



ARTHROPOD-BORNE VIRUSES INFORMATION EXCHANGE

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

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Death of Professor Dionýz Blaškoviè (November 17, 1998)

Professor Dionýz Blaškoviè, the founder of Czech and Slovak virology, passed away November 17, 1998 after a long illness. Dionýz was born August 2, 1913 in Jablonica, a small town in western Slovakia, the second of three children of a high school teacher. Soon thereafter the family moved to Banská Štiavnica in the heart of the mountains of Central Slovakia. There Dionýz received his basic education, strongly influenced by the natural beauties and ancient history of the nearby medieval gold mining areas.

Dionýz Blaškoviè studied medicine at Charles University in Prague where he became a Doctor of Medicine in February, 1931. During his studies and after graduation he became involved in microbiological research and in the diagnosis of infectious diseases. He worked at the Institute of Bacteriology and Serology of the Faculty of Medicine, Charles University. This was an important period of his life, one which oriented him towards microbiology. In the period June, 1939 - September, 1946 he became a research assistant of the Institute of Hygiene in Bratislava. In November, 1945 he was appointed Associated Professor of Microbiology of the Medical Faculty of Comenius University, Bratislava.

Then came another crucial period in his scientific life. In 1946-47 he obtained a one-year Rockefeller Foundation for Public Health fellowship in the USA. He arrived there as an ambitious microbiologist and returned home as a well educated virologist. He considered himself fortunate to have been able to visit and work in the best US virological laboratories of that time and to meet such virologists as Max Theiler, William C. Reeves, and Robert E. Shope. After his return home he organized and established the Central Institute of Biology in Bratislava with the Department of Virology within the Institute. It became the basis for the later Institute of Virology, which was founded in January, 1953. The Institute of Virology was one of the institutes of the Czechoslovak Academy of Sciences and later became a part of Slovak Academy of Sciences. Professor Blaškoviè was the director of the Institute of Virology from its foundation until 1978, when he retired. He also founded the Department of Virology at the Faculty of Natural Sciences, Comenius University, Bratislava, where he became Professor and Head of the Department.

The main scientific interest of Professor Blaškoviè was the ecology and epidemiology of viruses, principally influenza viruses and of tick-borne encephalitis (Central European Encephalitis) virus. These two areas were those that brought fame to the Institute of Virology. However, he took an active part in many other areas of virology. For example one of his latest research activities resulted in the discovery of a new virus of the family Herpesviridae, mouse herpesvirus. He produced 5 monographs and books and more than 250 original scientific papers.

Professor Blaškoviè was named a World Health Organization expert in the period of 1960-1983. He was the member of UNESCO International Council of Scientific Union (ICSU). In 1961-1963 he was General Secretary of ICSU. He became the member of several Academies and Scientific Organizations throughout the world. There are a great many scientists, students, co-workers, and family members who will remember Prof. Blaškoviè as a personality who strongly, and in a positive way, influenced their lives and their work, mostly research in biology and virology.

To remember Professor D. Blaškoviè, a foundation bearing his name has been established. The main goal of the Dionýz Blaškoviè Foundation for the Development of Virology is to support young scientists in their virological research, to help them contact the best virological laboratories, and to establish links for future collaboration. Contribution can be sent to:

The bank: Všeobecná úverová banka, Bratislava-mesto, Namestie SNP 14, 815 79 Bratislava, Slovakia

The name of the account: Nadácia Dionýza Blaškovièa pre rozvoj virológie

Account No.: 34833-428351-012/0200

Swift Code: SUBA SKBX

Milan Labuda and Charles Calisher

[Milan Labuda (Director, Institute of Zoology, Slovak Academy of Sciences, Dubravska cesta 9, 842 06 Bratislava, Slovakia) is married to the former Hana Blaskovic, Dionyz' charming and accomplished daughter. I met Dino Blaskovic more than 30 years ago and, through all that time, until his recent illness, he kindly provided detailed information and remarkable insight and was a gentleman par excellence. I shall miss him greatly. – C.H. Calisher]

A Comprehensive Site on Hantavirus Pulmonary Syndrome is Available on the Web

(<http://www.cdc.gov/ncidod/diseases/hanta/hps/index.htm>)

The "All About Hantavirus" web page includes comprehensive, regularly updated information on hantavirus pulmonary syndrome, a rare but deadly disease caused by several species of New World hantaviruses and carried by several species of rodents. The page was developed and is maintained by Special Pathogens Branch, Division of Viral and Rickettsial Diseases, in the National Center for Infectious Diseases at The Centers for Disease Control and Prevention.

"All About Hantavirus" is divided into two major sections: Public Information and Technical Information.

The former is designed and written to appeal to users who want general information on the disease, its transmission and prevention. This section is easy to read, conversational in style, and full of images large and small which illustrate the information.

The Technical Information section is written for a professional audience, and is formatted as a research article. Users can read the contents sequentially, like an article, or can skip from topic to topic. Thumbnail links to chest x-ray and pathology slides allow users to view full-color, high-resolution images if they wish.

Additional information includes a list of state health department contacts, continually updated basic case information with a case map and aggregate demographics, an extensive and detailed glossary and resources list, a special section on El Nino, plus information on hantaviruses in South America.

Please visit the website and leave your impressions, critiques, and questions via email. Email can be sent directly from the web page or using nbd2@cdc.gov. The nature of the "All About Hantaviruses" page has been shaped largely by user comments and questions, and will continue to evolve according to what you ask for.

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

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

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

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

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

If sending reports by mail, please use this address:

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

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

Previous Editors of the Arbovirus Information Exchange

Telford H. Work 1960-1972

Roy W. Chamberlain 1972-1981

W. Adrian Chappell 1981-1984

Barry R. Miller 1984-1989

Charles H. Calisher 1989-1996

CENTRE COLLABORATEUR OMS
DE REFERENCE ET DE RECHERCHE
POUR LES ARBOVIRUS ET LES VIRUS POUR
DE FIEVRES HEMORRAGIQUES

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POUR LE DEVELOPPEMENT EN
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PARIS

UP TO DATE CRORA REPORT ON THE WEB

During January 1999, a new version of the interactive report from CRORA, Pasteur Institute of Dakar, will be installed on the WEB (www.pasteur.fr/Bio/CRORA/ and www.orstom.fr/crora/).

Pasteur Institute and ORSTOM are associated to collect data and to create this report, using HTML language.

As usual, this new report involves all the data collected by Pasteur Institute and ORSTOM, since 1962.

For each virus, all the observed hosts or vectors are given, with the number of collected strains in each country, and the bibliographical data concerning all the strains of the same viral species.

As previously, for each host, or vector, the number of collected strains of each virus in all the countries is given. A special listing is given for the virus isolated from male mosquitoes.

In this new version, we add another improvement concerning molecular biology. A new line is added in the first HTML page: "BIOLOGIE MOLECULAIRE". This HTML anchor gives an access to a special listing of all the stains which had been studied using molecular biology. In this listing, an HTML anchor, "Voir biblio." gives the access to bibliographical data. All the end of each reference, another HTML anchor, "Conclusions", allows to see the conclusions of the authors about the studied strains of the CRORA.

As for the first version, for each isolated and identified strain, listings give the exact place (latitude and longitude), date of collect and the host (or vector). As previously, an HTML anchor is connecting with technical data: used methods to identify the strains, comments and conclusions, and, for some strains, results of complete identification tests.

The last bibliographical data are added for each study strain from CRORA. If the reference concerns molecular biology, the HTML anchor "Conclusions" appears in each listing of bibliographical data.

As previously, for all the viruses, hosts or vectors, and countries, HTML anchors give the opportunity to have an immediate access to the concerned subject.

Actually, we are including the last data from CRORA and up to date bibliographical references. The next version of our data base will be on the WEB during January 1999.

We are specially concerned by the opinion of the users of our data base, thanks to them to leave messages in our email boxes, in Pasteur Institute.

