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Monitoring Early Season Mosquito and Bird Populations: Implications for West Nile Virus in Lancaster County, Nebraska

Cover Page Footnote

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1. Introduction

Interest in mosquito biology has increased over the last several years largely because of the introduction of West Nile virus (WNV) to North America (Lanciotti et al. 1999). Since the virus was first reported in the United States in 1999, at least 1131 people have died from West Nile encephalitis and/or meningitis and thousands more have contracted non-fatal West Nile fever (Centers for Disease Control and Prevention 2009).

Many states have implemented monitoring programs to document outbreaks of WNV. In Nebraska, the WNV monitoring program is conducted by the Department of Health and Human Services (HHS), which collects and tests mosquitoes for the presence of WNV to assess the risk to humans throughout the state. The HHS concentrates its sampling efforts on the late summer and early fall months because most human cases of WNV occur between July and October (Hayes et al. 2005). However, the mosquitoes that transmit WNV are abundant before human WNV infections occur. Therefore, we were interested in monitoring mosquito population levels and prevalence of WNV in the mosquito community during the late spring and early summer. *Culex tarsalis* is the most important vector of WNV in the majority of states west of the Mississippi River (Hayes et al. 2005); therefore, it was chosen as a focal species for the study. However, some other common mosquito species are capable of transmitting the virus, particularly other mosquitoes of the genus *Culex* (Turell et al. 2005), so we also monitored populations of other species. Because birds are the main reservoirs for the virus (Komar et al. 2003, Ezenwa et al. 2005, Hayes et al. 2005), the composition of bird communities during the early WNV season was another component of the study.

2. Methods

Six locations for collecting mosquitoes and conducting bird surveys were chosen, according to habitat type, in and around the city of Lincoln in Lancaster County, Nebraska (Figure 1). Two sites were located in residential areas of Lincoln. Urban Site A was located near the intersection of North Hazelwood Drive and Sycamore Drive. Urban Site B was located near the corner of 44th Street and Sherman Street. Two sites were agricultural areas located east of Lincoln. Agricultural Site A was located along a fencerow between a pasture and a horse corral. Agricultural Site B was in a small wetland area in the middle of a dryland cornfield. The final two sites represented native grassland ecosystems. Prairie Site A was located in Nine Mile Prairie, a University of Nebraska-owned property located on the west side of Lincoln. Prairie Site B was in Spring Creek Prairie, located southwest of Lincoln near the town of Denton and managed by the Audubon Society of America.

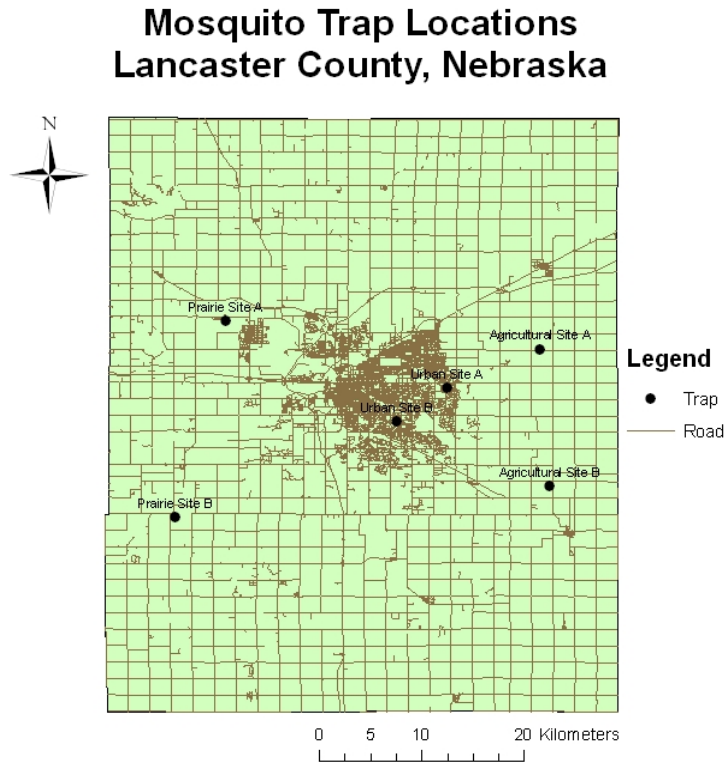


Figure 1. Map of Lancaster County, Nebraska with locations of trap sites.

