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Habitat Comparisons and Geographic Distribution of La Crosse Encephalitis in Eastern Tennessee Utilizing Geographic Information Systems

Howard Joe Morton

University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Howard Joe Morton entitled "Habitat Comparisons and Geographic Distribution of La Crosse Encephalitis in Eastern Tennessee Utilizing Geographic Information Systems." 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 Jones, Jerome Grant

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

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

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Anne Mayhew
Vice Provost and
Dean of Graduate Studies

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

Habitat Comparisons and Geographic Distribution of La Crosse Encephalitis in Eastern Tennessee Utilizing Geographic Information Systems

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Howard Joe Morton II

May 2003

Abstract

In eastern Tennessee there was a total of 17 cases of a La Crosse (LAC) encephalitis viral infection reported to the East Tennessee Department of Health in 2000. During that same time, 25 cases of other Central Nervous System Infection (CNSI) that were not La Crosse were also reported. Out of those 42 cases 11 were chosen to be revisited (6 Control and 5 LAC) and the nearby wooded habitats were surveyed. Geographic Information Systems (GIS) was utilized to plot each case location on various maps.

The sites were all in eastern Tennessee, in the Appalachian Valley and Ridge geographical area, across four counties. There was no difference in amount of precipitation that the LAC and Control Sites received. Control Sites had 8 species of exotic trees but the same soil categories as the LAC Sites. LAC Sites were mixed hardwood habitats and more mosquitoes were collected from them than the Control Sites which were not mixed hardwood.

There was a total of 24 cases of a LAC encephalitis viral infection reported to the East Tennessee Department of Health from 1999-2001. During that same time, 78 cases of other CNSI that were not LAC were also reported. Geographic Information Systems was utilized to plot each case location on various maps. Case site locations were divided into LAC Sites for La Crosse cases and Control Sites for CNSI cases that were not due to LAC.

The sites fell into one of the four physical regions of eastern Tennessee: Blue Ridge, Appalachian Valley and Ridge, Cumberland Plateau, and Eastern Highland Rim. There was no difference in amount of precipitation that the LAC and Control Sites received.

A High LAC Area and a Low LAC Area were noted and included parts of four counties. The High Lac Area had a similar incidence as the Low LAC Area for CNSI but the High LAC Area had 12 cases of La Crosse while the Low LAC Area had none. The difference between the two areas was the LAC case site proximity to larges wooded areas. In all counties except Hamblen County, LAC Sites were found to be in close proximity to large wooded areas unlike the Control Sites that occurred whether or not they were in close proximity to large wooded areas.

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CHAPTER I

Introduction

i. La Crosse Encephalitis

The La Crosse (LAC) virus is a member of the California Encephalitis Group of arboviruses, which includes California Encephalitis (CE), San Angelo (SA), Tahyna (TAH), Snowshoe Hare (SSH), South River (SR), Jamestown Canyon (JC), and Keystone (KEY) viruses (Sudia et al. 1971). La Crosse was discovered in and named after La Crosse, Wisconsin, where the first known case was reported in 1960 (Henderson and Coleman 1971). Since then, cases of La Crosse Encephalitis (LACE) have been reported in 29 states from Wisconsin to New York and as far south as Texas, Alabama, and Georgia (CDC 2002).

Cases increased during the 1960s and 1970s, with most reported cases coming from Illinois, Indiana, Iowa, Minnesota, Ohio, and Wisconsin (Henderson and Coleman 1971). Reported cases in West Virginia increased during the 1990s, and by 1997 West Virginia accounted for more than half of the reported cases of LACE (Jones et al. 1999). In September 1997, an apparent cluster of LAC cases was reported by a children's referral hospital in eastern Tennessee (Jones et al. 1999). The same pattern that occurred in West Virginia also was occurring in eastern Tennessee. During the summer of 1997, the total number of cases reported for that year rose to 10. During the previous 33 years since the first LACE case was reported in Tennessee in 1964, a total of 9 cases had been reported (Jones et al. 1999). During the following years, 9 cases were reported in 1998, 6 in 1999, 17 in 2000, 9 in 2001, and 15 in 2002 in eastern Tennessee (Jones et al. 1999, Smith 2001, Erwin et al. 2002, Stanich 2002).

The LAC virus has a classical zoonotic transmission cycle, going between small mammals (squirrels and chipmunks in most cases) in the woods and the mosquito vectors (typically in the genus *Aedes* and *Ochlerotatus*) similar to the other viruses in the California Group of arboviruses (Henderson and Coleman 1971). The small mammals that are susceptible to infections of the California Group viruses have low or absent pathogenicity and mortality, suggesting a long history or association between the viruses and hosts in nature in a balanced host-pathogen relationship (Henderson and Coleman 1971, Downs 1974). Human infection is incidental and is generally caused by an intrusion into the natural habitat of the hosts and vectors of the virus (Downs 1974).

A major feature of the epidemiology of arboviral diseases when compared to non-arthropod-borne virus disease is the high focality of arboviral disease. Arboviruses have specific requirements that are unique to each virus. They are dependent on the presence of suitable hosts, the virus, efficient vectors, and the host specificity of vectors (Downs 1974). Henderson and Coleman (1971) suggest LAC and other California Group arboviruses are more prevalent in the areas they are found than is indicated by the number of human cases reported. The problem is in the diagnosis of the viruses. LAC virus has probably been in eastern Tennessee since long before 1964 when the first case was identified (Jones et al. 1999). However, diagnostic tests for LAC virus were not available until 1964 (Henderson and Coleman 1971). Children who get LAC virus and other viral infections from the California group react to the infection differently. Children can acquire anything from mild, undifferentiated febrile illness to influenza-like syndrome or acute central nervous system disease with high fever, vomiting, seizure, and coma (Chun et al. 1968, Erwin et al. 2002). Most California Group virus diagnoses are from children (Henderson and Coleman 1971). More people test positive for California viruses in endemic areas than is indicated by the reported infection rate. The percent positive in an area varies depending on the daily activity of the people, location, and how far they lived from the vectors of the disease organism (Henderson and Coleman 1971). Also, the more time they spent outside and near wooded areas, the higher their chances were of having antibodies (Henderson and Coleman 1971).

Previously, LAC virus has been dependent on the presence of the eastern treehole mosquito (*Ochlerotatus* (=*Aedes*) *triseriatus* (Say)), wooded areas with treeholes, and presence of chipmunks, rabbits, and squirrels (Gauld et al. 1974, Loor and DeFoliart 1970, Moulton and Thompson 1971). Another mosquito, Ae. *albopictus* (Skuse) that was introduced into Texas in 1985 with shipments of tires from Asia, shares many of the same habitats as *Oc. triseriatus* (Reiter 1998). *Aedes albopictus* was incriminated as a possible vector for LAC virus when adults reared from eggs tested positive for the LAC virus, and more *Ae. albopictus* were associated with LAC positive cases when compared to the children with other central nervous system infections (CNSI) (Gerhardt et al. 2001, Erwin et al. 2002). Both species are treehole and container-inhabiting mosquitoes and prefer oak hickory forests, which contain trees with multiple stems. The places in which the stems diverge provide sites for pools of water in which the mosquitoes oviposit and the larvae develop (Watts et al. 1974, Hawley 1988). Those mosquitoes will also oviposit in man-made containers of waters, such as tires, bottles, cups, etc (Loor and DeFoliart 1969, Hawley et al 1987, Reiter 1998). Oak forests are the preferred habitat for squirrels, rabbits, and chipmunks, which, combined with the treehole mosquitoes, provide the necessary conditions for LAC virus transmission (Hanson and Hanson 1970).

ii. Ochlerotatus triseriatus

Ochlerotatus triseriatus is native to North America and has been incriminated as a primary vector of LAC virus (Watts et al. 1972). *Ochlerotatus triseriatus* overwinters in the egg stage in which the virus is maintained by transovarial transmission from infected females to the eggs (Watts et al. 1974). Eggs hatch in the spring, and emerging adults are infective to mammals (Pantuwantana et al. 1974). *Ochlerotatus triseriatus* readily feeds on chipmunks, rabbits, and squirrels with high engorgement rates (Loor and DeFoliart 1970). Virus multiplication and resultant high viremias occur in chipmunks, rabbits, and squirrels that have been inoculated with LAC virus. Duration and magnitude of the viremias in these small mammals are able to provide sufficient virus to infect virus free *Oc. triseriatus* females that subsequently feed on them (Gauld et al. 1974).

Humans are readily bitten when they enter the natural habitat of *Oc. triseriatus*. *Ochlerotatus triseriatus* are attracted to humans and feed upon them at the same rate as nearby rabbits, making it a good vector for virus transmission between small mammals and humans (Loor and DeFoliart 1970). *Ochlerotatus triseriatus* does not prefer the lower extremities of the human body but prefers the upper extremities, such as the arms and face (Loor and DeFoliart 1970).

A study in Iowa County, Wisconsin, showed that biting activity of the mosquito typically starts in the second half of June and increases steadily to the second half of August. It is a diurnal mosquito which attacks hosts most often in the afternoon, from the hours of 3 p.m. to 6 p.m., with low activity after 6 p.m. and little to no activity after 8 p.m. In the morning hours between 5 a.m. and 9 a.m. biting activity was moderate compared to the biting activity in late afternoon (Loor and DeFoliart 1970).

iii. Aedes albopictus

Aedes albopictus is a competent experimental vector of seven Alphaviruses: Chickungunya, eastern equine encephalitis (EEE), Mayaro, Ross River (RR), western equine encephalitis (WEE), Venezuelan equine encephalitis (VEE), and Sindbis viruses (Moore and Mitchell 1997). *Aedes albopictus* also is a competent experimental vector of the following Flaviviruses: dengue (DEN 1-4), Japanese encephalitis (JE), West Nile (WN) and Yellow Fever (YF) (Mitchell 1991). In the Bunyaviridae family, *Ae. albopictus* showed vector competence in the laboratory for JC, Keystone (KEY), Oropouche, Posti, Rift Valley fever (RVF), San Angelo (SA), and LAC, with KEY, LAC, and SA being transmitted vertically in the lab (Moore and Mitchell 1997). *Aedes albopictus* was introduced into Houston, Texas, from shipments of mosquito infected tires sent from Asia infested with the mosquito. Once in the United States it has spread from Houston, Texas to most of the southeastern United States and eastern coast in 29 states (CDC 2002, Henderson and Coleman 1971, Hawley et al. 1987, Reiter 1998). *Aedes albopictus* is not host specific, is diurnal like *Oc. triseriatus,* and shares many of the same larval habitats (Watts et al. 1974, Pantuwantana et al. 1974, Hawley 1988, Moore 1998). LAC virus has been isolated from this mosquito in the field and been shown to be transmitted transovarilly in Tennessee making it a good candidate for contributing to the increase of LAC virus transmission in Tennessee (Gerhardt et al. 2001, Erwin et al. 2002).

iv. Geographic Information Systems

Kitron et al. (1997) utilized Geographic Information Systems (GIS) and Geographic Positioning Systems (GPS) to compare areas of Illinois that had reported cases of LACE. The information on the site of each LACE case can be fused together and compared with GIS, which is a computer-based tool for solving problems that integrate information in a way that helps understand and find solutions to problems. It generates maps that reflect the real three-dimensional world in a two-dimensional environment. GIS is capable of assembling, storing, manipulating, and displaying geographically referenced information, or data identified according to their locations (ERSI 2000).

In GIS, three different types of geographic data are available: geometry, attributes, and behaviors. Geometry is the component that is the representation of geographical features associated with real world locations either in the form of a line, polygon, or point. Attributes contain the descriptive information that distinguishes a geometric shape, such as a line as being a river, road, utility line, border, etc. The behavior determines what the graphic representation of geographic feature can and cannot do when it is edited, displayed, or analyzed depending on the limitations that are placed on it. For example, a river cannot act as a road for cars (ERSI 2000).

Geographic Information Systems can use information from many different sources, in many different forms in order to help with analyses. The primary requirement for the source data is that the locations for the variables are known. Location may be annotated by x, y, and z coordinates of longitude, latitude, and elevation, ZIP codes, highway mile markers, etc. Any variable that can be located spatially can be entered into a GIS program allowing different kinds of data to be displayed in a map form (ERSI 2000). It then organizes that data and stores it as a collection of thematic layers that are linked by geography. Existing digital information that is not in a map form also can be converted into GIS format and added to a map. While a computer-aided mapping system may represent a road simply as a line, a GIS aided program can be set to also recognize that road as the border between wetland and urban development, or as the link between one street and the next (ERSI 2000).

Identities of the objects on the maps must be specified, as well as their spatial relationships. GIS links information that is difficult to associate through any other means by using combinations of mapped variables to build new variables, which can be analyzed (ERSI 2000). When one map is in a different scale from another map, the two maps can be warped which is a way to modify the maps until they match up to where the coordinates on one map are the same as the coordinates on the other map, making them geographically identical. Once that is done, the information from each map or layer can be combined, and locations can be compared in the same projection. A projection is a mathematical means of transferring information from the Earth's three-dimensional curved surface to a two-dimensional computer screen. Different projections are used for different types of maps because each projection is particularly appropriate to certain uses (ERSI 2000).

Since much of the information in a GIS comes from existing maps, a GIS uses the processing power of the computer to transform digital information, gathered from sources with different projections to a common projection. GIS can convert a satellite image map to a vector structure by generating lines around all cells with the same classification, while determining the cell spatial relationships, such as adjacency or inclusion (ERSI 2000).

Thus, a GIS can be used to analyze land use information in conjunction with property ownership information. GIS allows a person to "point" at a location, object, or area on the screen and retrieve recorded information about it from off-screen files. This gives a GIS the ability to produce graphics on the screen or on paper that convey the results of analysis, allowing the viewer to visualize and understand the results of analyses or simulations of potential events (Hurn 1993).

v. Global Positioning System

Global Positioning System (GPS) replaces the older forms of positioning, such as landmarks, dead reckoning, celestial, OMEGA, LORAN, and SatNav. Each had their own problems and is limited (Hurn 1993). Landmarks only work for the local area and are subject to change on a regular basis. Dead Reckoning is complicated and uses crude instruments and errors accumulate quickly. Celestial only works at night, in good weather, and is also complicated. OMEGA has limited accuracy and subject to radio interference. LORAN has variable accuracy, limited coverage to mostly coastal regions, and is easy to jam. SatNav is based on low-frequency Doppler measurements and is sensitive to small movements of the receiver, and has few satellites with infrequent updates (Hurn 1993).

Global Positioning System is a worldwide radio-navigation system formed from a constellation of 24 satellites and their ground stations. GPS uses the satellites as reference points to calculate positions within a couple of centimeters. Each satellite is called a NAVSTAR, weighs 861.82 kg (in orbit), is 5.18 m long with solar panels extended, lasts 7.5 years, have an orbital period of 12 hours, has an orbital plane of 55 degrees to equatorial plane, and stay at an altitude of 20186.8 km which is under the geosynchronous orbit of communication satellites (Hurn 1993).

Ground stations work in conjunction with the satellites. The ground stations job is to check the operational health and the exact position in space for each satellite. Master ground stations transmit corrections for the satellites ephemeris constants (errors caused by the atmosphere that slows the speed at which the radio waves travel giving a distance that is longer than the true distance) and clock offset back to the satellites.

Geographical Positioning Systems uses the satellites in space as reference points for locations on the earth's surface by using geometry and physics. The geometry part of GPS consists of four measurements that are needed to get an accurate reading on a location on the earth's surface. The first measurement puts the receiver on the surface of a sphere that has the satellite as the center of the sphere. The second measurement puts the location that is between where the two spheres merged. The third measurement puts the location in one of two places, in which one of the two locations is the correct position while the other position is impossible for the receiver to be located (Hurn 1993).

Three different types of GPS are available: Augmented, Carrier-Phase, and Differential GPS. Augmented GPS was developed by the aviation industry and involves the use of a geostationary satellite as a relay station for the transmission of differential corrections and GPS satellite status information. The corrections from the geostationary satellite provide corrections across an entire continent so that it can be used for instrument landings. Carrier-Phase GPS takes advantage of the GPS's carrier signal to improve accuracy. The carrier frequency is much higher than the GPS signal and can be used for more precise timing (Hurn 1993).

Differential GPS involves the use of two ground-based receivers with one monitoring variations in the GPS signal and communicates those variations to the other receiver while the second receiver can then correct its calculations for better accuracy. The satellites are so far out in space that the distances traveled on earth are insignificant. So if two receivers are within a few hundred kilometers the signals that reach both receivers will have traveled through the same slice of atmosphere and contain the same errors. One receiver in a stationary position measures the timing errors and then provides the corrections to the other receivers that are moving around in the field. Virtually all errors can be eliminated from the system. The receiver in the stationary position does not move so any change in position that it is receiving is corrected to put it back in the original position. The stationary receiver then transmits the errors to the mobile receiver so that it can correct its position. The stationary receiver, however, doesn't distinguish between satellites like the mobile receiver, so it sends separate correction for each satellite that is visible in the sky at the given time (Hurn 1993).

CHAPTER II

Habitat Comparisons of La Crosse Encephalitis to Other Central Nervous System Infections in Eastern Tennessee Utilizing Geographic Information Systems

i. Abstract

In eastern Tennessee, 17 cases of La Crosse encephalitis (LACE) were reported to the East Tennessee Department of Health in 2000. During that same time, 25 cases of other central nervous system infection (CNSI) that were not LACE also were reported. Out of those 42 cases, 11 were chosen to be revisited (6 Control and 5 LAC) and the nearby wooded habitats of all 11 sites were surveyed. Geographic Information Systems (GIS) were utilized to plot each case location on various rainfall, topographical, and vegetative maps.

