in the Pilbara and one case at Carnarvon. The conclusion is that MVE is endemic in the Kimberley region and is sporadically epidemic in the Pilbara and further south (Figure 2).

The 1981 Outbreak

MVE caused scattered cases of encephalitis in North West Australia in 1981. The first sign of unusual activity was the sero-conversion of five out of ten sentinel chickens at Karratha on February 10th. The first human case was confirmed on the 13th of March, and a total of eight cases were subsequently diagnosed. The period of exposure was from mid-February to the end of March. No pattern is discerned from the sequence of case exposures (see Figure 1).

It is of great interest to note that all the cases in aboriginals were in very young children (four cases with an average age of six months) whilst all caucasian cases were in adults (four cases, average age 32 years). If all the cases since 1978 are analysed, the pattern remains with the average age for aboriginal cases being 2.5 years and for caucasians 27 years (Figure 3). This reflects the disparity between living conditions of the different groups with the aboriginal population being exposed and becoming immune at a very early age. On the other hand, the higher levels of sanitation and protection (e.g. insect screening in houses) of the caucasian population is seen in the high average age of cases. Three caucasians who had lived the majority of their lives in the Kimberley, contracted Australian encephalitis at the ages of 20, 23 and 23 respectively. Furthermore, only two of the caucasians had moved to endemic or epidemic areas within the past three years whilst all the others had been resident in the North West for longer.

The clinical disease of the North West appears to be less severe than that recorded in epidemic situations in South East Australia. Of the eight cases recorded in 1981, only one had severe sequelae. The clinical manifestation of the disease in endemic areas often presents as an aseptic meningitis rather than a classic encephalitis.

Samplings in the Pilbara during and following the 1981 outbreak have shown intense and widespread activity throughout the region with high sero-positivity extending as far south as Carnarvon. Further south there are fewer positives (see Figure 4) while samples closer to Perth are all negative. We cannot fully determine whether there was a movement of the virus into the Pilbara from the endemic regions of the Kimberley, or whether it was activated in the Pilbara. Nor can we determine the dynamics of dissemination in the Pilbara. However, it is clear that the activity in the Pilbara, whilst not being apparent each year, is a regular occurrence and that the risk of human involvement must always be considered.

Mosquito studies during these investigations again confirmed the predominance of *Culex annulirostris* throughout North West Australia. This species was often associated with man-made water habitats - e.g. sewage disposal ponds and outflows, as is frequently observed in the more arid regions. The case at Carnarvon was shown to be exposed on the edge of a salt marsh breeding area for *Aedes vigilax*. The lack of rainfall at Carnarvon and mosquito monitoring data suggest that *Ae. vigilax* a species shown to be capable of MVE transmission in the laboratory, may have been responsible in this instance.

Aedes aegypti was widespread in most small coastal towns in North West Australia in the decade following the Second World War. However, widespread trapping and monitoring programs through the whole of this area over the last four years have failed to yield any *Ae. aegypti*.

(N.F. STANLEY, P.F.S. LIEHNE, A. WRIGHT, H. SAMBRAILO, J. KILIAN)

A HISTORY OF AUSTRALIAN ENCEPHALITIS - IN NORTH WEST AUSTRALIA

FIGURE 1

1: Human infection

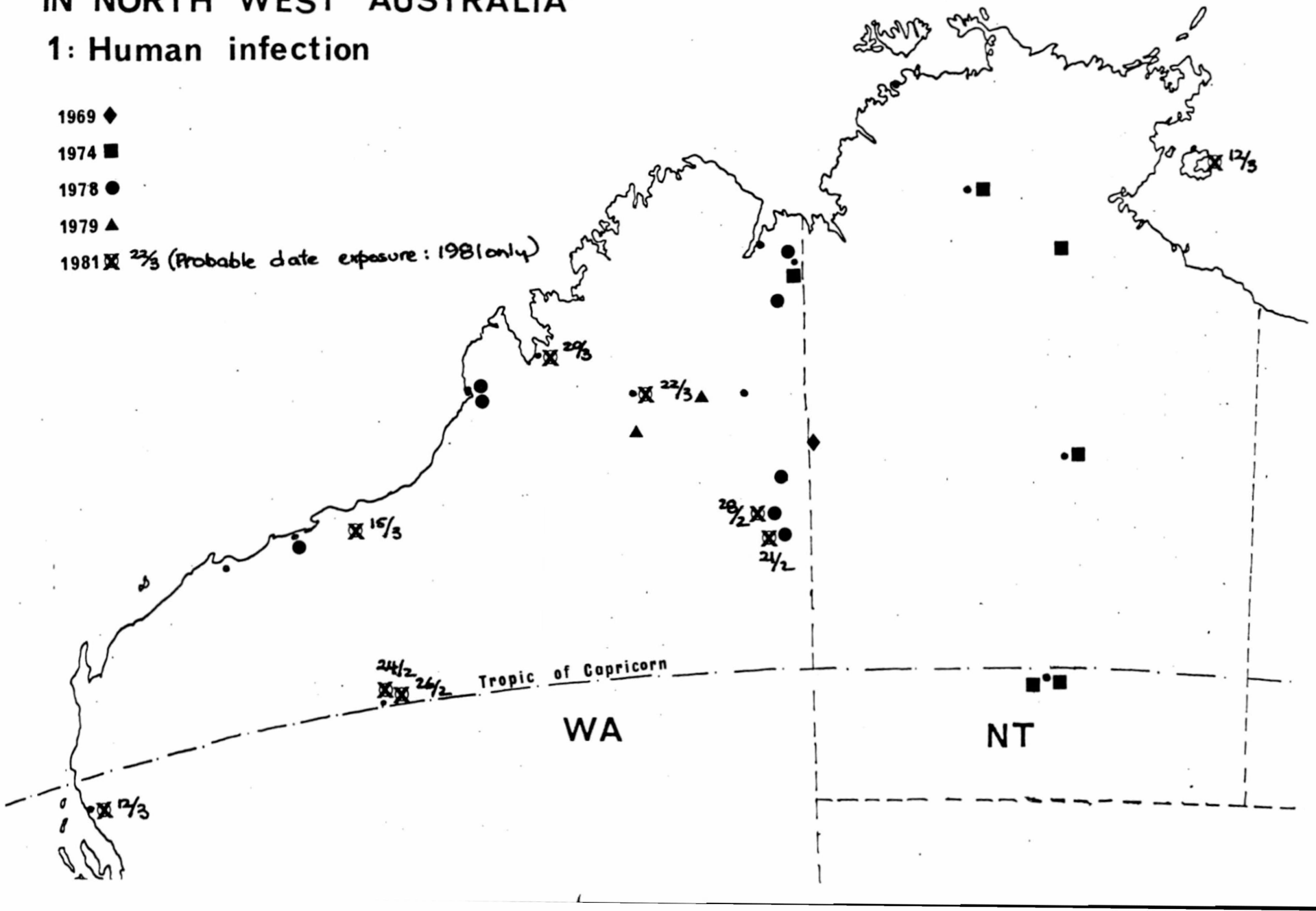


FIGURE 2: MVE in WESTERN AUSTRALIA.

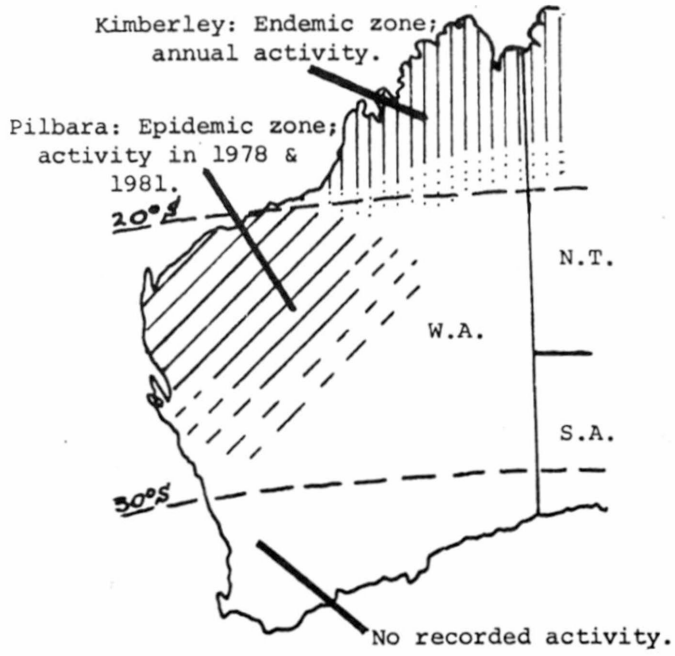
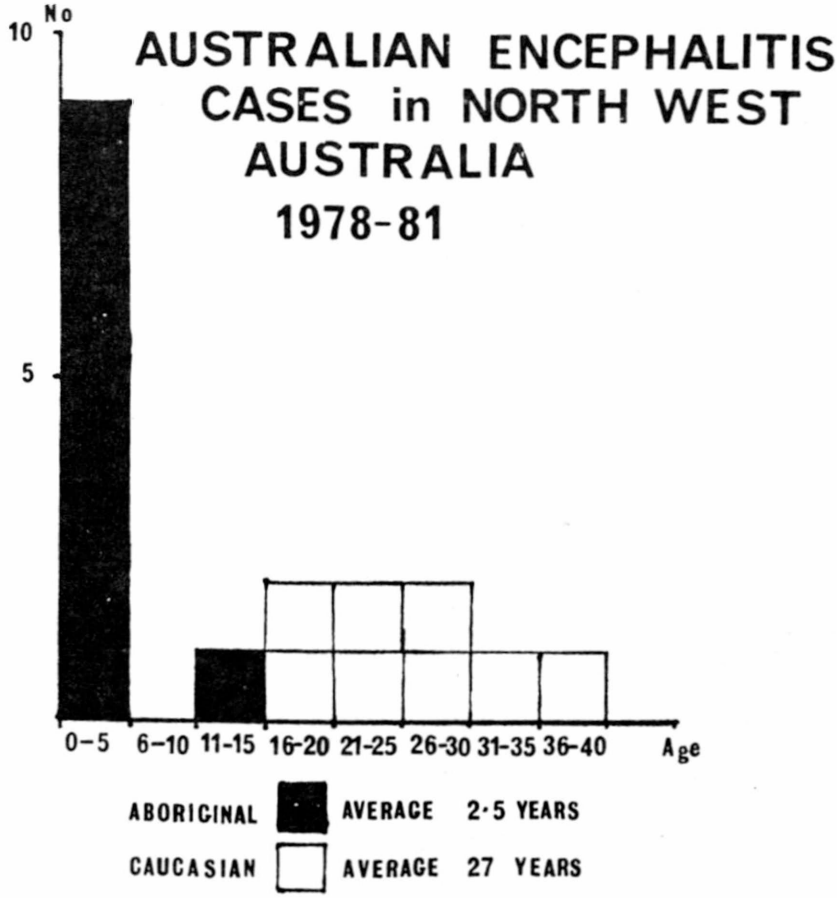
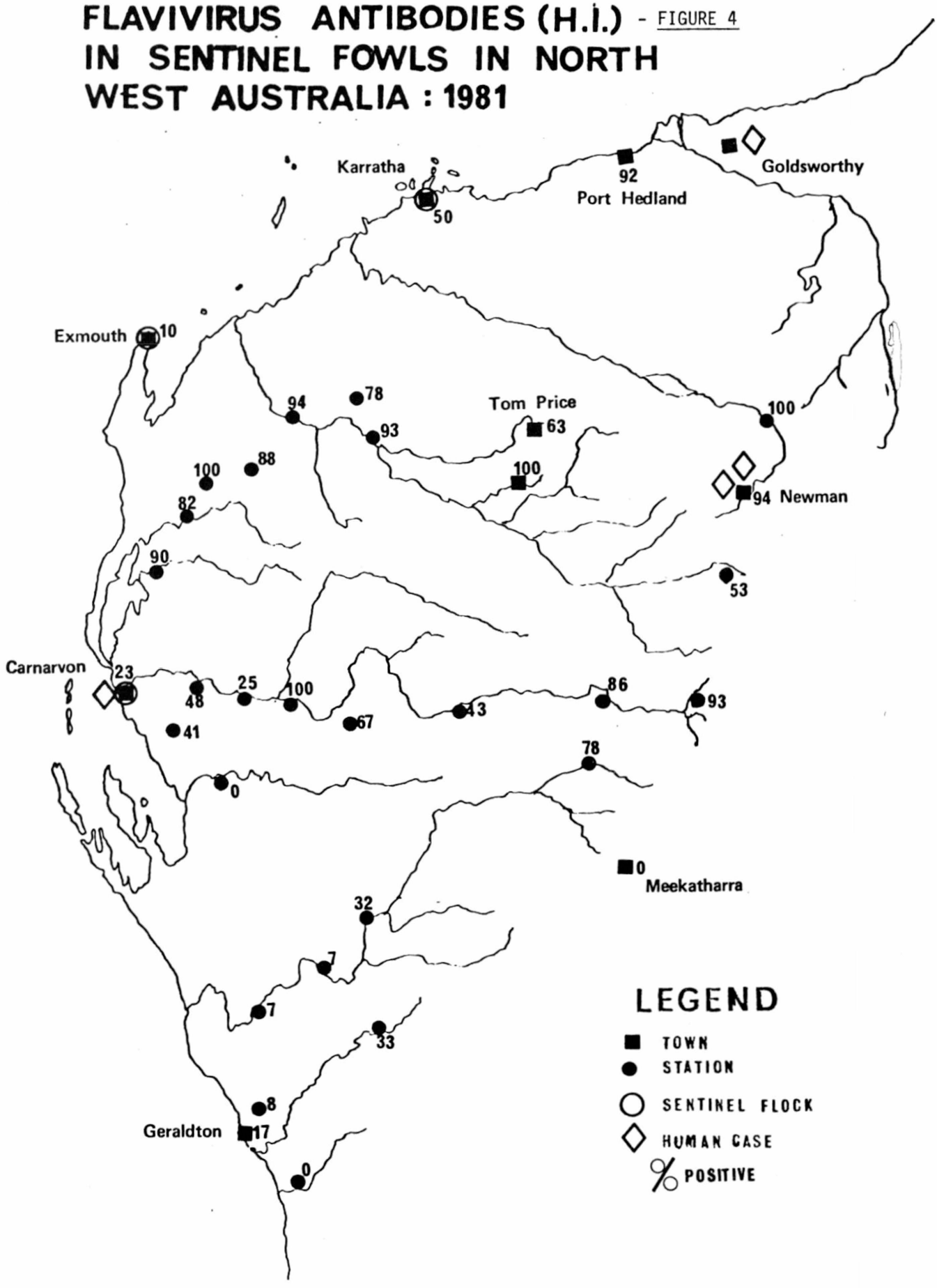


FIGURE 3:


No
10
5
AUSTRALIAN ENCEPHALITIS CASES in NORTH WEST AUSTRALIA 1978-81
0-5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
Age
ABORIGINAL
AVERAGE 2·5 YEARS
CAUCASIAN
AVERAGE 27 YEARS

FLAVIVIRUS ANTIBODIES (H.I.) - FIGURE 4 IN SENTINEL FOWLS IN NORTH WEST AUSTRALIA : 1981



REPORT FROM THE DEPARTMENT OF MEDICAL ZOOLOGY,
KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

Enzyme-linked Immunosorbent Assay for detection of Antibodies to Japanese Encephalitis Virus in Swine Sera

In order to evaluate enzyme-linked immunosorbent assay(ELISA) for the detection of antibodies to Japanese encephalitis(JE) virus in swine sera which conventionally play an important part in surveillance of JE in Japan, we examined several conditions of the technique. Iron beads coated with polycarbonate were used as the solid phase. These were sensitized with two kinds of viral antigens(sonicated JEV-infected cells and sonicated purified virus) and their control antigens(sonicated mock-infected cells and the coating buffer, respectively). Alkaline phosphatase and p-nitrophenyl phosphate were chosen as the enzyme-substrate system. After the reaction the absorbance at 400 nm was measured and the specific activity(ELISA titer) was calculated from the differences between the values obtained with viral-antigen-sensitized beads and those with control beads. Tests were done by using three beads per sample to give satisfactory reproducibility.

Because, as reported by other workers dealing with swine serum, nonspecific reaction(NSR) was remarkably observed, conditions for reducing NSR were examined first of all. Bovine serum albumine(BSA) and Tween 20 which are generally said to be reagents to reduce NSR, were added to the diluent of serum or conjugate to make final concentrations of 4% and 0.05%, respectively. Tween 20 was also applied to washing fluid at the same concentration as above. At the sensitization, pH 7.4 of the coating buffer is superior in reducing NSR to pH 9.6 and BSA treatment after sensitization was effective in the experiment using sonicated cells as antigen. When purified antigen was used, however, NSR was negligible, irrespective of the pH at sensitization or BSA treatment. As to specific reaction, sensitization by purified antigen at pH 9.6 without the following BSA treatment was the best condition, which was adopted in the subsequent experiments.

Parameters in ELISA procedure were determined as follows: (i) Beads were sensitized by 100-fold diluted antigen(corresponding to 2.2×10^6 PFU/ml) at 37 C for 1 hr. (ii) At the first reaction, sensitized beads were incubated with 10-fold diluted test sera at 37 C for 2 hr. (iii) The second reaction with 100-fold diluted conjugate was also at 37 C for 2 hr. (iv) The third reaction with substrate was at 37 C for 1 hr.

Using 87 serum samples of 6- to 8-month-old swine obtained from July to September 1980 in Hyogo Prefecture, the correlation between ELISA and HI titers were examined with good results(correlation coefficient: 0.705). From these results, ELISA was considered to be applicable to seroepidemiology of JEV, particularly from the viewpoint of its rapidity, simplicity, and economic efficiency.
(Investigations conducted by E. Konishi and M. Yamaoka)
(Reported by T. Matsumura)

Dengue Virus Replication in Cultures of Mouse Peritoneal Macrophages

DEN-2 virus (Trinidad 1751 strain) replicated in cultures of methylcellulose-induced peritoneal macrophages of BALB/c mice. The cultivated macrophages from DEN-1 virus-immune mice produced more amounts of DEN-2 virus than those from non-immune controls.

Effects of macrophage activation and inhibition were examined. DEN-2 virus replication in non-immune macrophages was enhanced when the cells had been treated for 3 days with certain macrophage activators, such as bacterial lipopolysaccharide (LPS, from S. enteritidis; 1.0 μg/ml), cell wall preparations of Nocardia corynebacteriodes (10 or 100 μg/ml) and Lactobacillus plantarum (10 or 100 μg/ml) or phytohemagglutinin (PHA; 10 μl/ml). Continual treatment of the macrophages with PHA before and after virus inoculation brought about the most remarkable enhancement of DEN-2 virus replication. In contrast, treatment with concanavalin A (Con A), pokeweed mitogen (PWM) or synthetic muramyl dipeptides (MDP), which have also been reported to activate mouse peritoneal macrophages, had little enhancing effect on the multiplication of the same virus at least under our experimental conditions.

By an infectious center assay method, the number of cells supporting infective virus production was estimated to be about 0.01 % or less of total macrophages when the virus replication enhancement was observed.

By the indirect fluorescent antibody technique, DEN-2 viral antigen was demonstrated in the cytoplasm of PHA-treated macrophage-like cells which had been inoculated with the virus.

Treatment with carrageenan (100 μg/ml), a specific macrophage inhibitor, markedly suppressed DEN-2 virus production in cultures of non-activated macrophages. In PHA-treated cultures, however, the suppressing efficiency of the same agent on virus replication varied from one experiment to another, i.e., marked suppression in some experiments, mild suppression in the other.

Our observations can be interpreted with respect to a possible role of macrophage activation for severe outcome of virus infections, for examples dengue hemorrhagic fever and/or dengue shock syndrome.

(Hak Hotta and Susumu Hotta)

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
FACULTY OF SCIENCE, MAHIDOL UNIVERSITY
RAMA VI ROAD, BANGKOK 4, THAILAND

Studies on Microbial Control Agents for Vector Control of Dengue

Dengue continues to be endemic and epidemic in wide areas of south and southeast Asia and in the southern and western Pacific. In these regions all four serotypes are found, and hemorrhagic manifestations of the disease are relatively common. Because of the absence of any form of specific treatment or immunization for dengue, control of the disease relies entirely on the control of the mosquito vector, on the early identification of cases, and, if possible, the isolation of cases from contact with vector mosquitoes.

Aedes aegypti and *Ae. albopictus* are important vectors of dengue fever in nature in Thailand and are the important species in the epidemic transmission of the disease. Successful and economically feasible biological control of certain important agricultural and forest insect pests with pathogens, used alone or in combination with other control agents, has been thoroughly documented. Several pathogens for use against agricultural pests have been approved by the Food and Drug Administration and the Environmental Protection Agency of the United States of America and are currently being produced commercially and used in the United States. The great progress recently made in the development and experimental use of certain spore-forming bacteria such as the serotype H-14 of *Bacillus thuringiensis* and the strain 1593 of *B. sphaericus* combined with widespread environmental interest and the development of insecticide resistance has provided impetus in recent years for more vigorous investigation of their potential value to medical entomology.

The larvicidal activity of *B. thuringiensis* var. *israelensis* (serotype H-14 or B.t.i.) and *B. sphaericus* strain 1593 on several species of mosquito larvae both reared in the laboratory and field-collected larvae was studied. The larvicidal activity of B.t.i. on several species of mosquito larvae showed the greater sensitivity of *Ae. aegypti* with 100% mortality in 20-40 minutes at high dose whereas *Culex quinquefasciatus* and *Armigeres subalbatus* demonstrated the moderate sensitivity to B.t.i. *An. dirus*, *An. maculatus*, *Mansonia uniformis* and *Mansonia indiana* showed relatively low sensitivity. The 3rd instar larvae of *Toxorhynchites splendens* were resistant to B.t.i.

The larvicidal activity of *B. sphaericus* strain 1593 on several species of mosquito larvae showed the greater sensitivity of *Cx. quinquefasciatus* with 100% mortality in 24 hours at high doses with LC_{50} in

2 days about 3.55 x 10^3 organisms/ml. The 2nd instar larvae of *Ae. aegypti* demonstrated less sensitive to *B. sphaericus* strain 1593 with LC_{50} in 2 days was about 4.2 x 10^5 organisms/ml. The 3rd and 4th instar larvae of *Armigeres subalbatus*, *Mansonia uniformis* and *Mansonia indiana* were resistant to *B. sphaericus* strain 1593.

It is generally accepted that in considering the economic of vector control it is not the cost of the agent (whether chemical or biological) that is critical, but rather the cost of the application. Therefore it is important to examine the longevity of the biological agents keeping in mind potential savings in number of applications. *Bacillus sphaericus* strain 1593 and *B. thuringiensis* serotype H-14 (B.t.i.) were evaluated for the stability of toxicity against two species of mosquito larvae, *Cx. quinquefasciatus* and *Ae. aegypti* in a selected simulating plot in Bangkok. Both strains of bacteria demonstrated an effectively killing activity towards both species of mosquito larvae. The toxicity of *B. sphaericus* strain 1593 was found to be more stable towards *Cx. quinquefasciatus* larvae than *Ae. aegypti* larvae in tap water, whereas the toxicity of B.t.i. was found to be more stable towards *Ae. aegypti* larvae than *Cx. quinquefasciatus* larvae. The longevity of the effectiveness of toxicity of these two bacteria was found to be different. B.t.i. demonstrated that the lethal concentration against *Ae. aegypti* decreased from LC_{90} to below LC_{50} in about 15 weeks when tested in tap water and the stability of toxicity was reduced faster when tested in polluted water. *B. sphaericus* strain 1593 demonstrated that the toxicity towards *Cx. quinquefasciatus* larvae was very stable with the level of lethal concentration was remained in the same level for at least 9 months when tested in tap water. The stability of toxicity was not affected by polluted water.

