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MOSQUITO PRODUCTIVITY AND SURVEILLANCE FOR ST. LOUIS ENCEPHALITIS VIRUS IN CHICAGO DURING 1993

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ABSTRACT. The City of Chicago's Department of Health monitors weekly deposition of egg rafts of *Culex* species, prevalence of St. Louis encephalitis (SLE) virus-specific antibodies in feral birds, and prevalence of the virus in mosquito pools. The total number of *Culex* egg rafts collected in 1993 (4,623) was 2-fold greater than for the 1992 mosquito season. Virtually all of the early summer egg rafts were identified as *Culex restuans*. After the week of July 18, *Culex pipiens* accounted for 20–70% of the total rafts collected weekly. The prevalences for SLE viral antibodies (avian) and RNA (mosquitoes) were 0.2% and 0.02%, respectively. Both values were about 25-fold less than normally occur in epidemic years. It is important for practical considerations to continue this surveillance in order to recommend time- and site-specific mosquito abatement.

INTRODUCTION

The principal vector of St. Louis encephalitis (SLE) virus in the Midwest is a member of the *Culex pipiens* complex (Monath 1979). Passerine birds serve as the primary or amplifying host in the maintenance and replication of the virus and are considered the source of its reintroduction during spring migration (Mitchell et al. 1979). Mosquitoes that successively feed on birds and mammals are thought to account for human infection.

The Illinois Department of Public Health began its annual arbovirus surveillance program in 1976, as a result of the 1975 epidemic of SLE virus, which was responsible for 578 diagnosed cases and 47 deaths in Illinois (Bowen and Francy 1979). The City of Chicago Department of Health (CDOH) began its own SLE virus surveillance program in 1981. These are 2 components in the latter program: a *Culex* monitoring and collection study and a surveillance of avian hosts and vectors for the presence of anti-SLE virus antibodies and SLE virus, respectively.

As a preventive measure, management of the mosquito population with targeted vector control is practiced, but only when a human health risk is demonstrated. There is no vaccine against SLE virus nor is a specific treatment available for amelioration of symptoms or neurological sequelae of SLE. There are no scientifically valid criteria to predict the occurrence of an epidemic. Data collected during the interepidemic period will allow the establishment of a baseline to more scientifically reach a decision for vector control.

MATERIALS AND METHODS

Avian surveillance: Passerine birds were trapped with mist nets throughout cemeteries in metropolitan Chicago. After 0.5–1.0 ml of whole blood was withdrawn by jugular venipuncture, the birds were released. After clotting and centrifugation, sera were analyzed for the presence of SLE virus-specific antibodies by the hemagglutination inhibition (HI) assay (Clarke and Casals 1958). Inhibitors and nonspecific agglutinins were removed from the sera by extracting with 0.167% protamine sulfate in 93.75% cold acetone. Various dilutions of the sera were incubated in triplicate with a previously titered stock of either an SLE virus or 2 other arboviral antigen preparations (provided by the Centers for Disease Control, Atlanta, GA). The above mixtures were then incubated with a 0.25% preadsorbed goose erythrocyte suspension. Agglutination was scored in the microtiter plate with a microplate absorbance reader. The presence of specific antibodies at 1:40 or greater was interpreted as a previous infection by SLE virus.

Mosquito collection: Culex oviposition was monitored weekly at 19 sites throughout Chicago using buckets baited with either sod (Madder et al. 1980) or rabbit chow (Steinly and Novak 1990). Egg rafts were reared until the 3rd instar for species identification. Adult mosquitoes were collected with CDC light traps from the city of Chicago as well as the South Cook County, Des Plaines Valley, and Northwest Chi-

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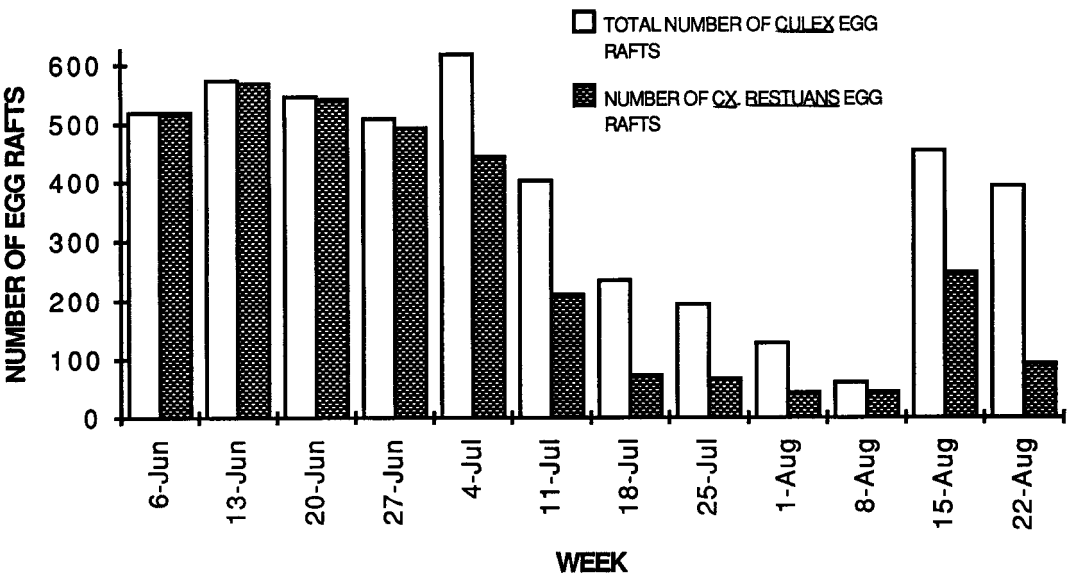