All 11 sites were in Knox, Anderson, Blount, and Loudon Counties, which are located in the Appalachian Valley and Ridge Province in eastern Tennessee. Tree species and size, percent canopy cover and numbers of artificial containers were determined for each of the 10 quadrants (10 m x 10 m) established at each site. Quadrants at LAC Sites had higher proportions of hardwoods and significantly more host seeking *Aedes albopictus*. Precipitation, numbers of containers and soil categories differed between LAC and Control Sites. No arboviruses were isolated from reared and caught mosquitoes that were tested. Comparisons between these counties and Nicholas County, West Virginia, are made.

ii. Introduction

La Crosse was discovered in and named after La Crosse, Wisconsin, where the first known case was reported in 1960 (Sudia et al. 1971). Since then LAC viral infections have been reported in 29 states from Wisconsin to New York and as far south as Texas, Alabama, and Georgia (CDC 2002).

In September of 1997, an apparent cluster of LAC cases was reported by a regional children's referral hospital in eastern Tennessee (Jones et al. 1999). During the summer of 1997 the total number of cases reported for that year rose to 10 (Jones et al. 1999). The following years in 1998 there were 9 cases, in 1999 6 cases, in 2000 16 and in 2001 9 cases and 15 cases in 2002 (Gottfried 2000, Smith 2001, Stanich 2002).

The purpose of this study is to quantify any differences between areas in which children acquired La Crosse virus infection and those where other types of central nervous system infection (CNSI) were acquired. Utilizing GPS and GIS technology and mosquito surveillance, wooded areas near LAC Sites and CNSI sites reported to the 2000 East Tennessee Regional Health Department La Crosse Surveillance Study are analyzed. This study strives to duplicate many of the parameters used by Nasci et al. (2000) in Nicholas County, West Virginia, to compare and contrast these two localities. A major difference between this study and the one in West Virginia is the presence of another mosquito, *Aedes albopictus (*Skuse*),* that is an invasive species and possible vector of LAC (Moore 1998, Nasci et al. 2000, Gerhardt et al. 2001, Erwin et al. 2002, Stanich 2002).

iii. Materials and Methods

Site Selection and Setup

In the spring of 2001, addresses were obtained for children who had been enrolled in the East Tennessee Regional Health Department La Crosse Surveillance Study for 2000 (Erwin et al. 2002). The addresses for each child were plotted on a map and sites were labeled as either La Crosse Positive or negative (Control). Once placed on maps, each owner of the property where each LAC positive or Control case site occurred was asked for permission to make season-long mosquito collections on their property. Sites were then chosen based on whether or not permission was obtained. Also, the proximity of the sites to each other was considered; only one site was picked if two sites of the same status (case or control) were closer then 2 km. Twelve sites in total were chosen, six where children with confirmed LACE and six where children with similar symptoms but other infections were documented (Erwin et al. 2002). Each site was given a two or three letter designation based upon the location. The LAC positive site identifications were CCR, FHC, PL, SR, SWC, and YR, and the Control Site identifications were BL, CR, MC, LL, PRR, and WHB.

Transects of varying configurations consisting of 10 quadrants (10 m x 10 m) were placed in wooded areas near each child's house. The quadrant was the primary sampling unit within each site. The data taken at each site were number and species of mosquito eggs collected, total number and species of adults reared from eggs, total number and species of adult host-seeking mosquitoes collected, tree species, number of trees, diameter breast height (DBH) of each tree, number of tree holes below 2 m, canopy density, longitude, latitude, number of artificial containers, and elevation. Precipitation, human population, soil types, and vegetative cover were obtained from different GIS data sources (Nasci et al. 2000).

One of the LAC Sites, SWC, was dropped after the third week of the study, because it had been clear-cut, bringing the number of sites to eleven, five LAC and six Control Sites. The closest wooded area that could be used as a replacement site was less than 2 km from another LAC Site.

Egg Collection and Rearing

Two ovipostion traps with one 5 cm X 28 cm piece of folded seed germination paper in a black 473 ml stadium cup were attached to a sturdy object (i.e. tree or fence) about 0.5 m above the ground in each quadrant and filled with water (Loor and DeFoliart 1969). Each trap was assigned a number that identified the site and quadrant in which it was placed. Ovitraps were left in the quadrants for one week starting the week of June 5^{th} and continuing on alternate weeks through October 21st in 2001. The water was poured out of the cup after the germination paper had been collected to kill any larvae that may have hatched. All strips were returned to the laboratory where the eggs were identified to species and counted (one strip per quadrant). All strips were then placed into 4.3-liter sterlite containers with bovine liver powder and water and allowed to hatch for a day, after which the strips were removed from the water (Loor and DeFoliart 1969, Gottfried et al. 2002). The larvae were maintained at 26.7^{o} C and allowed to mature and pupate. Once the first few mosquitoes had pupated, liquid from the containers was transferred to Mosquito Breeding Chambers® with all of the mosquito larvae and pupae. Adults were allowed to emerge and the top portion of the breeding chamber was exchanged and placed in the freezer daily if there were pupae that had yet to eclose. Adults were placed in a Baxter ® Cryo-Fridge at –70^{o} C, so that the virus, if present, did not degrade. Adults were sexed, identified to species, and counted on BioQuip® chill tables and then placed into vials in pools no greater than 50 mosquitoes per vial for virus isolation.

Adult Mosquito Collection

A CO_2 baited Faye Omni directional Trap was placed in a random quadrant at each site for approximately 24 hours on the same schedule as ovitrap collections to collect adult host-seeking mosquitoes. Live mosquitoes collected in the Faye trap were placed into a cooler with dry ice until they could be placed into the Cyro-Fridge. The mosquitoes were separated by species and sex before being placed into vials of pools of 50 mosquitoes or less. The differences in mosquito egg and adult collections within and between sites were evaluated by performing Analysis of Variance (ANOVA) with an alpha value of 0.05 using SAS.

All pools of mosquitoes were shipped overnight in coolers with dry ice to maintain a subzero temperature inside to the Centers for Disease Control Divisions of Vector-Borne Infectious Diseases (DVBID) in Fort Collins, Colorado. Virus isolation and identification was performed on the mosquitoes to determine if they carried the LAC virus according to the methods described in Gerhardt et al. (2001).

Tree Data

The trees in each quadrant were counted and identified to species and the diameter measured at breast height (DBH). Spherical densiometer readings were taken at each site in the center of the odd numbered quadrants to estimate canopy density for each site. The treeholes below two meters in all trees were counted for each quadrant. Any artificial container that was inside the confines of the quadrants was counted if it were capable of holding water.

GPS Data Collection

A Trimble GeoExplorer 3® was used to obtain the GPS coordinates (longitude and latitude) for each site. Geopathfinder Office® was used to generate a Data Dictionary® to use in the GeoExplorer 3®. The Data Dictionary was set up to record point and polygon features. The point feature was used to record the location of each site.

The almanac of satellites for Knoxville, Tennessee, was obtained by downloading the constellations of satellites from the GeoExplorer 3® into GPS Pathfinder Office®. The application Quick Plan® that is a part of GPS Pathfinder Office® was used to analyze the elevation masks for the sites. Once the elevational masks were entered into the Quick Plan program, it then calculated which satellites were going to be available, if any. Once the satellite constellations were established, the percent dilution of precision (PDOP) was calculated according to the elevation masks, available satellites, and satellite positions. Sites were visited when the almanac indicated that PDOP for the area was 3.9% or lower to obtain accurate readings. After GPS coordinates were collected they were uploaded into GPS Pathfinder Office® and corrected using information downloaded from the nearest ground station. Once the positions were corrected they were recorded and transferred to Environmental Systems Research Institute (ERSI) ® GIS program ArcView 3.2®.

GIS Data collection

Soil maps for Tennessee were obtained from Tennessee Spatial Data Server (http://www.tngis.org/soils250k.html). Maps of human population, county, and percent area devoted to farmland were found in ERSI's® program ArcView 3.2®. Vegetative cover maps of Tennessee containing information on the size of patches of woods were obtained from GIS Data Depot (http://www.gisdatadepot.com). Precipitation Map and Physical Region Map were downloaded and warped from the Tennessee Geographic Alliance (http://web.utk.edu/~tga/) using spatial and warping extensions found in the ArcView 3.2 ® program. GPS coordinates of the sites were placed into GPS Pathfinder Office 2.80® and transferred into ArcView® along with the downloaded maps. The spatial extension add-in was used to generate grids and finished maps. The relational join feature was used to tie information from maps together for analysis. All maps were analyzed and compared using ArcView 3.2® in the NAD 1983 Zone 16 projection.

iv. Results

Mosquito Egg Data

Ochlerotatus triseriatus females in LAC Sites produced an average of 306 more eggs per collection period than the *Oc. triseriatus* females in Control Sites (Figure 2.1). The maximum number of eggs was collected during the third collection period for LAC Sites and during the fourth collection period for the Control Sites. Eggs collected at

Control and LAC Sites decreased after these peaks reaching zero in collection period eleven (Figure 2.1). No significant differences in the number of eggs collected among the sites in either LAC or Control, except that YR, a LAC Site was significantly higher than the Control Sites MC, PRR, and WHB (Figure 2.2).

Aedes albopictus females in Control Sites produced an average of 38 more eggs per collection period than the *Ae. albopictus* females in LAC Sites (Figure 2.3). The maximum number of eggs per collection period collected for the season was during collection period four in the Control Sites and period five for the LAC Sites. Eggs collected at all sites began to decrease after the maximum egg collection period, dropping to zero on collection period eleven when egg count was zero (Figure 2.3). None of the sites in either Control or LAC was significantly different nor were LAC and Control Sites as a whole significantly different for *Ae. albopictus*.

Mosquito Adult Rearing Data

An average of 99 more *Oc. triseriatus* adults emerged per collection period from the eggs collected at LAC Sites than from the Control Sites. No adults emerged from eggs collected in the third period, probably because of fungal contamination in rearing containers. Successful rearing started again with eggs from collection period four (Figure 2.5). No significant differences in the numbers of adults reared between the LAC and Control Sites or among the individual LAC and Control Sites. A total of 211 pools of *Oc. triseriatus* (117 LAC 94 Control) were sent to the CDC DVBID, but none of the virus isolation attempts was positive for LAC virus.

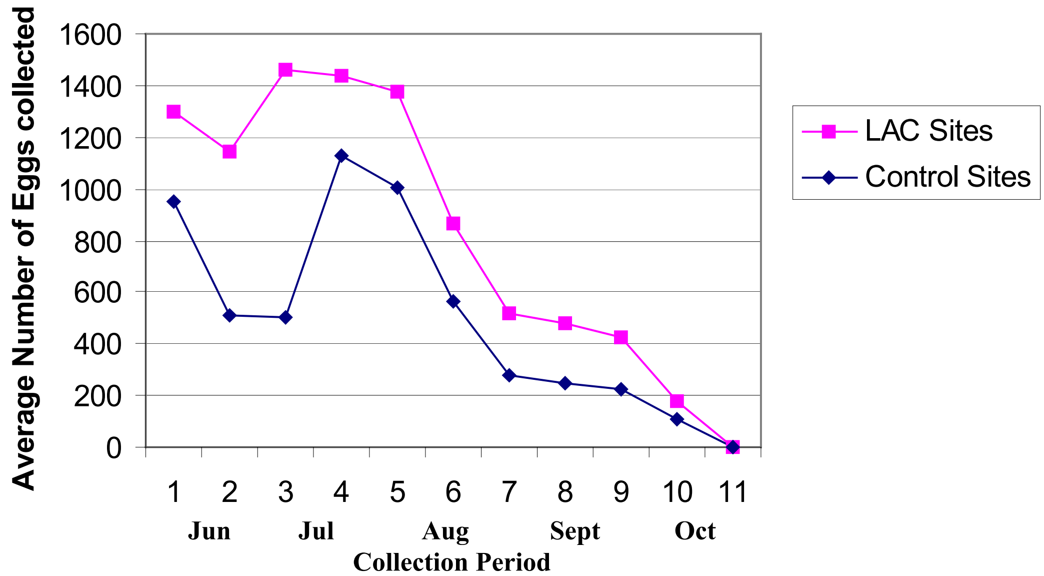

Figure 2.1: Average number of *Ochlerotatus triseriatus* eggs collected from ovipostion traps in La Crosse (LAC) and Control Sites, 2001

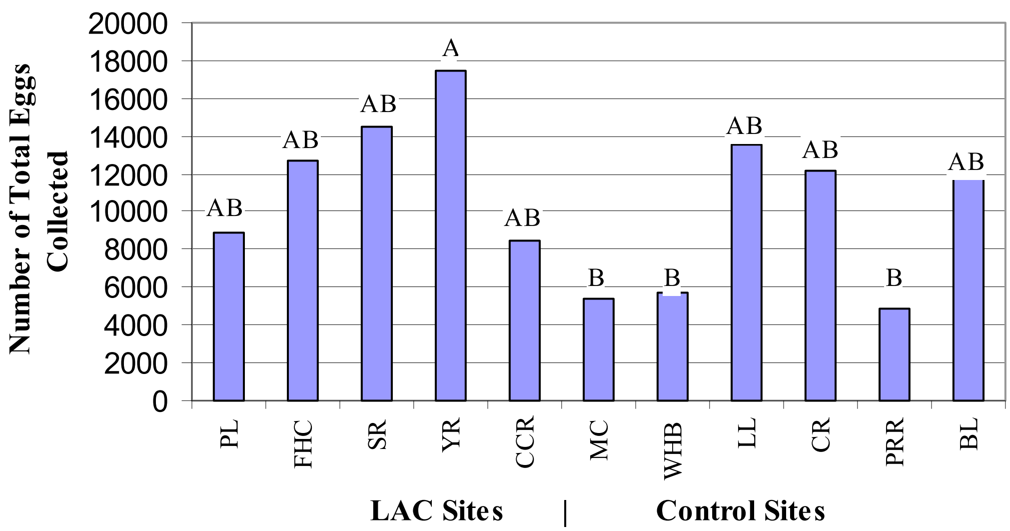

Figure 2.2 Total *Ochlerotatus triseriatus* eggs collected from oviposition traps in La Crosse (LAC) and Control Sites, 2001.

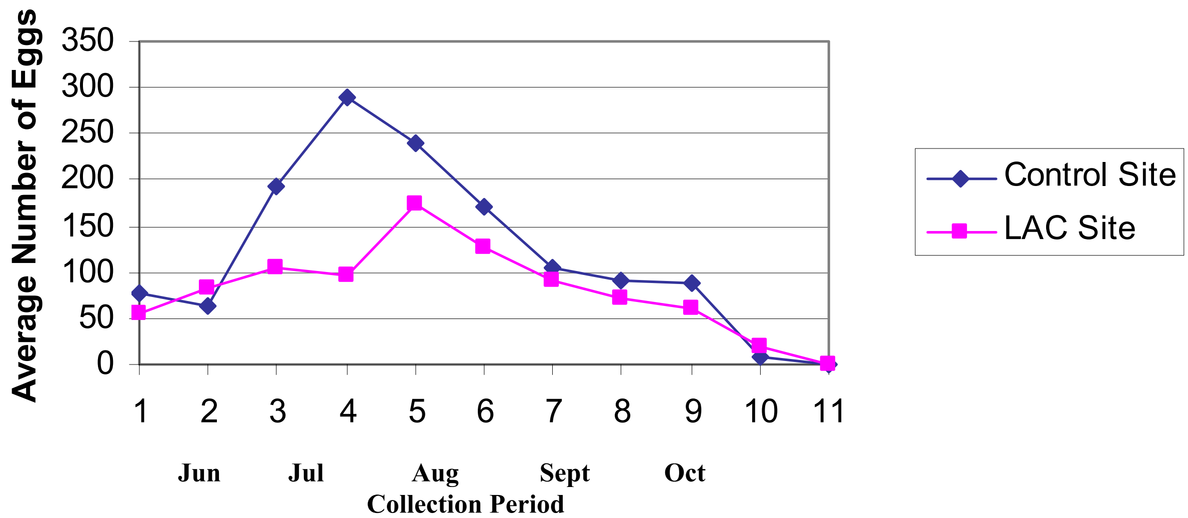

Figure 2.3: Average number of *Aedes albopictus* eggs collected from oviposition traps in La Crosse (LAC) and Control Sites, 2001