(Somsak Pantuwatana, Suthep Silapanantakul and Amaret Bhumiratana)

REPORT FROM THE DEPARTMENT OF VIROLOGY, U.S. COMPONENT, ARMED FORCES RESEARCH INSTITUTE OF MEDICAL SCIENCES, BANGKOK, THAILAND

AND

CHILDREN'S HOSPITAL, BANGKOK, THAILAND

(1) Dengue Virus Serotypes from Cases of Dengue Hemorrhagic Fever and Dengue Fever at Bangkok Children's Hospital, 1980.

1980 was the most severe DHF year in Bangkok since 1964; over 4000 cases were reported from the metropolitan area. During the calendar year, 787 children were assigned an initial diagnosis of DHF at Bangkok Children's Hospital; seven children died. All four serotypes were isolated from acute blood specimens from DHF patients in 1980. Of the 169 strains typed to date, there are 23 DEN-1, 139 DEN-2, 6 DEN-3, and 1 DEN-4. Also during the calendar year paired sera from 295 children with undifferentiated fever were examined by HAI serology; 81 (28%) showed serologic evidence of acute dengue infection. From these patients 39 virus strains have been isolated and typed to date: 16 DEN-1, 21 DEN-2, 2 DEN-3, and 0 DEN-4. Further data analysis is in progress.

(D.S. Burke, A. Nisalak, S. Nimmannitya)

(2) Detection of Japanese encephalitis Virus IgM Antibodies in Human Serum and CSF by "Antibody Capture" Immunoassay.

We developed a solid phase RIA using the "antibody capture" principle first described for hepatitis A virus (Duermeyer et al, J Med Virol 4:25-32, 1979). Steps in performance of the test are shown in the attached figure.

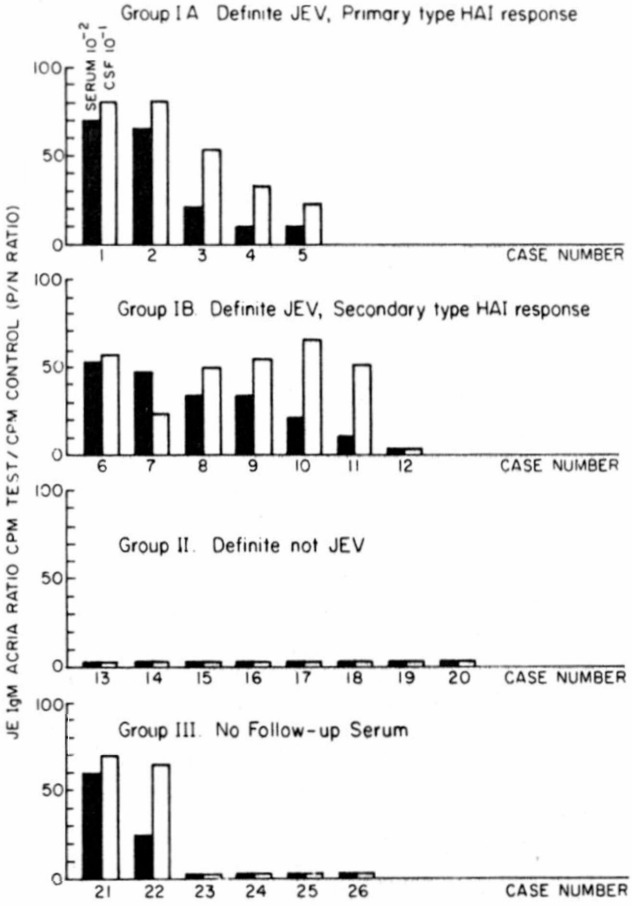
The "antibody capture" immunoassay approach has the following advantages over conventional "antigen first" assays (1) competition between IgM and IgG is not a problem because all the IgG is wasted from the system before antigen is added (2) crude antigens can be used (we use our crude HAI sucrose and acetone extracted mouse brain antigen) because only the antigens of interest are bound to the "captured" antibody (3) the same test can be used for detection of IgG anti-JE simply by replacing the goat anti-mu anti-serum in the sensitization step with goat anti-gamma anti-serum.

We detected JE IgM antibody capture RIA (JE MAC RIA), antibodies in the acute serum specimen of 16 of 20 acute JE patients, and in 19 of 20 convalescent sera. We also detected JE MAC RIA antibodies in several unpaired acute serum samples. None of 30 serum specimens from healthy adults and children with JE HAI antibodies had JE MAC RIA antibodies.

One peculiar yet powerful property of "antibody capture" solid phase immunoassays is that the intensity of the final reaction (CPM in RIA or absorbance in ELISA) is proportional to the fraction of the total of immunoglobulin molecules which have antigen-specific activity, rather than the absolute concentration of immunoglobulin molecules (this is true in the range of test immunoglobulin concentrations $\geq$ 1 microgram per milliliter, the concentration required to saturate all the "capture antibody sites"). Normal serum total IgM concentration is approximately 1000 micrograms per milliliter, so all "capture antibody sites" are occupied by IgM at test serum dilutions from undilute to 10^{-3}, and the CPM obtained are therefore the same at all these dilutions. At dilutions of serum greater than 10^{-3}, CPM bound to the solid phase decrease in proportion to the dilution.

We then hypothesized that the AC approach should be useful in examining cerebrospinal fluid (CSF) specimens from encephalitis patients, in which the absolute IgM concentration is low (usually 1-50 micrograms per milliliter) but in which the proportion of molecules with anti-JE activity should be high. Eleven of twelve patients with JE infections subsequently proven by HAI serology had JE MAC RIA antibodies easily detectable in the acute CSF specimens (GMT=1:2,500) as compared to none of eight with acute encephalitis proven not to be due to JE (see figure). Specific IgM anti-JE activity (units per microgram) was greater in CSF than in simultaneous sera specimens in all eleven positive cases, by a mean factor of more than four fold (range 1.4 to 13 fold). This data has been submitted for publication. The AC method for detecting virus specific antibodies in serum and CSF should prove useful in establishing a rapid etiologic diagnoses for most encephalitides caused by arboviruses.

(D.S. Burke, A. Nisalak, M.A. Ussery).



STEP	REAGENT ADDED	SCHEMATIC
1. Sensitization	Goat anti-human mu chain (Y)	
2. Test sample	Human serum containing IgM (☆)	
3. Antigen	Japanese encephalitis virus antigen (●)	
4. Label	Iodine-125 labelled human IgG anti-flavivirus (☤)	
5. Count		

REPORT FROM THE DEPARTMENT OF MEDICAL MICROBIOLOGY, UNIVERSITY OF MALAYA, KUALA LUMPUR, MALAYSIA

The Arbovirus Research Unit of the University of California International Centre for Medical Research (Hooper Foundation), San Francisco, concluded their programme of research in early 1980 after many years of invaluable service to this department and to the country as a whole. It is appropriate that we acknowledge their dedicated service under the leadership of Dr. A. Rudnick and for furthering our knowledge of arbovirus epidemiology, especially dengue. The transition of the workload has been smooth and there was no interruption to the diagnostic service.

Diagnostic Service

In 1980, 357 patients were investigated serologically for arbovirus infections. Most of the patients were seen or admitted to the University Hospital with a few from neighbouring hospitals and private clinics. The clinical diagnosis ranged from viral fevers to dengue haemorrhagic fever and no recorded case of dengue shock syndrome.

In the months of January and February, the haemagglutination-inhibition (HI) test was used in testing the patients' sera. Since this department took over the unit, it was decided to change over to the use of the complement-fixation (CF) test for the following reasons:

(1) The CF test is more specific

(2) It can be used for the typing of virus isolates

(3) The results can be obtained within 24 hours as there is no necessity of extraction of sera.

Prior to changing over to the use of CF, the two tests were run concurrently and the results were found to be compatible.

Table 1 shows the monthly distribution of positive dengue cases for 1980. The peak months of dengue virus activities were in June and July and the activity was at its lowest in November and December. 82 or 23.0% of the 357 patients were diagnosed as positive or presumptive positive Flavivirus infection. 195 or 54.6% of the patients did not submit second blood specimens and the results obtained were inconclusive. Through the co-operation of the Epidemiology Unit, Ministry of Health, steps are being taken to make house-calls if necessary to collect follow-up specimens in future.

Table 2 shows the age distribution of confirmed dengue cases. There appeared to be an unusually low incidence of dengue in children under 10 years of age as compared with previous years.

There was fairly even distribution of cases in males and females in the various age groups.

Table 3 presents the data based on sex and ethnic distributions. Again, there was no apparent difference in dengue incidence between the two sexes. As in previous years, the percentage of Chinese affected by dengue was higher than in other groups and reflected the fact that there is a larger Chinese population in the crowded urban area.

Virus Isolation

Intracerebral inoculation of 1 - 2 day old suckling mice is still being used for the isolation of arboviruses from clinical specimens. Routinely a blind pass of 2 suckling mouse brains harvested day 8 post-inoculation is done. Mice that survived for 21 days are bled and their sera tested for antibodies against JE and dengue viruses.

We have also introduced two additional host systems for routine virus isolation, viz. Aedes pseudoscutellaris (AP-61) cells and Toxorhynchites splendens mosquitoes. Preliminary results indicated that AP-61 cells are more sensitive than suckling mice for dengue isolation. A few strains were isolated from specimens taken 5-days after onset of illness and from blood samples which have high CF antibody levels ($\geq$1024). Some strains, although not showing cytopathic effect (CPF) in the mosquito cells after 14 days, were detected by direct immunofluorescence using conjugated human convalescent sera. The CPE often showed up subsequently on passage. Virus isolation is still in progress and the results will be analysed to determine the sensitivity and rapidity of dengue isolation in these 3 host systems.

Research

Two main lines of investigations are being pursued. Firstly, attempts are being made to produce monoclonal antibodies to dengue-4 virus by the hybridoma technique. Preliminary experiments succeeded in producing hybrid clones secreting anti-dengue-4 antibodies but these clones proved unstable as antibody-producing activity was lost upon subsequent cloning of the hybrids. These studies are continuing. Secondly, a study of cell-mediated immune (CMI) responses to dengue-4 virus is being carried out in mice. Results to date indicate that infection with dengue virus suppresses the CMI response as indicated by a depressed delayed type hypersensitivity response to sheep red blood cells in infected animals. Studies are in progress to further investigate and characterize this phenomenon.

(S.K. Lam and T. Pang).

Table 1

Monthly Distribution of DENGUE CASES, 1980

Month	No. of Patients	No. of Dengue Positive	Percentage of Dengue Positive
January	30	6	20.0
February	26	8	30.8
March	38	6	15.8
April	26	5	19.2
May	36	10	27.8
June	36	14	38.9
July	23	8	34.8
August	21	5	23.8
September	28	5	17.9
October	33	9	27.3
November	25	2	8.0
December	35	4	11.4
Total	357*	82	23.0

*195 (54.6%) patients did not provide second specimens

Table 2

Age Distribution of Laboratory Confirmed Dengue Cases, 1980

Age Group (in years)	No. of Male Positive	No. of Female Positive	Total Positive	Percentage Positive
< 1	0	1	1	1.2
1 - 4	3	2	5	6.1
5 - 9	1	1	2	2.4
10 - 14	6	6	12	14.6
15 - 19	8	4	12	14.6
20 - 24	12	12	24	29.3
25 - 29	7	4	11	13.4
30 - 34	4	4	8	9.8
35 - 39	1	3	4	4.9
40 - 44	0	2	2	2.4
45 - 49	0	1	1	1.2
Total	42 (51.2)	40 (48.8)	82	

Table 3

Sex and Ethnic Distribution of 83 Laboratory-confirmed Cases of Dengue, 1980

		Number Positive	Percentage Positive
SEX	Males	42	51.2
	Females	40	48.8
ETHNIC	Malay	32	39.0
	Chinese	39	47.6
	Indian	6	7.3
	Others	5	6.1

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

Serological survey of Japanese encephalities (JE) and related flaviviruses among certain domestic animals in West Bengal, India.

Following the first Japanese encephalities (JE) epidemic in West Bengal in 1973 and its recurrences in subsequent years, attempts were made to search for amplifier and/or reservoir of JE virus among various species of animals, birds and small mammals by virus isolation and serological surveys.

In the present study, an attempt has been made to find out the activity of JE virus including a few related flaviviruses in the sera of cattle, buffalo, pig and goat collected from a number of districts of West Bengal, India (Table-I). One hundred and fifty blood samples from each of these species were collected and tested for the presence of haemagglutination- intibiting (HAI) antibodies against JE, dengue-2 (DEN-2) and west Nile (WN) antigens (Table-II).

Highest number of sera of all the species of animals were found to possess HAI antibody to JE than other related flavivirus antigens. Moreover cattle and buffalo sera were found to be significantly higher reactors to JE antigen (62 to 63.3%) than pigs (42%).

Results of neutralisation test (NT) with 30 HAI positive (against JE and WN antigens) cattle, buffalo, pig and 10 goat sera (Table-III) revealed that 17(56.7%) pig, 14(48.7%) buffalo and 13(43.3%) cattle sera had N antibody to JE virus (JEV), while 7(23%) pig and each of 5(16.7%) buffalo and cattle sera had N antibody to WN virus including JEV. One out of 10 HAI positive goat sera was positive to JEV only. Proportion of positivity of pig, buffalo and cattle sera to JEV in N test did not differ significantly ($P>0.01$).

The results of these tests suggest the important role of cattle and buffalo along with pigs in the epidemiology of JE in West Bengal.

Table-I

Distribution of different animal sera according to the place of origin in West Bengal.

Districts	Cattle	Buffalo	Pig	Goat.
24-Parganas	20	22	17	15
Nadia	10	7	9	16
Murshidabad	13	16	18	7
Burdwan	36	37	31	32
Bankura	30	35	28	28
Midnapur	11	8	20	12
Hooghly	12	9	11	21
Howrah	18	16	16	19
	150	150	150	150

Table - II.

HAI antibodies to different flavivirus antigens in the sera of domestic animals in West Bengal (150 sera of each species Tested)

	Den-2	JE	WN	Total Group-B
Cattle	24(16)*	93(62)	59 (39.3)	100(66.7)
Buffalo	51(34)	95(63.3)	75(50)	105(70)
Pig	29(19.3)	63(42)	39(26)	45(43.3)
Goat	5(3.3)	16(10.3)	15(10)	23(15.3)

Table - III.

Results of N test with HAI positive sera of different species of animals to JE and WN Viruses.

	No of sera tested	JE positive	WN positive
Cattle	30	17(56.7)*	7(23.3)
Buffalo	30	14(48.7)	5(16.7)
Pig	30	13(43.3)	5(16.7)
Goat	10		

* Figures in the parenthesis indicate percentages.

M.S. Chakraborty
S.K. Chakravorty
K.K. Mukherjee
A.C. Mitra
A.K. Nandi.

REPORT FROM WHO COLLABORATING CENTRE FOR ARBOVIRUS REFERENCE AND RESEARCH, INSTITUTE OF VIROLOGY, BRATISLAVA, CZECHOSLOVAKIA

ISOLATION AND SEROLOGICAL SURVEYS OF TICK-BORNE ENCEPHALITIS (TBE) VIRUS IN SLOVAKIA

Two foci of TBE virus were studied. The first natural focus occurs in the Low Tatras at an altitude of 900-950 m. The natural focus of TBE virus in the Low Tatras belongs to the montanne type. Neutralizing antibodies against TBE virus were found in 10.7% of small rodent population. Two strains of TBE virus were isolates from 2 subadult bank vole, Cl. glareolus, (Tab. 1). (J. Nosek, O. Kožuch, J. Lysý; Čs. Epidem. Mikrobiol. Immunol. in press).

The second natural focus occurs at an altitude of 175 m in Southern Slovakia.

Six strains of TBE have been isolated from I. ricinus ticks during 1979 at Gbelce: 2 strains from nymphs, 1 strain from females and 3 strains from males. Virophory of ticks amounted to 1.4%. The next year 7 strains were isolated during 1980 at the same locality: 5 strains from nymphs, 1 strain from females and 1 strain from males. Virophory amounted to 0.7%.

One strain of TBE virus has been isolated from female I. ricinus ticks collected at Pavlova. Virophory of ticks amounted to 0.2%.

Isolation experiments carried out from the blood and organs of small mammals were negative. Neutralizing antibodies against TBE virus have been found in 35% of small mammals. In addition to TBE virus, 1 strain of Uukuniemi virus was isolated from I. ricinus ticks collected at Gbelce during 1977.

The natural foci of TBE in the examined region occur in ecotones: forest edge - field, forest edge - vineyard, and forest edge - pasture.
(Tab. 2). (O. Kožuch, J. Nosek, J. Lysý; Biologia, in press)

Tab. 1. N-antibodies against TBE virus in small terrestrial mammals

Species	20.-21.IX.77 n	1.-3.X.80 n	Σ	Number of positive individuals	% of positive individuals
Clethrionomys glareolus	3	25	28	3	10.7
Microtus arvalis	1	-	1		
Microtus agrestris	1	-	1		
Pitymys subterraneus	3	15	18	2	11.1
Apodemus flavicollis	20	13	33	6	18.2
Sorex araneus	7	5	12		
Sorex alpinus	2	4	6		
Sorex minutus	2	-	2		
Mustela nivalis	-	2	2		
9 species	39	64	103	11	10.7

Tab. 2.

Isolation of TBE virus from Ixodes ricinus ticks in the localities Gbelce and Pavlová

Date of collection	Total number of ticks examined	Number of examined ticks			Number of isolated virus strains			% of viruliferous ticks
		N	♀♀	♂♂	N	♀♀	♂♂	
27.4.1977	172	147	9	16	-	-	-	-
24.4.1978	693	636	27	30	-	-	-	-
10.7.1979	422	249	100	73	2	1	3	1,4
10.4.1980	780	660	61	59	4	-	1	0,6
1.7.1980	238	130	53	55	1	-	1	0,8
2.9.1980	20	13	3	4	-	1	-	5,0
10.4.1980+	475	434	17	24	-	1	-	0,2

+ Locality Pavlová

REPORT FROM WHO COLLABORATING CENTRE FOR ARBOVIRUS REFERENCE AND RESEARCH, INSTITUTE OF VIROLOGY, BRATISLAVA, CZECHOSLOVAKIA

Studies on 9-(R,S)-(2,3-dihydroxypropyl) adenine on tick-borne encephalitis virus strains

9-(S)-(2,3-dihydroxypropyl) adenine, a nucleoside analogue with alipathic chain resembling a portion of ribose moiety, was shown to inhibit replication of several RNA and DNA viruses. In the presents we report that this analogue has not shown any protective effect in mice infected with TBE virus strains when applicated at the time of infection and/or after the infection.

S enantiomer of DHPA is the active substance in inhibition of virus replication in these experiments the racemic form (R,S)-DHPA was used since practical factors such as ease and the cost of preparation give it some advantage over E enantiomer. A double dose of that required with S enantiomer was used throughout these experiments.

White mice Velaz (Czechoslovak radom breed) weighing 10-12 g were infected with 10 - 10,000.000 LD_{50} of Hypr and Ir13 strains, respectively. Hypr strain is the prototype strain of TBE virus and Ir13 strain was isolated from Ixodes ricinus ticks in 1978.

(R,S)-DHPA was administered perorally in the form of a 0.2 % water solution given as drinking water.

Table 1 shows that the substance did not affect mortality of mice when administered at the time of infection and/or after the infection. The only inhibitory effect was observed with both viruses, when the substance was administered 24 hours before virus infection in one experiment only.

In our previous experiments the inhibition of haemagglutination (HA) with several arboviruses by other analogue 6-azauridine was detected. Under conditions of optimal pH the inhibitory effect of (R,S)-DHPA on haemagglutinin in four TBE viruses was studied. Viral haemagglutinin prepared by sucrose-acetone extraction was exposed to a maximal nontoxic concentration (3 mg/ml) of (R,D)-DHPA for 18 hrs. at +4°C. As shown in Table 2, evident inhibitory effect of DHPA on the Skalica haemagglutinin was observed.

The analogue did not exert any direct effect on the virion outside the cell. Mouse brain suspension of Hypr virus was exposed to (R,S)-DHPA (3 mg/ml) for 18 hrs. at +4°C; there was no difference in the titre between the virus material incubated in the presence or absence of the analogue.

The presented data showed that the inhibition of haemagglutination may differ in various tick-borne encephalitis virus strains.

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Table 1. The effect of DHPA administered with drinking water to protect mice infected with TBE virus

Experiment No	Virus	Control	DHPA administered on day p.i. -1	0	1	2
I	Hypr	$10^{8.5}$	$10^{7.7}$	$10^{8.5}$	$10^{8.5}$	$10^{8.5}$
II	Hypr	$10^{9.5}$	$10^{9.5}$	$10^{9.5}$	$10^{9.5}$	$10^{9.5}$
I	Ir 13	$10^{8.5}$	$10^{7.5}$	$10^{8.5}$	$10^{8.5}$	$10^{8.5}$
II	Ir 13	$10^{9.5}$	$10^{9.5}$	$10^{9.5}$	$10^{9.5}$	$10^{9.5}$

Virus titres expressed as log LD_{50}

Table 2. The effect of DHPA on some tick-borne encephalitis haemagglutinins

Virus	pH+	HA titres Untreated control	DHPA 2 mg/ml
Hypr	6.4	2560	1280
Ir 13	6.4	1280	640
Skalica	6.6	160	20
Powassan	6.6	160	80

+Optimal pH used in the haemagglutination test

REPORT FROM THE ARBOVIRUS LABORATORY, MEDICAL INSTITUTE OF ENVIRONMENTAL HYGIENE, AUF'M HENNEKAMP 50, D-4000 DUESSELDORF, WEST GERMANY

A sensitive method for titration of Tahyna virus in tissue culture

The titration of Tahyna virus in tissue culture usually is difficult as the differentiation between cytopathogenic altered and degenerated cells often is rather impossible. Therefore we have tried to employ the indirect immunofluorescence technique for staining the virus infected cells.