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Francois ADAM
ORSTOM

Sequence Comparisons of Cache Valley Virus Medium Segment Among Several Isolates and Other Bunyaviridae

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The complete nucleotide sequences of the medium (or middle) segment of the prototype Cache Valley virus (CVV) as well as for two additional isolates, one from Michigan and one from Texas, have been determined. In addition, the sequence for a human isolate from North Carolina is also very near completion. Sequences were determined using a series of overlapping cDNA clones, and were sequenced from both strands in part using a series of primers deduced from conserved amino acid sequence among Clustal W aligned Bunyamwera and California serogroup viruses [Bunyamwera (BUN), Germiston (GER), La Crosse (LAC), Snowshoe Hare (SSH), Jamestown Canyon (JC), Inkoo, (INK), and Melao (MEL)(Genbank database)]. Partial sequences were assembled into a single contig using the Fragment Assembly System of GCG. Hydrophobicity and antigenicity profiles were plotted using PeptidePlot of GCG. The viral-complementary sense RNA is comprised of 4463 nucleotides which encodes a polyprotein precursor of 1435 amino acids. The base composition for the prototype virus is 34.9% A, 17.0% C, 19.4% G, and 28.7 % U. The putative G2 protein is 286 amino acids long with a predicted non-glycosylated weight of 32 kDa; NS_m is 175 amino acids and 19.6kDa; G1 is 958 amino acids long and has a predicted weight of 108 kDa.

Analysis of predicted glycosylation sites suggests that two found within the G1 protein at amino acids 246 and 307 (as based on presumed cleavage points for G1) are unique to CVV, using the Clustal W aligned sequences for comparison. Other amino acids also differ in CVV in all isolates analyzed from otherwise conserved regions within G2, NS_m and G1 among the California and Bunyamwera serogroup viruses. One of these falls within the predicted cleavage site at the carboxy terminus of G2. The presumed conserved sequence of KSLR(V/A)AR is substituted with an isoleucine at the fifth position, consistent among all isolates of CVV sequenced thus far. All cysteines are conserved for all sequences compared, but several switches to phenylalanine are found within the G1 coding region. Interestingly, amino acid comparisons between the four isolates (Prototype, Michigan, Texas, and North Carolina) indicates several substitutions; further analysis is in progress to determine possible significance of these differences.

Comparison of the prototype nucleotide sequence to that of BUN virus indicates an identity of 63%, but only 58% or 53% identity when compared to GER (Bunyamwera serogroup) or SSH (California serogroup) viruses, respectively. Most of the non-identity falls within the sequence encoding NS_m and G1, as determined by dotplot analysis (GCG).

The recent occurrence of a fatal human case in North Carolina (1) suggests the potential for dramatically increased virulence of this virus in humans, and recent corroboration of macroencephaly in infants born to mothers demonstrating antibody titers against CVV (2) supports the idea that CVV may be responsible for a number of unresolved cases of CNS disease. The variability of the isolates sequenced thus far may ultimately be a means of identifying and characterizing future incidences of CVV infection and transmission.

Additional CVV isolates of differing geographic or host sources may be considered for further investigation. Anyone with such isolates may contact Paul Grimstad at <grimstad.1@nd.edu> to discuss adding to this growing database.

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NATURAL HISTORY OF SIN NOMBRE VIRUS IN WESTERN COLORADO

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A mark-recapture longitudinal study of IgG antibody to Sin Nombre virus (SNV) in rodents in western Colorado was begun at one site (Fort Lewis, southwest) in June 1994 and at another (Molina, west central) in October 1994. Results, summarized to October 1997, indicate the presence of SNV or a closely related hantavirus at both sites. Most individual rodents (principally deer mice, *Peromyscus maniculatus*, and pinon mice, *P. truei*) did not persist on the trapping webs much beyond a month after they were first captured. That some persisted for more than a year suggests that longevity of even a few infected deer mice could serve as transeasonal (i.e., year-to-year) reservoirs, providing a mechanism for over-winter virus maintenance. A positive association between wounds and antibody in adult animals at both sites suggests that amplification of virus in appropriate rodent populations likely occurs when infected rodents fight with uninfected rodents. At Fort Lewis, males comprised 48.8% of the deer mice (and 47.8% of recaptured deer mice) but 58.3% of the seropositives; at Molina, males comprised 45% of the deer mice (and 46.3% of the recaptured deer mice) but 60% of the seropositives. However, incidence of antibody acquisition at each site appeared to be about the same for males and females.

Fluctuations in IgG antibody prevalence in deer mice at Fort Lewis and Molina were positively associated with deer mouse population fluctuations. Most seroconversions (recent infections) in deer mice at Fort Lewis and at Molina occurred during late summer and mid-winter. Furthermore, the rates of seroconversion in deer mice at both sites were higher than the seroprevalence, suggesting that the longer deer mice live, the greater the probability they eventually will become infected with SNV; one deer mouse had antibody to SNV detected for the first time 14 months after it had been initially captured.

(A complete and detailed manuscript on this work has been accepted for publication by *Emerging Infectious Diseases*)

West Nile flavivirus activity in South Moravia, Czechland, 1997

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Human cases of West Nile (WN) fever have not yet been reported from Central Europe (1), but the virus was isolated from *Aedes cantans* mosquitoes in W. Slovakia (2) and from migrating birds in SE. Slovakia (3,4). In Czechland, WN antibodies were detected in <5% of cattle, horses and hares (S. Bohemia: 5), 8% of game animals and 10% of cormorants (S. Moravia: 6,7). Circulation of WN virus in S. Moravia was first indicated in 1985 by detection of WN antibodies in 17 young (hatching year) wetland passerines on the Nesyt fishpond, Břeclav district (8). This natural focus was confirmed later during a serosurvey of 110 young domestic ducks kept over the summer season on this pond: antibodies against WN virus appeared in 29% of the birds (9).

In July 1997, devastating floods occurred after heavy rains at many places along the River Morava (March), Czech Republic. Populations of *Aedes* mosquitoes increased abruptly during July and August in the flooded area. Therefore we carried out surveillance for mosquito-borne virus infections in Břeclav area, S. Moravia. A total of 11,334 female culicine mosquitoes (9100 *Aedes vexans*, 917 *A. cinereus*, 11 *A. cantans*, 1074 *A. sticticus* and 232 *Culex pipiens pipiens*), collected between July and September 1997, were examined in 117 pools by inoculation of suckling mice (SM). Virus isolates were passaged twice in SM and the infectious brain homogenates were then used in the constant serum (1:5)-tenfold diluted virus neutralization tests on XTC-2 cells incubated at 28°C. Immune mouse sera, prepared against homologous isolates and the viruses TAH, BAT (CVO), SIN and WN, were inactivated at 56°C for 30 min. The results were expressed as log neutralization indices (NI) vs. normal mouse serum. Identification of the isolate 97-103 was also carried out in complement-fixation test (CFT) with optimum dilution of the borate antigen against a battery of diagnostic mouse sera and ascitic fluids to different arboviruses.

Seven mosquito suspensions yielded virus: 97-20 (52 *Aedes vexans*); 97-27 (100 *A. vexans*); 97-28 (45 *A. cinereus*); 97-30 (80 *A. vexans*); 97-41 (100 *A. vexans*); 97-46 (100 *A. vexans*); 97-103 (57 *Culex p. pipiens*). The first six strains were identified as Tšahyňa virus, whereas the isolate 97-103 was found to be West Nile virus (10). In neutralization test, log NI of diagnostic antisera with 97-103 isolate were ≤ 0.5 for TAH, BAT, SIN, DEN-1, TBE (CEE) and TYU antisera, while 0.8 for JE, 3.5 for WN and 2.9 for homologous antisera. In CFT, antigen 97-103 only reacted (1:128) with the homologous antiserum and diagnostic sera against Poly-B and WN virus; a slight reaction (1:8) was observed with JE antiserum, whereas it did not react with DEN-1, TYU, TBE, SIN, Poly-A, TAH, BAT and normal mouse sera. Further study compared 97-103 and Eg-101 WN virus strains in cross-PRNT (plaque reduction neutralization test) and revealed their great antigenic similarity: the mean difference of cross-titres was twofold.

