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Chapter

Survey for a Vector of Zika Virus and Two Other Mosquito Species in Four Ecoregions of Missouri: An *A Posteriori* Analysis

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Abstract

In 2015, Zika emerged as a vector-borne disease in the Americas, causing a variety of health issues ranging from Guillain-Barre syndrome in adults to microcephaly in newborns. Following the documentation of mosquito-borne transmission of the disease in the southern United States, the Missouri Department of Health and Senior Services contracted with researchers at Missouri State University to complete a survey of possible mosquito vectors of the Zika in the state. The primary vector of the disease, *Aedes aegypti*, had been reported from Missouri in previous surveys from several decades ago, but a comprehensive survey of the state mosquitoes and never been completed. Researchers focused on mosquitoes that spend the immature stages in artificial containers because this is descriptive of the most important Zika vectors. The large survey over three years provided an opportunity for post hoc analysis of mosquito occurrence data across a variety of ecoregions inside the state, documenting changes in the vector populations as a result of invasive species. The survey also allowed an analysis of different trapping techniques for important species in the state. The results are reported in this chapter along with a discussion of the potential impact on human health of changes to the mosquito population.

Keywords: mosquito traps, *Aedes albopictus*, Missouri, ecoregion

1. Introduction

Following the 2015 emergence of the Zika virus in Brazil, the virus rapidly spread through much of the Americas. Although historically associated with a relatively mild, self-limiting disease, the modern pandemic was linked to the severe manifestations of Guillain-Barre syndrome in adults and microcephaly in babies born to infected mothers [1]. Primarily mosquito-borne, the virus is unusual in that it can also be transmitted between humans sexually. Most cases detected in the United States were associated with travel to infected areas and some perhaps by sexual transmission; however, several cases of mosquito-borne Zika virus were reported in Puerto Rico and other American territories. In 2016, probable mosquito borne transmission involving the mosquito *Aedes aegypti* was reported in Florida [2].

The introduction of the Zika virus into North America and reports of mosquito-borne transmission in 2016 prompted public health officials in the state of Missouri to investigate potential vectors of this virus in the state. Previous mosquito surveys in the state were old and tended to cover only small geographic areas. In addition, many changes to the mosquito fauna had occurred with the introduction of invasive species thus increasing potential for disease transmission, so an extensive survey of mosquitoes associated with artificial containers was initiated in the summer of 2016, then continued in the summers of 2017 and 2018. Due to concerns about the Zika virus and the potential for local transmission, state public health officials focused the survey on mosquitoes that inhabit artificial containers during the larval and pupal stages in areas, especially those near human habitation or businesses. This focus was justified by the fact that the primary vector of the Zika virus in the Western Hemisphere is *Aedes aegypti*, a mosquito that is well known for developing in artificial containers near human habitations and one that has historically been reported in Missouri [3]. In addition, there was some concern that the invasive species and vector of the Zika virus, *Ae. albopictus*, might be widely distributed in the state. This latter species was also associated with artificial containers during immature stages.

The survey emphasized automobile salvage yards, used tire dealerships and cemeteries because these environments have historically provided large numbers of container-inhabiting mosquitoes. A complete list of the species obtained in both adult and larval surveillance and species occurrence by county for the first two years of the survey is available in Claborn et al. [4]. Two important findings from that survey were the absence of *Ae. aegypti* and the ubiquitous presence of *Ae. albopictus*. The latter of these two findings confirmed a potential for vector-borne transmission of Zika virus in the state, though no such transmission has been confirmed at the time of writing for this chapter.

Due to the original purpose of the survey, the traps were not used in an experimental design specifically suitable for comparing effectiveness between trap types, such as the Greco-Latin Square design often used to compare trap efficacy [5]. We used an analysis of variance with a protected mean separation test to analyze all data for this study. The extensive survey provides an *a posteriori* opportunity to compare results of trapping potential vectors of Zika and other species using different trap types in Missouri. The comparison allows an analysis of how trap type may affect the results of a survey. In addition, the traps were used in a variety of Missouri ecoregions as described by Nigh and Schroeder [6]. There is no current data on the difference in mosquito fauna between ecoregions in Missouri.

