To the Graduate Council:

I am submitting herewith a thesis written by Anthony Brent Smith entitled "A case comparison study and arboviral surveillance of LaCrosse encephalitis in eastern Tennessee." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Masters of Science, with a major in Entomology and Plant Pathology.

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We have read this thesis
and recommend its acceptance:

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Associate Vice Chancellor
and Dean of the Graduate School

A Case Comparison Study and Arboviral Surveillance of LaCrosse Encephalitis in Eastern Tennessee

A Thesis

Presented for the

Masters of Science

Degree

The University of Tennessee, Knoxville

Anthony Brent Smith
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ABSTRACT

The eastern region of Tennessee has experienced an increase in the occurrence of LaCrosse encephalitis (LACE) since 1996. The virus that causes the disease (LACV) is known to be endemic in West Virginia and North Carolina. Due to the recent increase in the number of cases of LACE it is suspected that a new endemic focus has been established in eastern Tennessee. This increase has occurred at the same time that *Aedes albopictus* (Suske) became established in the region. *Aedes albopictus* has been shown to be capable of transmitting the virus under laboratory conditions, and LACV has been isolated from *Ae. albopictus* reared from wild collected eggs from a known LACE site in Anderson County, Tennessee. This finding indicates that *Ae. albopictus* may be playing a role in the transmission of LACV.

Surveys of the residences and other areas frequented by the 38 potential LACE patients were conducted from May-October, 2000. Data about the habitat, home and yard maintenance, distance from the tree line and road, and numbers of containers present at each of the sites were collected. Larval mosquitoes and eggs were collected and reared, and host seeking adult mosquitoes were captured using CO^2 -baited Centers for Disease Control (CDC) light traps. The number of male *Ae. albopictus* males collected with CDC traps were significantly higher at LACE sites than at control sites. The combined numbers of host seeking *Ae. albopictus* adults and larvae collected from LACE sites were significantly higher than at control sites. No other factor was found to be significantly different between LACE and control sites.

Continued surveillance of a known LACE site in Anderson County, Tennessee was conducted from June - October, 2000. Three groups of ten oviposition traps were placed in the woods at the site and the eggs were collected weekly. Significantly fewer *Ae, triseriatus* eggs were found the further the traps were placed into the woods. No differences were found with the number of *Ae. albopictus* eggs collected at different distances into the woods. Adult host seeking female mosquitoes were collected weekly using a CO^2-baited Centers for Disease Control (CDC) light trap and dissected to determine parity rates. Parity rates for *Ae. albopictus*, as determined by examination of the tracheoles in the ovaries, ranged from 28% - 75% parous. The survival rate as calculated increased later in the summer, from 73% in June to 93% in September, but still did not reach levels found in similar studies. *Aedes triseriatus* parity rates ranged from 33% - 66%, but there were not enough *Ae. triseriatus* captured to accurately calculate survival rates. The parity rate for both species were lower than those found in similar studies.

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Chapter I

An Introduction to LaCrosse Encephalitis in Eastern Tennessee

From 1964 to1996, a total of nine cases of LaCrosse encephalitis (LACE) were recorded from the eastern region of Tennessee (Jones et al. 1999). Since 1997, an average of ten cases per year (Figure 1.1) has been reported to the Tennessee Department of Health (Jones et al. 1999, P. Erwin pers. communication). This increase in the number of LACE cases, in addition to the isolation of LaCrosse virus (LACV) and Jamestown Canyon virus (JCV) from *Aedes albopictus* (Skuse) (Gottfried 1999, Gerhardt et al. in press) has raised the health concerns of public health officials. Understanding the effects of the introduction of a competent vector of these disease organisms is currently limited, as is the role that changes in the local and regional landscape ecology have on the spread of the viral organisms, and populations of their vectors. The need for understanding and control of these mosquitoes in virus endemic areas has been brought to the attention of public health officials and entomologists.

i - Container Breeding Mosquitoes

Two mosquito species that complete larval development in temporary water found in small containers are known to carry the LACV. These are *Aedes triseriatus* (Say) (Watts et al. 1972) and *Ae. albopictus* (Gerhardt et al. in press).

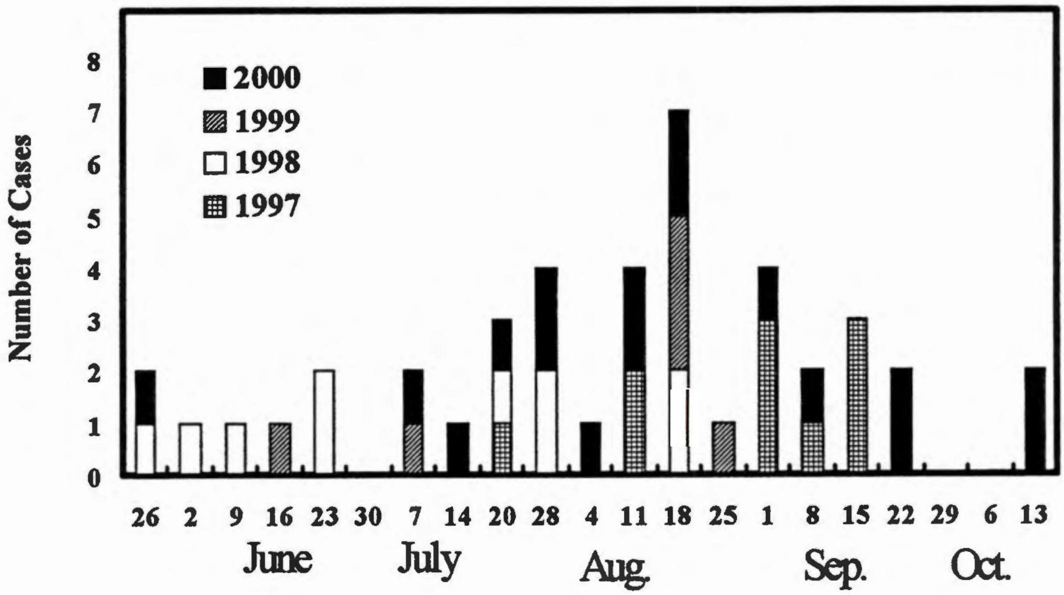

Figure 1.1 Date of onset by year, LaCrosse encephalitis cases in eastern Tennessee from 1997-2000.

Aedes triseriatus, the eastern tree hole mosquito, is a diurnal species that develops in natural cavities of trees, but will also use artificial containers (tires, buckets, garbage) for breeding purposes (Hedberg et al.1979). *Aedes triseriatus* is a low density mosquito common to the woodlands of eastern North America. Despite having a short range of flight (<300m) (Mather and DeFoliart 1984) *Ae. triseriatus* has an extensive distribution extending from New Brunswick to Florida, west to central Texas and the lower Great Lakes region (Walker 1992). *Aedes triseriatus*, which overwinters in the egg stage, has generally been believed to be the primary vector of LACV. It is believed the LACV also overwinters in the egg (Watts et al 1972, Pantuwatana et al. 1974). *Aedes triseriatus* bites humans vigorously and will freely feed upon other vertebrates, primarily the eastern gray squirrel, *Sciurus carolensis* (Gmelin), western fox squirrel, *Sciurus niger* (Linnaeus), and chipmunk, *Tamias striatus* (Illiger). The virus is transovarially transmitted from the female mosquito to her offspring (Watts et al. 1973, Miller et al. 1977). Male mosquitoes do not take blood meals, but transovarially-infected males can play a role in LACV transmission via venereal transmission of the virus to the uninfected females (Watts et al. 1973, Thompson and Beaty 1977). Sudia et al. (1971) found that *Ae. triseriatus* fed on virimic hosts in the lab attained a 100 % rate of infection after a seven-day incubation period, and a transmission rate of 50 % after 21 days.

Aedes albopictus, the Asian tiger mosquito, is indigenous to the Orient and Australia. It was found to have established a breeding population in Houston, Texas in 1985 (Sprenger and Wuithiranygool 1986) and is now widely distributed throughout much of the southeastern United States (Moore and Mitchell 1997), and in every county in

Tennessee (Moore 1998). Though *Ae. albopictus* is considered a non-migratory species, with a flight range <0.5km (Goddard 1996), it has spread rapidly. The transport of used tires has been implicated as the dispersal mechanism of *Ae. albopictus* (Reiter and Sprenger 1987). Though known to carry at least 22 arboviruses, many known to be of public health importance worldwide (Moore and Mitchell 1997), *Ae. albopictus* had only been proven to carry four human pathogens, Cache Valley Virus (CV), JCV, and eastern equine encephalomyelitis virus (EEE) (Moore and Mitchell 1997), in the United States. LACE was isolated for the first time from *Ae. albopictus* reared from eggs taken from a human LACE case site in Anderson County, Tennessee in 1999 (Gerhardt et al. in press). They suggest that this finding may indicate a possible reason for the increase in the number of LACE cases in humans.