The Tahyna virus strain was supplied to us by the courtesy of Dr. R. SHOPE, Yale Arbovirus Research Unit, New Haven, USA. It was used in the 18 th i.c. mouse passage, the brain being suspended in Dulbecco's Modification of Eagle's Medium (D-MEM) and diluted tenfold between 10^{-2} and 10^{-7}. Green monkey kidney (Vero) cells were grown in Costar flasks (150 cm^2) using D-MEM. After trypsination the cells were suspended in D-MEM with 20 mM Hepes buffer and adjusted to 2×10^5 cells/ml. 1.9 ml of this suspension containing 10 % of fetal bovine serum were seeded into each well of a plastic plate ("Multiplate" Lux, 8 wells, each well 26 x 33 mm, with coverslips), 3 plates being used for each virus suspension group. The plates were incubated at +37°C in a moisture chamber. On the following day the growth medium was replaced by the maintenance medium containing 5 % of fetal bovine serum. 72, 96 and 120 hours after inoculation the coverslips of one plate per dilution were removed, washed 2 x in D-MEM and 1 x in phosphate buffered saline, dried at room temperature and fixed in acetone at -20°C. For the demonstration of virus foci these coverslips were stained by the indirect fluorescence technique using a 1 : 20 dilution of a mouse ascitic fluid as a specific hyperimmune antiserum against the Tahyna virus strain employed in this experiment and fluoresceinisothiocyanate labelled anti-mouse goat gamma-globulin (Nordic). Coverslips were considered as positive when showing at least one cell with characteristic cytoplasma fluorescence. In parallel 1 and 6 days old mice were inoculated i.c. with 0.02 ml of each virus suspension.

The titres were calculated according to the method of SPEARMAN and KÄRBER (3). As shown in the following table the titre in the tissue culture groups increased from 5.8 lg $I.D._{50}$ 72 h to 6.6 lg $I.D._{50}$ 120 h after inoculation, whereas in the mouse groups they ranged between 6.2 lg $L.D._{50}$ using 1 day old mice and 5.8 lg $L.D._{50}$ using 6 days old mice for i.c. inoculation.

GMK cells				mice		
incubation period (hours)	72	96	120	age (days)	1	6
lg $I.D._{50}$/ml	5.8	6.1	6.6	lg $L.D._{50}$/ml	6.2	5.8

In the past several authors have titrated Tahyna virus in tissue culture in comparison with the mouse brain inoculation technique. In all cases lower titres were obtained in tissue cultures (4,5,6). Although ADAMCOVA-OTOVA & MARHOUL (1) and ADAMCOVA-OTOVA (2) have employed the indirect immunofluorescence technique for the identification of Tahyna virus, this method was not used for the titration of the virus until now. Our method seems to be a suitable alternative for titration of Tahyna virus in comparison with the mouse brain inoculation technique.

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(J. Pilaski, H. Mackenstein and F. Nelles)

REPORT FROM THE SECTION OF ARBOVIRUSES
LABORATORY OF VIROLOGY
NATIONAL INSTITUTE OF HEALTH
LISBON - PORTUGAL

1. SEROLOGICAL SURVEY FOR ANTIBODIES AGAINST ARBOVIRUSES IN THE HUMAN POPULATION FROM THE DELTA OF RIVER EBRO. SPAIN.

In cooperation with Dr. Alvaro Lozano-Olivares, research virologist from the National Center for Sanitary Microbiology, Virology and Immunology, Majadahonda, Madrid, Spain, we started recentely a programme of study of arboviruses in Spain.

The information we have about the presence, activity and responsability on public health of the arboviruses in Spain is at this moment incomplete and fragmented.

The delta of river Ebro is situated about 200 km south of Barcelona. It is a area which brings together very favourable ecological conditions to the introduction and support of the arboviruses transmitted by mosquitoes as for the life of wild rodents, migratory and resident birds as well.

In these conditions 1037 sera of the population living in various small towns of the region, have been studied. The preliminary results already obtained show that the population living inside the delta have hemagglutination-inhibition antibodies against the antigens of some Alfaviruses (4.1% of positive sera) and also against some Flaviviruses antigens (8.0% of positive sera) used in this survey. The study of the IgG and IgM of some sera of young people was made and it showed that some of them have had recent infections with arboviruses. It is very probable that a Flavivirus (maybe the West Nile virus) and a Alfavirus have recently been or are still active in the area. Studies in progress will help to clarify this situation.

This work about arboviruses which has been included in a more extensive study about the delta of river Ebro is part of a research project carried out with the colaboration between the laboratories of arboviruses of the National Center for Sanitary Microbiology, Virology and Immunology of Majadahonda, Madrid, Spain and the National Institute of Health of Lisbon, Portugal. We therefore follow a recommendation approved during the VI FEMS Symposium about the "Arboviruses in the mediterranean countries" held at Supetar, Yugoslavia, 1978, about the colaboration between laboratories of arboviruses of areas which are geographical very near. (A.Lozano-Olivares and A.R.Filipe)

2. GENETIC STUDIES WITH THE LABORATORY WHITE MICE

Since the beginning of this century the laboratory mice namely the inbred strains have been used in the genetic, biological experiments and biomedical studies.

It is well known, how important it is that when experiments are carried out with laboratory animals the results obtained be provided by genetic strains that differ as little as possible from one another. Although the white Swiss mice is one of the animals which has been more widely studied, there is still a big controversy about the greater or lesser variability of the inbred strains and their hibrids. Some authors think that the litter from inbred crossing are less variable, while others consider the hibrids from the F1 among inbred strains the ones that show less variability.

At the moment, work is in progress in our laboratory intended to study the genetic variability of the mice of the Charles River, the IHMT, the Balb C and C57 strains. The Charles River, Balb C and C57 strains were obtained from the Animal Laboratory Unit of the Center of Biology, Gulbenkian Foundation, Oeiras. The IHMT strain come from the Institute of Tropical Medicine, Lisbon, and was obtained in 1973 from wild mice and mice from the Charles River strain.

Studies that are being carried out include the study of the morphological and phisiological characteristics, namely the biometrical manifestations of the jaw and of the other bony structures, variability of the weight of the litter and the study of genetic resistance to the infection by arboviruses.
(Victor Almeida and A.R. Filipe)

Armindo R. Filipe

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF MEDICINE,
AL-AZHAR UNIVERSITY, CAIRO, EGYPT

Investigations were carried out to determine the effect of cyclophosphamide (CP) upon chicks infected with Quaranfil virus (QRV). Table 1 shows the effect of time that CP was injected into chicks. Table 2 shows virus titers in brain, blood, and spleen at various time intervals after injection. Table 3 shows the effect of virus dose on viremia when CP is injected 24 hours after QRV. Table 4 shows the effect of CP on QRV titer in leucocytes four to seven days after infection.

(Kouka S.E. Abdel-Wahab)

Table 1. Temporal relationship between Cyclophosphamide immune-supression of chicks and Quaranfil virus titer

Time of injection of cyclophosphamide[a] and QRF virus (b)	QRF virus infecting dose dex $SMLD_{50}$[b]	QRF virus titer after 5 days of infection[c]			
		Serum	Blood lymphocytes	Spleen lymphocytes	Brain
24 hours before virus	3.5	5.5	5.0	5.0	5.5
24 hours after virus	3.5	5.0	5.0	3.0	4.0
48 hours after virus	3.5	5.5	N.D.	6.0	6.0
72 hours after virus	3.5	5.5	5.0	5.5	4.0
NO drug	3.5	5.5	4.0	4.5	5.0

a) 2 mgm of drug per chick injected IM. Drug induced deaths counted for 10 to 50% deaths of the birds.
b) Quaranfil virus dose was 3.5 dex $SMLD_{50}$ injected SC per chick using thirty chicks as virus control, , thirty for combined QRF virus and CP drug and thirty as drug control only.
c) Titer in dex TCD_{50}/0.1 ml of 10% suspension (v/v or wt/v) of specimens assayed in secondary duck embryo fibroblast cultures. The figures represent the average readings from at least three samples.

Table 2. Relation between Cyclophosphamide immune-suppression and Quaranfil virus titer in chick tissues

Cyclophosphamide injected	Post-infection time in days	QRF virus titer [a] Brain	Blood	Spleen
24 hours after Quaranfil virus	5	4.0	6.0	3.0
	9	4.0	6.5	4.0
	16	4.0	5.0	4.0
	22	4.0	4.5	4.0
	29	6.0	2.5	6.0
Quaranfil virus only	5	5.0	4.0	4.5
	9	terminated by virus-induced encephalitis.		

a) See footnote of Table 1.

Table 3. Quaranfil viraemia in chicks after cyclophosphamide (CP) injection

	Virus dose[a]		CP dose[b]	QRF virus titer of chick serum[c] Infection time- Day							
	Dex	$SMLD_{50}$		4	5	9	14	16	22	29	33
Exp. 1	3.5		2 mgm CP	4	6.0	6.0	8.2	terminated			
	3.5		none	3.6	5.1	5.1	5.6				
	0.5		none	3.0	5.0	2.0	1.5				
Exp.11	6.45		2 mgm CP	N.D.6		6.5.	N.D.	5.0	4.5	2.5	No antibody
	6.45		none	N.D.	5.0	induced	terminated by virus induced encephalitis.				

a) See footnote of table 1.

b) Cp injected IM 24 hours after QRF virus.

c) Titration of infectivity by IC inoculation of suckling mice. Titers expressed in dex $SMLD_{50}$ in experiment I. In experiment II titration was by cytopathogenicity in DEF expressed in dex TCD_{50}.

Table 4. Effect of cyclophosphamide injections on Quaranfil virus titer in chick blood leucocytes.

Cyclophosphamide schedule[a]	Virus dose[b] Dex $SMLD_{50}$	Virus titer of blood leucocytes[c] Infection time - Day			
		4	5	6	7
24 hours before virus	3.5	5.0	5.0	5.5	5.7
24 hours after virus	3.5	5.5	5.0	5.0	4.7
Two injections one day before and one day after QRF virus	3.5	5.5	5.0	5.5	4.0
No drug	0.5	4.0	4.7	4.0	5.0

a), b), c) See footnotes of Table 1

During this first half-year 1981, the arbovirus laboratory achieved the working on mosquitoes caught in Kedougou during the 1980 rainy season, by connecting the suckling mouse inoculation to the parallel test of intrathoracic enrichment into breeding mosquitoes, Toxorhynchites or Aedes aegypti. Then 4 days after, the inoculated mosquitoes are crushed and inoculated I.C. into new borne mice. This technic allowed to get numerous viral strains that otherwise would be unknown.

After that, rodents caught in Bandia and the ticks collected in the rodent burrows in the same area during 1980 and 1981 were inoculated. The isolation of very numerous strains of Bandia virus indicates the virus circulation density in this ecosystem.

Finally, few monkeys were caught in Kedougou and Toubakouta, and investigated in the laboratory from the virological and the immunological points of view to detect as soon as possible a yellow fever reapparance.

1°) - VIROLOGICAL STUDIES

1.1. Human blood samples

Nine sera only were examined, but no strain was got.

1.2. Wild vertebrate samples

1.2.1. - Rodents : 76 rodents were inoculated in 1981 and 2 strains isolated = a Koutango virus strain and a BORNU strain (or Fika or Nafada or Sud An 754-61 not yet registrated) isolated in Sudan for the first time and found again repeatdly in Central African Republic and Senegal from rodents (Arvicanthis, Tatera and Taterillus).

Rodents caught and inoculated during the 1981 first half-year

	MASTOMYS	MYOMYS	TATERA	TATERILLUS	TOTAL
January.....	7	1	1	23	32
February.....	8		1	6	15
March.....	2			3	5
April	4		2	18	24
TOTAL.....	21	1	4	50	76

In 1979, 11 Bandia virus strains (10 from Mastomys and 1 from Taterillus) and one Saboya virus strain (from Mastomys) were isolated.
In 1980, 8 Bandia virus strains (4 from Mastomys and 4 from Taterillus), 2 Koutango virus strains (from Mastomys and Taterillus) and a new strain (from Mastomys), yet in study, were got.

1.2.2. - Monkeys : Caught and inoculated during 1981

CAPTURE (Date and country)	Cercopithecus aethiops	Erythrocebus patas	Papio papio	Colobus badius	TOTAL
February (Toubakouta) (and Kedougou)	7	2			9
March (Toubakouta) (and Kedougou)	15	5	3	1	24
May (Kedougou)	8	5	1		14
TOTAL.....	30	12	4	1	47

No strain was isolated when two zika virus strains were got in 1980 from Erythrocebus patas in September and from Cercopithecus aethiops in November.

1.2.3. A sick dog was examined but no viral strain was isolated and the examinations proved the leishmaniasis aetiology.

1.3. Arthropods :

1.3.1. Mosquitoes caught in 1980 mainly during the rainy season. 35.686 mosquitoes processed in 1276 pools were worked and 45 strains of zika virus were isolated.

	September	October	November	December	TOTAL
From Aedes luteocephalus	3	8	1		12
From Aedes gr. furcifer-taylori	1	23	7	2	33
TOTAL.....	4	31	8	2	45

Besides, 4 other strains were isolated and yet in study = 2 Bunyamwera group strains from Aedes vittatus in July and from Aedes argenteo punctatus in September, one Simbu group strain from Aedes vittatus in September and maybe a new strain from Aedes vittatus in June.

1.3.2. Ticks : After the inoculations of mosquitoes, the laboratory could undertake the workings upon the numerous ticks, Alectorobius sonraï, caught during 1980 and 1981 in the rodent burrows in Bandia.

In 1980, 9.426 ticks, processed in 198 pools, allowed the isolation of 96 viral strains = one Koutango virus strain, 58 Bandia virus strains and 37 other strains not yet identified. In 1981, 2.726 ticks were already collected in the rodent burrows and grouped in 58 pools. 33 viral strains were isolated, (27 Bandia virus and 6 not yet identified).

TICKS INOCULATED DURING THE FIRST 1981 HALF-YEAR

	BURROWS											
	ARVICANTHIS		MASTOMYS		TATERA		TATERILLUS		UNKNOWN		TOTAL	
1980	Pool	Number	Pool	Number	Pool	Number	Pool	Number	Pool	Number	Pool	Number
January									6	227	6	227
February									18	860	18	860
April									24	1155	24	1155
October	7	325	8	367	6	264	6	267			27	1223
November	12	578	13	627	12	583	12	576			49	2364
December	10	475	10	481	8	402	9	419			37	1777
?	9	448	10	502	9	448	9	422			37	1820
TOTAL 80.....	38	1826	41	1977	35	1697	36	1684	48	2242	198	9426
1981												
January	2	89	2	62	2	88	3	142	1	54	10	435
February	8	379			6	276	6	305			20	960
March			5	237	4	174	6	305	5	225	20	941
April	2	81	2	93	2	108	2	108			8	390
TOTAL 81.....	12	549	9	392	14	646	17	860	6	279	58	2726
TOTAL 80 + 81	50	2375	50	2369	49	2343	53	2544	54	2521	256	12152

2°) - SEROLOGICAL STUDIES :

2.1. Human sera

2.1.1. From Casamance (Senegal)

1547 sera were harvested from children vaccinated within the framework of a P.E.V. and are yet in study.

2.1.2. From North Cameroon :

The serologic survey carried out after an outbreak of febrile jaundices is now achieved and 337 sera from children were investigated against chikungunya, yellow fever, west-nile, zika, wesselsbron, and Bunyamwera viruses by I.H.A. and C.F. tests.

In the towns or villages unvaccinated or vaccinated against yellow fever eight years ago, we have found few heterologous reactions in the B group (maybe wesselsbron virus), but yellow fever virus could not be implicated.
In the villages vaccinated five months ago, the heterologous reactions against flaviviruses are numerous but at a low level (especially in the C.F test), expressing repeated flavivirus infections or infections by one or many B group viruses among vaccinated. There also, no evidence for yellow fever virus infection was detected and the covering vaccination was satisfactory. Chikungunya virus circulated sporadically, but involved relatively late outbreak in two villages.

2.2. Vertebrate sera :

2.2.1. From Bandia

140 goat sera and 119 rodent sera were harvested to study the Bandia virus influence on rodents.

2.2.2. From Kedougou

21 monkey sera were investigated by Hi test (C.F test is now in progress). Heterologous reactions were found against flaviviruses, particularly against zika virus, and there was no evidence for yellow fever virus infection.

Dr. J. J. SALAUN (Institut Pasteur)
Dr. M. GERMAIN, Dr. M. CORNET and Dr. J. L. CAMICAS
(ORSTOM)

REPORT FROM ARBOVIRUSES LABORATORY - INSTITUT PASTEUR AND ORSTOM
01 - B.P.490 - ABIDJAN 01 -IVORY COAST*

/ ARBOVIRUSES IDENTIFIED IN 1979 (second half-year).

TAI Forest (Ivory Coast - West region)

-From *Culex nebulosus* : "probable new strain whose antigen (Ar A 94/79) gives a positive reaction at the lowest titer with immune-serum Burg-el-Arab" (I.P.DAKAR Reference Center)

-From *Culex guiarti* : Virus OUBANGUI (Ar A 28/79)

-From *Aedes taylori* (mâles): Virus ORUNGO (Ar A 142/79).

DABAKALA Savana (Ivory Coast - North-East)

-From *Aedes aegypti* : Virus ORUNGO (Ar 217/79).

./ ARBOVIRUSES ISOLATED IN IVORY COAST AND UPPER VOLTA (second half year).

A. Isolations from mosquitoes pools (1316 pools tested)

1.TAI Forest

226 pools tested among which 41 were from potential yellow fever vectors (P.Y.F.V.) NO STRAIN ISOLATED.

2.DABAKALA Savana

689 pools tested among which 416 were from P.Y.F.V.
yielded 2 STRAINS from non-P.Y.F.V. *Aedes gr.palpalis* : MIDDELBURG
Culex gr.decens : BAGAZA

3.ATTIEKOI (Degraded forest,40 km North from Abidjan)

18 pools tested from which 6 were from P.Y.F.V.
NO STRAIN ISOLATED.

B. Human blood samples

NO STRAIN ISOLATED.

./ CLINICAL SERO-DIAGNOSIS.

25 serum specimens from hospital patients were investigated;they showed no evidence of recent infections.

J.C.ROCHE,N.MONTENY,J.C.ARTUS,N.ARON,V.AKRAN,B.DIACO Institut Pasteur
R.CORDELLIER,J.P.HERVY,B.BOUCHITE ORSTOM

./ ARBOVIRUSES ISOLATED IN IVORY COAST AND UPPER VOLTA IN 1980.

A.- 1119 POOLS FROM MOSQUITOES WERE TESTED

1. TAI Forest (Ivory Coast - West region)

Intracerebral inoculation of suckling mice with 367 pools of mosquitoes from which 62 were potential yellow fever vectors (P.Y.F.V.) yielded

4 STRAINS (from non-P.Y.F.V.):

Aedes domesticus.....probable arbovirus
Culex perfuscus.....identification in
Aedes argenteoventralis..... process
Culex albiventris..... " "

2. DABAKALA Savana (Ivory Coast - North-East)

Inoculation of suckling mice of 517 pools of mosquitoes among which 321 were from P.Y.F.V. yielded

25 STRAINS (from P.Y.F.V.):

Aedes taylori.....14 strains
" *luteocephalus*..... 7 "
" *opok*..... 3 "
" *africanus*..... 1 "

These strains have an haemaglutinin and show cross-reactions whith our immune-sera of group B in H.A.inhibition test;no reaction in complement fixing test with our reference sera; sent to the Reference Center of I.P.DAKAR for further identification;

3 STRAINS from non-P.Y.F.V.:

Culex perfuscus..... 2 strains
" *decens*..... 1 "

3. BOBO DIOULASSO Savana (Upper Volta)

Inoculation of suckling mice of 235 pools of mosquitoes
210 were from P.Y.F.V. yielded
34 strains ,all from P.Y.F.V.:

Aedes luteocephalus.....32 strains
" *africanus*..... 2 "

These strains have an haemagglutinin;they give cross-reactions with our reference immune-sera of B-group in H.A.inhibition test;no specific reaction in complement fixing test with our reference sera;sent to the Reference Center of I.P.of DAKAR for further identification.

B.- TICKS.

91 pools yielded 4 strains (identification in process).

C.- HUMAN BLOOD SPECIMENS.

All inoculations to suckling mice from human sera were negative.

./ SEROLOGICAL STUDIES.

Only human blood samples were tested.

A.- EPIDEMIOLOGICAL SURVEYS.

1. ATTIEKOI (40 km North from Abidjan - degraded forest)

One case of amaril hepatitis was histologically confirmed.
870 human sera were collected (380 were paired samples).
It has been observed:

- a lack of vaccinal immunisation beetween 0 and 15 years;
- fluctuations of antibodies status against yellow fever (rising and decreasing titers);
- a probable circulation of CHIKUNGUNYA virus (H.A.antibodies titers ranging from 1/20 to 1/1280 or more);
- serological protection of the children due to the vaccination campain following the yellow fever case.

2. TAI (primary forest - West part of Ivory Coast)

The 110 human sera studied have evidenced the lack of vaccinal protection among children and the low antibodies status against flaviviruses of the population in the two villages tested.

B.- CLINICAL SERO-DIAGNOSIS.

70 serum specimens for antibody determinations were collected from hospital patients but showed no evidence of recent infections.

(JEAN-CLAUDE ROCHE)

REPORT FROM THE VIRUS RESEARCH CENTER, MEDICAL RESEARCH CENTRE,
P.O. Box 20752, Nairobi, Kenya.

MARBURG - EBOLA VIRUS INVESTIGATIONS IN KENYA PRIMATES.

Following the 1980 human Marburg virus disease cases in Kenya, investigations were undertaken to determine the extent of virus activity in Kenya. Preliminary human serology suggested that both Marburg and Ebola viruses were present.

Lacking any zoonotic link to man other than monkeys, as was reported in the 1967 outbreak, we tested serum from sub-human primates held at the Institute of Primate Research (IPR) at Tigoni. The IPR is an institute of the National Museums of Kenya and holds approximately 600 primates of 11 species. Serum of 260 animals, diluted 1:8 in PBS, were screen by indirect immunofluorescence for antibodies against Marburg, Ebola, Congo haemorrhagic fever, Rift Valley fever and Lassa viruses, using slides kindly supplied by the Special Pathogens Branch of the Centres for Disease Control, Atlanta, GA. Sera proving positive on the screening test were titrated to determine an end-point.

Two of 98 vervet monkeys Cercopithecus aethiops spp. had titres against Marburg virus (1:128 and 1:64). When rebled and tested approximately seven months later, the animals had Marburg titres of 1:64 and 1:16. One of the positive vervet monkeys was captured at Naivasha in the Rift Valley. The origin of the other is not known.

Two of 100 baboons Papio cyanocephalus anubis were positive against Ebola virus at 1:128 and 1:64. When retested seven months later, titres of 1:128 and 1:16 were recorded. Both baboons were members of a troop, 12 of whom were captured on 14 March, 1979, on the southern shore of Lake Naivasha. Serum from 61 Sykes and blue C. mitis spp., colobus Colobus gureza and de Brazza's Cercopithecus neglectus monkeys were all negative.

The positive sera, which have been confirmed as positive by both CDC and the National Institute for Virology, Sandringham, South Africa, indicate that primates acquire and survive infection with Marburg and Ebola viruses. The results show a tendency for antibodies in primates (vervets and baboons) which spend part of their time on the ground compared to more arboreal species which seldom leave the trees. It could indicate that the source of infection is on the ground.

