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Comparison of Mosquito Abundance, Distribution, and Parity Between a High and a Low Prevalence Site for La Crosse Encephalitis in Eastern Tennessee

Sabra Lee Scheffel

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To the Graduate Council:

I am submitting herewith a thesis written by Sabra Lee Scheffel entitled "Comparison of Mosquito Abundance, Distribution, and Parity Between a High and a Low Prevalence Site for La Crosse Encephalitis in Eastern Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Reid R. Gerhardt, Major Professor

We have read this thesis and recommend its acceptance:

Carl J. Jones, John K. Moulton

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Major Professor

We have read this thesis
and recommend its acceptance:

Carl J. Jones

John K. Moulton

Accepted for the Council:

Anne Mayhew

Vice Chancellor and
Dean of Graduate Studies

(Original signatures are on file with official student records.)

COMPARISON OF MOSQUITO ABUNDANCE, DISTRIBUTION AND PARITY BETWEEN A HIGH AND A LOW PREVALENCE SITE FOR LA CROSSE ENCEPHALITIS IN EASTERN TENNESSEE

A Thesis

Presented for the

Master of Science

Degree

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Sabra Lee Scheffel

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ABSTRACT

A three-year investigation of the seasonal distribution, abundance and diversity of mosquitoes at a high and a low prevalence area for La Crosse (LAC) encephalitis was conducted in eastern Tennessee, USA. We identified a high LAC prevalence site (Knox County) from which two cases of LAC encephalitis were confirmed, one in 1997 and the other in 2000, and an ecologically similar low prevalence site (Blount County) with no confirmed LAC cases. Mosquitoes were collected at each site using 2 Center for Disease Control (CDC) miniature light traps baited with carbon dioxide, 1 Omni-directional Fay trap baited with carbon dioxide, 2 gravid traps and 25 oviposition traps. At both sites, mosquitoes were collected weekly between late May and early November 2003-2005. The traps that attracted host-seeking and gravid adults were operated for a 24-hour time period each week, while the oviposition traps were left out for the entire week. Throughout the 2003, 2004, and 2005 collection periods, a total of 7,593 adult female mosquitoes were collected and identified to species (Knox County (n=3,646), Blount County (n=3,946)). *Aedes albopictus*, *Ochlerotatus triseriatus*, and *Ae. vexans* were the most commonly collected mosquitoes at both sites. The proven and suspected LAC vectors, *Oc. triseriatus* and *Ae. albopictus,* comprised 19.1% and 46.6% of all adult female mosquitoes collected and identified, respectively. *Ochlerotatus triseriatus* was collected most often in the early summer (June) with fewer numbers collected in the late summer, whereas *Ae. albopictus* collections tended to be largest in the late summer to early fall (August and September). In 2003, egg and adult collections fluctuated in a similar manner between sites, but not among species. *Aedes vexans* comprised 26.6% of all adult female mosquitoes collected in 2003, but only 8.3% and 14.5% in 2004 and

2005, respectively. There were no significant differences in egg or adult collections of *Ae. albopictus* and *Oc. triseriatus* between the high and low prevalence LAC sites. Weather patterns also appeared to be similar between the two sites. The total average monthly parity rate for *Ae. albopictus* and *Oc. triseriatus* at each site was between 40-48% parous in 2003, between 35-49% parous in 2004 and between 8-24% parous in 2005. Parity rates did not significantly differ between sites or species. The carbon dioxide baited CDC light traps collected most of the adult mosquitoes used in parity determination. Small mammals were sampled multiple times at each site with live traps. Each site contained sufficient populations of squirrels for LAC virus amplification, but no chipmunks were collected from the low prevalence area for LAC encephalitis. La Crosse antibodies were found at both sites, but the Knox County site had a higher prevalence of 2.65% compared to the Blount County site with a prevalence of 0.44%. Out of a total of 226 serum samples tested from both sites, 5 squirrels and 2 chipmunks tested positive for LAC antibodies.

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CHAPTER I

INTRODUCTION

i - California Serogroup

Within the California (CAL) serogroup (Bunyaviridae: *Bunyavirus* spp.), two antigenic complexes are recognized, California encephalitis and Guaroa (Calisher 1983). The California encephalitis complex is comprised of 3 viruses: California encephalitis (CE), Melao (MEL) and trivittatus (TVT) (Calisher 1983). The first recognized member of the CAL serogroup, CE virus, was isolated from mosquitoes in 1943 and 1944 and was implicated in three cases of human encephalitis in 1945 in the San Joaquin Valley, California (Hammon and Reeves 1952). California serogroup viruses that have been reported to cause human disease include CE, La Crosse (LAC), Jamestown Canyon (JC), snowshoe hare (SSH), TVT, Tahyna (Lumbo), Inkoo (INK), and Guaroa (GRO); of these viruses, CE, LAC, JC, and TVT are found only in North America (Wilson 1991). Snowshoe hare virus causes disease in North America and China (Wilson 1991).

Small mammals such as rodents are usually the primary vertebrate hosts of CAL serogroup viruses, and mosquitoes, mainly within the genera *Aedes* and *Ochlerotatus*, are the primary arthropod vectors (Sudia et al. 1971). Field and laboratory studies have shown that a number of small mammals, such as chipmunks (*Tamias striatus* Linnaeus), gray and fox squirrels (*Sciurus* spp.), cottontail rabbits (*Sylvilagus floridanus* Allen), red foxes (*Vulpes fulva* Desmarest), and gray foxes (*Urocyon cinereoargenteus* Schreber) are susceptible to the California group viruses, but mortality in these animals is low or absent (Henderson and Coleman 1971, Yuill 1983). The low pathogenicity of these viruses for the host mammals suggests a long history of association between the viruses and these hosts, resulting in a balanced virus-host relationship (Sudia et al. 1971). Human infection is incidental, producing an insufficient viremia to infect other mosquitoes, thus making them dead-end hosts. Clinical manifestations of CAL serogroup viruses range from asymptomatic to mild, undifferentiated febrile illness, an influenza-like syndrome, or an acute central nervous system disease. These infections are generally restricted to children and young adults (Henderson and Coleman 1971). Only a low proportion of infected individuals develop severe encephalitis and fatalities are rare (Lindsey et al. 1976).

ii - La Crosse Virus

La Crosse virus is the most commonly diagnosed virus of the California serogroup of bunyaviruses and is the primary cause of pediatric arboviral encephalitis in the United States (Calisher 1994, Jones et al. 1999). The virus was first isolated in 1964 from the brain tissue of a child who died in La Crosse, Wisconsin after developing encephalitis in 1960 (Thompson et al. 1965). From 1964 to1986, a total of 1,726 cases of LAC encephalitis were reported to the CDC, an average of 75 cases per year; most of these cases came from midwestern states such as Illinois, Indiana, Iowa, Minnesota, Ohio, and Wisconsin (Nasci et al. 2000). From 1987 to 1997, there were 744 LAC cases reported nationwide, an average of 68 cases per year. The geographic distribution remained similar, but the greatest percentage of cases was reported from West Virginia (36% of the total cases) (Nasci et al. 2000); during this time, LAC encephalitis had also been increasingly recognized in North Carolina and was newly identified in eastern Tennessee after 1997 (Erwin et al. 2002). La Crosse encephalitis is primarily a disease of children below the age of 15 and is found to occur more commonly in males (64%) most likely due to greater exposure to mosquitoes while camping, hiking, or participating in other outdoor activities (Kappus et al. 1983). Most infections occur during the period between July and September (Calisher 1994). Common symptoms include fever, headache, vomiting and behavioral changes, while loss of consciousness and seizures are observed less frequently (Erwin et al. 2002). Case fatality rate for LAC encephalitis is <1% (Wilson 1991). La Crosse encephalitis may be under diagnosed because most infections are asymptomatic or mildy symptomatic and also because infections are difficult to distinguish from other viral infections of the central nervous system (Jones et al. 1999).

From 1963 to 1996 (a 33-year period) only nine cases of pediatric LAC viral encephalitis were reported in Tennessee. However, since 1997, there have been between 6-19 cases a year, except in 2005, when there were only 2 cases (Jones et al. 1999, Erwin et al 2002, unpublished data). This recent increase of confirmed cases has made LAC encephalitis the most common arboviral disease in eastern Tennessee. It has been shown that the total medical costs associated with LAC encephalitis range from $7,521-$175,586 (n=24) over 89.6 life years and the projected cost of a case with lifelong neurologic sequelae ranged from $48,775 to $3,090,798 (n=5) (Utz et al. 2003). This economic and sociological burden resulting from LAC virus is substantial and demonstrates the need for active surveillance, reporting and prevention programs (Utz et al. 2003, 2005).

iii - Container-Inhabiting Mosquitoes

Ochlerotatus triseriatus (Say), the eastern tree-hole mosquito, is a native container-inhabiting mosquito found throughout the eastern half of the U.S., primarily in deciduous wooded areas. It is diurnal and typically bites during the afternoon before 6 p.m. (Loor and DeFoliart 1970). It readily attacks humans, sometimes becoming a pest in wooded areas. Water-retaining cavities in a wide variety of deciduous trees provide suitable larval habitats for *Oc. triseriatus*, but this species will also utilize man-made containers. Eggs are attached just above the water surface on the sides of the cavity and hatch after submergence by rising water. Larvae feed on microorganisms in the tree-hole water and graze on organic debris on the sides and bottom of the cavity (Wilton 1968).

Ochlerotatus triseriatus is considered the primary vector of LAC virus. Four criteria deemed necessary to establish vector status have been met for this mosquito and La Crosse virus: (1) the virus strain has been isolated from this mosquito collected in the field; (2) there is field evidence confirming association of the infected arthropod with a vertebrate population in which the infection is occurring; (3) it has been infected after feeding upon a viremic vertebrate experimentally; and, (4) it has been shown experimentally to be capable of transmitting the virus to a susceptible vertebrate host by bite after a period of extrinsic incubation (Sudia et al. 1971). La Crosse infected females are capable of transmitting the virus to their progeny by transovarial transmission (Watts et al. 1973). Due to transovarial transmission, males can become infected with LAC virus even though they do not bloodfeed; these males are then able to transmit the virus venereally to female mosquitoes (Thompson and Beaty 1978). La Crosse virus can overwinter in diapausing eggs that have been transovarially infected (Watts et al. 1973).

Aedes albopictus (Skuse), the Asian tiger mosquito, has been implicated as a secondary vector of LAC virus. This diurnal, container-inhabiting species is indigenous to the Oriental and Australian regions. The first record of breeding populations established in the United States was reported from Harris County, Texas in 1985

(Sprenger and Wuithiranyagool 1986). This mosquito was probably introduced into the U.S. in shipments of scrap tires from northern Asia. The early dispersal pattern followed the interstate highway system, which suggests further dispersal by human activities (Moore and Mitchell 1997). It is primarily a forest edge inhabiting species that has readily adapted to the container habitats produced by humans (Moore et al. 1988). It is an opportunistic bloodfeeder and may selectively feed on an array of hosts depending on the season and microhabitat sampled (Niebylski et al. 1994).

Aedes albopictus is both a nuisance and potential disease vector. Laboratory studies show that it is susceptible to and can transmit many arboviruses that are of public health importance (Moore and Mitchell 1997). It is a documented vector of dengue viruses in Asia and 14 strains of eastern equine encephalitis (EEE) virus were isolated from field-collected mosquitoes in Florida (Mitchell et al. 1992). A JC-like virus was isolated from a naturally occurring population of *Ae. albopictus* in Tennessee (Gottfried et al. 2002). Potosi (POT) virus, Tensaw (TEN) virus, and Keystone (KEY) virus have also been isolated from naturally infected mosquitoes of this species, but these viruses are not known to cause disease in humans (Mitchell et al. 1992). This mosquito has been shown to be a competent laboratory vector of LAC virus (Grimstad et al. 1989) and can transmit the virus vertically by transovarial transmission (Tesh and Gubler 1975). In the laboratory, it is capable of transmitting LAC virus at rates equal to or greater than some reported field populations of *Oc. triseriatus* (Grimstad et al. 1989). La Crosse virus has also been isolated from naturally infected *Ae. albopictus* collected in Tennessee and North Carolina in 1999 (Gerhardt et al. 2001).

Ochlerotatus japonicus (Theobald) is a container-inhabiting mosquito that is common throughout Japan and also known from Korea (Tanaka et al. 1979). The larvae occur in a wide variety of natural and artificial containers. They usually prefer shaded places and water containing rich organic matter; rock holes seem to be the favorite larval habitat. The adults are diurnal, live in forested areas, and in their native range seem reluctant to bite humans (Tanaka et al. 1979). However, it was later shown that females were attracted to human bait, and in the laboratory, caged adults readily fed on human hosts and guinea pigs (Andreadis et al. 2001). This species is known to overwinter in the egg stage in the northeastern areas of Japan and in the larval stage in southwestern areas of Japan (Tanaka et al. 1979).

Ochlerotatus japonicus was first collected in the United States from New York and New Jersey in 1998 (Peyton et al. 1999). The most likely mode of introduction into the United States may have been through the used tire trade (Peyton et al. 1999). Since its introduction, this species has been expanding its geographic range within the United States. The first collection of *Oc. japonicus* in Tennessee occurred in Knox County on 9 June 2003 (Caldwell et al. 2005). The introduction of this mosquito into the United States is of public health concern as it has been shown to be a competent vector of West Nile virus (WNV) in the laboratory (Sardelis and Turell 2001, Turell et al. 2001). West Nile virus has also been detected from multiple pools collected in the northeast U.S. during the summer of 2000 (CDC 2000). It has been proven to efficiently transmit LAC virus in the laboratory (Sardelis et al. 2002), but there is not yet any field evidence to indicate that it is involved in the natural transmission of LAC virus.

iv - Techniques For Monitoring Container-Inhabiting Mosquitoes

Measuring mosquito abundance with acceptable accuracy and precision is a priority in epidemiology, surveillance, and control programs. Standardized techniques are necessary to monitor changes in the relative abundance and age structure of mosquito populations, for surveillance of arbovirus activity in the mosquito population, and to monitor the effectiveness of mosquito control programs (Jensen et al. 1994). There are various collecting methods for all life stages of container-inhabiting mosquitoes.

An ovitrap serves as an artificial oviposition site and consists of a black water-filled cup attached to a tree about 0.5 meters above the ground (Loor and DeFoliart 1969). Seed germination paper is clipped to the inside of the cup serving as the oviposition substrate (Steinly et al. 1991). The ovitrap is a useful detection tool, although results are influenced by rainfall and the availability of competing oviposition sites and it provides only a qualitative measure of population (Landry and DeFoliart 1986). Expanded polystyrene (EPS) floats can be used as an alternative technique to collect *Oc. japonicus* eggs (Scott and Crans 2003). Compared to larval surveys, ovitraps are extremely sensitive and have a low operating cost (Furlow and Young 1970). Larvae of container-inhabiting mosquitoes can be collected from naturally occurring tree-holes and artificial containers by siphons or dippers.