Coordinates (NAD83):

Prairie Site A	N 40°52'3.5", W 96°48'26.0"
Prairie Site B	N 40°41'40.4", W 96°51'5.8"
Agricultural Site A	N 40°50'35.8", W 96°31'54.6"
Agricultural Site B	N 40°43'20.4", W 96°31'24.8"
Urban Site A.....	N 40°48'30.7", W 96°36'47.7"
Urban Site B.....	N 40°46'45.7", W 96°39'27.5"

We placed the traps in shaded areas near woody vegetation at all sites. At least one large pool of standing water was located within 200 meters of each trap at the agricultural and prairie sites. Large, permanent or semi-permanent pools of standing water were not visible near the urban sites, indicating that mosquitoes in those areas were most likely breeding in small, temporary pools, such as in discarded tires, which were abundant in a lot adjacent to Urban Site B.

We captured mosquitoes using standard CDC light traps (John Hock[®] model 512), which attracted mosquitoes using a light bulb and a plume of carbon dioxide (CO₂) calibrated to be released from the tank at the rate of 50 mL/min. Each trap

had a light-sensitive switch, which at dusk turned on the bulb, CO₂ plume, and a fan to pull in and hold the catch. At dawn, the plume and the light switched off, but the fan stayed on to keep the mosquitoes from flying out of the trap. We trapped six nights per week from May 14 to July 11, 2007. We collected the mosquitoes early each morning after a trap night and then reset the traps. We sampled three sites, one of each habitat type, for three consecutive nights before moving the traps to the other three locations.

We freeze-killed the mosquitoes with dry ice, sorted them according to species and catch location, and counted them. We counted only female mosquitoes. Mosquitoes that were too damaged to identify were placed in an “unknown” category. We sorted *Culex* mosquitoes into pools to test for presence of WNV. We pooled *C. tarsalis* separately from other *Culex* species. When few *Culex* were captured at a site, we combined all individuals captured during a three-night trapping period into the same pool. The maximum number of mosquitoes in a pool was 50.

We tested mosquito pools for the presence of WNV using the Rapid Analyte Measurement Platform (RAMP[®]) test method. The RAMP method returns a quantitative reading of RAMP units, which is based on a comparison of fluorescence emitted by control particles and fluorescence emitted by WNV-specific antibodies bound to a fluorescent latex (Burkhalter et al. 2006). We used a reading of 30 as the threshold for a positive pool as recommended by the manufacturer (Tom Janousek, Nebraska Department of Health and Human Services, personal communication). This threshold is higher than the previously recommended threshold of 15 (Burkhalter et al. 2006).

Using a RAMP threshold value, however, may not represent the true dynamics of WNV. It is likely that there are two underlying distributions—one for negative pools and one for positive pools—that describe the distribution of RAMP readings. We used maximum likelihood methods to fit two models to the data and estimated negative log likelihood values. One model was a normal probability distribution function with a single mean. The second model was two normal probability distribution functions with different means but equal variances. All RAMP readings had 0.05 added and were then log transformed before analysis. We used a Likelihood Ratio test to determine if a two-distribution model better described the data than a single-distribution model. We performed all analyses using the R statistical language (R Foundation for Statistical Computing 2007).

We conducted double-observer point counts once per three-day trapping period to obtain an index of the bird species at each of the six sites. To obtain representative samples across the varying landscapes of the agricultural and prairie/riparian sites, we placed the survey points 50 m apart in transects that passed through both grassland areas and the wooded areas where the traps were set. At the urban sites, limited space was available for placing the points in straight tran-

sects, so we spread the points as far apart as possible to avoid double-recording birds. All surveys were conducted within three hours of sunrise. The designated primary observer counted all of the birds he/she saw or heard in a three-minute period, while the secondary observer recorded the birds counted by both observers; then roles were reversed and birds were counted for another three minutes at the same point. We systematically varied the combinations of observers so that all observers visited each site approximately the same number of times over the course of the study.

3. Results and Discussion

Mosquito Trapping

We captured 12,325 female mosquitoes during the study period, representing 20 species. Agricultural Site A produced the most mosquitoes (3812), followed by Prairie Site A (2931), Urban Site B (2498), Prairie Site B (1880), Agricultural Site B (937), and Urban Site A (267). The three most numerous species—*Aedes vexans*, *Ochlerotatus trivittatus*, and *C. tarsalis*—made up 58.2%, 12.4%, and 11.7%, respectively, of all the mosquitoes captured during the study. No other single species accounted for more than 3% of the total catch.