The choice of trap and bait types has an obvious effect on the results of a mosquito survey. Numerous studies have demonstrated differing results of trap effectiveness. To date, most trapping studies in Missouri have relied largely on the use of the venerable Centers for Disease Control Miniature Light trap and its variations [3, 7]. Development of newer traps and baiting technologies provides the opportunity to obtain more complete knowledge of the species composition and abundance in the state as well as the effect of ecoregion habitat on the abundance of mosquito species.

2. Materials and method

2.1 Study areas

We chose the survey sites based on the theoretical range of the primary vector of Zika virus, *Ae. aegypti,* as described by the CDC [8]. The surveyed area including most of Missouri south of the Missouri River as well as a few places north of the river on the western side of the state near and inside Kansas City, MO. This large region included four ecoregions: the Central Dissected Till Plains, the Osage Plains, the Ozark Highlands and the Mississippi River Alluvial Basin [6]. Only two surveyed counties were in the Central Dissected Till Plains, both near Kansas City. Most of the surveyed region lies within the Ozark Highlands, a region south of the Missouri River and covered with heavily forested hills. The western part of the surveyed region includes part of the Osage Plains region, a fertile prairie land with several streams and rivers. The southeastern portion of the surveyed region (the "Bootheel" of Missouri) includes parts of the Mississippi Alluvial Plain and is bordered by the Mississippi river. It supports large tracts of agriculture, including rice, soybean and cotton crops. The counties included in the survey (as well as the ecoregion for each) are depicted in **Figure 1**. Due to interest in potential vectors of human disease, the survey focused on locations near human habitations with many artificial containers, especially automobile salvage yards, used tire shops, and cemeteries. We also collected larvae from these sites and those data will be reported elsewhere. We re-surveyed some sites as many as six times during three summers. Trapping occurred between June 3 and September 23 in 2016, between July 17 and October 29 in 2017, and between June 19 and August 19 in 2018.

We used three types of traps: the Fay-Prince Omnidirectional trap, the BG Sentinel trap and the Centers for Disease Control miniature light trap. All traps were baited with approximately five pounds of dry ice in a plastic cooler with a hole in the bottom to let the gas disperse, but the BG Sentinel trap also used a