One vervet monkey and three baboons exhibited low antibody titres against Rift Valley fever virus (1:16-1:32). All other antigens proved negative

(B.K. Johnson, L.G. Gitau, A. Gichogo and P.M. Tukei
Virus Research Centre, P.O. Box 20752, Nairobi

J. Else, Institute of Primate Research,
P.O. Box 114, Tigoni).

REPORT FROM THE SPECIAL PATHOGENS UNIT NATIONAL INSTITUTE FOR VIROLOGY SANDRINGHAM 2131, REPUBLIC OF SOUTH AFRICA.

CRIMEAN-CONGO HAEMORRHAGIC FEVER (CCHF):

A 13 year old boy who had attended a school camp in a nature reserve near Bloemhof in the western Transvaal from February 5-13, 1981, became ill at his home near Johannesburg on February 14, with headache and chills. A tick found on his scalp was identified as an adult Hyalomma but discarded before more specific identification could be made. His condition deteriorated over the next few days and he went into shock with epistaxis, haemoptysis, haematemesis, melaena and bleeding at venipuncture sites. He died on February 19 and permission for autopsy was refused.

Blood collected on February 18 was inoculated into suckling mice which started dying on February 25. Sucrose-acetone extracted antigen from the brains of these mice fixed complement with NIH/YARU Congo grouping fluid. Calf kidney cell cultures infected with mouse brain showed fluorescence four days after inoculation in an indirect test with reference Congo positive human serum from CDC, Atlanta. Serial culture in mice has not reduced the minimum survival time below seven days and titres have not exceeded 10^{-4} per 0.02 ml in mice.

Indirect fluorescent antibody tests on Congo antigen slides from CDC, Atlanta, and from the PHLS, Porton Down, revealed antibody titres in 5 out of 74 employees and their families at the nature reserve. Three of the seropositives were related but none had a history of illness suggestive of CCHF infection. Several people were aware of having been bitten by ticks in the past.

A team from this Institute (Dr. P. Jupp and Mr. A. Shepherd) visited the nature reserve from February 20-27 and collected 2844 questing ticks plus some blood and organ samples from small mammals. The ticks were overwhelmingly Hyalomma marginatum rufipes and H. truncatum adults. Immatures did not appear to be active. At least one tick pool yielded a confirmed CCHF isolate and further material is still being passaged. H.m. rufipes and H. truncatum are widely distributed in the western two-thirds of the country while a third Hyalomma, H.m. turanicum, is confined to the inland Karoo area of the western half of the Cape Province. Other genera which have been associated with CCHF elsewhere, are well represented and widely distributed in South Africa.

The reserve is stocked with a variety of antelope and hares, ground squirrels and mongooses appeared to be abundant, while the results of trapping suggested that small rodents were sparse.

Some 400 sheep and cattle sera have been collected on surrounding farms and from the few cattle and sheep kept in the reserve. It was found that sheep and cattle sera showed strong non-specific staining of the cells used in indirect immunofluorescence tests and alternative serological tests are being investigated. It is intended to extend the observations on sheep and cattle sera into a country-wide survey of the possible distribution of the virus and to follow this with attempts to isolate virus from ticks and tests on human sera in areas where livestock seropositives are recorded.

This is the first record of CCHF virus in South Africa and appears to be the furthest south that it has been found in Africa, the nearest other evidence apparently being antibodies in Tanzania.

R. SWANEPOEL, J.K. STRUTHERS & G.M. McGILLIVRAY.

REPORT FROM THE LABORATORY OF ENZYMOLOGY, DEPARTMENT OF MOLECULAR BIOPHYSICS, INSTITUT OF BIOPHYSICS, FEDERAL UNIVERSITY OF RIO DE JANEIRO, R.J., BRASIL.

The generation of defective interfering particles of Marituba virus in L-A_9 cells

Marituba (MTB) virus was found to multiply efficiently in mouse fibroblasts L-A_9 cells, with a growth cycle wich was essentially completed within 24h. after infection. Growth characteristics in confluent L-A_9 cells shown a maximal viral production with a multiplicity of 0.1 plaque forming units per cell and at higher m.o.i. virus yield was depressed. We have examined the generation and propagation of MTB virus defective-interfering (DI) particles produced during high multiplicity serial passaging in L-A_9 cells. In repeated high-multiplicity. serial undiluted virus passages, the titer is inhibited in 90%. The specificity of the MTB virus inhibitory response was investigated in relationship to interference within homologous and heterologous viral classes. In the homologous system, MTB-defective stock inhibited MTB standard virus by 63%. In the heterologous system employing vesicular stomatitis virus only 8% of inhibition was obtained by addition of MTB-DI virus. In cultures co-infected with Apeū virus there was 44% inhibition of infections virus. These results clearly indicate that during the replication of MTB virus in L cells a population of defective interfering particles (DI) is induced.

(N. Volkmer and M.A. Rebello).

REPORT FROM ARBOVIRUS LABORATORY
INSTITUT PASTEUR
BP 304
97305 - CAYENNE CEDEX - FRENCH GUIANA

DENGUE IN GUADELOUPE , MARTINIQUE AND FRENCH GUIANA

Following the detection by the Center for Disease Control (CDC) of dengue type 4 activity in Saint-Barthelemy, surveillance was intensified and reports of dengue like illness occurring from early march 1981 were obtained from Guadeloupe and Saint-Martin. Sera were collected from Guadeloupe, Martinique and French Guiana patients.

Few specimens were submitted for virus isolation : two isolates are still under investigations.

Serological tests (HI and CF) were done on serum specimens, using 5 Flavivirus antigens : Yellow fever (FNV), Saint-Louis Encephalitis, dengue 2, dengue 3 and Ilheus. All responses were of the secondary type, A titer of 1: 64 or more with any of the antigen was condidered as indication of recent flavivirus infection.

1. GUADELOUPE.

76 serum samples were received from 42 patients (34 paired sera) from january to mid august. Results were as follows :

Month	N° of Patients	FC Titer ≥ 1:64
January to April	4	0
May	4	3(2)[a]
June	7	4(1)[a]
July	24	6(3)[a]
August	3	0

(a) Number of seroconversion in paired sera.

2. MARTINIQUE.

134 serum samples were collected from 126 patients (8 paired sera) from january to mid august and studied with the following results:

Month	N° of Patients	FC Titer ≥ 1: 64
January-April	45	2
May	10	0(1)[a]
June	14	1
July	22	7(1)[b]
August	35	11

(a) Significant fall in antibody. (b) Seroconversion.

3. FRENCH GUIANA.

294 serum samples were collected from 262 patients (16 paired sera) from january to mid august. Results were as follows.

Month	N° of Patients	FC Titer ≥ 1: 64
January-April	181	15
May	16	2
June	13	1
July	28	1
August	24	3

It is obvious that a Flavivirus which in all likelihood is a dengue virus was active in each of the three French Departments in the Americas in 1981. The virus was more active in Guadeloupe (31 %) than in Martinique (17 %) and in French Guiana (8,4 %).

No case of hemorrhagic syndrome was reported.

Aedes aegypti surveillance and control activities were increased in French Guiana, especially in the town of Cayenne where 3 fenitrothion ULV ground spraying at 15 days cycles were shown to be very effective in lowering both larval and adult mosquito number.

(M.LHUILLIER, G.GIRAULT, Y. ROBIN).

I. St. Louis Encephalitis Virus Surveillance in Panama

A surveillance program to monitor SLE virus activity in three ecologically distinct habitats in Panama was outlined in a previous report (Arthropod-Borne Virus Information Exchange 40:156-157, 1981). Since the initiation of this project in February, 1980, a number of viruses have been isolated from mosquitoes and the tissues of sentinel hamsters and chickens (Table 1). The two SLE isolates from Puerto Gago complement the detection of virus activity by sero-conversions in sentinels between July-November, 1980 (see previous report). SLE virus activity in sentinels has been detected at Puerto Gago again this season; sero-conversions in chickens have occurred in June, July and August. Mosquito collections have been initiated at this site using red-boxes, sweep nets and chicken baited traps.

(C. G. Hayes, A.J. Adames, P.H. Peralta, B.Dutary and P.Galindo).

Table 1. Virus isolations from mosquitoes and sentinel animals in Panama.

Virus (No.of Strains)	Isolated From	Location	Date Collected
Punta Toro (2)	Hamster	Maje, Bayano	July,1980
Chagres (1)	Hamster	El Llano-Carti Road	Oct.,1980
Chagres-related (1)	Chicken	Puerto Gago	Oct.,1980
SLE (1)	Hamster	Puerto Gago	Oct.,1980
Unknown (1)	Hamster	Puerto Gago	Oct.,1980
Unknown (1)	*Cx.* (*Melanoconion*) sp.	Puerto Gago	Nov.,1980
EEE (1)	*Ae. taeniorhynchus*	Puerto Gago	Dec.,1980
EEE (1)	*Ma. titillans*	Puerto Gago	Dec.,1980
Pacora (1)	*Cx. dunni*	Puerto Gago	Dec.,1980
Itaporanga-related (1)	Hamster	Majé, Bayano	Dec.,1980
Unknown (1)	*Cx. dunni*	Puerto Gago	Dec.,1980
SLE (1)	Chicken	Puerto Gago	Dec.,1980
Chagres-related (1)	Hamster	El Llano-Carti Road	Jan.,1981
VEE (22)	Hamster	Majé, Bayano	May-June, 1981

Table 2. Yellow fever virus infectivity of sloth cell fluid harvests.

Days Post-Infection	Titers in PFU/ml*	
	Wild	17-D
0	1-5 x 10^4	1-5 x 10^4
17	6.0 x 10^2	2.0 x 10^2
28	1.8 x 10^3	2.0 x 10^2
35	1.2 x 10^2	0
39	9.0 x 10^2	6.0 x 10^1
42	3.4 x 10^2	1.4 x 10^2
45	4.0 x 10^1	NT**
49	NT	2 x 10^1
52-53	0	0
55-56	0	4 x 10^1
60-62	0	1 x 10^1
63	NT	0
66-67	0	9 x 10^1
70-73	0	1 x 10^1
$\geq$74	0	NT

* Plaque titrations in Vero cells.

** NT= Not Tested

II. Virus susceptibility of fetal sloth cell culture

A fetal sloth (Choloepus hoffmanni) spleen cell culture initiated in our laboratory was tested at passage 26 for its susceptibility to VSV-Indiana, VEE (I-D) 3880, Punta Toro, St. Louis encephalitis, and yellow fever strains 17-D and wild. As shown in Table 1 the sloth cells were highly susceptible to VSV, VEE, SLE and Punta Toro viruses. Both strains of yellow fever caused nonlytic infections in the sloth cell culture with infectious virus being released in low levels (Table 2). Inoculation of supernate from the sloth cell cultures infected with the 17-D strain of YF virus onto Vero cell cultures produced plaques through day 70, the last day the supernate was tested. The supernate from culture infected with wild YF virus only produced plaques for 45 days. However, supernates from the sloth cell cultures infected with the 17-D and wild viruses produced CPE in Vero cell tube cultures for 112 days and 83 days, respectively. Samples from the sloth cell cultures infected with the 17-D and wild YF strains tested on days 116 and 90, respectively, were negative for CPE in Vero cultures. No later samples were available for assay because of deterioration of the sloth cells apparently caused by ageing. In a second experiment with the wild strain of YF, infectious virus was detected in the sloth cell supernate for 2 months. These results suggest that YF virus can establish a persistent infection in fetal sloth spleen cells.

(G. Oro)

Table 1. Comparison of onset of virus-induced CPE in sloth and Vero cells.

Virus	Day of Onset	
	Sloth	Vero
VSV-Indiana	1	1
VEE (ID) 3880	1	1
Punta Toro	3	3
SLE	2	4
YF 17-D		4
YF Wild		6

REPORT FROM THE OFFICE OF LABORATORY SERVICES AND ENTOMOLOGY
DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES
JACKSONVILLE, FLORIDA

Arbovirus surveillance in Florida for the period January through June, 1981 was conducted in the same manner as the previous year.

A total of 791 patients' sera with CNS symptoms were tested by HI against EEE, VEE, SLE, Dengue, and CAL antigens. There were over twenty patients with constant titers to Group B antigens, indicating infection at some undetermined time. There were three confirmed Dengue I infections, two of which were acquired while visiting St. Martin, Caribbean, and one while visiting in Puerto Rico.

2119 chicken sera were tested in the first six months of 1981. Six chickens developed antibody to SLE, these conversions occurred in late December 1980.

There were 260 small mammal sera tested with no detectable antibody to SLE. It is interesting to note that in a 4 county area in the west central part of Florida, a mammal study detected SLE antibody in recaptured animals. A total of 351 raccoons were tested, 191 were recaptured with 22 yielding SLE antibodies. Of the 229 opossums tested, 69 were recaptured with 32 yielding SLE antibodies.

For the first six months of 1981, light trap data indicated that populations of Culex nigripalpus were low. No mosquito pools were tested in this time period.

E.C. Hartwig, F.M. Wellings, E.E. Buff, J.A. Mulrennan

REPORT FOR ARBOVIRUS EXCHANGE
JANUARY 1, 1981--JUNE 30, 1981

MOSQUITO ISOLATES

For the period indicated, 281 litters of mice were inoculated for arbovirus isolation. This represents 1389 pools totaling 17,591 mosquitoes. Listed below are the isolates:

Locality	Collection Date	Pooled Species	Number of Isolations	Virus
Dallas	May 21	C. salinarius C. tarsalis C. quinquefasciatus A. aegypti	1	Hart Park
Dallas	May 21	C. tarsalis C. quinquefasciatus	1	Hart Park
Brazoria Co.	May 21	C. salinarius C. quinquefasciatus A. quadrimaculatus A. crucians A. taeniorhynchus	1	Hart Park
Thornton	June 9	C. quinquefasciatus	1	Hart Park
Kosse	June 9	P. cyanescens A. aegypti C. tarsalis C. quinquefasciatus	1	Hart Park
Fort Bend	June 10	A. quadrimaculatus C. quinquefasciatus A. aegypti A. taeniorhynchus	1	Hart Park
Dallas	June 10	C. tarsalis C. restuans C. quinquefasciatus A. crucians	1	Hart Park
Dallas	June 10	C. tarsalis C. salinarius C. quinquefasciatus	1	Hart Park
Dallas	June 10	C. quinquefasciatus	1	Hart Park

Locality	Collection Date	Pooled Species	Number of Isolations	Virus
Denton Co.	June 16	A. quadrimaculatus C. tarsalis C. quinquefasciatus A. punctipennis A. epactius	1	Hart Park
Corpus Christi	June 16	A. crucians Culex species C. quinquefasciatus A. taeniorhynchus C. salinarius	1	Hart Park
Hidalgo Co.	June 18	C. quinquefasciatus	1	Hart Park
Kosse	June 18	A. quadrimaculatus A. aegypti A. triseriatus P. cyanescens C. quinquefasciatus	1	Hart Park
Dallas	June 18	C. quinquefasciatus	1	Hart Park
Dallas	June 20	C. tarsalis C. salinarius C. quinquefasciatus	1	Hart Park
Hill Co.	June 20	C. tarsalis C. quinquefasciatus A. quadrimaculatus C. restuans	1	Hart Park
Corpus Christi-Nueces Co.	June 27	A. taeniorhynchus A. sollicitans C. quinquefasciatus Culex (Melanoconion) sp. C. salinarius C. quinquefasciatus	1	Hart Park

SEROLOGY

Sera for arbovirus surveillance were submitted from two regions during the period indicated: Public Health Region 11 (Rosenberg), 20 canine sera and Public Health Region 5 (Canyon), one rabbit serum and one pooled chicken serum. The hemagglutination inhibition test was performed with the following positive results:

Locality	Collection Date	Species	# Positives	Antibodies Detected
Texas City	April 10	canine	1	SLE 1:40
Canyon	June 16	rabbit	1	WEE≥1:80

BIRD BLOODS FOR ISOLATION

Wild bird bloods were submitted for arbovirus isolation from San Antonio. Seven specimens were submitted and all were found to be negative.

NOTE: During July 1981 two isolates of CEV (La Crosse) were obtained from mosquitoes. One isolate was obtained from Randall Co., the second was from the Dallas area.

(Charles E. Sweet)

REPORT FROM THE VIROLOGY DEPARTMENT, AMERICAN TYPE CULTURE COLLECTION, ROCKVILLE, MARYLAND

ARBOVIRUS REAGENTS AVAILABLE FROM AMERICAN TYPE CULTURE COLLECTION

Seed viruses and their corresponding immune ascitic fluids of reference arbovirus strains, formerly distributed by the Research Resources Branch of The National Institute of Allergy and Infectious Diseases, have been transferred to the American Type Culture Collection for cataloging, storage and distribution.

Reagents prepared under this program are now catalogued in the ATCC Catalogue of Strains II. The Catalogue is available without fee for shipment within the USA. A shipping fee of $10.00 is charged for Canada, South America, Mexico and Europe; $15.00 for Asia, Pacific, Africa and USSR.

In order to help defray expenses for maintenance of cultures a fee of $13.00 is assessed for each reagent shipped. All orders should be addressed to the American Type Culture Collection, C/O Professional Services Department, 12301 Parklawn Drive, Rockville, Maryland 20852, USA.

(David A. Stevens)

REPORT FROM THE DIVISION OF CLINICAL MICROBIOLOGY
BUREAU OF LABORATORIES
PENNSYLVANIA DEPARTMENT OF HEALTH
LIONVILLE, PENNSYLVANIA

Arbovirus Surveillance in Pennsylvania, 1981

The Pennsylvania Departments of Health and Environmental Resources reinstituted an arbovirus surveillance program similar to ones conducted during the summers of 1978 - 1980.

Sentinel flocks of four (4) chickens, mostly hens, were placed at 65 sites throughout the state at the locations shown in the Figure. This compares with 68 sentinel sites in 1980. This year, there was at least 1 sentinel flock in 32 of the 67 counties as compared with coverage in 36 counties in 1980. The chickens were bled weekly and the sera tested, after protamine sulfate-acetone extraction, for hemagglutination-inhibition (HI) antibodies against St. Louis Encephalitis (SLE), Western Equine Encephalitis (WEE), Eastern Equine Encephalitis (EEE), and California Encephalitis (CE) viruses. There were no seroconversions in 3532 HI tests performed through August 26, 1981.

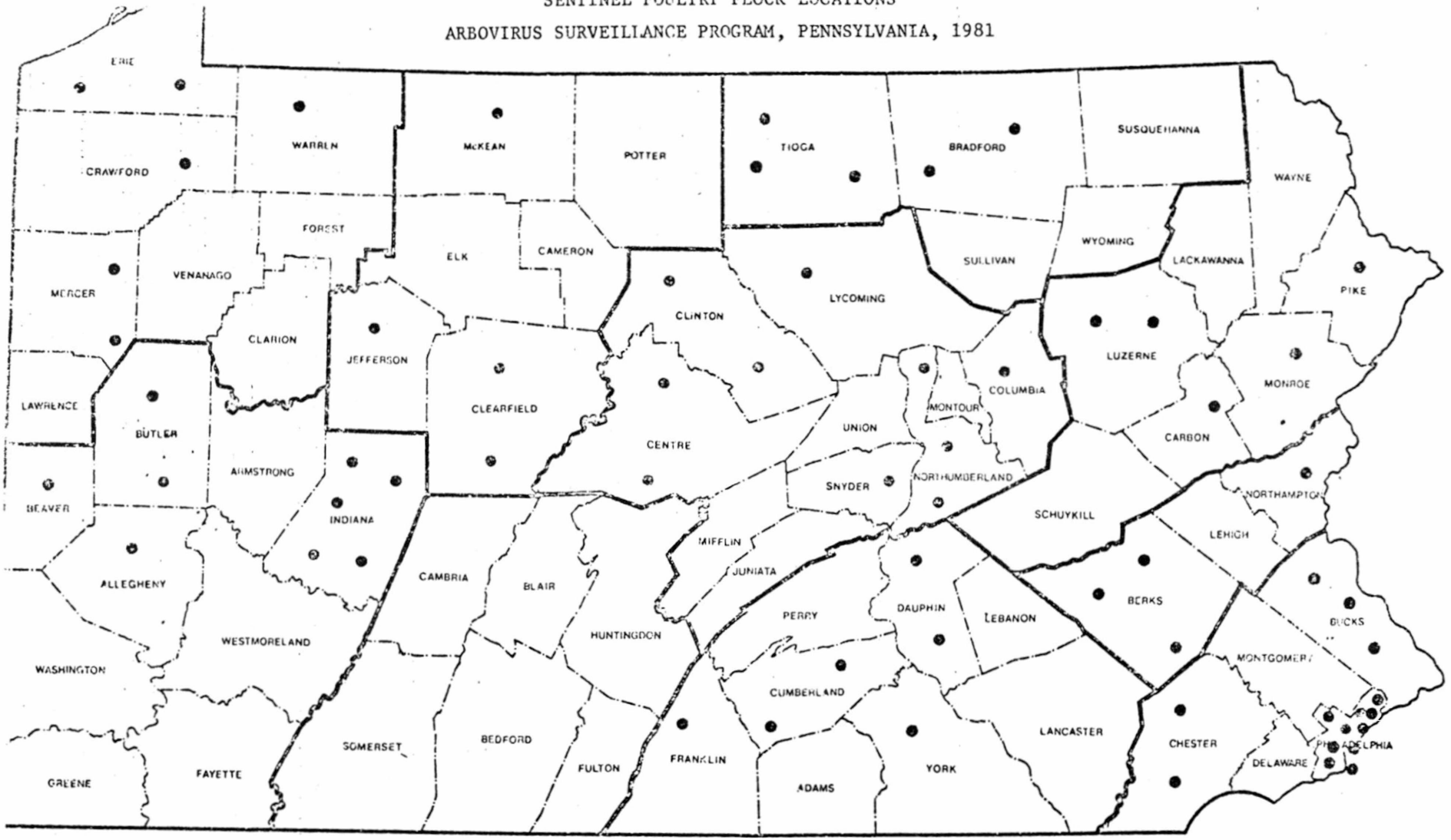
Through August 26, 1981, sixteen (16) patients with a clinical diagnosis of central nervous system disease were tested for serologic evidence of infection with SLE, WEE, EEE, and CE viruses. There was no evidence of infection with arboviruses in any of the cases.

The surveillance program will be continued through September 1981.

(Bruce Kleger, Vern Pidcoe, and Gisela Fischer)

SENTINEL POULTRY FLOCK LOCATIONS

ARBOVIRUS SURVEILLANCE PROGRAM, PENNSYLVANIA, 1981



REPORT FROM VIROLOGY PROGRAM, STATE OF NEW JERSEY DEPARTMENT OF HEALTH, TRENTON, N. J

Following is a report of arbovirus activity in New Jersey in the summer of 1981.

Isolations from Arthropods in New Jersey*

EE	WE	Isolated from	Collected in	Month
	1	Cs. melanura	Green Bank	June
	5	Cs. melanura	Green Bank	July
	1	C. restuans	Green Bank	July
	1	Cs. melanura	Green Bank	August
	1	Cs. melanura	Dennisville	July
1		Cs. melanura	Dennisville	July
	2	Cs. melanura	Dennisville	August
	2	Cs. melanura	Woodbine	July
	1	C. salinarius	Woodbine	July
2		Cs. melanura	Woodbine	July
Totals: 3	14			

*These include collections from the New Jersey Agricultural Experiment Station, Rutgers University

Sentinel chicken flocks of ten (10) cockerals were placed at 12 sites throughout the State in June. The flocks are bled weekly and St. Louis encephalitis neutralization tests are conducted in mice. To date there have been no sero conversions in 290 test bleedings.

