

## ABSENCE OF LA CROSSE VIRUS IN THE PRESENCE OF *Aedes triseriatus* ON THE DELMARVA PENINSULA<sup>1</sup>

GARY G. CLARK, C. L. CRABBS AND BROOKE T. ELIAS

Department of Arboviral Entomology, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701-5011

**ABSTRACT.** Between 1980 and 1984, field studies were conducted in 2 areas on the Delmarva Peninsula to identify the presence of La Crosse (LAC) virus. Ovitrap traps were used to collect eggs of *Aedes triseriatus* complex mosquitoes. No virus was obtained from 969 pools containing 22,370 adult mosquitoes reared from eggs. Only 1 of 143 raccoon serum samples had neutralizing antibody to LAC virus; 36 sentinel domestic goats, and 99 other wild mammal serum samples were negative. The apparent absence of LAC virus may be due to the uncommon occurrence of the eastern chipmunk, a species which has been shown to be an amplifying host for this virus, on the Peninsula.

### INTRODUCTION

La Crosse (LAC) virus, a member of the California virus serogroup, is a human pathogen (Kappus et al. 1983) transmitted primarily by the mosquito *Aedes triseriatus* (Say) (Watts et al. 1972). Grimstad and coworkers (1977) delineated "the endemic region" for LAC virus in the United States. This crescent-shaped area extended eastward from southeastern Minnesota around the southern edge of the Great Lakes up to western New York state. However, with increasing interest in this arbovirus, virus isolations and human cases have been identified from areas well beyond this region.

In northeastern U.S., following confirmation of human encephalitis cases and recovery of virus, a LAC virus endemic area was identified in extreme east-central New York (Grayson et al. 1983). Evidence of three human LAC virus infections has been reported from nearby northern New Jersey (Kappus et al. 1983), and in southeastern Pennsylvania a serosurvey of approximately 1,000 people suggested the existence of a LAC-endemic area there (Anonymous 1970). In southeastern U.S., evidence of LAC virus activity has been reported from North Carolina (Kappus et al. 1983), based on both occurrence of human cases and isolation of virus, and from South Carolina, where a human case occurred in 1982 (Centers for Disease Control, 1983). In summarizing California group virus isolations from mosquitoes in

North America for the period from 1943 to 1970, Sudia et al. (1971) reported 3 LAC virus isolations from Georgia where recent human infections have been documented by Sikes et al. (1984). In south Florida, Prather (1969) reported that 38% of 343 native Indians surveyed had neutralizing (N) antibody to LAC virus, although LAC virus has never been isolated in this area. Kappus et al. (1983) also report a single California encephalitis case from Florida.

In contrast to LAC virus activity documented to the north and the south, isolation of LAC virus has not been reported from the mid-Atlantic states of Delaware, Maryland and Virginia (Sudia et al. 1971, Parkin et al. 1972), and no human cases have been recorded from this area (Calisher 1983). Serological evidence of California group virus infection was reported from Virginia (Parkin et al. 1972), but the specific viral etiology was not determined.

In the area in which we were working, the Delmarva Peninsula, no LAC virus was isolated from relatively small numbers, 637 (Saugstad et al. 1972) and 347 (LeDuc et al. 1975), of *Ae. triseriatus* tested from 1969 to 1972. Serologic tests for N antibody to LAC virus from sera of 25 research personnel involved in field studies on the Peninsula were negative (Watts et al. 1982). As a result of the expansion in the recognized geographic distribution of LAC virus in the U.S., intensive efforts were made to determine if this virus, which can be transovarially maintained, occurred on the Delmarva Peninsula.