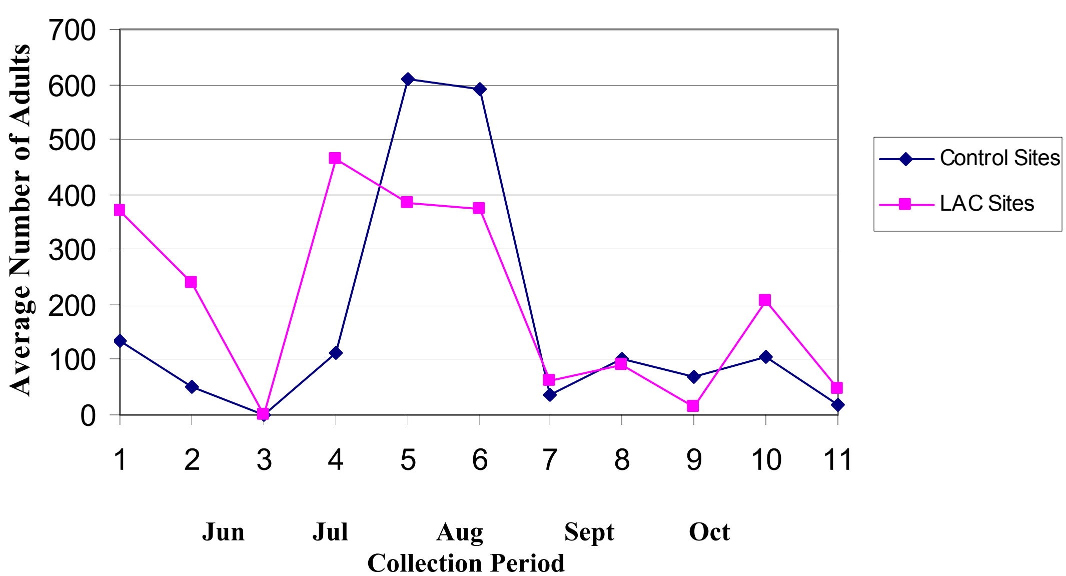

Figure 2.4: Average number of emerged *Aedes albopitus* adults from eggs collected in ovipostion traps from La Crosse (LAC) and Control Sites, 2001

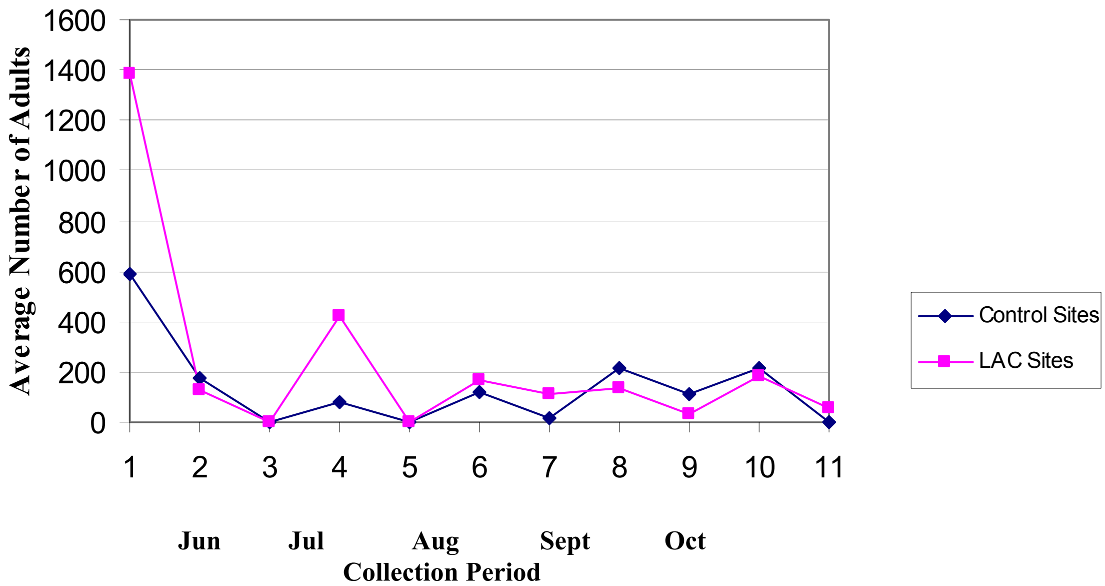

Figure 2.5: Average number of emerged *Ochlerotatus triseriatus* adults from eggs collected in ovipostional traps from LaCrosse (LAC) and Control Sites, 2001

An average of 42 more *Ae. albopictus* adults emerged per collection period from the eggs collected at Control Sites than and LAC Sites. No adults emerged from eggs collected in the third period, probably because of fungal contamination in rearing containers. Emergence started again with eggs in collection period four (Figure 2.4). There were a total of 202 (83 LAC, 119 Control) pools sent to the sent to the CDC DVBID. None of the virus isolation attempts were positive for LAC virus. No significant differences were observed in the number of adults reared from eggs between the LAC and Control Sites nor among the individual LAC and Control Sites (Figure 2.4).

Adult Collection Data

The LAC Sites had an average of 17 more *Oc. triseriatus* adults collected by the Faye Omnidirectional Traps than the Control Sites per collection period (Figure 2.6). La Crosse and Control Site collections both peaked in collection period seven. A total of 19 (11 LAC, 8 Control) pools of mosquitoes were sent to the CDC DVBID. None of the virus isolation attempts was positive for LAC virus. There were so significant differences between the LAC and Control Sites.

The LAC Sites had an average of 46 more *Ae. albopictus* adults collected from Faye Traps than the Control Sites per collection period than at LAC Sites (Figure 2.7). La Crosse and Control Site collections both peaked in collection period eight. A total of 52 (22 LAC, 30 Control) pools sent to the sent to the CDC DVBID. None of the virus isolation attempts were positive for LAC virus. There was a significant difference between the number of adults caught in LAC (mean=25.1) and Control Sites (mean=6.9), (ANOVA-F=11.21, df=1, $P<0.0029$) but not individually among LAC and Control Sites.

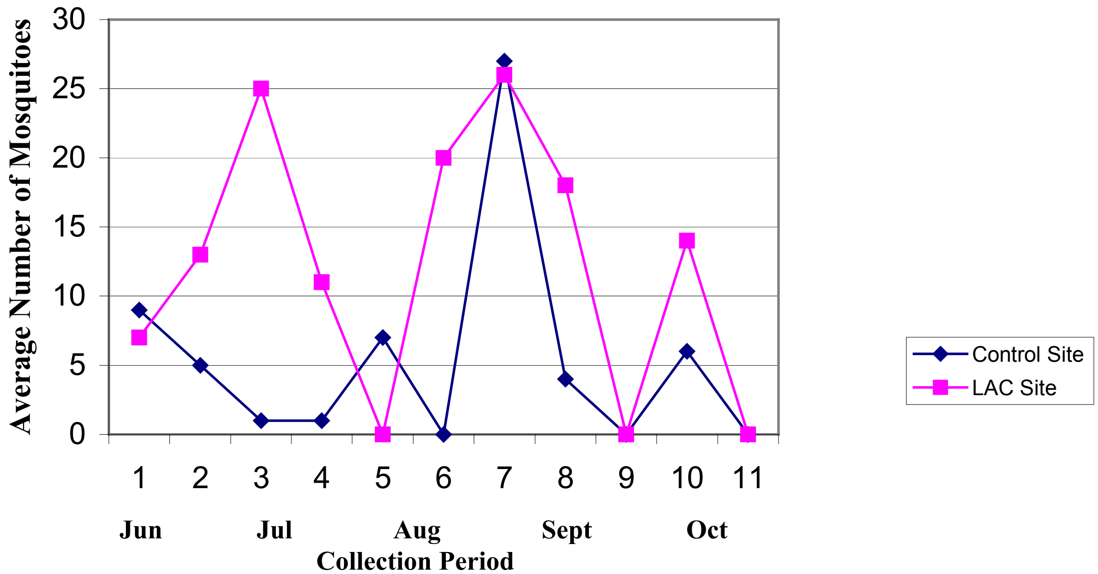

Figure 2.6: Averages of *Ochleratatus triseriatus* adults collected using Omidirectional Faye Trap in La Crossee (LAC) and Control Sites, 2001

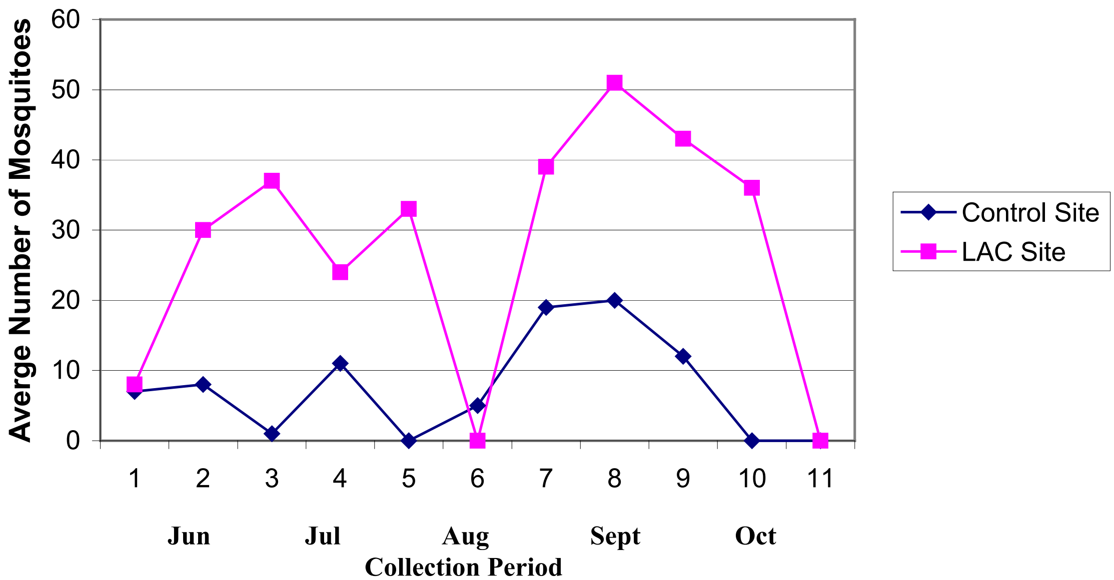

Figure 2.7: Averages of *Aedes albopictus* adults collected using Omidirectional Faye Trap in La Crosse (LAC) and Control Sites, 2001

Site Data

A total of 2,138 trees in 23 different families of 44 species were identified, measured at DBH, and tree holes below 2 m counted from all quadrants in the eleven sites. La Crosse sites contained 1,105 trees composed of 31 different species, 29 species native to Tennessee and 2 exotic species (Table 2.1). The average DBH of trees in LAC sites was 13.4 cm (Table 2.1). The most abundant species present in LAC Sites was sugar maple (*Acer rubrum (*Anther)), which accounted for 12% of the trees (Figure 2.8). An average of 4.8 treeholes and 6.4 artificial containers per site were documented (Table 2.2). Average densiometer reading for the LAC Sites was 88% canopy cover (CC). The distance from the children's homes to forested areas ranged from 0.0 to 0.02 km away (Table 2.3).

Control Sites had 1,033 trees composed of 39 different trees species, with 33 species native to Tennessee and 6 exotic species in the Control Sites (Table 2.1). The average DBH of the tree in Control Sites was 14.2 cm (Table 2.1). The most abundant tree types in Control Sites were dead and accounted for 19% of the trees (Figure 2.9). An average of 2.3

treeholes and 9.2 artificial containers per site were recorded (Table 2.2). Average densiometer reading for the Control Sites was 79% CC. The distance from the children's homes to forested areas where the quadrats were located ranged from 0.05 to 1.10 km (Table 2.3).

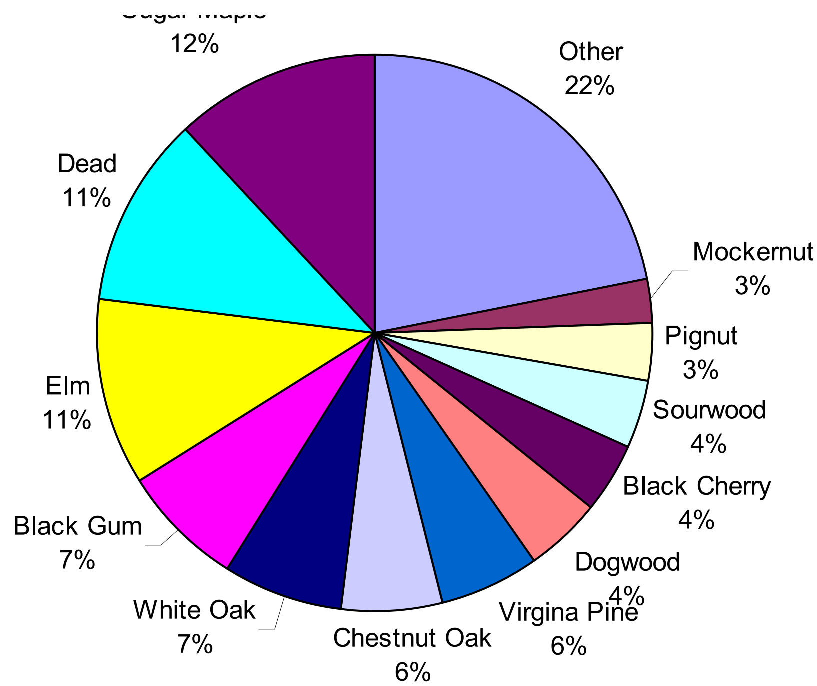

Figure 2.8: Compostion of forested areas in La Crosse (LAC) Sites by tree species

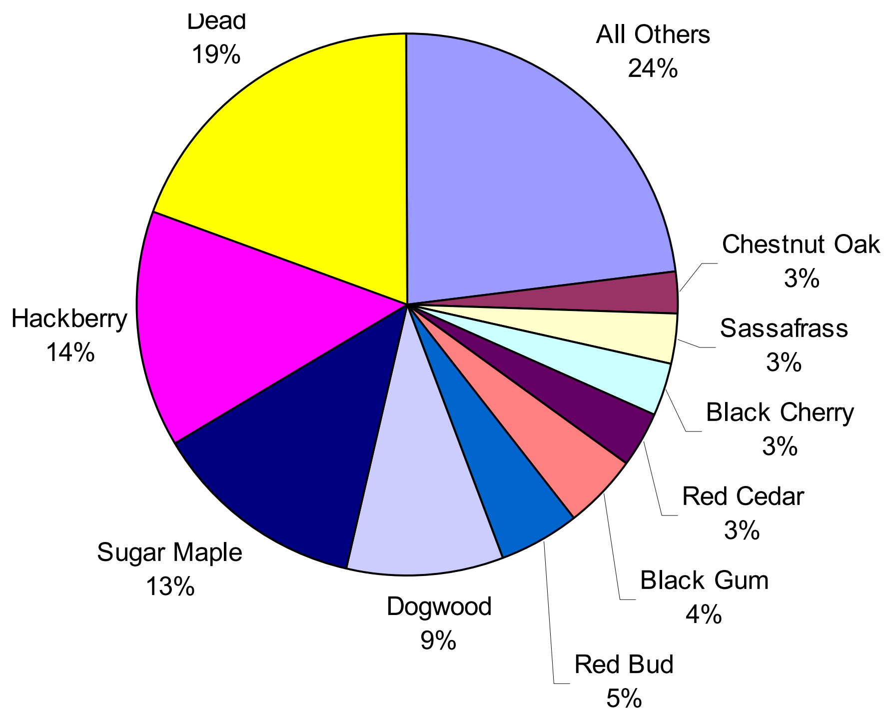

Figure 2.9: Compostion of forested areas in Control Sites by tree species

Table 2.1: Average diameter breast height (DBH) of each tree species within each La Crosse (LAC) and Control Site

	LAC Sites					Control Sites					
Tree	CCR	FHC	PL	SR	YR	BL	CR	LL	MC	PRR	WHB
American Beech	0.0	0.0	0.0	0.0	12.1	0.0	3.6	0.0	0.0	0.0	3.3
American Elm	0.0	0.0	6.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
American Red Cedar	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.1
Apple	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.0	0.0	0.0	0.0
Ash	0.0	0.0	20.6	0.0	0.0	0.0	16.1	0.0	7.2	0.0	0.0
Basswood	0.0	4.0	12.2	0.0	0.0	5.9	7.3	0.0	0.0	0.0	0.0
Beech	0.0	0.0	0.0	0.0	4.1	0.0	0.0	0.0	0.0	0.0	0.0
Black Cherry	8.5	3.3	8.1	0.0	14.6	0.0	2.1	16.9	16.8	10.7	19.2
Black Gum	0.0	10.7	0.0	10.7	6.4	5.4	0.0	9.3	13.3	4.8	0.0
Black Oak	10.3	37.6	0.0	33.1	11.6	42.2	0.0	21.0	0.0	40.5	0.0
Black Walnut	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.3	0.0	0.0
Blue Beech	0.0	0.0	0.0	3.8	0.0	7.6	0.0	4.3	0.0	0.0	0.0
Box Elder	0.0	0.0	10.1	0.0	0.0	0.0	11.0	0.0	5.2	0.0	0.0
Carolina Buckthorn	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.5	0.0	3.0
Cherry	7.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chestnut Oak	24.3	0.0	0.0	13.4	12.5	46.8	28.4	8.0	0.0	0.0	0.0
Dead	12.3	13.2	8.9	6.9	9.5	9.4	6.6	17.8	6.7	9.0	18.1
Dogwood	6.5	8.3	14.7	7.4	6.6	5.3	6.2	10.5	0.0	8.3	7.7
Eastern Red Cedar	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5
Elm	0.0	0.0	8.9	0.0	0.0	0.0	8.2	0.0	0.0	0.0	0.0
Grapevine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0
Hackberry	0.0	0.0	0.0	0.0	0.0	7.0	11.5	7.5	10.2	0.0	0.0
Honey Suckle	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.3	0.0	0.0
Mamossa	0.0	0.0	12.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mockernut	13.6	0.0	0.0	8.4	10.4	3.9	0.0	18.5	0.0	34.0	0.0
Mulberry	0.0	0.0	0.0	0.0	0.0	0.0	3.5	0.0	5.8	0.0	3.9
Northern Red Oak	45.4	32.3	0.0	0.0	24.3	45.4	40.4	0.0	0.0	54.0	0.0
Persimmon	16.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.4	0.0	0.0
Pignut	15.6	15.6	0.0	17.4	8.2	11.2	0.0	14.1	0.0	0.0	0.0
Pin Oak	41.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Plum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5	0.0	0.0	0.0
Poplar	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	41.0
Red Bud	0.0	0.0	0.0	0.0	0.0	3.0	6.2	0.0	6.2	0.0	0.0
Red Cedar	5.1	0.0	11.2	8.8	3.0	0.0	20.7	0.0	10.1	0.0	13.4
Red Elm	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0
Red Maple	7.0	5.4	8.0	12.5	0.0	2.7	0.0	0.0	0.0	0.0	0.0
Sassafras	3.2	0.0	0.0	0.0	0.0	3.0	0.0	10.4	6.6	0.0	3.4
Shagnut	0.0	6.1	0.0	0.0	0.0	33.8	0.0	18.0	0.0	0.0	27.3
Sourwood	12.5	11.7	0.0	9.3	7.7	11.5	0.0	13.5	0.0	8.3	9.5
Southern Red Oak	31.9	34.9	0.0	35.4	0.0	0.0	0.0	14.8	0.0	0.0	0.0
Sugar Maple	5.1	7.9	0.0	4.5	10.5	5.2	5.0	4.7	0.0	11.0	13.2