There are a number of techniques for monitoring adult container-inhabiting mosquitoes such as the Center for Disease Control (CDC) light trap, the CDC light trap baited with carbon dioxide (Stryker and Young 1970, Takken and Kline 1989, Reisen et al. 1999), the Omni-directional Fay trap (Jensen et al. 1994), infusion-baited gravid traps (Reiter 1983, Reiter 1987, Scott et al. 2001) and resting boxes (Crans 1989). Carbon dioxide has been found to be a suitable attractant for monitoring biting activity, but several trap locations should initially be tested so that traps at low-yielding locations can be removed (Landry and DeFoliart 1986). A variety of collection methods should be used in order to make the best predictions about the true mosquito population.

v - Vertebrate Hosts

An important vertebrate host is present in large numbers and readily accessible to vectors in time and space, attractive to and fed upon by vectors, susceptible to virus infection, experiences low mortality from infection, and becomes viremic with a titer of sufficient magnitude and duration to infect susceptible blood-feeding vectors (Scott 1988). The primary vertebrate hosts of La Crosse virus are the eastern chipmunk, the eastern gray squirrel (*Sciurus carolinensis* Gmelin), and the western fox squirrel (*Sciurus niger* Linnaeus) (Moulton and Thompson 1971). Due to the ecological habitat requirements of these species, they are often in contact with *Oc. triseriatus*, the primary LAC vector. Gray and fox squirrels are diurnal and arboreal, spending time in the forest canopy as well as the on the forest floor. Chipmunks spend most of their time on the forest floor and are active throughout the day and during the crepuscular periods, when they most frequently encounter *Oc. triseriatus*. Antibody prevalence rates were highest in populations of the eastern chipmunk (53%) and tree squirrels (39%) compared to other mammals that were tested, such as cottontail rabbits, flying squirrels (*Glaucomys volans* Linnaeus), and white-footed mice (*Peromyscus leucopus* Rafinesque) (Moulton and Thompson 1971). When only adult chipmunks and squirrels were considered, antibody prevalence rates were even higher at 65-75% (Moulton and Thompson 1971). The presence of antibodies in wild mammals indicates that they become infected in nature, but does not provide evidence that these animals develop viremias of adequate magnitude and duration to infect the mosquito vectors (Yuill 1983). Chipmunks and gray squirrels have both been shown to be susceptible to experimental infection with LAC virus, with 5 of 6 chipmunks and 4 of 5 squirrels developing high titer viremias, which persisted for up to 3 to 4 days (Pantuwatana et al. 1972). The comparatively high viremias in those inoculated with LAC virus provides evidence that they could contribute to the summer cycle and annual buildup of LAC virus activity in their hardwood deciduous forest habitat (Pantuwatana et al. 1972).

Secondary vertebrate hosts susceptible to infection of LAC virus include the red fox, the gray fox, the white-tailed deer (*Odocoileus virginianus* Zimmermann) and the cottontail rabbit (Yuill 1983). These secondary hosts are of limited significance to the horizontal transmission of the virus because viremic levels may be too low to infect the mosquito vector. Flying squirrels, white-footed mice, raccoons (*Procyon lotor* Linnaeus), and oppossums (*Didelphis virginiana* Linnaeus) have been ruled out as possible vertebrate hosts because they either do not come into contact with the arthropod vector or they do not develop detectable viremia (Yuill 1983).

CHAPTER II

SEASONAL DISTRIBUTION, ABUNDANCE AND DIVERSITY OF MOSQUITOES IN A HIGH AND A LOW PREVALENCE AREA FOR LA CROSSE ENCEPHALITIS

i – Abstract

A three-year investigation of the seasonal distribution, abundance and diversity of mosquitoes at a high and a low prevalence area for La Crosse (LAC) encephalitis was conducted in eastern Tennessee, USA. We identified a high LAC prevalence site (Knox County) from which two cases of LAC encephalitis were confirmed, one in 1997 and the other in 2000, and an ecologically similar low prevalence site (Blount County) with no confirmed LAC cases. Mosquitoes were collected at each site using 2 Center for Disease Control (CDC) miniature light traps baited with carbon dioxide, 1 Omni-directional Fay trap baited with carbon dioxide, 2 gravid traps and 25 oviposition traps. At both sites, mosquitoes were collected weekly between late May and early November 2003-2005. The traps that attracted host-seeking and gravid adults were operated for a 24-hour time period each week, while the oviposition traps were left out for the entire week. Throughout the 2003, 2004, and 2005 collection periods, a total of 7,593 adult female mosquitoes were collected and identified to species (Knox County (n=3,646), Blount County (n=3,946)). *Aedes albopictus*, *Ochlerotatus triseriatus*, and *Ae. vexans* were the most commonly collected mosquitoes at both sites. The proven and suspected LAC vectors, *Oc. triseriatus* and *Ae. albopictus,* comprised 19.1% and 46.6% of all adult female mosquitoes collected and identified, respectively. *Ochlerotatus triseriatus* was collected most often in the early summer (June) with fewer numbers collected in the late summer, whereas *Ae. albopictus* collections tended to be largest in the late summer to

early fall (August and September). In 2003, egg and adult collections fluctuated in a similar manner between sites, but not among species. *Aedes vexans* comprised 26.6% of all adult female mosquitoes collected in 2003, but only 8.3% and 14.5% in 2004 and 2005, respectively. There were no significant differences in egg or adult collections of *Ae. albopictus* and *Oc. triseriatus* between the high and low prevalence LAC sites. Weather patterns also appeared to be similar between the two sites.

ii – Introduction

La Crosse (LAC) virus has been an important emerging pathogen in eastern Tennessee since 1997. From 1963 to 1996, there had only been a total of 9 LAC cases reported in Tennessee. However, since 1997, there have been between 6-19 cases a year, except in 2005, when there were only 2 cases (Jones et al. 1999, Erwin et al 2002, unpublished data). La Crosse virus is the most medically important virus in the California (CAL) serogroup of bunyaviruses occurring in North America (Calisher 1994) with most infections occurring in children under the age of 15 (Kappus et al. 1983) during the period between July and September (Calisher 1994). La Crosse encephalitis may be under diagnosed because many infections are asymptomatic and also because infections are difficult to distinguish from other viral infections of the central nervous system (Jones et al. 1999). However, the financial and sociological burden resulting from LAC virus is substantial and demonstrates the need for active surveillance, reporting, and prevention programs (Utz et al. 2003, 2005).

Ochlerotatus triseriatus (Say), the eastern tree-hole mosquito, is a native container-inhabiting mosquito found throughout the eastern U.S. and is considered to be the primary vector of LAC virus. This species has been shown to be an effective vector of LAC virus in the laboratory (Watts et al. 1972) and LAC virus has been repeatedly isolated from field-caught *Oc. triseriatus* (Watts et al. 1973, Pantuwatana et al. 1974, Gerhardt et al. 2001). *Aedes albopictus* (Skuse), the Asian tiger mosquito, is a non-native container-inhabiting species that has been implicated as an important vector of LAC virus. It has been shown to be a competent laboratory vector of LAC virus (Grimstad et al. 1989) with naturally infected mosquitoes collected from Tennessee and North Carolina in 1999 (Gerhardt et al. 2001). In the laboratory, it is capable of transmitting LAC virus at rates equal to or greater than some reported field populations of *Oc. triseriatus* (Grimstad et al. 1989). It is thought that the increase in confirmed LAC cases in Tennessee may be related to the introduction and expansion of *Ae. albopictus* into eastern Tennessee (Gerhardt et al. 2001, Erwin et al. 2002). *Ochlerotatus japonicus* (Theobald) is another non-native container-inhabiting mosquito whose introduction into the United States is of public health concern. The first collection of this mosquito in Tennessee occurred in Knox County on 9 June 2003 (Caldwell et al. 2005) and has since been collected from Blount County (unpublished data). It has been shown to efficiently transmit LAC virus in the laboratory (Sardelis et al. 2002), but there is not yet any field evidence to indicate that this newly introduced mosquito is involved in the natural transmission of LAC virus.

The objectives of this study were to determine the species diversity and relative abundance of mosquitoes, as well as to determine the seasonal distribution and abundance of the proven and suspected LAC vectors, *Oc. triseriatus* and *Ae. albopictus*, in a high and a low prevalence site for LAC encephalitis. We also attempted to determine mosquito composition, abundance and seasonal distribution at each site due to environmental factors to detect any differences between the two areas and to better explain why one site has had several LAC cases and the other has had none.

iii – Materials and Methods

Collection Sites: Mosquito surveillance was conducted at one site each in Knox (high prevalence LAC area) and Blount (low prevalence LAC area) Counties, Tennessee. Both sites were located in mixed hardwood forests within close proximity to human dwellings and contained numerous natural as well as artificial containers to support mosquito larval production.

The Knox County site (US Postal Service ZIP code: 37931[1]) was located in the Karns community near the northwestern boundary of Knox County (Figure 1) and was established as the high prevalence area for LAC encephalitis. Previous research to determine the geographic distribution of LAC encephalitis in eastern Tennessee showed that from 1997-2001, there were a total of 24 LAC cases reported from 9 counties. Knox County had the highest number of cases (n=9), with the majority of these cases occurring in a cluster in the northwestern area of that county and the adjoining Anderson Co. (Erwin et al. 2002, Morton 2003). The specific Knox County site was chosen because two children, both living within 150m of the site, had been diagnosed with LAC encephalitis, one in 1997 and the other in 2000. The average elevation is 305m above sea level. The primary overstory species were white oak (*Quercus alba* L.), black oak (*Quercus velutina* Lam.), tulip poplar (*Liriodendron tulipifera* L.), and Virginia pine (*Pinus virginiana* Mill.). The primary understory species were red maple (*Acer rubrum*

1. A more specific location cannot be provided due to the privacy provisions of the Health Insurance Portability and Accountability Act (HIPAA).

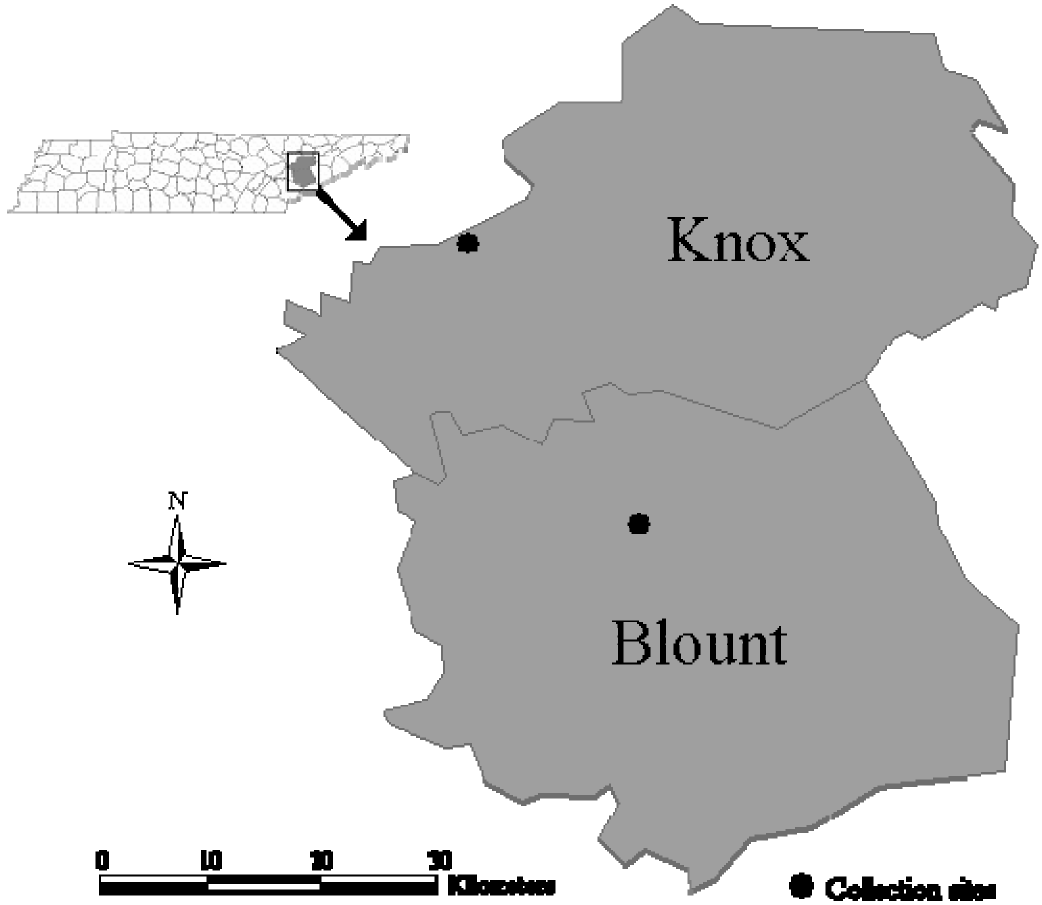


Figure 1. Sites in Knox and Blount County, Tennessee, where mosquito surveillance was conducted from 2003-2005.

L.), eastern redbud (*Cercis Canadensis* L.), red elm (*Ulmus rubra* Muhl.), and Virginia creeper (*Parthenocissus quinquefolia* [L.] Planch.).

The Blount County site (35°, 44'N: 83°, 57'W) was established as the low prevalence LAC site because there has been only one case reported in the county since 1999 despite an active surveillance program (Erwin et al. 2002, Morton 2003). This site was located in the north central area of the county (Figure 1) in the Stewardship Forest, on the campus of Maryville College, Maryville, TN. The average elevation is 287m above sea level. The main overstory species in this area were white oak, black oak, pignut hickory (*Carya glabra* [Mill.] Sweet.), and tulip poplar. The main understory species were American beech (*Fagas grandifolia* Ehrh.), red maple, mimosa (*Albizia julibrissin* Durazz.), bigleaf magnolia (*Magnolia macrophylla* Michx.), common privet (*Ligustrum vulgare* L.), American holly (*Ilex opaca* Ait.), English ivy (*Hedera helix* L.), and poison ivy (*Rhus radicans* L.).

Weather patterns: Temperature and rainfall data were obtained from the NOAA National Climactic Data Center (NCDC 2005). Weather data for the Knox County site was supplied from a weather monitoring station located in Oak Ridge, Tennessee (36°, 01'N: 84°, 14'W). Weather data for the Blount County site was supplied from a weather monitoring station located at McGhee Tyson airport (35°, 49'N: 83°, 59'W). Deviations from the norm (30-year monthly averages computed by the NCDC from 1971-2000) were obtained for April through November 2003-2005, at both collection sites (NCDC 2005).