### METHODS AND MATERIALS

**MOSQUITOES.** In 1980, ovitraps with black flannel liners (Loor and DeFoliart 1969) were placed at ground level on trees in the Pocomoke Cypress Swamp (PCS), Worcester County, Maryland, and approximately 36 km east-southeast of the PCS in the Chincoteague National Wildlife Refuge (CNWR) at the southern end of Assateague Island, Accomack County,

<sup>1</sup> The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Virginia. The PCS and its vegetation have been described by Saugstad et al. (1972), while Buescher et al. (1970) have characterized CNWR. In 1983, ovitraps, with the balsa substrate modification of Novak and Peloquin (1981), were again used in these two study areas. As part of a study of the oviposition patterns of *Ae. triseriatus* and *Aedes hendersoni* Cockerell (Clark and Craig 1985), individual ovitraps were placed at 0, 3 and 6 m on 20 trees at both the PCS and CNWR. A total of 120 traps were utilized. Ovitrap inserts were removed and replaced after 2 weeks in the field.

In the laboratory, eggs were hatched and reared to the adult stage. Adult mosquitoes were stored at  $-70^{\circ}\text{C}$  and identified on a chill table at  $4^{\circ}\text{C}$  as being members of the *Ae. triseriatus* complex. Mosquito pools containing a maximum of 25 individuals were sorted by sex and site and date of collection. Mosquito pools were triturated in prechilled tissue grinders containing medium 199, supplemented with glutamine, 20% fetal bovine serum (FBS), and antibiotics (200 units/ml penicillin, 200  $\mu\text{g}/\text{ml}$  streptomycin, and 10  $\mu\text{g}/\text{ml}$  fungizone). One ml was used for pools with 1 to 12 mosquitoes and 2 ml for pools with 13 to 25 mosquitoes. Suspensions were clarified by centrifugation at  $800 \times g$  for 20 min at  $4^{\circ}\text{C}$  and assayed immediately for virus or stored at  $-70^{\circ}\text{C}$  until assayed. Samples were tested for virus by plaque assay in Vero cells. Aliquots of 0.1 ml of each supernatant were inoculated in triplicate onto Vero 12-well trays, adsorbed for 1 hr at  $35^{\circ}\text{C}$ , and then overlaid with 2 ml of an agar overlay medium consisting of equal volumes of 1.5% agarose and 2X Eagle's basal medium in Earle's salts with HEPES buffer (4 g/liter), 10% heat-inactivated FBS and antibiotics. Cell cultures were incubated in a humidified atmosphere with 5%  $\text{CO}_2$  for plaque development. After 4 days, the overlay medium (1 ml/well), containing a 7% solution (stock 1:300) of neutral red, was added to the cells. Cultures were incubated for 24 hr and examined for plaques.

**MAMMALS.** During 1983 and 1984, wild mammals were trapped alive at CNWR, anesthetized, ear-tagged and bled via cardiac puncture. With recaptured mammals, the last sample collected was assayed. Fluid was obtained from the thoracic cavity of white-tailed deer brought to state deer hunter checking stations. Blood specimens were allowed to clot, kept at  $4^{\circ}\text{C}$  and centrifuged at  $800 \times g$  for 20 min. Serum was decanted and stored at  $-20^{\circ}\text{C}$ . Pairs of young, seronegative domestic goats were housed in 5 goat-baited insect traps. In 1983, these traps were situated in various locations at CNWR. The distances between the closest traps and farthest traps were approx-

imately 2 km and 15 km, respectively. Arthropods were collected 2-3 times per week and goats were bled via venipuncture of the jugular vein once per week. Blood was processed as described above. At the PCS, 5 goats were in a corral and had a Magoon trap with the door removed for shelter. Arthropods and goats were sampled as at CNWR. In 1984, goats were again utilized at PCS and the goat-baited traps were moved from CNWR and situated near brackish water marshes of the Chesapeake Bay in Somerset County, Maryland, and Accomack County, Virginia, on the western border of the Delmarva Peninsula. The final serum sample, obtained from each goat in late October, was assayed for N antibody to LAC virus.