Due to the opportunistic and aggressive blood feeding behavior, and ability to reproduce rapidly and inhabit many microhabitats, *Ae. albopictus* is a potentially very dangerous introduction to the habitats of North America (Niebylski et al. 1994, Hawley 1988). This species is widely distributed in rural, urban, and suburban areas, and is responsible for the majority of mosquito complaints received by the Tennessee Cooperative Extension Service (K. Vail, pers. communication).

ii - California Serogroup

The California (CAL) serogroup viruses (Bunyaviridae: *Bunyavirus* spp.) contain several antigenically similar arthropod-borne viruses that are reported to cause human disease. Five of the viruses, California encephalitis (CE), LACV, JCV, snowshoe hare

(SSH), and trivittus (TVT) are endemic to North America (Wilson 1991). JCV was isolated from *Ae. albopictus* mosquitoes collected in Knox County, Tennessee in June 1998 (Gottfried 1999).

With the recent increase in the number of LACE cases, it is possible that eastern Tennessee may be a new natural focus for the disease (Jones et al. 1999). In the 33-year period from 1964 to1996, only nine cases were reported in Tennessee. Since then, ten cases in 1997, ten cases in 1998, six cases in 1999, and 17 cases in 2000 have been reported (Gottfried 1999, P. Erwin pers. communication). The LACV is considered endemic to eastern Tennessee due to the presence of human cases, vectors, amplifying hosts, and suitable habitat.

Serological tests conducted by Thompson and Inhorn (1967) and Gauld et al.(1979) in Wisconsin, and Henderson and Coleman (1979) in California have found that 6 - 60% of rural residents had antibodies to the CAL-serogroup. Grimstad and Haramis (1984) found viral antibody levels of LACV ranging up to 12.8% in residents in several areas of Indiana. Szumlas et al. (1996) found that 9.6% of the residents of the Cherokee Indian Reservation in western North Carolina were positive for the LACV antibody. These results indicate that this virus could be more widespread than previously believed. The potential for viral transmission to humans increases as we travel further and move into woodland areas where natural hosts and vectors are present (Sudia et al 1971).

iii - LaCrosse Encephalitis

Of all the CAL - serogroup viruses in the United States, LACV is the most medically important, and is known to be endemic to the north central and eastern United States

(Mandell et al. 1990). Encephalitis caused by LACV is primarily a disease of children <15 years of age. The symptoms of this disease range from mild to, in rare cases, death (<1%) (Wilson 1991). It has been estimated that there are 26 to 1,571 asymptomatic cases for each symptomatic case (Kappus et al. 1983, Grimstad and Haramis 1984). To confirm diagnosis of LACE, infectious disease personnel use detection of a fourfold change in virus-specific immunoglobulin G (IgG) antibody titer levels between acute and convalescent sera (Beaty et al. 1982). Approximately 94% of human LACV infections have a disease onset in late July, August and September (Kappus et al. 1983, Berry et al. 1983) with the highest incidence occurring in the 5-9 year age group (Szumlas et al. 1996).

A study in West Virginia by Woodruff et al. (1992) found that the presence of natural breeding sites (tree holes) in peridomestic areas is an important risk factor for LACE. This result was supported by a study by Garry and DeFoliart (1975) in which the authors found that oviposition activity decreased 66-91% in the wood lots after closure of all tree holes. These studies also indicated that the presence of artificial containers and other factors may increase disease risk. Hanson and Hanson (1970) found that land use practices could exacerbate this problem. Their study in Wisconsin found that the cessation of fires that would reduce a continuous canopy encouraged shading leading to dying of smaller stems. This disturbance allowed an invasion of the tree by fungus that led to tree hole formation. They also found that damage to the trees from logging, pruning, or other activities that injured the tree also led to an increase in the number of tree holes present.

Field studies of LACV have shown that the eastern gray squirrel, western fox squirrel, and chipmunk, had the highest antibody prevalence rate (65-75% in adults) of the most common species of woodland animals (Moulton and Thompson 1971). These species have been generally accepted as being the amplification hosts for LACV in North America. Both the red fox, *Vulpes vulpes* (L.), and gray fox, *Urocyon cinereoargenteus* (Schreber), have high virus antibody rates in endemic areas (68%) and should also be considered a potential amplification host and potential means of possible spread of the disease (Amundson and Yuill 1981).

iv-Landscape Ecology

Landscape ecology is the study of the structural mosaic of landscapes and the structure, function, and change of spatial patterns of ecosystems (Forman and Godron 1986). Landscapes are the dynamic interactions between biotic and abiotic components and processes. Using landscape ecology, scientists can study these interactions from many view points, and at different spatial and temporal scales (Turner 1989). Landscape features have long been recognized as important factors in the transmission of vector-borne diseases (Kitron 1998). Resent studies have shown that changes in these features by both humans and natural forces can increase the possibility of disease occurrence. Studies in Africa have shown that human modification of the landscape has created increasingly favorable conditions for *Anopheles gambiae* (Giles), a known malaria vector (MacCormack 1984). Similar studies in Mexico with *Plasmodium* spp. transmitted by *An. albimanus* (Wiedemann) (Rodriguez et al. 1996) and LACV in north central United States

(Hanson and Hanson 1970) have shown similar results. This problem is compounded by the introduction of artificial containers brought into an area by humans. Many species of mosquitoes known to transmit disease organisms will quite readily use these containers to deposit their eggs (Hedberg et al.1979). The purpose of this study was to determine if any ecological or entomological factors present at locations where LACE has been confirmed could be indicators that would help predict future cases of LACE.

v - Research Objectives

The intent of this study is to ascertain any possible cause for the continued increase in the number of LACE cases in the eastern region of Tennessee. The introduction of *Ae. albopictus*, different landscape patterns, and the effect of human behavior in areas of mosquito habitat were all studied to gain more understanding of the development and spread of this disease. In particular, to determine the effects of development of homes in recently cleared or partially cleared woodland areas, and the use of artificial containers in these areas were studied. A risk comparison analysis was conducted between the confirmed LACE cases diagnosed during 2000, and central nervous system infection patients not ill with LACV. Surveys were made of the patients' residences, and other locations that a large part of the patients time was spent at during the 2-3 weeks before the onset of symptoms.

Chapter II

A Case Comparison Study of LaCrosse Encephalitis in Eastern Tennessee

i - Abstract

An increase in the number of pediatric cases of LaCrosse encephalitis (LACE) has occurred in eastern Tennessee over the last four years. This increase corresponds with the arrival of *Aedes albopictus* (Skuse) into the region, and the isolation of LaCrosse virus (LACV) from *Ae. albopictus* reared from eggs collected at a confirmed LACE site in Anderson County in 1999. This finding has led to the need for a better understanding of the factors that occur when LACE cases appear.

Site surveys of the residences and areas frequented by 36 children diagnosed with central nervous system (CNS) infections in the eastern Tennessee region were conducted. Data on the maintenance condition of homes and yards, distances from roads and tree lines, numbers of artificial containers and tree holes, and the type of habitat were collected to determine if any differences could be found between LACE and non-LACE (control) sites. Oviposition traps and CO_2-baited Centers for Disease Control (CDC) miniature light traps were placed at each study sites. Containers present at the sites were examined for mosquito larvae. Collected larvae were transported to the laboratory and reared to adults. Adult mosquitoes reared from larval collections were identified to species, sexed, counted, placed into lots of ≤ 50 individuals, and frozen at -80°C. These were sent to the Centers

for Disease Control Division of Vector-Borne Infectious Diseases (DVBID) in Fort Collins, Colorado, for viral isolation. Eggs recovered from the sites were reared to the adult stage, and prepared the same as stated above. Adults were counted, and identified to species, and sexed. Results from LACE and control sites were compared to determine if any possible differences could be found.

ii - Introduction

From 1963 to1996, nine cases of LACE were recorded from the eastern region of Tennessee. Since 1997, an average of ten cases per year has been reported to the Tennessee Department of Health (Jones et al. 1999, P. Erwin pers. communication). This increase in the number of LACE cases in addition to the isolation of LACV and Jamestown Canyon Virus (JCV) from *Aedes albopictus* (Skuse) (Gottfried 1999, Gerhardt et al. in press) has raised the health concerns of public health officials. Understanding the effects of the introduction of a competent vector of these diseases is currently limited, as is the role that differences in the local and regional landscape ecology have on the spread and persistence of the viral organisms, and populations of their vectors.

Recent improvement in technology has allowed the study of several vector borne illnesses to be conducted more effectively than in the past. Geographic Information Systems (GIS), Global Positioning Systems (GPS), remote sensing, and spatial analysis are the tools now used to analyze and integrate the spatial components in epidemiology (Kitron 1998). With these tools, all factors can be examined from a variety of scales and multiple view points. Also this information can then be combined with ecological data.

This information can allow physicians and epidemiologists to quickly pinpoint disease foci and possibly even predict areas that have a high probability of disease occurrence.

iii - Materials and Methods

Disease Surveillance: Active surveillance for potential cases of LACE was conducted by the East Tennessee Regional Health Department primarily at a large childrens' hospital in eastern Tennessee. The hospital is the primary pediatric referral center in the region. Any patient that met the following criteria was entered into the surveillance system.

- Age 6 months to 15 years.
- Having a physician-diagnosed febrile central nervous system (CNS) infection.
- Receiving a lumbar puncture for the presumed CNS infection.
- Alternative diagnosis for the illness not immediately available.