Ovitrapping and rearing: Aedes albopictus and *Oc. triseriatus* eggs were collected weekly from the Knox and Blount County sites using oviposition traps

(Gottfried et al. 2002). At each site, 25 ovitraps (five sets of five) were placed on the north side of the tree approximately 0.5 meters above the base and about 10 meters apart from each other (Loor and DeFoliart 1969). Ovitraps consisted of 473ml black plastic cups with two holes cut below the rim of the cup for drainage of excess water. The cups were filled with tap water and placed on trees using aluminum nails. Seed germination paper (76 lb) (Anchor Paper Company, Saint Paul, MN) was cut into strips ca 4cm x 27cm, then labeled and attached to the inside of the cup with a paperclip, to serve as an oviposition substrate (Steinly et al. 1991). The oviposition strips were replaced weekly and the cups were refilled with clean tap water. Strips from the previous week were placed in plastic bags and transported to the laboratory. Throughout the 2003 collection period and the beginning of the 2004 collection period, the eggs were individually counted and identified to species using a binocular dissecting scope (Pratt and Kidwell 1969).

Oviposition strips were placed in 4.3-liter plastic tubs and submerged in 2.0-liters of tap water. Approximately 6.0ml of a bovine liver powder (ICN Biomedicals Inc., Aurora, OH) and guinea pig food mixture (1:1 ratio) was added to each tub serving as the larval food supply. Water temperature was maintained at 27± 2°C. Oviposition strips were removed from the containers after three days and larvae were allowed to develop to 4^{th} instar and pupal stages. At the first sign of mosquitoes in the pupal stage, they were transferred to mosquito breeders (BioQuip®, Gardena, CA). The bottom half of the breeder was covered in dark construction paper and newly emerged adults that went to the top half of the breeder were killed by freezing, then sorted by species (Darsie and

Ward 1981) and sex on a BioQuip® chill table or an ice tray. Sorted mosquitoes were placed into pools of less than 50 individuals and stored at -80°C for future virus isolation.

Adult mosquito collections: Weekly collections of adult host-seeking and gravid mosquitoes were made from late May to early November of the 2003 and 2004 mosquito seasons. In 2005, collections began in late May and ended in late October due to cold temperatures. At each site, mosquitoes were collected using 2 dry ice-baited Center for Disease Control (CDC) miniature light traps (Stryker and Young 1970) and 1 dry ice-baited Omni-directional Fay trap (Jensen et al.1994). Two gravid traps were also placed at each site to collect gravid female mosquitoes (Reiter 1983). The ovipositional attractant used in the gravid traps consisted of an aged mixture of tap water, cow manure and oak leaves. The CDC miniature light traps (Model 512), Omni-directional Fay traps (Model 112), and gravid traps (Model 1712) were constructed by the John W. Hock Company in Gainesville, Florida. Dry ice for the traps that attracted host-seeking adults was placed into 1.5-gallon water coolers and suspended next to the traps with the nozzles of the coolers open in order to allow the carbon dioxide to escape. All traps were operated for 24 hours. The collection cups/nets from each trap were removed and chilled during transportation back to the laboratory. Once in the laboratory, mosquitoes were identified to species and sex. All adult mosquitoes collected by various trapping techniques were stored at -80°C for future virus isolation.

Statistical analysis: To test for differences in the abundance of adults and eggs collected at each site, all data were analyzed with PROC MIXED of SAS 9.1, 2004. The density of both adult and egg collections was log transformed to normalize the distribution before analyses. Raw means and standard deviations of adult and eggs were determined with PROC MEANS of SAS 9.1, 2004. Least square means were back transformed to the original scale so that they would be consistent with the statistical tests and the means would be less affected by a few skewed values.

iv – Results

Weather Patterns: Weather patterns observed from 2003, 2004 and 2005 were similar for both sites. The normal temperature and precipitation at both sites were fairly similar (Figure 2). The normal precipitation was higher (generally about 1-2 cm per month) at the Knox County site than at the Blount County site, whereas the normal temperature was essentially identical at both sites (Figure 2). Throughout the 8 months observed from each of the 3 different years, the trend in temperature between both sites was different in only 3 of the months (Figure 3). Average monthly temperatures were higher than normal at both sites; the monthly average temperature deviations from the norm were 0.9°C in 2003, 1.4°C in 2004, and 1.0°C in 2005 in Knox County, and 0.3°C in 2003, 0.7°C in 2004, and 0.8°C in 2005 in Blount County. Overall monthly average temperature deviations from the norm were smaller in Blount County than in Knox County. In 2005, seven out of the eight months were warmer than normal at both sites; May was the only month that was colder than normal (Figure 3).

Rainfall patterns were similar at both sites. Throughout the 8 months observed from each of the 3 different years, the trend in rainfall between both sites was different in 4 of the months (Figure 4). Rainfall at the Knox County site was higher than the norm in 2003 and 2004, but lower in 2005, with overall monthly average precipitation deviations from the norm being 3.5cm, 4.1 cm, and –1.3cm, respectively. Rainfall at the Blount County site was about normal in 2003, higher in 2004 and lower in 2005, with overall

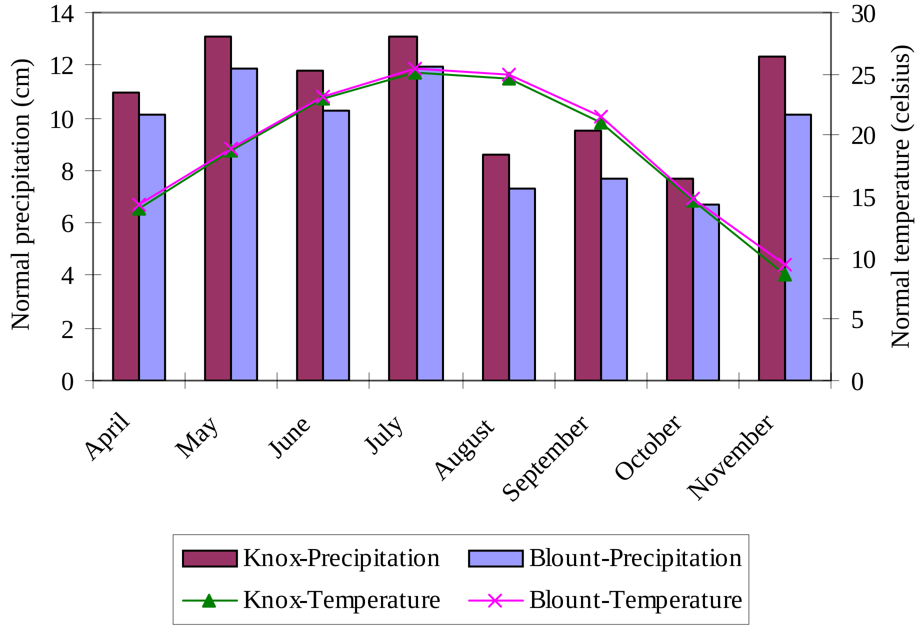
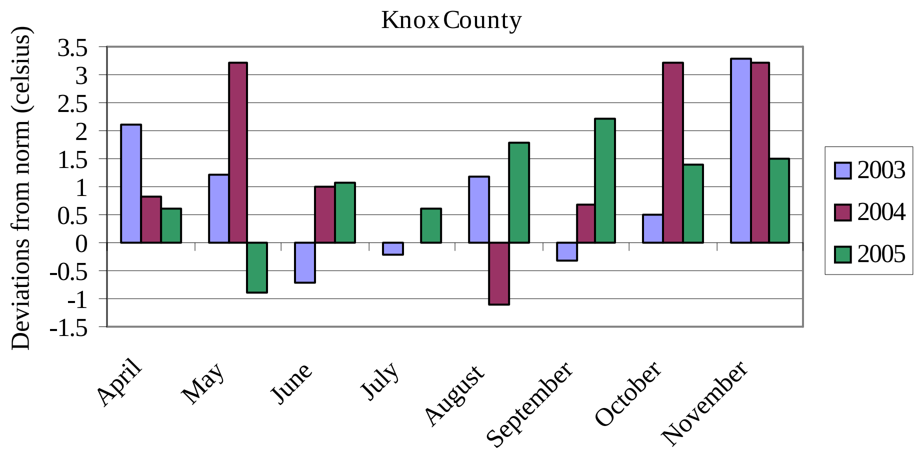


Figure 2. Normal precipitation (cm) and temperature (°C) per month collected from NOAA National Climactic Data Center from April through November, at each site in Knox and Blount County, TN.



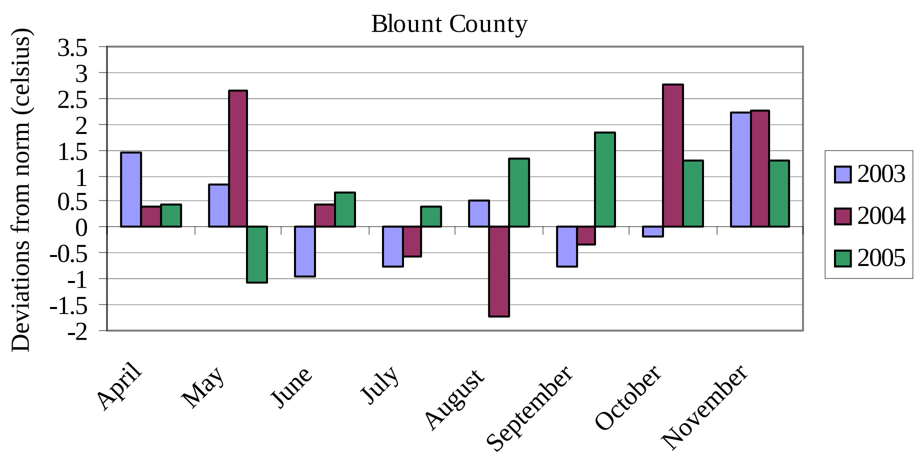


Figure 3. Mean monthly temperature deviations from the norm for April through November, 2003-2005 at each site in Knox and Blount County, TN.

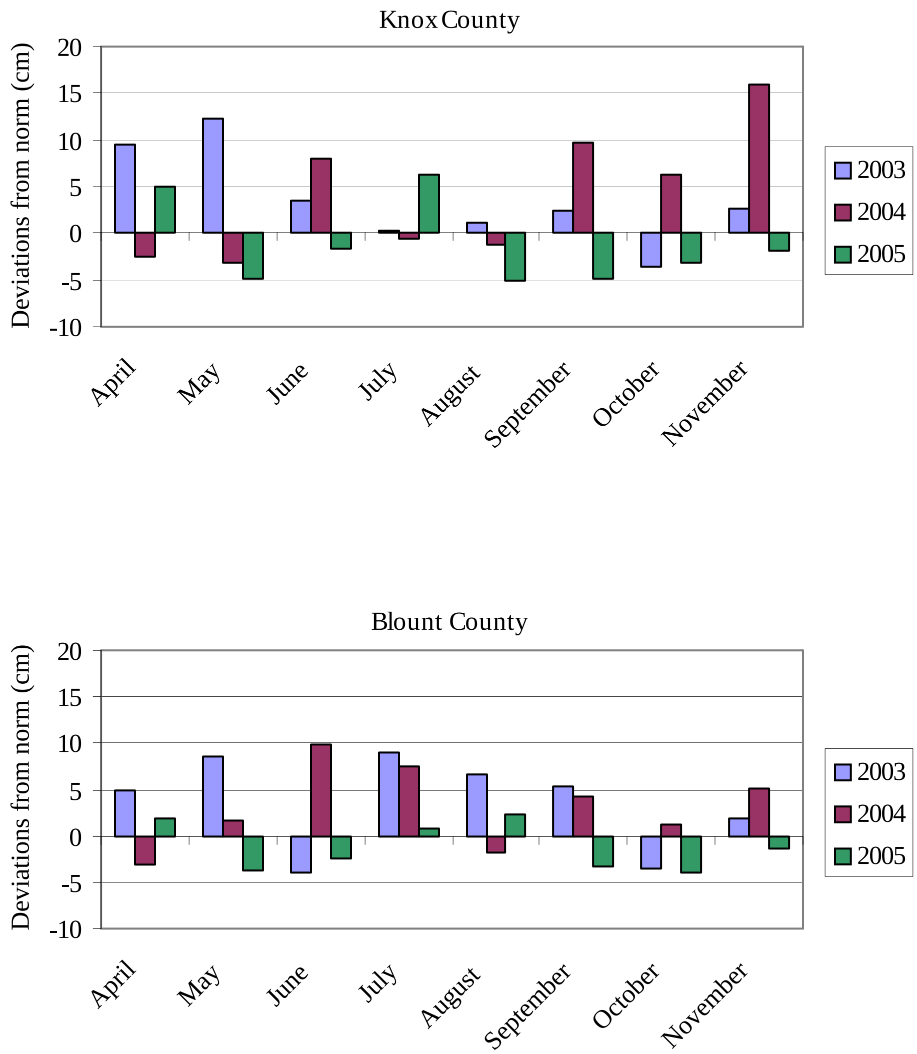


Figure 4. Mean monthly rainfall deviations from the norm for April through November, 2003-2005 at each site in Knox and Blount County, TN.

monthly average precipitation deviations from the norm being 0.4cm, 3.1cm, and –1.3 cm, respectively. At both sites, the 2003 season started out very wet during the months of April and May, with a drop in precipitation in June, whereas the 2004 season started out with very low precipitation and had a large increase in June (Figure 4). Rainfall in April and May of 2003 exceeded the norm by 21.8cm and 13.6cm in Knox and Blount Counties, respectively. Precipitation throughout the entire 2005-collection season was slightly below the norm. Rainfall from September to November 2005 was especially low being 9.8cm and 8.7cm below the norm in Knox and Blount Counties, respectively. During the 27-week collection period in 2003, the total accumulation of precipitation was 70.5cm in Knox County and 68.9cm in Blount County. The total accumulation of precipitation during the 24-week collection period in 2004 was 88.13cm in Knox County and 77.9cm in Blount County. The total accumulation of precipitation during the 23-week collection period in 2005 was 42.4cm in Knox County and 36.0cm in Blount County.

Ovitrapping and rearing: During the 2003 collection period, a total of 88,261 *Oc. triseriatus* eggs and 20,712 *Ae. albopictus* eggs were collected and counted from both sites with *Oc. triseriatus* comprising approximately 81% of all eggs collected. Eggs of *Oc. triseriatus* and *Ae. albopictus* were collected in similar numbers at both the Knox (n=42,447 and 10,112) and Blount County sites (n=45,814 and 10,600). There were no significant differences between sites when comparing egg counts of both species over the season. There was a large and significant difference in the total egg counts of *Oc. triseriatus* and A*e. albopictus* within both sites (P<0.0001). The egg ratio in Knox County was 4.2:1 in favor of *Oc. triseriatus* and in Blount County was 4.3:1 in favor of

Oc. triseriatus. At both sites, egg collections for *Oc. triseriatus* were bi-modal with a peak in June followed by a decrease, then another peak in mid-late August (Figure 5). At both sites, there was an increase in *Ae. albopictus* egg collections in late June to early July with another rise throughout August and early September (Figure 5).

Egg counting and identification to species ceased during the 2004 collection period; the purpose of collecting and rearing eggs following this date was solely to gain more mosquitoes for virus isolations.

