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Habitat Associations of Eastern Equine Encephalitis Transmission in Walton County Florida

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Abstract

Eastern Equine Encephalitis virus (EEEV; family *Togaviridae*, genus *Alphavirus*) a highly pathogenic mosquito-borne virus is endemic to eastern North America. The ecology of EEEV in Florida differs from that in other parts of the United States; EEEV in the northeastern United States is historically associated with freshwater wetlands. No formal test of habitat associations of EEEV in Florida has been reported. Geographical Information Sciences (GIS) was used in conjunction with sentinel chicken EEEV seroconversion rate data as a means to examine landscape features associated with EEEV transmission in Walton County, FL. Sentinel sites were categorized as enzootic, periodically enzootic, and negative based on the number of chicken seroconversions to EEEV from 2005 to 2009. EEEV transmission was then categorized by land cover usage using Arc GIS 9.3. The land classification data were analyzed using the Kruskal–Wallis test for each land use class to determine which habitats may be associated with virus transmission as measured by sentinel chicken seroconversion rates. The habitat class found to be most significantly associated with EEEV transmission was tree plantations. The ecological factor most commonly associated with reduced levels of EEEV transmission was vegetated nonforest wetlands. *Culiseta melanura* (Coquillett), the species generally considered to be the major enzootic EEEV vector, was relatively evenly distributed across all habitat classes, while *Aedes vexans* (Meigen) and *Anopheles crucians* Weidemann were most commonly associated with tree plantation habitats.

Keywords

Eastern Equine Encephalitis virus; habitat; arbovirus; GIS; transmission

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Eastern Equine Encephalitis virus (EEEV; family *Togaviridae,* genus *Alphavirus*) is a pathogenic arbovirus with a high case fatality rate among infected humans and horses (Villari et al. 1995, Reimann et al. 2008). Human symptomatic cases of EEE exhibit a case fatality rate of roughly 50%, and many survivors suffer residual neurological sequelae (Calisher et al. 1986, Martin et al. 2000). EEEV is endemic in many of the states of the eastern United States and cases of neuroinvasive EEE have been reported in 20 states (Bigler et al. 1976). Since 1964, human cases of EEEV in Florida have accounted for 70 (25%) of the total 270 fatalities observed because of EEEV in the United States (CDC 2010).

EEEV is widespread across Florida, with transmission reported in 64 of Florida's 67 counties. Studies of EEEV in Florida suggest that its ecology is distinct from that found in other parts of the United States. For example, EEEV circulates year-round in Florida, while it is dormant during the winter months elsewhere (Wellings et al. 1972, Day and Stark 1996b). EEEV isolates from Florida appear to be genetically similar to those found in the northeastern United States (Weaver et al. 1991, 1994; Armstrong et al. 2008; Young et al. 2008; White et al. 2011). However, specific ecological niches may exist that enhance amplification and transmission of EEEV in Florida, making the state heterogeneous with respect to EEEV activity. For example, higher sentinel chicken seroconversion rates have been reported in the last five years in Florida's panhandle region than in the southern half of the Florida peninsula (Florida Department of Health 2010a), suggesting that the ecological conditions present on the panhandle may be more suitable for EEEV than those in the more tropical southern parts of the state.

Most counties in Florida maintain active surveillance and vector control programs aimed at reducing the threat of arboviral transmission. Many of these programs rely upon mosquito collections to monitor levels of probable vectors and serological surveillance of sentinel chickens to detect arboviral transmission. Historically, sentinel chicken flock locations have been determined based upon the proximity to human cases of St. Louis encephalitis virus (SLEV) during major human outbreaks of SLEV in Florida, or based on operational convenience and practicality (Nelson et al. 1983, Monath and Tsai 1987). These flocks have subsequently been used for surveillance of West Nile virus (WNV), a Flavivirus related to SLEV, that was introduced to the United States in 1999, and to Florida in 2001 (Blackmore et al. 2003). While the ecological predictors associated with WNV transmission have been extensively studied (Gibbs et al. 2006, Ruiz et al. 2007, Tran et al. 2007, Brown et al. 2008, DeGroote et al. 2008), ecological factors influencing the spread of EEEV have not been fully defined, particularly in Florida. As a result, Florida's sentinel chicken surveillance and vector control programs have limited information for placement of surveillance sites for monitoring EEEV, and a more targeted approach would improve the efficacy for detection of EEEV activity.

Landscape and spatial epidemiology are important variables to consider when investigating the connection between pathogens and their hosts, vectors, and the environment (Brown et al. 2008, Mammen et al. 2008). Synergistic elements are involved in realizing an optimal disease transmission cycle; some of these elements include appropriate habitats for vector and host reproduction, population densities, and meteorological influences. The advancement of remote sensing technology and geographic information science (GIS) has given researchers a greater opportunity to explore these habitat elements and their role in vector-borne disease transmission (Kitron 1998, Lian et al. 2007, Eisen and Eisen 2008, Lambin et al. 2010, Krefis et al. 2011). With respect to EEEV, remote sensing and GIS have been successfully used to characterize associations between vector abundance and landscape variables in the northeastern United States (Moncayo et al. 2000).