The strain 97-103 is characteristic by a very low peripheral virulence for mice: it does not kill young adult (3-4 wk) ICR mice inoculated subcutaneously or intraperitoneally, and only 7 of 24 SM were killed after intraperitoneal inoculation of 1% infectious SMB suspension. In contrast to Eg-101 strain, adult ICR mice survive when given 97-103 virus intracerebrally.

Blood samples were obtained from 619 persons attending clinical wards for various

complaints between 23 June and 29 September 1997. Paired blood samples were taken mainly from febrile children, and, to a lesser degree, from adult persons. Blood sera were inactivated at 56°C for 30 min, and tested for antibodies against WN viruses (Eg-101; 97-103), after an incubation with the virus for 60 min at 37°C, in PRNT on XTC-2 cells. Virus test dose used was *c.* 30 PFU per well, and a 50% reduction of PFU number was regarded as the serum titre. All sera that had been reactive against WN virus, revealing at least a 90% reduction of PFU in the 1:8 serum dilution, were additionally tested to TBE virus (strain Hypr) in PRNT on SPEV pig kidney embryo cells. All individuals with detectable WN antibody were contacted and questioned retrospectively about their health history during last 5 years, in addition to consulting their written medical records. Antibodies neutralizing WN virus were detected in the blood sera of 13 individuals out of 619 examined (i.e., 2.1%) in PRNT (reciprocal titres):

Serum no.	Person	Sex	Age yr.	Date coll.	WN Eg-101	WN 97-103	TBE Hypr
P5	M.G.	m	9	4 August	512	256	<8
P15	B.G.	f	9	20 August	256	128	<8
R124	D.U.	f	35	3 July	512	512	<16
R137	R.V.	f	73	7 July	512	256	<16
R179	V.M.	f	31	15 July	512	256	16
R216	M.B.	f	74	22 July	512	256	8-16
R277	V.D.	m	51	31 July	512	256	16
R319	B.C.	f	42	6 August	256	128	<16
R337	A.J.	m	71	11 August	256	256	≤16
R344	M.B.	m	71	12 August	512	256	<16
R370	A.P.	f	72	14 August	256	128	<16
R554	L.F.	m	70	18 September	512	256	<16
R581	K.S.	f	42	24 September	128	128	<16

The difference between males (2.8% with antibodies) and females (1.8% with antibodies) was statistically insignificant (Fisher's exact test). The age effect could not be analyzed statistically because of the low number of seroreactors. None of these persons admitted suffering from TBE (the agent is the only other flavivirus present in this country) in the past, or being subjected to vaccination against TBE or yellow fever. Only two were abroad during last 5 years: B.G. in Dalmatia (Croatia) in 1996, and M.B. lived in South Australia between 1951 and 1994.

Paired samples of blood sera were obtained from 72 individuals. A significant increase in antibody titre against WN virus between the first (acute-phase) and second (convalescent-phase) sera were detected four times. Clinical symptoms compatible with WN fever were found in two children: **Case 1.** M.G., m, 9 y.: fever (around 39°C) for 4 days, sore throat, headache, muscle ache, pronounced fatigue, nausea. The illness lasted for about 6 days, recovery complete after 7 additional days. Serological examination for zoonoses (leptospirosis, tularemia), cytomegalovirus, respiratory syncytial virus and chlamydial infections all negative. PRNT titres (Eg-101/97-103 WN virus): 22

July 1:64/1:32; 4 Aug. 1:512/1:256.

Case 2. B.G., f, 9 y.: fever (38-39°C) for 3 days, sore throat, headache, muscle ache, pronounced fatigue, nausea, maculopapular rash (flushed face), slightly enlarged inguinal lymph nodes. The illness lasted for about 7 days, recovery was complete after 10 additional days. Serological examination for Lyme borreliosis negative. PRNT titres: 6 Aug. 1:64/1:32; 20 Aug. 1:256/1:128. Of the remaining nine seropositive persons lacking paired serum samples, B.C. claimed to be affected with pronounced headache, muscle ache, prolonged fatigue, nausea, pain on the movement of eyes, maculopapular rash and insomnia in summer 1997. Two other persons (V.M., R.V.) had got 'summer fever' in 1997, with sore throat and lymphadenitis in V.M., and headache combined with pain on the movement of eyes in R.V. The clinical symptoms in B.C., V.M. and R.V. were suggestive of WN fever. D.U. reported sore throat without fever, and L.F. was subjected to cholecystectomy in summer 1997. All the other persons did not report any significant illness occurring in summer 1997.

This report describes the second isolation of WN virus from mosquitoes and the first observation of WN fever in patients in Central Europe. In the study area, antibodies neutralizing WN virus were detected in 2.1% of inhabitants. Of the 13 seroreactors, 5 revealed clinical symptoms compatible with WN fever in summer 1997; in two of them (children), acute infection with WN virus was confirmed by a significant increase of antibody titre between the acute and convalescent serum sample. WN virus should not be underestimated as a potential agent of human epidemics even in Central Europe locally. Environmental factors including human activities that enhance vector population densities (irrigation, heavy rains followed by floods, higher temperature caused by, e.g., global warming) or warm air currents carrying infected mosquitoes northwards (11) might produce increased incidence of emerging mosquito-borne diseases. Arbovirus surveillance should therefore be performed; it should consist of monitoring population density and infection rate of principal vectors, serosurveys on vertebrates and exposed human groups, and a routine diagnostic of human infections.

Acknowledgments: This study was made possible through valuable cooperation of the District Public Health Service (Dr. V. Žáková), medical staff of the biochemical laboratory in Valtice hospital (Dr. Z. Příkazský), pediatric clinic in Břeclav hospital (Dr. P. Vajčner), and outpatient wards in Břeclav, Lednice and Lanžhot. The work has been funded by the Department for Environmental Protection of the Czech Republic (PPŽP/610/7/97, PPŽP/610/3/98).

REMARK: *We should be very grateful for notes about West Nile virus occurrence (including human and animal cases) elsewhere in Europe.*

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TICK BORNE ENCEPHALITIS VIRUS IN NORTHERN ITALY: Molecular analysis and the potential importance of roe deer (*Capreolus capreolus* L.)

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Introduction

The flavivirus that causes tick borne encephalitis (TBE) is distributed widely throughout northern Eurasia, associated with the two tick species, *Ixodes ricinus* and *I. persulcatus*. The virus occurs as one of several serotypes which can be clearly identified through molecular sequencing of the envelope gene (Gao *et al.*, 1993). Tick borne encephalitis is usually observed in distinct pockets of infection within the forestry ecosystem where it circulates through the tick and vertebrate host populations. It has also been recorded in the human population in Italy since 1967 in at least four areas, three in the north east provinces of Belluno, Trentino and Friuli and another in the central region of Lazio e Toscana. Studies of people at risk have identified about 1% seroprevalence in both Belluno and Trentino although clinical evidence indicates higher infections in Belluno (18 cases in 3 years) than Trentino (10 cases in 5 years: Basetti *et al.*, 1993, Caruso *et al.*, 1997). Professional people at risk of infection are now vaccinated as a matter of course in both provinces.

The objective of this study was to identify the virus serotype in ticks from north east Italy and to make ecological comparisons between areas of infection and areas of no infection. We believe the rise in the roe deer population plays a significant role in multiplying ticks and maintaining TBE virus even though there may be no direct involvement in virus multiplication. We have therefore compared roe deer numbers from the relevant areas. These data form part of a larger study investigating the landscape epidemiology of tick borne diseases in Trentino Province (Furlanello *et al.*, 1997).