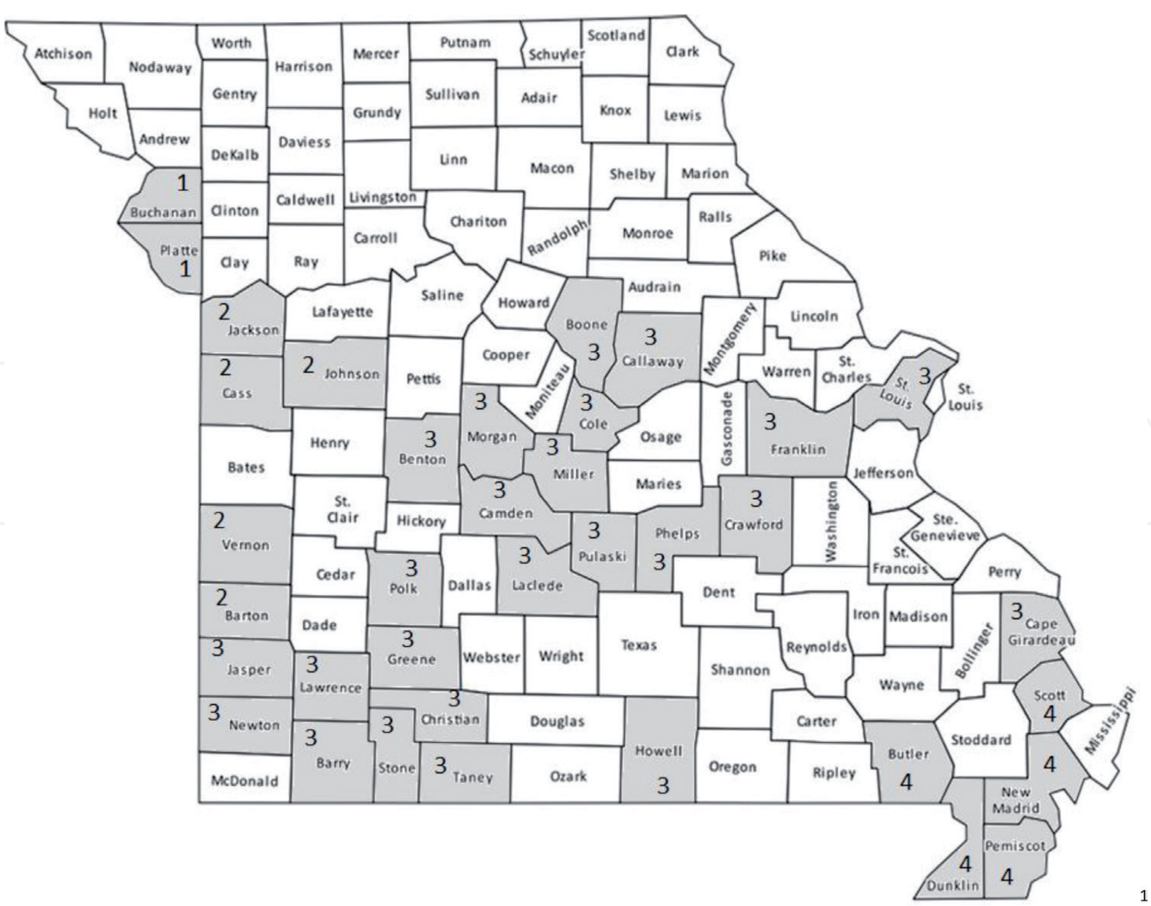

Figure 1.
Missouri counties where mosquito survey was performed are shaded gray (summers 2016–2018) (counties noted with a 1 are in the dissected till plains ecoregion. Those noted with a 2 are in the Osage Plains Ecoregion. Counties noted with a 3 are in the Ozarks Highlands ecoregion and those noted with a 4 are in the Mississippi River Alluvial Plain ecoregion).

commercial lure (BG Lure) as it was designed to do. We placed the traps with baits on the sites in early afternoon and retrieved them in late morning. All traps were at least 50 paces apart from other traps. Trap contents were placed in a large cooler with a small amount of ice, then transported to the laboratory in Springfield, Missouri, where all mosquitoes were killed by freezing. Laboratory workers then pinned the female specimens and identified them microscopically using dichotomous keys in Darsie and Ward [9] and Burkett-Cadena [10].

2.2 Analysis

Data points consisted of the number of mosquitoes from each of the three species caught in a given trap on a given date. The unit of trap-night is used to describe the number of traps over the number of nights used to survey for each site.

Trap type	**n**	*Aede albopictuss*	*Culex eraticus*	*Anopheles quadramaculatus*
BG Sentinel[1]	57	55.24	1.54	18.18
		28.10a[2]	0.47a	1.04a
Fay-Prince Omnidirectional	271	24.66	5.83	27.6
		12.15b	1.32a	3.07a
CDC Light trap	325	11.75	3.40	10.20
		2.90c	0.84a	1.24a

[1]All traps except BG Sentinel traps were baited with approximately five pounds of dry ice in a plastic cooler with a hole in the bottom. The BG Sentinel traps were baited with dry ice and a commercial attractant (BG lure).
[2]Data were transformed as the square root of (x + 0.1). The means in the lower position of each couplet are the back-transformed means of the transformed data. When means in a column are followed by the same letter, the means of transformed data are not statistically different (Tukey's mean separation test; alpha = 0.05).

Table 1.
Arithmetic mean (upper value of each couplet) and back transformed mean of transformed trapping rates of three abundant mosquito species caught in three types of trap in southern Missouri (2016–2018).

Ecoregion	**n**	*Aedes albopictus*	*Culex eraticus*	*Anopheles quadramaculatus*
Mississippi River Alluvial Basin	53	24.03	6.13	36.15
		8.54a[1]	2.79a	12.22a
Osage Plains	54	29.83	5.11	19.04
		5.66ab	1.04ab	0.96b
Ozark Highlands	207	4.60	2.35	1.68
		1.6b	0.46b	0.26b
Central Dissected Till Plains	9	0.56	0.10	0.56
		0.31ab	<0.01	0.18b

[1]Data were transformed as the square root of (x + 0.1). Statistical analysis was done on transformed data but the means reported here are the arithmetic means of the original data and the back-transformed means of the transformed data. Means of transformed data (represented by backtransformed means) within each column followed by the same lower case letter are not significantly different (Tukey's mean separation test; alpha = 0.05).