Mammalian sera were tested for N antibody to LAC, Jamestown Canyon (JC), and Keystone (KEY) viruses in Vero cells. The LAC stock virus used in the plaque reduction neutralization (PRNT) was originally received from Dr. Wayne H. Thompson at the University of Wisconsin and has undergone 8 passages in suckling mouse brain and 1 Vero cell passage. The JC stock virus was isolated from a pool of *Aedes canadensis* (Theobald) collected at the PCS in 1979 and had undergone 2 passages in tissue culture. The KEY stock virus was isolated from *Aedes atlanticus* Dyar and Knab collected at the PCS in 1972 and had been passed 3 times in tissue culture. Sera were heat treated at  $56^{\circ}\text{C}$  for 30 min and a 1:10 dilution was prepared in the medium described above. Mixtures of an equal volume of each diluted serum and a virus suspension containing 50-100 PFU were incubated for 1 hr at room temperature and inoculated onto Vero cells (0.1 ml per each of 3 cultures). Subsequent assay procedures were according to those described above. A reduction of 80% or more of the virus dose by a 1:10 serum dilution was our criterion for the presence of N antibody.

## RESULTS

A total of 22,370 *Ae. triseriatus* complex mosquitoes in 969 pools from PCS and CNWR was assayed as adults for virus (Table 1). No virus was obtained from these mosquitoes. Most of the mosquitoes (73% of 16,349) were collected and assayed in 1983, with the remaining 6,021 collected from the same localities in 1980. Collections from the PCS accounted for approximately 71% of the mosquitoes assayed. Overall, samples were fairly evenly distributed (21-28%) from the 4 monthly periods beginning on June 4 and concluding on September 26. Additionally, no virus was isolated from 109, including 100 (91%) bloodfed, *Ae.*

Table 1. Adult *Aedes triseriatus* complex mosquitoes collected as eggs and assayed for virus, Delmarva Peninsula, 1980 and 1983.

Area Year	Collection interval				Total
	June 4- July 6	July 7- August 1	August 2-31	September 1-26	
PCS <sup>1</sup>					
1980	0	613/25 <sup>2</sup>	716/31	2305/93	3634/149
1983	3323/149	3105/142	4555/163	1230/78	12213/532
CNWR <sup>1</sup>					
1980	0	710/30	565/23	1112/47	2387/100
1983	1859/82	1954/84	271/16	52/6	4136/188
Total	5182/231	6382/281	6107/233	4699/224	22370/969

<sup>1</sup> PCS=Pocomoke Cypress Swamp, Maryland and CNWR=Chincoteague National Wildlife Refuge, Virginia.

<sup>2</sup> No. mosquitoes/no. pools.

*triseriatus* complex mosquitoes collected from goat-baited traps.

From the mammal trapping program at CNWR in 1983, 170 sera were available for assay of N antibody to LAC virus (Table 2). Including the wild mammals trapped there in 1984, white-tailed deer sera from checking stations, and sera from domestic goats, 277 of the 278 sera assayed did not have detectable N antibody to LAC virus. The single exception was a CNWR raccoon, captured and bled on July 7, which had a 1:20 PRNT to LAC virus. This raccoon had no detectable titers to JC and KEY viruses. Three raccoons and 1 eastern cottontail had N antibody to KEY virus.

## DISCUSSION

The presence of *Ae. triseriatus*, the principal vector for LAC virus in the upper Midwest (Watts et al. 1972), and the absence of viral isolates from 22,479 *Ae. triseriatus* complex

mosquitoes from 2 study sites suggest that LAC virus is not present on the Delmarva Peninsula. In a related study, Clark and Craig (1985) found that all of the *Ae. triseriatus* complex specimens they examined from CNWR and 95% of those from the PCS were *Ae. triseriatus*. The remaining 5% at PCS were *Ae. hendersoni*, a sympatric species that does not transmit LAC virus.

Earlier reports from LAC-endemic areas revealed minimum field infection rates (MFIRs), per 1,000 female *Ae. triseriatus* tested. These MFIRs ranged from 2.5 in Wisconsin and Iowa (Thompson et al. 1972) and 9.9 in Illinois (Clark et al. 1983) up to 12.3 in Ohio (Berry et al. 1975). In studies of this transovarially maintained virus, where eggs or immature *Ae. triseriatus* were collected and reared to the adult stage for viral assay, MFIRs (per 1,000) ranging from 1.0 in North Carolina (Kappus et al. 1983) and 1.2 in Illinois (Clark et al. 1983) to 6.4 in Wisconsin (Beatty and Thompson 1975) have been observed. By extrapolating from these reports, in which LAC virus was isolated from *Ae. triseriatus*, we might have obtained from 24 to 143 isolates of LAC virus, if the above MFIRs were directly applicable and if our study areas were part of endemic foci.