(Wayne Pizzuti)

REPORT FROM THE MOSQUITO RESEARCH CONTROL, NEW JERSEY AGRICULTURAL EXPERIMENT STATION, HEADLEE RESEARCH LABORATORIES, NEW BRUNSWICK, N. J.

*Mosquito Surveillance Report**

Vol. 9 *No.* 2 *Period* July 9-29, 1981

Sporadic and widely spaced thundershowers have resulted in a patchwork of mosquito problems throughout New Jersey. Sufficient rainfall has been received by some areas to produce sizeable mosquito populations, while other districts have shown a steady decline in the number of mosquitoes collected.

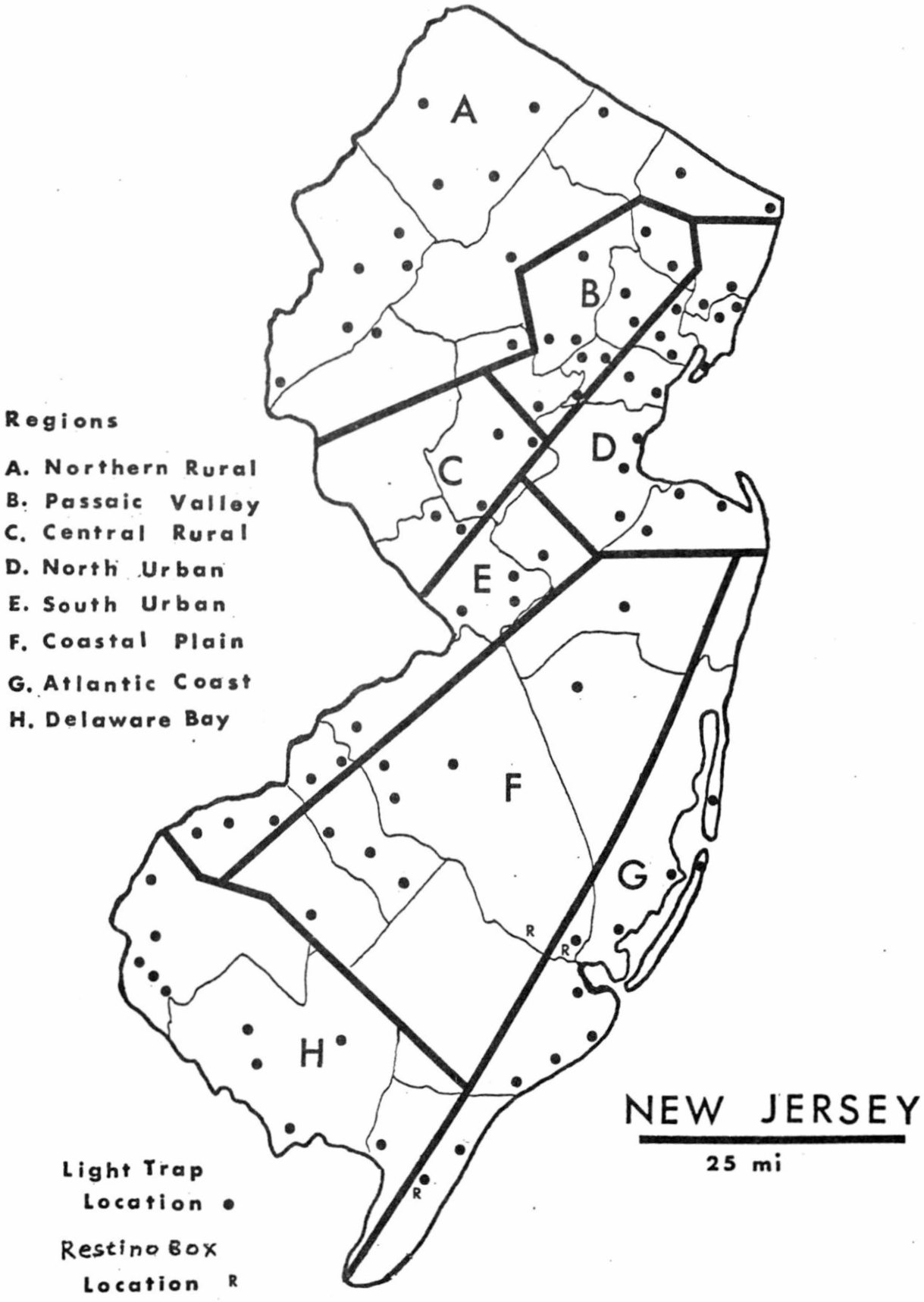
Aedes vexans levels have decreased in all regions where populations of the species are plotted. During mid July, Ae. vexans reached over 10 per light trap per night in the South Urban Region E. The species has now dropped below 5 per trap night in Region E, a level that is about average for this time of year.

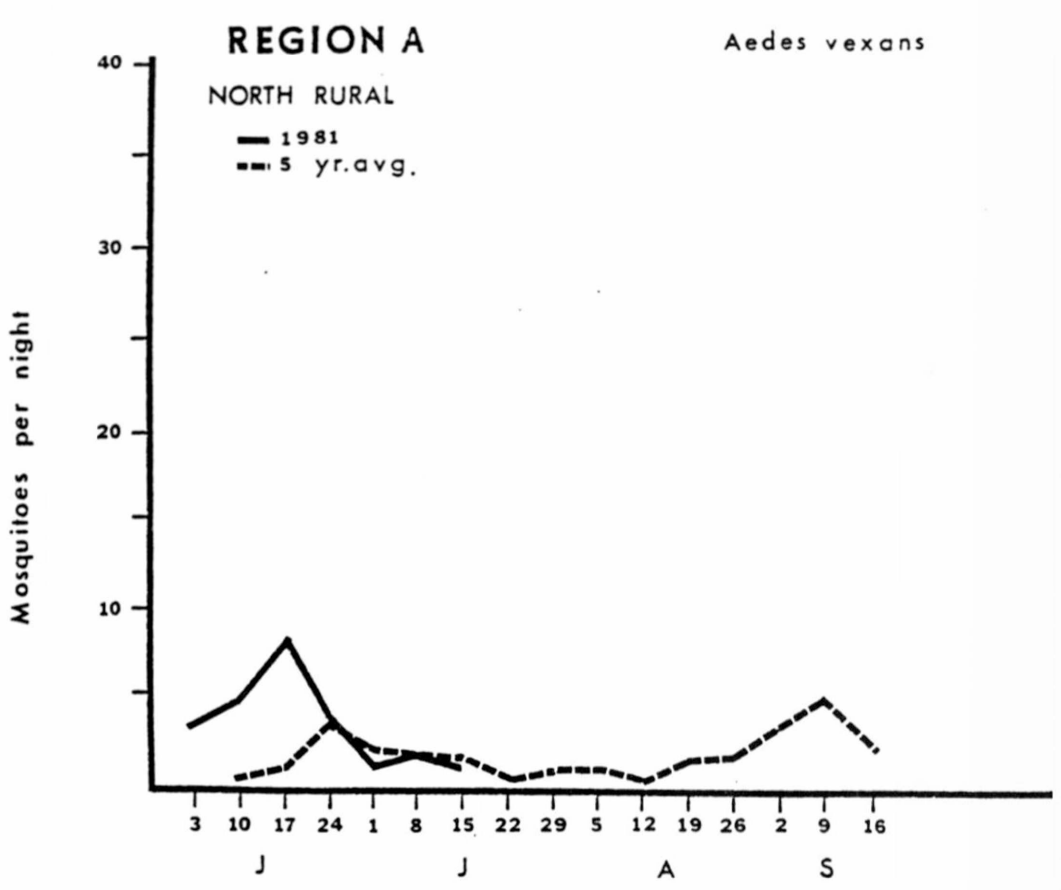
Members of the Culex complex have not increased in either the North Urban Region D or the South Urban Region E in the past few weeks. In addition, Culex populations in the Delaware Bay Region H plummeted during the current reporting period and are now well below normal for the area. The decrease in Culex is heartening, since these mosquitoes generally peak around this time of year. Lower levels of the Culex complex are likely to reduce the risk of an outbreak of St. Louis encephalitis in New Jersey.

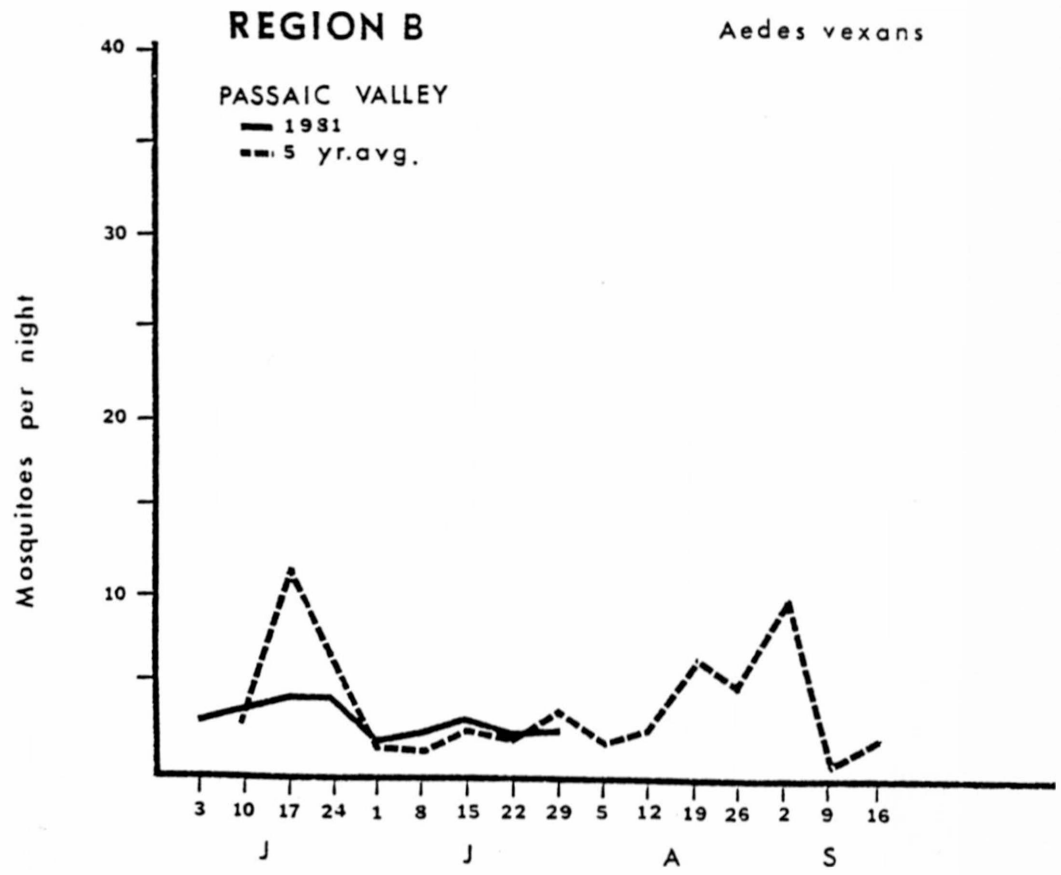
Culiseta melanura presents somewhat of a paradox this year. Although the species is averaging less than 3 mosquitoes per resting box, a widespread enzootic of Highlands J virus is currently underway. In addition, eastern encephalitis has been isolated from Cs. melanura at a sampling site. Past research has indicated that relatively high levels of Cs. melanura are essential to initiating virus activity, but evidently such activity is possible with only small numbers of the species present.

Aedes sollicitans reached levels not seen for 2 years in coastal New Jersey. In the Atlantic Coast Region G, the species topped 20 mosquitoes/trap night during mid July, causing severe nuisance problems throughout the area. In general, these levels are now greatly reduced, although a small secondary brood emerged later in July in some sections of Region G.

*This work was performed as a part of NJAES Project 40506. Supported by the New Jersey Agricultural Experiment Station and the State Mosquito Control Commission.


Regions
A. Northern Rural
B. Passaic Valley
C. Central Rural
D. North Urban
E. South Urban
F. Coastal Plain
G. Atlantic Coast
H. Delaware Bay
A
B
C
D
E
F
G
H
R
R
R
NEW JERSEY
25 mi
Light Trap Location •
Restino Box Location R


REGION A
Aedes vexans
NORTH RURAL
1981
5 yr.avg.
Mosquitoes per night
40
30
20
10
3 10 17 24 1 8 15 22 29 5 12 19 26 2 9 16
J J A S


REGION B
Aedes vexans
PASSAIC VALLEY
1981
5 yr.avg.
Mosquitoes per night
40
30
20
10
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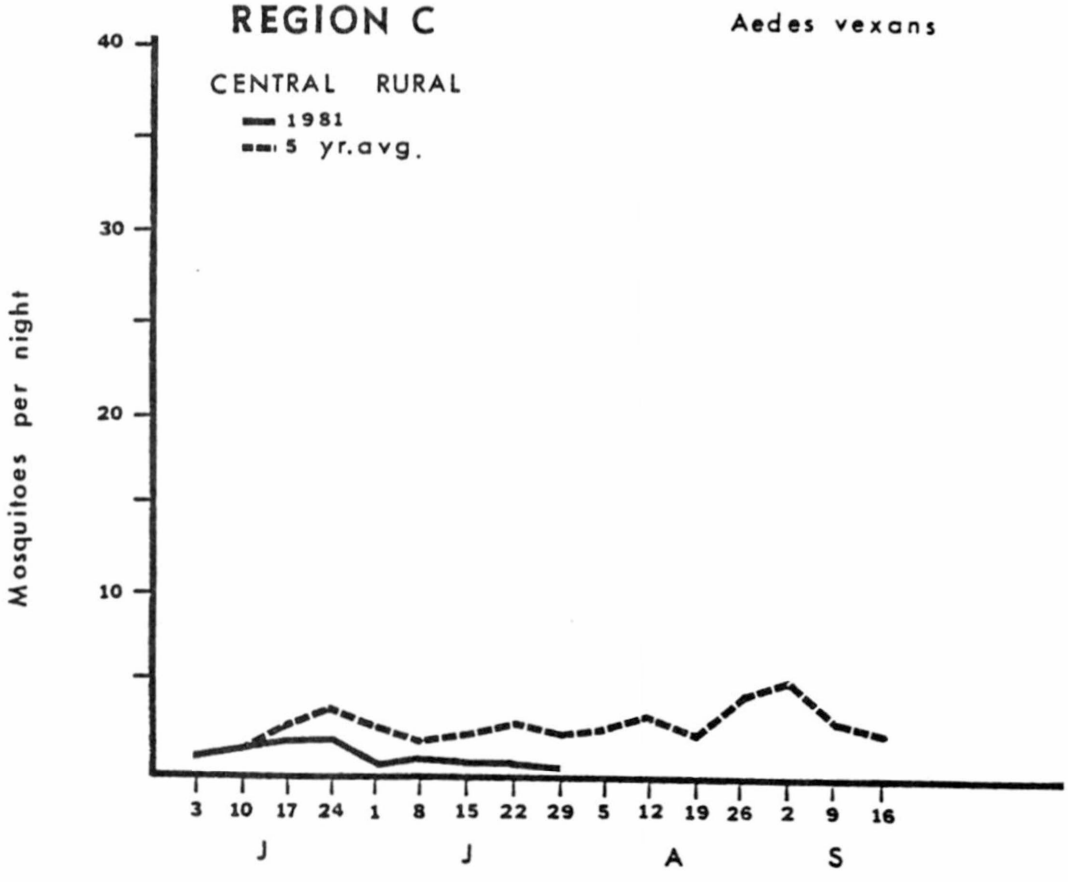
J J A S


REGION C
Aedes vexans
CENTRAL RURAL
1981
5 yr.avg.
Mosquitoes per night
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30
20
10
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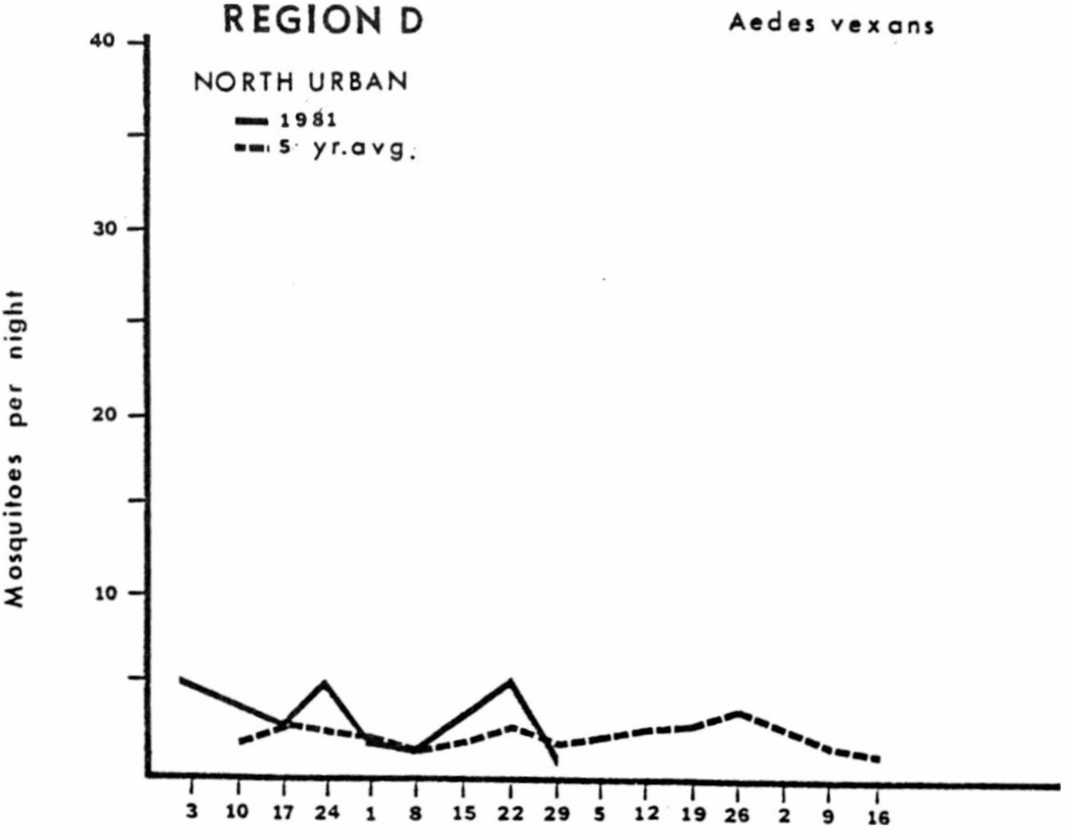
J J A S


REGION D
Aedes vexans
NORTH URBAN
1981
5 yr.avg.
Mosquitoes per night
40
30
20
10
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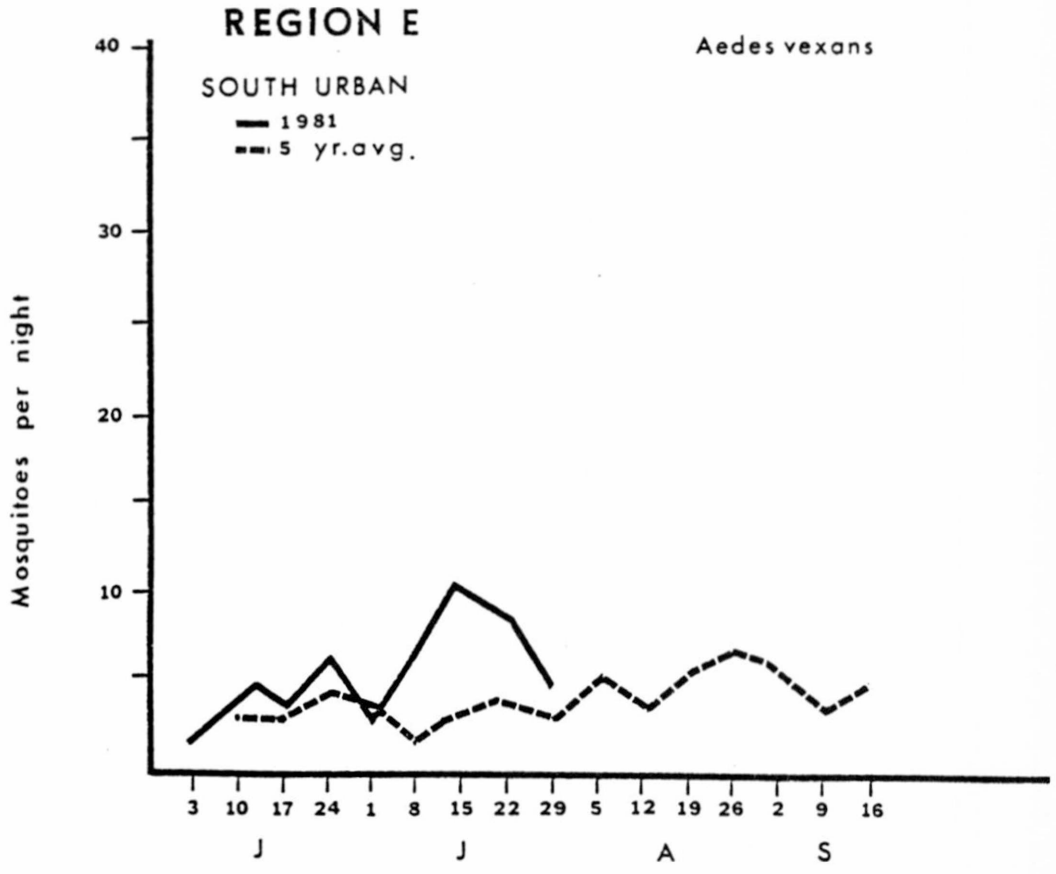
REGION E
Aedes vexans
SOUTH URBAN
1981
5 yr.avg.
Mosquitoes per night
40
30
20
10
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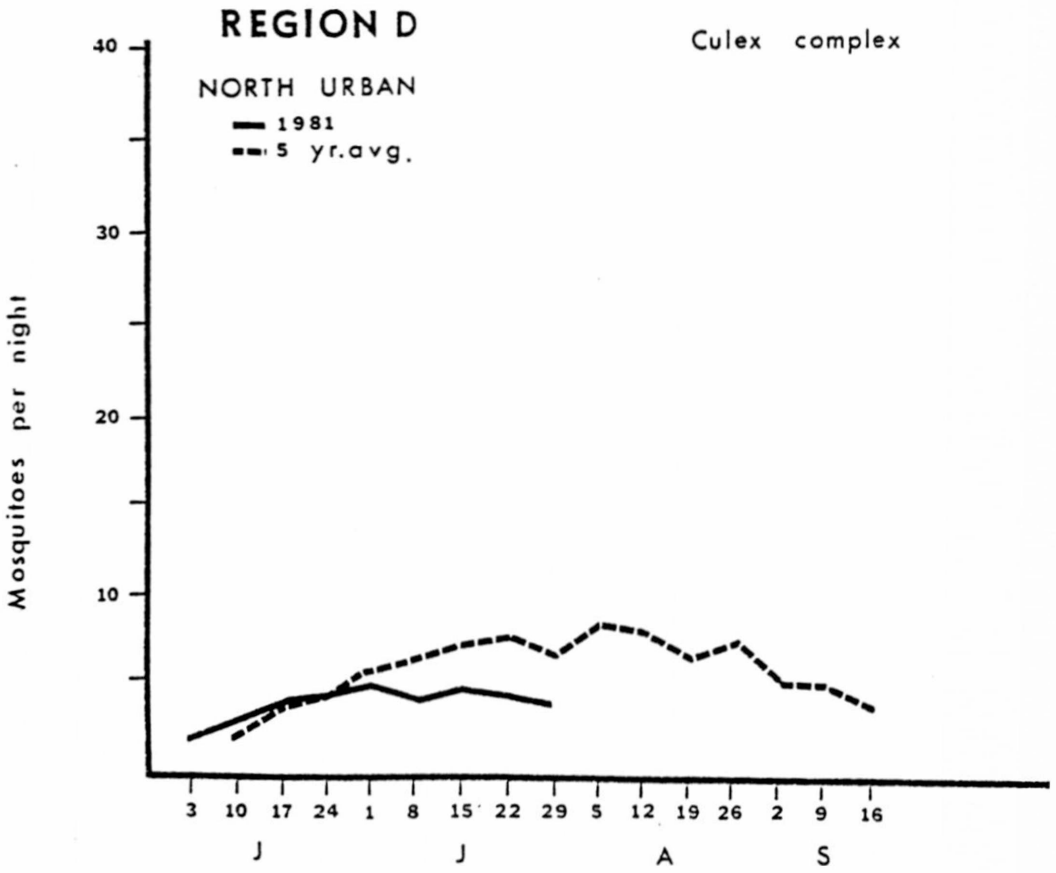
J J A S