Table 2.
Arithmetic mean (upper value of each couplet) and back-transformed mean of trapping rates (mosquitoes/trap-night) for three species caught in CO_2-baited CDC traps in four ecoregions of southern Missouri (summer, 2016–2018). The value in the lower position of each couplet is the back-transformed mean of the transformed data for trapping rate.

The survey consisted of a total of 653 trap-nights. The number of trap-nights for each trap type and ecoregion combination is reported in **Tables 1**–**4**. The data did not display a normal distribution and many data points reflected no catch ("0"); therefore, all data were transformed by taking the square root of (x + 0.1) where x was the number of mosquitoes of a given species caught in a single trap. This transformation appeared to improve the distribution of the data. For instance, the non-transformed data for *Ae. albopictus* caught in the CDC light trap had a skewness score of 2.92 and a kurtosis score of 11.23. After transformation, those scores were reduced to 1.21 and 1.01, respectively. Both transformed scores are below the recommended maximum thresholds recommended by West [11]. We used transformed data for all subsequent analyses and reported the results with back transformed

Ecoregion	n	*Aedes albopictus*	*Culex eraticus*	*Anopheles quadramaculatus*
Mississippi River Alluvial Basin[1]	44	33.20	8.68	153.12
		17.54a	2.12a	51.60a
Osage Plains	43	31.79	2.55	0.28
		15.11a	0.80a	0.13b
Ozark Highlands	161	21.81	6.59	5.41
		10.70a	1.51a	0.67b
Central Dissected Till Plains	21	13.61	0.23	0.42
		8.02a	0.07a	0.16b

[1]*Data were transformed as the square root of (X + 0.1). Statistical analysis was done on transformed data but the means reported here are the arithmetic means of the original data and the back-transformed means of the transformed data. Means of transformed data within each column followed by the same lower case letter are not significantly different (Tukey's mean separation test; alpha = 0.05).*

Table 3.
Arithmetic mean (upper value of each couplet) and back-transformed mean of trapping rates (mosquitoes/trap-night) for three species caught in CO_2-baited fay-prince omnidirectional traps in four ecoregions of southern Missouri (summer, 2016–2018). The value in the lower position of each couplet is the back-transformed mean of the transformed data.

Ecoregion	n	*Aedes albopictus*	*Culex eraticus*	*Anopheles quadramaculatus*
Mississippi River Alluvial Basin[1]	17	60.7	1.29	60.41
		44.24a	0.40a	6.66a
Osage Plains	15	84.27	3.33	0.26
		45.32a	1.32a	0.14a
Ozark Highlands	25	34.12	0.64	0.16
		12.9a	0.18a	0.04a

[1]*Data were transformed as the square root of (X + 0.1). Statistical analysis was done on transformed data but the means reported here are the arithmetic means of the original data and the back-transformed means of the transformed data. Means of transformed data within each column followed by the same lower case letter are not significantly different (Tukey's mean separation test; alpha = 0.05).*
[2]*The BG Sentinel trap was not used in the Dissected Till Plans during any of the three years of the survey.*

Table 4.
Arithmetic mean (upper value of each couplet) and back-transformed mean of trapping rates (mosquitoes/trap-night) for three species caught in BG sentinel traps in three[2] ecoregions of southern Missouri (summer, 2016–2018). The value in the lower position of each couplet is the back-transformed mean of the transformed data.

means. Because trap catches for each trap type were significantly different for at least one species, we analyzed the trap catches by ecoregion separately for each trap type. We used the data for the most abundant species from each of three genera for the comparison: *Anopheles, Aedes* and *Culex*. These were also the three most abundant species in the entire survey regardless of genus. We calculated the mean trap catch for each species by ecoregion using an unbalanced analysis of variance in the PROC GLM of SAS, with mean separation using a Tukey's HSD test.

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3. Results

Table 1 displays the means of trapping rates for three trap types over all three summers for the three most abundant species, one from each of three genera. Due to the non-normal distribution and the large number of traps with no adult mosquitoes (zeroes), the back-transformed means of the transformed data are also reported. Analysis indicates a statistically significant difference in the trapping rates only for *Ae. albopictus*, with the BG Sentinel using the BG Lure capturing the most mosquitoes. The Fay-Prince Omni Directional trap captured fewer than did the BG Sentinel, but more than the CDC Light trap, with all comparisons of traps for this species being statistically significant at the 0.05 probability level. The latter two traps used a carbon dioxide attractant only. For the other two species compared in this study, statistically significant differences in trap rates between traps were not detectable, though the arithmetic means for both *Cx. eraticus* and *An. quadramaculatus* were highest for the Fay-Prince trap.

