AEDES CANADENSIS, A VECTOR OF LA CROSSE VIRUS (CALIFORNIA SEROGROUP) IN OHIO[1]

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ABSTRACT. *Aedes canadensis* was shown to be a vector of La Crosse (LAC) virus in Ohio through isolation of LAC virus from field-collected specimens, infection of 54 of 72 (75%) individuals when fed on viremic suckling mice and transmission of LAC virus by 29 (54%) of infected individuals. Frequent identification of *Ae. canadensis* as a human biting species implicates it as an auxiliary vector of LAC virus to man. A possible regional association in Ohio of *Ae. canadensis* and Type C LAC virus is discussed.

INTRODUCTION

In 1971, we reported results from virus assay of field-collected mosquitoes and identified *Aedes triseriatus* (Say), *Aedes canadensis* (Theobald), *Aedes vexans* (Meigen) and *Aedes sollicitans* (Walker) as potential vectors of La Crosse (LAC) virus in Ohio (Masterson et al. 1971). In subsequent publications (Berry et al. 1974, 1975, 1983), we confirmed studies published elsewhere that *Ae. triseriatus* is the primary vector of and served as an overwintering host for LAC virus (Watts et al. 1972, 1973a; Pantuwatana et al. 1974). In the 1975 and 1983 publications, we cited additional evidence that *Ae. canadensis* was a potential vector of LAC virus in Ohio. This was in contrast to other published studies (Watts et al. 1973b).

In Wisconsin, studies with caged animals showed that *Ae. canadensis* had high engorgement rates on several medium-to-large size mammals (whitetail deer, raccoon, woodchuck, etc.) and, also, on three species of turtles and a leopard frog. Engorgement rates were very low for grey and fox squirrels and chipmunks (Wright and DeFoliart 1970). In the laboratory, it was demonstrated that *Ae. canadensis* was readily infected with LAC virus, but had poor virus replication and low transmission rates (Watts et al. 1973b). These studies concluded that *Ae. canadensis* had a low potential as an important biological vector of LAC virus in nature.

The purpose of this report is to present field and laboratory findings which tend to incriminate *Ae. canadensis* as a vector of LAC virus in Ohio.

MATERIALS AND METHODS

MOSQUITOES. The techniques used for the field collection and laboratory processing of mosquitoes for virus isolation were described previously (Berry et al. 1974, 1975). Since 1976, primary isolation of California serogroup viruses has been done using a continuous line of Vero (African green monkey kidney) cells.

On May 6, 1975, *Ae. canadensis* were collected as 4th instar larvae or pupae from a woodland pool in Stow, Summit County, Ohio. They were transported to the laboratory with water and debris from the woodland pool. Small amounts of ground Purina guinea pig chow supplemented the natural nutrient material. Insectary environmental conditions included: temperature, 24 ± 3°C; 75 ± 5% RH; and 16 hr photoperiod. Adults reared from these samples were provided with a 5% sucrose solution and soaked raisins for food. Adult females, held in pint ice-cream cartons with fine nylon screen tops, were 11–12 days old at the beginning of the infectivity-transmission experiment. A control sample of 319 *Ae. canadensis* was found to be negative for naturally acquired arbovirus infections.

EXPERIMENTAL VIRUS. The LAC virus used to infect the mosquitoes was isolate number ODH 69–1015 (=ODH-P76-4263), CDC No. R-15804. It was originally isolated from a pool of 50 female *Ae. canadensis*, collected in a CDC miniature light trap set in Stow, Summit County, Ohio, on July 9–11, 1969. The virus was a 3rd suckling mouse passage. Virus assays of mosquitoes, suckling mouse brain and suckling mouse and chipmunk blood samples were prepared according to methods described by Sudia and Chamberlain (1967) and Sudia et al. (1970) and assayed or titrated by plaque technique on normal human skin cell cultures in 25 cm^2 plastic bottles (Perry et al. 1956).

Viruses recovered on cell cultures from chipmunk blood samples and mosquitoes were inoculated into litters of suckling mice. Recovered virus in brain material from mice dying after these inoculations and from mice dying after mosquito bite were identified by complement fixation (CF) test as described earlier (Berry et al. 1974). The identity of two isolates recovered from mosquitoes was confirmed by CF test by Dr. C. Calisher, Centers for Disease Control, Fort Collins, Colorado.