For the purpose of this study, GIS was used in conjunction with sentinel chicken EEEV seroconversion rate data as a means to examine the landscape features associated with EEEV transmission in Walton County, FL, located in the Florida panhandle. Thus, our investigation focused on determining landscape risk factors associated with elevated EEEV transmission as monitored by seroconversions among sentinel chicken flocks located in Walton County.

Materials and Methods

Study Area

The sites selected for inclusion in this study were in Walton County, located in the Western Panhandle region of Florida (Fig. 1). The county's landscape is dominated by five major land cover classes, which together account for 86% of the total county area; these habitats include upland forests, tree plantations, wetlands, cropland, and residential (Table 1). Walton County was chosen for two reasons. First, an examination of records maintained by the state of Florida over the past 5 yr (2005–2009) indicate that Walton County is one of the most active in the state in terms of EEEV transmission (Florida Department of Health 2010a). Second, Walton County has actively participated in the statewide arbovirus surveillance program that monitors sentinel chickens for arbovirus activity. Thus, substantial archived sentinel data covering several years was available from Walton County.

Site Selection

Twenty four sentinel sites were selected from a total of 29 possible sites in the county for inclusion in the study. Five sites were excluded from this study because they were only operational for 1 yr, and therefore comparisons of yearly virus activity during 2005–2009 were not possible at these sites. Sentinel flocks in areas surrounding Choctawhatchee Bay were maintained by South Walton County Mosquito Control and used 2–3 chickens per flock. Sentinel flocks located in the inland areas of the county were maintained by North Walton Mosquito Control and contained six birds per flock. Chickens in both districts were maintained in the field in cages that protect them from predators and weather. Blood samples from the sentinels were collected weekly by county mosquito district personnel and then shipped to the Florida Department of Health Bureau of Laboratories for testing. Sera were screened using a hemagglutination inhibition (HI) assay to detect alphavirus antibodies (Florida Department of Health 2009). If the sentinel sera HI assay was positive, a confirmatory test for IgM antibody to EEEV was then conducted, as described by Calisher et al. (1986) and Martin et al. (2000) to differentiate EEEV from Highlands J virus. Chickens that were confirmed EEEV positive were removed from the flock and replaced with a serologically naïve bird.

Sentinel sites were categorized based on the number of chicken seroconversions to EEEV from 2005 to 2009. Sites with at least one confirmed positive EEEV seroconversion per year for three or more of the five total years were classified as EEEV enzootic sites. Sites with at least one EEEV positive sentinel per year for less than three of the five total years were classified as periodically EEEV enzootic. Finally, sites with no EEEV seroconversions were classified as EEEV negative (Fig. 2). Seroconversion rates were calculated by dividing the total amount of confirmed seroconversions per site by the number of susceptible chickens exposed at that site during the 5 yr study period.

Habitat Analysis

The habitats comprising the study sites were characterized using the level 2 land cover usage classifications taken from the Northwest Florida Water Management District Land Use Land Cover 2004 (Florida Department of Environmental Protection 2009). In addition, a habitat

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analysis was conducted using Arc GIS 9.3 on all 24 sites. The Florida Department of Environmental Protection's Bureau of Watershed Restoration has developed land use and land cover maps using Digital Ortho Quarter Quad Aerial Imagery program Color Infrared and True Color photography (Florida Department of Environmental Protection 2009).

Habitat descriptions for the land use land cover are based on the Florida Department of Transportation schema and encompass four different levels, with level 1 being the most general and level 4 the most specific (Florida Department of Transportation 1999). Level 2 descriptions were used in this study because they differentiated between wetland types (Fig. 3). Out of the 42 subclassifications found in the level 2 categories, 11 were used in this study, which were selected based on their habitat importance to mosquito vectors associated with EEEV or possible hosts. Several of the classes were combined, such as lakes, streams, and reservoirs. The remaining classes were excluded because they were not suitable habitats for EEEV (e.g., large paved areas, beaches, ocean, and airports).

Previous studies have shown that the average mosquito dispersal distance is between 0.97–3.21 km for *Culex erraticus* (Dyar and Knab) (Estep et al. 2010), 1 km for *Cx. quinquefasciatus* Say (Reisen et al. 1992), and 4 km for *Culiseta melanura* (Coquillett) (Howard et al. 1989). Ecological spatial scales of a 1–2 km radius are considered appropriate when trying to capture mean mosquito flight distances in GIS (Rochlin et al. 2008). Therefore, a 1.5 km buffer was created in GIS around the chicken sentinel sites to provide an appropriate ecological scale that could account for the average mosquito movement during the collection period. Habitat features were then extracted from each buffer area using the intersect function and hectarage composition was summarized. The land classification data were analyzed using the Kruskal–Wallis test (Sokal and Rohlf 1981) for each land use class to determine which habitats may be associated with virus transmission and comparisons were made between enzootic, periodically enzootic, and negative sites.

