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La Crosse Encephalitis Virus Habitat Associations in Nicholas County, West Virginia

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ABSTRACT *Aedes triseriatus* (Say) population density patterns and La Crosse encephalitis virus infection rates were evaluated in relation to a variety of habitat parameters over a 14-wk period. Ovitraps and landing collections were used in a La Crosse virus-enzootic area in Nicholas County, WV. Study sites were divided into categories by habitat type and by proximity to the residences of known La Crosse encephalitis cases. Results demonstrated that *Ae. triseriatus* population densities were higher in sugar maple/red maple habitats than in hemlock/mixed hardwood habitats or in a site characterized by a large number of small red maple trees. Sites containing artificial containers had higher population densities than those without. La Crosse virus minimum infection rates in mosquitoes collected as eggs ranged from 0.4/1,000 to 7.5/1,000 in the 12 study sites, but did not differ significantly among sites regardless of habitat type or proximity to human case residences. La Crosse virus infection rates in landing *Ae. triseriatus* mosquitoes ranged from 0.0/1,000 to 27.0/1,000. La Crosse virus was also isolated from host-seeking *Ae. canadensis* (Theobald) in two study sites, at rates similar to those found in the *Ae. triseriatus* populations. The *Ae. triseriatus* oviposition patterns and La Crosse virus infection rates suggest that this mosquito species disperses readily in the large woodlands of central West Virginia. The La Crosse enzootic habitats in Nicholas County, WV, are contrasted with those studied in other geographic regions where La Crosse virus is found.

KEY WORDS *Aedes triseriatus*, *Aedes canadensis*, La Crosse encephalitis virus, mosquito ecology, habitat structure

LA CROSSE (LAC) encephalitis virus is distributed from Minnesota eastward to New York, and south to Texas, Alabama, and Georgia (Calisher 1983). Confirmed human cases of LAC encephalitis have been documented from 29 of the lower 48 states (CDC 1993). The geographic distribution of LAC virus is associated with the distribution of hardwood forests and the distribution of the vector, *Aedes triseriatus* (Say) (Calisher 1983, 1994; Grimstad 1988).

From 1964 to 1986, a total of 1,726 cases of LAC encephalitis was reported to CDC (G. R. Campbell, Centers for Disease control and Prevention, unpublished data) for an average of 75 cases per year; most of these cases were from midwestern states (Fig. 1). West Virginia accounted for <1% of the total cases reported during that period. From 1987 to 1997, some 744 LAC cases (average 68 cases per year) were reported nationwide. The geographic distribution of cases remained approximately the same during the latter period, but a greater percentage of the cases was reported from West Virginia (36% of total cases). During each year from 1994 to 1997, at least half of the total LAC cases reported nationwide were from that state. The increase in LAC cases reported from West Virginia indicates that the region contains habitat suitable for maintaining LAC virus enzootic foci and raises concern that LAC encephalitis cases may be increasing in frequency and are under-reported in the region.

Within West Virginia, LAC encephalitis cases have been reported from 18 of the state's 55 counties, mainly in the southern half of the state (E.S.B., unpublished data). Nicholas County accounted for 25% of the cases reported from West Virginia during 1987–1997, and an average of 10 cases per year during 1992–1997 has been reported from the county.

Though LAC virus transmission is broadly associated with hardwood forests (Calisher 1994) the influence of habitat on the LAC virus transmission cycle is not fully understood, particularly outside areas of the upper midwestern United States. Factors related to suitability of a habitat for the eastern chipmunk (*Tamias striatus* Linn.) were related to the prevalence of antibody to LAC virus in resident chipmunk populations and to *Ae. triseriatus* population densities in southeastern Wisconsin woodlots (Gauld et al. 1974). However, LAC cases occasionally are associated with sites that do not appear to be optimum chipmunk or *Ae. triseriatus* habitats. Records of LAC cases in Nicholas County, WV (Woodruff et al. 1992), and subse-

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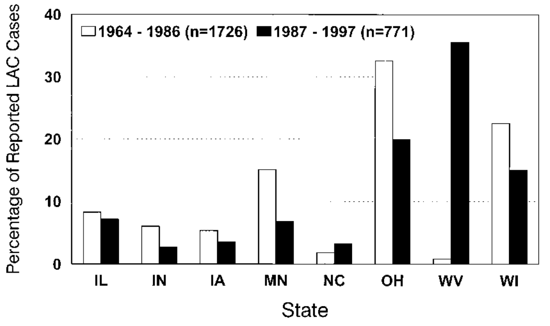

Fig. 1. States accounting for >90% of reported La Crosse encephalitis cases during the periods 1964–1986 and 1987–1997. Percentage of reported cases is shown for each state.

quent visits to LAC case residences in the county by the authors indicate that LAC cases and the presence of LAC virus in *Ae. triseriatus* mosquitoes are associated with a variety of habitat types.

Nicholas County is ecologically diverse and contains pine, hemlock, oak-hickory, and maple-beech-birch forests (DiGiovanni 1990). This article describes *Ae. triseriatus* population density and LAC virus infection rates throughout the 1996 transmission season in a variety of habitat types in Nicholas County. This study is part of a larger project to determine if habitat type influences LAC virus distribution in predictable ways and to determine how habitat is associated with the risk of acquiring LAC encephalitis.

Materials and Methods

Study Areas. Using information provided by the West Virginia Department of Health and Human Resources, 32 confirmed LAC encephalitis cases that occurred in Nicholas County, WV, from 1987 to 1995 were visited and the geographic location (latitude, longitude) of each was determined using by a GPS receiver (ProMark X, Magellan, San Dimas, TX). The locations were plotted on a map of the county and wooded areas for use as study sites were selected based on proximity to the known cases. The three A sites, the single B site, and the two C sites were in wooded areas adjacent to known LAC case residences. The D sites, E sites, and F sites were in wooded areas at least 2 km from the nearest known LAC case residence but were near residential areas.

Habitat Quantification. Each of the 12 sampling sites contained a single transect consisting of a straight line of contiguous quadrats. Transects in sites A1, A2, A3, C1, C2, D1, D2, E1, E2, and F1 contained 10 quadrats each. Transects in sites B1 and F2 were limited to five quadrats each because of the small size of the plot. Each quadrat measured 10 by 10 m. The transects were located at least 50 m from the edge of the wooded area in locations where open field bordered the forest. Habitat measurements were taken between 30 May and 4 June 1996.