TRANSMISSION TRIALS. Two to three-day-old suckling mice were inoculated intracranially

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with 100 TCID-50 of the LAC virus. *Aedes canadensis* were allowed to obtain a viremic blood meal from these suckling mice 40 hr after inoculation. Individual suckling mice were restrained on the nylon mesh tops of the mosquito cups, which contained 20 female *Ae. canadensis*. When mosquito feeding had terminated, blood samples (0.02–0.10 ml) were obtained from each of the suckling mice, diluted in 1.9 ml normal rabbit serum and stored at −70°C. Serial 10-fold dilutions of suckling mouse blood samples were assayed in triplicate and plaque numbers averaged to determine virus titers.

Fully engorged *Ae. canadensis* were held individually in pint cartons. Virus transmission was attempted daily from day 7 to day 14 after engorgement. Two or three-day-old uninfected suckling mice, fed upon by a single mosquito, were observed for 14 days for signs of illness and/or mortality. Littermates that were exposed to mosquitoes were given identifying marks by subcutaneous injection of india ink in their tails prior to *Ae. canadensis* feeding. The transmission experiment was terminated and all mosquitoes frozen by the 22nd day after acquiring the viremic blood meal.

Thirteen chipmunks trapped in Richland County, Lucas, Ohio, during the fall of 1974 were held in a laboratory isolated from the mosquito and mouse-rearing facilities. A blood sample (0.2 ml) was taken from each chipmunk 1 month prior to the transmission experiment and tested by hemagglutination inhibition technique for evidence of previous infection with California serogroup viruses. On day 13 post-blood feeding, 12 chipmunks were anesthetized with carbon dioxide and 42 *Ae. canadensis*, in lots of 1 to 8, were allowed to re-feed through a nylon mesh screen secured on a small plastic cup. One chipmunk was fed upon by previously unfed *Ae. canadensis* as a control. On days 3 and 7 after mosquito feeding, blood specimens (0.2 ml) were obtained from the chipmunks, diluted in 1.9 ml normal rabbit serum, and the sera stored at −70°C for virus and antibody assay. Uninfected *Ae. triseriatus* were allowed to feed on the chipmunks 3 days after the *Ae. canadensis* had fed. These *Ae. triseriatus* were held in the insectary for 14 days, then frozen at −70°C for virus assay.

RESULTS

VIRUS ISOLATION FROM VECTORS. Between 1965–81, 82,885 adult *Ae. canadensis* were collected from 64 Ohio counties and tested for arboviruses, yielding 41 LAC virus isolations. During that same period, 27,828 *Ae. triseriatus* adults were assayed and produced 32 LAC virus isolates.

The months when *Ae. canadensis* were collected and the number of isolates were: June (12); July (20); August (8); and September (1). Multiple isolates were obtained on separate occasions from several small areas—woodlots of 5–20 acre size. These included 30 isolates from small collections made between June 19 and August 12. Minimum field infection rates (MFIR) among those collections with multiple isolates ranged from 2.8 to 42.6/1,000 (Table 1).

During 1978 and 1979, 4,776 and 2,426 *Ae. canadensis* larvae were collected from Summit and Lake counties, respectively. They were reared to the adult stage and tested for LAC virus, with negative results.

From 1965 to 1981, LAC virus isolates from adult *Ae. canadensis* were obtained from 6 Ohio counties: Defiance (1); Knox (1); Lake (14); Lorain (3); Mahoning (7); and Summit (15). La Crosse virus isolates obtained from adult *Ae. triseriatus* during the same time interval were from 9 counties: Allen (7); Cuyahoga (1); Defiance (2); Delaware (1); Huron (1); Knox (17); Lake (1); Summit (1); and Wyandot (1). Summit and Knox counties were intensively studied due to the recurrence of human LAC virus infections. Greater numbers of LAC isolates from *Ae. canadensis* occurred in the northeast part of Ohio, whereas isolates from *Ae. triseriatus* showed a more widespread distribution (Fig. 1).