Scatter plots were produced with best-fit lines to visualize the relationships between selected habitat covariates and EEEV seroconversion rates of chickens in sentinel flocks. Six of the 11 total habitat types were selected a priori, which were predicted to have the greatest potential for biological influence on transmission potential of EEEV.

Mosquito Collection and Analysis

Mosquitoes were collected twice weekly from May through August 2009 at all 24 sites with CO_2-baited light traps. Upon collection, mosquitoes were identified using morphological keys (Darsie and Ward 2004). Mosquitoes were pooled by species, collection date, and site. Each pool contained a maximum of 50 individuals. Pools were tested for the presence of EEEV using a real time reverse transcription polymerase chain reaction (RT-PCR) assay (Cupp et al. 2003, Lambert et al. 2003). Positive mosquito pool homogenates were centrifuged and supernatant (≈1 ml) was collected, filtered and added to confluent Vero cells in individual T-25 flasks for virus isolation, as previously described (Day and Stark 1996a). Mosquito collections were normalized by trap night across habitats to determine relative species abundance within habitat types during the collection period.

Results

Habitat Associations

Of the 24 sites in Walton County included in the study, 9 were classified enzootic, 10 were periodically enzootic, and 5 were negative using the criteria described in Materials and Methods. The difference in spatial extent of each land cover type among the three EEEV classes was statistically compared. These data were first examined for homogeneity of

variance and normality, two critical assumptions of normal parametric statistics. These tests revealed that the data strongly violated each of these assumptions, with variance ratios exceeding five and highly significant deviations from normality in the Kolmogorov–Smirnov tests in 92% of the cases. As a result of these violations, differences were tested using the Kruskal– Wallis test, a nonparametric equivalent of a one-way analysis of variance (ANOVA). The Kruskal–Wallis test results suggested significant associations of particular habitats and EEEV activity (Table 2). Three habitat classifications (cropland and pastureland, tree plantations, and vegetated nonforested wet-land) were significantly associated with EEEV activity ($P < 0.05$) while three (lakes and streams, upland coniferous forest, and wetland forested mixed) were marginally associated with EEEV activity, that is, the associations seen did not reach statistical significance ($0.05 < P < 0.1$).

Scatter plots were prepared comparing the incidence of seroconversions to the number of hectares of each habitat for the habitats shown to be significantly or marginally associated with EEEV activity (Fig. 4). Wetland mixed forest and tree plantation habitats were found to be positively associated with EEEV transmission. For example, enzootic sites had an average of 122.765 ha of tree plantation habitat per site, while the negative sites averaged only 9.18 ha per site (Table 2). Two habitat classes (cropland and pasture-land, and lakes and rivers) had little or no association with EEEV activity when analyzed by scatter plot. This divergence in the results produced in the scatter plot and nonparametric analyses was at least in part because of the bimodal distribution in habitats among the three EEEV activity classification groups. For example, cropland and pastureland were present in similar amounts in enzootic and negative sites (41.4 and 65.36 ha, respectively) while periodically enzootic sites averaged only 2.02 ha of this habitat (Table 2). The two remaining classes (upland coniferous forest, and vegetated nonforested wetland) were negatively associated EEEV transmission to varying degrees (Fig. 4).

Sentinel Seroconversions

In total, 242 chickens seroconverted to EEEV in Walton County from 2005 to 2009 (Table 3), as determined by HI assay and IgM antibody confirmation as described in Materials and Methods. The average EEEV seroconversion rate in the sentinel chickens across all sites over the study period was 18.5%, with the most active sentinel flock averaging a seroconversion rate of 42.2%. During 2009, 64 sentinel chickens seroconverted in Walton County, resulting in an 18.7% overall seroconversion rate (Table 3). Two enzootic sites in 2009 had high seroconversion rates (58.6 and 45.8%) that were greater than the 5 yr average rate of 42.2% for the most active site in Walton County (Fig. 5). In 2009, sentinel seroconversion rates in 9 out of 19 positive sites exceeded the 18.5% average for the study period (Fig. 5).

Mosquito Abundance

In total, 7,653 mosquitoes were collected during the course of this study (May through August 2009), representing 30 different mosquito species. The most abundant species in this region were *Anopheles crucians* Weidemann, *Cs. melanura, Cx. erraticus,* and *Culex nigripalpus* Theobald (Fig. 6). Greater numbers of *Aedesvexans*(Meigen) and *Aedes infirmatus* Dyar and Knab were collected in the tree plantation habitat than other habitat types (Table 4). When normalized by trap night, far more *Ae. vexans* females (80% of the total) were collected in tree plantation habitat than all other habitat types. Similarly, 74% of all *Ae. infirmatus* were collected in tree plantations.

In contrast, collections of *Cs. melanura,* the mosquito thought to be the primary enzootic vector of EEEV (Scott and Weaver 1989), were relatively evenly distributed across all habitat classes (Table 4). EEEV was detected in five separate pools of *Cs. melanura* with a

minimum infection rate of 13.24/1000. EEEV was isolated from four of these positive pools. Additionally, the EEE viral genome was detected by RT-PCR in one pool of *Coquillettidia perturbans* (Walker) and one pool of *Psorophoraferox*(von Humboldt). However, virus was not recovered from either of these pools when cultured.