Table 2.1 Continued

Sumac	4.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.8	0.0	0.0
Sweet Gum	0.0	36.4	0.0	0.0	12.2	0.0	0.0	30.1	0.0	0.0	0.0
Sycamore	0.0	0.0	13.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tree of Heaven	4.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.2	0.0
Tulip poplar	5.0	4.3	0.0	24.5	0.0	0.0	0.0	19.5	0.0	36.7	24.9
Virginia Pine	22.7	0.0	0.0	27.4	23.8	0.0	33.4	19.5	0.0	34.7	0.0
White Ash	4.8	0.0	24.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
White Oak	23.9	10.5	0.0	18.8	15.2	18.2	14.0	17.9	0.0	18.8	3.1
White Pine	0.0	0.0	0.0	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Yellow birch	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0
Yellow Poplar	9.2	3.2	0.0	4.1	0.0	3.2	0.0	43.3	0.0	51.8	0.0
White Elm	0.0	0.0	0.0	0.0	0.0	0.0	4.7	0.0	0.0	0.0	0.0
Average per site	14.2	14.4	12.2	13.9	11.3	14.2	11.8	15.4	9.4	22.5	12.8
Average for Sites	13.4					14.2					

Table 2.2: Average densities of treeholes and artificial containers per site, 2001

	LAC Sites					Control Sites					
	CCR	FHC	PL	SR	YR	BL	CR	LL	MC	PRR	WHB
Number of Treeholes	2	6	5	3	8	3	2	2	5	0	2
Average Number of Treehole	4.8					2.3					
Number of Artificial Containers	8	16	6	1	1	0	0	19	4	8	24
Average Number of Containers	6.4					9.2					

Table 2.3: Distance of Collection Sites from children's homes in km, 2001

	LAC Sites					Control Sites					
	CCR	FHC	PL	SR	YR	BL	CR	LL	MC	PRR	WHB
Distance	0.00	0.00	0.02	0.00	0.00	1.10	0.60	0.4	0.06	0.05	0.30
Averages	0.00					0.42					

Table 2.4: Eastern Tennessee Counties and Nicholas County Information from ArcView 3.2

	County				
	Anderson	Blount	Knox	Loudon	Nicholas
sq km	886	1474	1357	647	673
Population	71171	102934	368503	40012	27622
Pop per sq km	200	151	641	125	41

GIS Data

GIS data for the ovitraps within quadrants could not be obtained in mid summer. The trees and foliage blocked the signal from the satellites. The canopy of all the sites was too dense to obtain any reading inside the wooded areas. The GeoExplorer 3® was tried again in October and still readings could not be obtained in the forested areas even with the majority of the foliage having fallen from the trees. Readings could be obtained from just outside the wooded areas but adjacent to the sites. Those readings were uploaded into ArcView 3.2® and soil maps showed that LAC and Control Sites fell into one of five of the general soil categories, one general soil type had only a LAC Site and two general soil categories had only Control Sites (Figure 2.10). LAC Sites occurred in soils that were moist, rich, Dewey loam, Fullerton, bodine, rock outcrops and well drained soils. Control Sites occurred in dry, abandoned, Dewey loam, Tellico, rock outcrops and eroded soils.

Slight but insignificant differences in rainfall were noticed on maps. Most LAC Sites (four out of five) had average rain fall amounts of 139.7 cm - 152.3 cm per year and one had 127.0 cm - 139.6 cm of rain per year. Control Sites (four out of six) had average rainfall amounts of 127.0 cm - 139.6 cm and two had 139.7 cm - 152.3 cm (Figure 2.11). All sites fell into the Appalachian Valley and Ridge area of Tennessee that is characterized by its numerous narrow, heavily forested parallel ridges and broad valleys that run northeast to southwest (Figure 2.12).

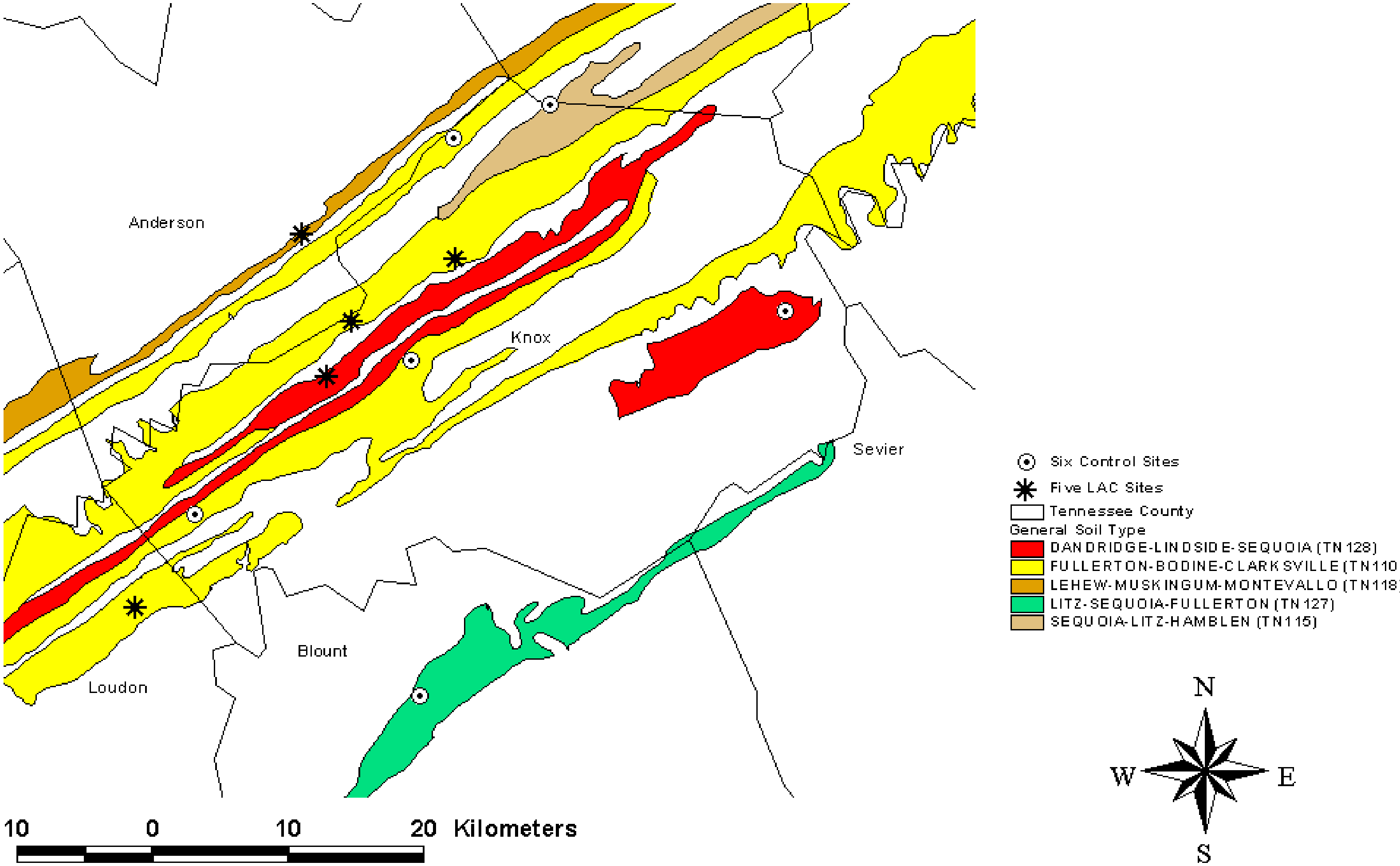

Figure 2.10: La Crosse (LAC) and Control Site locations in relation to general soil types

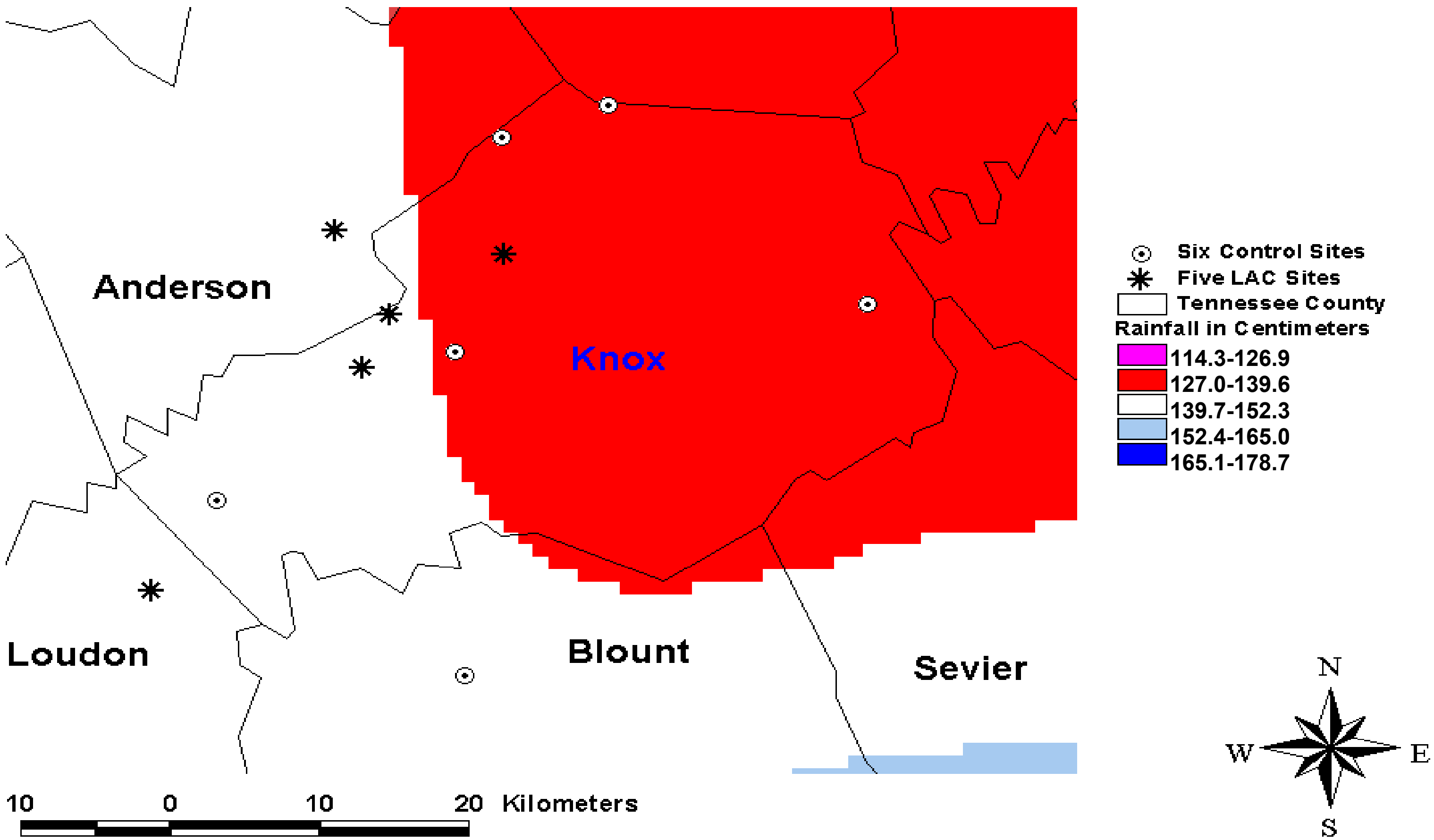

Figure 2.11: Average annual rainfall for La Crosse (LAC) and Control Sites from Tennessee Geographical Alliance (http://web.utk.edu/~tga/)

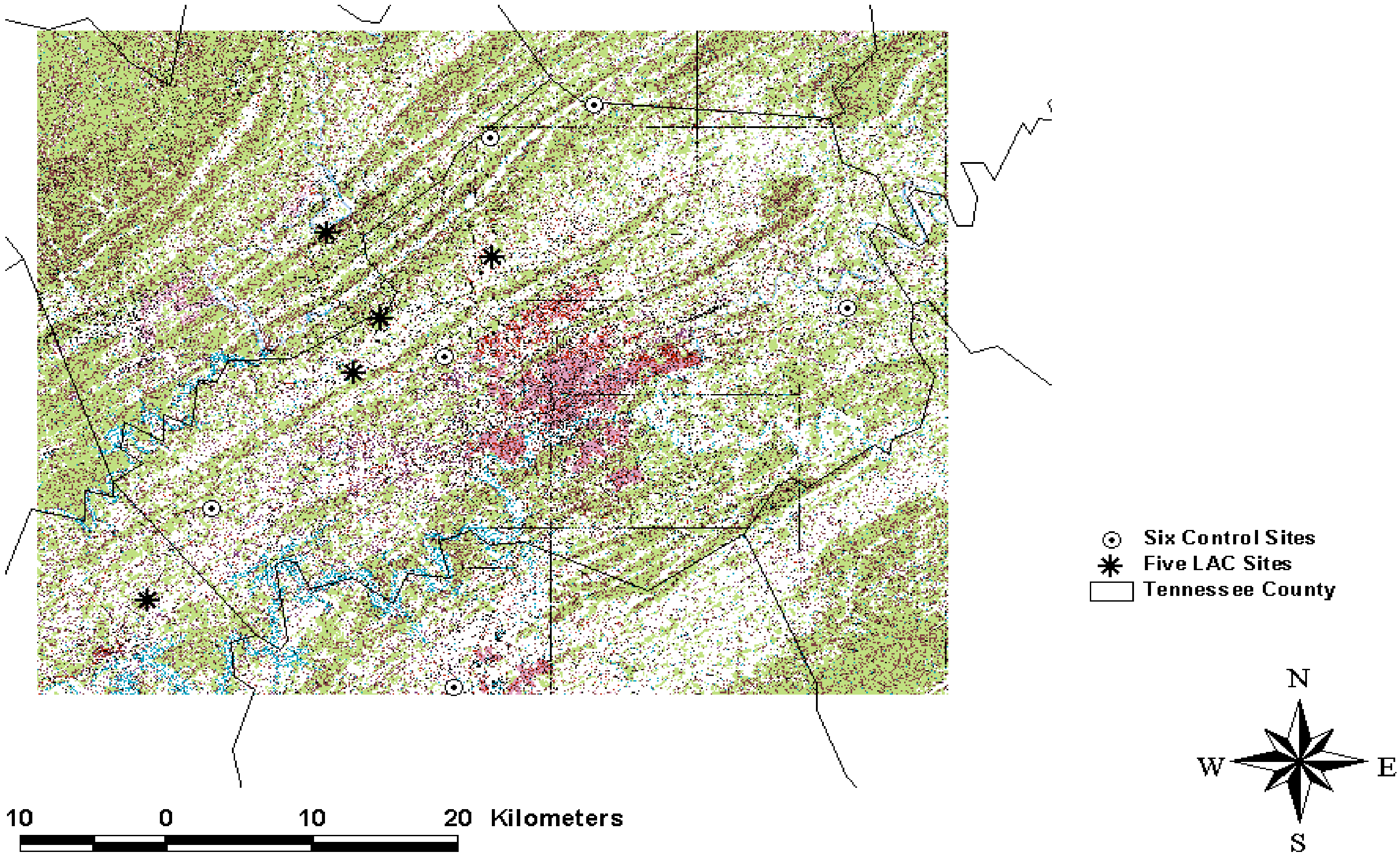

Figure 2.12: La Crosse (LAC) and Control Site locations in relation to ridges in eastern Tennessee

v. Discussion

Comparison to Nicholas County, West Virginia

Nicholas County, West Virginia, has a very large amount of uninterrupted forested land consisting of a contiguous matrix of different forest types (mixed hardwood, hemlock, and maple orchard) (Nasci et al. 2000). The only difference found in the two categories (LAC and Control) of sites in Nicholas County, West Virginia, was that the forested areas associated with LACE had higher numbers of *Oc. triseriatus* adults. The major differences between Nicholas County and the eastern Tennessee area are the habitat and human population densities. The population density of Nicholas County, West Virginia, is 41 people per sq km while, those in the counties of eastern Tennessee are higher: Knox County is 641 people per sq km, Anderson County is 200 people per sq km, Blount County is 151 people per sq. km, and Loudon County is 125 people per sq km (Table 2.4). Nicholas County is a rural area when compared to Knox, Anderson, Blount and Loudon Counties which are more urban and suburban. Nasci (et al. 2000) was able to lay out eight of his quadrants in straight lines of ten quadrants in the forest, and only two sites were limited to five quadrants in a straight line. It was not possible to do that in this study, because the areas that were used, particularly as Control Sites, were in heavily populated areas with small wood lots, unlike Nicholas County that had continuous forested areas in all sites. The quadrants in the current study area had to be laid out with the quadrants in short lines of less than 10 adjacent to each other.