INFECTION-TRANSMISSION EXPERIMENTS. Seventy-two *Ae. canadensis* engorged on viremic

Table 1. Multiple isolates of La Crosse virus from field-collected *Aedes canadensis*[1]

Date	County (City)	No. collected	No. isolates	Infection rate/1000
July 26–29, 1966	Mahoning (Austintown)	664	7	10.5
Aug. 8–12, 1966	Summit (Stow)	163	3	18.4
July 8–11, 1969	Summit (Stow)	1,659	6	3.6
Aug. 4–7, 1970	Summit (Stow)	215	2	9.3
July 27–30, 1971	Lake (Mentor)	47	2	42.6
June 19–21, 1978	Lake (Eastlake)	1,162	8	6.9
June 19–25, 1980	Lorain (N. Ridgeville)	703	2	2.8

[1] Collected in CDC miniature light traps supplemented with dry ice.

suckling mice (log_{10} 3.26–5.77 pfu/0.03 ml of blood) and refed on uninfected suckling mice. The number of mosquitoes which fed each day of the 8-day transmission trials was, respectively: 19, 10, 10, 3, 5, 11, 9, 5. Fifty-four (75%) of the *Ae. canadensis* became infected. Twenty-nine (53.7%) of the 54 transmitted virus, based upon virus isolations from brain tissue of the sick and dead suckling mice.

During the course of the transmission trials, the transmission rate appeared to be increasing. The average transmission rate for the first 4 days of the trials was 16/34 (47.1%), whereas the average rate during the last 4 days was 13/20 (65.0%).

TRANSMISSION OF LAC VIRUS TO CHIPMUNKS. Thirty of 42 (71%) *Ae. canadensis* used in this transmission trial were infected. Two of the 12 chipmunks were fed upon by uninfected *Ae. canadensis*. Three of the remaining 10 (30%) became infected, as demonstrated by virus isolation from blood samples drawn 3 days after feeding of *Ae. canadensis*. Of the 3 infected chipmunks, 2 were fed upon by single *Ae. canadensis*, and the 3rd chipmunk was fed upon by 2 *Ae. canadensis* and bitten by a third. Both of