Adult mosquito collections: A total of 7,593 adult female mosquitoes was collected and identified to species (Knox County (n=3,646), Blount County (n=3,947)) from 2003-2005. Nineteen species in 7 genera were collected and identified at both sites combined, with 15 of the 19 mosquito species found at both sites. The Blount County site had two more mosquito species (n=18) than the Knox County site (n=16) (Appendix 1). The most commonly collected species were *Ae. albopictus*, *Ae. vexans* (Meigen), and *Oc. triseriatus* (Table 1). There were no significant differences between sites when comparing adult collections of *Ae. albopictus* and *Oc. triseriatus* over all seasons, but there were differences between the two species (P=0.0015). The total number of female mosquitoes collected and identified in 2004 and 2005 was much smaller than the total number collected and identified in 2003 (Table 1), primarily because *Ae. vexans* comprised 26.6% of all adult female mosquitoes collected in 2003, but only 8.3% and 14.5% in 2004 and 2005, respectively. Collections of *Ae. vexans* were largest in the early summer with more than half of these mosquitoes collected from one 24-hour collection period on 2 June 2003 at Knox County.

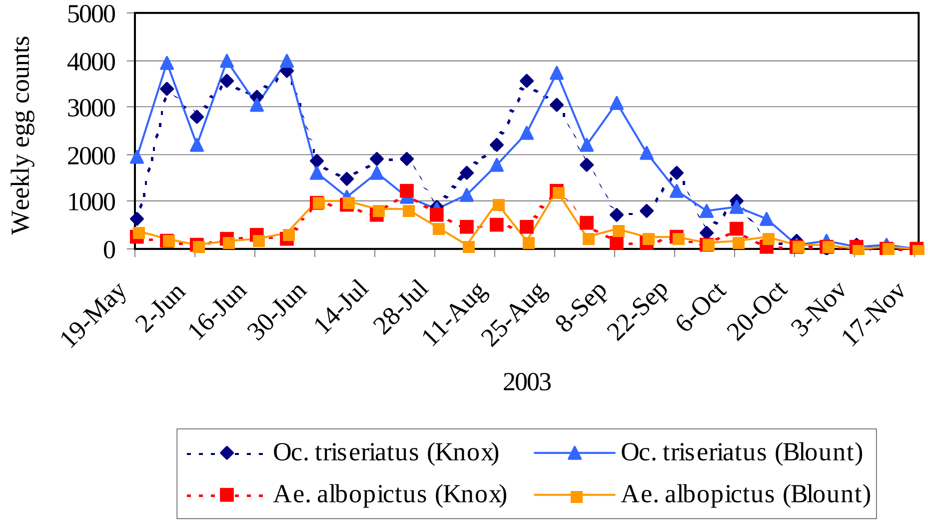


Figure 5. Number of *Ochlerotatus triseriatus* and *Aedes albopictus* eggs collected weekly at each site in Knox and Blount County, TN between 19 May and 24 November, 2003.

Table 1. Total number of adult female mosquitoes collected from 2 CO_2-baited CDC miniature light traps and 1 CO_2-baited Omni-directional Fay trap at each site in Knox and Blount County, TN, 2003-2005. Traps collected host-seeking mosquitoes for a 24-hour time period each week. See text for dates.

	2003		2004		2005	
Species	Knox County	Blount County	Knox County	Blount County	Knox County	Blount County
Ae. albopictus	555	1328	285	416	322	432
Oc. triseriatus	449	270	126	220	79	197
Ae. vexans	922	314	44	75	131	73
An. punctipennis	227	142	12	31	30	28
Cx. restuans	38	56	4	2	0	1
Oc. trivittatus	22	65	2	56	0	0
Cx pipiens	10	4	0	0	0	0
Oc. japonicus	11	0	34	1	22	3
Others	11	42	28	34	6	13
Total	2245	2221	535	835	590	747

The most commonly collected species at both sites was *Ae. albopictus*, comprising over 46% of all adult females collected and identified with more collected from the Blount County site than from the Knox County site (Table 1). More *Ae. albopictus* were collected in the late summer (August and September) than in the spring and early summer (Figure 6 and 7). Seasonal patterns for *Ae. albopictus* fluctuated in a similar manner for both the CO_2-baited CDC miniature light trap and the CO_2-baited Omni-directional Fay trap (Figure 6). The majority of host-seeking *Ae. albopictus* were collected from a single CDC miniature light trap (labeled CDC#1) at the Blount County site throughout all years, with this CDC light trap collecting an uncharacteristically large number of *Ae. albopictus* from a few weeks in late August to early September, 2003 (Figure 7). This species was the most commonly collected species in the Omni-directional Fay trap, except in 2003, at the Blount County site, when *Ae. vexans* was most numerous. Collections of *Ae. albopictus* at the Knox County site increased from 2003 to 2004 and stayed higher in 2005, comprising 26.7%, 53.7%, and 54.9% of all adult female mosquitoes collected, respectively. Collections of *Ae. albopictus* at the Blount County site remained similar among all years and comprised 58.1%, 49.9% and 58.3% of all adult female mosquitoes collected and identified.

Similar numbers of *Oc. triseriatus* were collected in the two sites, comprising approximately 19% of all adult female mosquitoes collected and identified. This species was collected most often in the spring and early summer (June), decreasing throughout the summer, and collected in very small numbers in the fall (Figure 8 and 9). Collections were bi-modal with peaks in late May and August in 2003 at the Blount County site (Figure 9). This species comprised 20.7%, 22.0%, and 14.6% of all adult female

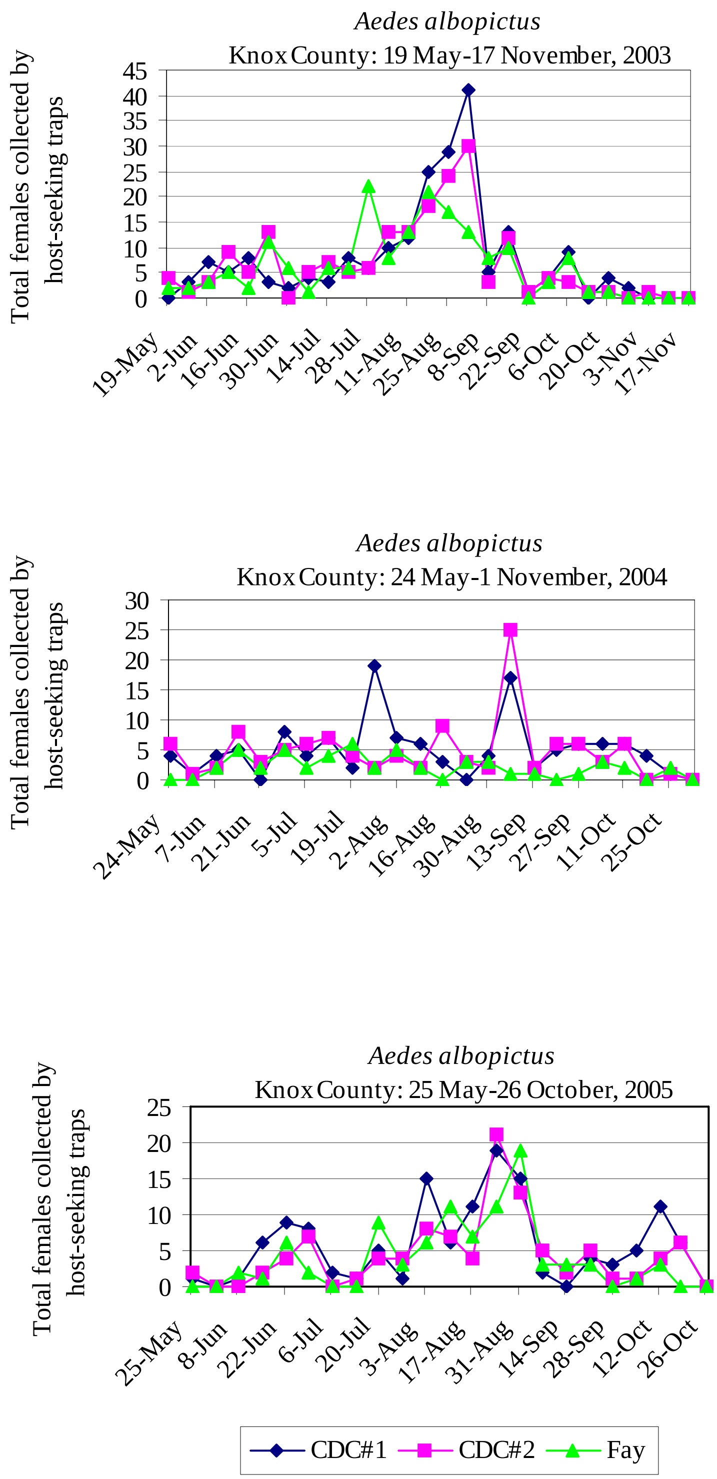


Figure 6. Total number of *Aedes albopictus* females collected by 2 CO_2-baited CDC miniature light traps and 1 CO_2-baited Omni-directional Fay trap in Knox County, TN, 2003-2005.

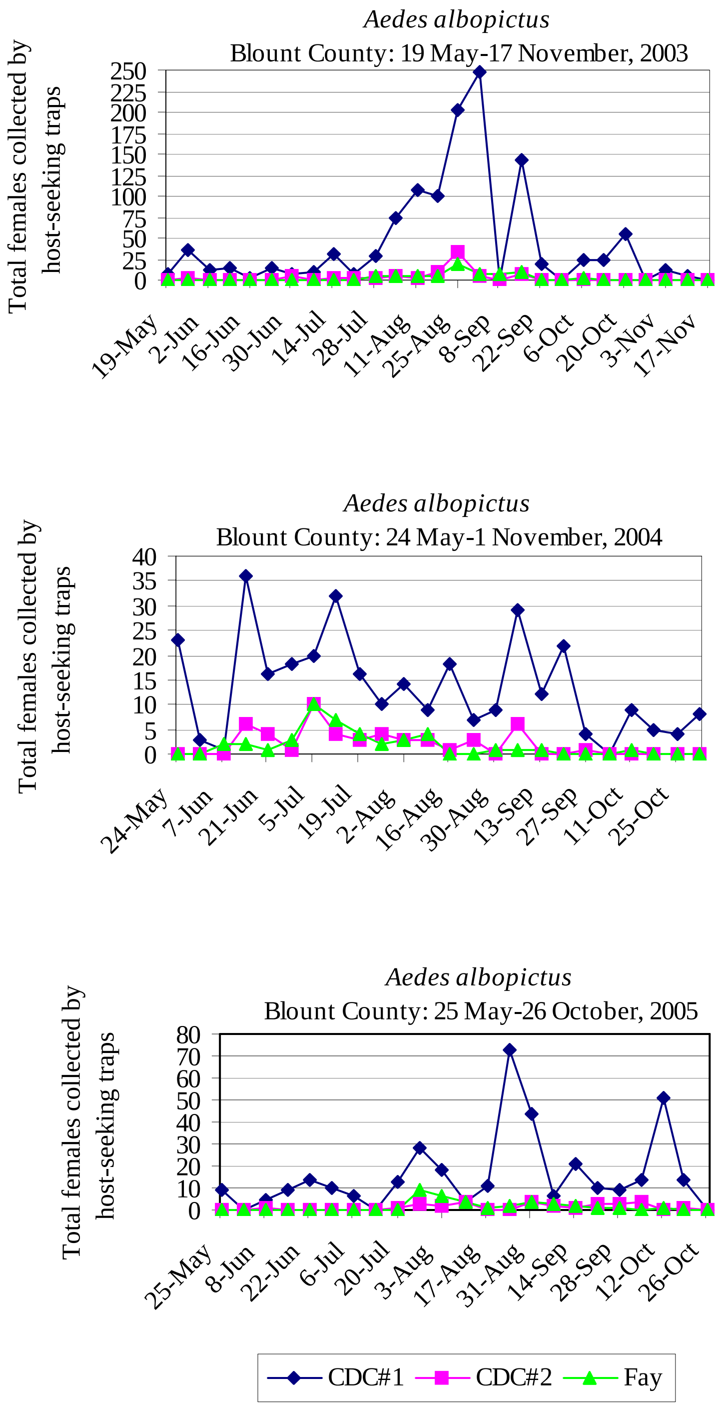


Figure 7. Total number of *Aedes albopictus* females collected by 2 CO_2-baited CDC miniature light traps and 1 CO_2-baited Omni-directional Fay trap in Blount County, TN, 2003-2005.

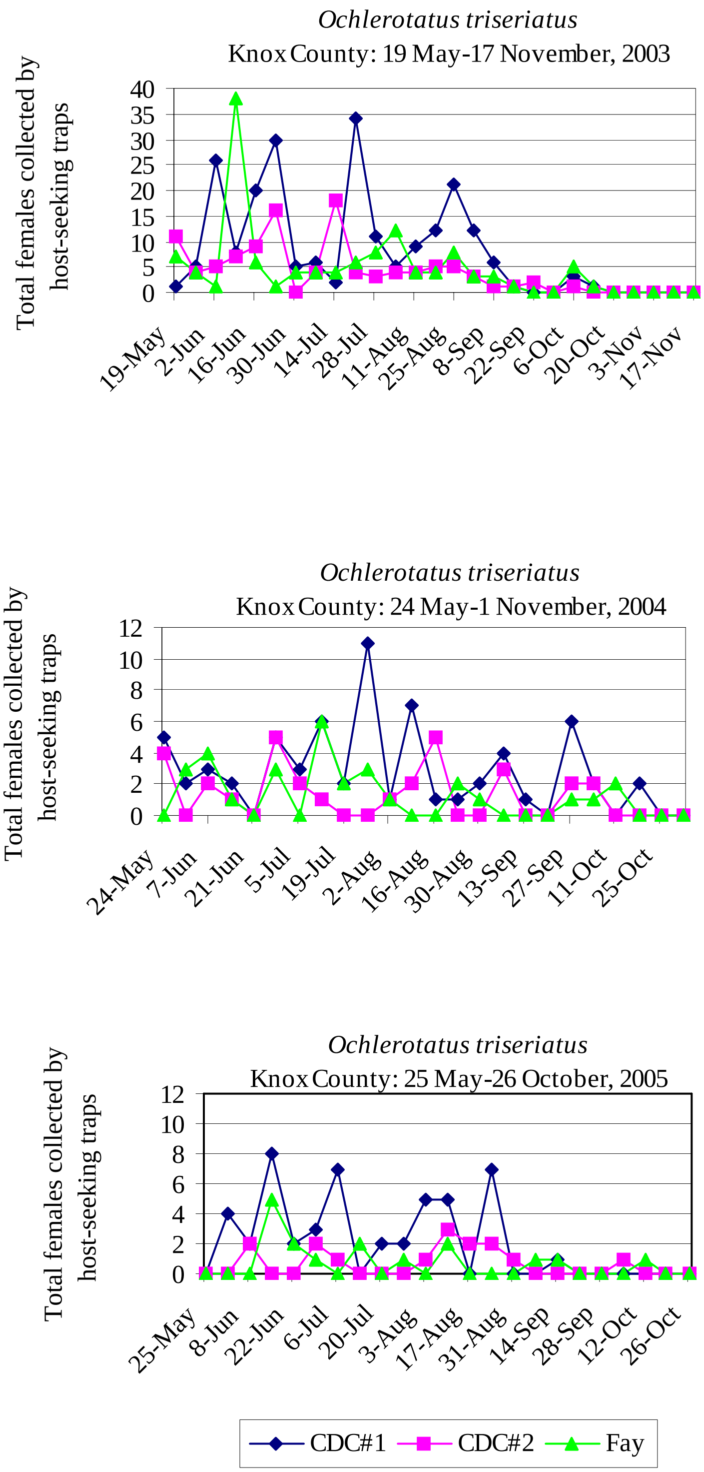


Figure 8. Total number of *Ochlerotatus triseriatus* females collected by 2 CO_2-baited CDC miniature light traps and 1 CO_2-baited Omni-directional Fay trap in Knox County, TN, 2003-2005.

