Published in final edited form as:

J Med Entomol. 2007 January ; 44(1): 117–125.

West Nile Virus Infection in Mosquitoes in the Mid-South USA, 2002–2005

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Abstract

West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) was first detected in the Tennessee Valley and in Alabama in August 2001. In summer 2002, intensive viral activity was seen, but in subsequent years, viral activity settled into an enzootic pattern. Here, we report an analysis of viral activity in the mosquito fauna in the Mid-South from 2002 (the first year viral activity was detected in mosquitoes) through 2005. Eight mosquito species were infected with WNV during 2002. However, viral activity was only detected in four species—*Culex salinarius* Coquillett, *Culex erraticus* Dyar & Knab, *Coquillettidia perturbans* Walker, and *Aedes vexans* Meigen—in multiple years. The greatest number of positive pools was in *Cx. erraticus* and *Cx. salinarius*. Despite being specifically targeted for collection, *Aedes albopictus* Skuse was only found to be infected during the epizootic year (2002), suggesting that under enzootic transmission conditions its role as a bridge vector in the region may not be significant. Virus-positive pools of *Cx. erraticus* were identified from winter-resting and early season dry ice-baited trap collections in 2005, implicating this species in WNV overwintering in Alabama. Molecular analysis of individuals initially identified as members of the *Culex pipiens* L. complex suggested that alleles characteristic of *Cx. pipiens* predominated in mosquitoes collected in Huntsville, AL, whereas alleles in the Auburn, AL, population were predominately characteristic of *Culex quinquefasciatus* Say. The southern boundary of the overlap zone of the two species seems to be located primarily between Huntsville and Auburn, a distance of 350 km.

Keywords

West Nile virus; Alabama; overwintering; *Culex*

West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) has spread rapidly across the United States and southern Canada since its introduction in New York in 1999 (Hayes et al. 2005), and it seems to be adapting to a variety of different habitats and bioclimates throughout the United States. Thus, it is likely that the ecological components of WNV transmission cycles will vary in different regions. In general, upon its introduction into a given region in North America, WNV has followed a similar pattern of introduction, amplification, and subsidence. The virus usually becomes apparent late in the season in its first year, and there is an epidemic

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pattern of transmission that occurs in the summer after the first year of introduction, with large numbers of avian and human cases reported. In the years after the epidemic year, viral activity declines and enters a more endemic pattern, with lower, but relatively stable levels of transmission and cases in vertebrate animals (Hayes et al. 2005).

One important variable in determining the dynamics of WNV transmission in different areas of the country is the ability of the virus to use local mosquito species as vectors that are unique or uncommon in other regions of the United States. Sixty species of mosquitoes have been shown to harbor WNV by various assays (CDC 2006b), of which roughly 20 have been shown to be competent vectors for the virus (Turell et al. 2005). Species belonging to the genus *Culex* are the major vectors for WNV in the United States (Turell et al. 2005), with members of the *Culex pipiens* L. species complex the major vector throughout the eastern portion of the United States (Andreadis et al. 2001, 2004; Godsey et al. 2005b; Loftin et al. 2006). In the continental United States, the *Cx. pipiens* complex consists of at least two species, with *Cx. pipiens* occurring in the north and *Culex quinquefasciatus* Say occurring in the south (Fonseca et al. 2004). The ranges of these two species overlap, with hybridization between the species occurring within the overlap zone (Smith and Fonseca 2004). It is thought that the southern boundary of this hybridization zone is near the northern border of the state of Alabama; however this has not been precisely determined.