Culex tarsalis catch numbers peaked very early in the study period and then generally declined, with a few spikes in catch numbers at several dates and locations (Figure 2). The number of *C. tarsalis* caught at Prairie Site A peaked on May 22, with 194 mosquitoes. At the same site, we only caught three *C. tarsalis* on May 23; however, we caught 90 *C. tarsalis* on May 24. After June 11, we never caught more than two *C. tarsalis* per night at this site. May 20 was the peak night at Prairie Site B, with 60 *C. tarsalis* captured. After May 20, the number of *C. tarsalis* in the trap at this location was never above 10 individuals. The numbers of *C. tarsalis* caught at the other sites also peaked in May and then fell to very low levels in June and July. At Agricultural Site A, we did observe an increase in *C. tarsalis* on July 5, which was the last night that we trapped at the site. We caught 23 *C. tarsalis*, which was the greatest catch since we had trapped 27 on June 10. The *C. tarsalis* catch at Urban Site B increased every night during the first trapping period and peaked on May 21 with 322 mosquitoes. After May 21, catches of *C. tarsalis* at Urban Site B were very low, with a peak of 22 mosquitoes on June 12.

Aedes vexans was the most abundant species, but it was relatively uncommon during the first half of the study period (Figure 2). *Aedes vexans* catch numbers peaked from mid-June to early July at five of the sites. The dates for peak *A. vexans* catches were June 21, June 26, July 8, June 17, and July 9 at Prairie Site B, Agricultural Site A, Agricultural Site B, Urban Site A, and Urban Site B, respectively. Prairie Site A was the exception, with the *A. vexans* catch peaking

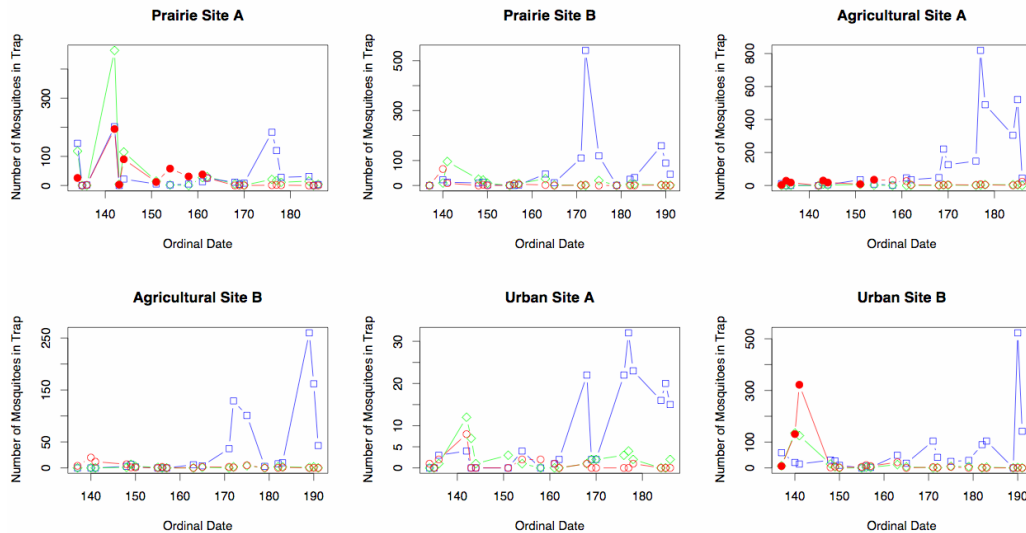


Figure 2. *Aedes vexans* (squares), *Culex tarsalis* (circles), and *Ochlerotatus trivittatus* (diamonds) caught each trap night from May 14–July 11, 2007. Solid circles represent days when potentially WNV-positive *C. tarsalis* were captured.

on May 22. However, we also caught large numbers of *A. vexans* later in the study period at Prairie Site A. *Aedes vexans* was captured more than any other species at all sites during June and July.

At every site, the catch numbers of *O. trivittatus* peaked in late May and fell to relatively low levels for the rest of the study period (Figure 2). The maximum *O. trivittatus* catches were noted on May 22 at Prairie Site A, May 23 at Agricultural Site A, May 22 at Urban Site A, May 21 at Prairie Site B, May 29 at Agricultural Site B, and May 20 at Urban Site B. *Ochlerotatus trivittatus* was the most abundant species caught at Prairie Site A, but 54.4% of the *O. trivittatus* caught at the site were collected on May 22 and 83.9% were collected between May 14 and May 31. Few *O. trivittatus* were collected at any site in June and July.