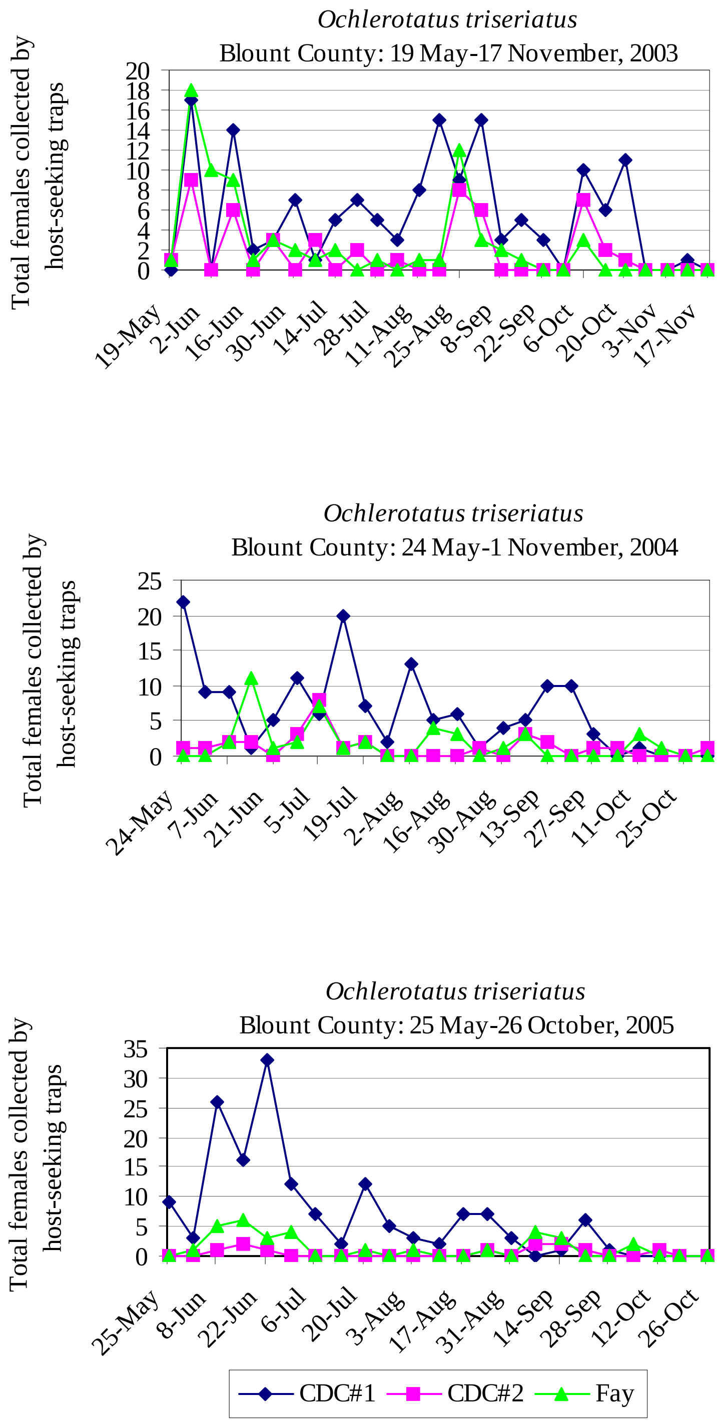


Figure 9. Total number of *Ochlerotatus triseriatus* females collected by 2 CO_2-baited CDC miniature light traps and 1 CO_2-baited Omni-directional Fay trap in Blount County, TN, 2003-2005.

mosquitoes identified in 2003, 2004 and 2005 at the Knox County site, and 13.0%, 26.5% and 26.1% of all adult female mosquitoes identified in 2003, 2004, and 2005 at the Blount County site, respectively. Collections of *Oc. triseriatus* were larger in 2003, than in 2004 and 2005 at the Knox County site (Figure 8). Seasonal patterns fluctuated in a similar manner between the CDC light trap and the Omni-directional Fay trap. Collections of *Oc. triseriatus* were outnumbered by *Ae. albopictus* collections in 2003, 2004 and 2005 by a ratio of 1:2.6, 1:2 and 1:2.7, respectively.

The majority of mosquitoes were collected by traps for host-seeking adults (n=7,173), rather than those attracting gravid females (n=420). The CDC miniature light traps baited with dry ice generally collected more adult mosquitoes as well as the widest variety of mosquito species. Gravid traps consistently collected fewer mosquitoes throughout all years at both sites.

v - Discussion

Weather patterns: Weather patterns between the two sites were very similar. The trend in rainfall and temperature being above or below the norm varied between the three years, but appeared similar between sites. The high rainfall in the spring of 2003 could account for the higher number of *Ae. vexans* that were collected due to the increase in suitable larval habitats (depressed areas filled with water) for this mosquito.

Ovitrapping and rearing: Overall egg collections did not significantly differ between the high prevalence and low prevalence LAC sites, but there were differences in the numbers of eggs between *Ae. albopictus* and *Oc. triseriatus*. Throughout the 2003 collection period, *Oc. triseriatus* eggs outnumbered *Ae. albopictus* eggs in the oviposition traps. A previous study from western North Carolina and two previous studies from southwestern Virginia reported that over 98%, 84%, and 90% of mosquitoes collected in ovitraps were *Oc. triseriatus*, respectively (Szumlas et al. 1996, Barker et al. 2003a, 2003b). In the southwestern Virginia studies, *Ae. albopictus* comprised only 15% and 9.9% of all mosquitoes collected in ovitraps (Barker et al. 2003a, 2003b). The seasonal distribution of *Oc. triseriatus* and *Ae. albopictus* egg collections fluctuated in a similar manner in comparison to adult collections.

Previous egg and adult collections were made at the Knox County site in 1998 (Gottfried et al. 2002) with the ratio of *Oc. triseriatus* to *Ae. albopictus* eggs being 4.8:1 in 1998 and 4.2:1 in 2003. *Ochlerotatus triseriatus* was the most abundant species collected in the oviposition traps during both years, whereas *Ae. albopictus* was the most abundant species collected in the traps that attracted host-seeking adults. In 1998, egg collections of *Oc. triseriatus* were largest in July, whereas egg collections of *Ae. albopictus* were largest in August. In 2003, egg collections of *Oc. triseriatus* were largest in June and egg collections of *Ae. albopictus* were largest in July. Seasonal patterns in oviposition at the high prevalence site were similar between the two different years.

Adult mosquito collections: Mosquito collections were similar between the Blount and Knox County sites with a total of 7,593 adult female mosquitoes collected and identified to species (Knox County (n=3,646), Blount County (n=3,947)) from all years. Adult collections of *Ae. albopictus* and *Oc. triseriatus* differed between species, but not between sites. Collections of *Oc. triseriatus* were lower than those of *Ae. albopictus* at both sites and every year. The apparent failure of adult traps to collect *Oc. triseriatus* may be because this species is not attracted to these traps, or it could possibly have a higher larval mortality, resulting in fewer adults compared to *Ae. albopictus* (Gottfried 2000). Our results differ from a previous study in a LAC-endemic area in western North Carolina where *Oc. triseriatus* was the most abundant species collected, comprising over 98% of mosquitoes collected by CO_2-baited suction trapping (Szumlas et al. 1996). In this previous study, 8 species were collected by CO_2-baited suction trapping, but only *Oc. triseriatus* and *Anopheles punctipennis* (Say) were well represented throughout the season, and no *Ae. albopictus* were collected with this trap, which could be because it had been recently introduced, but was not yet capable of overwintering in the area (Szumlas et al. 1996). The seasonal distribution of *Oc. triseriatus* was similar between the two studies with adult females being collected in the highest numbers in June and July, and declining from August to October. The lack of differences in mosquito abundance and seasonal distribution between the high and low prevalence sites leads us to believe that other factors may be responsible for the differences in disease prevalence between the sites that are unrelated to mosquito distribution.

The week of onset for confirmed cases of LAC encephalitis in eastern Tennessee, between 1997 and 2003, has ranged from 26 May to 13 October, with the majority of cases occurring in July and August (Figure 10). The week of onset for LAC cases follows a similar trend to that of the seasonal abundance of *Ae. albopictus*. The highest numbers of confirmed cases were seen in the late summer to early fall, which coincides with when the highest numbers of *Ae. albopictus* were collected. An association between the increase in incidence and distribution of LAC virus in eastern Tennessee with the

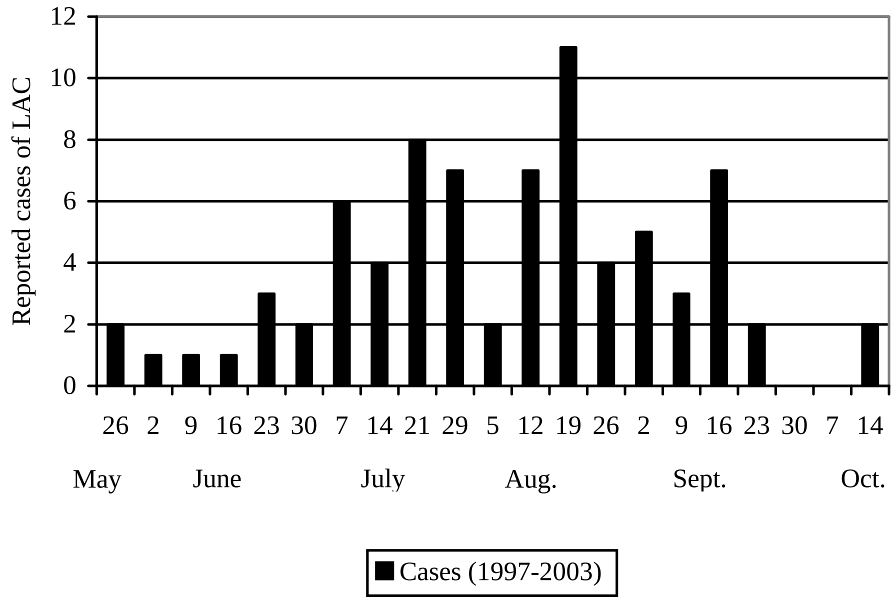


Figure 10. Week of onset for 78 confirmed cases of La Crosse encephalitis in eastern Tennessee between 1997 and 2003 (provided by the Tennessee Department of Health).

introduction and range expansion of *Ae. albopictus* has been previously described (Gerhardt et al. 2001). The association seen between the week of onset of LAC cases and the seasonal abundance of *Ae. albopictus* may further implicate this mosquito as an important LAC vector.

CHAPTER III

PARITY OF CONTAINER-INHABITING MOSQUITOES, LA CROSSE ANTIBODY PREVALENCE IN VERTEBRATE HOSTS AND HOST AVAILABILITY IN A HIGH AND A LOW PREVALENCE AREA FOR LA CROSSE ENCEPHALITIS

i – Abstract

A three-year investigation of the seasonal distribution, abundance and parity of mosquitoes at a high and a low prevalence area for La Crosse (LAC) encephalitis was conducted in eastern Tennessee. Population estimates and LAC antibody prevalence were determined for the primary amplification hosts, eastern gray squirrels (*Sciurus carolinensis*) and chipmunks (*Tamias striatus*), at each site. We identified a high LAC prevalence site (Knox County) from which two cases of LAC encephalitis were confirmed, one in 1997 and the other in 2000, and an ecologically similar low prevalence site (Blount County) with no confirmed LAC cases. Mosquitoes were collected at each site using 2 Center for Disease Control (CDC) miniature light traps baited with carbon dioxide, 1 Omni-directional Fay trap baited with carbon dioxide, 2 gravid traps, and 25 oviposition traps. At both sites, mosquitoes were collected weekly between late May and early November 2003-2005. The total average monthly parity rate for *Aedes albopictus* and *Ochlerotatus triseriatus* at each site was between 40-48% parous in 2003, between 35-49% parous in 2004 and between 8-24% parous in 2005. Parity rates did not significantly differ between sites or species. The carbon dioxide baited CDC light traps collected most of the adult mosquitoes used in parity determination. Small mammals were sampled multiple times at each site with live traps. Each site contained sufficient populations of squirrels for LAC virus amplification, but no chipmunks were collected

from the low prevalence area for LAC encephalitis. La Crosse antibodies were found at both sites, but the Knox County site had a higher prevalence of 2.65% compared to the Blount County site with a prevalence of 0.44%. Out of a total of 226 serum samples tested from both sites, 5 squirrels and 2 chipmunks tested positive for LAC antibodies.

ii – Introduction

La Crosse (LAC) virus has been an important emerging pathogen in eastern Tennessee since 1997. From 1963 to 1996, there had only been a total of 9 LAC cases reported in Tennessee. However, since 1997, there have been between 6-19 cases a year, except in 2005, when there were only 2 cases (Jones et al. 1999, Erwin et al 2002, unpublished data). La Crosse virus is the most medically important virus in the California (CAL) serogroup of bunyaviruses occurring in North America (Calisher 1994) with most infections occurring in children under the age of 15 (Kappus et al. 1983) during the period between July and September (Calisher 1994). La Crosse encephalitis may be under diagnosed because many infections are asymptomatic and also because infections are difficult to distinguish from other viral infections of the central nervous system (Jones et al. 1999). However, the financial and sociological burden resulting from LAC virus is substantial and demonstrates the need for active surveillance, reporting and prevention programs (Utz et al. 2003, 2005).