WNV first was detected in Alabama in a red-tailed hawk collected in Birmingham, AL, on August 7, 2001. Virus continued to be detected in dead birds from that date through 23 October 2001, with a total of 33 birds confirmed as WNV infected. Beginning in early July 2002, intensive transmission of WNV was seen in both Alabama and the Tennessee Valley area, with laboratory confirmed WNV infection in 85 of 139 dead birds tested from when the first WNV positive bird was detected on 2 July 2002 through 26 September 2002. As part of a broad scale surveillance program in the Southeastern USA, mosquitoes were systematically collected throughout the Tennessee Valley over the period of 2002–2004 and examined for WNV. The Valley area spans seven states and is formed by a major river basin and its tributaries in the southeastern USA. The principal river in the valley, the Tennessee, is some 1,050 km long and flows through four states. These collections offered the opportunity to describe the progression of infection of WNV in rural mosquito species in the epidemic year of 2002, and continuing through 2003 and 2004, as the virus settled into an enzootic pattern of transmission. We have also conducted field studies that focused more narrowly on transmission dynamics of WNV in an urban/periurban setting where the mixture of mosquito species was representative of the region. To complement these studies, overwintering mosquitoes also were collected in February in Huntsville, AL, where WNV activity had been detected during the previous transmission season. Because some of the mosquitoes collected were identified using morphological characters as *Cx. pipiens/quinquefasciatus*, this material also was identified to species or hybrid level and evaluated for the presence of WNV. We report here the outcome of these studies and interpret these results in regard to the probable development of endemicity and transmission of WNV in the southeastern United States.

Materials and Methods

Tennessee Valley Collections

CDC light traps using a CO₂ attractant were placed at 64 sites in the Tennessee Valley, as described previously (Cupp et al. 2004). Locations stretched from the Kentucky Dam area near Gilbertsville, KY, eastward along major Tennessee Valley Authority (TVA) reservoirs through western Tennessee, northern Mississippi, and northern Alabama into eastern Tennessee. Areas sampled included flood plains and bottomlands subject to periodic flooding, permanent standing water habitats, and recreational facilities located along the river. Trapping was conducted from 29 May to 11 September 2002, from 24 May to 29 October 2003, and from

25 May to 26 October 2004. Mosquitoes were processed for species identification and virus detection as described previously (Cupp et al. 2004).

Alabama Collections

The studies conducted in the Tennessee Valley were carried out to detect region-wide WNV activity. This permitted us to identify the major mosquito species infected with WNV in the Mid-South and to monitor the shifts that occurred in infection in the mosquito fauna as the virus moved from an epizootic to an enzootic pattern of infection. However, these studies left several questions unanswered concerning the biology of the virus. For example, the number of mosquito species collected as part of the surveillance effort was relatively limited. Second, no attempt was made in these studies to classify mosquitoes belonging to the *Cx. pipiens/quinquefasciatus* complex to the species level. Finally, because collections in the TVA area commenced in mid-May and ran for 4–5 mo, the data could not be used to address the question of how the virus might overwinter in the region. To address these questions, detailed studies were conducted in Alabama during 2005, which involved the collection of overwintering mosquitoes as well as extensive collections of mosquitoes by using a variety of methods in an urban/periurban area in and around Auburn, AL. In the latter studies, emphasis was placed on collecting *Aedes albopictus* Skuse by using a species-specific method because, as described below, the results obtained from the TVA area in 2002 from a small number of individuals suggested that this species might be frequently infected with WNV late in the season. Recent laboratory studies also have shown that *Ae. albopictus* is a competent vector for WNV (Sardelis et al. 2002) and that it may serve as a significant bridge vector for the virus (Sardelis et al. 2002, Turell et al. 2005, Richards et al. 2006). Thus, it was of interest to collect relatively large numbers of this species to further evaluate its role as a potential bridge vector for WNV.

Overwintering mosquitoes were collected from storm drains and culverts in Huntsville, AL. Eight sites were sampled repetitively on 3, 17, and 25 February 2005 by using a backpack vacuum to collect resting adults. Live mosquitoes were transported to the Auburn University Vector Biology Laboratory, sorted on a cold plate, identified, and then frozen at -80°C for virus identification.

Trapping at the Auburn site began on 23 March 2005 in Auburn, AL, and continued through 30 September 2005. CDC light traps baited with CO_2 were placed in four locations on the Auburn University campus and in three nearby locations in a residential urban setting. The campus locations were adjacent to heavily wooded areas with standing water nearby. Each light trap was accompanied by three resting boxes placed in proximity to collect mosquitoes not attracted to light or CO_2 . Light traps were collected twice per week and resting boxes were vacuumed using the same schedule. Duplex cone traps designed specifically to collect *Ae. albopictus* (Freier and Francy 1991) also were used when this species first occurred in early May and continued to 30 September 2005. Traps were placed at four wooded locations in an urban area in which there were tires and other artificial containers.