REGION D
Culex complex
NORTH URBAN
1981
5 yr.avg.
Mosquitoes per night
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30
20
10
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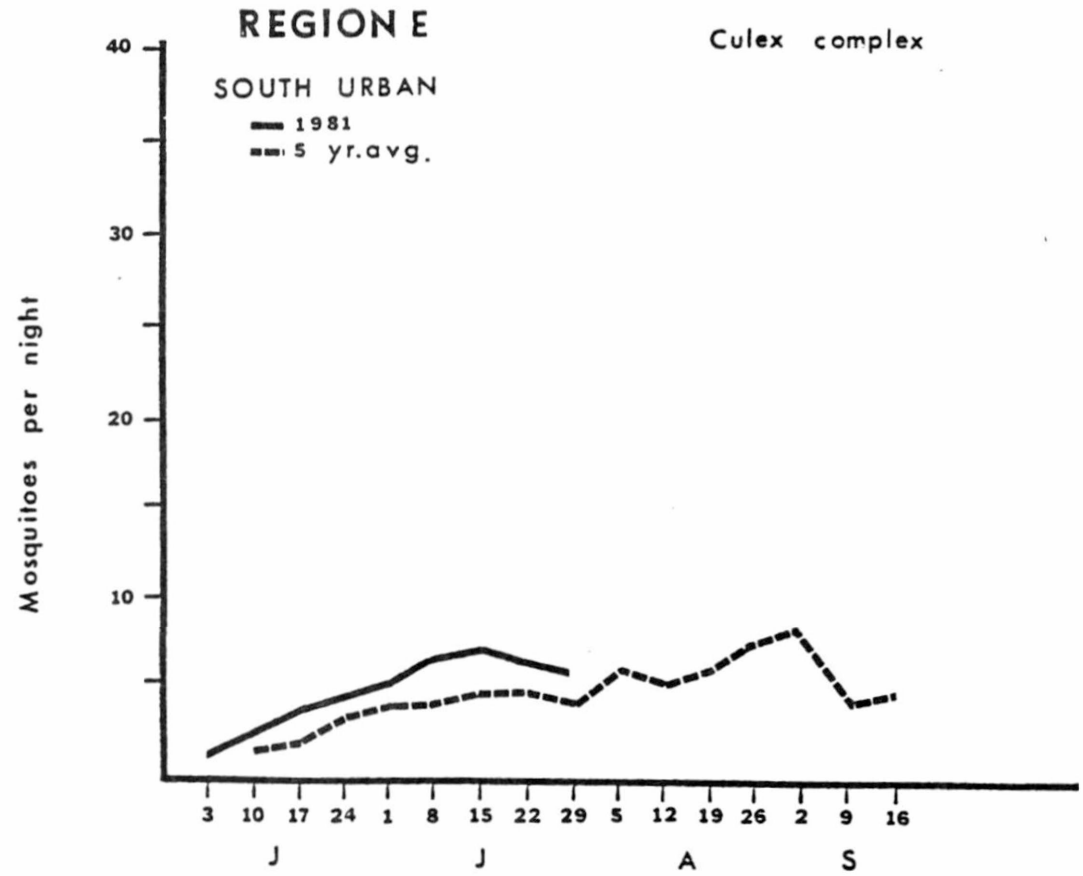
J J A S


REGION E
Culex complex
SOUTH URBAN
1981
5 yr.avg.
Mosquitoes per night
40
30
20
10
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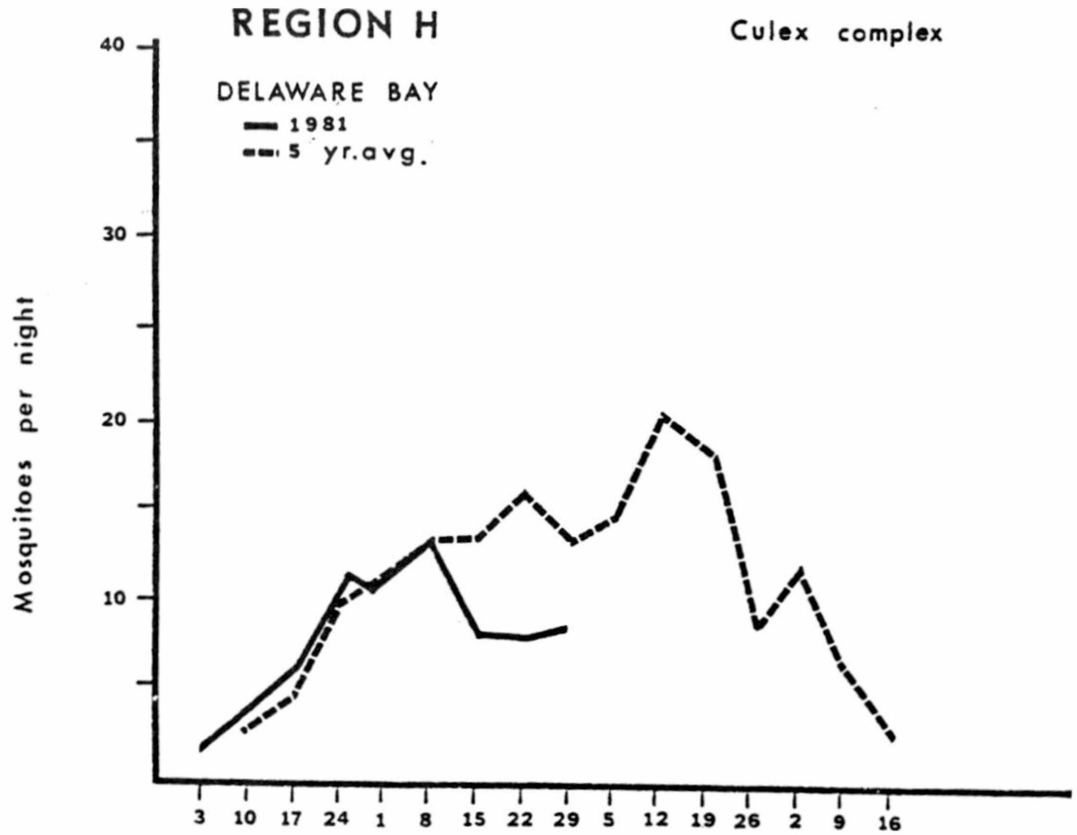
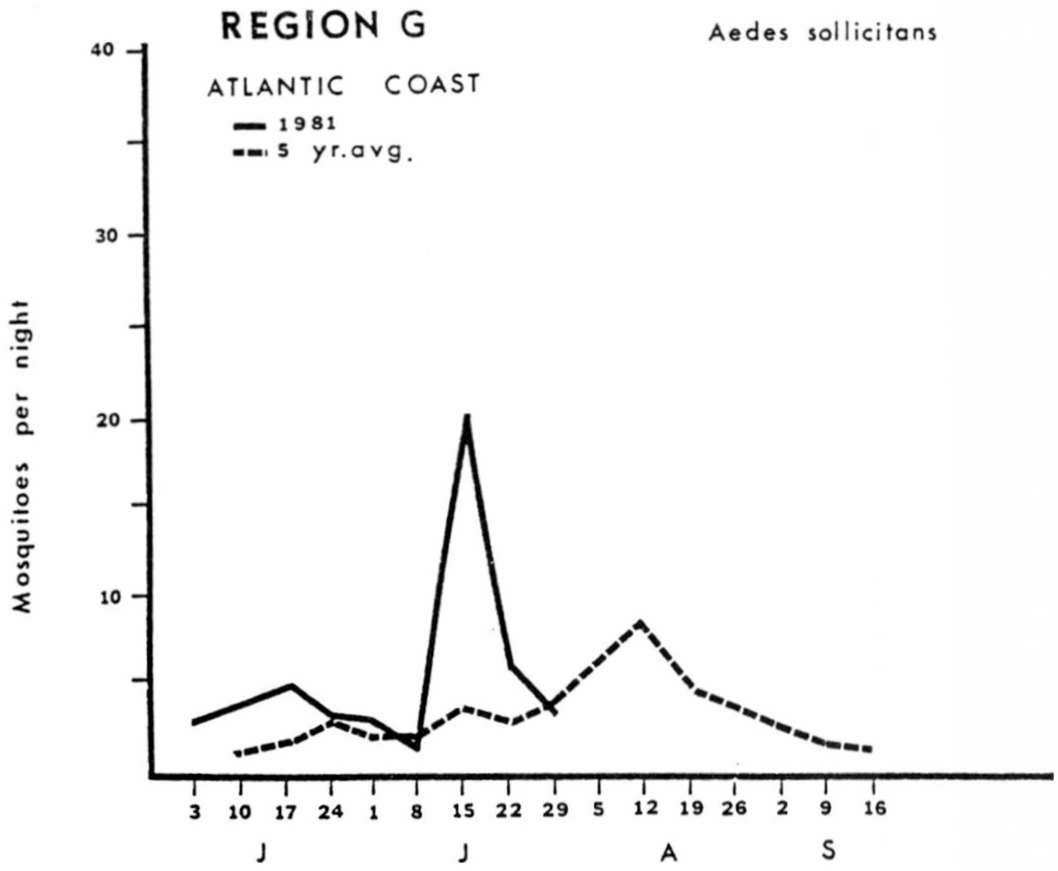
J J A S


REGION H
Culex complex
DELAWARE BAY
1981
5 yr.avg.
Mosquitoes per night
40
30
20
10
3 10 17 24 1 8 15 22 29 5 12 19 26 2 9 16


REGION G
Aedes sollicitans
ATLANTIC COAST
1981
5 yr.avg.
Mosquitoes per night
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30
20
10
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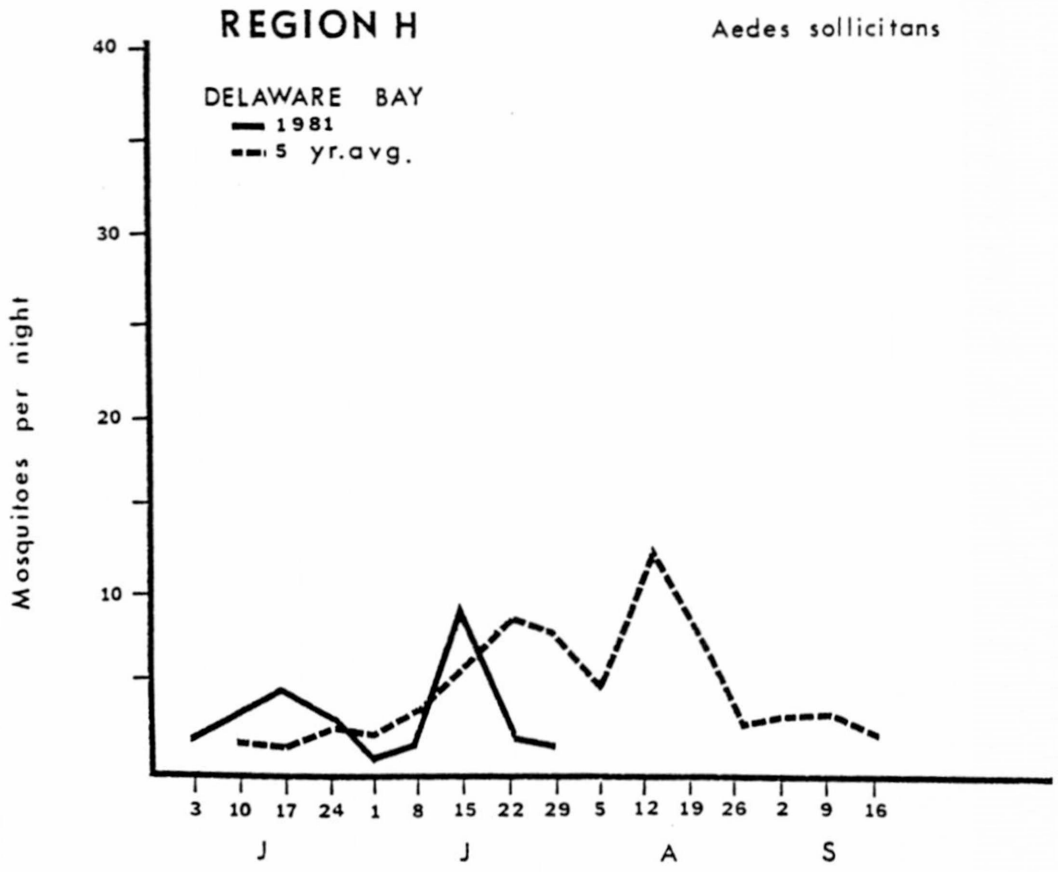
J J A S


REGION H
Aedes sollicitans
DELAWARE BAY
1981
5 yr.avg.
Mosquitoes per night
40
30
20
10
3 10 17 24 1 8 15 22 29 5 12 19 26 2 9 16
J J A S

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	Wayne J. Crans	Roy Gonzalez
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	Robert B. Kent	
Cooperating County Mosquito Control Commissions:	Atlantic	Middlesex
	Bergen	Monmouth
	Burlington	Morris
	Camden	Ocean
	Cape May	Passaic
	Cumberland	Salem
	Essex	Somerset
	Gloucester	Sussex
	Hudson	Union
	Mercer	Warren

REPORT FROM THE STATE OF NEW YORK DEPARTMENT OF HEALTH
DIVISION OF LABORATORIES AND RESEARCH, ALBANY, NEW YORK

Arbovirus Surveillance, 1981

From May through August 20 a total of 1,155 pools of up to 200 mosquitoes each were received and tested. Aedes mosquitoes accounted for 761 of these pools. California encephalitis complex virus was isolated from 15 of the Aedes pools; 11 of the isolates were from 451 pools received from the eastern regions and 4 from 310 pools from the western region of the state. Species from which isolates were obtained were Aedes communis (6), Aedes stimulans (3), Aedes sollicitans (3), Aedes canadensis (1) and Aedes spp. (2).

Serum samples collected during the same period from 145 patients with signs of central nervous system infection or with fever of unknown origin were tested with California encephalitis, Powassan, St. Louis encephalitis, Eastern and Western equine encephalitis antigens. A presumptive diagnosis of California encephalitis was obtained for 7 patients (eastern regions: 6; western region: 1) with signs of CNS infections.

(Sunthorn Srihongse, Margaret A. Grayson and Rudolf Deibel)

Report from the State Laboratory Institute

Massachusetts Department of Public Health

305 South Street

Boston, Massachusetts 02130

MOSQUITO SURVEILLANCE IN MASSACHUSETTS, 1971-1980

Our previous report summarized eastern equine encephalitis surveillance data gathered on mosquitoes, humans, and equines during the period 1976-1980. The current report presents additional results of trapping and virus testing of mosquitoes, 1971-1980.

Table 1 summarizes the total captures and minimum infection rates observed in 21 species of mosquitoes. In addition to those cited, a total of 7 species were captured but no species exceeded 100 specimens and no virus was isolated. The additional species were Aedes communis, Ae. taeniorhynchus, Ae. trivittatus, Ae. sollicitans, Anopheles crucians, Psorophora ciliata and Ps. ferox.

Culiseta melanura was the most abundant species, totalling more than 69 per cent of all the mosquito captures (Table 1). Although our traps are set mainly on the "ecological edge" of Cs. melanura habitat, wooded fresh water swamps, the high proportional representation of this species is consistent with the fact that 70 per cent of the entire wet lands area is wooded fresh water swamp.

Culiseta melanura was also the species from which both eastern equine encephalomyelitis virus (EEE virus) and Highlands J, western equine encephalomyelitis virus (HJ, WEE virus) were isolated most frequently, accounting for more than 94 per cent of all isolations. In nearly every season, the first isolation of HJ, WEE virus and the subsequent isolation of EEE virus occurred in Cs. melanura. Furthermore Cs. melanura was the only species that was infected with both viruses in each of the ten years.

We have also found that the total numbers of Cs. melanura captured co-vary significantly with total precipitation recorded during an 18 month period (May through October of the previous year and November through October of the immediate year; unpublished data, Encephalitis Field Station). Our data indicate that Cs. melanura is the principal enzootic vector of EEE virus and HJ (WEE) virus in the trapping area. As such, Cs. melanura is a valuable indicator of annual enzootic virus transmission activity. The relationship of precipitation and the annual numbers of Cs. melanura partially explain the cyclic distribution of the viruses.

(H. Maxfield, R. Gilfillan, B. Rosenau, G. Grady, L. Marcus, W. Andrews)

TABLE 1

Total number of adult female mosquitoes captured by C.D.C., light traps in eastern Massachusetts and total number of EEE virus and HJ (WEE) virus isolations 1971-1980. Minimum infection rate calculated per 1000 mosquitoes.

Species	Total Number Adult Female Mosquitoes	Total Virus Isolations and Minimum Infection Rate per 1000 Mosquitoes	
		EEE Virus	HJ (WEE) Virus
Culiseta melanura	266,099	323 (1.2)*	944 (3.5)
Coquillettidia perturbans	20,930	1 (0.05)	8 (0.4)
Aedes canadensis	16,714	0	8 (0.5)
Culex salinarius	15,668	1 (0.06)	7 (0.4)
Aedes aurifer	11,155	0	1 (0.1)
Culiseta morsitans	9,858	8 (0.8)	7 (0.7)
Culiseta silvestris	8,442	1 (0.1)	3 (0.4)
Aedes cinereus	6,628	0	0
Culex pipiens	4,637	2 (0.4)	3 (0.6)
Aedes vexans	4,277	0	4 (0.9)
Culex restuans	3,961	2 (0.5)	4 (1.0)
Uranotaenia sapphirina	3,798	1 (0.3)	1 (0.3)
Culex spp.	3,075	0	3 (1.0)
Aedes abserratus	2,988	0	0
Anopheles walkeri	1,860	0	0
Culex territans	1,524	2 (1.3)	5 (3.3)
Aedes cantator	1,186	0	0
Aedes excrucians Aedes Fitchii	1,159	0	1 (0.9)
Anopheles quadrimaculatus	848	0	1 (1.2)
Aedes triseriatus	428	0	0
Anopheles punctipennis	92	0	1 (10.9)
Total	385,330	341	1001

*Total number virus isolations (minimum infection rate per 1000 mosquitoes)

REPORT FROM THE UNIVERSITY OF NOTRE DAME'S LABORATORY FOR ARBOVIRUS RESEARCH AND SURVEILLANCE (UNDLARS), NOTRE DAME, INDIANA 46556

The Indiana State Board of Health-University of Notre Dame cooperative mosquito-borne encephalitis surveillance program in Indiana, now in its 5th year, began this season in early March and will continue into late October. To date (28 August) we have tested (by hemagglutination-inhibition) 6954 individual birds' sera for the presence of antibodies to eastern equine encephalomyelitis, western equine encephalomyelitis, and St. Louis encephalitis viruses. Of these, 37 were found by HI and confirming neutralization tests to have antibody specific to SLE virus and 1 to Highlands J virus (Figure 1)(a complete report of this season's work will be submitted for the March issue).

The most significant SLE virus activity to date has occurred in Knox County in the southwestern area of the state. Historically this region has seen a majority of human infections with this virus and Knox was the county of residence of all 7 persons with confirmed SLE virus infections in 1980 in Indiana. All resided in the town of Vincennes. This season the vast majority of house sparrows collected having significant HI and N antibody titers were taken in the Vincennes stockyards. Several other sparrows were collected in 2 other Knox County towns (at a hogmarket and 2 grain elevators).

The first seropositive sparrow was collected on 17 March in Vincennes; its reciprocal HI serum antibody titer was 320-640 (N titer was 128). Based on our previous 4 years of seroconversion and overwintering records of banded sparrows it is highly unlikely that this bird was infected in 1980 and we were seeing overwintering antibody. Climatic conditions may suggest why this is the earliest we have captured a seropositive sparrow with a recent infection evident. The weather in southwestern Indiana the preceeding few weeks was unseasonably warm with temperatures in the 60°F to 70°F range. The possibility of an overwintering infected mosquito feeding on this bird in the early warm March days must be considered as a likely explanation. After the middle of March temperatures returned to seasonal normals. No further seropositives were found in Vincennes until the first week in May; seropositives were then found for seven consecutive weeks thereafter.

Health authorities in Vincennes responded in May to the 10-25% seropositive rate in sparrows with an intensive Culex larvicide and a limited adulticide (thermal fog) program. Subsequently a 4-week sampling period (essentially the month of July) failed to detect further seropositive sparrows. However, beginning in early August a low-level seropositivity rate has been detected on a weekly basis in Knox County sparrows although the antibody prevalence rate has not approached the high rates observed in May and June.