La Crosse Sites in the current study area did have forested areas around the homes. They were located on edges or the numerous narrow, heavily forested parallel ridges in eastern Tennessee that run northeast to southwest (Figure 2.9). Even given this, we were unable to place the quadrants in a straight line (as in Nicholas County) in the LAC Sites with the exception YR and SR. The other three LAC Sites were too steep to safely place cups and traps for collecting eggs and adults or the wooded areas were not large enough to put quadrats in a straight line.

Mosquito Data

Ochlerotatus triseriatus was the only mosquito monitored in ovitrap and adult traps in Nicholas County (Nasci et al. 2000). *Aedes albopictus* and *Oc. triseriatus* were both found in each of the eleven sites in the Knox County area. Nicholas County and the eleven sites in Tennessee had more adult mosquitoes and eggs collected near the LAC Sites. The reason that the LAC Sites produced more eggs could be because of a trend stated by Nasci (1982) that *Oc. triseriatus* eggs represent the density of gravid females in the area. A higher density of eggs was positively correlated to more ideal habitats for *Oc. triseriatus*. The same may be true for *Ae. albopictus* since the two species of mosquito utilize many of the same habitats (Hawley 1988). Gravid *Oc. triseriatus* females search extensively for ovipositional sites and lay large numbers of eggs. In their ideal habitat, it has been found that there will be a high density of eggs throughout the area (Nasci 1982). Gravid *Oc. triseriatus* females, however, will also go outside of the wooded areas to other shaded areas in search of more ovipositinal sites if they find none in their ideal habitat.

The collection of eggs outside of the typical mixed hardwood habitat were found to be from gravid females venturing out of the mixed hardwood habitat that found an ovipositional site in a area where they normally would not oviposit (Nasci 1982). LAC Sites seemed to have a more ideal habitat for the mosquitoes because of the higher numbers of eggs and adults collected from them. By having an increased number of adults and eggs the risk for contracting LAC virus may be increased in the areas as well even though the virus isolation attempts were all negative (Woodruff et al. 1992)

Tree Data

In Nicholas County, the LAC Sites were associated with maples and other mixed hardwoods, such as oaks, that were believed to contribute to the higher populations of mosquitoes than in the control areas (Nasci et al. 2000). In the eastern Tennessee study, maples were the most abundant tree in the LAC Sites along with other hardwoods such as oaks (Figure 2.8). In contrast the Control Sites in Nicholas County were mainly hemlock forest where lower numbers of eggs were obtained. In this study the Control Sites had a higher percentage of dead trees, exotic, and Hackberry with very few if any oaks or maples (Figure 2.9).

A total of 35 species were identified in Nicholas County all of which were native species. All of the trees found in Nicholas County were also native species to eastern Tennessee. Nicholas County and the Tennessee sites shared 28 tree species. There were also eight native tree species in the eastern Tennessee study that was not found in Nicholas County sites. The shared species were typically found in hardwood forest. Eight species found in the eastern Tennessee study were exotic to the United States and not found in Nicholas County. Most of theses were found in the ecotone of the Control Sites in eastern Tennessee.

Soil Data

The soils in Nicholas County were moist, rich, and acidic soils according to the tree species that were identified (Little 2002). LAC and Control Sites within eastern Tennessee study occurred in five different soil categories in four different counties. The majority of the LAC Sites (60%) and half of the Control Sites occurred on the Fullerton-Bodine-Clarksville Soil Category. There was no difference between LAC and Control Soil Categories. Each Soil Category had a one to one ratio of Control and LAC Sites with the exception of the Litz-Sequoia-Fullerton Soil Category. The Litz-Sequoia-Fullerton Soil Category only contained a Control Site; however the soil type is capable of sustaining forested habitats like the ones in the LAC Sites.

vi. Conclusions

The two categories (LAC and Control) of sites didn't have any significant differences between the soils, precipitation, trees, and mosquitoes collected. However, if more data such as time of day and amount of time spent outside, type of clothing worn, play area of children, location of play area, time spent in adjacent wooded areas, lotions and insecticide worn, and densities of mosquitoes in varying distances from the home may result in significant differences between the two categories of sites.

In the Control Sites the wooded areas were in close proximity to the living areas. The LAC Sites contained the ecotone between grass yard and woodlot within the boundaries of the backyard and was more likely to be played in by the children unlike the Control Sites. In two of the Control Sites there were barriers that may have prevented the children from going into the wooded areas adjacent to their homes. For example, in the lot next to the home in WHB there was construction site for a new house between the residence and the wooded area. The construction site may have served as a barrier keeping the children outside of the wooded area and may have decreased or eliminated the risk of contracting a LAC viral infection. However the mosquitoes may venture out of the wooded area and into the living area of the children. Ovitrap and adult mosquito traps placed at certain distances from the home and into the wooded area could indicate the density in which the mosquitoes are traveling out of the wooded area.

There is still much to learn about this disease, such as, how it is transmitted and what factors increase and decrease risk of contracting it. Many variables seem to come into play on a person's susceptibility to the disease from their daily activity to each person individual health and DNA. This study touched on some of those variables but not all of them. Further work needs to be done covering different areas to better understand the epidemiology of this disease.

CHAPTER III

Geographic Distribution of La Crosse Encephalitis in Eastern Tennessee

i. Abstract

In eastern Tennessee, 24 cases of LACE were reported to the East Tennessee Department of Health from 1999-2001. During that same time, 78 cases of other Central Nervous System Infections (CNSI) that were not LAC also were reported. Geographic Information Systems was utilized to plot each case location on various maps. Case site locations were divided into LAC Sites for LAC cases and Control Sites for CNSI cases that were not due to LAC.

All sites were in eastern Tennessee and were categorized into one of the four physical regions of eastern Tennessee: Blue Ridge, Appalachian Valley and Ridge, Cumberland Plateau, and Eastern Highland Rim. There were no difference in amount of precipitation that the LAC and Control Sites received.

High LAC Area and Low LAC Areas were noted and included parts of four counties. The High LAC Area had a similar incidence as the Low LAC Area for CNSI but the High LAC Area had 12 cases of LAC while the Low LAC Area had none. The difference between the two areas was the case site proximity to larges wooded areas. In all counties except Hamblen County, LAC Sites were found to be in close proximity to large wooded areas unlike the Control Sites that occurred whether or not they were in close proximity to large wooded areas.

ii. Introduction

La Crosse (LAC) virus was discovered in and named after La Crosse, Wisconsin, where the first known case was reported in 1960 (Sudia et al. 1971). Since then, it has been reported in 29 states from Wisconsin to New York and as far south as Texas, Alabama, and Georgia (CDC 2002).

The majority of La Crosse encephalitis (LACE) cases occurred in the upper Midwestern United States, during the 1960s and 1970s with most cases reported from Illinois, Indiana, Iowa, Minnesota, Ohio, and Wisconsin (Henderson and Coleman 1971). Reported cases in West Virginia increased during the 1990s, and by 1997 West Virginia accounted for more than half of the nation's known cases of LACE (Nasci et al. 2000).

La Crosse virus was first detected in 1964, and only nine cases of LACE were reported in eastern Tennessee from 1964 to 1996. However, by 1997, the number of cases of LACE reported in Tennessee went from nine to 19, with 10 new LACE cases being reported. Then in 1998 there were nine cases with six in 1999, 16 in 2000, nine in 2001, and 15 in 2002 (Jones et al. 1999 Gottfried 2000, Smith 2001 Stanich 2002).

La Crosse Virus is historically known to occur in areas near hardwood forests that contain *Ochlerotatus triseriatus* (Say) and small mammals, such as chipmunks, squirrels, and rabbits (Loor and DeFoliart 1970, Kitron et al. 1997, Henderson and Coleman 1971). When humans enter into this habitat they become incidental hosts for LAC virus and interrupt the normal vector-host relationship.

Enzootic transmission of viruses have foci from which they seem to be transmitted within certain areas dictated by the location of vectors, host species and human invasion of an area (Clark et al. 1982)   A cluster of cases in Illinois was in close proximity to forested areas with numerous artificial containers and a tire dump site in which *Oc. triseriatus* were found to be present (Kitron et al. 1997).

Rural residents of southeastern Minnesota had a significantly higher prevalence of antibodies to LAC virus than people in urban areas (Monath et al. 1970). The number of people with antibodies to LACE was significantly higher than the number of reported cases of a viral infection because symptoms are typically mild or sub clinical (Chun et al. 1968). Due to the higher amount of time that is spent in the natural habitat, people who live near wooded areas have a higher chance of exposure to the virus than those that live in cities. The people in cities are further removed from the native habitat and encounter the mosquito-small mammal cycle of the arbovirus less frequently (Monath et al. 1970).

The purpose of this study was to determine the relative risk factors of contracting LAC encephalitis among children of eastern Tennessee using Geographic Information Systems (GIS) by comparing the geographic characteristics of homes of children who had contracted LAC encephalitis, with those of children who had contracted other CNSI.

iii. Materials and Methods

In the summers of 1999, 2000, and 2001, children from six months of age or older that had a physician-diagnosed febrile CNSI, had a lumbar puncture performed, and had no evidence of a bacterial CNSI or other documented disease to explain their illness were enrolled into the East Tennessee Regional Health Department La Crosse Surveillance Study. After parental permission was obtained, each child's home was designated as either a La Crosse Site or Control Site based on a four-fold increase in LAC antibodies and Polymerase Chain Reaction (PCR) tests (Erwin et al. 2002, Gottfried 2000, Smith 2001, Stanich 2002).

Latitude, longitude, and elevation of each site was obtained using a Garmin GPS 12 CX® in 1999 and 2000 and a Trimble GeoExplorer 3® in 2001 (Erwin et al. 2002, Gottfried 2000, Smith 2001, Stanich 2002). Each location was entered into ERSI's ArcView® 3.2. Elevational maps were made using the Surfacing feature in ArcView® to create an elevation map.

All sites were in eastern Tennessee and were categorized into one of the four physical regions of eastern Tennessee: Blue Ridge, Appalachian Valley and Ridge, Cumberland Plateau, and Eastern Highland Rim. The Blue Ridge area borders the entire eastern edge of Tennessee and is characterized by several mountain ranges with an average elevation of 1,500 m. The Unika Range defines the eastern edge of the Blue Ridge, and the western border is defined by the Chilhowee Range (Moore 1994).

The Appalachian Valley and Ridge is part of a larger province that stretches from the Gulf Coastal Plain of Alabama to the Hudson Valley. It consists of numerous narrow, heavily forested parallel ridges and broad valleys that generally run northeast to southwest. In Tennessee the region is about 322 km long and 72 km wide. The area is noted for its fertile farmland, and is the best place for agriculture in eastern Tennessee (Moore 1994).

The Cumberland Plateau is the southern part of the Appalachian Plateau. It is a high tableland composed of sedimentary rock that has an average elevation of close to 610 meters. High mountains break the northern extent of the plateau, while the southern two-thirds is nearly level to rolling. The Sequatchie Valley is a long linear valley that is carved into the Cumberland Plateau (Moore 1994).

The Highland Rim is divided by the Central basin into eastern and western halves. The Eastern Highland Rim is a karst area composed of elevated plains with steep slopes, underground caverns, and streams throughout, flat to slightly rolling terrain because the limestone strata underlying the area are almost flat or dip toward the east (Moore 1994).

Maps of eastern Tennessee counties were contained within the ArcView® program and had been updated in 1999. Information found within the maps included population of children ages 0-17 years of age, population of all ages, highways, area, population per square km, farms, and amount of land used for agriculture.

Soil maps were downloaded from the Tennessee Spatial Data Server (http://www.tngis.org/soils250k.html) that gave the general description of soil types as described by the National Resources Conservation Service (NRCS). Vegetative cover maps obtained from the United States Department of Agriculture (USDA) were downloaded from GIS Data Depot (http://www.terraserver.com/). Precipitation Map and Physical Region Map were downloaded and warped from the Tennessee Geographic Alliance (http://web.utk.edu/~tga/).

All maps were viewed in Universal Transverse Mercator (UTM) 1983 Zone 16 and 17 projections so that the coordinates of the maps would line up with one another. The combined maps were examined to determine the differences and similarities among the La Crosse Sites and the Control Sites.

iv. Results

The distribution of the 24 LAC Sites (4 1999, 12 2000, 8 2001) and 78 Control Sites (11 1999, 19 2000, 48 2001), within thirteen counties in eastern Tennessee that had at least one child entered into the case comparison study in 1999-2001 is presented in Figure 1. All 35 of the reported LAC cases in eastern Tennessee were not included in the three years for various reasons such as permission not being given or cases were received too late in the season. The number and case rates of LACE and other CNSI, population of children 0-17 years of age, and area by county are presented in Table 3.1. The combined three year LACE Case rate for the 13 counties was 12.8 children per 100,000 children and overall CNSI Case rate (excluding LACE Cases) was 31.5 children per 100,000 children. Blount, Jefferson, Monroe, and Morgan counties had only Control Sites, while Cocke and Hamblen counties had only LAC Sites and no Control Sites. The remaining counties had at least one LAC and one Control Site (Table 3.1).

The distribution of all cases by one of the five phsyiogeographic regions is presented in Figure 3.2. The Valley and Ridge area contained 93 % of the Control Sites and 75% of the LAC Sites and had the highest LACE (1.7 per 100,000 people) and CNSI (6.3 per 100,000 people) infection rate and the second highest population density of 72.5 people per sq. km. The Blue Ridge area had the highest population density with 79.1 people per sq km., however it has the lowest population and smallest area of the four regions with a LACE infection rate (0.5 per 100,000 people) of all the areas.

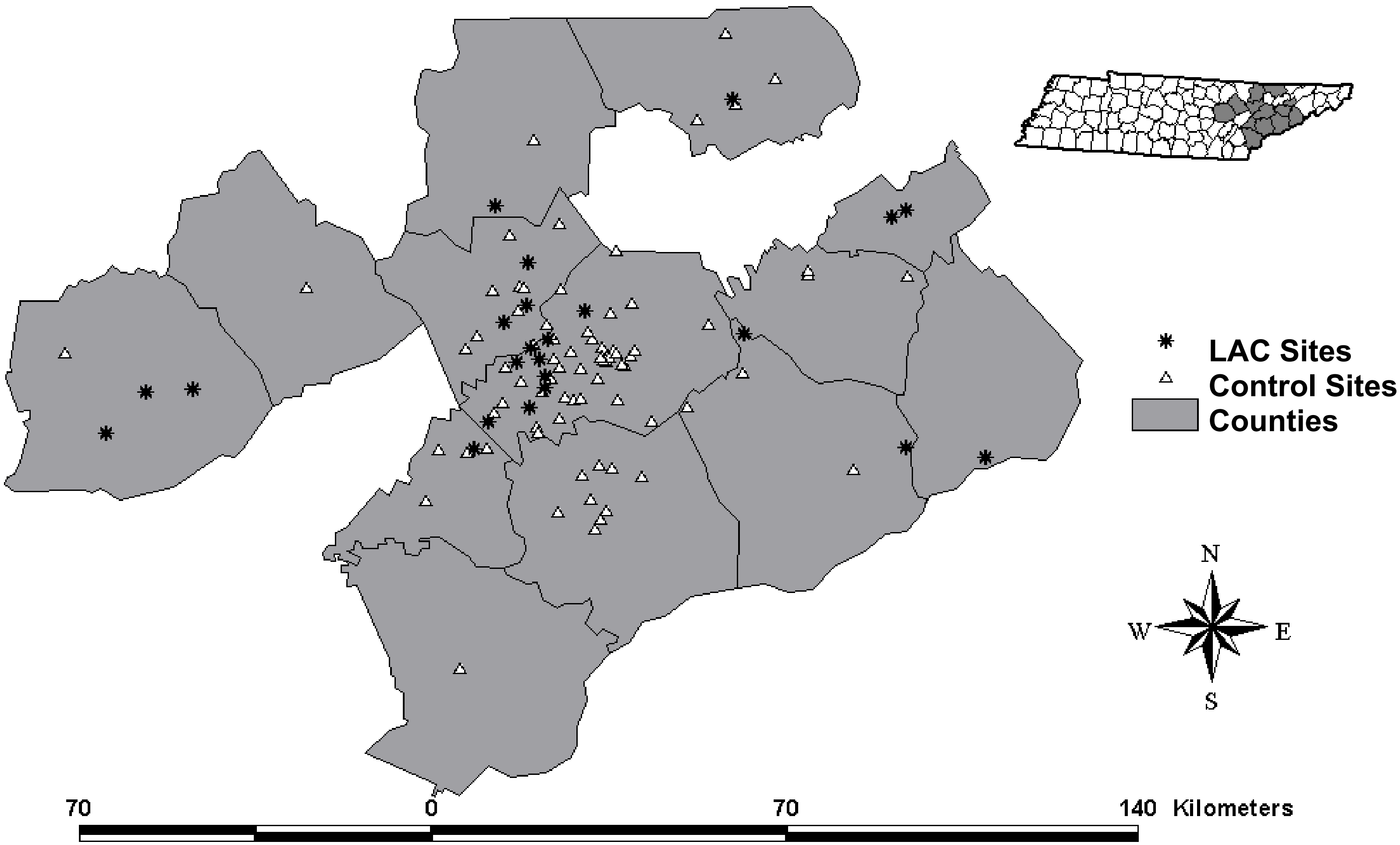

Figure 3.1: Control and LAC Site Location in eastern Tennessee for years 1999-2001 from ArcView county data