The quadrat was the primary sampling unit. Within each quadrat the following measurements were made: each tree was identified to species, the diameter at breast height (dbh) of each tree was measured (Avery 1967), and the number of holes in each tree below 2.5 m above the ground was counted. All treeholes were counted, whether or not they contained water at the time of the survey. Forest canopy height was measured by using a Suunto PM-5 Clinometer (Suunto, FIN-02920 Espoo, Finland) and expressed as the distance to the highest point of the tree canopy in the center of the quadrat. Forest canopy density was measured at the center of each quadrat by using a spherical densiometer (Forest Densiometers, Bartlesville, OK) and expressed as the proportion of the canopy occupied by leaf cover. Shrubby vegetation and ground cover were measured as described by James and Shugart (1970). Shrub density is expressed as the number of small branches (<4.0 cm diameter at waist height, not originating from a larger tree) per 100 m^2. Ground cover is expressed as the proportion of the total area with low (<0.5 m), herbaceous vegetation. Transect values for numbers of trees and treeholes were determined by totaling the number of trees and treeholes in the quadrats within each transect. Transect values for canopy height, canopy density, shrub density, and proportion ground cover were determined by calculating an average of the quadrat values within each transect. The number of artificial containers was counted in each quadrat. Transect values for artificial containers were determined by totaling the number within the transect.

Weather data (daily minimum/maximum temperature and daily precipitation) were obtained from the Beckley-Raleigh County Memorial Airport Weather Station in Beckley, WV (National Oceanographic and Atmospheric Administration, National Climatic Data Center), ≈60 km from the study sites.

Vector Sampling. Mosquitoes were collected during seven sampling periods from 31 May through 10 September 1996 (Fig. 2). Specimens were collected approximately every other week during this period. Oviposition traps (Loor and DeFoliart 1969) with seed germination paper as an oviposition substrate (Steinley et al. 1991) were used to evaluate *Ae. triseriatus* population densities in the sites. Two ovitraps were placed in each quadrat in each transect and left in place for 7–10 d. The total number of eggs on each ovitrap substrate was counted and the number of eggs collected per trap day at each sampling site, during each sampling period, was calculated and used as the basis for comparing ovipositing population densities across sites and periods.

Host-seeking adults were collected once during each sampling period using CO_2 enhanced, human bait collection. A collector with a small cooler of dry ice stood in the center of each quadrat in each transect for 10 min and, using a battery-powered aspirator, collected all mosquitoes landing during that period. Specimens were immediately placed in a plastic screw-cap vial and stored on dry ice.

Vector Processing. Eggs on the paper strips from the oviposition traps were counted; then the eggs were hatched by placing them in a container holding 200 ml

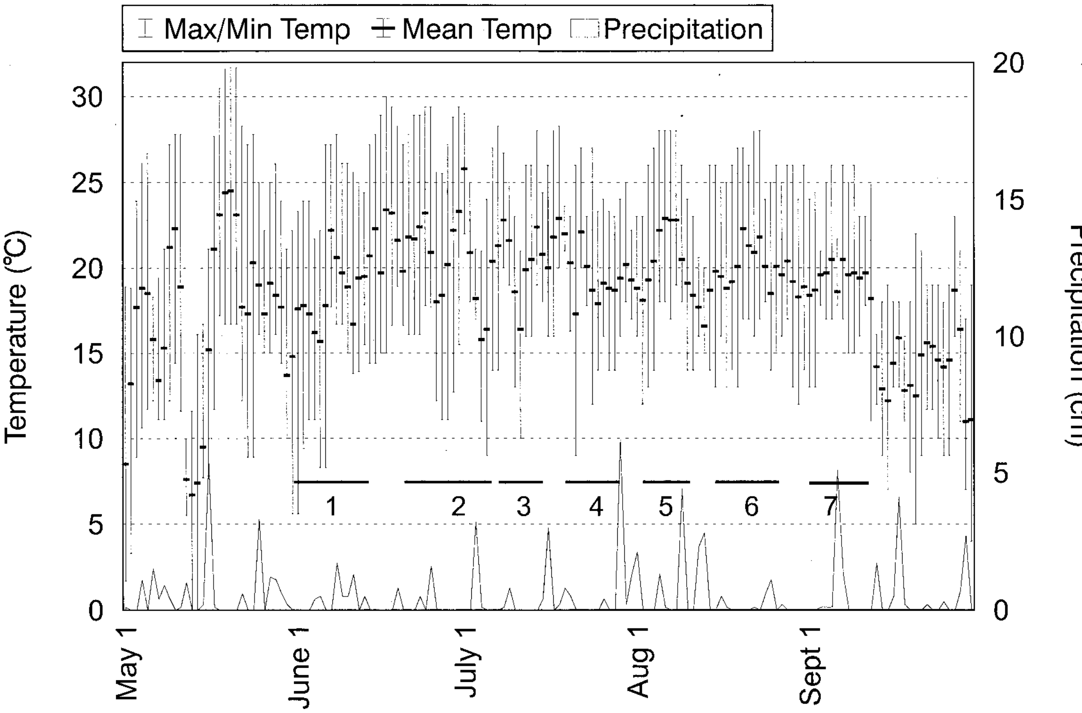

Fig. 2. Weather conditions from 1 May through 30 September 1996, showing daily temperature (mean, maximum, minimum) and daily precipitation levels. The duration of the seven sampling periods used during the study are displayed as the labeled horizontal bars.

water and placing the container in a vacuum chamber for 15 min to reduce oxygen concentration. Larvae were placed in pans and reared at 37°C and a photoperiod of 16:8 (L:D) h, with a slurry of dried liver powder and water as a food source. Fourth instars were examined to separate *Ae. hendersoni* Cockerell from *Ae. triseriatus* (Hedeen 1963, Darsie and Ward 1981) At eclosion, the adult specimens were killed by freezing and stored at −80°C. To approximate the number of *Ae. triseriatus* eggs in the ovitrap collections, the total number of eggs collected was reduced by the proportion *Ae. hendersoni* reared from eggs collected in the site during the time. The average number of *Ae. triseriatus* eggs collected in ovitraps per trap day over the entire season in each site was calculated by dividing the total number of eggs collected (corrected for *Ae. hendersoni*) by the number of trap days in the site. None of the eggs collected during period 3 (5–12 July) hatched because they were inadvertently exposed to high temperatures and were killed. However, eggs from period 3 were counted and used in the ovitrap data analyses, without a correction for *Ae. hendersoni.*

The adult mosquitoes were sorted by species and sex, placed in pools of not >50 individuals and stored at −80°C until tested for the presence of virus. Pools of mosquitoes were placed in 12 by 75-mm (5-ml) polypropylene, round-bottom, snap-top tubes (Falcon #352063, Becton Dickinson Labware, Franklin Lakes, NJ) with 2 ml BA-1 diluent (1× M199 with Hanks' balanced salt solution, 0.05 M Tris buffer (pH 7.6), 1% bovine serum albumin, 0.35 g/liter sodium bicarbonate, 100 μg/liter streptomycin, 1 μg/ml Fungizone). Pools were ground by placing four 4.5-mm-diameter, copper-clad steel beads (BB caliber airgun shot) into the tube with the mosquitoes and diluent, and vortexing on a laboratory mixer for 20–30 s. The homogenate was centrifuged to remove suspended solids, without removing the beads. Specimens were tested for virus using a Vero cell culture plaque assay in six-well plates (Beaty et al. 1989).