Detection of West Nile Virus in Mosquito Pools

Mosquitoes were separated by date of collection, location, and species and combined into pools with each pool containing a maximum of 50 individuals. In the samples collected in the Tennessee Valley in 2002–2004 and in Huntsville in 2005, samples were tested for the presence of WNV by reverse transcription-polymerase chain reaction (RT-PCR), by using a modified version of a published protocol (Lanciotti et al. 2000). In brief, pooled mosquitoes were homogenized by adding 2 ml of M-199 culture medium and four copper-clad BBs to each tube and by mixing on a vortex mixer. The insoluble material was pelleted by brief centrifugation. RNA was extracted from 140 μl of the supernatant by using the QIAmp Viral RNA extraction kit (QIAGEN, Valencia, CA). The RNA was eluted into a final volume of 60 μl , and 2.4 μl of

this preparation was used as a template in an RT-PCR, by using the one-step RT-PCR reagents provided by QIAGEN. The RT-PCR solution contained 1× Qiagen RT-CR buffer, 0.4 mM each dNTP, 0.6 μM each WNV primer, and 1.2 μl of QIAGEN one-step RT-PCR enzyme mixture on a total volume of 30 μl. The primers used were WNc233 (5'-TTGTGTTGGCTCTCTTGGCGTTCTT-3') and WNnc640 (5'-CAGCCGACAGCACTGGACATTCATA-3'). Cycling conditions consisted of 50°C for 30 min, 95°C for 15 min, followed by 40 cycles consisting of 94°C for 30 s, 58°C for 20 s, and 68°C for 2 min. The reaction was completed with a single extension step consisting of 72°C for 10 min. Products were analyzed by electrophoresis on a 2% agarose gel. Reactions producing an amplicon of the expected size (408 bp) were scored as putatively positive. Putatively positive samples were retested using a second RT-PCR, with a different primer set (WNc9504: 5'-CACCTACGCCCTAAACACTTTCACC-3' and WNnc9815: 5'-GGAACCTGCTGCCAATCATACCATC-3'). The cycling conditions in the confirmatory RT-PCR were the same as those used in the initial PCR. Putatively positive samples that produced an amplicon of the expected size (312 bp) in the confirmatory assay were scored as confirmed WNV positive. The identity of these amplicons was further confirmed by direct DNA sequence analysis.

In the samples collected in Auburn in 2005, initial screening for WNV was conducted using the RAMP test (Response Biomedical, Burnaby, British Columbia, Canada). Extracts were prepared following the manufacturer's recommend protocols and were scored as putatively positive following the manufacturer's recommendations. Any sample that was scored as putatively positive was then confirmed using the two RT-PCR assays described above.

Calculation of Infection Rates

Minimum infection rates were calculated by assuming that each positive pool contained a single infected mosquito, following standard procedures (Nasci and Mitchell 1996). Point estimates and the associated 95% confidence intervals (CIs) for the actual rates of infection were determined using the nonequal pool size algorithms contained in the computer package Poolscreen, version 2.0 (Barker 2000). Poolscreen, version 2.0 is available from Dr. Charles R. Katholi at the University of Alabama at Birmingham (ckatholi@uab.edu). The Bayesian estimates provided by Poolscreen are reported here, because recent simulations have suggested that these represent the most accurate estimates of the infection rate and the associated 95% confidence interval (unpublished data). Upper bounds for the 95% confidence interval for species in which no infected pools were identified also were calculated using the unequal pool size algorithm in Poolscreen, version 2.0. Upper bounds for the prevalence of infection in species for which no positive pools were found were only determined for species for which >1,000 individuals were collected, because confidence intervals calculated on samples less than this are too wide to be meaningful (Katholi and Unnasch 2006).