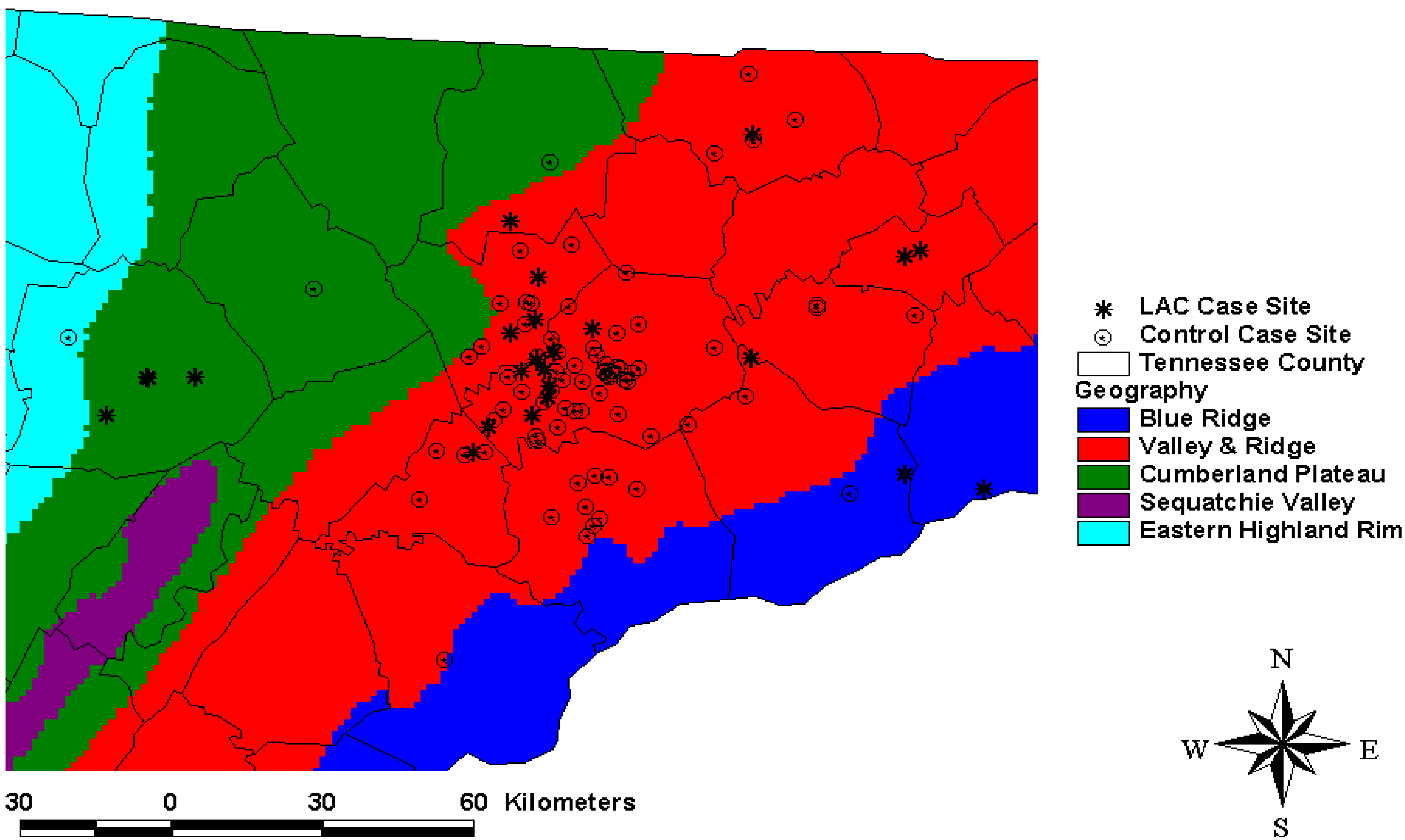

Figure 3.2: 1999-2001 LAC and Control Site location according to geographic regions from Tennessee Geographic Alliance Image (http://web.utk.edu/~tga/) warped with county map

Table 3.1: Number of La Crosse Sites, number of Control Sites, area, total number of children age 0-17 years of age, La Crosse and Central Nervous System Infection case rate from counties who had a child entered into La Crosse Surveillance Study in the years 1999-2001

County Name	# LAC	# Control	Area Sq. Km	Pop. Age 0-17	LAC Case Per 100,000*	LAC Case Per 100,000+	Other CNSI Case Per 100,000*	Other CNSI Case Per 100,000+
Anderson	3	9	886	16334	18.4	4.2	55.1	12.6
Blount	0	9	1474	19662	0.0	0.0	45.8	8.7
Campbell	1	1	1285	9003	11.1	2.6	11.1	2.6
Claiborne	1	5	1178	6668	15.0	3.3	75.0	16.7
Cocke	1	0	1178	6984	14.3	3.1	0.0	0.0
Cumberland	4	1	1766	8121	49.3	8.8	12.3	2.2
Hamblen	2	0	451	12082	16.6	3.7	0.0	0.0
Jefferson	0	3	813	7238	0.0	0.0	41.4	6.6
Knox	9	41	1357	75112	12.0	2.5	54.6	11.1
Loudon	1	4	647	7332	13.6	2.5	54.6	10.0
Monroe	0	1	1717	7731	0.0	0.0	12.9	2.8
Morgan	0	1	1339	4462	0.0	0.0	22.4	5.3
Sevier	2	3	1567	12209	16.3	3.0	24.6	4.5
Total	24	78	15658	192938	166.6	33.7	409.8	83.1
Averages	2	6	1204	14841	12.8	2.6	31.5	6.4

* Among only children age 0-17 years of age
+ Among all age groups

Table 3.2: Soil categories of Control and La Crosse (LAC) Sites as described by the National Resources Conservation Service (NRCS) in each county reporting a case of CNSI or LACE infection from the years 1999-2001

County	Soil Categories-Control	Soil Categories-LACE
Anderson	ARMUCHEE-COLLEGEDALE-MONTEVALLO (TN155), DANDRIDGE-LINDSIDE-SEQUOIA (TN128), FULLERTON-BODINE-CLARKSVILLE (TN110), COLLEGEDALE-DUNMORE-ETOWAH (TN119), WAYNESBORO-ETOWAH-HOLSTON (TN156)	ARMUCHEE-COLLEGEDALE-MONTEVALLO (TN155), FULLERTON-BODINE-CLARKSVILLE (TN110), LEHEW-MUSKINGUM-MONTEVALLO (TN118)
Blount	DUNMORE-FULLERTON-GREENDALE (TN134), LITZ-SEQUOIA-FULLERTON (TN127), DANDRIDGE-LINDSIDE-SEQUOIA (TN128), CUMBERLAND-ETOWAH-TALBOTT (TN132), DECATUR-DEWEY-EMORY (TN121)	Not Applicable
Campbell	FULLERTON-BODINE-CLARKSVILLE (TN110)	FULLERTON-BODINE-CLARKSVILLE (TN110)
Claiborne	FULLERTON-BODINE-CLARKSVILLE (TN110),TALBOTT-ROCK OUTCROP-COLBERT (TN131)	FULLERTON-BODINE-CLARKSVILLE (TN110)
Cocke	Not Applicable	JUNALUSKA-SPIVEY-TSALI (TN140)
Cumberland	LILY-MUSKINGUM-RAMSEY (TN098)	CANEYVILLE-BOULDIN-RIGLEY (TN095), LILY-MUSKINGUM-RAMSEY (TN098)
Hamblen	Not Applicable	FULLERTON-BODINE-CLARKSVILLE (TN110)
Jefferson	DUNMORE-FULLERTON-GREENDALE (TN134)	Not Applicable
Knox	ARMUCHEE-COLLEGEDALE-MONTEVALLO (TN155), DANDRIDGE-LINDSIDE-SEQUOIA (TN128),DECATUR-DEWEY-EMORY (TN121), FULLERTON-BODINE-CLARKSVILLE (TN110), LEHEW-MUSKINGUM-MONTEVALLO (TN118), SEQUOIA-LITZ-HAMBLEN (TN115), SEQUOIA-LITZ-HAMBLEN (TN115)	DANDRIDGE-LINDSIDE-SEQUOIA (TN128), DECATUR-DEWEY-EMORY (TN121), FULLERTON-BODINE-CLARKSVILLE (TN110)
Loudon	DANDRIDGE-LINDSIDE-SEQUOIA (TN128), CUMBERLAND-PEMBROKE-ALLEN (TN101), DECATUR-DEWEY-EMORY (TN121), FULLERTON-BODINE-CLARKSVILLE (TN110)	FULLERTON-BODINE-CLARKSVILLE (TN110)
Monroe	TELLICO-CHRISTIAN-ALCOA (TN120)	Not Applicable
Morgan	KIMPER-SHELOCTA-HAZLETON (TN160)	Not Applicable
Sevier	RAMSEY-ROCK OUTCROP-BARBOURVILLE (TN139), DUNMORE-FULLERTON-GREENDALE (TN134), LITZ-SEQUOIA-FULLERTON (TN127)	RAMSEY-ROCK OUTCROP-BARBOURVILLE (TN139), DUNMORE-FULLERTON-GREENDALE (TN134)

Table 3.3: Number of La Crosse (LAC) and Control Sites from 1999-2001 per soil category as described by the National Resources Conservation Service (NRCS) for eastern Tennessee.

Soil Type	LAC	Control
ARMUCHEE-COLLEGEDALE-MONTEVALLO (TN155)	1	4
DANDRIDGE-LINDSIDE-SEQUOIA (TN128)	1	13
FULLERTON-BODINE-CLARKSVILLE (TN110)	12	28
COLLEGEDALE-DUNMORE-ETOWAH (TN119)	0	3
WAYNESBORO-ETOWAH-HOLSTON (TN156)	0	1
LEHEW-MUSKINGUM-MONTEVALLO (TN118)	1	3
DUNMORE-FULLERTON-GREENDALE (TN134)	1	5
LITZ-SEQUOIA-FULLERTON (TN127)	0	3
CUMBERLAND-ETOWAH-TALBOTT (TN132),	0	1
TALBOTT-ROCK OUTCROP-COLBERT (TN131)	0	1
LILY-MUSKINGUM-RAMSEY (TN098)	3	1
DECATUR-DEWEY-EMORY (TN121)	2	6
SEQUOIA-LITZ-HAMBLEN (TN115),	0	1
CUMBERLAND-PEMBROKE-ALLEN (TN101)	0	1
TELLICO-CHRISTIAN-ALCOA (TN120)	0	5
KIMPER-SHELOCTA-HAZLETON (TN160)	0	1
RAMSEY-ROCK OUTCROP-BARBOURVILLE (TN139)	1	1
JUNALUSKA-SPIVEY-TSALI (TN140)	1	0
CANEYVILLE-BOULDIN-RIGLEY (TN095	1	0
Total	24	78

In the 13 counties where the LAC and Control Sites occurred, 19 different soil categories were found (Table 3.2). The sites fell into one of the 19 general soil categories, 17 for Control Sites and 10 for the LAC Sites (Table 3.3). La Crosse (50%) and Control (36%) Sites most often occurred in the Fullerton-Bodine-Clarksville soil category (Table 3.3).

Anderson, Blount, Knox, and Loudon County

Anderson, Blount, Loudon and Knox Counties had as many or more Control (64%) and LAC Sites (50%) as all of the other counties combined. Anderson, Blount, Knox, and Loudon Counties had 61.4% of the total population of children in the 13 county study area. Areas of eastern Anderson, western Knox, and northwestern Loudon counties (Figure 3.3), with a combined area of 521.6 square km contained 50% of the LAC Sites and 23% of the Control Sites and was designated as a High LAC Area. Other portions of Knox and Blount Counties with a combined area of 556.3 square km that contained 41% of the Control Sites but no LAC Sites were designated as a Low LAC Area. The High LAC Area had a higher rate of LACE between the two areas with 10.9 cases of LAC virus per 100,000 people of all ages and a CNSI rate of 14.6 cases per 100,000 people of all ages. The Low LAC Area had a zero LAC case rate and a CNSI rate of 14.0 control cases per 100,000 people (Table 3.4).

The High LAC Area consists of three valleys between forested ridges that are the largest unbroken blocks of hardwood forest in the area. The Low LAC Areas are in a heavily populated area in a valley (Figure 3.3). The Control and LAC Sites in the High LAC Area were less than 0.5 km from the wooded ridges (Figure 3.3) (Table 3.3). The Low LAC

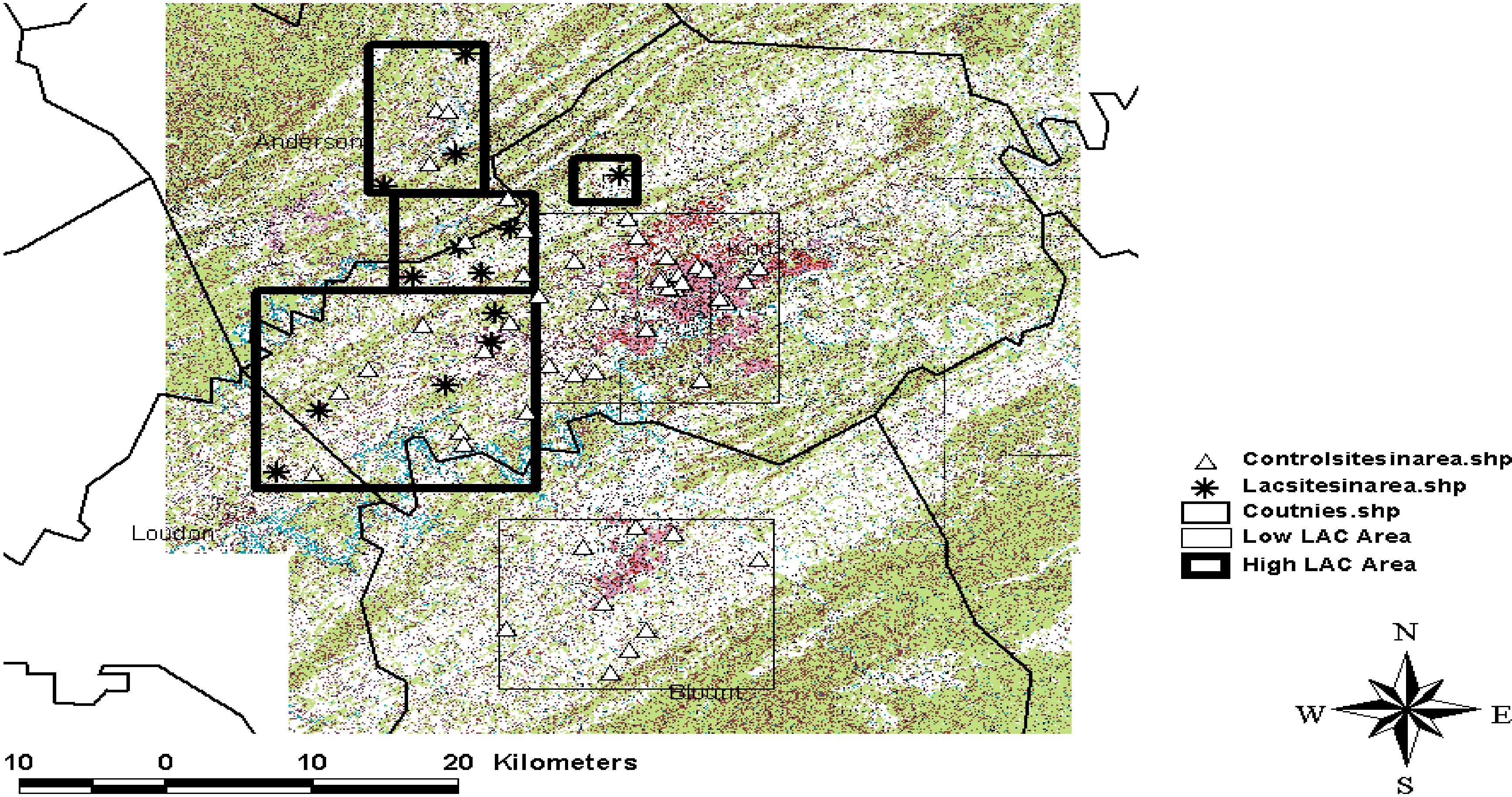

Figure 3.3: High and Low LAC Area Comparisons in eastern Tennessee for the years 1999-2001 using DRG from USDA (http://terraserver.homeadvisor.msn.com) added to county map from ArcView 3.2

Table 3.4: Number of La Crosse Cases, number of Control Cases, area, population, population of area per square kilometer, La Crosse and Central Nervous System Infection incidence from low and high LAC areas who had a child entered into La Crosse Surveillance Study in the years 1999-2001