Virus isolates were identified with an indirect immunofluorescence assay (IFA) using a battery of National Institutes of Health (Bethesda, MD) and CDC polyvalent mouse hyperimmune ascitic fluids (Tsai 1995) and with a reverse transcriptase polymerase chain reaction (RT-PCR) procedure. The RT-PCR procedure used a LAC virus-specific primer pair to amplify a 120-bp fragment from the S segment of the La Crosse virus genome (Kuno et al. 1996).

Statistical Analysis. Associations among habitat parameters were evaluated by using the Spearman rank correlation and the Kruskal–Wallis rank sum test (Sokal and Rohlf 1981, S-Plus 4 Guide to Statistics 1998). Patterns in *Ae. triseriatus* egg density were evaluated by performing analysis of variance (ANOVA) on the square root-transformed number of eggs per trap day in each sampling site and sampling period (S-Plus 4 Guide to Statistics 1998). The square-root transformation was used to stabilize variation across

Table 1. Summary of all trees identified in the 12 transects, showing the number of trees identified, number of treeholes counted on the species, and the calculated number of treeholes per tree

Tree species	No. of trees	No. of treeholes	No. treeholes/tree
Red maple, *Acer rubrum* L.	177	12	0.07
Standing, dead, various unidentified sp.	176	10	0.06
Sugar maple, *Acer saccharum* Marsh.	159	15	0.09
Hemlock, *Tsuga canadensis* (L.) Carr.	135	4	0.03
Tulip tree, *Liriodendron tulipifera* L.	91	1	0.01
Ironwood, *Caprinus caroliniana* Walt.	55	1	0.02
Black birch, *Betula lenta* L.	43	3	0.07
Beech, *Fagus grandifolia* Ehrh.	39	4	0.10
Black cherry, *Prunus serotina* Ehrh.	39	1	0.03
Hawthorn, *Crataegus sp.*	35	0	0.00
Black gum, *Nyssa sylvatica* Marsh.	29	3	0.10
White oak, *Quercus alba* L.	27	0	0.00
Witch hazel, *Hamamelis virginiana* L.	24	3	0.13
Black locust, *Robinia pseudo-acacia* L.	23	0	0.00
Dogwood, *Cornus florida* L.	19	1	0.05
Pignut hickory, *Carya glabra* (Mill.) Sweet	15	0	0.00
Downy serviceberry, *Amelanchier arborea* (Mich. f.) Fern.	15	9	0.60
Slippery elm, *Ulmus rubra* Muhl.	12	3	0.25
Red oak, *Quercus rubra* L.	10	1	0.10
Chestnut oak, *Quercus prinus* L.	7	0	0.00
Domestic apple, *Pyrus malus* L.	7	0	0.00
Shagbark hickory, *Carya ovata* (Mill.) K. Koch	6	0	0.00
Ash, *Fraxinus sp.*	5	0	0.00
Yellow birch, *Betula lutea* Michx. f.	4	0	0.00
Sumac, *Rhus* sp.	3	0	0.00
Black hickory, *Carya texana* Buckl.	2	0	0.00
Ten additional species, 1 specimen each	10	0	0.00
Total (number of tree species = 35)	1,170	71	0.06

periods (Sokal and Rohlf 1981). La Crosse virus minimum infection rates were expressed as number infected/1,000 specimens tested, but were analyzed as transformed proportions (arcsine $\sqrt{p}$ using the Spearman rank correlation and Fisher exact test (Sokal and Rohlf 1981).

Results

Weather Patterns. Over the 153-d period from 1 May through 30 September 1996, average daily temperatures ranged from a low of 6.7°C (13 May) to a high of 25.8°C (1 July) (Fig. 2). During this period, measurable rainfall occurred on 66 d, totaling 76.1 cm. There were no extended periods of either unseasonably high or low temperatures, or prolonged periods of rainfall or drought.

Habitat Parameters. Thirty-five tree species were identified in the 12 sites in the study (Table 1). Red maple, *Acer rubrum* L.; sugar maple, *Acer saccharum* Marsh.; hemlock, *Tsuga canadensis* (L.); and tulip tree, *Liriodendron tulpifera* L., were the most frequently encountered species. Standing dead trees were also common, but species identification could not be determined for these specimens. Of the 61 treeholes found in identifiable tree species, most were in sugar maple (24.5%) and red maple (19.6%), likely because these species were so common in the area. Red maple and sugar maple trees produced an average of only 0.07 and 0.09 treeholes per tree, respectively. Though they were relatively rare species, the downy serviceberry, *Amelanchier arborea* (Mich. f.), produced an average of 0.6 treeholes per tree and the slippery elm, *Ulmus rubra* Muhl., produced an average of 0.25 treeholes per tree.

Tree species distribution varied considerably among the 12 sites, even among sites that were in close proximity to each other (Table 2). For example, sites A1, A2, and A3 were within 300 m of each other in a continuous wooded area, but tulip tree was the predominant tree species in A1 and sugar maple was predominant in A2 and beech in A3. Also, site E1, which was located in the bottom of a ravine adjacent to a stream, consisted mainly of hemlock and birch species, and site E2, located 320 m uphill from E1, consisted of a large number of small red maple trees.

Other quantified characteristics of the 12 sites are shown in Table 3. Average percent ground cover ranged from 9.5 to 92%. There was little variation in the density of shrubby vegetation among the habitats. Average canopy density ranged from 36.3% to above 76%. Average canopy height ranged from 7.5 to 33.4 m. The average tree dbh was lowest in E2, the site with the large number of small red maples, and greatest in A2. The calculated number of treeholes per hectare of habitat ranged from 0 in F2 (characterized by a large number of small ironwood and hawthorne trees in a stand of hemlocks next to a stream) to 180 in C2, a predominantly red maple habitat.