The parent/guardian of these patients was then asked for permission to conduct site surveys of the area around the home, and any possible sites where the child may have spent a significant (≥5 hours) amount of the daylight hours during the three weeks preceding the onset of symptoms (P. Erwin pers. communication). In such cases the site that had the highest numbers of mosquito adults and larvae present at the time of the survey was chosen for use during the statistical analysis. Site surveys were conducted as soon as possible (average 5 days) after enrollment of the patient into the surveillance system, and prior to the determination of LACV as the source of infection using convalescent titers. Conducting the site surveys prior to a final diagnosis was to help insure a lack of bias on the part of the persons conducting the survey, and to increase the chances of encountering conditions similar to when infection most likely occurred.

Site surveillance: The location and elevation of the survey sites were determined using a hand-held GPS receiver produced by GARMIN® (Garmin International, Inc.1200 E. 151st Street, Olathe, KS.). Surveys included evaluating the maintenance condition of the home and yard, and distance of the home from the tree line and roads. Each of these evaluations were placed into one of three categories. For the condition of the home and yard, category 1=well-maintained homes, category 2= moderately well maintained (a few cans or garbage present), and category 3= poorly maintained (large amounts of trash present, lawn overgrown). For the distance of the home to the tree line and road category 1= home ≥30 m from tree line or road. Category 2= home 15-30 m from tree line or road, and category 3= home 0-15 m from tree line or road. The presence and number of artificial and natural containers, general type of habitat surrounding the site, identification of the primary vegetation types and the species of plants in the canopy, and understory were also determined. A search for mosquito larvae and pupae was conducted in any water-bearing container at each site. All of these data were then placed on a survey form (Appendix 1) for entry into the computer database.

Larval mosquito collections: Any mosquito larvae and/or pupae that were collected from the containers at the sites were transferred to the laboratory in 3.8 liter plastic freezer bags. These specimens were placed into Mosquito Breeders® (Bioquip, Gardena, CA.) until emergence. Newly-emerged adults were killed by freezing and were immediately sorted by species and sex. Sorted mosquitoes were placed in lots of ≤50 individuals/lot, and stored at -80° C until tested for the presence of LACV (Gottfried 1999).

Oviposition activity: Aedes triseriatus and *Ae. albopictus* eggs were collected for one week from each of the sites in the surveillance system. Ten, 473 mL black (inside and outside) plastic cups were placed at each site, attached to the north side of trees approximately 0.5 meters from the base. These cups were spaced ca. 10 meters apart (Loor and DeFoliart 1969). Two holes were cut below the rim of the cup with a paper hole punch for drainage and the cups were filled with 400 mL of tap water (Gottfried 1999). A 5 cm x 25.5 cm strip of 76 lb seed germination paper (Anchor Paper Company, Saint Paul, MN.) was attached to the inside of each cup with a paper clip to serve as an oviposition substrate (Sternly et al. 1991, Gottfried 1999). After one week, the paper strips were collected and placed in plastic bags for transport to the laboratory where they were air dried for one week before the eggs were counted and identified to species (Pratt and Kidwell 1969). The total number of eggs collected for each species at the sites was then converted to eggs/trap day by dividing the total number of eggs collected by the number of strips recovered times the number of days the traps were in place.

Rearing success: Eggs were reared to adults by submerging the ten strips in 2.0 liters of tap water with 2.5 g of dried liver powder/liter in a plastic tray. Larvae were maintained at 27±2°C until most reached the pupal stage at which point all developmental stages were placed in Mosquito Breeders® (Bioquip, Gardena, CA.). Newly-emerged adults were killed by freezing and immediately sorted/counted by species and sex. Rate of survival was determined by dividing the total number of adults reared successfully by the total number of eggs captured on the strips. This survival rate is clearly at optimal conditions and would not reflect natural conditions (Gottfried 1999).

Processing for viral analysis: The mosquitoes reared from the oviposition strips were sorted by species and sex and placed into lots of no more than 50 individuals/lot. These pools were placed in 12 x 75 mm (5ml) polypropylene, round bottom, snap top tubes (Falcon #352063, Becton Dickson Labware, Franklin Lakes, NJ.), and maintained at -80°C for transport to the CDC Division of Vector-Borne Infectious Diseases (DVBID) in Fort Collins, CO. for processing and viral isolation (Gottfried 1999). The processing of these mosquitoes was done by personnel of the DVBID. The author of this manuscript played no further part in this procedure

Host-seeking adult collections: Host-seeking mosquitoes were collected using dry ice baited CDC light traps that had the lights removed. The dry ice was contained in a 1.89 liter water cooler suspended next to the trap with the nozzle of the cooler dispenser opened to allow the carbon dioxide to escape into the trap vicinity (Gottfried 1999). One trap was placed at each site for approximately 24 hours. The mosquitoes collected were chilled and returned to the laboratory for counting and species determination and parity determination.

Statistical analysis: After confirmation of the diagnosis using convalescent titers and a final diagnosis for each patient was determined, the LACE and control (non-LACE) cases were then analyzed to determine if any differences could be detected between them. Survey data from both LACE and control sites were entered into and tested using EPI-INFO Version 6®, an epidemiological database developed by The Centers for Disease

Control (CDC) and The World Health Organization (WHO) (EPI-INFO 1990). All data were tested for normality using the Bartlett test for homogeneity. Qualitative data, such as the condition of the residences, were analyzed using the Chi-square function provided by the program. All continuous data were analyzed using a one way analysis of variance (ANOVA) or the Kruskal - Wallis test. All analysis were conducted using $\alpha = 0.05$.

iv - Results

During this study, a total of 42 sites (residences plus four sites where patients spent time) were surveyed for 38 potential cases of LACE. The location of the sites ranged from the North Carolina border west to the Cumberland Plateau (Figure 2.1). A total of 17 cases of LACE were confirmed during this study. Twenty-six patients tested negative for the LACV (Figure 2.2). Two of the LACE and one of the control cases were not included in the analysis because notification occurred too late in the season for mosquito trapping. Sixty percent of the LACE cases were male with a mean age of 7.4 yrs. (range 2-13 yrs.), while the control group were 76% male with a mean age of 6.7 yrs. (range 7 mo-15 yrs.).

The survey of the areas around the residences revealed that 54% of the patients that tested positive for LACE lived in suburbs, 33% lived in homes surrounded by successional forests, and 13% were in cultivated pasture lands (Table 2.1) Seventy-two percent of the control patients lived in suburbs, 16% lived in areas of successional forests, and 6% in uncultivated pasture lands. Another 6% lived in other habitat types (Table2.2).

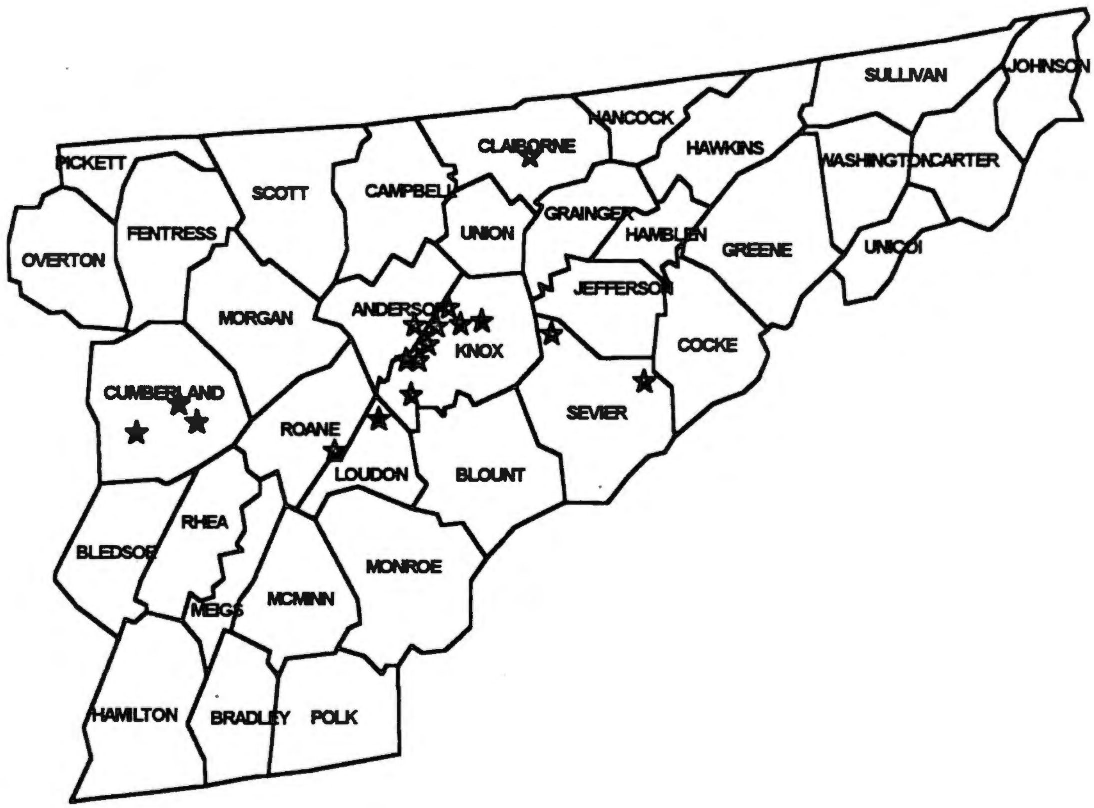

Figure 2.1 Locations of confirmed LaCrosse encephalitis cases in eastern Tennessee in 2000.