Three TBE virus isolates were recovered from small pools of ticks approximately 8,000 ticks in total collected by blanket dragging, all from one district within Belluno. The sequence of a 300 nucleotide region of the envelope (E) gene was identical to that of a typical western European TBE virus e.g. Neudorfl (Table 1). While the clinical evidence suggests that TBE is a recent arrival in this part of Italy, it is possible that the virus has been circulating within the forest for a longer period.

In addition to the possibility that roe deer may have a significant influence in the introduction of the virus to northern Italy, an alternative explanation for the presence of TBE virus is that the virus was introduced following the release of brown hares (*Lepus europaeus* L) from the former Czechoslovakia and this will form part of the follow up study. The recovery of 3 virus isolates from Belluno and none from Trentino implies that the prevalence of the disease amongst ticks is probably greater in Belluno than Trentino. This could be considered a minimum estimate of prevalence since most of the ticks collected were unfed questing ticks and virus is probably harder to find in such ticks than in ticks which have been collected from animals.

Interestingly, two different methods were used to identify the presence of virus in the tick pools (average 5 ticks per pool). In the first, homogenised ticks were inoculated onto porcine kidney (PS) cell monolayers and after incubation at 37 °C for 4 days, the cells were washed in phosphate buffered saline, fixed in cold acetone and tested for the presence of TBE complex virus using an appropriate monoclonal antibody conjugated with fluorescein isothiocyanate. In the second method, a small region of the envelope gene was reverse transcribed (RT) and the cDNA amplified by polymerase chain reaction (PCR) using the procedures described by McGuire *et al.*, 1998. The two methods proved equally sensitive at identifying infected ticks. The advantage of the RT-PCR procedure was that the PCR product could be immediately sequenced to confirm the identity of the virus.

In summary, TBE in the northern Italy region of Belluno is caused by a strain of virus that not surprisingly closely resembles the western European subtype, previously recorded in Austria. Comparative data on hosts (not presented) suggest that the roe deer has a significant role to play in maintaining the ticks and virus in some of these areas. We are continuing to search for virus in ticks in parts of Trentino where there have been human cases of TBE.

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Table 1: Partial envelope gene sequence for the 3 isolates from Belluno in comparison with the Neudorfl strain of virus that represents a typical western European virus subtype. The 3 Belluno isolates are identical and differ from the Neudorfl strain by just one amino acid (emboldened). *Amino acid number commencing for first amino acid of the envelope gene

Isolate	
	152*
Belluno1	AANQTHSGR KTASFTVSSE KTILTMGEYG DVSLLCRVAS G VDLAQTVIL
Belluno2	AANQTHSGR KTASFTVSSE KTILTMGEYG DVSLLCRVAS G VDLAQTVIL
Belluno3	AANQTHSGR KTASFTVSSE KTILTMGEYG DVSLLCRVAS G VDLAQTVIL
W.TBE	AANQTHSGR KTASFTVSSE KTILTMGEYG DVSLLCRVAS G VDLAQTVIL
	201
Belluno1	ELDKTVEHLP TAWQVHRDWF NDLALPWKHE GAQNWNNAER LVEFGAPHAV
Belluno2	ELDKTVEHLP TAWQVHRDWF NDLALPWKHE GAQNWNNAER LVEFGAPHAV
Belluno3	ELDKTVEHLP TAWQVHRDWF NDLALPWKHE GAQNWNNAER LVEFGAPHAV
W.TBE	ELDKTVEHLP TAWQVHRDWF NDLALPWKHE GAQNWNNAER LVEFGAPHAV
	251
Belluno1	KMDVYNLGDQ TGVLLKALAG VPVAHIEGTK YHMK
Belluno2	KMDVYNLGDQ TGVLLKALAG VPVAHIEGTK YHMK
Belluno3	KMDVYNLGDQ TGVLLKALAG VPVAHIEGTK YHMK
W.TBE	KMDVYNLGDQ TGVLLKALAG VPVAHIEGTK YHLK

PATHOGENICITY OF TICK-BORNE ENCEPHALITIS VIRUS HOKKAIDO STRAIN IN MICE

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In order to explain the severity of a tick-borne encephalitis case found in Hokkaido and to construct mouse infection model for the evaluation of the vaccine to the prevalent tick-borne encephalitis (TBE) virus strain, we examined the pathogenic potential of the TBE virus Hokkaido strain. The pathogenic characteristics of TBE virus strain (Oshima 5-10) isolated from a sentinel dog in Hokkaido, Japan was compared by use of mouse model with several inoculation routes to other strains of TBE virus (the Far Eastern subtype; Sofjin strain and the Western subtype; Hochosterwitz strain) and TBE complex virus (Langat virus; TP-21 strain). The degree of neuroinvasiveness was Sofjin--Hochosterwitz--Oshima--TP-21 in mice subcutaneously (s.c.) inoculated. Neuro-virulence, as determined after intracerebral inoculation was the highest in Sofjin strain followed by Oshima, Hochosterwitz and TP-21 strains, respectively. Virus replication in the brains of mice s.c. or intracerebrally inoculated with Oshima strain was slower and of lower titer than that of Sofjin strain. Histopathological findings indicated that subarachnoid infiltration of mononuclear cells prior to necrosis of cerebrum was characteristic in Oshima strain. Consequently, these findings indicated that the Oshima strain had a pathogenic potential common among TBE viruses and is less virulent for mice as compared with these two other TBE virus strains.

This mouse infection model of TBE virus Oshima strain established is useful for future study to develop and/or evaluate vaccines to the prevalent form of TBE virus in Japan.

Incidence from Coincidence: Patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus

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Endemic cycles of tick-borne encephalitis (TBE) virus, which have a highly focal distribution through Eurasia, appear to depend on the transmission of non-systemic infections between ticks co-feeding on the same rodent hosts. Large samples of rodents and their feeding ticks from four regions within TBE foci in Slovakia, show particular features of seasonal dynamics and infestation patterns of larval and nymphal *I. ricinus*, but not *Dermacentor reticulatus*, that promote TBE virus transmission. Coincident aggregated distributions of larvae and nymphs on their principal rodent hosts result in the same c.20 % of hosts feeding about three quarters of both larvae and nymphs. This consistently doubled the number of infectible larvae feeding alongside potentially infected nymphs compared with the null hypothesis of independent distributions of these two tick stages. Overall, co-feeding transmission under these circumstances brings the R_0 value for TBE virus to a level that accounts quantitatively for maintained endemic cycles. Essential for coincident aggregated distributions of larvae and nymphs is their synchronous seasonal activity. Preliminary comparisons support the prediction of a greater degree of coincident seasonality within recorded TBE foci than outside. The particular climatic factors that permit such patterns of tick seasonal dynamics may be the primary predictors for the focal distribution of TBE.

SITUATION OF DENGUE IN FEDERAL DISTRICT, BRAZIL AND FIRST ISOLATION OF DEN 1 VIRUS FROM *Aedes aegypti*

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Imported Dengue cases are notified and laboratory-confirmed in Federal District (FD) since 1991. From 1993 onwards, the number of cases increased (fig. 1), with the first autochthonous cases appearing in 1997.