When all of the sites were examined together, there were significant negative correlations between percent ground cover and canopy density and canopy height, and between shrub density and canopy density (Table 4). Canopy density was positively correlated with canopy height. There was no significant difference in average percent ground cover, average shrub

Table 2. Species distribution of living trees in 12 sampling sites evaluated in Nicholas County, West Virginia

Site	No. of Trees[a]	No. of tree species[a]	Proportion of trees in transect										
			Sugar Maple	Red Maple	Beech	Yellow Poplar	Oaks	Hickories	Hemlock	Birches	Black Cherry	Ironwood	Hawthorn
A1	48	10	0.17	0.00	0.06	0.27	0.10	0.13	0.02	0.00	0.00	0.00	0.00
A2	54	9	0.43	0.00	0.04	0.06	0.22	0.06	0.06	0.00	0.00	0.00	0.00
A3[b]	116	13	0.15	0.00	0.21	0.03	0.04	0.03	0.00	0.04	0.09	0.00	0.00
B1	27	10	0.07	0.22	0.07	0.00	0.26	0.04	0.22	0.00	0.04	0.00	0.00
C1	77	9	0.43	0.25	0.00	0.05	0.00	0.08	0.00	0.06	0.00	0.00	0.00
C2	93	10	0.15	0.44	0.00	0.31	0.02	0.00	0.00	0.00	0.00	0.00	0.00
D1	64	11	0.06	0.08	0.00	0.03	0.14	0.00	0.61	0.03	0.00	0.00	0.00
D2	50	5	0.38	0.00	0.00	0.04	0.10	0.00	0.48	0.00	0.00	0.00	0.00
E1	119	10	0.06	0.00	0.03	0.06	0.00	0.02	0.50	0.28	0.04	0.00	0.00
E2	110	10	0.00	0.64	0.00	0.05	0.00	0.01	0.00	0.00	0.15	0.00	0.00
F1	147	15	0.19	0.24	0.00	0.16	0.00	0.01	0.00	0.01	0.03	0.05	0.02
F2	89	5	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.53	0.36

The major species shown account for >73% of the total number of living trees in each transect except site A3. See Table 4 for a list of tree species.
[a] Total number of trees and tree species in the transect.
[b] Site A3 also contained Black Gum (0.13), Witch Hazel (0.08), and Slippery Elm (0.07).

density, average canopy density, or average canopy height when compared between sites adjacent to LAC encephalitis cases versus those ≥2 km away from LAC encephalitis cases (Kruskal–Wallis rank sum test $F > 1.0$, df = 1, $P > 0.05$ for each comparison).

Despite their proximity to residential areas, artificial containers were absent or rare in most sites (Table 3). Only three of the 12 sites had more than two containers. Within these sites the artificial containers were usually clustered within one or two quadrats, apparently resulting from a small area being used as a trash dump.

Because tree species appears to be the factor most influencing the characteristic of the habitats, for subsequent analysis the 12 sites were divided into three categories, based on tree species composition. Sites A1, A2, A3, C1, C2, and F1 are categorized as mixed northern hardwood habitats (Table 5). Sites B1, D1, D2, E1, and F2 are categorized as hemlock/mixed hardwood habitats. Site E2 is an early succession red maple habitat developing in an abandoned orchard. It was characterized by a high density of small-diameter red maple trees.

Vector Population. Over the entire collection period from 31 May through 10 September 1996, ovitrap collections yielded a total of 317,257 mosquito eggs in 11,811 ovitrap days. From the total number of eggs collected, 73,984 mosquitoes were reared to the adult stage and tested for virus, of which 73,479 (99.32%) were *Ae. triseriatus*, and 505 (0.68%) were *Ae. hendersoni*. Though *Ae. hendersoni* were reared from eggs collected in all sites except F2, and were found during four of the seven sampling periods, this species was not uniformly distributed throughout the collection sites and sampling periods. Depending on period and site, *Ae. hendersoni* represented from 0.0 to 5.4% of the adults reared from eggs.

The average number of *Ae. triseriatus* eggs collected in ovitraps per trap day over the entire season (corrected for *Ae. hendersoni*) ranged from 10.4 in site E1 to 52.6 in site B1 (Table 5). The number of trap days was not equal among sites because of differences in the number of quadrats per transect and because of lost and damaged ovitraps.

The square root-transformed numbers of *Ae. triseriatus* eggs per trap day and the results of a nonpara-

Table 3. Habitat characteristics of the 12 sampling sites in Nicholas County, West Virginia

Site	Avg % ground cover	Avg shrub density/100 m^2	Avg canopy density, %	Avg Canopy ht, m	No. of trees[a]	Avg tree DBH, cm	No. treeholes/ha[b]	No. artificial containers
A1	46.5	0.0	83.0	24.2	64	18.4	70.0	0
A2	77.5	0.0	81.8	33.4	59	25.4	70.0	0
A3	43.0	0.2	77.8	23.9	139	14.3	140.0	8
B1	39.0	0.2	79.9	27.5	28	23.8	80.0	17
C1	52.5	0.2	76.4	16.9	107	13.6	50.0	1
C2	58.5	0.2	82.4	20.9	107	13.7	180.0	15
D1	27.5	0.2	81.8	28.5	77	19.0	50.0	0
D2	9.5	0.0	86.3	27.7	61	21.3	20.0	0
E1	20.0	0.2	83.2	25.6	134	19.3	30.0	0
E2	92.0	0.1	36.3	7.5	110	7.2	10.0	2
F1	75.0	0.1	77.4	20.3	175	11.7	50.0	0
F2	53.0	0.1	78.2	12.8	109	9.1	0.0	1

[a] Including standing, dead, but unidentifiable trees.
[b] Calculated from number of treeholes found in 1,000-m^2 or 500-m^2 transect.

Table 4. Correlation among habitat parameters measured in the 12 sampling sites in Nicholas County, West Virginia

	Spearman's rank correlation r		
	Shrub density	Canopy density	Canopy height
Ground cover	0.16	−0.45[a]	−0.36[a]
Shrub density		−0.29[a]	−0.20
Canopy density			0.47[a]

[a] Correlation coefficients are statistically significantly different from 0 ($P < 0.01$).

metric, locally weighted regression analysis (Cleveland and Devlin 1988) are shown in Fig. 3. The regression curve represents a smoothing of the data and permits visualization of structure in the underlying data points. The regression curve indicates that, except in site E1, the egg densities rise to a fairly stable level by period 3, which is maintained throughout the remainder of the sampling periods. In site E1, a hemlock/hardwood site, the number of *Ae. triseriatus* eggs per trap day appeared to rise gradually throughout the season.