Ochlerotatus triseriatus (Say), the eastern tree-hole mosquito, is a native container-inhabiting mosquito found throughout the eastern U.S. and is considered to be the primary vector of LAC virus. This species has been shown to be an effective vector of LAC virus in the laboratory (Watts et al. 1972) and LAC virus has been repeatedly isolated from field-caught *Oc. triseriatus* (Watts et al. 1973, Pantuwatana et al. 1974, Gerhardt et al. 2001). *Aedes albopictus* (Skuse), the Asian tiger mosquito, is a non-native container-inhabiting species that has been implicated as an important vector of LAC virus. It has been shown to be a competent laboratory vector of LAC virus (Grimstad et al. 1989) with naturally infected mosquitoes collected from Tennessee and North Carolina in 1999 (Gerhardt et al. 2001). In the laboratory, it is capable of transmitting LAC virus at rates equal to or greater than some reported field populations of *Oc. triseriatus* (Grimstad et al. 1989). It is thought that the increase in confirmed LAC cases in Tennessee may be related to the introduction and expansion of *Ae. albopictus* into eastern Tennessee (Gerhardt et al. 2001, Erwin et al. 2002). A previous study conducted in eastern Tennessee compared parity rates of *Oc. triseriatus* and *Ae. albopictus* (Gottfried et al. 2002). The monthly mean proportion of parous *Ae. albopictus* from July to October ranged from 0.78 to 0.92; although the numbers of *Oc. triseriatus* individuals were too low for comparison to *Ae. albopictus*, the proportion of parous *Oc. triseriatus* adults ranged from 0.71 to 0.80 (Gottfried et al. 2002).

The primary vertebrate hosts of LAC virus are the eastern chipmunk (*Tamias striatus* Linnaeus), the eastern gray squirrel (*Sciurus carolinensis* Gmelin), and the western fox squirrel (*Sciurus niger* Linnaeus) (Moulton and Thompson 1971). Due to the ecological habitat requirements of these species, they are often in contact with *Oc. triseriatus*, the primary LAC vector. Gray and fox squirrels are diurnal and arboreal, spending time in the forest canopy as well as the on the forest floor. Chipmunks spend most of their time on the forest floor and are active throughout the day and during the crepuscular periods, when they most frequently encounter *Oc. triseriatus*. Antibody prevalence rates were highest in populations of the eastern chipmunk (53%) and tree squirrels (39%) compared to other mammals that were tested, such as cottontail rabbits (*Sylvilagus floridanus* Allen), flying squirrels (*Glaucomys volans* Linnaeus), and white-footed mice (*Peromyscus leucopus* Rafinesque) (Moulton and Thompson 1971). When only adult chipmunks and squirrels were considered, antibody prevalence rates were even higher at 65-75% (Moulton and Thompson 1971). The presence of antibodies in wild mammals indicates that they become infected in nature, but does not provide evidence that these animals develop viremias of adequate magnitude and duration to infect the mosquito vectors (Yuill 1983). Chipmunks and gray squirrels have both been shown to be susceptible to experimental infection with LAC virus, with 5 of 6 chipmunks and 4 of 5 squirrels developing high titer viremias, which persisted up to 3 and 4 days (Pantuwatana et al. 1972). The comparatively high viremias in those inoculated with LAC virus provides evidence that they could contribute to the summer cycle and annual buildup of LAC virus activity in their hardwood deciduous forest habitat (Pantuwatana et al. 1972).

The objectives of this study were to determine monthly parity rates of *Oc. triseriatus* and *Ae. albopictus* at a high prevalence and a low prevalence area for LAC encephalitis in eastern Tennessee. We also attempted to conduct population estimates of the primary amplification hosts in each area, as well as to determine LAC antibody prevalence in these hosts to detect any differences between the two areas and to better explain why one site has had several LAC cases and the other has had none.

iii – Materials and Methods

Collection Sites: Mosquito surveillance was conducted at one site each in Knox (high prevalence LAC area) and Blount (low prevalence LAC area) Counties, Tennessee.

Both sites were located in mixed hardwood forests within close proximity to human dwellings and contained numerous natural as well as artificial containers to support mosquito larval production.

The Knox County site (US Postal Service ZIP code: 37931[1]) was located in the Karns community near the northwestern boundary of Knox County (Figure 1) and was established as the high prevalence area for LAC encephalitis. Previous research to determine the geographic distribution of LAC encephalitis in eastern Tennessee showed that from 1997-2001, there were a total of 24 LAC cases reported from 9 counties. Knox County had the highest number of cases (n=9), with the majority of these cases occurring in a cluster in the northwestern area of that county and the adjoining Anderson Co. (Erwin et al. 2002, Morton 2003). The specific Knox County site was chosen because two children, both living within 150m of the site, had been diagnosed with LAC encephalitis, one in 1997 and the other in 2000. The average elevation is 305m above sea level. The primary overstory species were white oak (*Quercus alba* L.), black oak (*Quercus velutina* Lam.), tulip poplar (*Liriodendron tulipifera* L.), and Virginia pine (*Pinus virginiana* Mill.). The primary understory species were red maple (*Acer rubrum* L.), eastern redbud (*Cercis Canadensis* L.), red elm (*Ulmus rubra* Muhl.), and Virginia creeper (*Parthenocissus quinquefolia* [L.] Planch.).

The Blount County site (35°, 44'N: 83°, 57'W) was established as the low prevalence LAC site because there has been only one case reported in the county since 1999 despite an active surveillance program (Erwin et al. 2002, Morton 2003). This site

1. A more specific location cannot be provided due to the privacy provisions of the Health Insurance Portability and Accountability Act (HIPAA).

was located in the north central area of the county (Figure 1) in the Stewardship Forest, on the campus of Maryville College, Maryville, TN. The average elevation is 287m above sea level. The main overstory species in this area were white oak, black oak, pignut hickory (*Carya glabra* [Mill.] Sweet.), and tulip poplar. The main understory species were American beech (*Fagas grandifolia* Ehrh.), red maple, mimosa (*Albizia julibrissin* Durazz.), bigleaf magnolia (*Magnolia macrophylla* Michx.), common privet (*Ligustrum vulgare* L.), American holly (*Ilex opaca* Ait.), English ivy (*Hedera helix* L.), and poison ivy (*Rhus radicans* L.).

Parity determinations: Mosquitoes were collected at each site using 2 Center for Disease Control (CDC) miniature light traps baited with carbon dioxide, 1 Omni-directional Fay trap baited with carbon dioxide, 2 gravid traps, and 25 oviposition traps. At both sites, mosquitoes were collected weekly between late May and early November 2003-2005. Adult host-seeking females that had been previously collected and identified were submerged in 70% ethyl alcohol, and then placed in saline solution (Hayes 1953). Using forceps, the tip of the abdomen was torn and pulled away from the body, causing the ovaries and gut to emerge. The ovaries were removed and examined under a compound microscope. The skeins of the ovarian tracheal system were evaluated and determination of parity was based on the condition of the tracheal system (Detinova 1962). An individual was assumed to have experienced at least one gonotrophic cycle and considered parous if the majority of the ovarian tracheal skeins were elongate and not tightly knotted. Specimens with tightly coiled ovarian tracheal skeins were considered nulliparous and it was assumed that they had not experienced a gonotrophic cycle (Detinova 1962). The monthly parity rate was calculated by dividing the total number of parous mosquitoes by the total number of mosquitoes that were dissected (Haramis and Foster 1990, Gottfried et al. 2002). Using previously published parity data from eastern Tennessee (Gottfried et al. 2002), a power analysis using a modified version of SAS: UnifyPow (O'Brien 1998) determined that a sample size of 36 females was necessary at an alpha=0.05 (Sanders 2005). Therefore, we attempted to dissect 10 females of each species per week at both collection sites to achieve a rounded sample of 40 females total per month. The remains of all dissected mosquitoes were stored at -80°C for future virus isolation.

Vertebrate host and population assessment: Live animal Tomahawk® traps were deployed at each site in an attempt to catch gray squirrels and eastern chipmunks. All animal procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee (No.1255). The small mammals were trapped on alternating days between 27-31 May 2003, 20-22 October 2003, 17-21 November 2003, 13-17 December 2004, 16-18 May 2005, and 5-9 December 2005. At each site, 50 collapsible, single door, Tomahawk® traps (25 traps = Model: TLT12, L-W-H: 16 x 5 x 5 inches, 25 traps = Model: TLT13, L-W-H: 19 x 6 x 6 inches) were wired open and pre-baited with sunflower seeds for three consecutive days prior to the initial trapping date. All trap lines were positioned in the vicinity of adult mosquito trap sites, and spaced approximately 10 meters apart from each other. On trapping dates cages were baited with sunflower seeds and set at dawn. Traps were checked every 2 hours throughout the day until dusk. At dusk the traps were wired open to avoid animals being trapped for the entire night and also to avoid trapping nocturnal animals. An attempt was made to trap and tag 25 squirrels and/or chipmunks at each site during both the spring and fall months. Trapped animals were anesthetized with isoflurane to ease handling and reduce stress levels (Imai et al. 1997, Kreeger et al. 1998). Animals were sexed, checked for ectoparasites, and tagged on both ears, then released at the same place where they were originally trapped.

Antibody testing: Blood samples from the caudal veins of anesthetized animals were collected on Nobuto® strips (Advantec Manufacturing Inc., CA) to be tested for LAC antibodies. Dried Nobuto® strips were placed into 2ml vials and eluted with 200ul of phosphate-buffered saline (pH 7.4). Eluted sera was heat-inactivated at 56ºC for 30 minutes then screened for neutralizing antibodies reactive to La Crosse virus in a 96-well format. Twenty-five microliters of each eluted sample was incubated with 25ul of LAC virus (250 $TCID_{50}$/25ul) for 1 hour. To each well, 150ul of Vero cells (65,000 cells/ml) were added and the plates were incubated for 4 days at 37ºC in a 5% CO_2 environment. Using a microtiter serum dilution assay (Pantuwatana et al. 1972), protected samples were titrated against LAC virus and 3 other viruses within the California serogroup, which are known to occur in the southeast United States; these three viruses are Keystone (KEY), South River (SR), and Jamestown Canyon (JC) viruses. After the incubation period, the cells were fixed using a crystal violet/formalin solution. Titers were calculated (Reed and Muench 1938) and expressed as the highest dilution showing <50% cytopathic effects. Homologous and heterologous mouse hyper-immune ascetic fluids were used as positive and negative controls, respectively. A fourfold difference in titer between the homologous and heterologous viruses was required to make a positive determination. If a fourfold difference could not be demonstrated, then determination of a specific viral infection was not possible.

Statistical analysis: To test for differences in parity rate between sites and species, the data was analyzed using a univariate analysis of variance test (ANOVA), with PROC MIXED of SAS 9.1, 2004. All assumptions of the ANOVA were met. Raw means and standard deviations of the parity data were determined with PROC MEANS of SAS 9.1, 2004. Population estimates for the potential amplification hosts were conducted using the program "CAPTURE", which allowed for population estimates of capture-recapture data on closed populations (Otis et al. 1978, White et al. 1982). The null (Mo) model in the program "CAPTURE" was continuously selected as the most appropriate model to determine population sizes for the capture-recapture data at both the Knox and Blount County sites.

iv – Results

Parity determinations: Monthly parity rates for host-seeking adults of *Ae. albopictus* (n=1,126) and *Oc. triseriatus* (n=788) were determined at the high and low prevalence areas for LAC encephalitis between 2003-2005. Parity rates did not significantly differ between sites or species (P>0.05). The monthly percentage of parous *Oc. triseriatus* in Knox County ranged from 0.10 to 0.64 in 2003, 0.18 to 0.57 in 2004, and 0.00 to 0.13 in 2005 (Table 2). The monthly percentage of parous *Oc. triseriatus* in Blount County ranged from 0.05 to 0.79 in 2003, 0.28 to 0.80 in 2004, and 0.00 to 0.23 in 2005 (Table 2). The percentage of parous *Ae. albopictus* in Knox County by month varied from 0.08 to 0.77 in 2003, 0.20 to 0.52 in 2004, and 0.00 to 0.25 in 2005 and Blount County parity rates varied from 0.00 to 0.75 in 2003, 0.32 to 0.61 in 2004, and 0.11 to 0.50 in 2005 (Table 3). The total mean monthly parity rate for both species at

Table 2. Monthly proportion of parous *Ochlerotatus triseriatus* collected in Knox and Blount County, TN, from 2003 to 2005.

	2003 Knox County				2003 Blount County			
Month	Total dissected[1]	Parous	Nulliparous	Parity rate	Total dissected	Parous	Nulliparous	Parity rate
May	30	3	27	0.10	19	1	18	0.05
June	52	8	44	0.15	42	14	28	0.33
July	40	19	21	0.48	25	16	9	0.64
August	41	19	22	0.46	40	15	25	0.38
September	31	19	12	0.61	38	30	8	0.79
October	11	7	4	0.64	35	24	11	0.69
Total	205	75	130	0.41	199	100	99	0.48

	2004 Knox County				2004 Blount County			
Month	Total dissected[1]	Parous	Nulliparous	Parity rate	Total dissected	Parous	Nulliparous	Parity rate
June	26	13	13	0.50	36	21	15	0.58
July	28	10	18	0.36	32	12	20	0.38
August	20	5	15	0.25	29	8	21	0.28
September	11	2	9	0.18	26	11	15	0.42
October	7	4	3	0.57	5	4	1	0.80
Total	92	34	58	0.37	128	56	72	0.49

	2005 Knox County				2005 Blount County			
Month	Total dissected[1]	Parous	Nulliparous	Parity rate	Total dissected	Parous	Nulliparous	Parity rate
June	22	6	16	0.27	49	17	32	0.35
July	14	0	14	0.00	25	6	19	0.24
August	16	2	14	0.13	19	0	19	0.00
September	2	0	2	0.00	13	3	10	0.23
October	1	0	1	0.00	3	1	2	0.33
Total	55	8	47	0.08	109	27	82	0.23

[1]Months with fewer than 36 total mosquitoes dissected are below the necessary sample size, thus preventing an accurate determination of parity.

Table 3. Monthly proportion of parous *Aedes albopictus* collected in Knox and Blount County, TN, from 2003 to 2005.

	2003 Knox County				2003 Blount County			
Month	Total dissected[1]	Parous	Nulliparous	Parity rate	Total dissected	Parous	Nulliparous	Parity rate
May	12	1	11	0.08	20	0	20	0.00
June	48	6	42	0.13	43	10	33	0.23
July	43	18	25	0.42	44	21	23	0.48
August	43	15	28	0.35	42	16	26	0.38
September	44	28	16	0.64	47	29	18	0.62
October	30	23	7	0.77	40	30	10	0.75
Total	220	91	129	0.40	236	106	130	0.41

	2004 Knox County				2004 Blount County			
Month	Total dissected[1]	Parous	Nulliparous	Parity rate	Total dissected	Parous	Nulliparous	Parity rate
June	41	15	26	0.37	43	22	21	0.51
July	40	16	24	0.40	40	17	23	0.43
August	41	11	30	0.27	50	16	34	0.32
September	30	6	24	0.20	33	12	21	0.36
October	27	14	13	0.52	18	11	7	0.61
Total	179	62	117	0.35	184	78	106	0.45

	2005 Knox County				2005 Blount County			
Month	Total dissected[1]	Parous	Nulliparous	Parity rate	Total dissected	Parous	Nulliparous	Parity rate
June	32	8	24	0.25	31	12	19	0.39
July	18	3	15	0.17	25	4	21	0.16
August	42	2	40	0.05	44	5	39	0.11
September	16	2	14	0.13	40	8	32	0.20
October	21	3	18	0.14	38	13	25	0.34
Total	129	18	111	0.15	178	42	136	0.24

[1]Months with fewer than 36 total mosquitoes dissected are below the necessary sample size, thus preventing an accurate determination of parity.

each site was between 40-48% parous in 2003, between 35-49% parous in 2004 and between 8-24% parous in 2005.