Allelic Analysis

Mosquitoes collected in Auburn and Huntsville and initially identified as *Cx. pipiens/quinquefasciatus* were further characterized using molecular assays based upon polymorphisms in the *ace 2* gene (Aspen and Savage 2003). In brief, DNA was prepared using a commercial kit (DNAeasy, QIAGEN) from a severed leg of each mosquito identified as *Cx. pipiens/quinquefasciatus* complex by morphological criteria. The resulting DNA was employed as a template in PCR amplifications targeting a portion of the *ace 2* gene, by using previously published protocols (Aspen and Savage 2003). The resulting amplicons were analyzed by agarose gel electrophoresis, and the allelic pattern of each individual was recorded.

Results

WNV Infection in Mosquitoes in Tennessee Valley, 2002–2004

In total, 42,451 mosquitoes were collected during 4.5 mo of sampling in the Tennessee Valley in 2002. West Nile virus was detected in 25 pools of mosquitoes collected during this period. The initial detection was made from pools of *Ae. albopictus* Skuse and *Culex erraticus* Dyar & Knab collected on 24 July 2002 from the Big Sandy Wildlife Management Area near Paris, TN, and the Tennessee National Wildlife Refuge near Hustburg, TN, respectively. Both of these areas are located on Kentucky Lake in northwestern Tennessee. The virus was detected a week later at Benton, KY, in two pools of *Culex salinarius* Coquillett. The linear distance between the two farthest points where positive pools were initially seen (Benton, KY, and Hustburg, TN) is ≈ 140 km. Viral activity was then recorded during the following 2-mo period in northwestern Alabama (Muscle Shoals in Colbert County and Red Bank in Lawrence County). The last positive pool in 2002 came from mosquitoes collected on 18 September 2002 in the Muscle Shoals, AL, area and in Benton, KY.

Following the high level of transmission in 2002, the numbers of positive pools dropped precipitously during 2003 and 2004. In 2003, three pools were WNV positive (single pools of *Aedes vexans* Meigen, *Coquillettidia perturbans* Walker, and *Psorophora ferox* Humbolt), from a total of 44,147 mosquitoes collected over a 6-mo period. WNV-positive collections were made on 18 June 2003 from Brown's Creek in northern Alabama and the Tennessee National Wildlife Refuge. A third positive pool was detected from mosquitoes collected on 12 August 2003 from Brown's Creek. In 2004, a single positive pool (of *Cq. perturbans*) was detected from a total of 48,029 mosquitoes collected over a 6-mo period. That pool came from mosquitoes collected on 10 August 2004 near Kentucky Dam in western Kentucky.

From 2002 to 2004, WNV was detected in nine species of mosquitoes collected in the Tennessee Valley, of 12 species for which >100 individuals were collected. Minimum infection rates (MIRs), point estimates of the actual rate of infection, 95% confidence intervals for the infection rates, and the upper bound of the 95% confidence interval for those species for which no infections were detected were calculated as described in Materials and Methods (Table 1). The most commonly infected species were *Cx. erraticus* and *Cx. salinarius*. *Cx. erraticus* was the most commonly collected species during the 3-yr period, representing 49% of total number of mosquitoes collected. Ten positive pools were seen in this species in 2002 (Table 1), with the overall infection rate calculated at 0.49 per 1,000 individuals in 2002 (95% CI 0.24–0.86 per 1,000), with a peak MIR of 2.0 per 1,000 in the second half of September 2002. *Cx. salinarius* composed 15% of the total number of mosquitoes collected and produced seven WNV positive pools in 2002 (Table 1). Semimonthly MIRs for this species in 2002 varied from 1.49 to 3.46 per 1,000, with an overall infection rate of 1.67 per 1,000 in 2002 (95% CI 0.61–2.94 per 1,000).

Ae. vexans and *Cq. perturbans* were found to be infected with WNV during multiple years (Table 1). *Cq. perturbans*, which composed 16% of all mosquitoes collected, was infected each year, with annual infection rates of 0.14, 0.25, and 0.09 per 1,000 respectively, and an overall infection rate over all 3 yr of 0.16 per 1,000 (95% CI, 0.02–0.33 per 1,000). *Ae. vexans* was infected in 2002 and 2003 with annual MIRs of 0.25 and 0.13 per 1,000 respectively, and an overall infection rate of 0.21 per 1,000 (95% CI, 0.01–0.46 per 1,000). This species composed 9% of the total collection.