The number of eggs per trap day over all seven sampling periods was significantly greater in those sites adjacent to LAC encephalitis cases (mean = 23.3) versus those ≥2 km away from LAC encephalitis cases (mean = 17.0) (ANOVA on square root of eggs per trap day $F = 22.0$; df = 1, 1,481; $P < 0.0001$). This indicates that the density of *Ae. triseriatus* eggs collected at sites adjacent to LAC cases was 38% larger than at sites that were not adjacent to cases. When the same comparison was done using only data from sample periods 3 through 7, when the egg densities appeared to stabilize, the same pattern was seen. The number of eggs per trap day was significantly greater in sites adjacent to LAC encephalitis cases (mean = 32.8) versus those ≥2 km away from LAC encephalitis cases (mean = 22.6) (ANOVA on square root of eggs per trap day $F = 38.7$; df = 1, 1,067; $P < 0.0001$). After mid-July, the density of *Ae. triseriatus* eggs collected at sites adjacent to LAC cases was 46% larger than at sites that were not adjacent to cases.

Over the entire season (all seven sampling periods), the number of *Ae. triseriatus* eggs per trap day did not differ between the mixed hardwood sites (mean = 21.8) and the hemlock sites (mean = 19.7), but both of these were significantly higher than the number of *Ae. triseriatus* eggs per trap day found in site E2, the red maple old orchard site (mean = 11.39) (ANOVA on square root of eggs per trap day $F = 11.8$; df = 2, 1,480; $P < 0.0001$). When the same comparison was done using only data from sample periods 3 through 7, when the egg densities appeared to stabilize, there were significant differences among all three habitat types (mixed hardwood sites, mean = 31.1 eggs per trap day; hemlock sites, mean = 25.3 eggs per trap day; red maple site, mean = 16.3 eggs per trap day) (ANOVA on square root of eggs per trap day $F = 17.1$; df = 2, 1,066; $P < 0.0001$).

The number of eggs per trap day was also compared among sites with and without artificial containers. Over the entire season, sites with artificial containers had significantly more eggs per trap day (mean = 21.7) than sites without artificial containers (mean = 18.5). When the comparison was limited to data from sample periods 3 through 7, the same pattern occurred. Sites with artificial containers contained ≈22% more eggs per trap day (mean = 30.3) than sites without artificial containers (mean = 24.9) (ANOVA on square root of eggs per trap day $F = 10.37$; df = 1, 1,067; $P < 0.0001$). The number of eggs per trap day over the entire season was not correlated with the number of treeholes per hectare in the site (Spearman $r = 0.16$, $P = 0.05$).

Data from the landing collections are of limited value in comparing population density among the study sites because host seeking activity is not constant throughout the day and host seeking adults could not be collected simultaneously in all quadrats. Also, the sample sizes of landing mosquitoes were small (Table 6). Throughout the season, 1,055 *Ae. triseriatus* and 1,863 *Ae. canadensis* (Theobald) were obtained in the

Table 5. Proximity to confirmed LAC encephalitis cases and habitat type designations for the 12 study sites in Nicholas County, West Virginia, season average number of *Ae. triseriatus* eggs collected per trap day, and season total LAC virus minimum infection rates for *Ae. triseriatus* adults reared from eggs for each site

Site	Proximity to case	Habitat type	Trap days	Total eggs[a]	Mean no. eggs/trap day	No. positive pools / No. specimens tested	MIR per 1,000
A1	Adjacent	Mixed hardwood	1,104	30,137	27.3	17/5,951	2.9
A2	Adjacent	Mixed hardwood	1,018	28,693	28.2	4/5,667	0.7
A3	Adjacent	Mixed hardwood	1,123	36,294	32.3	37/7,889	4.7
B1	Adjacent	Hemlock	480	25,273	52.6	23/5,724	4.0
C1	Adjacent	Mixed hardwood	971	30,770	31.7	27/4,811	5.6
C2	Adjacent	Mixed hardwood	1,100	26,818	24.4	45/5,989	7.5
D1	≥2 km	Hemlock	1,005	25,704	25.6	2/5,209	0.4
D2	≥2 km	Hemlock	996	28,156	28.3	33/7,323	4.5
E1	≥2 km	Hemlock	1,152	11,973	10.4	5/3,298	1.5
E2	≥2 km	Red maple old orchard	1,160	19,901	17.2	15/5,207	2.9
F1	≥2 km	Mixed hardwood	1,140	31,823	27.9	28/10,044	2.8
F2	≥2 km	Hemlock	562	19,411	34.5	5/6,370	0.8
Total			11,811	314,953	26.6	241/73,479	3.3

[a] Number of *Ae. triseriatus* eggs determined by reducing total number of eggs collected by the proportion *Ae. hendersoni* adults reared from the eggs.