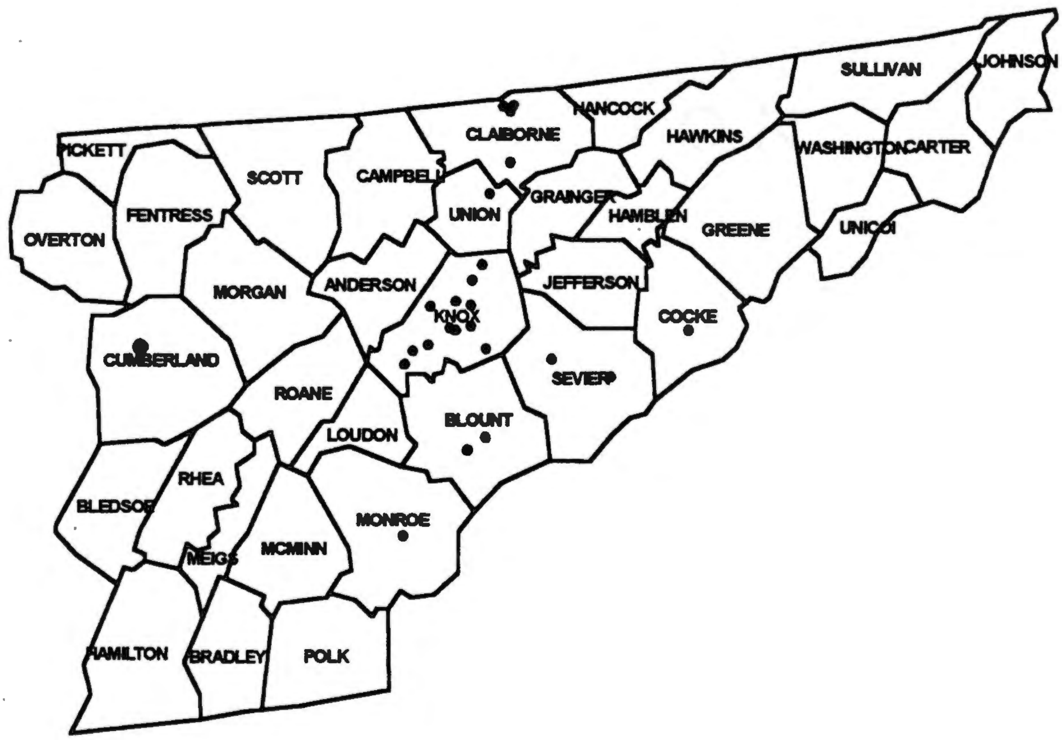

Figure 2.2 Location of control (non-LACE) cases in eastern Tennessee in 2000.

Table 2.1 Numbers and type of containers and habitat found at LACE sites.

Case #	Perm. Cont.[1]	Disp. Cont.[2]	Tree holes[3]	Habitat
5	10	80	1	Successional forest
14	4	30	6	Suburban
17	28	16	2	Successional forest
23	8	0	1	Successional forest
24	20	6	1	Suburban
30	15	300	1	Successional forest
32	12	0	2	Suburban
37	9	0	6	Suburban
40	6	12	3	Suburban
44	4	4	0	Suburban
46	20	80	0	Suburban
49	5	7	2	Cultivated pasture
53	5	1	3	Successional forest
56	8	3	0	Successional forest
mean	9.0	99.1	2.2	

1. Examples of permanent containers are wheelbarrows, buckets, wading pools, gutters, toys, etc. with or without water present.

2. Examples of disposable containers are trash, cans, bottles, tires, etc. with or without water present.

3. Tree holes 2m or less above ground level with or without water present.

Table 2.2 Numbers and types of containers and habitat found at control sites.

Case #	Perm. Cont.[1]	Disp. Cont.[2]	Treeholes[3]	Habitat
3	7	9	2	Suburban
6	6	0	0	Suburban
9	23	200	3	Unmanaged Pasture
11	8	6	1	Suburban
12	30	250	4	Suburban
18	9	0	7	Suburban
20	9	0	4	Suburban
21	5	6	1	Successional Forest
25	7	27	2	Suburban
26	8	200	6	Nursery
27	10	0	0	Suburban
31	8	30	6	Suburban
34	11	50	0	Unmanaged Pasture
36	45	1000	1	Successional Forest
39	9	11	0	Unmanaged Pasture
41	3	3	0	Suburban
45	8	1	0	Suburban
47	11	100	0	Suburban
50	4	0	0	Suburban
51	3	0	0	Suburban
52	6	0	0	Cultivated Pasture
54	3	0	0	Suburban
57	4	0	0	Suburban
mean	7.5	88.0	1.7	

1. Examples of permanent containers are wheelbarrows, buckets, wading pools, gutters, toys, etc. with or without water present.

2. Examples of disposable containers are trash, cans, bottles, tires, etc. with or without water present.

3. Tree holes 2m or less above ground level with or without water present.

Analysis with EPI-INFO indicated that no significant (p=0.64) differences existed among the types of habitat surrounding the homes of the two groups. Analysis indicated that no significant difference existed between LACE and control sites for house condition (p = 0.33), yard condition (p = 0.48), distance to tree line (p = 0.15), distance from road (p = 0.40), and elevation (p = 0.20). The number of permanent containers (p = 0.46), disposable containers (p = 0.88) and tree holes (p = 0.47) were found to not be significantly different between LACE and control sites (Table 2.3).

Larval mosquito collection: A total of 180 *Ae. albopictus* and 156 *Ae. triseriatus* larvae/pupae was collected and successfully reared to the adult stage during the study. *Aedes albopictus* averaged 9.07 (range 0 - 44) larvae/pupae from confirmed LACE sites and 2.19 (range 0 - 22) larvae/pupae were collected at the control sites (Table 2.3). No significant (p=0.076) difference was found between the two groups. No *Ae. triseriatus* larvae/pupae were recovered from the LACE sites while a mean of 6.50 (range 0 - 125) were successfully reared from control sites. Analysis with EPI-INFO found no significant (p=0.30) difference between the two groups (Table 2.3).

Oviposition activity: Aedes albopictus (n=11,501) and *Ae. triseriatus* (n=22,238) eggs were collected at every site that oviposition trap were placed at during this study. LACE sites accounted for 38.01% (4,372) of the *Ae. albopictus* eggs collected with a mean of 4.4 eggs/trap day (range 0.24-8.59). Control sites averaged 4.39 *Ae. albopictus* eggs/trap day with 7,129 (61.9%) collected. No significant difference (p=0.85) was found between the two groups (Table 2.3).

Table 2.3 Mosquito breeding containers and mosquito rearing and collection data from LACE positive and LACE negative sites in eastern Tennessee 2000.

Site (mean numbers)	**LACE positive**	**Lace negative**	**P[1]**
Permanent containers	12.47	9.96	0.46
Disposable containers	99.07	88.00	0.88
Tree holes	2.20	1.67	0.47
Aedes albopictus **(mean numbers)**			
Eggs/trap day collected	4.40	4.39	0.85
Adults females reared from eggs	37.20	38.45	0.90
Adults males reared from eggs	37.87	28.96	0.42
Adult females collected	6.87	3.24	0.26
Adult males collected	0.27	0	0.046
Adults reared from larvae	9.07	2.19	0.076
Aedes triseriatus **(mean numbers)**			
Eggs/trap day collected	9.22	7.71	0.56
Adult females reared from eggs	7.87	2.75	0.156
Adult males reared from eggs	11.8	4.5	0.29
Adult females collected	1.60	2.67	0.53
Adult males collected	0	0	-
Adults reared from larvae	0	7.43	0.30

1. Significance level as determined by EPI-INFO.

Aedes triseriatus eggs collected at LACE sites averaged 9.22 eggs/trap day (range 0.086-33.84) with 9,002 (40.5%) eggs collected. Control sites yielded 7.71 eggs/trap day (range 0.029-31.97) with 13,236 (59.52%) *Ae. triseriatus* eggs collected. No significant difference (p=0.56) was found between the two groups (Table 2.3).

Rearing success: The rearing success for *Ae. albopictus* was calculated to be 25.75% from eggs collected at LACE sites (n=1,126). Eggs collected from control sites had a rearing success rate of 20.80% (n=1,483). *Aedes albopictus* females (n=558) averaged 37.20 individuals (range 0-112) reared from LACE sites, while an average of 35.52 individuals (reared 0-156) were reared from control sites (n=817). No significant difference (p=0.90) was found between the two groups.