Vertebrate host population assessment: Population estimates were made from collection dates in May and November 2003. The number of potential amplification hosts (capture-recapture) collected at both sites is shown in Tables 4, 5, and 6. Sources of variation including time, behavior, and heterogeneity did not appear to affect capture probabilities; however sample sizes for captures were low and may have affected population estimates (White et al. 1982). Since population assessments are based on a closed population, animals that were tagged in May and recaptured in November were not considered recaptures. In Knox County, the population of squirrels in May was estimated to be 31 individuals (SE = 9.02) with the approximate 95% confidence interval (CI) ranging from 22 to 68 total individuals. The November population of squirrels at Knox County was estimated to be 11 individuals (SE = 1.82) with a 95% CI ranging from 11 to 20. The Knox County population of chipmunks in May was estimated to be 5 individuals (SE = 0.96) with no range (95% CI). In November, collections of chipmunks depicted the population to be at 28 individuals (SE = 14.67) with the 95 % CI ranging from 16 to 86.

More squirrels were collected from the Blount County site than from the Knox County site, but no chipmunks were collected from the Blount County site. The population of squirrels at the Blount County site in May was estimated to be 85 individuals (SE = 48.73) with the 95% CI ranging from 40 to 267 individuals. The

Table 4. Capture-recapture data for gray squirrel (*Sciurus carolinensis*) collections in 2003, at a site in Knox County, TN.

Collection Date	*Sciurus carolinensis* Animals Caught	Newly Caught	Accumulated # Tagged
27-May-2003	8	8	8
29-May-2003	5	3	11
31-May-2003	10	8	18
18-Nov-2003	5	5	5
20-Nov-2003	7	3	8
21-Nov-2003	5	3	11

Table 5. Capture-recapture data for chipmunk (*Tamias striatus*) collections in 2003, at a site in Knox County, TN.

Collection Date	*Tamias striatus* Animals Caught	Newly Caught	Accumulated # Tagged
27-May-2003	3	3	3
29-May-2003	4	3	6
31-May-2003	2	1	7
18-Nov-2003	4	4	4
20-Nov-2003	4	4	8
21-Nov-2003	6	4	12

Table 6. Capture-recapture data for gray squirrel (*Sciurus carolinensis*) collections in 2003, at a site in Blount County, TN.

Collection Date	*Sciurus carolinensis* Animals Caught	Newly Caught	Accumulated # Tagged
28-May-2003	11	11	11
30-May-2003	16	14	25
17-Nov-2003	13	13	13
19-Nov-2003	18	8	21

November population of squirrels was estimated to be 27 individuals (SE = 4.6) with a range from 23 to 43 individuals (95% CI).

Antibody testing: A total of 226 squirrel and chipmunk blood samples were tested from the Knox and Blount County sites with 10 samples testing positive for LAC antibodies (Table 7). One positive sample was collected from a squirrel at the Blount County site and the other 9 positive samples were collected from the Knox County site. Three of the 9 positive samples collected from the Knox County site were from a single chipmunk that had been recaptured twice and 2 of the 9 positive samples were from a single squirrel that had been recaptured once. Of the remaining 4 positive samples collected from the Knox County site, 3 were samples collected from squirrels and 1 was from a chipmunk. Altogether, there were 5 squirrels and 2 chipmunks that tested positive for LAC antibodies. La Crosse antibody prevalence at the Blount County site was 0.44% and prevalence at the Knox County was approximately 2.65%. Although not significantly different, the antibody prevalence in chipmunks was higher (6.25%) than the antibody prevalence in squirrels (2.58%).

v – Discussion

Parity: Collections of *Oc. triseriatus* and *Ae. albopictus* were not always large enough to achieve the goal of dissecting 40 females per month, thus preventing an accurate determination of parity for certain months. Parity rates from May 2004 and 2005 were not included because it was the beginning of the collection season and too few mosquitoes were collected. Parity rates did not differ between the high prevalence and low prevalence LAC sites or between species. Parity rates did differ among years with

Table 7. Serum saples that tested positive for La Crosse antibodies.

Collection site	Date Captured	Species	Antibody level
Blount County	8 December 2005	*Sciurus carolinensis*	160
Knox County	29 May 2003	*S. carolinensis*	40
Knox County	20 November 2003	*Tamias striatus*	20
Knox County	21 November 2003	*S. carolinensis*	40
Knox County	16 December 2004	*S. carolinensis*	80
Knox County	29 May 2003	*T. striatus*	160
	21 November 2003		80
	16 May 2005		40
Knox County	17 December 2004	*S. carolinensis*	320
	16 May 2005		10

2003 having the highest parity rates and 2005 having the lowest. The general increase in parity from May to October in 2003 for both species could have resulted in increased opportunities for vertical and horizontal transmission throughout the mosquito season. In most years, there was a decrease in the proportion of parous *Oc. triseriatus* and *Ae. albopictus* females around August or September, which could be due to a large recruitment of emerging adults during periods of high rainfall in late July and early August; this may have increased the proportion of potentially infected females by transovarial transmission. The parity rates that we observed were very high compared to parity rates observed in Lousiana, where the percentage of parous *Ae. albopictus* ranged from 3.5 % to 26.7% from April to November (Willis and Nasci 1994).

It is interesting to compare the number of confirmed LAC cases that were reported in Tennessee with parity rates for 2003-2005. There were 19 confirmed LAC cases reported in Tennessee in 2003, which is the highest number of cases reported since the emergence of this virus in 1996; parity rates were also highest in 2003. The number of confirmed LAC cases and parity rates were a little lower in 2004, with 13 cases reported. In 2005, there were only 2 LAC cases reported in Tennessee and parity rates were at their lowest, never reaching 40%; this was also the driest of the three years studied. A lower parity rate could result in a smaller proportion of mosquitoes infected by transovarial transmission and thus fewer chances of transmission to humans since transovarial transmission is the primary mode of maintaining the virus within the mosquito population (Watts et al. 1973, Pantuwatana et al. 1974).

Vertebrate host population assessment: Population estimates of squirrels varied between the high prevalence and low prevalence LAC sites, with larger populations occurring at the Blount County site (low prevalence LAC site). Population estimates at both sites were larger in May than in November, which may be due to the breeding cycle and gestation period of squirrels. In Tennessee, squirrels breed from January to February and again in May to early July (Whitaker and Hamilton 1998). The increased number of squirrels captured in May could have resulted from an increase in ground activity and foraging.

Chipmunks were only collected from the Knox County site. Unlike the squirrel population estimates, chipmunk population estimates were much larger in November than in May. In eastern Tennessee, chipmunks mate in late February to early April and have a gestation period of 31 to 32 days (Whitaker and Hamilton 1998), which could have caused the low collection numbers in May. The increased population estimate in November is most likely a closer estimation of the actual density of chipmunks in Knox County. The absence of chipmunks in the low prevalence area for LAC encephalitis could result in a lower incidence of horizontal amplification of LAC to the mosquito vectors, but due to the large population of squirrels at this site, it appears that there are sufficient numbers of potential amplification hosts.

Antibody testing: La Crosse antibodies were found at both sites, but there was a higher prevalence at the Knox County site with 9 out of the 10 serum samples that tested positive for antibodies to LAC collected from this site. Both sites had a low antibody prevalence (Blount County = 0.44%, Knox County = 2.65%), but the higher prevalence at the Knox County site could help to explain the larger number of human LAC cases at this site. The single positive sample from the Blount County site was collected during the final trapping date (December 2005), which could possibly mean that LAC virus has only recently been introduced into the area. La Crosse antibodies can persist in chipmunks for at least 200 days (Pantuwatana et al. 1972). In another study, antibody levels peaked at about 21 days, but were still detectable 256 days post-viremia (Ksiazek et al. 1977). The 3 positive samples that were collected from a single chipmunk were collected from May 2003, November 2003 and May 2005, which showed that this chipmunk maintained LAC antibodies (although at a decreasing level) for at least 2 years (Table 7). The 2 positive samples collected from a single squirrel were collected from December 2004 and May 2005, but antibody levels decreased much more quickly than those of the chipmunk (Table 7).

CHAPTER IV

RESULTS

Mosquito composition and abundance appeared to be similar between the established low prevalence and high prevalence sites among the 2003, 2004, and 2005 collection seasons. There were also no differences in the seasonal occurrence or parity rates of mosquitoes between the two sites. Out of a total of 226 serum samples that were tested for antibodies to LAC virus, 9 samples from the high prevalence site tested positive, whereas only 1 sample from the low prevalence site tested positive.

Weather patterns observed from the three collection seasons appeared similar between the high and the low prevalence LAC areas. The 2003 collection season started out very wet in April and May with a decrease in precipitation in June, whereas the 2004 collection season started out dry in April and May with an increase in precipitation in June. The entire 2005 collection season was fairly dry compared to 2003 and 2004. During the 27-week collection period in 2003, the total accumulation of precipitation was 70.5cm in Knox County and 68.9cm in Blount County. The total accumulation of precipitation during the 24-week collection period in 2004 was 88.13cm in Knox County and 77.9cm in Blount County, and the total accumulation of precipitation during the 23-week collection period in 2005 was only 42.4cm in Knox County and 36.0cm in Blount County. The total number of female mosquitoes collected and identified in 2004 and 2005 was much smaller than the total number collected and identified in 2003, which could be a result of the large amount of rainfall in April and May of 2003. The high rainfall in the spring of 2003 could also account for *Ae. vexans* comprising 26.6% of all adult female mosquitoes collected in 2003, but only 8.3% and 14.5% collected in 2004 and 2005, respectively, due to the increase in suitable larval habitats for this species. Collections of *Ae. vexans* in 2003 were largest in the early summer after the high rainfall with more than half of these mosquitoes collected from one 24-hour collection period on 2 June at the high prevalence site.

There were no significant differences in egg or adult collections of *Ae. albopictus* and *Oc. triseriatus* between the high and the low prevalence LAC sites, but there were differences between the two species. Egg collections of *Oc. triseriatus* greatly outnumbered egg collections of *Ae. albopictus* (generally by a ratio of over 4:1, respectively), whereas adult collections of *Ae. albopictus* greatly outnumbered adult collections of *Oc. triseriatus* (generally by a ratio of over 2:1, respectively). Previous studies have similarly found that *Oc. triseriatus* is collected in much higher proportions than *Ae. albopictus* in oviposition traps (Gottfried et al. 2002, Barker et al. 2003a, 2003b). The failure of adult traps to collect *Oc. triseriatus* may be because this species is not attracted to these traps, or it could possibly have a higher larval mortality, resulting in fewer adults compared to *Ae. albopictus* (Gottfried 2000). Both egg and adult collections of *Oc. triseriatus* were generally largest in the spring and early summer (June), whereas egg and adult collections of *Ae. albopictus* tended to be largest in the late summer to early fall (August and September). A previous study conducted in a LAC-endemic area in western North Carolina similarly collected the largest numbers of *Oc. triseriatus* in June and July, with collections declining from August to October (Szumlas et al. 1996). There was an uncharacteristically large number of *Ae. albopictus* collected from a single CDC miniature light trap in a few weeks from late August to early September of 2003 at the low prevalence site.

The week of onset for LAC cases reported in Tennessee follows a similar trend to that of the seasonal abundance of *Ae. albopictus*. The highest numbers of confirmed cases from 1997 to 2003 were seen in the late summer to early fall, which coincides with when the highest numbers of *Ae. albopictus* were collected; this may further implicate *Ae. albopictus* as an important LAC vector.

Based on the results from this 3-year study, the CDC miniature light trap baited with carbon dioxide is the best trapping technique for studying LAC virus transmission because this trap generally collected the largest numbers of *Oc. triseriatus* and *Ae. albopictus*. The CDC miniature light traps generally collected the largest numbers of adult mosquitoes (n = 5,988) as well as the widest variety of mosquito species, whereas the gravid traps consistently collected the smallest numbers of mosquitoes (n = 420). The Omni-directional Fay trap most commonly collected *Ae. albopictus*, but collections were very small.

Parity rates did not significantly differ between the two sites, or between the two species. We were not always able to collect enough mosquitoes to achieve the goal of dissecting 40 females per month, which prevented us from making an accurate determination of parity for certain months. Parity rates were highest in the 2003 collection season, dropped a bit in 2004, and were the lowest in 2005. The total mean monthly parity rate for both species at each site was between 40-48% parous in 2003, between 35-49% parous in 2004 and between 8-24% parous in 2005. The parity rates that we observed were very high compared to parity rates observed in Lousiana, where the percentage of parous *Ae. albopictus* ranged from 3.5 % to 26.7% from April to November (Willis and Nasci 1994). It is interesting to compare the number of confirmed

LAC cases that were reported in Tennessee with parity rates for 2003-2005. In 2003, there were 19 confirmed LAC cases reported in Tennessee, which is the highest number of cases reported since the emergence of this virus in 1996; parity rates were also highest in 2003. The number of confirmed LAC cases decreased to 13 cases in 2004, and parity rates were also a little lower. In 2005, although there was a comparable amount of active surveillance, there were only 2 LAC cases reported in Tennessee and parity rates were at their lowest; this was also the driest of the 3 years studied. Transovarial transmission is the primary mode of maintaining the virus within the mosquito population (Watts et al. 1973, Pantuwatana et al. 1974), so a lower parity rate could have resulted in a smaller proportion of mosquitoes infected by transovarial transmission and thus fewer chances of virus transmission to humans. It will be interesting to compare parity rates with the number of LAC cases reported in the upcoming 2006-mosquito season.

There were no chipmunks collected from the low prevalence site, but more squirrels were collected from this site than from the high prevalence site. The absence of chipmunks in the low prevalence area could result in a lower incidence of horizontal amplification of LAC to the mosquito vectors, but due to the large population of squirrels at this site, it appears that there are sufficient numbers of potential amplification hosts. Population estimates of squirrels at both sites were larger in May than in November, which may be due to the breeding cycle and gestation period of squirrels. In Tennessee, squirrels breed from January to February and again in late May to early July (Whitaker and Hamilton 1998). The increased number of squirrels captured in May could have resulted from an increase in ground activity and foraging. Population estimates of chipmunks at the high prevalence site were larger in November than in May. In eastern

Tennessee, chipmunks mate in late February to early April and have a gestation period of 31 to 32 days (Whitaker and Hamilton 1998), which could have resulted in the low collection numbers in May. The larger population estimate in November is most likely a closer estimation of the actual density of chipmunks at the Knox County site.