Discussion

In an effort to reduce the exposure of EEE to both human and equestrian populations, consideration needs to be taken as to which habitats have an increased risk for disease transmission and how much of that habitat is currently being monitored. Historically, EEEV transmission has been associated with freshwater wetlands, swamps, and marshes (Komar and Spielman 1994). Wetland habitats comprise 23% of Walton County's land area, and 23% of the total wetland habitat falls within their buffer area surrounding the sentinel sites. This indicates that the sentinel surveillance program in Walton County covers the wetland habitat in proportion to its actual abundance, so that historically at-risk habitats (swamps, marshes) are covered by the activities at their current sentinel sites.

Tree plantation and wetland forested mixed habitats were found to be positively associated with EEEV activity in Walton County. The finding that wetland forest mixed habitat was positively associated with EEEV activity was in keeping with the understanding of the ecology of EEEV developed from studies in the Northeast, where wetland habitats have been shown to be associated with EEEV activity (Moncayo et al. 2000). More surprisingly, this analysis suggested that tree plantation habitat was the habitat classification most significantly positively associated with EEEV activity. Tree plantations primarily consist of forest regeneration areas and coniferous plantations. Florida's climate allows for rapid growth of several different types of tree species, and because of the productive timber industry, Florida allocates large portions of land for tree plantations. In Walton County, tree plantations were shown to be a positively associated with EEEV transmission. Tree plantations make up 23% of Walton County's habitat; however, this habitat only comprises 7% of the area currently monitored by sentinel flocks. Within the sentinel sites, the greater the area of tree plantations correlated with an increase in sentinel seroconversions to EEEV. Enzootic sites had 3.5 times more area of tree plantations per site then the negative sites and two times more area then the periodically enzootic sites.

One possible reason behind this positive association is that the tree plantations often have a high number of trees per hectare (Florida Department of Transportation 1999). The density and availability of these trees may make the habitat more attractive to nesting and roosting birds. This would increase the potential population and density of reservoir hosts, thereby increasing the risk of virus transmission. A second possible reason for this positive association is the large amount of *Ae.vexans*and *Ae.infirmatus*that were seen in the tree plantation habitats as compared with all other habitats. As a possible bridge vector for EEEV (Vaidyanathan et al. 1997, Andreadis et al. 1998), *Ae. vexans* may play a role in EEEV transmission seen within the tree plantation areas.

We found that vegetated nonforested wetland (primarily treeless hydric savanna) and upland coniferous forests were negatively associated with the transmission of EEEV to sentinel chickens. These habitat types comprise 18% of the county's total land use and are represented in the sentinel site habitats at 30% of the total area monitored. The reason for the negative association of these wetland types with EEEV transmission is not clear, given the historical connection between wetland habitats and epizootics of EEEV in the northeastern United States. As wetlands are generally conducive to mosquito breeding, and Florida contains a large amount of wetlands, mosquito densities in these habitats are generally high (Rochlin et al. 2008). It may be that the negative association may therefore relate more to

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the availability of competent reservoir hosts than to vector densities. For example, past studies have shown that there tends to be higher production of seed and fruit producing plants, as well as insects, in hardwood swamps as opposed to coniferous swamps (Ewel and Atmosoedirdjo 1987). The difference in food resources between wetlands may play a role in habitat preference or foraging areas for potential avian hosts. Similarly the lack of trees in this vegetated wetland may reduce the number of roosting birds, thereby limiting the contact opportunities of vectors with potential hosts. Regardless of the explanation for this negative association, it is clear that the relationship is not because of an under-representation of these wetland habitats in the buffer area surrounding sentinel sites.

Cu.melanura is typically considered a wetland mosquito; however, our results indicate that in the Florida panhandle this species is not as habitat specific as reported in the northeastern United States (Morris and Zimmerman 1981). *Cu. melanura* appeared in all habitat types within this study. Because *Cs. melanura* is considered the primary vector of EEEV (Scott and Weaver 1989, Armstrong and Andreadis 2010), its abundance in a wide range of habitats, especially residential areas, could explain (in part) why there are almost twice as many human cases of EEE between 1964–2010 reported in Florida than any other state (CDC 2010). This widespread distribution would allow enzootic EEEV to be transmitted in or nearby areas frequented by humans, thus increasing the possibility of epizootic transmission by bridge vectors to humans.

Mosquito species distributions among the different habitats were fairly uniform, except for the tree plantations. In tree plantations, seven times more *Ae. vexans* (14.57) were collected per trap night than any other habitat type (2.88). Four times more *Ae. infirmatus* (13.14) were also collected in the tree plantations, in relative to other habitats (3.44). *Aedes infirmatus* and *Ae.vexans* have been implicated as potential bridge vectors for EEEV (Wellings et al. 1972, Cupp et al. 2003, Hassan et al. 2003) and their greater abundance in the tree plantation habitat points toward their possible roles in EEEV transmission. Despite the lack of EEEV isolations in *Ae.infirmatus* and *Ae.vexans* in this study, virus isolations have been made in previous studies from wild caught specimens (Vaidyanathan et al. 1997, Cupp et al. 2003, Florida Department of Health 2010b). This idea is supported by the seroconversions of chickens during the time of increased abundance of these two mosquitoes. Furthermore, their lack of abundance in the wetland habitats may be a factor contributing to the lower transmission rates within those areas.