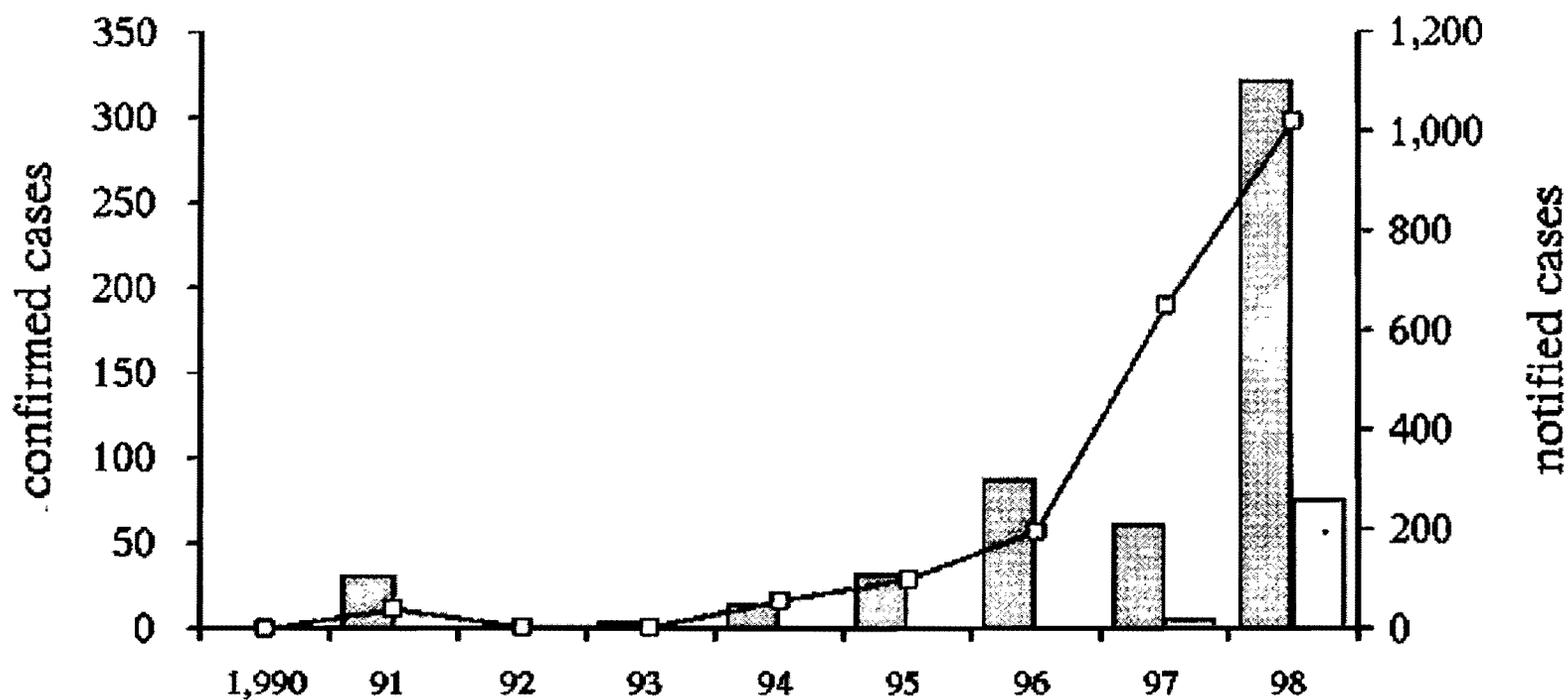
During the first half of 1998, 296 and 13 cases were confirmed serologically (MAC ELISA) and by viral isolation, respectively. Of these, 75 were autochthonous, infected either by DEN 1 or DEN 2 viruses, with 32 from the Gama county located at the border of Goiás State. Entomological surveys were preferentially conducted in houses where cases were suspected to be autochthonous.

One strain of DEN 1 virus has been isolated from a pool of 4 *Aedes aegypti* females, attracted to people, on 27 April 1998 (3 other pools, made of 4 females and 21 males, were negative) in Engenho das Lages, located 30 km from Brasilia on road BR060. The owner of this house begun to be ill on 1st April, thus appr. 4 weeks before the capture of the mosquitoes. This fact denotes their great survival despite their low density (0.45 mosquitoes/ man*hour in mid afternoon). However, such a high infection rate may not be an artefact because another 3 human cases were diagnosed in the same area during the 2nd half of April.

The isolation was done on C6/36 cell line after one blind passage. Serotype was determined by indirect immunofluorescence (monoclonal antibodies against DEN 1-4) as no cytopathic effect was noticed. This result shows that some populations of *Ae. aegypti* in Federal District may be highly susceptible to natural infection by dengue viruses. Thus outbreaks of dengue will depend mainly upon the control of mosquito densities, coupled with rapid diagnostic of cases.

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Dengue cases in Federal District, Brazil, 1990 - July 1998. Line = notified cases; bars = confirmed cases, solid: imported; blank: autochthonous



Oral susceptibility to dengue type 2 virus of *Aedes aegypti formosus* from Franceville (Gabon, Central Africa).

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INTRODUCTION

Dengue is widely distributed in the tropics. Except from the outbreak of 1927-28 in South Africa, dengue epidemic activity was not reported in Africa before the 1980's despite the first isolation of dengue viruses on this continent which occurred in Nigeria in 1971. In the past 15 years, increased epidemic dengue fever has been reported both in East and West Africa and all four serotypes have been active in Africa, bringing out concern about the ability of local populations of *Aedes aegypti* to transmit dengue viruses. *Ae. aegypti* is present in two forms in Africa: *Ae. aegypti aegypti* and *Ae. aegypti formosus*. This latter form, much darker, is supposed to represent the native non-domestic species but is now colonizing artificial breeding sites in cities. The aim of this study was to estimate the oral susceptibility to dengue type 2 virus of *Ae. aegypti formosus* collected in Franceville (Gabon, Central Africa) and to compare it to that of a positive control, *Ae. aegypti* Paea strain, originating from French Polynesia.

MATERIALS AND METHODS

Mosquitoes. The *Aedes aegypti formosus* (F0) were collected as larvae in artificial breeding sites around the houses in the Akou district of Franceville, Gabon in July 1997. These field collected mosquitoes (F0 generation) were raised to the adult stage in the laboratory at $28 \pm 1^\circ\text{C}$ with 80% relative humidity and a 16h:8h photoperiod. Adults were given 10% sucrose solution and females were allowed to feed on a restrained mouse to obtain eggs. For infection experiments, we have tested females from the F2 and F3 generations.

The Paea strain of *Ae. aegypti*, provided by Institut Louis Malarde (Tahiti, French Polynesia) and reared in Paris since 1994, was used as a control of mosquito susceptibility.

Virus. The dengue type 2 virus strain, provided by L. Rosen, was isolated in 1974 from a human sera from Bangkok (Thailand). This virus had been passed only in different mosquito species (*Toxorhynchites amboinensis*, *Aedes albopictus* and *Ae. aegypti*) by intrathoracic inoculation ¹. Viral stocks were produced by inoculating *Ae. albopictus* cells C6/36 clone ² with triturated infected mosquitoes. The mosquito cells were maintained at 28°C on RPMI-1640 medium supplemented by non-essential amino-acids, penicillin and streptomycin and 10% heated (56°C for 30 min) fetal calf serum.

The percentage of infected cells was monitored during the incubation period by the indirect fluorescent antibody assay (IFA)³. When 100% of the cells were infected, the supernatant fluid was collected, and the pH adjusted to 7.5 with 10% sodium bicarbonate. The virus stock was divided into aliquots and stored at -80°C until used. Titration of the virus stock was carried out in *Ae. aegypti* (Paea strain) by inoculating serial dilutions of the supernatant intrathoracically. Mosquito infection was detected by IFA on head squashes. Titers were calculated by the 50% endpoint method⁴ and expressed as 50% mosquito infectious doses (MID₅₀) per ml.

Oral infection of mosquitoes. The oral susceptibility of females was tested by an artificial feeding protocol. Briefly, 5-7 day-old females were deprived of sucrose solution 24h prior to the infectious meal and then allowed to feed for 20 min through a chicken skin membrane covering an apparatus containing the feeding mixture maintained at 37°C. The infectious meal consisted of 2/3 washed rabbit erythrocytes, 1/3 virus suspension, and ATP (as a phagostimulant) at a final concentration of 5×10^{-3} M. Rabbit arterial blood was collected and erythrocytes washed 24 to 48h before the infectious meal. All the meals yielded $10^{8.2}$ MID₅₀/ml. Only fully engorged females were transferred to small cardboard containers and maintained at $28 \pm 1^\circ\text{C}$ for 14 days. Surviving females were sacrificed and tested for the presence of dengue virus by IFA on head squashes.