An average of 37.87 *Ae. albopictus* males (range 0-87) were reared (n=568) from eggs collected at LACE sites, while a mean of 28.96 (range 0 - 117) were reared (n=666) from eggs collected at the control sites. No significant difference (p=0.42) was found between the groups (Table 2.3).

LACE sites had a rearing success of 3.14% (n=283) for *Ae. triseriatus*, while control sites had a rearing success of 0.89% (n=129). *Aedes triseriatus* females averaged 8.4 individuals (range 0-62) successfully reared from eggs collected at LACE sites, while 42 *Ae. triseriatus* females, averaging 2.7 individuals (range 0-29) were reared from eggs collected at control sites. No significant difference (p=0.156) was found between the two groups (Table 2.3).

An average of 11.8 *Ae. triseriatus* males (range 0-120), were successfully reared (n=165) from eggs collected at LACE sites, while an average of 4.5 *Ae. triseriatus* males (range 0-46) were successfully reared (n=77) from eggs collected at control sites. No significant difference (p=0.29) was found between the two groups (Table 2.3).

Host seeking adult collections: A total of 176 host seeking *Ae. albopictus* females were captured in CO^2 baited CDC traps. A mean of 6.867 (range 0 - 12) *Ae. albopictus* females were captured from the confirmed LACE sites, while a mean of 3.042 (range 0 - 29) females were captured from the control sites. No significant difference (p=0.26) was found between the groups. A mean of 0.267 (range 0-2) males were captured at LACE locations (n=4), while no male *Ae. albopictus* were captured at the control sites (Table 2.3). A significant difference (p=0.046) was found between the two groups. When the host seeking adults and larvae/pupae from each site were combined a significant difference (p=0.035) was found.

A total of 84 host seeking adult *Ae. triseriatus* females were captured during the study. Twenty-four of these were captured at LACE sites averaging 1.6 (range 0 - 9). Control sites accounted for 60 *Ae. triseriatus* females with a mean of 2.5 (range 0 - 16). No significant difference (p=0.53) was found between the groups. No *Ae. triseriatus* males were captured during the study. No significant difference (p=0.15) was found when the host seeking adults and larvae/pupae from each site were combined.

v-Discussion

Site survey: Though Hedberg et al. (1985) in Minnesota and Woodruff et al. (1992) in West Virginia found that the presence of both artificial and tree holes at a site was an important risk determinant when looking at LACE, they were not found to be a significant factor ($p>0.05$) in this study. Hanson and Hanson (1970) in Wisconsin, and Woodruff et al. (1992) in West Virginia found that the distance the tree line was from the residence was correlated with viral transmission at LACE sites. These findings were not supported during this study.

During the study in West Virginia, Woodruff et al.(1992) also found that the condition of the home was not a significant risk factor ($p>0.05$). This finding was confirmed during this study even with the presence of *Ae. albopictus* which unlike *Ae. triseriatus* is known to frequent domestic areas to a greater extent (Busvine 1984).

Vector populations: Both *Ae. albopictus* and *Ae. triseriatus* were found at every site surveyed. Analysis found that *Ae. albopictus* adults (male and female) were captured in higher numbers using the CO_2 baited CDC light traps at the LACE sites than at the control sites. *Aedes triseriatus* adults were in similar numbers at both LACE and control sites. Due to the low density nature of both of these mosquito species, and differential trapping success it may be necessary to use multiple traps or human host collections in future adult collections (Hawley 1988).

The numbers of *Ae. albopictus* larvae/pupae found at the LACE sites were not significantly higher ($p>0.05$) than those found at the control sites. *Aedes triseriatus* larvae/pupae were found only at control sites, but they were not significantly different ($p>0.05$) from LACE sites. More larvae could have been collected if the water from the oviposition traps had been collected and returned to the laboratory, and it is recommended that these should be collected in any future research. The number of *Ae. albopictus* host seeking adults and larvae/pupae when combined were significantly higher($p=0.035$) at

LACE sites than at control sites. This analysis was done in an attempt to better understand vector populations present at the time of exposure.

The numbers of both *Ae. albopictus* and *Ae. triseriatus* eggs collected during this study were similar at both LACE and control sites. Lower numbers of *Ae. triseriatus* eggs were captured in eastern Tennessee than in the LACE study in West Virginia (Nasci et al. 2000). Egg collections in eastern Tennessee ranged from 0.096 - 33.84 eggs/trap day while those in West Virginia ranged from 10.4 to 52.6 eggs/trap day. Dry weather in the late-summer months could be a reason for lower than expected egg collections. This could have been compounded by wildlife searching for water sources.

Rearing success: The rearing success for *Ae. triseriatus* (3.14%) was much lower than expected for this species. In a LACE study by Nasci et al. (2000) a rearing success rate of 24% was found for this species. Gottfried (1999) also had poor rearing success of 1.6% for *Ae. triseriatus* in a LACE study in Tennessee. Analysis of the egg strips under a dissecting microscope revealed that most of the eggs were flattened. The flattened eggs from five randomly selected strips were removed from each egg strip and placed in a saline solution as described by Novak and Shroyer (1978). All the flattened eggs sank in the saline solution, indicating that they were non-viable. The reason for such a high number of non-viable eggs is not known. The rearing success for *Ae. albopictus* was found to be 21.7%. This rate of success was higher than the 16% rearing success found by Gottfried (1999) in Tennessee. This finding indicates that even though fewer eggs of this species are being deposited, a higher percentage are successfully reaching the adult stage. This would explain the abundance of host-seeking adults present at every site visited.

Chapter III

Continued Arboviral Surveillance of a Known LaCrosse Site in Anderson County, Tennessee

i - Abstract

Researchers from The University of Tennessee and The Centers for Disease Control isolated LaCrosse virus (LACV) from *Aedes albopictus* (Skuse) reared from collected eggs at a LaCrosse encephalitis (LACE) site in Anderson County, Tennessee, in 1999. The isolation of the LACV from this site was the first time LACV had been found in a natural population of *Ae. albopictus,* and has demonstrated the need for better understanding of the biology and life cycle of this species. Centers for Disease Control (CDC) light traps baited with CO_2, and 30 oviposition traps were placed at the Anderson County site weekly to monitor egg and adult populations, parity and survival rates of both *Ae. albopictus* and *Ae. triseriatus* (Say) from 1 June to 4 October, 2000. Host seeking *Ae. albopictus* (n = 121) and *Ae. triseriatus* (n = 35) as well as eggs of *Ae. triseriatus* (n = 12,095) and *Ae. albopictus* (n = 1,223) were collected from the site. Monthly parity rates for both species were lower than in previous studies indicating a lower than expected survival rate.

ii - Introduction

In 1999, while monitoring a known LaCrosse encephalitis (LACE) site in Anderson County, Tennessee, entomologists from the University of Tennessee collected and reared eggs of the Asian tiger mosquito, *Aedes albopictus* (Skuse), that tested positive for the presence of LaCrosse virus (Gerhardt et al. in press). The isolation of LACV from this site was the first time LACV had been found in natural populations of *Ae. albopictus*, although Hawley (1988) had shown that they were capable of maintaining and transovarially transmitting LACV under laboratory conditions. Although *Ae. albopictus* only recently arrived in the United States, it has become well established over a large area (Moore and Mitchell 1997, Moore 1998). The discovery of the LACV organism in an introduced mosquito species, in addition to its presence in *Ae. triseriatus* (Say), the native vector of LACV (Watts el al 1972, Pantuwatana et al. 1974), has added a new dynamic to the epidemiology of this disease. This study was undertaken in an effort to better understand the survival, parity rates, prevalence of arbovirus, and laboratory rearing success of these mosquitoes.

iii - Materials and Methods

Collection site: Eggs and adults of *Ae. albopictus* and *Ae. triseriatus* were collected from a known LACV site in Anderson County, Tennessee, from 13 July to 18 October, 2000. The site is located at N36° 11' 08",W84° 6' 14". This area consists of mixed hardwood forests and grazing lands. The primary canopy species are black oak, *Quercus velutina* (Lambert), white oak, *Quercus alba* (Lambert), and yellow pine, *Pinus* *virginiana* (Miller). Understory species include dogwood, *Cornus florida* (L.), mountain laurel, *Kalmia latifolia* (L.), and various wild grapes. Several natural cavities (treeholes) are present at the site, as well as various artificial containers (soda bottle and cans) along the roadside.

Oviposition activity: Aedes albopictus and *Ae. triseriatus* eggs were collected weekly from the site using oviposition traps. Three sets of ten 473 ml black (inside and outside) plastic cups attached to the north side of trees approximately 0.5 meters from the base and spaced ca.10 meters apart were placed at the site (Loor and DeFoliart 1969). The first set (line A) was placed ca. 5 meters inside the tree line paralleling the route of the county road. The second and third sets were placed at 15 (line B) and 25 (line C) meters, respectively, inside the tree line. Two holes were cut below the rim of each cup with a paper hole punch for drainage. A 5 cm x 25.5 cm strip of 76 lb seed germination paper (Anchor Paper Company, Saint Paul, MN.), was attached to the inside of each cup with a paper clip to act as the oviposition substrate (Steinly et al. 1991). Cups were filled with 400ml of tap water. Each week the oviposition traps were replenished by replacing the oviposition strips and supplying fresh water. The strips from the previous week were then placed in labeled plastic bags and taken to the laboratory where they were air dried for one week.