The data presented here suggests that increasing monitoring of tree plantation habitat may be a way to increase the efficiency of EEEV surveillance activities in the Florida panhandle. The unique orderly arrangement of trees in tree plantations allows them to be easily recognized through GIS. Tree plantations fall into the industrial forestry category and therefore are not typically environmentally protected, as are many wetlands. Targeting tree plantations for surveillance could improve the protection of both human and horse populations in the Panhandle region of Florida by providing earlier warnings of transmission activity and more effective opportunities for resource management and prevention measures.

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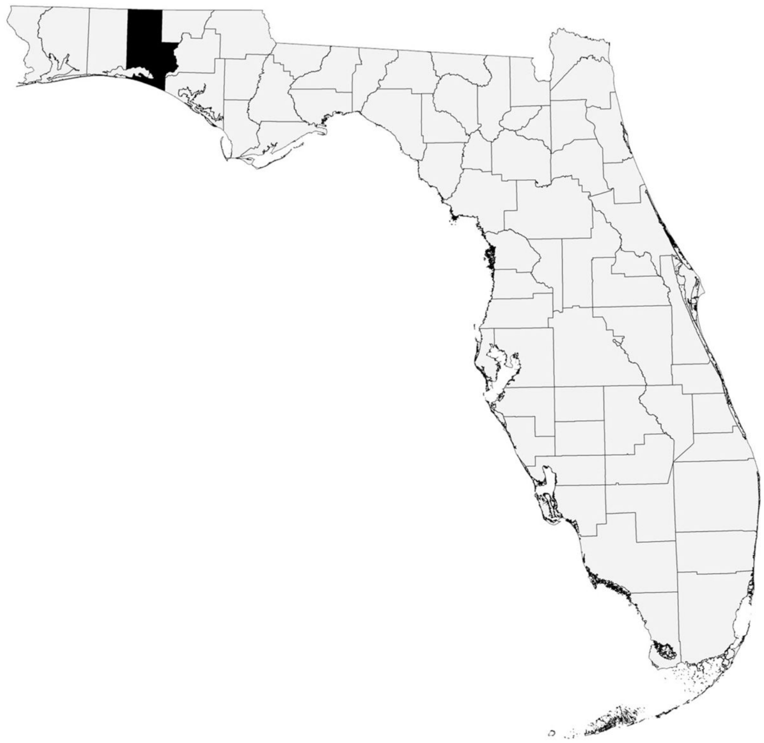

Fig. 1.
Location of Walton County.