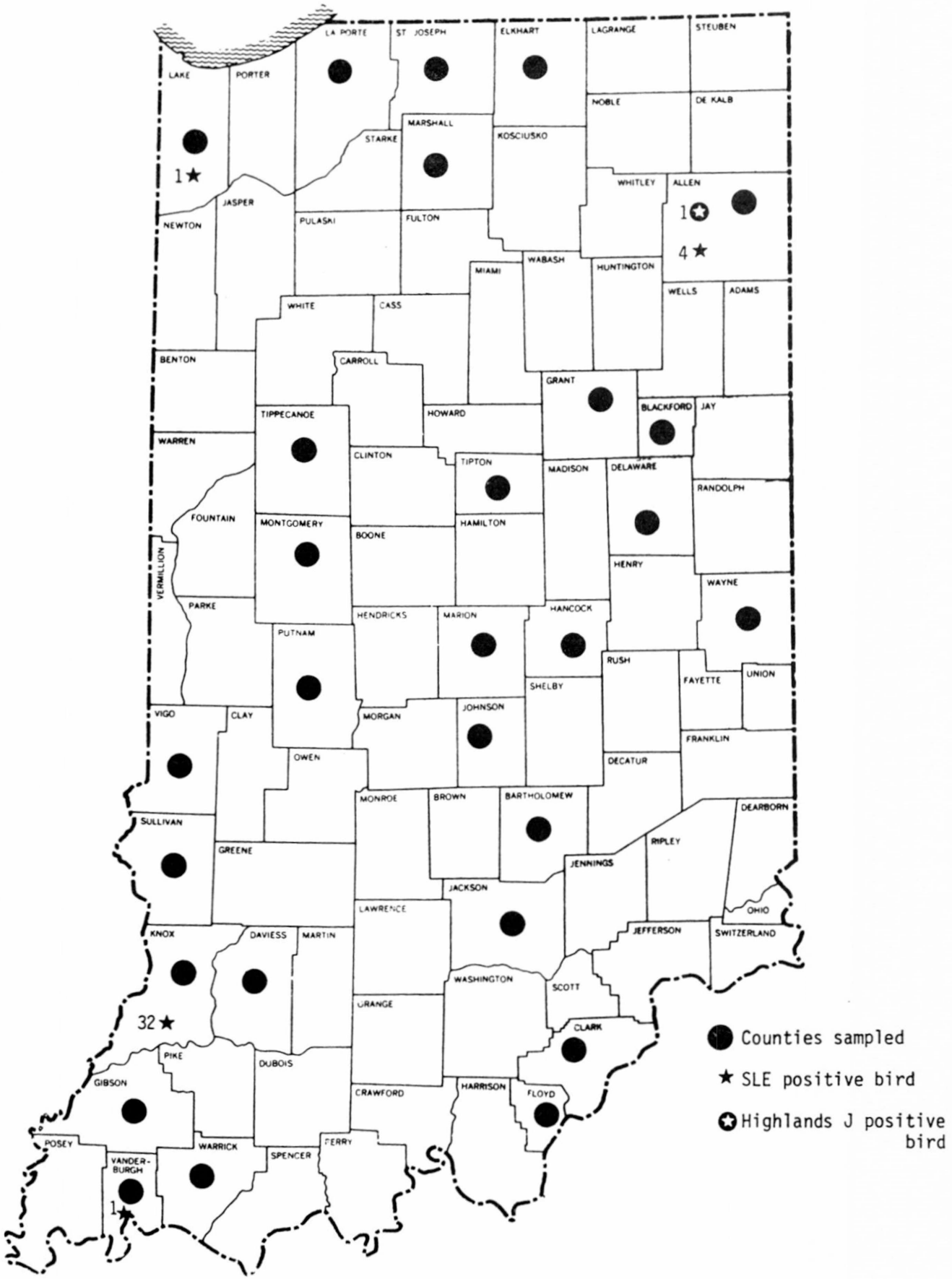
We are now beginning to see a suggestion of SLE virus activity in juvenile sparrows in northeastern Indiana (Allen County-Ft. Wayne) in late August. It remains to be seen if this will amount to any-

thing significant in the final weeks of this season.

The single sparrow with antibodies to Highlands J virus is the first evidence we have that this virus is present in the state. The sparrow was captured in late March and had low HI and N antibody titers suggestive of an infection in the past. This virus was detected in southern Michigan last fall during the outbreak of EEE. Perhaps this sparrow was infected at that time; the lack of movement of adult birds suggests the bird was infected in Indiana. Again this season EEE in equines is evident in southern Michigan. Although this virus has historically occurred in northern Indiana, and as recently as last fall when we made several isolations from horse brains, we have so far failed to see any EEE virus activity in Indiana this season. Virus isolation attempts have focused on Culiseta melanura collected from resting boxes in areas where EEE virus has been noted before.

Paul R. Grimstad (University of Notre Dame

Michael J. Sinsko (Indiana State Board of Health-Sanitary Engineering)


LAKE
PORTER
LA PORTE
ST JOSEPH
ELKHART
LAGRANGE
STEUBEN
NOBLE
DE KALB
MARSHALL
STARKE
KOSCIUSKO
WHITLEY
ALLEN
1★
1✪
4★
JASPER
NEWTON
PULASKI
FULTON
WABASH
HUNTINGTON
MIAMI
WELLS
ADAMS
WHITE
CASS
BENTON
CARROLL
GRANT
BLACKFORD
JAY
TIPPECANOE
HOWARD
WARREN
CLINTON
TIPTON
MADISON
DELAWARE
RANDOLPH
FOUNTAIN
MONTGOMERY
BOONE
HAMILTON
VERMILLION
HENRY
WAYNE
PARKE
HENDRICKS
MARION
HANCOCK
PUTNAM
RUSH
FAYETTE
UNION
VIGO
CLAY
MORGAN
JOHNSON
SHELBY
FRANKLIN
OWEN
DECATUR
MONROE
BROWN
BARTHOLOMEW
DEARBORN
SULLIVAN
GREENE
RIPLEY
JENNINGS
JACKSON
OHIO
LAWRENCE
KNOX
DAVIESS
MARTIN
JEFFERSON
SWITZERLAND
WASHINGTON
SCOTT
ORANGE
32★
CLARK
PIKE
DUBOIS
GIBSON
CRAWFORD
HARRISON
FLOYD
POSEY
WARRICK
SPENCER
PERRY
VANDER-
BURGH
1★
Counties sampled
★ SLE positive bird
✪ Highlands J positive bird

REPORT FROM THE ARBOVIRUS SURVEILLANCE PROGRAM

Division of Laboratories
Illinois Department of Public Health
Chicago, Illinois

As a result of budgetary difficulties arbovirus surveillance in Illinois during 1981 was restricted to collection and testing of avian sera for hemagglutination-inhibition (HI) antibodies to St. Louis encephalitis (SLE), Western Equine encephalitis (WEE), and Eastern Equine encephalitis (EEE) viruses. Through August 15, 25 of 3653 (0.7%) birds tested had HI antibodies to SLE virus (Table 1). This group consisted of 17 juvenile house sparrows (Passer domesticus)and 8 adult house sparrows. Antibodies were detected in Richland and St. Clair (adjacent to St. Louis, Missouri) Counties 3 weeks earlier than in 1980. Three human cases of SLE were identified in the St. Clair County area last year. The percentage positive from those two counties subsequently decreased through late July and mid-August. No human cases of SLE have been identified.

Two birds had antibodies to WEE virus. One was from Cook County (extreme northeastern Illinois) and the other was from St. Clair County.

A single human case of California (LaCrosse) Encephalitis has been confirmed. The individual was a 10 year-old female from Peoria County with an onset of July 21.

(Gary G. Clark & Harvey L. Pretula)

Table 1.

AVIAN SEROLOGY -ILLINOIS- 1981

REGION	COUNTY	Collection Interval: MAY		JUNE 1- 6		JUNE 21-27		JULY 5-18		JULY 19-25		Aug 2 - 8		Aug 9 - 15					
		HY*	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages	HY	All Ages
NORTH	COOK	0/2**	1/26	0/3	0/5	1/36	1/84***	0/278	0/375	0/3	0/6	0/339[a]	0/378						
	WILL																		
CENTRAL	CHRISTIAN			0/95	0/101	0/57	0/98			0/71	0/106			1/67	1/78				
	CUMBERLAND			0/84	0/105	0/43	0/93			0/36	0/85			1/118	1/122				
SOUTH	MADISON													0/119	0/120				
	ST. CLAIR			1/93	1/169	5/175	7/215			3/165	4/213			0/119[a]	0/135				
	RICHLAND			1/73	2/100	2/67	3/96			1/123	1/132			0/89	0/105				
	WHITE			0/26	0/82	0/60	2/110			0/28	0/111			0/90	0/100				
	HAMILTON									0/77	0/100			0/53	0/58				
	SALINE					1/57	1/83							0/46	0/62				
	TOTAL (PERCENT POSITIVE)	0/2	1/26	2/374 (0.5)	3/562 (0.5)	9/495 (1.8)	14/779 (1.8)	0/278	0/375	4/503 (0.8)	5/753 (0.7)	0/339	0/378	2/701 (0.3)	2/780 (0.3)				

*Hatching year; includes older nestlings, fledglings, and juvenile birds.

**Number positive (HAI antibody titer to St. Louis Encephalitis (SLE) virus of 1:20 or greater)/Number tested.

***Includes 10 birds from June 14-20.

[a]One bird with HAI antibody titer to Western Equine Encephalitis (WEE) virus of 1:20 or greater.

ANTIGENIC ANALYSIS OF A VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS (TC-83) USING MONOCLONAL ANTIBODIES

J. T. Roehrig, J. H. Mathews, J. W. Day, and R. M. Kinney

Monoclonal antibodies directed against the surface glycoproteins of TC-83 virus were used to analyze the antigenic structure expressed in a natural viral infection. Epitope maps were constructed using both cross-reactivity assays with closely related viruses, and an enzyme-linked competitive binding assay (Table 1). Representative monoclonal antibodies directed against each viral epitope were analyzed for biologic activity (hemagglutination inhibition and virus neutralization). Only antibodies directed against the $gp56^{c}$ epitope effectively block hemagglutination or virus infectivity (Table 2). Anti-$gp-50^{b}$ antibodies had very low levels of *in vitro* biologic activity. This activity was most likely due to the close spatial arrangement of the $gp56^{c}$ and $gp50^{b}$ epitopes which could be demonstrated in the competitive binding assay (Table 3).

Representative monoclonal antibodies directed against each epitope were used as antibody sources for passive immunization of 3-week-old outbred Swiss mice. Only anti-$gp56^{c}$ and anti-$gp50^{b}$ antibodies were effective in protecting the animals from an intraperitoneal challenge with 100 LD_{50} (20 PFU) of Trinidad Donkey virus (Trd). As little as 5 ug of antibody/mouse protected the animals from virus challenge. Greater than 10 ug of antibody protected animals from challenge with 10^{7} LD_{50} (2×10^{8} PFU) of Trd. Animals surviving passive immunization followed by virus challenge, in general, mounted a significant anti-virus antibody response when monitored 25 da. post-infection. Viremia studies of immunized and non-immunized animals following virus challenge indicated that the virus was sequestered in the spleen of the immunized animals, and failed to establish virus titers in either the serum or brain of the infected animals. Further studies analyzing the protective characteristics of both antigen and antibodies are currently in progress.

Table 1. Epitopes on gp56 and gp50 determined with representative monoclonal antibodies.

Representative Hybrid Cell Line	Isotype of Monoclonal AB	Epitope	Reaction of Monoclonal Antibody with Virus Subtype[a/]										
			TC-83	Trd	1B	1C	1D	1E	1F	2	3	4	5[b/]
5B4D-6	IgG2A	gp56[a]	+	-	-	-	-	-	-	-	-	-	-
2A4B-12	IgG2B	gp56[b]	+	+	+	-	-	-	-	-	-	-	-
3B4C-4	IgG1	gp56[c]	+	+	+	+	+	-	-	+	-	-	-
3B2D-5	IgG2A	gp50[a]	+	+	+	±	-	-	-	-	-	-	-
3B2A-9	IgG2A	gp50[b]	+	+	+	+	+	-	-	+	-	-	-
5B6A-6	IgG2A	gp50[c]	+	+	+	+	+	+	+	+	+	-	+
3A5B-1	IgG2A	gp50[d]	+	+	+	+	+	+	+	+	+	+	+
Epitopes Shared			7	6	6	4-5	5	2	2	4	2	1	2

a/ Differences significant at an $\alpha = 0.01$ level by two-tailed t-distribution.

b/ Proposed subtype. Virus strains: TC-83 (1A), Trd (1A), PTF-39 (1B), P676 (1C), 3880 (1D), Mena II (1E), 78V-3531 (1F), Fe3-7c (2), Mucambo (3), Pixuna (4), and Cabassou (5).

Table 2. Competitive binding assay mapping of gp56 and gp50 epitopes using representative monoclonal antibodies.

Epitope Specificity of Competitor[a/]	Competition Reaction with Enzyme-Conjugated Monoclonal Antibody					
	3A3D-9	3B4C-4	3B2D-5	3B2A-9	5B6A-6	3A5B-1
Controls						
Anti-VEE MHIAF	+	+	+	+	+	+
Control MHIAF	-	-	-	-	-	-
Tissue culture media	-	-	-	-	-	-
Anti-gp56[a]						
3A3D-9	+	+	-	-	-	-
5B4D-6	+	+	-	-	-	-
Anti-gp56[b]						
2A4B-12	+	-	-	-	-	-
Anti-gp56[c]						
3B4C-4	+	+	-	+	-	-
4A1C-3	+	+	-	+	-	-
Anti-gp50[a]						
3B2D-5	-	-	+	-	-	-
Anti-gp50[b]						
3B2A-9	-	+	-	+	+	+
Anti-gp50[c]						
5B6A-6	-	-	-	+	+	-
Anti-gp50[d]						
3A5B-1	-	-	-	+	-	+
3A1C-12	-	-	-	+	-	+

a/ All competing antibodies were purified from ascitic fluids and standardized to 1 mg/ml concentration. Monoclonal antibody 2A4B-12 was a tissue culture fluid.

Table 3. Cross-reactivities of the hemagglutinin and neutralization epitope determined with monoclonal antibodies 3B4C-4 ($gp56^c$) and 3B2A-9 ($gp50^b$)

	TITERS OF ASCITES[a]			
	HI[b]		N[c]	
Subtype	$gp56^c$	$gp50^b$	$gp56^c$	$gp50^b$
1A (TC-83)	1×10^6	80	1×10^5	10
1A (Trd)	1×10^6	80	1×10^5	<10
1B	1×10^6	160	1×10^5	10
1C	1×10^6	160	1×10^5	10
1D	3×10^4	40	100	<10
1E	<10	40	10	<10
1F	<10	40	10	10
2	160	80	1×10^4	10
3	<10	<10	10	10
4	<10	40	<10	<10
5[d]	<10	20	<10	<10

a/ Ascites contained 7 mg/ml in the HI test and 0.7 mg/ml in the N test.

b/ HI was done against 4 HA units of antigen.

c/ N test was done by plaque assay with 20-60 plaque-forming units (pfu)/test and 70% plaque reduction end-points.

d/ Proposed subtype.

Comparison of the Effectivenss of Malathion Thermal Fog and ULV Applications and of Malathion ULV Applications with and without Addition of Heavy Aromatic Naptha (HAN) Against Caged Adult Female Aedes aegypti in Puerto Rico

During 1979 and 1980 seven applications each of ultra low volume (ULV) and thermal fogs of malathion were made in residential areas of Puerto Rico where dengue cases had been reported. Both methods of application were made in the same residential areas on different evenings to reduce the effect of the area on final results. The mean mortality of caged mosquitoes exposed to thermal fogs in outdoor locations was slightly higher than that recorded for ULV sprays (Table I) and in indoor locations the mortality obtained with thermal fogs was more than double that recorded with ULV sprays. Although the malathion dosage was greater with thermal fogs than with ULV applications, the increased volume of the thermal fog (40 gal/hour compared to 2 gals/hour with ULV) was considered to be the most likely factor contributing to their increased effectiveness.

In an attempt to improve the performance of ULV, especially in indoor locations, tests were conducted in Puerto Rico in 1981 in which the volume of ULV applications was increased by addition of HAN in a ratio of 1 part malathion:2 parts HAN. Comparison of mortalities obtained using caged adult Aedes aegypti exposed to malathion alone or to the malathion-HAN mixture (flow rate adjusted to provide the same dosage of malathion in both cases) are shown in Table II. There was a slight increase in mortality in outdoor cages and a substantial (69%) increase in mortality in indoor cages exposed to the malathion-HAN mixture compared to results obtained with malathion alone.

Detailed analysis of data is underway to evaluate the impact of wind velocity and position of cages in relation to their distance from the delivery point of the insecticide.

The data presented here indicate that especially in indoor locations, malathion thermal fogs were more effective than conventional ULV malathion applications against caged adult Aedes aegypti and that the addition of HAN to malathion improved the performance of ULV applications both outdoors and indoors.

(Donald A. Eliason)

I. Mortality of Caged Female Aedes aegypti Exposed to Malathion ULV and Thermal Fog Application. Puerto Rico, 1979 and 1980.

	Percent Mortality*	
	Cages Placed Outdoors	Cages Placed Indoors
ULV	66.6	31.1
Thermal Fog	79.4	66.9

*Mean % mortality from 7 applications with each type of application.

II. Mortality of Caged Female Aedes aegypti Exposed to Malathion ULV Applications with and without Dilution with HAN*. Puerto Rico, 1981.

	Percent Mortality**	
	Cages Placed Outdoors	Cages Placed Indoors
Malathion alone	63.6	37.6
Malathion + HAN (1:2)	82.5	63.7

* Heavy aromatic naptha.

**Mean % mortality from 6 applications wityh each type of formulation.

REPORT FROM THE VIRAL AND RICKETTSIAL DISEASE LABORATORY,
STATE OF CALIFORNIA DEPARTMENT OF HEALTH SERVICES, BERKELEY, CALIFORNIA

STUDIES OF A PERSISTENT FOCUS OF MODOC VIRUS

Addendum: ABV Information Exchange, Number 39, September 1980, pages 166-167

Subsequent studies of the isolate from the #5 Peromyscus maniculatus mouse trapped April 25, 1980, which was identified as Modoc virus by the FA test, has revealed that it contained also a paramyxovirus. The original mouse brain specimen that was positive for Modoc virus by the FA test, was not saved. The kidney cell cultures of the #5 Peromyscus mouse were maintained through the summer by occasional changes of the maintenance medium. The culture fluid was positive for a virus when it was tested in November 1980. Mice immunized with the late harvest material were completely susceptible to infection with Modoc virus. Although the cell culture tested at the 13th day was negative by the hemadsorption test with guinea pig red blood bells, the subculture of the virus in BHK cells was positive. It produced syncytia in this system. FA tests were negative for Modoc virus, Parainfluenza 1 and 2 viruses and for a paramyxovirus isolated previously in this laboratory.

In the study of small mammals at Hackamore Station in May 1980, primary kidney cell cultures were made from 6 small mammals, trapped the same day. Five were Peromyscus mice and one was a Perognathus parvus pocket mouse. The Perognathus mouse kidney cell culture was positive for a hemadsorbing virus. The other kidney cell cultures, including the #5 Peromyscus kidney cell culture, were negative by the hemadsorption test at two weeks. This suggests that there was an epizootic infection with a paramyxovirus, involving more than one species. We have not done any studies to compare the two hemadsorbing viruses at this time. A specimen of the #5 Peromyscus kidney cell culture fluid from the 29th day supernate was tested in infant mice in January 1981. The mice sickened at the 8th day and two were tested by the FA test. Both were negative for Modoc virus. Having had one definite FA positive for Modoc virus in the original test, it seems likely that Modoc virus was present in the kidney cell culture but we cannot consider this a valid isolation of Modoc virus.

It was noted in the previous report that Modoc virus was isolated from kidney cell cultures of Peromyscus mice collected at Hackamore Station, June 8, 1970. This isolate was identified by the serum-virus neutralization test, which has been used for the specificity test in all previous isolations of Modoc virus. We did isolate Modoc virus from a pool of salivary glands, lungs and kidneys of a Peromyscus mouse trapped at Hackamore Station, May 17, 1973. This was a direct test of the organ suspension. This isolate was identified as Modoc virus by the serum-virus neutralization test. This proved the persistence of Modoc virus at Hackamore Station for a period of 15 years.

(Dr. Harald N. Johnson)

REPORT FROM THE ARBOVIRAL RESEARCH UNIT
SCHOOL OF PUBLIC HEALTH
UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA

Vector Competence and Genetic Control of Mosquitoes of Public Health and Veterinary Consequence in California

Epidemiological surveillance

Surveillance of WEE and SLE viral activity in California assumed a high priority in our 1980 research. Surveillance had 2 principal applications: to anticipate and record factors that lead to epidemics and to document the need for a continuing effective level of vector control. Opportunities to observe the events associated with decreased vector control due to a loss of tax revenue added to the scientific data base and improved our epidemiological knowledge of encephalitis. To assure the continuation of an effective program, we assigned a technician into Dr. R. Emmons' laboratory for 6 months to help test 3,844 mosquito pools for virus infection from representative regions of California distributed from Imperial County to Shasta County.

Weekly reports of viral isolations were distributed by the State Department of Health Services. WEE virus was isolated from most areas that were sampled intensively. The first isolations were from 2 pools collected May 21 at Needles and WEE virus persisted in Southern California all summer. WEE virus first appeared in the San Joaquin Valley (Kern County) in June but was not detected in the Sacramento Valley area until July 16 in Colusa County. SLE viral isolations were limited to Southern California in July and August. Numerous isolations of California encephalitis (CE), Turlock and Hart Park viruses were made from mosquitoes collected in many parts of the state.

Tests of bloods from 31 flocks of sentinel chickens revealed that WEE virus had infected birds in Imperial County by July but was not widely active in the Central Valley until late August. The highest WEE antibody conversion rates were in flocks at sites in Kern and Imperial Counties, 86 and 69% respectively. Overall, the WEE antibody conversion rates were lower than in 1979, and this observation correlated with the reduced mosquito light trap indices and below normal temperatures that were reported almost statewide. The only bird to develop SLE antibody was in a sentinel flock in Riverside County.

No confirmed WEE or SLE cases in humans were reported from California in 1980 although there was 1 SLE case from Nevada. We will retest for a variety of antibodies the paired sera from the 69 suspect human clinical cases of encephalitis that were not diagnosed to be WEE or SLE. Two cases of WEE were confirmed in horses, 1 each from Placer and Kern Counties. We will retest sera from horse cases that could not be diagnosed for infection with other known causes of encephalitis in equines such as Turlock and Main Drain viruses.

An essential part of the surveillance program was to monitor vector population data weekly. A light trap station was maintained at each of the 31 sentinel chicken flock sites in 13 Mosquito Abatement Districts. Data were entered into a computer bank, summarized weekly and reports sent to the Districts. Culex tarsalis populations were below 1979 levels at most sites in 1980, and fewer chickens overall developed antibodies to WEE virus in 1980 (15% as compared to 40% in 1979). A general pattern of moderate Cx. tarsalis populations was reported throughout the state by mosquito control agencies.

In summary, the high level of viral activity predicted for 1980 did not occur even though it was a year of water surplus. Contributing factors to this low level were unusually cool temperatures in the early summer and a delayed production of high vector populations; the availability of over $3,000,000 in emergency funds for vector control provided by the state legislature; and a concentration of efforts to control Cx. tarsalis by the mosquito control agencies.