A total of 226 squirrel and chipmunk serum samples were tested from both sites combined, with 10 samples testing positive for antibodies to LAC. Only one of the positive samples came from the low prevalence site, and the other 9 were all from the high prevalence site. Altogether, there were 5 squirrels and 2 chipmunks that tested positive for LAC antibodies. Although both sites had a low antibody prevalence (Blount County = 0.44%, Knox County = 2.65%), the higher antibody prevalence at the Knox County site could help to explain the larger number of human LAC cases in this area. The single positive sample from the low prevalence site (Blount County) was collected during the final trapping date (December 2005), which could possibly mean that LAC has only recently been introduced into the area. It would be worthwhile to continue collecting serum samples from squirrels in this low prevalence area to see if in the future there are more animals testing positive for LAC antibodies. It will also be interesting to watch for any LAC cases reported in this area that has previously been known as a low prevalence area. Three of the 9 positive samples collected from the high prevalence site were from a single chipmunk that had been recaptured twice and 2 of the 9 positive samples were from a single squirrel that had been recaptured once. The 3 positive samples collected from the single chipmunk were collected from May 2003, November, 2003, and May 2005, which showed that this chipmunk maintained LAC antibodies (although at a decreasing level) for at least 2 years. The 2 positive samples collected from the single squirrel were collected from December 2004 and May 2005, with antibody levels decreasing much more quickly than those of the chipmunk. A previous study demonstrated that LAC antibodies could persist in chipmunks for at least 200 days (Pantuwatana et al. 1972). In another study, antibody levels peaked at about 21 days, but were still detectable 256 days post-viremia (Ksiazek et al. 1977).

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APPENDIX

Female mosquitoes collected by date from 2 CO_2-baited CDC miniature light traps in Knox County, TN, 19 May-17 November, 2003.

	Date																											
	May		June					July				August				September					October				November			Total
Species	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27	3	10	17	
Ae. vexans	151	43	415	45	41	10	4	5	4	14	14	6	16	3	17	16	5	5	3		1	4	2					824
Ae. albopictus	4	4	10	14	13	16	2	9	10	13	12	23	25	43	53	71	8	25	2	8	12	1	5	2	1			386
Oc. triseriatus	12	9	31	15	29	46	5	10	20	38	14	9	13	17	26	15	7	2	2		4	1						325
An.punctipennis	2	27	80	35	4	12	1	5	12	2	3		8	1	13	6	4	1										216
Cx. restuans	6	12	6	2	1					3	2																	32
Oc. trivittatus		2	5	1	1	5	1		1					1														17
Cx. pipiens			1						3				2		1	2			1									10
Oc. japonicus				1					1					2	3	1	2											10
Ps. ferox				2	1								1															4
Cx. tarsalis		1	2																									3
Ps. cyanescens																					1							1
Total	175	98	550	115	90	89	13	29	51	70	45	38	65	67	113	111	26	33	8	8	18	6	7	2	1	0	0	1828

Female mosquitoes collected by date from 1 CO_2-baited Omni-directional Fay trap in Knox County, TN, 19 May-17 November, 2003.

	Date																											
	May		June					July				August				September					October				November			Total
Species	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27	3	10	17	
Ae. albopictus	2	2	3	5	2	11	6	1	6	6	22	8	13	21	17	13	8	10		3	8	1	1					169
Oc. triseriatus	7	4	1	38	6	1	4	4	4	6	8	12	4	4	8	3	3	1			5	1						124
Ae. vexans	16	8	8	14	5	2	2	2	6	4	13			3		1		1		2		3	8					98
An.punctipennis		1	1	3					2		1		1		1	1												11
Cx. restuans	1			1	3						1																	6
Oc. trivittatus			1	4																								5
Ps. ferox				2																								2
An. barberi																		1										1
Oc. japonicus						1																						1
Total	26	15	14	67	16	15	12	7	18	16	45	20	18	28	26	18	11	13	0	5	13	5	9	0	0	0	0	417

Female mosquitoes collected by date from 2 CO_2-baited CDC miniature light traps in Knox County, TN, 24 May-1 November, 2004.

	Date																								
	May		June				July				August					September				October				November	Total
Species	24	31	7	14	21	28	5	12	19	26	2	9	16	23	30	6	13	20	27	4	11	18	25	1	
Ae. albopictus	10	2	6	13	3	13	10	14	6	21	11	8	12	3	6	42	4	11	12	9	12	4	2		234
Oc. triseriatus	9	2	5	3		10	5	7	2	11	2	9	6	1	2	7	1		8	4		2			96
Ae. vexans		2	11		1	1	2	1	1	1	1	1	2			2		1		1	3	1		3	35
Oc. japonicus	2	2	3							1	1		6			5		1	5	2	5			1	34
An.punctipennis	2	1	1							2					1	2		2	1						12
Ae. dupreei								4														3			7
Cx. restuans									4																4
Oc. trivittatus						2																			2
Ps. ferox							1														1				2
Cx.erraticus					1										1										2
Cx. tarsalis	1																								1
Ps. columbiae																1									1
Ur. sapphirina																		1							1
Total	24	9	26	16	5	26	18	26	13	36	15	18	26	4	10	59	5	16	26	16	21	10	2	4	431

Female mosquitoes collected by date from 1 CO_2-baited Omni-directional Fay trap in Knox County, TN, 24 May-1 November, 2004.

	Date																								
	May		June				July				August					September				October				November	Total
Species	24	31	7	14	21	28	5	12	19	26	2	9	16	23	30	6	13	20	27	4	11	18	25	1	
Ae. albopictus			2	5	2	5	2	4	6	2	5	2		3	3	1	1		1	3	2		2		51
Oc. triseriatus		3	4	1		3		6	2	3	1			2	1				1	1	2				30
Ae. vexans						3	1	1	1						1						1		1		9
Ae. dupreei								4													1		3		8
Ps. ferox									1		1	1								1					4
Cx.pipiens complex														1											1
An. barberi																			1						1
Total	0	3	6	6	2	11	3	15	10	5	7	3	0	6	5	1	1	0	3	5	6	0	6	0	104

Female mosquitoes collected by date from 2 CO_2-baited CDC miniature light traps in Knox County, TN, 25 May-26 October, 2005.

	Date																							
	May	June					July				August					September				October				Total
Species	25	1	8	15	22	29	6	13	20	27	3	10	17	24	31	7	14	21	28	5	12	18	26	
Ae. albopictus	3		1	8	13	15	2	2	9	5	23	13	15	40	28	7	2	9	4	6	15	12		232
Ae. vexans	43	16	2	4	5	10	1	1	3		1	1				1		4		3		2		97
Oc. triseriatus		4	4	8	2	5	8		2	2	6	8	2	9	1		1			1				63
An. punctipennis	2	1	1	8	2	5			1			2				2		4						28
Oc. japonicus		1		4	4	6				1	1	1			2							2		22
Ps. ferox										2														2
Cx. tarsalis			1	1																				2
Ur. sapphirina																					1			1
Total	48	22	9	33	26	41	11	3	15	10	31	25	17	49	31	10	3	17	4	10	16	16	0	447

Female mosquitoes collected by date from 1 CO_2-baited Omni-directional Fay trap in Knox County, TN, 25 May-26 October, 2005.

	Date																							
	May	June					July				August					September				October				Total
Species	25	1	8	15	22	29	6	13	20	27	3	10	17	24	31	7	14	21	28	5	12	18	26	
Ae. albopictus			2	1	6	2			9	3	6	11	7	11	19	3	3	3		1	3			90
Ae. vexans	19	1	2		1		3		6			1	1											34
Oc. triseriatus				5	2	1		2		1		2				1	1				1			16
An. punctipennis						1	1																	2
Ps. ferox									1															1
Total	19	1	4	6	9	4	4	2	16	4	6	14	8	11	19	4	4	3	0	1	4	0	0	143

Female mosquitoes collected by date from 2 CO_2-baited CDC miniature light traps in Blount County, TN, 19 May-17 November, 2003.

	Date																											
	May		June					July				August				September					October				November			Total
Species	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27	3	10	17	
Ae. albopictus	7	38	11	16	2	16	11	10	33	9	31	79	111	111	235	252	1	149	21	1	25	25	55		12	4		1265
Ae. vexans	23	9	13	18	4	1	8	4	5	25	7	6	25	2	2	15	2	2	7	2		9	35	1	3			228
Oc. triseriatus	1	26		20	2	6	7	4	5	9	5	4	8	15	17	21	3	5	3		17	8	12			1		199
An. punctipennis	6	46	8	7	6	11	3	6	1	2	1	1	1	7		4	1	1	1	1	1		1					116
Cx. restuans		27	8	2	3	2	1	1		5	1																	50
Oc. trivittatus	1	23	8	2					4	1			3	1														43
Oc. canadensis	4	10	8	2	1																							25
Ps. ferox									1	2					1													4
Cx. pipiens									1						1						1							3
Ae. dupreei												1																1
An.quadrimaculatus																1												1
Cx. erraticus																1												1
Or. signifera	1																											1
Ps. columbiae																		1										1
Ps. cyanescens																							1					1
Total	43	179	56	67	18	36	30	25	50	53	45	91	148	136	256	294	7	158	32	4	44	42	103	1	15	5	0	1938

Female mosquitoes collected by date from 1 CO_2-baited Omni-directional Fay trap in Blount County, TN, 19 May-17 November, 2003.

	Date																											Total
	May		June					July				August				September					October				November			
Species	19	26	2	9	16	23	30	7	14	21	28	4	11	18	25	1	8	15	22	29	6	13	20	27	3	10	17	
Ae. vexans	17	32		5	2	3		4			7	1	5	2		2	3		1		1				1			86
Oc. triseriatus	1	18	10	9	1	3	2	1	2		1		1	1	12	3	2	1			3							71
Ae. albopictus						1	1	1			5	4	4	5	18	6	7	9			2							63
An. punctipennis	1	16		3		2							1					1						1	1			26
Oc. trivittatus	6	10		2		2						1		1														22
Cx. restuans	1	1		1	1	1					1																	6
Oc. canadensis		2		1																								3
An.quadrimaculatus		1																										1
Cx. erraticus															1													1
Cx. pipiens																	1											1
Ps. ferox																		1										1
Ur. sapphirina																	1											1
Total	26	80	10	21	4	12	3	6	2	0	14	6	11	9	31	11	14	12	1	0	6	0	0	1	2	0	0	282

Female mosquitoes collected by date from 2 CO_2-baited CDC miniature light traps in Blount County, TN, 24 May-1 November, 2004.

	Date																							
	May		June				July				August					September				October			November	Total
Species	24	31	7	14	21	28	5	12	19	26	2	9	16	23	30	6	13	20	27	4	11	18	25	1	
Ae. albopictus	23	3	1	42	20	19	30	36	19	14	17	12	19	10	9	35	12	22	5		9	5	4	8	374
Oc. triseriatus	23	10	11	3	5	14	14	21	9	2	13	5	6	2	4	8	12	10	4	1	1			1	179
Ae. vexans	1	1	8	1	1		2	5	1	4			1			7		2	8	2	1	2	2	3	52
Oc. trivittatus							27	8		1		1													37
An.punctipennis			4	1				2		3						6			4				1		21
Ps. ferox								7	1			1	3										1		13
Ae. dupreei								1	1			1								1					4
Ur. sapphirina																					1	1		1	3
Oc. japonicus																						1			1
Oc. canadensis						1																			1
Or. signifera						1																			1
Total	47	14	24	47	26	35	73	80	31	24	30	20	29	12	13	56	24	34	21	4	12	9	8	13	686

Female mosquitoes collected by date from 1 CO_2-baited Omni-directional Fay trap in Blount County, TN, 24 May-1 November, 2004.

	Date																							
	May		June				July				August					September				October			November	Total
Species	24	31	7	14	21	28	5	12	19	26	2	9	16	23	30	6	13	20	27	4	11	18	25	1	
Ae. albopictus			2	2	1	3	10	7	4	2	3	4			1	1	1				1				42
Oc. triseriatus			2	11	1	2	7	1	2			4	3		1	3					3	1			41
Ae. vexans			2		1		3	2		2			1		1					5	2		2	2	23
Oc. trivittatus							17		1				1												19
An. punctipennis							1	1		3			1			2		2							10
Ps. ferox						2	1	1				2	1							1					8
Ae. dupreei								3																	3
Cx. restuans							2																		2
Ps. cyanescens																				1					1
Total	0	0	6	13	3	7	41	15	7	7	3	10	7	0	3	6	1	2	0	7	6	1	2	2	149

Female mosquitoes collected by date from 2 CO_2-baited CDC miniature light traps in Blount County, TN, 25 May-26 October, 2005.

	Date																							
	May	June					July				August					September				October				Total
Species	25	1	8	15	22	29	6	13	20	27	3	10	17	24	31	7	14	21	28	5	12	18	26	
Ae. albopictus	9		6	9	14	10	6		14	31	20	8	11	73	48	8	22	13	12	18	51	15		398
Oc. triseriatus	9	3	27	18	34	12	7	2	12	5	3	2	7	8	3	2	3	7	1		1			166
Ae. vexans	29	5	1	3	3	1			2		1						2	1	3	5		3		59
An. punctipennis	2		2	1		1			2						1	1	2		4	4		1		21
Oc. canadensis		1	1	1	1																			4
Ur. sapphirina																			1	2			1	4
Oc. japonicus					1					1	1													3
Ps. ferox									1					1										2
An. barberi											1													1
Total	49	9	37	32	53	24	13	2	31	37	26	10	18	82	52	11	29	21	21	29	52	19	1	658

Female mosquitoes collected by date from 1 CO_2-baited Omni-directional Fay trap in Blount County, TN, 25 May-26 October, 2005.

	Date																							
	May	June					July				August					September				October				Total
Species	25	1	8	15	22	29	6	13	20	27	3	10	17	24	31	7	14	21	28	5	12	18	26	
Ae. albopictus										9	6	4	1	2	4	3	2	1	1		1			34
Oc. triseriatus		1	5	6	3	4			1		1			1		4	3			2				31
Ae. vexans	4	2			1	1	2				1	1				1			1					14
An. punctipennis		3		1					1						1		1							7
Cx. restuans			1																					1
Ps. ferox									1															1
Ur. sapphirina																					1			1
Total	4	6	6	7	4	5	2	0	3	9	8	5	1	3	5	8	6	1	2	2	2	0	0	89

VITA

Sabra Lee Scheffel was born in Baltimore, Maryland on August 19, 1982. She graduated in May of 2004, from Virginia Polytechnic Institute and State University, Blacksburg, Virginia, with a B.S. in Biology, a minor in Sociology and a concentration in Entomology. In July of 2004, she entered the Department of Entomology and Plant Pathology at the University of Tennessee, Knoxville, as a graduate research assistant under the direction of Dr. Reid Gerhardt in the Medical and Veterinary Entomology Laboratory. In August of 2006, she was awarded a Master of Science degree in Entomology and Plant Pathology.

The author is a member of the American Mosquito Control Association, the Entomological Society of America, the Society of Vector Ecology, and Gamma Sigma Delta.