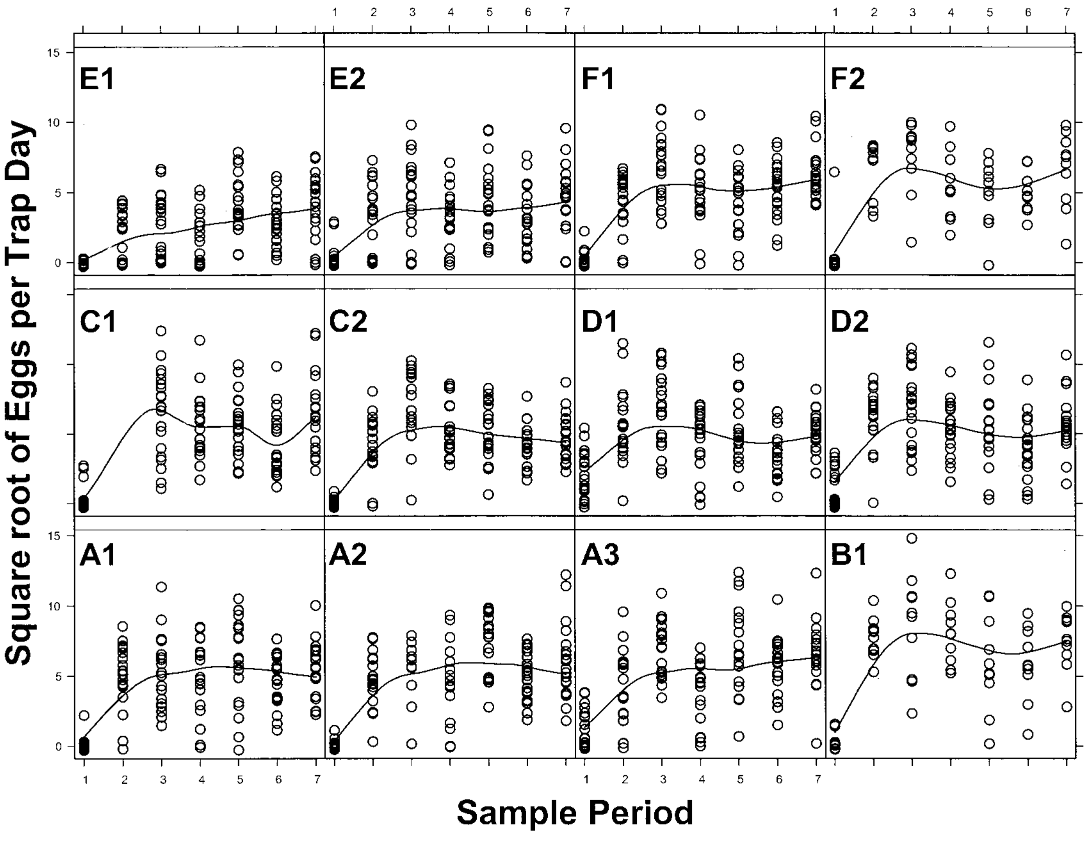

Fig. 3. Square root of the number of *Ae. triseriatus* eggs collected per trap day in each sampling site during each sampling period. Included in each panel is a curve representing a smoothing of the data by use of nonparametric, locally weighted regression (see text for description).

landing collections. Most of the *Ae. canadensis* were collected in sites A1, A2, and A3. Other species were collected in very small numbers in the landing collections throughout the season: *Ae. vexans* (Meigen) (n = 11), *Anopheles crucians* Wiedemann (6), *An. punctipennis* (Say) (3), *Coquillettidia perturbans* (Walker) (7), *Psorophora ferox* (Von Humbolt) (1).

LAC Virus Infection Rates. Season total LAC virus minimum infection rates (number infected per 1,000 tested) for *Ae. triseriatus* collected as eggs within a site ranged from 0.4 in site D1 to 7.5 in site C2 (Table 5). No statistically significant differences in season total LAC virus minimum infection rates were found when using a Student t-test to compare the arcsine-trans-

Table 6. Season total LAC virus minimum infection rate for *Ae. triseriatus* and *Ae. canadensis* collected in landing collections in each sampling site

Site	*Ae. triseriatus*		*Ae. canadensis*	
	No. positive pools / No. specimens tested[a]	MIR/1,000	No. positive pools / No. specimens tested[a]	MIR/1,000
A1	2/156	12.8	1/600	1.6
A2	0/70		0/418	
A3	1/230	4.3	2/820	2.4
B1	2/214	9.3	0/3	
C1	1/46	21.7	0/1	
C2	1/106	9.4	0/3	
D1	0/71		0/4	
D2	0/26		0/3	
E1	0/4		0	
E2	0/7		0	
F1	0/51		0/6	
F2	2/74	27.0	0/5	

[a] The number of specimens tested is the total number of landing mosquitoes of that species collected during the entire season in that site.

Table 7. LAC virus minimum infection rate in *Ae. triseriatus* reared from eggs in each sampling site

Site	Min. infection rate/1,000						
	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7
A1	—	0	No data	4.3	0	11.4	2.3
A2	0	0		0	0	5.3	0
A3	0	4.2		0	3.6	1.2	8.5
B1	0	0		20.8	8.0	8.5	0
C1	0	No data		0	7.7	10.1	0
C2	—	15.4		0	10.1	14.3	1.7
D1	0	0		5.4	0	0	0.3
D2	0	0		10.0	0	7.6	1.2
E1	—	0		6.2	3.1	0	0
E2	0	0		0	10.7	0.7	2.5
F1	0	0		5.6	0	5.6	2.8
F2	—	0		0	0	0	5.7

—, no specimens tested from that period; 0, indicates that specimens were tested, but no pools contained virus. No eggs from period 3 hatched.

formed proportions between sites adjacent to LAC cases versus those ≥2 km away from LAC cases (P = 0.12); or mixed hardwood sites (excluding site E2) versus hemlock sites (P = 0.21). In addition, there was no significant correlation between the season average number of *Ae. triseriatus* eggs collected per trap day and the season total LAC virus minimum infection rates in the site (Spearman $r = 0.18$, $P > 0.05$). None of the 505 *Ae. hendersoni* reared from eggs was infected with LAC virus.

Within-season patterns in the LAC virus minimum infection rates from *Ae. triseriatus* collected as eggs were examined by comparing sites within each sampling period (Table 7). There were no significant differences in minimum infection rates (arcsine-transformed proportions) between the sites adjacent to LAC cases versus those ≥2 km away from LAC cases within any of the sample periods. In addition, there were no significant differences in minimum infection rates between hardwood sites and hemlock sites within any of the sample periods (one Student t-test run for each period, $P > 0.05$ in all comparisons).

The season total LAC virus minimum infection rates in the host-seeking *Ae. triseriatus* collected in landing collections ranged from 0.0/1,000 in several of the sites that produced few landing mosquitoes to 27.0/1,000 in site F2 (Table 6). The small sample sizes, particularly from the sites ≥ 2 km away from LAC case residences, limit the use of these data in statistical analyses.

LAC virus-infected *Ae. triseriatus* were collected on seven separate occasions. Minimum infection rates (per 1,000) were 83.3 in A3, period 2 (number positive/number tested = 2/24); 28.5 in site F2, period 3 (1/35); 58.8 in site A3, period 4 (1/17); 37.0 in site C2, period 4 (1/27); 200 in site C1, period 6 (1/5); 26.6 in site B1, period 7 (2/75); and 76.9 in site F2, period 7 (1/13).

LAC virus was isolated from host-seeking *Ae. canadensis* collected in landing collections in sites A1 and A3. The season total LAC virus minimum infection rates in landing mosquitoes were not significantly different across the sites (Fisher exact test: *Ae. triseriatus* P value = 0.65, *Ae. canadensis* P value = 0.81). Further, the *Ae. triseriatus* and *Ae. canadensis* minimum infection rates did not differ significantly within sites A1 (Fisher exact test P = 0.11) or A3 (Fisher exact test P = 0.52).