The three-night rotational trapping method was intended to provide a weekly index of mosquito population levels. Using several different types of traps at each location could possibly provide a more representative sample of the mosquito community due to specific differences in feeding behaviors and responses to stimuli (Takken 1999, Anderson et al. 2004, Bradford 2005); however, the trapping method did seem to be effective for collecting *C. tarsalis* as well as a range of other mosquito species. We expected to see changes in catch numbers from week to week, but we found that catches were also highly variable on consecutive trap nights. On many occasions, the number of mosquitoes in a trap was quite high after one night and very low after the following night, or vice versa. In these cases, we were likely observing changes in mosquito feeding patterns influenced by

environmental factors rather than dramatic changes in the local population size. If we had trapped continuously at every site during the study period, we would probably have observed even more of these fluctuations.

Feeding behavior of mosquitoes is influenced by weather; *C. tarsalis* typically feed most heavily during the warmest, driest hours of the night (Reisen et al. 1997). We found that cool and/or rainy conditions during trap nights consistently produced low mosquito catches. Temporal and weather-related fluctuations are consistent with results reported by others (e.g., Lungstrom 1954 in Kansas, Meece et al. 2003 in Wisconsin).

Bird Surveys

The urban sites proved to have the lowest diversities of avian species. We recorded 12 species at Urban Site A and 13 at Urban Site B during the study period. However, bird abundance was greater at these sites than at any of the others except Agricultural Site A. The three most common species observed at Urban Site A were the common grackle (*Quiscalus quiscula*), American robin (*Turdus migratorius*), and house sparrow (*Passer domesticus*). These three species made up 63.5% of the birds observed or heard at the site (Table 1). The most abundant species recorded at Urban Site B were the common grackle, blue jay (*Cyanocitta cristata*), and American robin. Together these three species accounted for 68.5% of all birds recorded at the site (Table 1).

The agricultural sites had a greater diversity of species, with different dominant species as well. Agricultural Site A had the greatest abundance of birds among the sites with 513 birds of 27 different species (Table 1). The common grackle was the most abundant bird recorded, followed by the red-winged blackbird (*Agelaius phoeniceus*) and the barn swallow (*Hirundo rustica*). Agricultural Site B produced the fewest total birds (374), and it was fourth in number of species (24). Dominant species included the red-winged blackbird, American robin, and bobwhite quail (*Colinus virginianus*).

Prairie Site B and Prairie Site A ranked first (29) and second (28), respectively, in number of species recorded (Table 1). At Prairie Site B, the three most common species were the red-winged blackbird, mourning dove (*Zenaida macroura*), and house wren (*Troglodytes aedon*). At Prairie Site A, the most numerous species were the dickcissel (*Spiza americana*), European starling (*Sturnus vulgaris*), and American robin. Although avian diversity was high at both prairie sites, we did not record as many total birds at these sites as we did at the urban sites and Agricultural Site A.

The avian surveys provided us with an indicator of possible reservoirs of WNV at each site. The dominant bird species at a given location can influence the potential for WNV to be transmitted to other birds and possibly humans. Some

Table 1. Birds observed at Lancaster County study sites.