Several other species were infected with WNV in single years. Pools of infected *Ae. albopictus*, *Anopheles crucians* Weidemann, *Anopheles quadrimaculatus* Say, and *Cx. pipiens/quinquefasciatus* were detected in 2002, whereas an infected pool of *Ps. ferox* was detected in 2003 (Table 1). The MIRs for some of these species (especially *Ae. albopictus*) very quite high,

although the 95% confidence intervals surrounding these estimates were very wide, as a result of the small number of individuals collected (Table 1).

Analysis of WNV Infection in Mosquitoes: Alabama, 2005

In total, 177 overwintering adult mosquitoes were collected and analyzed for WNV (Table 2). Seven species were identified in the overwintering collection, with *Cx. pipiens/quinquefasciatus* being predominant (Table 2). A single WNV-positive pool was detected in these mosquitoes by RT-PCR as described in Materials and Methods, in a sample of overwintering *Cx. erraticus* collected on 25 February (Table 2).

In total, 10,145 mosquitoes representing 29 species were collected in Auburn from 23 March through 29 September 2005 from light traps, cone traps and resting boxes. Eight positive WNV pools were detected among these collections. The collection data and infection rates are summarized in Table 3, whereas the temporal pattern of the mosquito collections are summarized in Fig. 1. *Ae. albopictus* was the most numerous species collected ($n = 3,073$; 30%), in keeping with the emphasis on collection of this species mentioned above. However, it was not found to be infected with WNV, even though the bulk of the collection was made when WNV was active in the local mosquito population. *Ae. vexans*, the second most abundant species ($n = 3,064$; 30%), yielded three positive pools. Interestingly, the first positive *Ae. vexans* pool was seen in mosquitoes collected on 23 March 2005, which was coincident with the beginning of the major population peak of this species early in the season (Fig. 1). *Ae. vexans*-positive pools also were collected on 13 April and 24 June. A single positive pool of *Cx. erraticus* was detected on 26 March 2005, which was substantially before the peak in the population of this species, which occurred in late July (Fig. 1). The remaining four positive pools came from *Cx. salinarius* and occurred from June through early August. Overall infection rates for the three infected species varied from 0.98 per 1,000 for *Ae. vexans* to 4.76 per 1,000 for *Cx. salinarius* (Table 3).

A molecular method (Aspen and Savage 2003) was used to investigate the population structure of the Huntsville and Auburn populations of *Cx. pipiens/quinquefasciatus*. These experiments suggested that the Huntsville population was composed primarily of *Cx. pipiens* homozygotes, whereas the Auburn population was primarily *Cx. quinquefasciatus* homozygotes (Fig. 2). Overall, the Huntsville population contained 85% *pipiens* alleles and 15% *quinquefasciatus* alleles. In contrast, the Auburn population contained 4% *pipiens* alleles and 96% *quinquefasciatus* alleles. This suggests that although the Huntsville population was primarily *pipiens* in character, the population in Auburn is primarily *quinquefasciatus*. The distance between the two locations is ≈ 350 km, with Auburn probably representing the southernmost extension of a hybrid zone in the state of Alabama.

Discussion

There was a high degree of WNV activity in mosquito populations in the western Tennessee Valley region during midsummer 2002, with virus detected in nine species collected along a 330-km linear transect. Initial detection was in the Tennessee National Wildlife Refuge, an expansive resting and feeding area for wintering waterfowl and migratory birds. This intense activity was followed by sharply declining activity over the next 2 yr, with a reduction in number of positive pools from 25 to 3 to 1 positive pool. This pattern indicates that the virus was unable to maintain its intense transmission status after introduction and became enzootic during the following 2 yr. However, unlike the 2001 situation reported in northern Florida/southern Georgia (Godsey et al. 2005a), several genera of mosquitoes in addition to *Culex* seemed to be involved in the Tennessee Valley outbreak, which may account in part for its spread throughout the area during the summer 2002. *Ae. albopictus*, *Ae. vexans*, and *Cq. perturbans* are competent WNV vectors (Turell et al. 2005), and the latter two species are fairly

catholic in their feeding habits (Apperson et al. 2004, Gingrich and Williams 2005) and have a high dispersal capability (Breeland et al. 1961).