Fig. 1. Total and distribution of *Culex* egg rafts collected. Egg rafts were collected weekly from oviposition traps. Egg rafts were reared in the laboratory until the 3rd instar, when *Culex pipiens* could be distinguished from *Culex restuans.*

cago Mosquito Abatement districts. Up to 50 mosquitoes/site/wk were pooled and were maintained at −70°C until further processing. Thawed mosquitoes were triturated with a disposable plastic tissue grinder (Kontes, St. Louis, MO) in 20 μl of tissue culture fluid/mosquito. The virus was inactivated and the RNA released by diluting an aliquot of the homogenate 1:10 with 5 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% sodium dodecylsulfate and holding the mixture at 94°C for 5 min in a dry block (Vodkin et al. 1994). An aliquot of this mixture was diluted 1:100 in RNAase-free H_2O and held at −20°C until further analysis.

Reverse transcriptase (RT)–polymerase chain reaction (PCR): One microliter of sample was reverse-transcribed in a 10 μl reaction mix consisting of 10 mM Tris (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 1 mM of the 4 deoxyribonucleoside triphosphates, 2.5 μM random hexamers, 1 U/μl RNAase inhibitor, and 2.5 U/μl reverse transcriptase (Perkin Elmer Applied Biosystems, Inc., Foster City, CA). After mixing and overlaying with mineral oil, the reaction mixture was incubated sequentially at room temperature for 10 min, at 42°C for 15 min, at 99°C for 5 min, and at 5°C for 5 min (Vodkin et al. 1994). All runs included a known positive control (provided by Carl Mitchell, Centers for Disease Control, Fort Collins, CO).

The resultant cDNA (10 μl) was amplified in a 50-μl reaction mix of final concentrations: 10 mM Tris (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of the 4 deoxyribonucleoside triphosphates, 0.025 U/μl AmpliTaq polymerase, and 0.4 μM of the SLEV-specific primers (TTAATCTTGGCTCACCCC and ATCTCGGCATTCTGTTCC) that span a 232 base pair region of the NS2a and NS2b genes. Forty cycles at 94°C for 1 min, at 56°C for 1 min, and at 72°C for 1 min were employed for amplification. A final cycle consisting of 94°C for 1 min, 56°C for 1 min, and 72°C for 5 min was employed to ensure completion of a full strand equivalent.

Analysis of products: Ten μl aliquots of the product were electrophoresed through a 2.2% agarose gel, which was then stained with one μg/ml of ethidium bromide. In order to generate enough material for further analysis, an aliquot of the product was diluted 1:100 and subjected to another round of amplification. The products were purified over a QIAquick PCR purification spin column (QIAGEN, Chatsworth, CA) and sequenced by the dideoxynucleotide chain termination method (Sanger et al. 1977).

RESULTS

Avian surveillance: The City of Chicago Department of Health collected and tested the sera from 408 birds in 1993. A single juvenile *Passer domesticus* (house sparrow) was found to be seropositive for SLE viral antibody (also cited in Haramis et al. 1994).

Mosquito collection: A total of 4,623 *Culex* egg rafts was collected during the summer of