Species	Total count per site						Total
	Urban Site A	Urban Site B	Agricultural Site A	Agricultural ¹ Site B	Prairie Site A	Prairie Site B	
American Crow	4	10	0	0	0	0	14
American Goldfinch	0	0	1	8	0	15	24
American Robin	81	53	26	43	21	17	241
Baltimore Oriole	0	0	11	9	7	10	37
Barn Swallow	0	0	51	4	2	1	58
Belted Kingfisher	0	0	0	0	1	0	1
Black-capped Chickadee	1	0	0	0	0	0	1
Blue Jay	52	98	7	13	15	9	194
Bobolink	0	0	3	0	0	0	3
Brown Thrasher	0	0	6	21	6	13	46
Brown-headed Cowbird	0	0	0	5	10	9	24
Canada Goose	0	0	0	0	12	0	12
Chimney Swift	15	19	0	0	0	1	35
Common Grackle	136	155	129	18	16	28	482
Dickcissel	0	0	4	0	30	25	59
Downy Woodpecker	0	0	0	2	1	5	8
Eastern Bluebird	0	0	0	0	0	1	1
Eastern Kingbird	0	0	21	6	8	13	48
Eastern Towhee	0	0	0	0	2	0	2
European Starling	5	30	37	0	22	3	97
Gray Catbird	0	0	1	10	1	2	14
Great Blue Heron	0	0	1	6	1	0	8
Green-winged Teal	0	0	0	0	1	0	1
House Finch	2	0	0	1	0	4	7
House Sparrow	53	47	3	0	0	0	103
House Wren	0	2	23	8	16	32	81
Killdeer	0	2	4	6	1	6	19
Mallard Duck	0	0	2	0	0	0	2
Mourning Dove	48	18	40	26	16	32	180
Northern Bobwhite	0	0	10	27	0	18	55
Northern Cardinal	26	9	4	10	14	4	67
Northern Flicker	0	0	1	0	9	1	11
Orchard Oriole	0	0	1	0	0	0	1
Red-headed Woodpecker	0	0	0	0	3	3	6
Red-tailed Hawk	0	0	1	0	0	0	1
Red-winged Blackbird	0	0	88	126	0	113	327
Ring-necked Pheasant	0	0	9	5	15	7	36
Rock Dove	2	0	0	0	0	0	2
Rose-breasted Grosbeak	0	0	2	6	0	1	9
Tree Swallow	0	0	0	2	12	19	33
Turkey Vulture	0	2	0	0	0	0	2
Western Meadowlark	0	0	27	9	7	3	46
White-breasted Nuthatch	0	2	0	0	1	2	5
Wild Turkey	0	0	0	0	2	0	2
Yellow Warbler	0	0	0	3	0	0	3
Total Birds	425	447	513	374	252	397	2408
Number of Species	12	13	27	24	28	29	45

avian species develop very high titers of WNV, making them more likely to infect mosquitoes (Hayes et al. 2005). Komar et al. (2003) experimentally infected 25 species of birds to evaluate their potential as reservoirs for the virus and calculated a competency index (C_i) for each species. Several of the most abundant species recorded during our surveys received high C_i values in the Komar et al. (2003) study, including the blue jay, common grackle, American crow, house sparrow, American robin, and red-winged blackbird. We observed competent reservoir species at all sites throughout the study period. However, the vector competences of some other common species, such as the house wren and barn swallow, are still unknown (Komar et al. 2003).

Only certain species of birds are competent WNV reservoirs (Komar et al. 2003); therefore, an abundance of species that are poor reservoirs may reduce the risk of humans becoming infected. Ezenwa et al. (2005) found that the proportion of WNV-infected *Culex* mosquitoes decreased as the number of non-passerine bird species increased and as abundance of non-passerine birds increased. High non-passerine species richness was also associated with fewer human cases of WNV (Enzenwa et al. 2005). Non-passerines were poorly represented at the urban sites in our study. The agricultural and prairie sites provided better habitat for other orders of birds, such as Anseriformes, Falconiformes, Galliformes, and Piciiformes. The bird communities at the urban sites appeared to be well suited for WNV amplification.

While we recorded more total birds at the urban and agricultural sites, this does not necessarily mean that more birds were actually present in those habitats. Our bird surveys did not take detectability into account. The hilly terrain and the thick vegetation at the prairie sites made visual and aural detections of birds more difficult than at the other locations. In addition, some species of birds are easier to see and/or hear than others due to their habits. The four observers may have differed in their individual abilities to detect birds, but we minimized observer bias by rotating all four observers equally through all of the sites.

RAMP[®] Analysis

We tested 95 pools of *Culex* for the presence of WNV using the RAMP method. Only one pool returned a positive reading. The positive pool contained 50 *C. tarsalis* collected at Prairie Site A on May 22. This particular pool produced a RAMP reading of 51.1. Sixty (63.2%) of the pools returned readings of 0.0, and 75 (78.9%) of the pools returned readings ≤ 1.0 (Figure 3). The remaining 19 pools returned readings that ranged from 1.3 to 20.4.

The single normal distribution probability function of the RAMP readings had a mean of -1.7 with a variance of 3.7. The two normal distributions in the mixture model had means of -2.8 and 1.1. The variance for both distributions in the mixture model was 0.62. The negative log-likelihood values for the normal distribu-

tion and mixture distribution models were -197.1 and -164.5, respectively. A Likelihood Ratio test found that two normal probability density functions significantly improved the likelihood of the model ($p \leq 0.001$). The mixture model divided the RAMP readings into two distributions (Figure 3). Of the 95 readings, 27 were placed into the distribution describing the positive pools. All of these pools returned a RAMP reading ≥ 0.5 , which, according to the model, are potentially positive pools. Twenty-two of the 27 pools were made up of *C. tarsalis*. The remaining five pools contained other *Culex* species, including *C. restuans*, *C. pipiens*, and *C. erraticus*. None of the pools from Prairie Site B or Agricultural Site B returned a high enough reading to fall into the second distribution. A pool of two *C. pipiens* from Urban Site A returned a potentially positive reading of 1.6.