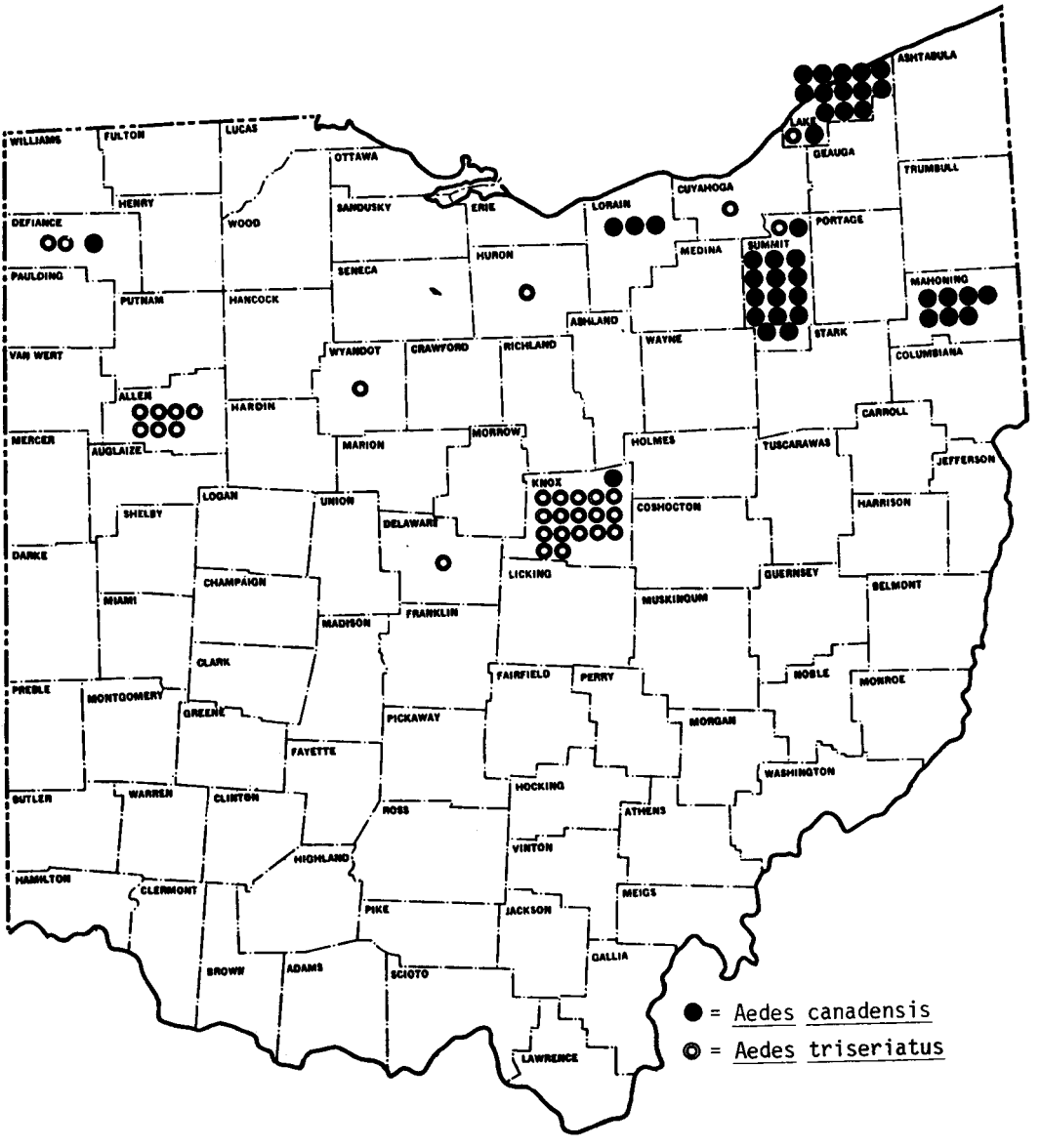

Fig. 1. La Crosse virus isolates from *Aedes canadensis* and *Ae. triseriatus*, Ohio, 1965–81.

the latter which fed were infected. The identity of the one which bit was not determined and may or may not have been infected. Virus was not isolated from the blood samples drawn 7 days after the *Ae. canadensis* feeding, nor from the blood samples obtained from the control chipmunk. One of the viremic chipmunks circulated sufficient virus to infect 2 of 3 *Ae. triseriatus* from the laboratory colony.

Tests of the sera, including the pretransmission sample, and the samples drawn on days 3 and 7 after *Ae. canadensis* feeding, were negative for HI antibody to LAC virus.

DISCUSSION

Four basic criteria necessary to establish the vector status of a mosquito species were outlined by Sudia et al. (1971): 1) isolation of the virus from field-collected mosquitoes; 2) field evidence associating the vector with the vertebrate population in which the infection is occurring; 3) successful infection of the vector when fed upon a viremic vertebrate; and 4) successful transmission of the virus to a susceptible vertebrate after a period of extrinsic incubation.

We recognized early in our studies of California serogroup viruses that *Ae. canadensis* was probably involved in the epidemiology of LAC virus (Masterson et al. 1971). We actually have more LAC virus isolates from field collected adult. *Ae. canadensis* (41) than from adult *Ae. triseriatus* (32). The overall statewide infection rates for field collected adults of the two species, for the period 1965–81, were 0.5 and 1.1/1,000, respectively. These data clearly fulfill the first requirement of a potential vector.

Aedes canadensis has long been recognized as one of the most important early breeding pest mosquitoes in Ohio (Venard and Mead 1953). *Aedes canadensis* is well represented in records of biting mosquitoes in Ohio, accumulated since 1965 by the Vector-borne Disease Unit (unpublished data, Ohio Department of Health).

The number of field collected *Ae. canadensis* found infected with LAC virus in Ohio is indirect evidence that it feeds on vertebrate reservoir hosts with a fair degree of regularity. Data on field infection rates gives further evidence that MFIRs of epizootic proportions may occur locally (Table 1). This is contrary to results of host preference studies in Wisconsin, where the absence of contact with reservoir mammals minimized a role for *Ae. canadensis* as a biological vector (Wright and DeFoliart 1970 and Watts et al. 1973b). The documented interaction with humans and the evidence of interaction with the vertebrate reservoir hosts satisfies the second criteria for vector status.

By laboratory experimentation, we satisfied the 3rd and 4th criteria for vector incrimination, demonstrating that *Ae. canadensis* is readily infected by feeding on a viremic host and is capable of transmitting LAC virus to suckling mice and chipmunks. In regard to the latter, we found LAC virus in blood samples of 3 chipmunks on day 3, but no virus on day 7 following *Ae. canadensis* feeding. This agrees with the results of Pantuwatana et al. (1972), who found chipmunk viremias on days 2–5 after infection, but no detectable viremias after day 5. One of the chipmunks infected by *Ae. canadensis* circulated sufficient virus to infect laboratory colony *Ae. triseriatus*, suggesting further that *Ae. canadensis* can play a role in the enzootic cycle of LAC virus.