LAC Area	# LAC	#Control	AREA Sq. km	POP/ Sq. km	Population	LAC Case per 100,000+	Other CNSI Case Per 100,000+
High	12	16	521.63	209	109435	10.9	14.6
Low	0	31	556.31	290	221937	0.0	14.0

+ Among all age groups

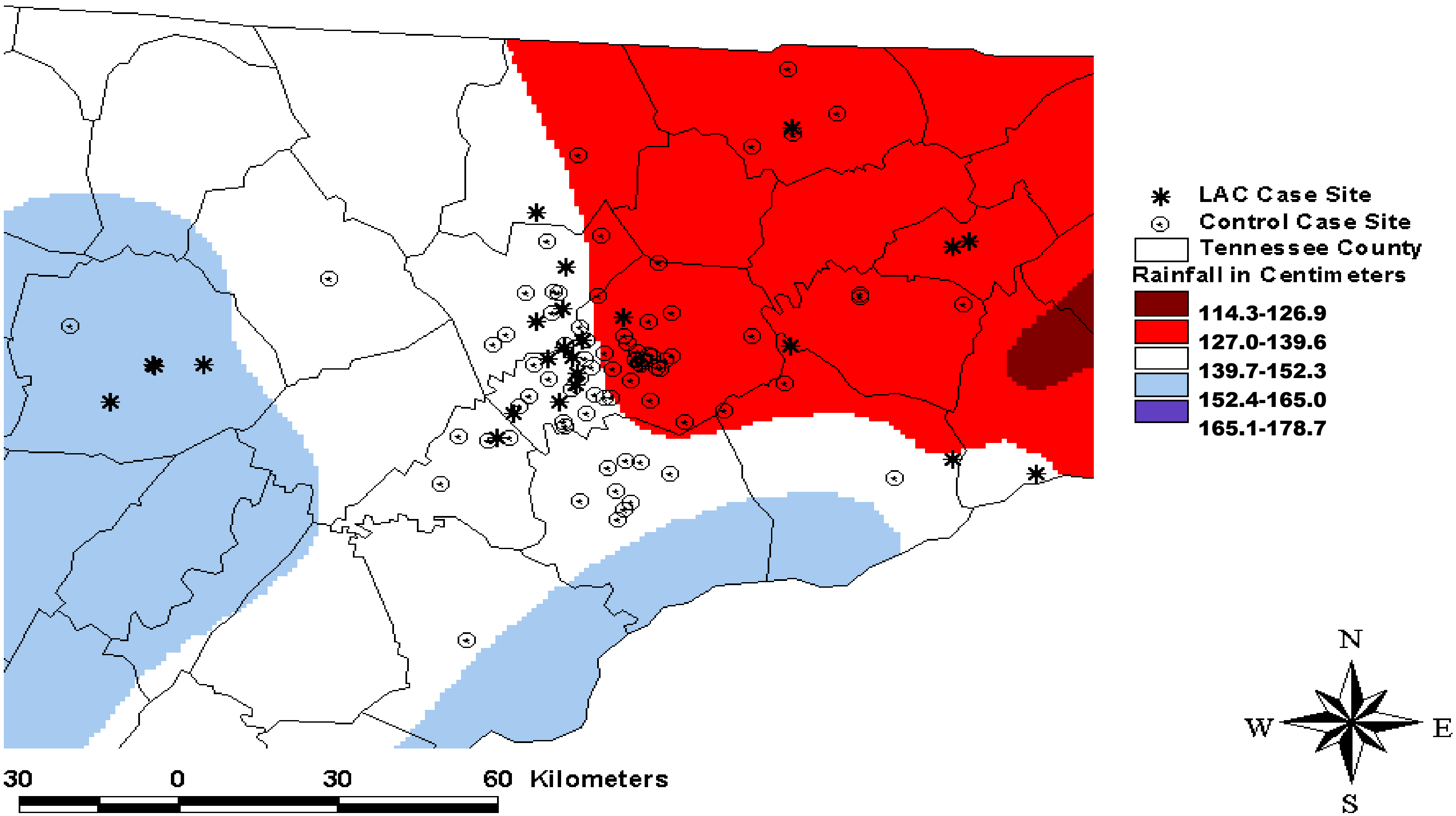

Figure 3.4: Annual Rainfall in centimeters of each site in eastern Tennessee according to annual precipitation map from the Tennessee Geographic Alliance (http://web.utk.edu/~tga/) warped with county map from ArcView 3.2

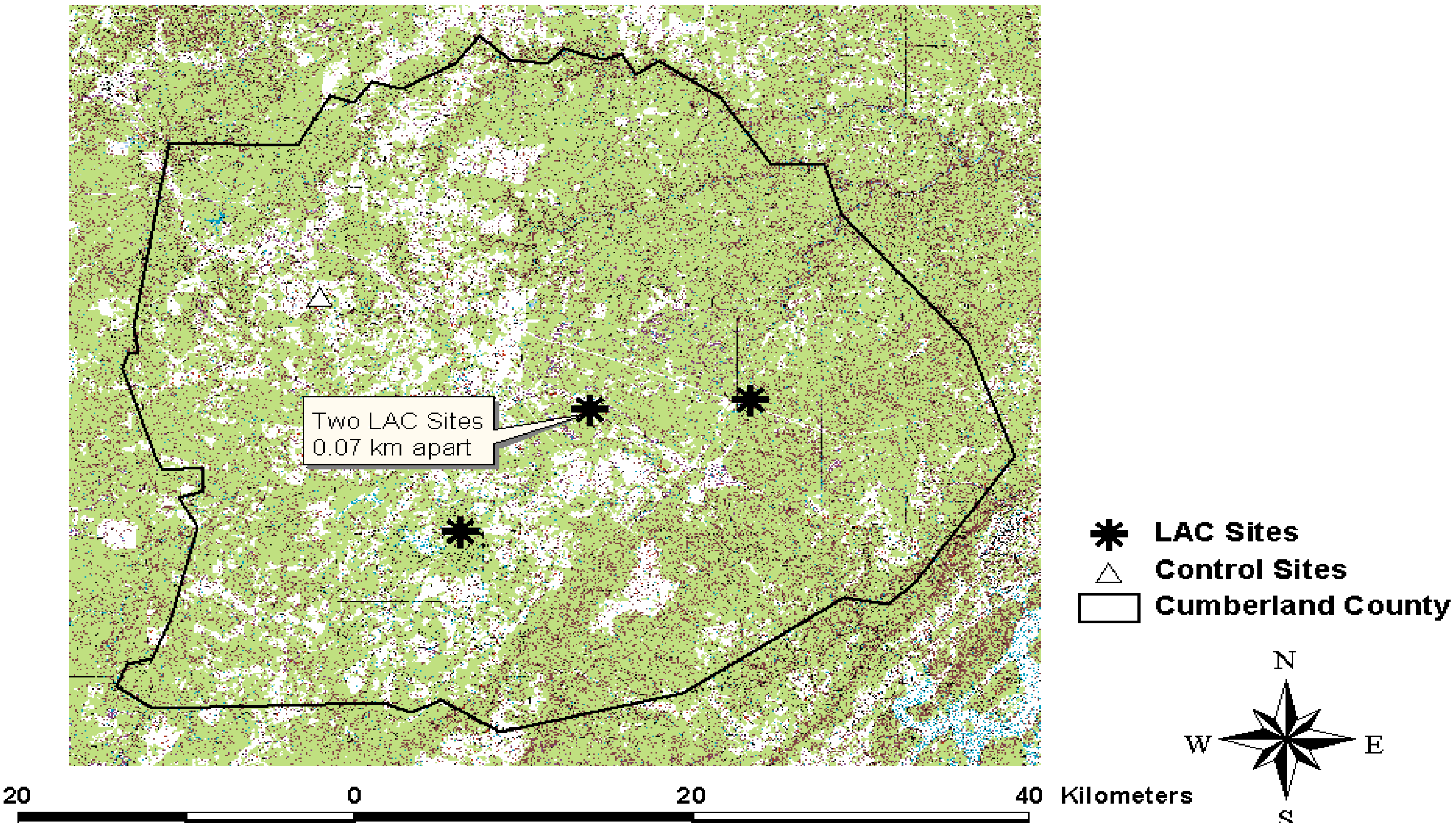

Figure 3.5: La Crosse (LAC) and Control Sites in Cumberland County using DRG from USDA (http://terraserver.homeadvisor.msn.com) added to county map from ArcView 3.2

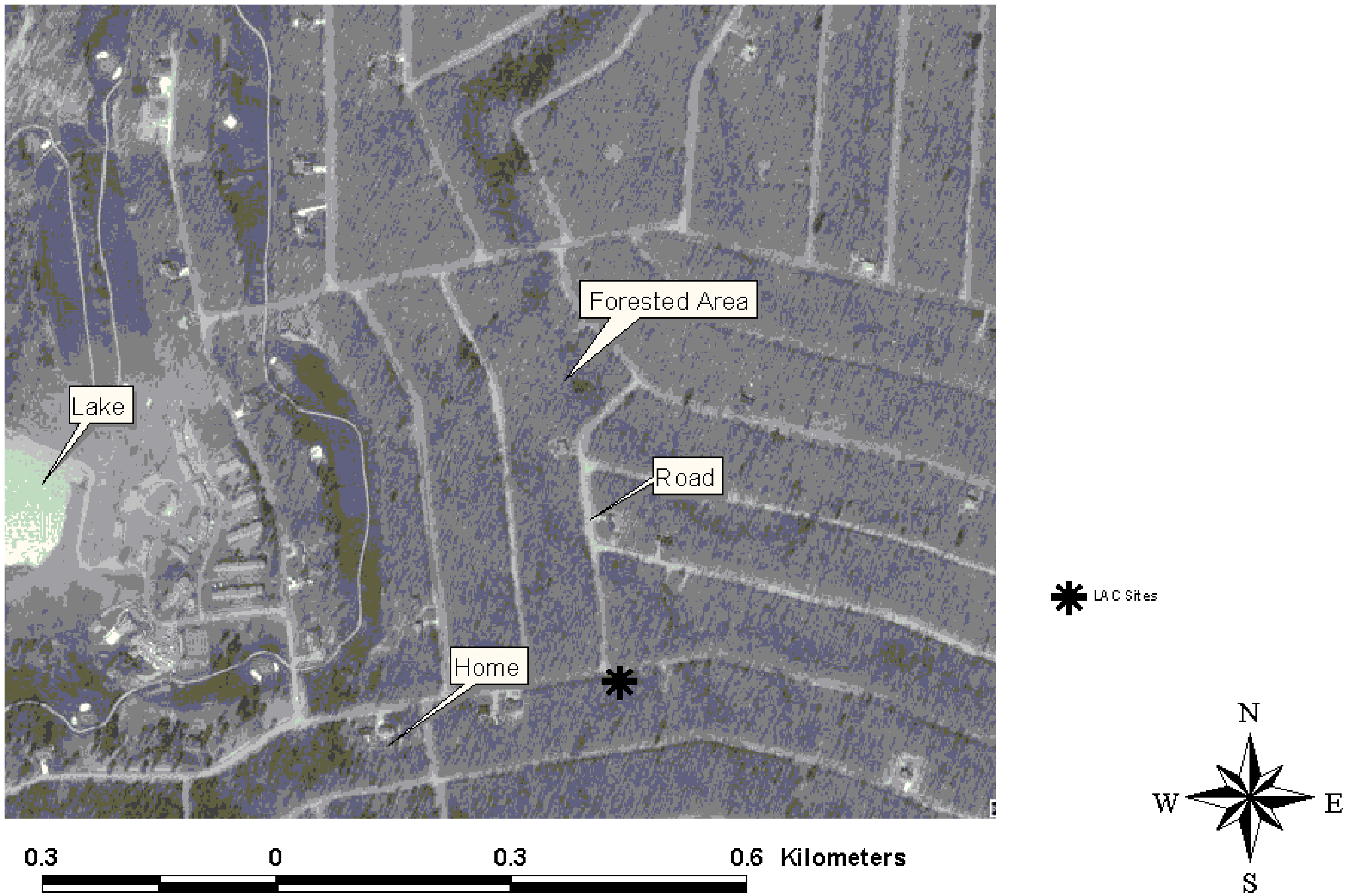

Figure 3.6: Cumberland County La Crosse (LAC) Site 1 and surrounding area satellite image from USDA (http://terraserver.homeadvisor.msn.com) added to county map from ArcView 3.2

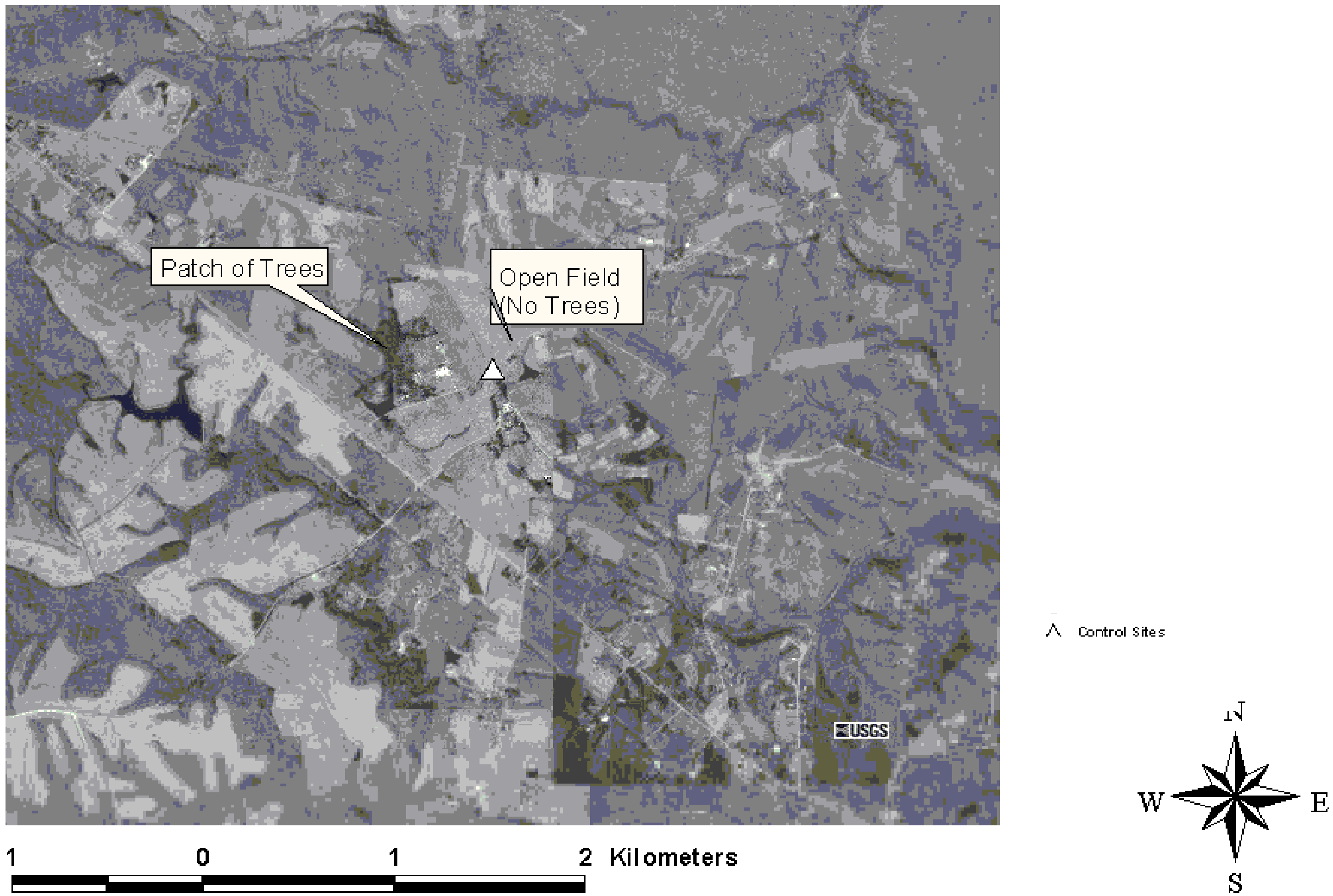

Figure 3.7: Cumberland County Control Site 1 and surrounding area satellite image from USDA (http://terraserver.homeadvisor.msn.com) added to county map from ArcView 3.2