The modeling results indicate that the threshold reading of 30 provided by the manufacturer may return a higher rate of false negatives when compared to mixture models. Further modification of the mixture model may be necessary to decrease the level of uncertainty about readings that fall below the threshold. The model assumed that both normal distributions had the same variance, but in reality, equal variance is unlikely.

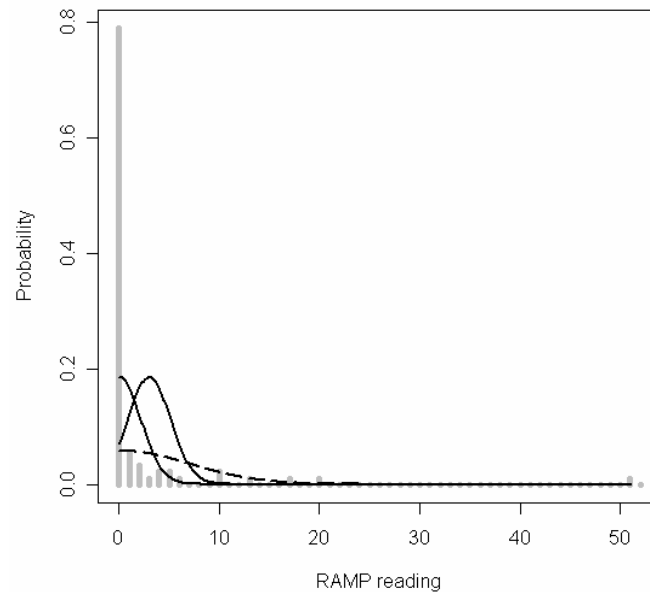


Figure 3. Probability distribution functions of RAMP readings with a single normal probability distribution function (dashed lined) and mixture model of two normal probability distribution functions (solid lines), along with observed distribution of RAMP readings (gray bars) from pools of *Culex* mosquitoes collected in Lancaster County, Nebraska from May 14-July 11, 2007.

Environmental Effects

Human cases of West Nile fever, meningitis, and encephalitis in Nebraska do not typically appear until late summer, and the reasons are not completely understood. The virus was present in the Lancaster county mosquito population in late May of 2007. The Nebraska Department of Health and Human Services (2007) reported the first WNV positive bird in Lancaster County on July 11. The first human case in Lancaster County was not reported until July 25. Other states have also detected WNV in birds before human cases were reported (McLean 2006). Kilpatrick et al. (2006) suggested that a late-summer switch in host preference from birds to mammals by *C. tarsalis* might account for increased WNV infection in humans.

Mosquito population dynamics are variable, but environmental conditions early in the season can influence the composition of species during the peak WNV season. Certain conditions favor some mosquito species more than others (Bradford 2005). Weather conditions early in the mosquito breeding season may be especially important in determining the risk of WNV. Abundant precipitation and warm temperatures in the spring produce a large mosquito population, especially of efficient vector species (DeGaetano 2005). Warm temperatures also lead to shorter transmission and amplification cycles for the virus (Dohm et al. 2002). Weather patterns of wet springs and hot summers are believed to be partly responsible for large WNV outbreaks in New York City in 1999 and in Colorado in 2003 and in the dispersal of WNV to previously uninfected areas (Marra et al. 2004, McLean 2006, Reisen et al. 2006). Cool summers have resulted in decreased or delayed WNV activity (Reisen et al. 2006).

4. Implications

Early season mosquito surveillance has a role in predicting future epidemics of WNV. The timing of peaks in the mosquito population, especially of efficient vector species like *C. tarsalis*, is an important part of the WNV transmission cycle. Weather is a key driving force behind WNV epidemiology because it alters the species compositions and abundances of mosquito communities, determines transmission times for the virus, and influences mosquito feeding behavior. Monitoring local bird communities can also be used to evaluate the risk of WNV. Further studies are needed to determine the initial source of WNV each year in temperate regions such as Nebraska. The methods used to capture and test mosquitoes should also be carefully analyzed to ensure that WNV-positive mosquitoes are accurately detected.

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