Cx. erraticus is of particular interest, because it has recently been identified as an important potential enzootic vector of eastern equine encephalitis virus in the southeastern United States (Cupp et al. 2003). This species yielded 10-positive pools of WNV during the 2002 epidemic and composed 49% of the total mosquitoes collected during the 3-yr study. WNV has previously been detected in *Cx. erraticus* (Hribar et al. 2004) and in a mixed pool of *Cx. erraticus* and *Cx. salinarius* from Denton County, TX (Bolling et al. 2005), suggesting that this species may be exposed to WNV in areas where it is common. Although the vector competency of *Cx. erraticus* for WNV is unknown, its large proportional representation in the mosquito fauna, its wide distribution throughout the region, and its feeding behavior (Hassan et al. 2003) make it a potentially important species in the WNV transmission cycle in the southeastern United States.

Two species—*Ae. vexans* and *Cx. salinarius*—are likely bridge vectors in WNV ecology in the Mid-South because of their feeding behavior and seasonal densities. *Ae. vexans* is an important nuisance mosquito in the region, and, as indicated by the RT-PCR data for the 2005 Auburn collections, is commonly infected in a periurban setting. The occurrence of a WNV-positive pool of this species very early in the season also suggests that vertical transmission by *Ae. vexans* may be involved in the maintenance of the virus in the southeastern United States. Vertical transmission of WNV in *Culex* has been demonstrated in laboratory-based studies (Goddard et al. 2003, Reisen et al. 2006b), and its potential role as an overwintering mechanism has been supported by data from field-based studies (Nasci et al. 2001a, Bugbee and Forte 2004, Farajollahi et al. 2005, Reisen et al. 2006b). The potential role of *Ae. vexans* in this process deserves further investigation.

Cx. salinarius has been suggested to be an important bridge vector in the northeastern United States (Andreadis et al. 2001, 2004, Molaei et al. 2006) and is likely playing a similar role in this region. Its MIR of 4.76 per 1,000 determined from the 2005 Auburn collection falls well within the range of MIRs calculated for *Culex* spp. during an outbreak of encephalitis owing to WNV in New York City and surrounding areas during 1999 (Nasci et al. 2001b). Earlier studies in Louisiana indicated that this species fed on a fairly wide array of avian and mammalian hosts in an urban/periurban setting (Cupp and Stokes 1973, 1976), a pattern that has been associated with WNV transmission in the northeastern United States (Molaei et al. 2006).

The *Cx. erraticus* WNV-positive pool collected in Huntsville in February implies that this species also may be involved in overwintering of the virus in the southeastern United States. Furthermore, a WNV-positive *Cx. erraticus* pool collected on 26 March in Auburn, well before significant numbers of this species became active, adds further evidence that overwintering mosquitoes in this region maintain the virus between transmission seasons. These two observations follow the general pattern of virus maintenance in overwintering *Culex* spp. recorded in the northeastern United States (Bugbee and Forte 2004, Farajollahi et al. 2005). In contrast, although Tesh and co-workers (Tesh et al. 2004) found that a low percentage of birds collected during winter (December–February) in eastern Texas and southern Louisiana were WNV positive, they did not see evidence of positive mosquito pools until May. However, the mosquito trapping methods used in that study differed from those reported here.

Cx. pipiens and *Cx. quinquefasciatus* are sympatric throughout much of the Mid-South and are capable of hybridizing throughout much of this region (Fonseca et al. 2004). Because of the difficulty in distinguishing between the two by using morphological criteria, recent research efforts have focused upon developing molecular tools that are capable of separating these

species. These methods are now being applied to map the distribution of the two species and of their overlap zone. These data, taken together, suggest that the southern extent of the sympatric zone in Alabama is located near the latitude of Auburn. Because the two species have different foraging strategies, with *Cx. quinquefasciatus* exhibiting a more mammalophilic host selection than *Cx. pipiens* (Breeland et al. 1961), studies comparing the feeding patterns of the two species and their hybrids in the sympatric zone would be of great interest and of potential importance to public health.