RESULTS

We were able to orally infect with dengue type 2 virus the *Ae aegypti formosus* from Akou. The percentage of infected females were 52.0 % (13/25) for the F2 and 69.6 % (16/23) for the F3. The infection rates obtained for the Paea control strain fed with the same meal were respectively 91.7 % (33/36) and 96.4 % (27/28). The difference in infection rates between the Paea and the Akou females were significant ($p = 0.0007$ for the F2 ; $p = 0.015$ for the F3) when compared by the Fisher's exact test. We have noticed that Akou mosquitoes digested blood meal more rapidly. At the end of the meal, the blood content of the Paea females was bright red whereas Akou females had brown-red blood.

DISCUSSION

We were able to demonstrate the oral susceptibility for dengue type 2 virus of *Ae. aegypti formosus* collected in Franceville. However these mosquitoes exhibit lower infection rates than that of a strain of *Ae. aegypti aegypti* originating from French Polynesia and of populations of French Guyana and Vietnam tested in our laboratory (unpublished data). Further investigations would be needed to explicit the role of the two different forms of *Ae. aegypti* in dengue outbreaks in Africa. Sampling more mosquitoes for a better characterization of their genetic variation and their ability to transmit dengue viruses might be helpful for a better understanding of dengue epidemiology in Africa.

ACKNOWLEDGMENTS

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SPECIAL REPORT FROM THE TEXAS STATE DEPARTMENT OF HEALTH
VIRUS LABORATORY, AUSTIN, TEXAS;
THE UNIVERSITY OF ILLINOIS CENTER FOR ZOOSES RESEARCH,
URBANA, ILLINOIS;
THE NEW JERSEY STATE HEALTH DEPARTMENT PUBLIC HEALTH
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THE COMMUNICABLE DISEASE CENTER ARBOVIRUS UNIT,
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The CDC Arbovirus Unit laboratory and field staff joined with personnel of Texas, Louisiana, Missouri, Tennessee, Illinois, Indiana, Kentucky, and New Jersey, to monitor and investigate what appears to be the most extensive dispersion of St. Louis encephalitis virus into human inhabitants on record in the United States. By far the largest number of human cases occurred in Houston, Texas, where the nature of the disease, which began early in July, was not recognized until the third week of August, when the City Health Officer, Dr. C.A. Pigford, moved by finding an unusual number of death certificates describing acute central nervous system disease in the elderly, submitted four acute-convalescent serum pairs from the Ben Taub Hospital for Dr. J.V. Irons, Director of the State Laboratory in Austin, where rise in titer of HI and CF antibodies for St. Louis encephalitis (SLE) virus was reported.

On request of the City of Houston and Dr. J.E. Peavy, Texas State Health Officer, on August 20, a CDC team went into operation the following day, with headquarters in the City Public Health Laboratory provided by Director Reuben Wende, and aided by the considerable personnel assistance of Dr. John R. Hall's immunization program staff. At the initiation of efforts to define the nature and extent of the epidemic, it appeared that most of the cases had entered Ben Taub Hospital. The accumulated specimens from these cases, submitted to Baylor School of Medicine Virus Laboratories, where they were examined for an enterovirus etiology, were on hand for tests with arbovirus antigens. In order to retrospectively obtain further etiological information on suspected cases in that area of the city, and to further define a distribution pattern which did not fit previous concepts of mosquito-dispersed SLE virus, antigens, other reagents, equipment, and technologist (Mr. Leo Chester) support were provided to the Baylor laboratory. Canvassing of hospitals and physicians for past and current cases reshaped the

geographical distribution to a more random distribution and stimulated submission of serological specimens.

The primary functions of the laboratory program initiated in Houston were to: 1) solicit and obtain acute and convalescent specimens for laboratory examination; 2) document the specimens according to case; 3) direct the specimens toward most expeditious examination in one of the three laboratories involved; 4) receive by telephone preliminary results for recording on the master record and interpretation; 5) prepare Xerox copies of the master form sheet for information of the epidemiologists, city health officials, and a report to the physician, often within 72 hours of submission of the specimen.

The utilization of the HI test for SLE was worked out along with the serial reporting mechanism during the 1962 SLE epidemic in Florida. It was learned there that SLE HI antibodies appear as soon as three days after onset of illness and in a population not generally exposed previously to Group B arbovirus infection, a presumptive diagnosis of SLE infection can be made early in the course of the disease. This served two useful purposes: 1) shaped early definition of the disease problem; and 2) attracted submission of specimens on suspected cases from private physicians seeking diagnostic clues to their patients' illness. This, of course, resulted in a more comprehensive assessment of the epidemic earlier in its ultimate course.

About ninety per cent of the presumptive diagnoses prove out on subsequent sera. About half of the reported cases were quickly put in the presumptive category. A shift to confirmed on the basis of rise in titer in convalescent sera is now occurring; so, of the more than 700 reported cases, it is expected that over 300 will prove valid--which amounts to one of the biggest SLE epidemics on record (Table I). There were 32 reported deaths, all but one over the age of 50. The epidemic onset and progress are illustrated in the series of histograms in Figure 1, issued by the CNS Disease Surveillance Unit of CDC.

Simultaneous with the epidemiological and laboratory investigation of cases shared between the three laboratories, an entomological team, supervised by Dr. Daniel Sudia, commenced work on August 22. Initial light-trapping efforts proved ineffective and sucking tube collections retrieved

Culex quinquefasciatus from culverts, chicken houses, and other peridomestic resting sites. To date, 38,278 mosquitoes have been processed. The last of 11 isolations of SLE virus from Houston mosquitoes was from collections made September 10. Actual number and source of isolations from mosquitoes appear in Table II. It indicates widespread dispersion and activity of SLE virus infected mosquitoes in the Houston epidemic situation.

Dr. Rex Lord initiated mist net bird collections, and animal trapping to obtain data on the possible vertebrate species involved was also initiated on August 22. Results of isolation attempts appear in Table III, and implied incidence of infection by HI screening appears in Table IV. With up to 25% of certain species possessing antibodies and virus isolations from peridomestic resident birds, the blue jay and mockingbird, it appears that the wild birds played a significant role in circulating the virus and supplying it to infect Culex quinquefasciatus mosquitoes, which appeared to be abundant because of the extensive amount of contaminated standing or slow-flowing water.

The vertebrate virus isolation, characterization, and identification, as well as serological studies, were under supervision of Dr. Philip Coleman. In attempting to convey an impression of the size of the investigative effort, there were times during the initial two weeks when as many as 25 CDC and related personnel were occupied in Houston, working with even more local medical and other professional personnel.

Although the first human case of encephalitis due to SLE in Illinois was recognized in McLeansboro in the southeast region of the state on August 5, it was not until the end of the month that an outbreak which eventually amounted to 19 suspect cases, 12 serologically proven, came under the scrutiny of Dr. R.H. Kokernot of the Illinois Center for Zoonoses Research. A histogram showing the cases by week of onset is presented in Figure I, the first known case having an onset during the week ending July 18.

Under the direction of Dr. Kokernot, intensive collections were undertaken of mosquitoes, bird bloods, and human survey sera. As of this date, over 1500 mosquitoes, representing 55 pools, have been processed, resulting in 30 possible isolates, one of which has been confirmed as SLE. This isolate is from a pool of Culex pipiens collected on September 4, 1964 (Table III).

In addition, tissues from over one hundred birds have been inoculated, resulting in the isolation of SLE virus from two house sparrows and a catbird collected in the McLeansboro area (Table III). As in Houston, there is evidence by HI test of a considerable number of birds collected having antibodies to SLE (Table V).

Dr. Norman Rose, Chief, Bureau of Epidemiology, Illinois Department of Public Health, has also reported eleven cases of serologically confirmed SLE from the Edwardsville-Alton area in Madison County in the western part of the state.