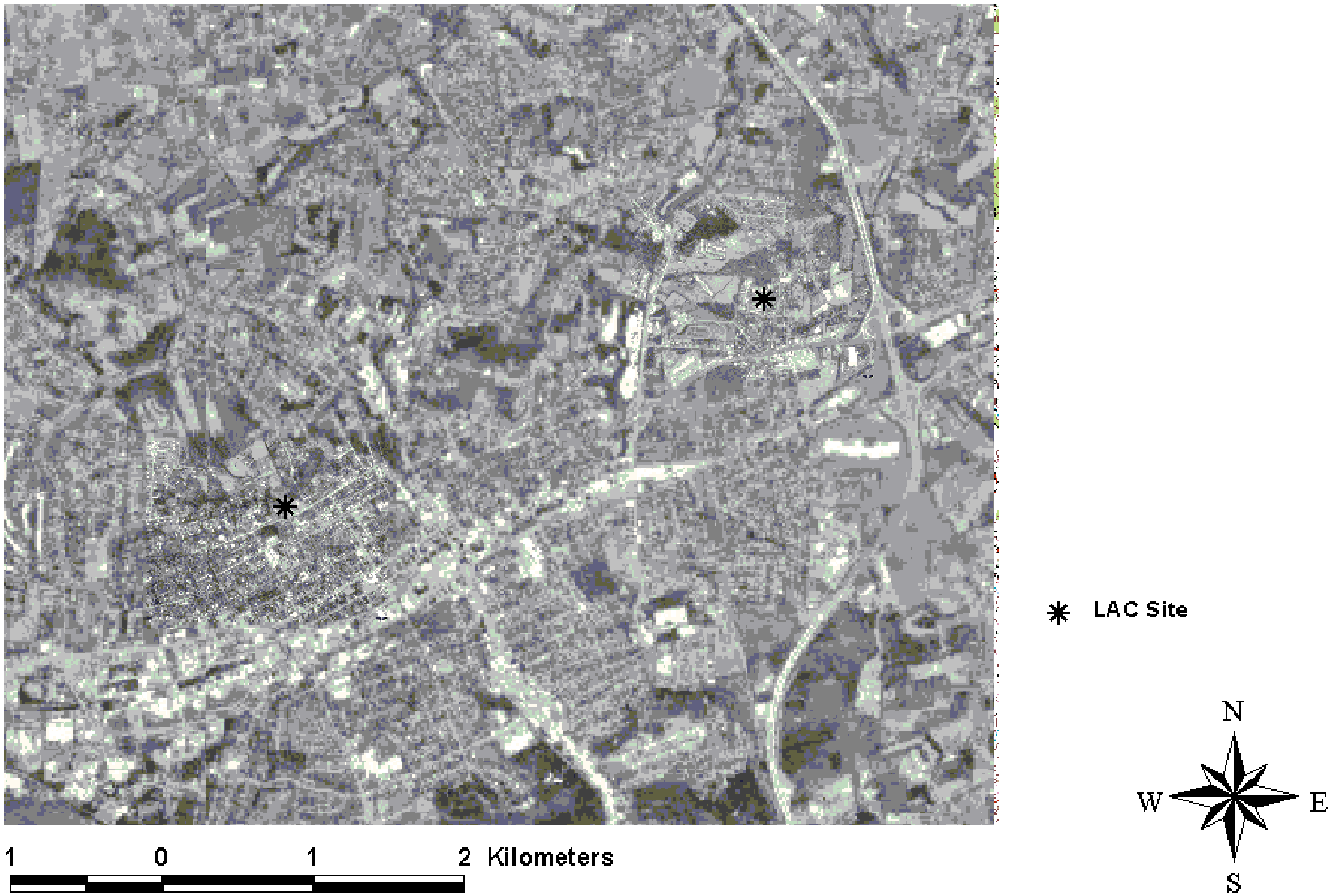

Figure 3.8: Hamblen County LAC Sites and surrounding area satellite image from USDA (http://terraserver.homeadvisor.msn.com) added to county map from ArcView 3.2

Area is more heavily urbanized and lacks the forested ridges. The elevation of the sites ranges from 248 m to 390 m. The High LAC Area has an average rainfall of 139.7 cm - 152.3 cm per year (Figure 3.4). The Low LAC Area has an annual rainfall of 127.0 cm - 139.6 (Figure 3.4).

Cumberland County

Cumberland County is the western most county in the study area reporting any cases of CNSI. It had by far the highest LACE case rate of all the counties in the study with an infection rate of 49.3 per 100,000 children for three years (Table 3.1). All four of the LAC Sites were in close proximity (<0.1 km) to large wooded areas (> 0.5 km x 0.5 km) with two of the homes adjacent to each other (0.07 km) (Figure 3.4, 3.5, and 3.6). The only Control Site in the county (Figure 3.7) was located 26.67 km From the nearest LAC Site and was in a rural grassland area with few trees and was 0.7 km away from the nearest block of trees (Figure 3.7).

Hamblen County

Hamblen County had only LAC cases (n=2), which occurred in a suburban area with no large blocks of unbroken forest (Figure 3.8). They were less than 4 km apart and the homes were close (> 0.1 km) to small wooded areas (approx. 0.2 km x 0.1 km).

Remaining Counties

Morgan, Monroe, and Jefferson Counties did not have a single confirmed LACE case. The majority of the Control Sites was not near any forested edges like the one in

Cumberland County that displays a typical Control Site (Figure 3.8). No CNSI of any type has been reported in the southern half of Monroe, Blount, Sevier, or Cocke Counties because the southern portions of those counties are National Forest and Parks where no families live on a permanent basis. Campbell, Loudon, and Jefferson Counties had Control Sites that did not have any of the surrounding wooded areas that would typically be associated with LAC virus transmission (Figure 3.1).

Cocke, Sevier and Campbell Counties had LAC Sites near wooded areas like the majority of the LAC Sites (Figure 3.7). The Cocke County and one Sevier County case were in the Blue Ridge area of eastern Tennessee while the LACE Cases in Sevier, Campbell, and Claiborne Counties were in the Appalachian Valley and Ridge area. All of the cases were within the area that receives an average of 114.3 cm - 126.9 cm of rain each year. Each of these counties had one LAC Site each except Sevier, which had two LAC Sites. The LAC Sites were within 0.5 km of large wooded areas.

v. Discussion

La Crosse sites occurred in nine of the 13 counties reporting CNSI in eastern Tennessee. The LAC Sites in eastern Tennessee from the past three years are associated with homes that encroached into wooded areas with higher populations of mosquitoes. Kitron et al. (1997) also reported that the places where LAC transmission occurred were in places in close proximity to wooded areas and illegal tire dumps and noted both as locations where *Oc. triseriatus* occurs. Most of the LAC Sites in this study were located within 0.5 km of large blocks of mixed hardwood forest. In the High LAC Area of Anderson, Knox, Blount and Loudon Counties these forested areas were the characteristic northeast to southwest running ridges in eastern Tennessee. La Crosse cases in Cumberland County were located on the Cumberland Plateau near large forested areas while the one Control Site in Cumberland County was in the Eastern Highland Rim and was not heavily forested. La Crosse Sites in Hamblen County were the only exceptions to having heavily forested areas in close proximity. The forested areas near these cases were smaller than those areas in other counties.

Although the primary difference between the sites studied in eastern Tennessee and those in Nicholas County, West Virginia, was that the West Virginia study was done in areas that were all forested (Nasci et al. 2000). No single or combined habitat parameters could be selected that could predict the absence, presence, and or risk of transmission (Nasci et al. 2000). The sites in eastern Tennessee were not all in forested areas, only the LAC Sites were always bordering forested areas but many of the Control Sites were not. There were no non-forested or urban areas studied in Nicholas County like there were in eastern Tennessee.

The GIS Data shows that all of the LAC cases occurred along the borders of or in heavily forested areas. In the survey done by Monath et al. (1970) the rural residents had a significantly higher rate of infection of LACE when compared to urban residents. The same is seen in the High and Low LAC Areas of Knox, Blount, Anderson and Loudon Counties. They both had the same level of CNSI rate but the more rural High LAC area had a much higher rate of LAC infection when compared to the Low LAC area. It seems the habitat parameter that is important is the proximity of living and playing areas to forested areas.

The soil categories differed when moving from one geographical region to the next. All of the counties except four have the Fullerton-Bodine-Clarksville soil category, a common soil category for the valley and ridge area and occurs throughout the counties in those areas. The four counties that do not contain Fullerton-Bodine-Clarksville category of soils had sites that occurred out of the valley and ridge area. Though half of the cases occurred on the Fullerton-Bodine-Clarksville category it does not seem that soil category plays a role for a site being more or less likely to be a suitable place for LACE transmission. All of the soil categories could sustain a maple oak or mixed hardwood forest with the amount of rain that is received annually in the different areas of eastern Tennessee (Little 2002).

Maples, oaks, and other mixed hardwoods are able to thrive in each of the soil categories with the annual rainfall that is received in eastern Tennessee. Soil category and rainfall do not seem that they could be linked to an increased or decreased risk of a child being infected with LACE. Risk of contracting LACE does seem to be linked with distance to wooded areas of mixed hardwood. Even though the soil, rain, and temperatures are ideal for each area LAC and Control, one quality that separates many of the Control from the LAC Sites is the proximity to wooded areas. In two studies one done in Illinois and one done in West Virginia LACE risk was found to be increased with a closer proximity to maple, oak, and mixed hardwood forest (Woodruff et al. 1992, Kitron et al. 1997).

There were some Control Sites that were in close proximity to maple or mixed hardwood forest but those children were not reported as having a LACE infection. The children enrolled in the study from the 1999-2001 in eastern Tennessee were not given an antibody screen like the children in West Virginia during the 1992 study (Woodruff et al. 1992). In the 1992 study in West Virginia 6% of the total patients were excluded because they tested positive on an antibody screen for LACE indicating a previous infection. The children from 1999-2001 were not given an antibody screen so some of the controls may have already had a LACE infection but had mild symptoms not requiring hospitalization (Henderson and Coleman 1974).

vi. Conclusions

There were nine counties in eastern Tennessee with reported cases of LACE from 1999-2001 with most homes in close proximity to the ecotone between the living area and forested area. The characteristic northeast to southwest running ridges in eastern Tennessee, forested areas of the Cumberland Plateau, and the forested areas of the Smokies composed the forested areas bordering the LAC Sites. La Crosse Sites in Hamblen County were the only exceptions to having heavily forested areas in close proximity. The LAC Sites were always bordering forested areas but many of the Control Sites were not. The Control Sites were scattered evenly throughout the urban and rural areas of eastern Tennessee.

Monath et al. (1970) found in their survey the rural residents had a significantly higher rate of infection of LAC virus when compared to urban residents. In eastern Tennessee the rate of infection for LAC virus was higher as well in the rural areas when compared to urban areas. In the High and Low LAC Areas the rate of CNSI were similar, however the LAC virus infection rate was much higher when the High LAC Area was compared to the Low LAC Area.

This disease seems to be confined to the rural forested areas in Tennessee. The people who have encroached into the forested area seem to be at the greatest risk for contracting this disease. However more needs to be done to pinpoint all of the factors that contribute to the risk of infection for this virus.

REFERENCES

REFERENCES

Centers for Disease Control. 2002. Confirmed California (La Crosse) Encephalitis Cases, Human, United States, 1964-1997, By State. http://www.cdc.gov/ncidod/dvbid/arbor/lac64_97.pdf

Chun, R. W. M., W. H. Thompson, J. D. Grabow, and C. G. Matthews. 1968. California Arbovirus Encephalitis in Children. Neurology 18: 369-375.

Clark, G. C., W. H. Rohrer, D. N. Robbins, H. L. Pretula, and R. N. Harroff. 1982. LaCrosse Virus Activity in Illinois Detected by Ovitraps. Mosq. News 42 [4]: 551-556.

Downs, W. G. 1974. Arboviral Infections: The Importance of Endemic and Importable Arboviruses. Am. Soc. Trop. Med. Hyg. 23[4]: 781-784.

Environmental Research Systems Institute. Introduction to ArcView. 2000. ERSI.

Erwin, P. C., T. F. Jones, R. R. Gerhardt, S. K. Halford, A. B. Smith, L. E. R. Patterson, K. L. Gottfried, K. L. Burkhalter, R. S. Nasci, and W. Schaffner. 2002. La Crosse Encephalitis in Eastern Tennessee: Clinical Environmental and Entomological Characteristics from a Blinded Cohort Study. Am. J. Epidemiol. 155[11]: 1060-1065.

Gauld, L. W., R. P. Hanson, W. H. Thompson, and S. K. Sinha. 1974. Observations on a Natural Cycle of La Crosse Virus (California Group) in Southwestern Wisconsin. Am. Soc. Trop. Med. Hyg. 23[5]: 983-992.

Gerhardt, R.R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. First Isolation of La Crosse Virus from Naturally Infected *Aedes albopictus*. Emer. Infect. Dis. 7[5]: 807-811.

Gottfried, K. L., R. R. Gerhardt, R. S. Nasci, M. B. Crabtree, N. Karabatsos, K. L. Burkhalter, B. S. Davis, N. A. Panella, and D. P. Paulson. 2002. Temporal Abundance, Parity, Survival Rates, and Arbovirus Isolation of Field-Collected Container-Inhabiting Mosquitoes in Eastern Tennessee. J. Am. Mosq. Control Assoc. 18[3]: 164-172.

Gottfried, K. L. 2000. Seasonal Distribution and Abundance of the Mosquito Species (Diptera: Culicidae) in Eastern Tennessee. M. S. Thesis. University of Tennessee, Knoxville.

Hanson, R. P. and M. G. Hanson. 1970. The Effect of Land Use Practices on the Vector of California Encephalitis in North Central United States. Mosq. News 30 [2]: 215-221.

Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G. B. Craig. 1987. *Aedes albopictus* in North America: Probable Introduction in Used Tires from Northern Asia. Science 236: 1114-1116.

Hawley, W. A. 1988. The Biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 4: 2-39.

Henderson, B. and P. Coleman. 1971. The Growing Importance of California Arboviruses in the Etiology of Human Disease. Prog. Med. Virol. 13: 404-461.

Hurn, J. 1993. Differential GPS Explained. Trimble.

Jones, T. F., R. S. Nasci, A. S. Craig, L. E. R. Patterson, P. C. Erwin, R. R. Gerhardt, X. T. Ussery, and W. Schaffner. 1999. Newly Recognized Focus of La Crosse Encephalitis in Tennessee. Clin. Infect. Dis. 28: 93-97.

Kitron, U., J. Michael, J. Swanson, and L. Haramis. 1997. Spatial Analysis of the Distribution of LaCrosse Encephalitis in Illinois, Using a Geographic Information System and Local and Global Spatial Statistics. Am. Soc. Trop. Med. Hyg. 57[4]: 469-475.

Little, E. 2002. The Audubon Society Field Guide to North American Trees: Eastern Edition. Knopf. pp. 714.

Loor, K. A., and G. R. DeFoliart. 1969. Oviposition Trap for Detecting the Presence of *Aedes triseriatus (SAY)*. Mosq. News 29[3]: 487-488.

Loor, K. A., and G. R. DeFoliart. 1970. Field Observation on the Biology of *Aedes triseriatus*. Mosq. News 30[1]: 60-65.

Mitchell C. J. 1991. Vector competence of North and South American strains of *Aedes albopictus* for certain arboviruses. J. Am. Mosq. Control Assoc. 7[3]:446-451.

Monath, T. P. C., J. G. Nuckolls, J. Berall, H. Bauer, W. A. Chappell, and P. H. Coleman. 1970.. Studies on California Encephalitis in Minnesota. Am. J. Epidemiol. 92[1]: 40-50.

Moore, C. G. and C. J. Mitchell. 1997. *Aedes albopictus* in the United States: Ten-Year Presence and Public Health Implications. Emerg. Infect. Dis. 3[3]: 326-334.

Moore, H. 1994. A Geologic Trip Across Tennessee by Interstate 40. University of Tennessee Press. pp 339

Moore, J. P. 1998. *Aedes albopictus* (Diptera: Culicidae) Occurrence Throughout Tennessee, with Biological Notes. Entomol. News. 109: 363-365

Moulton, D. W. and W. H. Thompson. 1971. California Group Virus Infections in Small, Forest-Dwelling Mammals of Wisconsin. Am. Soc. Trop. Med. Hyg. 20[8]: 474-482.

Nasci, R. S. 1982. Activity of Gravid *Aedes triseriatus* in Wooded Fencerows. Mosq. News 42[3]: 408-412.

Nasci, R. S., C. G. Moore, B. J. Biggerstaff, N. A. Panella, H. Q. Liu, N. Karabatsos, B. S. Davis, and E. S. Brannon. 2000. LaCrosse Encephalitis Virus habitat Association in Nicholas County, West Virginia**.** J. Med. Entomol. 37[4]: 559-570.

Pantuwantana, S., W. H. Thompson, D. M. Watts, T. M. Yuill, and R. P. Hanson. 1974. Isolation of La Crosse Virus from Field Collected *Aedes triseriatus*. Am. Soc. Trop. Med. Hyg. 23: 246-250.

Reiter, P. 1998. *Aedes albopictus* and the World Trade in Used Tires, 1988-1995: The Shape of Things to Come. J. Am. Mosq. Control Assoc. 14[1]: 83-94.

Smith, A. B. 2001. A Study of La Crosse Encephalitis in Eastern Tennessee. M. S. Thesis. University of Tennessee, Knoxville.

Stanich, D. M. 2002. Epidemiology of La Crosse Encephalitis in Eastern Tennessee, 2000 and 2001. M. S. Thesis. University of Tennessee, Knoxville

Sudia, W. D., V. F. Newhouse, C. H. Calisher, and R. W. Chamberlain. 1971. California Group Arboviruses: Isolations from Mosquitoes in North America. Mosq. News 31[4]: 576-600.

Watts, D. M., C. D. Morris, R. E. Wright, G. R. DeFoliart, and R. P. Hanson. 1972. Transmission of La Crosse Virus (California Encephalitis Group) by the Mosquito *Aedes triseriatus*. J. Med. Entomol. 9[2]: 125-127.

Watts, D. M., W. H. Thompson, T. M. Yuill, G. R. DeFoliart, and R. P. Hanson. 1974. Overwintering of La Crosse Virus in *Aedes triseriatus*. Am. Soc. Trop. Med. Hyg. 23: 694-700.

Woodruff, B. A., R. C. Baron and T. T. Tsai. 1992. Symptomatic La Crosse Virus Infections of the Central Nervous System: A Study of Risk Factors in an Endemic Area. Am. J. Epidemiol. 136[3]: 320-327.

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