Rearing success: Each week two oviposition strips were randomly chosen from each set, and the eggs were counted and identified to species (Pratt and Kidwell 1969). Each of these two strip subsets were placed in separate plastic trays from the remaining strips in the sets for determination of rearing success rates. The eggs were reared to adults by submerging the two strips in 2.0 l of tap water with 2.5 g of dried liver powder/liter in a plastic tray. Larvae were maintained at 27± 2°C until most reached the pupal stage at which point all developmental stages were placed in Mosquito Breeders® (Bioquip, Gardena, CA.). Newly emerged adults were killed by freezing and immediately sorted/counted by species and sex (Gottfried et al. in press). Rate of survival for each of the species was determined by dividing the total number of adults reared successfully by the total number of eggs captured on the strips. Oviposition and survival rates were also compared between the sets. This is a laboratory survival rate, and is clearly at optimal conditions and may not reflect natural conditions.

Processing mosquitoes for viral isolation: The remaining eight strips of each set were reared without counting the eggs and the adults sorted in the same manner as the subsets mentioned above. The individuals from the subsets were placed back into the larger pools and the mosquitoes were sorted by species and sex. The mosquitoes were then placed into pools of ≤50 individuals/pool. These pools were placed in 12 x 75 mm (5 ml) polypropylene, round bottom, snap top tubes (Falcon #352063, Becton Dickson Labware, Franklin Lakes, NJ.), and maintained at -80°C for transport to the CDC Division of Vector-Borne Infectious Diseases (DVBID) in Fort Collins, CO. for processing and viral isolation (Gottfried 1999, Gerhardt et al. in press). Virus isolation tests were conducted by personnel of the DVBID.

Host-seeking adult collections: Host-seeking mosquitoes were collected weekly from the site using a dry-ice baited omni-directional Fay trap (Fay and Perry 1965). The dry ice was contained in a 1.89 liter water cooler suspended next to the trap and the nozzle of the cooler dispenser was open to release the CO_2 in the trap vicinity. The trap was placed at the site for a 24 hours starting at 0900-1000 EDT. Mosquitoes were collected, were chilled, and transported to the laboratory for identification and parity determination (Gottfried 1999, Detinova 1962).

Parity determination and survival rate: Host-seeking adults were cooled in the field, transported to laboratory and immediately immobilized in a refrigerator prior to dissection. Specimens were placed in saline solution (Hayes 1953) and the ovaries were removed, placed in a drop of saline solution, and observed under a compound microscope to evaluate the skiens of the ovarian tracheal system using a compound microscope. Determination of parity was based on the condition of the tracheal system described by Detinova (1962). If most of the skiens were still tightly knotted, the individual mosquito was considered to be non-parous or a nullpar. If the majority of skiens were unknotted, indicating that the mosquito had produced a clutch of eggs, then the mosquito was considered parous. Gravid mosquitoes were considered to be parous. The weekly, monthly, and seasonal proportion of parous individuals was determined by dividing the number of parous individuals by the number of nulliparous individuals plus the number of parous individuals (Gottfried 1999, Haramis and Foster 1990). Since gonotrophic cycles vary with temperature throughout the season, *Ae. albopictus* survival rates were calculated using estimated gonotrophic cycle lengths of 4.6 days for 26°C and 10 days at 20°C (Davidson 1954, Gottfried 1999, Hawley 1988). Survival rates were determined by using the formula $\sqrt[x]{p}$, where x = the number of days of the gonotrophic cycle, and p = the parity rate of a given period of time.

Statistical analysis: Statistical analysis of the data in this experiment was conducted using the PROC ANOVA and Duncans MRT of SAS® SAS Institute Inc. 1990). Analysis were conducted at α=0.05.

iv - Results

Ovitrapping: Eggs were collected from the site from 1 June to 4 October, 2000. A total of 12,095 (90.8%) *Ae. triseriatus* and 1,223 (9.2%) *Ae. albopictus* were collected from the sub-samples taken from the site. The mean densities of eggs deposited by *Ae. triseriatus* (Figure 3.1) and *Ae. albopictus* (Figure 3.1) was 15.0 and 1.5 eggs/trap day, respectively. The mean number of *Ae. triseriatus* peaked in mid-June and continued to decrease until the end of the trapping season. Mean numbers of *Ae. albopictus* eggs peaked twice during the trapping season. The first peak occurred during early July with the second peak occurring in mid-August, followed by a gradual decline until the end of the trapping season. These findings are similar to those by Gottfried (1999) with the exception that the *Ae. triseriatus* at the Anderson County site peaked earlier in the trapping season.

The number of both *Ae. triseriatus* and *Ae. albopictus* eggs captured at the trap lines decreased the further into the tree line the traps were placed. The mean number of *Ae. triseriatus* eggs captured from line A (5 m from road) was 140.1 per trap week, and lines B (15 m) and C (25 m) having means of 87.6 and 61.7 eggs/trap week, respectively. Analysis with Duncan MRT indicated that significantly more eggs/trap week in line A than

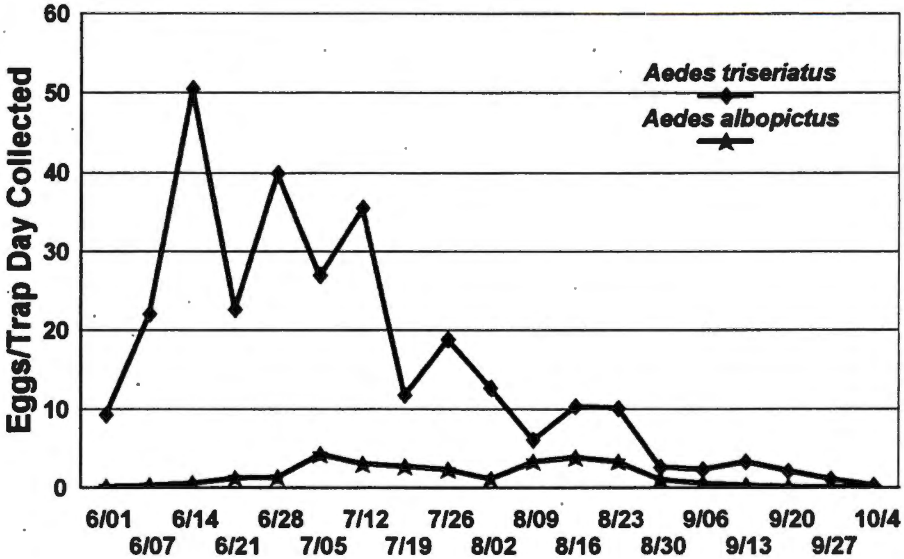

Figure 3.1 The number of eggs/trap day collected at Anderson County, Tennessee from 1 June-4 October, 2000.

line C. The number of eggs/trap week for line B was found to be the same as lines A and C.

Analysis of the *Ae. albopictus* eggs captured indicated that no significant differences existed among the three sets of traps. A mean of 12.91 eggs/trap week were collected from line A, with 9.43 and 6.73 eggs/trap week being taken from lines B and C, respectively.

Rearing success: Adult *Ae. albopictus* (n = 310) and *Ae. triseriatus* (n = 133) were reared from eggs collected from the randomly selected subsets. The rearing success for *Ae. triseriatus* was 0.35%, with a weekly range of 0 - 3% (Figure 3.2). The successful hatches occurred over a 5-week period from 6 June to 12 July when all attempts to get eggs to hatch failed (Table 3.1). Examination of the egg strips under a dissecting microscope revealed high numbers of flattened eggs. Some of these eggs were removed from the egg strips and placed in a saline solution as described by Novak and Shroyer (1978). All the flattened eggs sank in the saline solution indicating that they were non-viable. The reason for such a low number of viable *Ae. triseriatus* eggs present at the site is not known. The rearing success for *Ae. albopictus* eggs captured at the site was found to be 21.7% with a weekly range of 0-69% (Table 3.1). The successful hatches occurred over a fourteen-week period from 7 June to 6 September, after which all hatching ceased.

Parity determination and survival rate: Host seeking female *Ae. albopictus* (n = 121) and *Ae. triseriatus* (n = 35), were collected and dissected to determine parity. The first adults were captured 15 June and successful trapping continuing until 28 September, after which no further host seeking adults were collected (Figure 3.3). The

Table 3.1 Number of eggs collected, adults reared (male and female), and rearing success of container breeding mosquitoes by date in Anderson County, Tennessee, 2000.