During the first week of September, Mr. Cliff Todd, Epidemiologist with the State Health Department, requested assistance from the CDC to investigate an outbreak of encephalitis in Boyle County (Danville), Kentucky, located in the central part of the state. Specimens from human cases collected by Mr. Todd and an EIS Officer of CDC were processed at CDC and results to date are summarized in Table II. Of the 36 cases on whom specimens have been received, 13 show presumptive or confirmed serological evidence of recent SLE infection. Once again referring to Figure I, the first case appeared during the end of the first week in August, the peak during the first week of September, and last suspect case on September 22.

Simultaneous investigation of the mosquito vector by a CDC team resulted in the collection of close to 5000 mosquitoes. From those processed to date, there have been one confirmed and two probable isolations of SLE virus (Table III) from Culex pipiens mosquitoes.

Mr. Herbert Maxfield, using mist nets, collected serum from 259 birds. These specimens have not been tested at this time.

The scope of the dispersion of SLE virus into this area of the country was further widened by the finding of another focus of encephalitis in Evansville, Indiana, in the southwestern part of the state, across the Wabash River from McLeansboro. Specimens submitted to CDC by Dr. Josephine Van Fleet have shown eight cases of presumptive or confirmed SLE infection in this area, the dates of onset ranging from August 22 to September 4.

Over 5000 mosquitoes for virus isolation were collected by Dr. Verne Newhouse from the Evansville area during the end of September; these are being processed at this time at CDC.

An additional focus of SLE activity became apparent in Camden County, New Jersey, during the middle of September. Dr. Martin Goldfield reports 88 suspect cases to date, 61 of which have been serologically confirmed. A histogram is shown in Figure 1, and further available information on cases in Table II. This is the first known occurrence of encephalitis due to SLE in this area of the country.

Collection of over 15,000 mosquitoes was undertaken in the Camden County area. No confirmed isolates are reported at this early date.

Just west of New Jersey in Philadelphia, Pennsylvania, there are eight suspect cases of encephalitis, one confirmed as SLE.

Evidence of infection with SLE has also been obtained from Memphis, Tennessee, with one confirmed clinical case, and SLE was isolated from two pools of *C. pipiens* collected in Memphis during the second week of September (Table III).

Figure I

HISTOGRAM OF HUMAN CASES OF ENCEPHALITIS
BY WEEK OF ONSET FOR HOUSTON, TEXAS, ILLINOIS,
KENTUCKY, AND NEW JERSEY

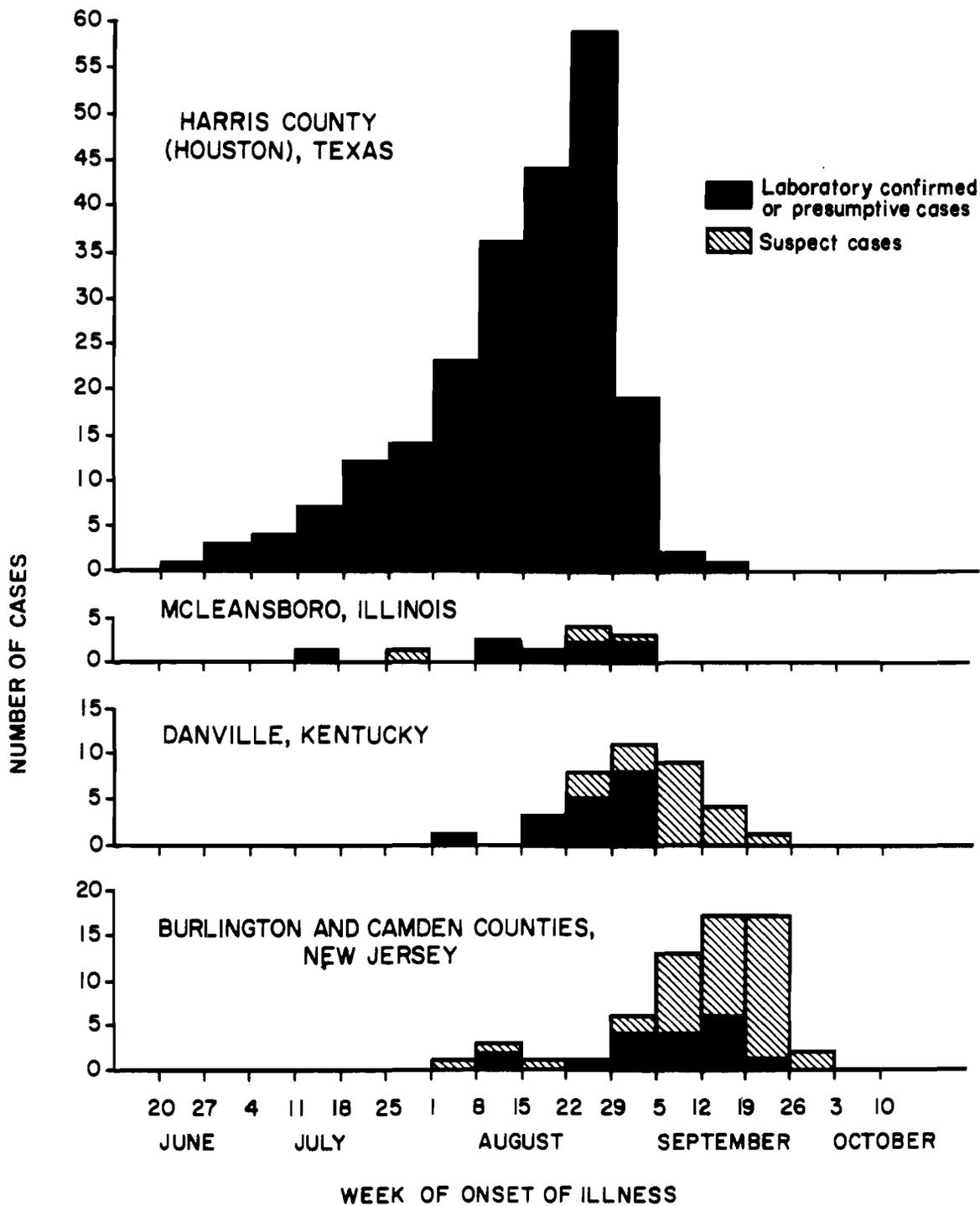


Table I

STATUS OF LABORATORY ANALYSIS OF SUSPECT HUMAN CASES IN HOUSTON, TEXAS, BY
SEROLOGICAL TESTING TO SLE, THROUGH 10 OCTOBER 1964

AGE	TOTAL SUSPECT CASES	SPECIMENS RECEIVED	NEGATIVE	INCON- CLUSIVE	CONFIRMED OR PRESUMPTIVE	% CONFIRMED OR PRESUMPTIVE OF SPECIMENS RECEIVED	ESTIMATED ACTUAL CASES
0-9	104	75	17	32	26	35%	36
10-19	101	79	10	45	24	30%	30
20-29	141	115	11	78	26	22%	31
30-39	92	79	9	41	29	37%	34
40-49	70	54	6	25	23	43%	30
50-59	70	50	4	21	25	50%	35
60-69	63	50	3	11	36	72%	45
70-79	44	34	0	10	24	70%	31
80+	22	15	0	1	14	93%	20
	707	551	60	264	227	41%	290

Table II

STATUS OF LABORATORY ANALYSIS OF SUSPECT HUMAN CASES IN DANVILLE, KENTUCKY, MCLEANSBORO, ILLINOIS, AND CAMDEN, NEW JERSEY, BY SEROLOGICAL TESTING TO SLE, THROUGH 10 OCTOBER, 1964

	SUSPECT CASES	SPECIMENS RECEIVED	NEGATIVE	INCON- CLUSIVE	PRESUMPTIVE OR CONFIRMED	% PRESUMPTIVE OR CONFIRMED OF SPECIMENS RECEIVED	ESTIMATED ACTUAL CASES
McLeansboro, Ill.	19	15	1	5	9	60%	11
Danville, Ky.	42	36	4	19	13	35%	15
Camden, N.J.	88	88	0	17	61	70%	61
Indiana	20	20	4	8	8	40%	8