Discussion

Habitat Parameters. The 12 study sites used in this project varied markedly, particularly in terms of tree species composition. Using tree species as a basis for differentiation, the sites were divided into three habitat categories (mixed northern hardwood habitats primarily containing large maples, hemlocks mixed with hardwoods, small-diameter red maples growing in an abandoned orchard). The sites were also divided into two categories, depending on their proximity to confirmed LAC encephalitis cases (adjacent to or ≥2 km away from confirmed LAC encephalitis cases). *Aedes triseriatus* and LAC virus were found in all of the sites studied, regardless of habitat structure or proximity to LAC cases.

Habitat parameters such as canopy height, canopy density, percent ground cover, and shrub density varied among habitats. In general, sites with a more dense tree canopy had a lower percent ground cover and a lower density of shrubby vegetation. However, ground cover, shrub density, canopy density, and canopy height did not differ among sites that differed in their proximity to LAC case residences.

The Nicholas County, WV, sites differed in tree species composition and density of treeholes from LAC virus enzootic sites studied in other areas. Red maples containing 0.07 treeholes per tree and sugar maples containing 0.09 treeholes per tree were dominant in Nicholas County sites. Oaks were the dominant species in sites with documented LAC virus transmission activity in southwestern Wisconsin (Hanson and Hanson 1970, Moulton and Thompson 1971), north-central Illinois (Clark et al. 1983), and western North Carolina (Szumlas et al. 1996). White oak and black oak (*Quercus velutina* Lam.) trees studied in one of the southwestern Wisconsin sites each contained 0.05 treeholes per tree (Hanson and Hanson 1970), suggesting that the oak species in these areas contained treeholes at a rate similar to that seen in the maples in Nicholas County. Though the major tree species in Wisconsin and Nicholas County appeared to contain treeholes at similar rates, the number of treeholes per unit area in many of the Nicholas County sites was considerably greater than reported from other LAC virus enzootic sites. The density of treeholes in the Nicholas County mixed hardwood sites ranged from 50 to 180 treeholes per hectare. The density was 10 treeholes per hectare in a southwestern Wisconsin LAC virus enzootic site (Hanson and Hanson 1970), and was calculated to be ≈2.5 treeholes per hectare in a LAC enzootic site in Peoria, IL (Clark et al. 1983). Although oak treeholes and maple treeholes may differ in their capacity to produce *Ae. triseriatus,* and the methods of quantifying treeholes probably varied across studies, these observations suggest that there was more larval habitat available in the Nicholas County maple forests than in the oak forests of other LAC virus enzootic sites.

Vector Populations. *Ae. triseriatus* eggs were collected in all of the habitats studied in Nicholas County. When the number of eggs in ovitraps was used as an index of population density (Craig 1983, Hanson et al. 1988, Szumlas et al. 1996), the data showed that *Ae. triseriatus* populations gradually increased in density from the beginning of the season to mid-July. From mid-July through early September, density was relatively stable. This pattern is generally similar to those seen in *Ae. triseriatus* populations sampled in southern Wisconsin (Gauld et al. 1974) and in western North Carolina (Szumlas et al. 1996). The duration of *Ae. triseriatus* activity was shorter in Wisconsin (Gauld et al. 1974), probably because the Summer season was shorter.

Aedes triseriatus population density in Nicholas County differed among site categories. The number of eggs per trap day was highest in the mixed northern hardwood habitats, intermediate in the hemlock/mixed hardwood habitats, and lowest in the site containing mainly small red maples. This trend probably reflects the productivity of the site for *Ae. triseriatus* and, because gravid *Ae. triseriatus* disperse extensively in search of oviposition sites (Nasci 1982), the dispersal of gravid females through the area. The northern hardwood sites containing a relatively high density of maples and their associated treeholes were the most productive sites. The hemlock/hardwood sites, which had fewer treeholes, likely produced fewer *Ae. triseriatus* but experienced a movement of gravid females, which encountered the ovitraps while dispersing through the site. The red maple/old orchard site contained few treeholes and probably produced very few *Ae. triseriatus,* so the eggs captured in the ovitraps mainly represented the density of gravid females dispersing through the area. The same is probably true of site E1, a hemlock/hardwood site that had few treeholes and a low number of eggs per trap day. Another hemlock/hardwood site (F2) with few treeholes had a larger *Ae. triseriatus* population density, which was probably because of the presence of artificial containers in the area. Hemlock/hardwood site B1 had both treeholes and artificial containers and had a high population density. The presence of artificial containers in a site was positively correlated with increased *Ae. triseriatus* egg density in ovitraps at the site. This association has also been observed in Wisconsin (Garry and DeFoliart 1975).

Comparison based on proximity to LAC encephalitis cases demonstrated that *Ae. triseriatus* population density was significantly greater at the sites adjacent to cases. Because LAC virus may produce inapparent-to-apparent case ratios reported to range from 26:1 to 1,571:1 (Monath et al. 1970, Grimstad et al. 1984) and residents living near the sites that were ≥2 km away from confirmed LAC cases were not tested for antibodies against LAC virus, it is not known if these sites actually represented areas where exposure to LAC virus was reduced (i.e., residents may have been exposed to LAC virus, but did not experience severe clinical illness). However, these observations suggest that relatively high *Ae. triseriatus* population densities increase the risk of severe illness caused by LAC virus in this area of West Virginia.

The number of *Ae. triseriatus* eggs collected per trap day over the entire season in Nicholas County sites was higher than those reported from a LAC virus enzootic site in western North Carolina (Szumlas et al. 1996). In Nicholas County, season total eggs per trap day ranged from 10.4 to 52.6, whereas in the North Carolina study sites, the number of eggs per trap day ranged from 2.2 to 7.7 in a low-density year and 8.9 to 19.3 in a higher density year.

LAC Virus Infection Rate. Though the season total LAC virus infection rates in *Ae. triseriatus* reared from eggs ranged from 0.4/1,000 to 7.5/1,000 among the 12 sites in Nicholas County, statistical analyses indicated that there were no significant differences in infection rate regardless of habitat type or proximity to confirmed LAC cases. These infection rates are similar to those reported in other enzootic areas. Infection with LAC virus in *Ae. triseriatus* reared from eggs was 0/1,000–5.9/1,000 (Lisitza et al. 1977) and 6.4/1,000 in Wisconsin (Beaty and Thompson 1975), 1.2/1,000 over 3 yr in Illinois (Clark et al. 1983), up to 12.3/1,000 in Ohio (Berry et al. 1975), and 1.0/1,000 in North Carolina (Kappus et al. 1983).