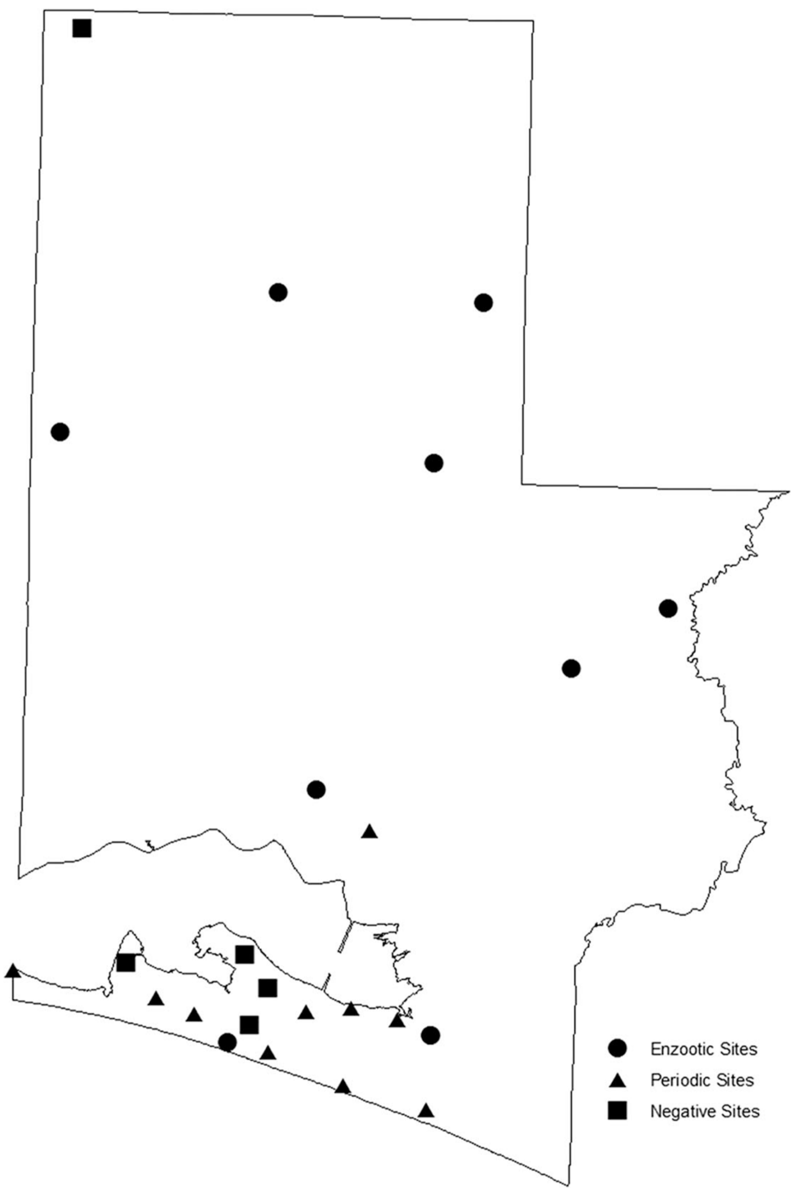

Fig. 2.
Study site classifications in Walton County based on sentinel seroconversion activity: circles, enzootic; triangles, periodically enzootic; and squares, negative sites.

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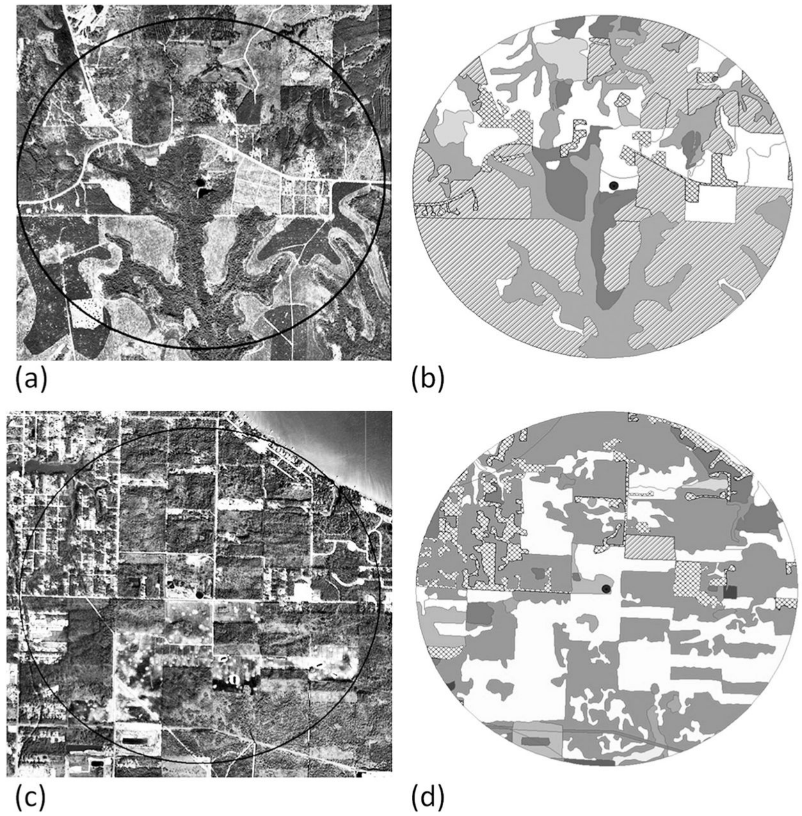

Fig. 3.

Satellite images and habitat classifications of sites in Walton County: Panel (a): Satellite image of an enzootic sentinel site in Walton County. Panel (b): Land use land cover data used to analyze the enzootic sentinel site habitat. Panel (c): A satellite image of a negative sentinel site in Walton County. Panel (d): Land use land cover data used to analyze the negative sentinel site habitat.