	Aedes triseriatus				*Aedes albopictus*			
	No.	Adults Reared		Rearing Success	No.	Adults Reared		Rearing Success
Date	Eggs[1]	Male	Female	(%)[2]	Eggs[1]	Male	Female	(%)[2]
6/01	391	0	0	0	3	0	0	0
6/07	919	0	0	0	10	2	1	30
6/14	2120	42	24	3.1	19	12	1	68.4
6/21	948	1	1	0.2	49	7	9	32.7
6/28	1676	0	0	0	59	8	11	32.2
7/05	1128	20	11	2.7	181	17	15	17.7
7/12	1489	1	6	0.5	128	2	0	1.6
7/19	495	0	0	0	115	7	19	22.6
8/02	532	0	0	0	41	15	7	53.7
8/09	257	0	0	0	136	8	5	9.6
8/16	431	0	0	0	161	28	19	29.2
8/23	425	0	0	0	135	13	6	14.1
8/30	111	0	0	0	36	5	9	38.9
9/06	96	0	0	0	28	2	1	10.7
9/13	140	0	0	0	18	0	0	0
9/20	88	0	0	0	2	0	0	0
9/28	46	0	0	0	3	0	0	0
10/04	12	0	0	0	4	0	0	0

1. Eggs collected in oviposition traps.

2. Rearing success = (numbers of adults reared/number of eggs collected) times 100.

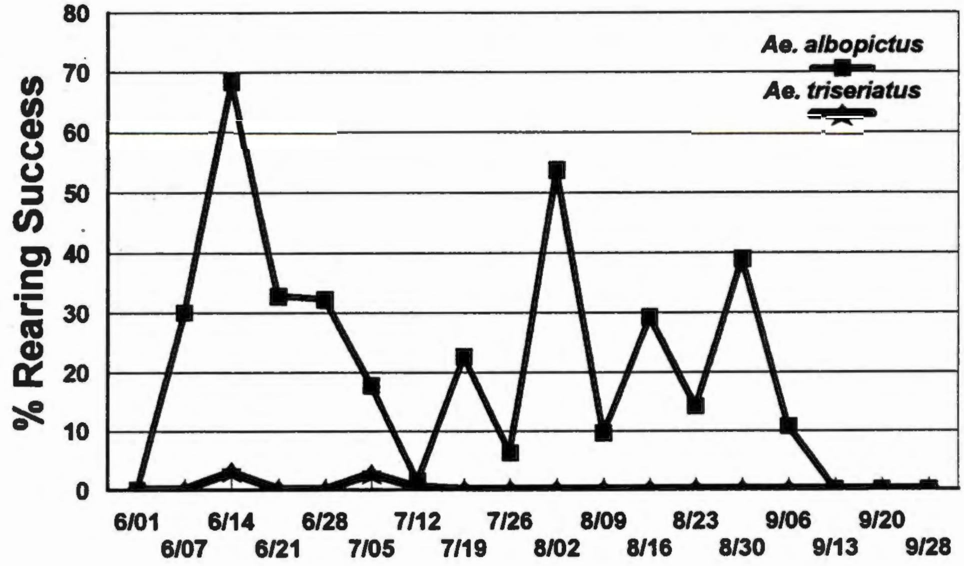

Figure 3.2 Rearing success of eggs collected at Anderson County, Tennessee from 1 June-28 September, 2000.

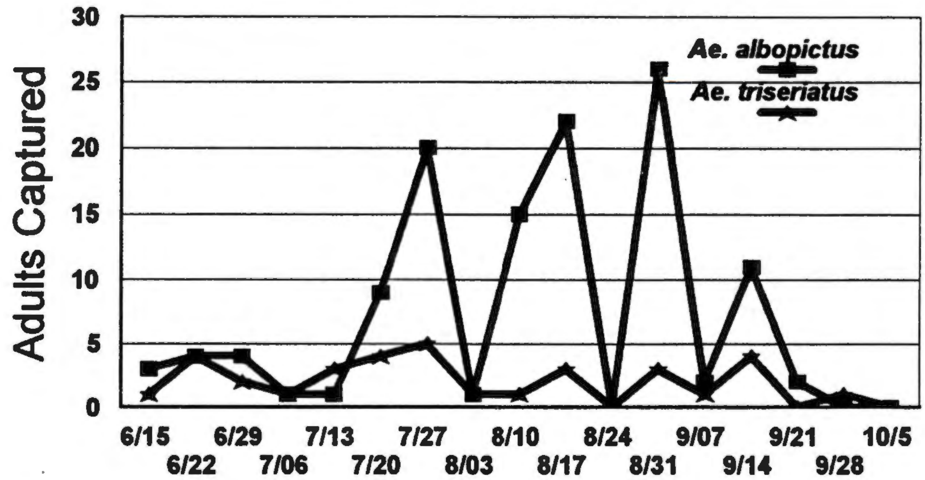

Figure 3.3 Host seeking adult females collected at Anderson County, Tennessee from 15 June-5 October, 2000.

seasonal mean of parous, host-seeking *Ae. albopictus* was 0.50 with a monthly range from 0.28 to 0.75 (Appendix 2). The seasonal mean of parous host seeking *Ae. triseriatus* was 0.58 with a monthly range of 0.33 to 0.66 (Figure 3.4, Appendix 2). The monthly parity rates for either species were not significantly different (*Ae. albopictus* p=0.075, *Ae. triseriatus* p=0.38). The calculated survival rates for *Ae. albopictus* ranged from 73% in both June and July (mean temp 23.3°C, 4.6 days estimated gonotrophic cycle) to 93% in September (mean temp 22.6°C, 4.6 days estimated gonotrophic cycle). The number of *Ae. triseriatus* collected were not sufficient to calculate survival rates from this site.

v - Discussion

Ovitrapping: Egg collections of *Ae. triseriatus* peaked in mid-June and continued to decrease until the end of the trapping season. The significant decrease in the number of *Ae. triseriatus* eggs collected at traps further away from the tree line is consistent with the known behavior of several mosquito species. Bidlingmayer and Hem (1981) found that the closer traps were placed to tree line areas, the more adult mosquitoes the traps would collect. They suggest that the mosquitoes are visually attracted to the increased abundance of vegetation and brush found at the tree line, possibly providing the mosquitoes an increased number of resting areas. Rodriguez et al. (1996) found that *Anopheles albimanus* (Weidemann) frequented areas that were near tree lines and the increased number of resting areas they provide. This area was the location of several artificial and natural containers that the female mosquitoes may have already been using for oviposition sites.

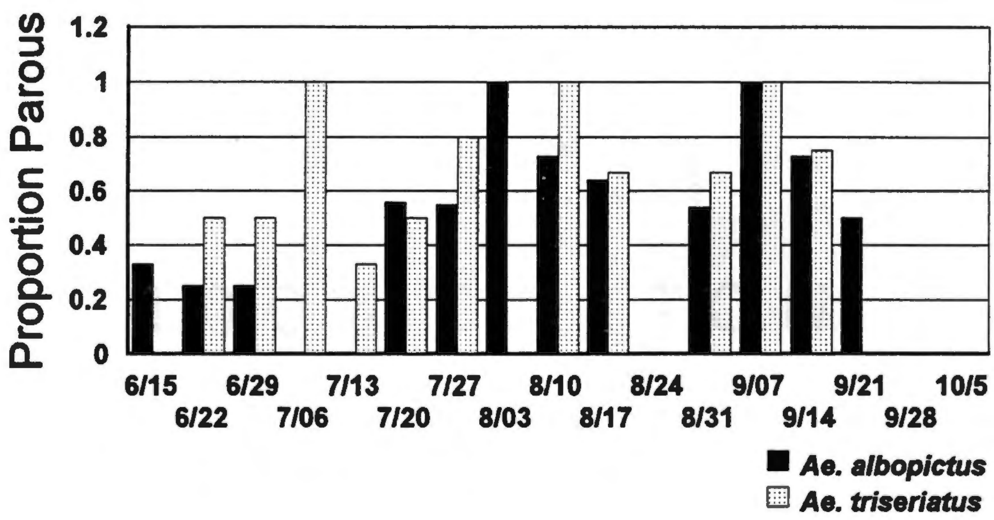

Figure 3.4 Proportion of female mosquitoes collected from Anderson County, Tennessee found to be parous from 15 June-5 October, 2000.

Rearing success: The rearing success for *Ae. triseriatus* from Anderson County was found to be 0.35%, and was lower than expected for this species. A LACV study in West Virginia in 1996 had a 24% rearing success for *Ae. triseriatus* (Nasci et al. 2000). Gottfried (1999) also had poor rearing success (1.7%) in a study in Tennessee. Analysis of the egg strips under a dissecting microscope revealed that most of the *Ae. triseriatus* eggs were flattened. Flattened eggs from five randomly selected strips were removed from the strips and placed in a saline solution as described by Novak and Shroyer (1978). All of the flattened eggs sank in the saline solution, indicating that they were non-viable. The reason for such a high number of non-viable eggs present at the site is not known. The drying of the eggs in the laboratory may have resulted in a high mortality rate in the eggs. Future studies should keep the eggs in an area of moderate to high humidity. The rearing success for *Ae. albopictus* at the site was found to be 21.7%. This rate was higher than the 16% rearing success found for this species by Gottfried (1999) in Tennessee.