Table III

STATUS OF LABORATORY EXAMINATION OF MOSQUITOES, WILD BIRDS, AND HUMAN AUTOPSY MATERIAL FOR ISOLATION OF SLE VIRUS IN TEXAS, TENNESSEE, ILLINOIS, INDIANA, KENTUCKY, AND NEW JERSEY (AS OF 7 OCTOBER 1964)

	LOCATION	NUMBER COLLECTED	INOCULATED	I S O L A T I O N S		
				NO.	SPECIES	DATE COLLECTED
Mosquitoes	Houston	58,831	35,278	9	<u>C. quinquefasciatus</u>	8/24-28/64
				1	<u>C. quinquefasciatus</u>	9/10/64
				1	<u>A. quadramaculatus</u>	8/27/64
	McLeansboro	2,500	1,500 (55 pools)	30	pools suspect isolates	
	Danville	4,724	3,745	1	<u>C. pipiens</u>	9/4/64
				2	<u>C. pipiens</u> " (probable)	9/12/64 9/13,23/64
	Camden	15,000	*	*		
Memphis	580	580	2	<u>C. quinquefasciatus</u>	9/8-11/64	
Evansville	5,200	*	*			
Wild birds	Houston	1,034	550	1	Blue Jay	8/24/64
				1	Mockingbird	8/27/64
	McLeansboro	171	125	2	House Sparrow	9/2-12/64
				1	Catbird	9/2-12/64
	Danville	259	*	*		
Human	Houston	7 (autopsy material)	7	0		
	Danville	0	0	0		
	Camden	0	0	0		

*Results of isolation attempts not available as of this date.

Table IV

STATUS OF EXAMINATION OF WILD BIRD SERA FOR INCIDENCE OF HI ANTIBODIES TO SLE IN HOUSTON,
TEXAS (AS OF 25 SEPTEMBER 1964)

SPECIES	D A T E S O F C O L L E C T I O N								TOTALS		% POS.
	8/24-9/1		9/2-9/9		9/10-9/16		9/17-9/21		COLL.	POS.	
	TOTAL	POS.	TOTAL	POS.	TOTAL	POS.	TOTAL	POS.			
Mockingbird	46	8	31	5	7	-	10	2	94	15	1
Cardinal	25	2	9	1	7	-	11	1	52	4	7.5
Tufted Titmouse	9	3							9	3	
Blue Jay	65	14	31	8	17	6	26	15	140	43	32
Unidentified flycatcher	14		35	5	17	1	9		75	6	8
Carolina wren	5	1	4		2		2		14	1	
Screech Owl	5	2	2						7	2	
Great-crested flycatcher	6	1	4		1		2	1	13	2	
Redbellied woodpecker	7	1	2	1			1		10	2	
Common grackle	12	1	1		19		23	3	55	4	7
Robin	3		9	2					12	2	16
House sparrow	22	4	66	20			33	7	121	31	26
Baltimore oriole			12	4	20		3		35	4	11
Boat-tailed grackle					3	1	7	1	10	2	
Pigeons	100	18			100	21			200	39	19.5
Other species (30)	19	4	26	0	23	0	14	0	85	4	
Grand Total	338	59	232	46	216	29	141	30	932	167	17.9

Table V

STATUS OF EXAMINATION OF WILD BIRD AND DOMESTIC FOWL SERA
FOR INCIDENCE OF HI ANTIBODIES TO SLE IN MCLEANSBORO,
ILLINOIS (AS OF 25 SEPTEMBER 1964)

Species	Total Collected	HI Positive to SLE or MVE	Per Cent Positive
Mourning Dove	14	0	0
Common grackel	11	0	0
Blue Jay	15	8	53
House sparrow	58	24	41
Chimney swift	101	11	11
Pigeon	37	28	76
Starling	10	1	10
13 other species	<u>25</u>	<u>9</u>	<u>36</u>
	271	83	30

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Quotes (Courtesy of Charlie Calisher)

Benjamin Franklin: "They that can give up essential liberty to obtain a little temporary safety deserve neither liberty nor safety."

Winston Churchill: "A fanatic is one who can't change his mind and won't change the subject."

Winston Churchill: "The inherent vice of capitalism is the unequal sharing of blessings; the inherent virtue of socialism is the equal sharing of misery."

Leo Burnet: "When you reach for the stars you may not quite get one, but you won't come up with a handful of mud either."

Humphrey Bogart (Casablanca): "Well everybody in Casablanca has problems. Yours may work out."

Woody Allen: "It shows what you can do if you are a total psychotic."

John Mortimer: "After all, Michelangelo was wise enough not to ask the Pope exactly how he came by every penny needed for the ceiling of the Sistine Chapel."

Mark Twain: "Whenever you find yourself on the side of the majority, it is time to pause and reflect."

Vladimir Lenin: "Why should freedom of speech and freedom of the press be allowed? Why should a government which is doing what it believes right allow itself to be criticized? It would not allow opposition by lethal weapons, and ideas are more fatal than guns."

Bert Brecht: "After the rising of the 17th June, the Secretary of the Writers' Union had leaflets distributed in the Stalinallee on which it could be read that the people have frivolously forfeited the confidence of the government and could win it back only by working twice as hard. Would it not be simpler for the government to get rid of the people and elect another?"

Matt Cartmill: "As an adolescent I aspired to lasting fame. I craved factual certainty, and I thirsted for a meaningful vision of human life -- so I became a scientist. This is like becoming an archbishop so you can meet girls."

John Snow ("On the Mode of Communication of Cholera - 1854): "The communicability of cholera ought not to be disguised from the people, under the idea that the knowledge of it would cause panic, or occasion the sick to be deserted."

Tim Owen: "We are facing a situation now in which the New York Times contains every day as much information as Renaissance Man encountered in a lifetime."

Nicholas Humphrey: "Why is music such a pleasure?"

G.K. Chesterton: "A woman uses her intelligence to find reasons to support her intuition."

Gloria Steinem: "I have yet to hear a man ask for advice on how to combine a marriage and a career."

Ambrose Bierce: "Death is not the end; there remains the litigation."

Dolly Parton: "I'm not offended by all the dumb blonde jokes, because I know I'm not dumb and I also know that I'm not blonde."

Gilda Radner: "I base most of my fashion taste on what doesn't itch."

Shirley Hibberd: "It would be an anomaly to find a student of nature addicted to the vices that cast so many dark shadows on our social life; nor do I remember among the sad annals of criminal history, one instance of a naturalist who became a criminal, or of a single gardener who has been hanged."

Ellen DeGeneres: "I ask people why they have deer heads on their walls. They always say 'Because it is such a beautiful animal'. I think my mother is attractive, but I have a photograph of her."

John Mendoza: "Ever wonder whether illiterate people get the full effect of alphabet soup."

"God is dead" -- Nietzsche

"Nietzsche is dead" -- God

King James I (1566-1625): "He was a bold man who first swallowed an oyster."

Herb Caen: "The trouble with born-again Christians is that they are an even bigger pain the second time around."

A. Bartlett Giamatti: "Baseball is one of the few enduring institutions in America that has been continuous and adaptable and in touch with its origins. As a result, baseball is not simply an essential part of this country; it is a living memory of what American culture at its best wishes to be."

Ralph Waldo Emerson: "To laugh often and much; to win the respect of intelligent people and the affection of children; to earn the appreciation of honest critics and endure the betrayal of false friends; to appreciate beauty, to find the best in others; to leave the world a bit better, whether by a healthy child, a garden patch or a redeemed social condition; to know even one life has breathed easier because you have lived. This is to have succeeded."

Voltaire: "God is a comedian playing to an audience of people afraid to laugh."