Vector competence studies during 1980

Four vector competence studies were undertaken in Kern County: 1) monthly monitoring of the Cx. tarsalis population in an isolated locality, Poso West, for susceptibility to infection with WEE and SLE viruses by the pledget feeding technique, 2) comparison of different geographic strains of Cx. tarsalis and Cx. pipiens pipiens or Cx. pipiens quinquefaciatus for susceptibility to infection with SLE virus, and occasionally to WEE virus, by pledget feeding, 3) simultaneous comparison of Imperial Valley and San Joaquin Valley populations of Cx. quinquefasciatus for susceptibility to infection with SLE virus and ability to transmit virus by bite after a 14-21 day extrinsic incubation period after feeding on viremic chickens infected with an Imperial Valley or a San Joaquin Valley isolate of SLE virus, and 4) simultaneous evaluation of collections of Cx. tarsalis and Cx. quinquefasciatus for susceptibility to and subsequent ability to transmit 5 strains of SLE virus that had been isolated from widely separated areas of the United States.

There was no difference in the susceptibility of San Joaquin Valley and Imperial Valley populations of Cx. quinquefasciatus to infection with 2 strains of SLE virus by the viremic chick feeding technique. These findings confirm earlier studies using the pledget feeding technique. Re-feedings of mosquitoes on normal chicks in viral transmission attempts were poor, but the data suggest that the 2 populations transmitted SLE virus with similar efficiency. Thus, differences in SLE viral activity in the San Joaquin and Imperial Valleys were not explained by differences in the vector competence of Cx. quinquefasciatus from the 2 areas.

Data on the infectivity and transmissibility of different geographic strains of SLE virus by mosquitoes were interesting. Cx. tarsalis was more susceptible than Cx. quinquefasciatus to infection with San Joaquin Valley (California), Imperial Valley (California), Texas and Maryland strains of SLE virus and was equally susceptible to infection with an Indiana strain of SLE virus. The Indiana, Texas and Maryland strains of SLE virus were more infectious for Cx. quinquefasciatus than were the 2 California strains of SLE virus, particularly the Imperial Valley strain. More data are needed to substantiate this observation. However, if true, these observations would partially explain the observation that Cx. quinquefasciatus and other members of the Cx. pipiens complex are not important vectors of SLE virus in California, whereas, they are the most important vectors of SLE virus in other areas of the country.

Studies at the Naval Biosciences Laboratory in Oakland continue to focus on intrinsic factors and mechanisms that affect the competence of mosquitoes to be vectors of arboviruses. We previously reported that a high proportion of female Cx. tarsalis that became infected after ingesting low concentrations of WEE virus were unable to transmit virus because 2 barriers prevented the escape of virus from the infected mesenteron (mid-gut) or the invasion of and/or multiplication of virus in the salivary glands. A study was done during the past year to determine if the incubation period in the vector affected the expression of the mesenteronal escape and salivary gland infection barriers. It was assumed that these barriers would not be expressed at higher temperatures even though this would increase the rate of viral multiplication. To our surprise, females incubated at 32C were less efficient transmitters of WEE virus than were females infected on the same virus source but incubated at 18C or 26C. In fact, females incubated at 32C eliminated the virus.

The observations add credence to our concept that mosquitoes possess mechanisms that enable them to modulate arboviral titers in their tissues/cells if they are not initially overwhelmed with a high infection dose of virus. Further, these observations explain earlier epidemiologic observations that WEE epidemics frequently occur in years when springtime temperatures are unusually low and that WEE viral infection rates in Cx. tarsalis and clinical cases in humans and horses decrease significantly during peak summer temperatures. Since just the opposite is true for SLE virus, one would expect the vector efficiency of mosquitoes for SLE virus to increase as the extrinsic incubation temperature is increased. Studies with SLE virus have been initiated to investigate this hypothesis. An early finding in this study is that transovarian transmission of SLE virus to larval Cx. tarsalis is as high as 1:50 larvae infected when incubated at 18C. Earlier studies at higher temperatures had indicated that transovarian infection was very rare.

California encephalitis (CE) virus was isolated originally from Kern County in 1943. Closely related viruses have caused significant numbers of encephalitis cases in persons in midwestern and eastern states. Representatives of the California complex of viruses were shown to be transmitted by infected Aedes mosquitoes to their progeny in studies in Wisconsin and Maryland. A PhD candidate (M. Turell) undertook a study to determine if this occurs in the vectors in California. CE virus was transmitted transovarially to approximately 20% of the progeny of infected female Aedes melanimon. This species is the primary vector of CE virus in the Central Valley of California. Infection rates in progeny were the same if parental females were infected by feeding on virus or were inoculated intrathoracicly. Storage of eggs at 4, 22 and 27C for up to 9 months, or freeze-thawing, did not affect the infection rates in the eggs. Thus, virus survived in transovarially infected eggs for sufficient time to allow infection to occur in generations that hatch from overwintering eggs the following spring or summer. No increases in mortality occurred in infected eggs, larvae, or adults; however, infected larval Ae. melanimon took slightly longer to develop into pupae than did uninfected larvae.

Aedes dorsalis from a colony derived from a field population collected in the salt marshes of the San Francisco Bay Area also transovarially transmitted CE virus whether females were infected orally, or by inoculation. An effort to select a high transmitting line of Ae. dorsalis was successful, the filial infection rate in the F_5 generation was 93%. Backcrosses with uninfected males indicated that this high transmission rate was due to cytoplasmic, rather than genomic inheritance. Female F_4 mosquitoes from this line successfully transmitted virus by bite, suckling mice died 4 days after they had been bitten, and virus was recovered from their brains. Thus, even after 4 consecutive transovarial passages, CE virus remained highly virulent for mice.

It was noted that Aedes and other mosquitoes infected with CE and some related viruses were sensitive to CO_2. This observation was published in Science and has attracted considerable interest.

Field studies on the bionomics of Culex tarsalis

Five mark-release-recapture studies were done at Breckenridge, a Kern County oil field waste-water disposal area, to estimate Cx. tarsalis life table characteristics and population size in the area where a genetic control evaluation with sterilized males was in progress. Mean daily loss rates from the population varied from 14 to 30% for females and from 18 to 24% for males. Sweep net collections of swarming males shortly after sundown markedly improved our ability to recapture marked males as compared to shelter and light trap collections, and when used routinely, accounted for as high as 46% of the total males recaptured. An unusually high ratio of females to males was observed in the Breckenridge population during this study and efforts to determine the cause led to material being referred to our colleagues in the genetic section, Drs. Asman and Stoddard. They determined that Amblyospora californica infection was a

possible cause of the aberrance.

Males that had been reared from pupae collected at Poso West and irradiated to sterilize them were released at Breckenridge daily or every other day from 17 June through 28 August. One-third of the 71,016 males released were marked to facilitate estimates of mortality, dispersal and the ratio of released to wild-type males. The mean daily loss rate from the field population for these sterile males was 18%, and the proportion of released males in the total male population varied from 3 to 13%. A total of 47,019 female and 8,465 male Cx. tarsalis were collected from the field population during the study. Egg rafts from 2,619 field-collected females were examined for hatch and embryonation rates: less than 10% had mated with sterile males. The principal reason for the failure to achieve significant decreases in population was low ratio of sterilized to native males in the populations.

There is a highly significant correlation between the wing length of Cx. tarsalis females and the size of their egg rafts. This observation is based on examination of more than 2000 raft-female pairs collected at the Breckenridge site in Kern County from late May through the end of September 1980. Wing length and raft size are both negatively correlated with temperature; when temperatures are high, females are smaller and lay fewer eggs. Earlier studies had indicated that adult mosquito size was influenced by population density. However, the Breckenridge population remained at moderate levels throughout the summer, and no significant relationship between density and wing length or raft size was detected. Hatch rate also appears to be correlated with raft size, wing length and temperature. Further investigations are needed to determine the nature of these relationships.

An extensive study was continued to determine if enzymes can be identified as markers to differentiate adult populations of Cx. tarsalis and Cx. pipiens that vary in their vector competence or that represent geographically isolated subpopulations. During 1980 these studies have included representative field samples collected for vector competence studies from Imperial Valley northward to Sutter-Yuba Counties, and all the colonies that are being maintained for vector competence studies or for genetic studies.

Computer and biostatistical consultation services

We have obtained data that will improve our ability to predict levels of viral activity that will occur in California. The 1980 experience further clarified the interplay of factors such as water availability and temperature. Vector competence studies provided new data on the effect of temperature on the multiplication and transmission of WEE virus in Cx. tarsalis and will add a major refinement in the prediction model. New information on the vector competence of Cx. tarsalis and Cx. pipiens for SLE and the influence of different viral strains on vector infection can now be included in a prediction model.

Finally, in the computerized projections of the efficiency of alternative approaches to control Cx. tarsalis populations, new data are available. Collaborative projects may provide life table data on Cx. tarsalis females from Imperial and Sutter-Yuba Counties. Studies in the Breckenridge study area of Kern County have revealed a reduced mortality or loss rate from vector populations where there is increased shade from trees and tall aquatic vegetation. The first good data on persistance of male Cx. tarsalis in a field population were obtained in 1980. A second year's data were obtained on the release of sterilized males in the field. A new variable was recorded and must be considered, namely, the effect of Am. californica in reducing the male to female ratio in Cx. tarsalis field populations.

(William C. Reeves and James L. Hardy)

REPORT FROM THE DIVISION OF MEDICAL MICROBIOLOGY, UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, CANADA V6T 1W5

During June and July 1981, unengorged female mosquitoes of 4 species were collected at locations throughout the Yukon Territory, Canada, in boreal forest terrain extending from 61 to 66°N. Of the initial 782 mosquitoes from 4 species which were collected at 4 locations, strains of snowshoe hare (SSH) virus (California encephalitis group) were recovered from 1 of 174 Aedes communis and 1 of 49 A. hexodontus collected at Marsh Lake (61°N, 134°N) on 20 July (week #29). Previous SSH isolations were achieved from A. communis collected there during 1972, 1973, 1978 and 1980, during week #24 through week #29, but this is the first occasion that A. hexodontus collected there has yielded an SSH isolate. Throughout the Yukon Territory and the adjacent Mackenzie Valley of the Northwest Territories from 1972 through 1980, 107,782 mosquitoes of 6 species have yielded 42 SSH isolates plus 4 isolates of Northway virus (Bunyamwera group).

(Donald M. McLean).

REPORT FROM THE NATIONAL ARBOVIRUS REFERENCE SERVICE,
DEPARTMENT OF MEDICAL MICROBIOLOGY,
UNIVERSITY OF TORONTO,
TORONTO, ONTARIO, CANADA.

Dogs as Monitors for Arbovirus Activity in Ontario.

Dogs have been identified as hosts of ticks associated with two disease producing agents isolated previously in Ontario - Franciscella tularensis and Powassan virus. In view of this fact, our laboratory initiated tests to determine the extent of infection of dogs in various parts of Ontario with F. tularensis and several arboviruses including Powassan, St Louis encephalitis, Snowshoe hare, eastern equine encephalitis and western equine encephalitis. To date, 407 sera have been tested.

Results of these tests are presented in Table 1. It may be seen that one of 157 sera tested against F. tularensis yielded complement fixing antibodies. This reactor was one of eleven dogs from the Six Nations Reserve in southern Ontario - an area in which five isolates of F. tularensis were obtained from D. variabilis ticks collected in 1979.

Flavivirus antibodies were detected in eight dogs with eight hemagglutination inhibition reactors to Powassan and five hemagglutination inhibition reactors to St Louis encephalitis antigen. However all five St Louis encephalitis reactors cross reacted at higher titer with Powassan antigen indicating that all eight dog reactors were infected with Powassan virus. Over ten per cent of the dogs tested had hemagglutination inhibiting antibodies to Snowshoe hare antigen, thus confirming the extensive distribution of this California group virus in Ontario. No alphavirus reactors were obtained.

The results of our study confirm that dogs may serve as monitors for the distribution of two arboviruses known to be endemic in Ontario - Powassan and Snowshoe hare. In addition the single dog reactor to F. tularensis in an area where the organism has been isolated previously is further evidence for F. tularensis activity in at least one localized area of Ontario.

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Table 1

Testing of dog sera from Ontario for *Franciscella tularensis* and arbovirus antibodies.

AGENT TESTED	SEROLOGICAL TEST[1]		
	HAI	CF	NEUT
Franciscella tularensis	--	1[2]/157[3](0.64)[4]	--
Powassan	8/407(1.97)	1/2[5]	--
St Louis encephalitis	5/407(1.23)	1/5	--
Showshoe hare	42/407(10.32)	20/23	7/8
Eastern equine encephalitis	0/407	--	--
Western equine encephalitis	0/407	--	--

1. HAI = hemagglutination inhibiting antibodies; CF = complement fixing antibodies; NEUT = neutralizing antibodies.
2. Number of sera positive for anitibodies.
3. Number of sera tested.
4. Per cent positive reactors.
5. For arbovirus antigens, CF and NEUT testing was undertaken only on HI positive sera.

REPORT FROM THE ARBOVIRUSES AND DANGEROUS EXOTIC PATHOGENS LABORATORY
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California and Powassan Virus Disease in Ontario, Canada 1977-80

California Encephalitis Virus (CE)

The widespread presence of California encephalitis (CE) virus - Snowshoe hare (SSH) serotype in Canada has been demonstrated. The virus was isolated from various species of mosquitoes in Ontario, Quebec, Alberta, British Columbia, Saskatchewan and the Northwest and Yukon Territories (1). In a neighbouring state, Alaska, 10/94 persons living near Northway showed seroconversion during the summer of 1970 (4).

Serological surveys for the natural distribution of SSH in its primary reservoir, the snowshoe hare (Lepus americanus), showed that antibodies to this virus can be detected in hares throughout Canada. Evidence has been reported for the natural infection of other small mammals which seem to play a role in the natural occurrence of SSH in Canada, and of other mammals such as marmots, porcupines, raccoons, coyotes, moose, cattle and horses. The virus has also been isolated from indicator rabbits in Ontario and from snowshoe hares in Alberta. Serological data indicate also that human exposure to the CE group virus occurs in many parts of this country.

In spite of all these findings the role of SSH in human disease was not identified until 1978 when Fauvel et al reported the association of SSH virus with acute CNS disease of three patients in Quebec (2).

We report here the first three cases of California encephalitis virus SSH serotype associated with acute CNS disease in Ontario. Table 1 is a summary of these cases.

A 12 year old brother of Case 3 had a history of headache following being at a park with the family. This brother had static HAI and neutralizing antibody titres to SSH. Fractionation of his sera by sucrose density gradient centrifugation showed that both HAI and neutralizing antibodies to SSH were resolved in the IgM fraction. Four other members of the family had no detectable antibodies to this antigen.

During the period between 1977 to 1980, 2492 cases with suspect or definitive acute CNS disease were investigated at the Central Laboratories, Ontario Ministry of Health; 24 individuals were identified as reactors to CE group virus (es) by the HAI, CF and/or neutralization tests. With the exception of Cases 2 and 3, discussed above (Table 1), none showed evidence of seroconversion by any of these three serological techniques except a case of a 50 year old male. He had a history of headache, myalgia and malaise; seroconversion to SSH antigen was demonstrated by HAI but could not be confirmed by complement fixation and neutralization of SSH, La Crosse, Jamestown Canyon and Trivittatus viruses. Neutralizing antibodies were detected however to SSH (1:80 and 1:40) and to LAC (1:20 and 1:40); this activity could not be resolved in the IgM fraction of the acute serum. Members of the patient's family had no detectable antibodies.

In view of these results, the data on abundance of mosquito species in the patient's area and the time of early onset of illness (May 12,1980), the patient's disease could not be considered as associated with recent infection with CE group virus.

Powassan Virus

Since 1977 we have identified or confirmed five cases of Powassan virus encephalitis; three of them occurred in Ontario. A summary of these three cases is given in Table 2. To our knowledge, this brings the total number of cases recorded in North America to 19; five in Ontario, three in Quebec, Canada; ten in N.Y. State (one presumptive and nine confirmed) and one in New Jersey, USA. One of the N.Y. State cases (No. 16) was imported to and identified in Canada. Table 3 updates our earlier communications on this tick-borne arbovirus disease (3, 5).

Twelve patients were males and seven were females. Fifteen out of the nineteen cases were children within fifteen years of age. In Case 1 (Table 2), two cats and one dog belonging to the patient's family had HAI antibodies to Powassan antigen and in Case 2 (Table 2) five chipmunks and nine dogs were bled and tested; four dogs had HAI antibodies. Case 3 (Table 2) occurred in December 1979, the most seasonally late record of this disease.

In the nineteen cases there were two fatalities and seven patients with severe sequelae. In the absence of follow-up information about five patients (Table 3); the percentage of cases resulting in

death or with serious neurological complications is 47% or higher. Therefore, Powassan virus should be considered as a highly neurovirulent agent. Serological surveys suggest that human exposure may be geographically spread beyond the areas where the disease was identified, however, results of studies in Northern Ontario, and in British Columbia, Canada and review of serological results on 3100 cases with acute CNS in New York State show that infection with Powassan is not widespread. The percentage of positive reactors to Powassan virus and/or antigen ranged from less than one percent in New York State to about 5% in Manitoulin Island in Northern Ontario.

The possibility of Powassan disease in cases of acute CNS with indeterminate aetiology should be considered. Furthermore, because of the potential severity of this disease, laboratory work involving live virus should be done under adequate containment of this hazard. Presently we are trying to investigate the incidence of Powassan antibodies in individuals with histories of tick bite and to obtain a pool of positive sera that might be needed in cases of laboratory exposure to Powassan virus.

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TABLE 1 HUMAN DISEASE WITH CALIFORNIA GROUP VIRUS(ES)
ONTARIO, 1978 - 80

CASE	DAYS AFTER ONSET	ANTIBODY TITRE					
		HAI		CF		NEUT	
		SSH[a]	LAC	SSH	LAC	SSH	LAC
(1) 1978 Male, 30 year old. Onset 19/6/78, frontal headache malaise, nausea, chills, rigors, photophobia and vomiting. Diagnosis: aseptic meningitis Area: Bracebridge	7	160	160	16	4	320	80
	11	320	320	32	4	640	320
	17	320	320	32	4	640	320
(2)[b] 1979 Male, 10 year old. Onset July 1979, fever, consistent headache, rash, may have had seizure. Diagnosis: headaches Area: Chatham	25/7/79[c]	20	NT[d]	8	NT	80	20
	15/8/79[c]	160	NT	8	NT	640	160
(3) 1980 Female, 9 year old. Onset 15/6/80, vomiting, Diagnosis: viral meningitis Area: Stratford	5	320	NT	4	NT	40	20
	22	640	NT	16	NT	1280	40
	45	320	NT	16	NT	80	10

Footnotes:

a. SSH: Snowshoe hare, LAC: La Crosse

b. Patient showed seroconversion to measles by CF (32 to 256). Did not receive measles vaccine

c. Date sera were obtained from patient, exact date of onset unknown

d. Not tested

TABLE 2 POWASSAN (POW) VIRUS ENCEPHALITIS IN ONTARIO, 1977 - 80

CASE	DAYS AFTER ONSET	ANTIBODY TITRE	
		HAI	CF
(1) Female, 13 month old, fever, anorexia, lethargy macular erythematous rash, stiff neck and seizures: Onset 18/8/77 Diagnosis: meningoencephalitis Tick bite: 28/7/77 Area: Kingston	6	<10	<8
	42	160	128
	63	160	128
(2) Female, 18 year old, headache, fever, speech difficulty, unable to communicate, mental confusion. Onset 30/5/79 Diagnosis: meningoencephalitis Tick bite: 21/5/79 Area:Northeast of Peterboro	4	<10	<4
	9	<10	16
	12	10	32
	19	20	32
(3) Male, 8 year old, fever, vomiting, anorexia, headache, lethargy, Diagnosis: encephalitis Onset: 25/12/79 No history of tick bite Area: Near Kingston	2	<10	<8
	17	80	64
	23	80	128

TABLE 3 REPORTED CASES OF POWASSAN VIRUS DISEASE IN NORTH AMERICA

Case	Date of onset	Place	Patient Age	Patient Sex	History of bite	Sequelae in 1 year after illness	Reference
1	Sept. 1958	Powassan, Ont.	5	M	?	Death	McLean, D.M., Donahue, W.L. Can. Med. Assoc.J.80:708, 1959
2	June 1970	New Jersey	57	F	?	Recurrent severe headaches	Goldfield, M. et al, Am. J. Trop. Med. & Hyg. 22:78,1973
3	July 1971	Green County, N.Y. State	7	M	Tick, 1 week incubation	None	Smith R. et al, Am. J. Dis. Child., 127:691, 1974
4	June 1972	Warren County, N.Y. State	1.2/12	M	?	Left hemiplegia & unable to walk	"
5	July 1972	Oneida County, N.Y. State	12	M	Blackfly	None	"
6[a]	July 1972	Washington County N.Y. State	5	F	?	None	"
7	Oct. 1972	Sherbrooke, Quebec	8	M	Tick,1 month incubation	Right hemiplegia	Rossier, E. et al, Can. Med. Assoc. J., 110:1177,1974
8[b]	Aug. 1974	N.Y. State	6-15	M	Not stated	?	Deibel, R. et al, N.Y. State J. Med. 75:2337, 1975
9	Oct. 1975	West Quebec	3	M	?	Right hemiplegia,right facial weakness, wasting right shoulder muscles.	Conway, D., Rossier, E. Can.Dis.Wkly.Rep.2,22:85, 1976

TABLE 3 cont'd.

Case	Date of onset	Place	Patient Age	Patient Sex	History of bite	Sequelae in 1 year after illness	Reference
10	Summer 1975	N.Y. State	6-15	M	Not stated	?	Deibel, R. et al, N.Y. State. J. Med.77:1398, 1977
11	Summer 1975	N.Y. State	6-15	M	Tick, week before illness	?	Deibel, R. et al, N.Y. State. J.Med. 77: 1398,1977
12	Summer 1975	N.Y. State	82	M	Not stated	Death	Deibel, R. et al, N.Y. State J. Med. 77:1398, 1977
13	Aug. 1976	Niagara, Ont.	15	F	? Mosquito	None	Rossier, E. Can. Dis. Wkly. Rep. 2:202, 1976
14	Aug. 1977	Kingston, Ont.	1.1/12	F	Tick 3 weeks incubation	Spastic quadriplegia	Wilson, M.S. et al, Can. Med. Assoc. J. 111:210, 1979
15	1977	N.Y. State	0-5	F	Not stated	Unknown	Deibel, R. et al, Am.J.Trop. Med & Hyg. 28:577, 1979
16	Before Aug. 1978	N.Y. State (Identified in Nova Scotia,Can)	8	M	None contacted woodchuck	Unknown	Embil, J.A. et al, 1980 Can. J. Pub.-Health, 71:135, 1980
17	June 1979	Peterborough, Ont.	19	F	Tick, 9 days incubation	Quadriplegia	Joshua, J.M. et al & Crapper, D.R. et al Can.Dis. Wkly.Rep. 5:129, 1979
18	Dec. 1979	Kingston, Ont.	7	M	Not stated	Expressive aphasia & spastic quadriplegia	Darlington, M.W. et al, Can. Med. Assoc. 123:603, 1980
19	June 1980	West Quebec	9	F	Insect	Paralysis	Fauvel, M. Personal Communication

a. Presumptive laboratory diagnosis not confirmed as follow-up specimens could not be obtained.

b. Presumptive diagnosis, defined by authors as four-fold or greater CF antibody decline or unusual antibody.