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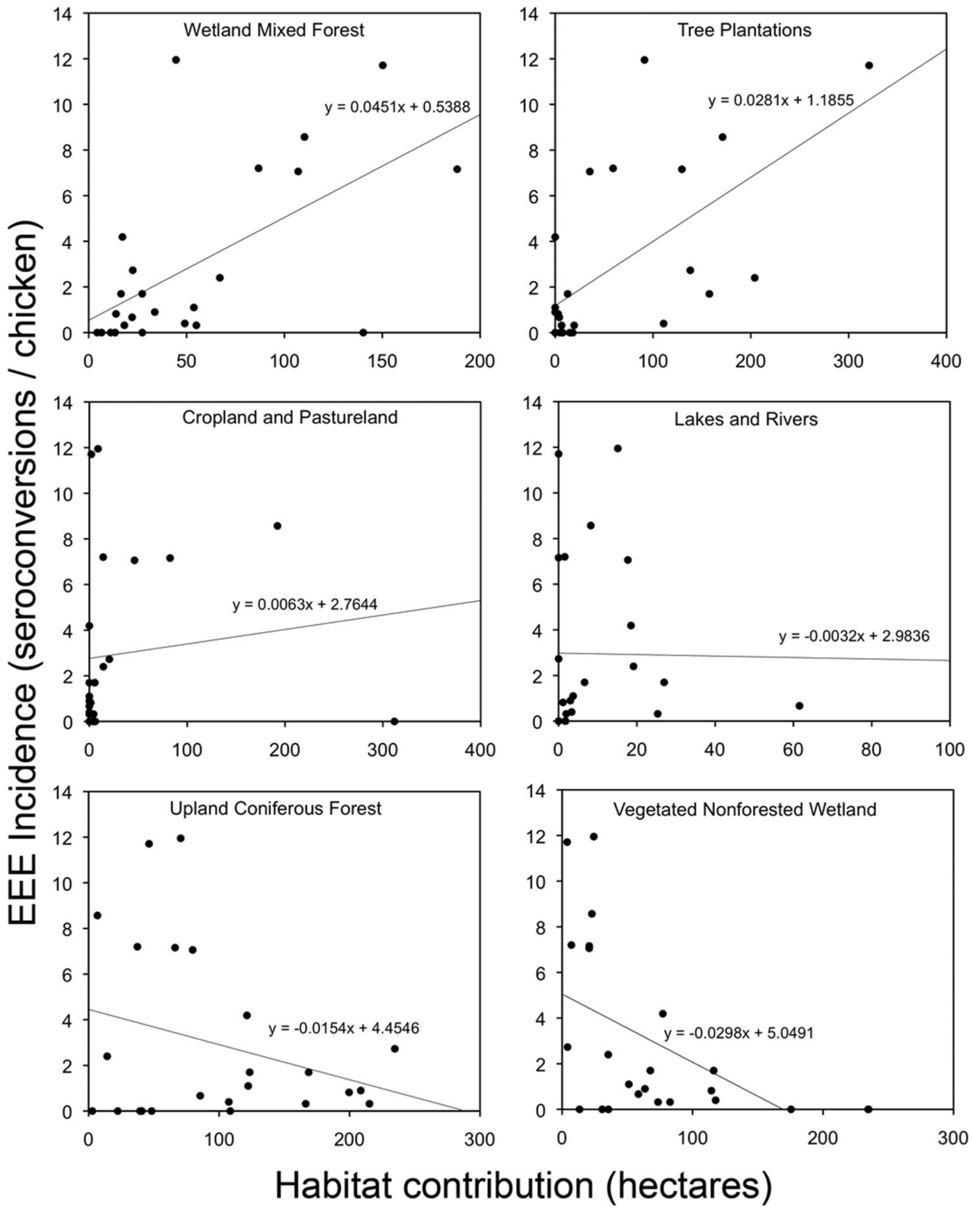

Fig. 4.

Scatter plots with best-fit lines and equation depicting the relationship between habitat contribution: X axis, acres of habitat present; Y axis, EEEV seroconversions in sentinel flocks.

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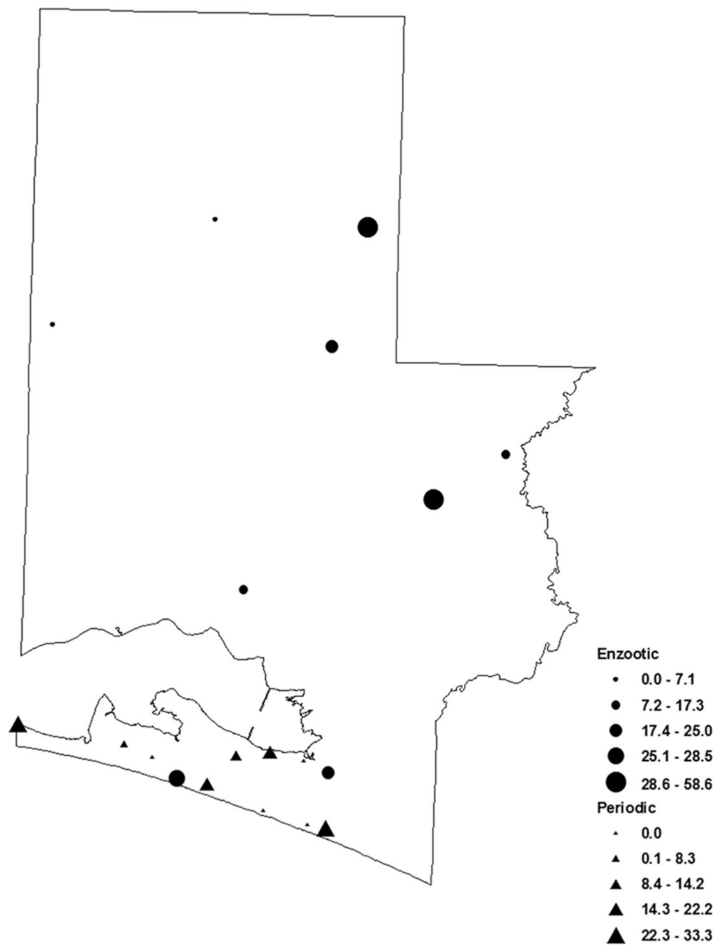

Fig. 5.
Seroconversion rates by site in 2009. Circles represent enzootic sites and triangles represent periodically enzootic sites. None of the sites classified as negative had any seroconversions in 2009.