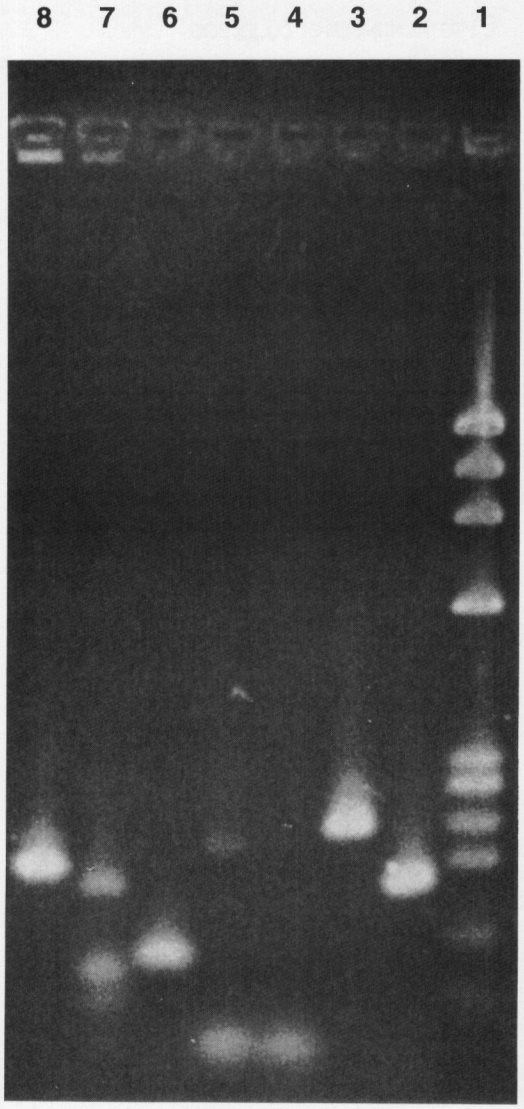

Fig. 2. Electropherogram of some RT-PCR products from the Chicago samples. After RT-PCR, 10 μl of the reaction mix was electrophoresed through a 2.2% agarose gel. Lane 1, ϕX 174-*Hae*III digest; lanes 2–8 various samples; lane 3, positive; and lane 4, positive control.

1993 at the 19 sites in Chicago. These collections represent more than double the 2,120 *Culex* egg rafts collected in 1992. Peak oviposition occurred between June 6 and July 3, averaging 550 egg rafts per week. A secondary peak also occurred during the week of August 15.

Culex restuans Theobald accounted for 75% of the identified rafts, and *Culex pipiens* Linn. composed 24%, with less than 1% from other *Culex* species. From May 30 to July 3, *Cx. restuans* accounted for almost 100% of the material collected (Fig. 1). After July 4, the percentage of *Cx. pipiens* increased until a crossover with *Cx. restuans* occurred during the week of July 18.

Vector surveillance: A sample set of 134 pools of adult mosquitoes was assayed for SLE virus by the RT-PCR. Seven of the pools yielded a visible band following amplification. Some of the products are shown in Fig. 2. However, only one band comigrated with that from the positive control. (This sample was collected at Calumet City on July 23, 1993.) A comparison of its sequence with that published for SLE virus (Trent et al. 1987) shows greater than 90% identity of the aligned nucleotides (Fig. 3). The nucleotide sequences from 2 of the other products were not similar to SLE virus or to any other DNA reported in public databases (data not shown).

DISCUSSION

From 1932 when SLE was first described until 1975, 4 epidemics have been documented in Illinois (Tsai and Mitchell 1987). No other epidemics have occurred in the 20 years since then. This rate translates to an average of one per 14.3 years with a standard deviation of 7.6. The factors that determine the occurrence of an epidemic are doubtless multifactorial and not fully understood.

The transmission cycle of SLE virus is known to involve a bidirectional transfer of virus from birds to mosquitoes. Humans and other mammals are considered dead-end hosts, because their viral titers may be insufficient to infect a mosquito. Some parameters that may be predictors of an epizootic cycle are now being monitored on an annual basis in Chicago. These include estimates of the population densities of *Culex* species and the prevalence of virus in both the reservoir and the vector.

The number of egg rafts collected in 1993 was double that of the previous year (1992). It is hypothesized that the dramatic spring flooding during 1993 disturbed the environment and may have contributed to the increase in *Culex* populations. It will be interesting to determine whether this increase will be sustained in the next few seasons.

The overall *Culex* abundance, defined on the basis of egg rafts, was higher during the first half of the summer and consisted primarily of *Cx. restuans.* This species is not considered the primary vector for either the enzootic or epizootic cycle. *Culex pipiens* accounted for the greater proportion of the overall abundance during the second half of the summer. Historically, all the epidemics have occurred during this period; this