Parity determination and survival rates: Monthly parity rates for both *Ae. albopictus* and *Ae. triseriatus* were lower than those found by Gottfried (1999) in Tennessee. The parity rates for *Ae. albopictus* increased throughout the summer (Table 4.2), indicating an increase in female survival rates. The increase in the parity rate also indicates that the rate of emergence and mortality became increasingly steady as the season continued (Haramis and Foster 1990). The estimated adult survival rate (0.76 - 0.98) was lower than that reported from a LACE survey in Tennessee (0.92 - 0.99) (Gottfried 1999). The low parity rates together and lower survival rates indicate that the female population at this site is not multiparous in nature, and are less likely to ingest and transmit a pathogen (Haramis 1983). Adult *Ae. albopictus* females were consistently collected at higher rates than *Ae. triseriatus* at this site. This difference is probably due to differential trapping success of *Ae. triseriatus*. Future studies should use multiple CO_2-baited Fay traps in an attempt to increase the number of *Ae. triseriatus* captured.

Chapter IV

Conclusions

i - Site Surveys

Forty-four sites associated with 38 suspected LaCrosse encephalitis (LACE) cases were surveyed during this study. Of these 38 suspected LACE cases, 17 were confirmed to be LACE using a fourfold increase in the number of virus specific antibodies in the patients' blood. Most of the study sites were visited within one week of the patients' admittance into the surveillance system in an attempt to increase the chance of finding infective mosquitoes, larvae, or eggs still present at the site.

Analysis of the number of permanent containers ($p=0.46$), disposable containers ($p=0.88$), and tree holes ($p=0.47$) found that these were not a significant risk determinant for LACE. This finding is different from the results found by Woodruff et al. (1992) in West Virginia, and Hedberg et al. (1979) in Minnesota, who found that sites with higher numbers of containers, both artificial and natural, had a higher risk of having LaCrosse virus (LACV). The condition of the home ($p=0.33$) and yard ($p=0.48$) was found not to be a significant risk determinants. This result was also found by Woodruff et al. (1992). The distance of the tree line from the residence was not found to be significant ($p=0.15$). This is different than the evidence found by Hanson and Hanson (1970) in Wisconsin, and Woodruff et al. (1992) in a LACE study in West Virginia. They found that residences close to a tree line had a higher chance of LACE.

Both *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say) were found at all the sites surveyed. Adult trapping with CO_2 baited CDC light traps (lights removed) resulted in significantly (p=0.046) higher number of *Ae. albopictus* males being captured at LACE sites than at the control sites though the numbers of *Ae. albopictus* females were not significantly different (p=0.26). Adult trapping found that the number of host-seeking *Ae. triseriatus* at LACE and control sites were not significantly different (p=0.53). Due to the differential trapping success it may be necessary to use more than one CDC light trap, or human bait surveys at future study sites (Hawley 1988).

Larval surveys of LACE and control sites found no significant difference (p=0.08) in the numbers of *Ae. albopictus*. Even though larval surveys found no *Ae. triseriatus* larvae/pupae at any of the LACE sites no significant (p=0.30) difference between LACE and control sites were found. Additional larvae can be included to future case comparison studies by collecting of the water in the oviposition traps, and rearing out of any larvae that may be present in them.

A combined analysis of the number of host seeking adults and larvae collected from the sites found a significant difference (p=0.035) between the number *Ae. albopictus* at LACE and control sites. The numbers of *Ae. triseriatus* larvae and host seeking adults at LACE and control sites were not found to be significantly different (p=0.15).

Ovitrapping results for both *Ae. albopictus* and *Ae. triseriatus* were lower than those found in other studies (Nasci et al. 2000). The limited time that the traps were in place may be the reason for this result. The traps may not have been discovered by a portion of the female population, and therefore had not yet been used as an oviposition site. The numbers of eggs of both species collected from LACE and control sites were not significantly different (*Ae. albopictus* $p=0.85$, *Ae. triseriatus* $p=0.56$).

The rearing success of *Ae. triseriatus* was far lower than expected for these studies. Nasci et al. (2000) observed a rearing success of 24% during a LACE study in West Virginia. Natural factors are believed to be the cause for this occurrence. Although rearing success for *Ae. albopictus* were higher than the 16% rearing success found by Gottfried (1999) during a LACE study in Tennessee the levels were still disappointing. It may be possible to increase rearing success in future by subjecting the eggs collected to the procedure described by Novak and Shroyer (1978) in which a 90% rearing success rate was achieved. An increased rearing success would assist entomologists in determining the true percentage of viable eggs, and provide additional mosquito adults for viral analysis.

ii - Parity and Survival Rates

The parity rates found for both *Ae. albopictus* and *Ae. triseriatus* were generally lower than those found by Gottfried (1999), but are still considered to be high enough to indicate good rates of survival. This level could indicate high levels of vector competence since female mosquitoes that survive longer periods of time are more likely to aquire a pathogen and pass it to a host at each gonotrophic cycle. Due to the low numbers of host-seeking *Ae. triseriatus* collected, survival rates could not be calculated with any degree of accuracy. Future studies should use multiple CO_2-baited Fay traps to increase the number of specimens collected. This information would be useful in future epidemiological studies of the diseases transmitted by these species.

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APPENDICES

Appendix 1

Case / Non-case ___/___/___ Date of visit:

Name:________________________ Case ID: ____________

Address: ______________________ Phone: _____________

____

____

Lat: _____°______'______" Elevation:__________________

Lon: _____°______'______"

Permanent Containers:	Type:	Total:
Disposable Containers:	Type:	Total:
House Condition:	1) Well maintained (new, newly painted) 2) Moderately-well maintained 3) Not well maintained (paint peeling, broken items visible) Generally dilapidated if old	
Yard Condition:	1) Tidy (no trash evident, lawn mowed, well maintained) 2) Moderately tidy 3) Untidy (trash abundant, lawn overgrown)	
Shade Condition:	1) Forest line greater than 100 ft from house 2) Forest line 50 – 100 ft from house 3) Forest line less than 50 ft from house	
Distance from road:	1) Road greater than 100 feet from home. 2) Road 50 – 100 feet from home. 3) Road less than 50 feet from home.	

Examples of permanent containers: Flower pots reservoirs, bird bath, toys, water bowl, wheel barrow, watering can, rain barrel, buckets

Examples of disposable containers: Trash, old tires, plastic tarps, other easily discarded items

Eggs Collected: Total:	*Ae. albopictus:*		*Ae. triseriatus*	
Mosquitoes collected:	*Ae. albopictus* females	*Ae. albopictus* males	*Ae. triseriatus* females	*Ae. triseriatus* males
Adults reared:				
Light trapping:				
Larval collection:				
Canopy Species:				
Understory species:				
Tree holes: Number:_______	Tree species with Treeholes:			
Classification of surrounding habitat:	1) Cultivated pasture 2) Unmanaged pasture 3) Fallow 4) Annual Crop 5) Permanent Crop 6) Mature forest		7) Successional forest 8) Nursery 9) Urban 10) Suburban 11) Riparian 12) Other ____________	
Comments: (Include small mammals, pets, condition of neighboring lots, etc.)				

Appendix 2

Appendix 2.1 The number of female *Aedes triseriatus* mosquitoes dissected, number parous and proportion parous collected from 15 June to 12 October, 2000 in Anderson County, Tennessee.

	Date	Dissected (n)	Parous (n)	Proportion Parous
June	15	1	0	0.00
	22	4	2	0.50
	29	2	1	0.50
	Total	7	3	mean 0.33
July	6	1	1	1.00
	13	3	1	0.33
	20	4	2	0.50
	27	5	4	0.80
	Total	13	8	mean 0.66
August	3	1	0	0.00
	10	1	1	1.00
	17	3	2	0.67
	24	0	-	-
	31	3	2	0.67
	Total	8	5	mean 0.63
September	7	1	1	1.00
	14	4	3	0.75
	21	0	-	-
	28	1	1	1.00
	Total	6	5	mean 0.92
October	5	0	-	-
	12	0	-	-
Total		34	21	mean 0.58

Appendix 2.2 The number of female *Aedes albopictus* mosquitoes dissected, number parous, and proportion parous collected from 15 June to 12 October, 2000 in Anderson County, Tennessee.

	Date	Dissected (n)	Parous (n)	Proportion Parous
June	15	3	1	0.33
	22	4	1	0.25
	29	4	1	0.25
	Total	11	3	mean 0.28
July	6	1	0	0.00
	13	1	0	0.00
	20	9	5	0.56
	27	20	11	0.55
	Total	31	16	mean 0.52
August	3	1	1	1.00
	10	15	11	0.73
	17	22	8	0.36
	24	0	-	-
	31	26	14	0.54
	Total	64	34	mean 0.66
September	7	2	2	1.00
	14	11	8	0.73
	21	2	1	0.50
	28	0	-	-
	Total	15	11	mean 0.74
October	5	0	-	-
	12	0	-	-
Total		121	64	mean 0.50

VITA

Anthony Brent Smith graduated Tennessee Technological University, Cookeville, Tennessee, with a B.S. in Biology in August of 1997. In January 1999 he entered the Department of Entomology and Plant Pathology at the University of Tennessee as a graduate research assistant under the tutelage of Dr. Reid R. Gerhardt. In May of 2001 he was awarded a Masters of Science degree with a major in entomology. The author is a member of the Society of Vector Ecology. He plans to stay in the entomology field, and play a role in the betterment of the discipline.