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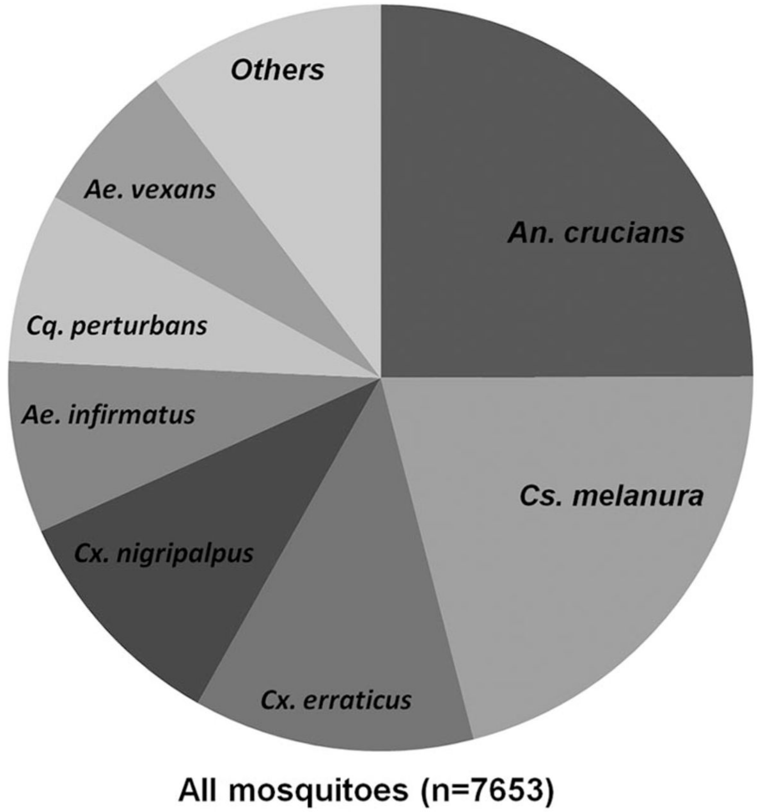

Fig. 6.
Total mosquito collections from May to August 2009, Walton County Florida.

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Table 1

Habitat and area composition of Walton County Florida

Habitat	Area (ha)	County %
Upland forest	70,973.36	26%
Wetland	63,751.75	23%
Tree plantations	48,089.20	23%
Cropland and pastureland	24,685.82	9%
Residential	11,077.86	4%
Other (x = 24)	48,045.49	15%
Total	283,164.21	100%

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Table 2

Analysis of habitats present at sites classified as enzootic, periodically enzootic, or negative for EEEV classifications

Land use description	Enzootic (mean area [ha])	Periodic (mean area [ha]))	Negative (mean area [ha])	H	*P* (df = 2)
Cropland and pastureland	41.40	2.02	65.22	8.57	0.014
Lakes and streams	7.54	14.63	0.69	5.54	0.063
Mixed rangeland	30.48	31.12	6.14	2.25	0.325
Residential	63.72	80.17	80.84	1.99	0.369
Tree plantations	122.77	36.13	9.18	7.12	0.028
Upland coniferous forest	92.56	128.58	44.37	5.12	0.077
Upland hardwood forest	70.25	27.95	11.01	4.28	0.118
Vegetated nonforested wetland	27.63	72.73	142.46	9.0	0.011
Wetland coniferous forest	38.66	70.98	172.73	4.1	0.129
Wetland forested mixed	83.94	34.09	38.47	5.26	0.072
Wetland hardwood forest	29.18	9.57	2.88	1.17	0.557

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Table 3

Sentinel EEEV seroconversion rates per year of the study

Year	EEE-positive chickens	Total no. susceptible chickens	Seroconversion rate (%)
2005	96	348	27.6
2006	20	250	8.0
2007	37	109	3.7
2008	25	254	9.8
2009	64	342	18.7
Total	242	1,303	18.5

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Table 4

Average mosquito collection per trap night per habitat type from May through Aug. 2009

Species	Residential	Tree plantation	Wetland forest	Wetland forested mixed	Upland forest
Ae. albopictus	0.12	0.1428	0.209	1.166	0.059
Ae. infirmatus	0.75	13.14	0.175	3.44	0.198
Ae. vexans	0.41	14.57	0.2975	2.88	0.112
An. crucians	1.08	6.28	0.429	2.5	0.68
Cq. peturbans	0.589	2.14	0.517	1.166	0.209
Cs. melanura	1.705	0.428	0.824	0.77	0.72
Cx. erraticus	0.3	1.28	0.443	3.27	0.424
Cx. nigripalpus	0.933	0	0.941	0	0.345
Cx. quinquefasiatus	0.187	0.142	0.078	0	0.066
Ps. ferox	0.125	1.57	0.117	0	0.101
Other	0.098	0	0.239	0.833	0.069