None of the chipmunk blood samples had demonstrable antibody to LAC virus on the days of serum collection. This is reasonably consistent with the results obtained by Pantuwatana et al. (1972), who found neutralizing antibody to LAC virus just beginning to develop in chipmunks on day 5 postinfection, with highest geometric mean titers reached 21 to 28 days postinfection. At the serum dilution of 1:40, we could not have been able to detect low titers of HI antibody. If we had monitored antibody levels for a longer period, we probably would have detected increasing titers and might have found evidence of a greater rate of transmission than that shown by virus isolation.

Although *Ae. canadensis* is generally regarded to have one generation per year, there is evidence that a second brood sometimes occurs during the summer. This has been observed in northeastern Ohio (R. Rings, personal communication), and we have found late-season (July–August) hatching and emergence of *Ae. canadensis* in association with *Ae. sollicitans* breeding in water of high salt content. These were isolated occurrences restricted to areas of industrial- and oil well-associated salt pollution. Evidence for second broods of *Ae. canadensis* was cited by Horsfall (1955). Also, Magnarelli (1977) documented a second *Ae. canadensis* emergence during July and August in New York. Multiple broods lengthen the season of adult *Ae. canadensis* activity and may enhance their involvement in LAC virus transmission during the epidemic season.

The data on field infection rates in *Ae. canadensis* may be evidence of vertical transmission, especially those documented during 1978 and 1980, in which isolates were obtained from collections made shortly after adult emergence (Table 1). Attempts to isolate LAC virus from adult *Ae. canadensis* reared from field collected larvae and pupae have been negative. Ad-

ditional sampling and testing should be done, especially in areas where there has been a history of LAC virus isolation from *Ae. canadensis.*

Although *Ae. canadensis* has been collected and tested from 64 Ohio counties, the preponderance of LAC isolates from this species were obtained from the northeastern part of the state (Fig. 1), suggesting a unique regional association of LAC virus. Recent studies by El Said et al. (1979) demonstrated distinct characteristics in a LAC virus isolate from this region. Analyses of the oligonucleotide fingerprints of LAC virus isolates from different regions of the United States showed that a distinctive Ohio isolate from *Ae. canadensis* (CDC No. R–15804, the same isolate used in the experiment reported here) had affinities to a LAC virus isolate from *Ae. canadensis* obtained near Albany, New York. Grayson et al. (1983) have reported LAC virus associations which are similar to those in Ohio; the highest LAC virus prevalence rates in New York were found in *Ae. triseriatus* and *Ae. canadensis.* Further studies by Klimas et al. (1981) have demonstrated that the *Ae. canadensis* isolate from Stow, Ohio, is a member (Type C) of a complex with representatives in New York, Georgia, North Carolina and Texas.

Our experiment showed infection and transmission rates of 75.0 and 53.7%, respectively. Watts et al. (1973b) reported average infection rates of 79%, but transmission rates of only 30% for LAC virus by *Ae. canadensis.* It is possible that the combination of LAC virus and *Ae. canadensis* from sympatric populations resulted in higher transmission rates due to compatibility factors. Watts et al. (1973b) used a LAC isolate originally obtained from *Ae. triseriatus* in Wisconsin. In addition, Gubler and Rosen (1976) and Grimstad et al. (1977) have demonstrated differences between regional populations of mosquitoes in their ability to become infected and to transmit arboviruses. Miller et al. (1982) found that differences also existed in geographic strains of *Ae. triseriatus* in their ability to transmit LAC virus transovarially.

We believe that our study provides another example of regional differences in virus-vector relationships. The elucidation of epidemiological facts in one region cannot be literally extrapolated to another region without scientifically gathered confirmatory evidence.

Based upon the results of our field and laboratory studies, we have shown that *Ae. canadensis* is involved in the enzootic cycle of LAC virus in Ohio. It is evident that LAC virus-vector associations are more complex than have been previously described and auxiliary amplifiers of LAC virus should be considered in mathematical models currently under development (Lisitza et al. 1977, Burkot and DeFoliart 1982, DeFoliart 1983).

It is not known if the other isolates of LAC virus from northeastern Ohio, including those from *Ae. triseriatus,* are of the A, B or C type, described by Klimas et al. (1981). Oligonucleotide studies of these isolates would resolve this question and might lead to evidence that LAC Type C is responsible for human infection in Ohio.

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