Although there was no significant difference in *Ae. triseriatus* LAC virus infection rates collected at sites adjacent to LAC cases and those sites ≥2 km away from confirmed LAC cases, the *Ae. triseriatus* population density was 38 to 46% higher at the sites adjacent to LAC cases. Therefore the total number of LAC virus-infected *Ae. triseriatus* is probably 38 to 46% higher at the sites adjacent to LAC cases.

A study in southwestern Wisconsin identified chipmunks, *Tamias striatus,* important amplifier hosts in the discontinuous transmission foci found in the area and defined the qualities of habitats supporting large chipmunk populations that were associated with high levels of LAC virus transmission activity (Gauld et al. 1974). We hypothesized that the *Ae. triseriatus* collected as eggs in the hemlock/hardwood sites and the red maple/old orchard sites in Nicholas County would have lower LAC virus infection rates than those collected in the mixed hardwood sites because the latter habitat type was more characteristic of the chipmunk habitat described by Gauld et al. (1974). The lack of differences in LAC virus infection rates in *Ae. triseriatus* among habitat types suggests that, in Nicholas County, *Ae. triseriatus* readily disperse from foci with many amplifier hosts to areas that are less than optimal chipmunk/LAC virus transmission habitats. This is consistent with the oviposition patterns we observed among habitat types. Sinsko and Craig (1979) suggested that dispersal by *Ae. triseriatus* between two isolated woodlots in Indiana was very limited. However, other studies suggest that gravid *Ae. triseriatus* disperse through wooded fencerows and other habitat structures providing cover (Nasci 1982), and that host-seeking females disperse across open areas (DeFoliart and Lisitza 1980). *Ae. triseriatus* dispersal range may be much greater than previously suspected, particularly in areas with large, uninterrupted wooded habitat. Nicholas County contains 82% wooded habitat compared with Iowa County, WI (location of the site in Gauld et al. 1974), which is 28% wooded, and Peoria County, IL (location of the site in Clark et al. 1983), which is only 14% wooded (Hansen et al. 1992). The large, continuous woodlands of Nicholas County would not limit *Ae. triseriatus* dispersal, as would the patchy woodlands of southwestern Wisconsin or central Illinois, where woodlots are islands surrounded by open fields (Levenson 1976). Rather, extensive, uninterrupted woods would facilitate *Ae. triseriatus* dispersal.

The season total LAC minimum infection rates in host-seeking *Ae. triseriatus* in Nicholas County ranged from 4.3/1,000 to 27/1,000 in sites where virus was found in landing mosquitoes. These rates are similar to those observed in host-seeking *Ae. triseriatus* in enzootic areas in Wisconsin (2.5/1,000; Thompson et al. 1972), Illinois (11.4/1,000; Clark et al. 1983), and Ohio (12.3/1,000; Berry et al. 1974), suggesting similar transmission dynamics. The rates of infection in host-seeking *Ae. triseriatus* found in the individual sites/periods when virus was detected were 10–50 times greater than the infection rates found in *Ae. triseriatus* collected as eggs during the same periods. Though sample sizes in the host-seeking collections were small in any given site/period, this difference suggests that horizontal amplification plays a very large role in the LAC virus transmission dynamics in this area.

The season total LAC virus minimum infection rates in host-seeking *Ae. canadensis* ranged from 1.6 to 2.4/1,000 in sites A1 and A3 in Nicholas County, which was similar to the season total infection rates found in *Ae. triseriatus* in the same sites. *Ae. canadensis* is a competent vector of LAC virus, though not as efficient a vector as *Ae. triseriatus* (Watts et al. 1973). Berry et al. (1986) reported LAC minimum infection rates in host-seeking *Ae. canadensis* in LAC virus enzootic areas in Ohio ranging from 2.8 to 42.6/1,000, and implicated *Ae. canadensis* as an auxiliary vector of the virus in the region. Given the infection rates we have observed and the large population densities that this species can achieve, our results also indicate that *Ae. canadensis* may be an important accessory vector of LAC virus in certain areas where it is in contact with an *Ae. triseriatus* maintained LAC virus transmission cycle.

La Crosse Virus Habitat Associations. The results of this project demonstrated that, within Nicholas County, there was a very large amount of uninterrupted forested land consisting of a contiguous matrix of different forest types. All of the forest types contained *Ae. triseriatus* populations and LAC virus, but higher *Ae. triseriatus* populations were associated with mixed sugar maple/red maple habitats than with other habitat types, and confirmed human LAC cases were associated with sites having higher *Ae. triseriatus* densities. The LAC virus infection rate in *Ae. triseriatus* did not appear to differ among habitat types, probably reflecting widespread dispersal of infected *Ae. triseriatus* throughout the continuous wooded habitat. These qualities are considerably different from other LAC virus enzootic areas that have been studied, which are predominantly a patchwork of oak woodlots, with or without LAC infected *Ae. triseriatus*, surrounded by open lands. One similarity in all of the studies is that the presence of artificial containers, which have been repeatedly associated with human LAC cases (Hedberg et al. 1985, Woodruff et al. 1992), is associated with higher *Ae. triseriatus* population densities.

Unfortunately, no single habitat parameter or set of parameters could be identified that would permit us to predict the presence or absence of LAC virus in an area, or the relative infection rates of *Ae. triseriatus* in different habitats. The dynamics of enzootic LAC virus transmission by *Ae. triseriatus* are very complex. To develop a model to predict risk of human exposure to LAC encephalitis virus, existing and new information about quantifiable habitat parameters associated with transmission dynamics must be accumulated and incorporated into the analysis. Needed information includes the effects of habitat and weather parameters on *Ae. triseriatus* population dynamics and the dynamics of amplifier and nonamplifier hosts in areas with and without LAC virus activity (e.g., Clark et al. 1986), information regarding geographic variation in vector competence (e.g., Grimstad et al. 1977), local dynamics of transovarial transmission of LAC virus in *Ae. triseriatus* (e.g., Miller et al. 1977), and variations in LAC virus that may influence virus/vector interactions. Only when such a data base is assembled and analyzed will a useful predictive model, such as that initiated by Kitron et al. (1997), be built and made available to the public health community.

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