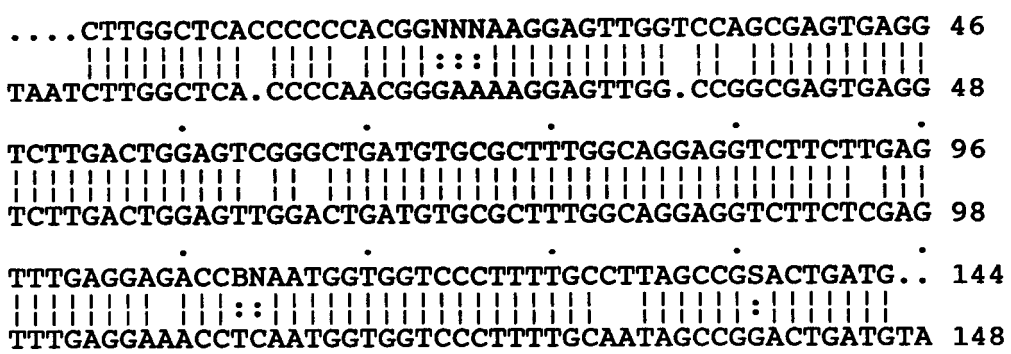

Fig. 3. Comparison of aligned sequences of the RT-PCR product of a Chicago sample (top) and the published SLE virus sequence (bottom). The sequences were aligned with GAP from the University of Wisconsin computer program package (Devereux et al. 1984). S = C or G; B = C, G, or T; N = A, C, G, or T.

is consistent with *Cx. pipiens* as the primary vector in human epidemic transmission. St. Louis encephalitis virus was indeed isolated from mosquitoes captured just prior to the 1975 epidemic (Mitchell et al. 1979).

One of 408 wild birds sampled had a prior exposure to SLE virus (0.2%). The sample was from a juvenile bird, which suggests an exposure during the 1993 season. Until more yearly data are collected, there is no basis to infer whether the observed SLE viral activity was high or low.

Although several of the mosquito pools yielded a signal, only the one that produced a band that migrated with the appropriate mobility was of SLE virus origin. It is important to note that a positive signal generated by RT-PCR is characterized by molecular weight, which is not the case for the other SLE virus assays. This information is a distinct advantage for distinguishing a spurious signal. Also, the assay generates information (sequence data) that is independent of the primers supplied. This information is valuable in verifying whether the amplified product originated from SLE virus.

In Chicago, the avian prevalence was 0.2% and the mosquito pool prevalence was 0.7% for SLE virus (on a per mosquito basis, possibly as little as 0.02%, assuming a maximum of 50 mosquitoes/pool). One human case was recorded in Chicago during this time period (Haramis et al. 1994). Prevalence of SLE virus has been estimated retrospectively in both hosts and vectors sampled just prior to or during an epidemic. Generally, 5–80% of birds were seropositive in association with an SLE virus epidemic (Bowen and Francy 1979). A prevalence of 0.06% was documented in mosquitoes during the 1975 epidemic in Memphis (Mitchell et al. 1979). Because so little data have been collected during interepidemic periods, it is difficult to place those from an epidemic year in perspective. By collecting during each season within Chicago, which has historically suffered epidemics, it may be possible to temporally trace the parameters that convert an enzootic cycle into an epidemic.

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REFERENCES CITED

Bowen, G. S. and D. B. Francy. 1979. Surveillance, pp. 473–499. *In:* T. P. Monath (ed.). St. Louis encephalitis. American Public Health Association, Washington, DC.

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

Devereux, J., P. Haeberli and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12:387–395.

Haramis, L., L. Dudley, C. Jones, B. O'Meara and G. Patschke. 1994. 1993 arbovirus surveillance activities. Vector Control Bull. North Central States 3: 106–110.

Madder, D. J., R. S. MacDonald, G. A. Surgeoner and B. V. Helson. 1980. The use of oviposition activity to monitor populations of *Culex pipiens* and *Culex restuans* (Diptera: Culicidae) Can. Entomol. 112: 1013–1017.

Mitchell, C. J., D. B. Francy and T. P. Monath. 1979. Arthropod vectors, pp. 313–379. *In:* T. P. Monath (ed.). St. Louis encephalitis. American Public Health Association, Washington, DC.

Monath, T. P. 1979. Epidemiology, pp. 239–312. *In:* T. P. Monath (ed.). St. Louis encephalitis. American Public Health Association, Washington, DC.

Sanger, F., S. Nicklen and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.

Steinly, B. A. and R. J. Novak. 1990. *Culex restuans* and *Culex pipiens* oviposition frequency characteristics during a drought year (1988) in east-central Illinois. Proc. Ill. Mosq. Vector Control Assoc. 1: 16–24.

Trent, D. W., R. M. Kinney, B. J. Johnson, A. V. Vorndam, J. A. Grant, V. Deubel, C. M. Rice and C. Hahn. 1987. Partial nucleotide sequence of St. Louis encephalitis virus RNA: structural proteins, NS1, ns2a, and ns2b. Virology 156:293–304.

Tsai, T. F. and C. J. Mitchell. 1987. St. Louis encephalitis, pp. 113–143. *In:* T. P. Monath (ed.). The arboviruses: epidemiology and ecology IV. CRC Press, Inc., Boca Raton, FL.

Vodkin, M. H., T. Streit, C. J. Mitchell, G. L. McLaughlin and R. J. Novak. 1994. PCR-based detection of arboviral RNA from mosquitoes homogenized in detergent. BioTechniques 17:114–116.