The overwhelming absence of N antibody to LAC virus in the mammalian sera assayed is a further reflection of the absence of LAC virus in this area, despite reports to the contrary from northeastern and southeastern U.S. The occurrence of N antibody to LAC virus detected in a single raccoon is not understood. This serum sample was assayed 3 times with the same result on each occasion. No antibody response to JC or KEY viruses, both endemic in the PCS, was detected. The collection of a high percentage of bloodfed females indicates that this species will readily feed on goats. Yet, no goats developed N antibody to LAC virus.

Table 2. Mammal sera, assayed for N antibody to LAC virus, from the Delmarva Peninsula.

Common name (Species)	1983	1984	Total
Wild mammals			
Raccoon ( <i>Procyon lotor</i> )	120	23	143
White-tailed deer ( <i>Odocoileus virginianus</i> )	43	4	47
Opossum ( <i>Didelphis virginianus</i> )	23	0	23
Eastern cottontail ( <i>Sylvilagus floridanus</i> )	14	0	14
Muskrat ( <i>Ondatra zibethicus</i> )	7	0	7
Rat ( <i>Rattus</i> spp.)	6	0	6
Red Fox ( <i>Vulpes vulpes</i> )	0	2	2
Domestic mammals			
Goat ( <i>Capra hircus</i> )	19	17	36
Total	232	46	278

While it is not possible to fully explain the apparent absence of LAC virus in our study areas, one plausible explanation involves the eastern chipmunk (*Tamias striatus*). Studies in midwestern U.S., reviewed by Yuill (1983), have described the close ecological association between the chipmunk, *Ae. triseriatus*, and LAC virus and the chipmunk's role as an amplifying host for LAC virus. LAC virus and/or N antibody to LAC virus have been detected in the chipmunk. Additionally, experimental studies demonstrated that LAC virus-infected chipmunks developed a viremia sufficient to infect *Ae. triseriatus* which could then biologically transmit LAC virus.

Paradiso (1969) reported that the chipmunk is "Uncommon on the Eastern Shore [i.e. the Delmarva Peninsula]" and, where present in Maryland, "... prefers to live on wooded hill-sides or mountain slopes." This mammal does not occur on CNWR (V.F. Flyger 1985, personal communication). The area where our study was conducted is part of the Mid-Atlantic Coastal Plain (U.S. Department of Agriculture 1981) and, with an elevation ranging from sea level to 25 m, is underlain by unconsolidated sand, silt, and clay. These soil characteristics may contribute to the lack of suitable habitat, thus precluding occurrence of the chipmunk, which spends considerable time in underground burrows.

The gray squirrel (*Sciurus carolinensis*) occurs in this area and has been associated with LAC virus in Wisconsin (Yuill 1983). Sera from approximately 30 gray squirrels collected at the PCS have been assayed for N antibody to LAC virus (D.M. Watts 1985, personal communication). No LAC virus specific antibody was detected. Analysis of a larger sample size of gray squirrel sera from this locality might clarify the negative evidence we have presented.

In summary, results of this study of *Ae. triseriatus* complex mosquitoes and serosurvey of wild and domestic mammals did not identify the presence of LAC virus on the Delmarva Peninsula. A possible explanation for the absence of this human pathogen in this area may be the uncommon occurrence of the eastern chipmunk, an important component of the LAC virus cycle in midwestern U.S. With the southern and eastern expansion of the recognized LAC endemic area, it may soon encompass the range of *Ae. triseriatus* (Grimstad et al. 1977), which is almost identical to that for the eastern chipmunk (Burt and Grossenheider 1964).

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