Tables 2–**4** display the trapping rates for all three summers by ecoregion, with each table reporting rates for one type of trap. **Table 2** reports the trap rates for the CDC miniature light trap with CO_2 bait. The CDC trap demonstrated a consistent difference in trap rates between the Mississippi River Alluvial Plain and at least one other ecoregion across all three species. The trap rate for *An. quadramaculatus* was the only one for which the Alluvial Plain was different from all other ecoregions; however, the trap rate for at least one ecoregion was different from that of the Alluvial Plain in all three species. **Table 3** displays the trapping rates for all Fay-Prince Omnidirectional traps across all three years. Unlike the CDC trap, the Fay-Prince did not demonstrate significant differences in trapping rates between ecoregions for two of the species: *Ae. albopictus* and *Cx. eraticus*. For *An. quadramaculatus*, however, a significant difference was noted in trap rates in the Mississippi Alluvial Plain and all three of the other ecoregions. **Table 4** displays the trapping rates for the BG-Sentinel trap using BG-Lure. None of these traps were used in the Central Dissected Till Plains ecoregion, so only three ecoregions were compared. No statistical differences in transformed trapping rates were detected between ecoregions for any of the three species as measured by the BG-Sentinel trap, despite very large differences in the arithmetic mean, reflecting great variation even in the transformed data. The means were somewhat similar between ecoregions for *Ae. albopictus*, as they were for the Fay-Prince trap; however, the means were widely separated for *An. quadramaculatus*. It should be noted that the number of trap-nights for Fay-Prince and CDC traps was five to six times that of BG Sentinel. The difference in findings suggests that the BG Sentinel were probably under-utilized in comparison to the other traps and sample numbers were probably insufficient.

The most productive trap for *Ae. albopictus* was the BG Sentinel. For the other two species compared here, no significant differences in average trap catch between trap types were apparent, though the Fay Prince trap demonstrated the highest arithmetic mean for both.

4. Discussion

This study suggests that the choice of traps affects conclusions about relative species abundances in different ecoregions. Though general conclusions by arithmetic mean are similar, detection of statistically significant differences in abundance may be dependent on trap type and is highly dependent on sample size. In this survey, there was an obvious difference between mosquito abundance, especially for *An. quadramaculatus*, in the Mississippi River Alluvial Plain and the other ecoregions, and this conclusion was consistent across trap types. Two trap types suggested higher abundance of *Ae. albopictus* in the Osage Plains ecoregion, though these differences were not statistically significant.

This *post hoc* analysis of trapping data confirms earlier studies demonstrating high trap effectiveness for the BG-Sentinel trap for *Ae. albopictus*, though the Fay-Prince Omnidirectional trap had somewhat similar results. This study suggests that the BG Sentinel is suitable for continued surveillance of container-inhabiting mosquitoes in Missouri, though it probably provides a disproportionate estimate of relative *Aedes* abundance. This finding will be important when interpreting survey results for *Ae. albopictus* and other vectors of Zika virus.

This survey is the largest mosquito survey in Missouri to date. It covered a much larger geographical area than any previous study and is the only one to include four different ecoregions. The survey utilized a variety of trap types. It does have several weaknesses. First, it was not originally designed as a comparison of different trap efficacies, but was instead a *post hoc* analysis of available data. In addition, due to the focus on potential vectors of Zika virus, the choice of surveillance sites emphasized habitats associated with artificial container-inhabiting species near human habitation and thus collected *Aedes* species in disproportionate numbers. Also, some of those sites were in urban habitats that may have masked some of the effect of ecoregion. Finally, the traps were not randomly assigned to sites and were at times placed in the exact same spot repeatedly over the trapping seasons. Finally, sample sizes varied greatly between the three trap types and were probably insufficient for at least one type, the BG Sentinel. Nevertheless, this survey provides consistent estimates of relative mosquito abundance by ecoregions and provides some evidence of trap type efficacy by species.

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