The pattern of WNV infection in the mosquito population changed as the virus moved from an epizootic to an enzootic pattern of infection. In 2002, many mosquito species were found to be infected in which no evidence for infection was seen in later years. These species included *Ae. albopictus*, *An. quadrimaculatus*, and *An. crucians*, and data suggest that these species became infected only when the level of virus transmission was relatively high; thus, they probably do not play an important role in enzootic maintenance transmission. This pattern follows previous reports concerning host preferences of these three species, all of which feed primarily upon mammalian hosts (Apperson et al. 2004, Richards et al. 2006). In contrast, WNV infections were detected in *Cx. erraticus*, *Cq. peturbans*, and *Cx. salinarius* in multiple years, suggesting that these species may play more significant roles in the transmission of WNV in the Mid-South. This, too, follows host preference for each species, all of which seem to target avian hosts a significant percentage of the time (Hassan et al. 2003, Apperson et al. 2004).

Although we were able to detect WNV activity in 2002–2004 in the Tennessee Valley area, few if any human cases of WNV infection were reported in the Tennessee Valley during this period (CDC 2006a). In contrast, in 2002–2004, 62 human cases were reported in Shelby County, TN, which includes the city of Memphis. The reason for the lack of human infections in rural areas, despite continuing enzootic viral activity, remains unclear. Recent studies in California have suggested that the density of competent bridge vectors and highly susceptible avian hosts are critical factors in permitting WNV to move from a strictly enzootic pattern of transmission to begin to infect humans (Reisen et al. 2006a). A similar situation might prevail in the southeastern United States, where enzootic transmission continues in rural areas, but where a critical combination of bridge vectors and highly susceptible avian reservoirs exists only in selected urban areas, such as Memphis, TN. More research on the critical factors that are necessary to permit WNV to escape from a strictly enzootic pattern of transmission will be needed to determine whether this is the case.

Acknowledgements

We thank the staff of the Entomology Unit of the Tennessee Valley Authority, especially Ken Tennesen and Kristy Gottfried, as well as Tyler Hicks of Auburn University for assistance with the fieldwork. This work was supported by a contract from the Tennessee Valley Authority to T.R.U. and by a grant from the Centers for Disease Control and Prevention to T.R.U. and E.W.C. (project R01 CI000226).

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Fig. 1. Mosquito collections by time, Auburn, 2005. Only species for which >100 individuals were collected are shown. Data are normalized to the number of trap nights in each semimonthly period.

Fig. 2. Genotypic frequencies in *Cx. pipiens/pienqufaciatus* collections from Huntsville and Auburn, AL. Genotypes were determined as described in Materials and Methods.

Table 1
Summary of collection and infection data from the Tennessee Valley Collections, 2002–2004

Species	Total collected	WNV-positive pools	Months positive pools found	MIR ^a	Prevalence point estimate ^b	95% CI lower bound ^b	95% CI upper bound ^b
<i>Ae. albopictus</i>	634	2	7/02, 9/02	3.15	3.92	0.23	8.76
<i>Oc. canadensis</i> ^c	8						
<i>Oc. fabyus</i> ^c	97						
<i>Oc. sticticus</i> ^c	760						
<i>Oc. thibaulti</i> ^c	1						
<i>Oc. triseriatus</i> ^c	155						
<i>Ae. vexans</i>	12,056	2	9/02, 6/03	0.17	0.21	0.01	0.46
<i>An. crucians</i>	10,213	1	9/02	0.10	0.15	0.0001	0.38
<i>An. punctipennis</i>	248						
<i>An. quadrimaculatus</i>	865	2	8/02, 9/02	2.31	2.86	0.17	6.4
<i>An. walkeri</i>	24						
<i>Coq. perturbans</i>	21,539	3	9/02, 8/03, 8/04	0.14	0.16	0.02	0.33
<i>Cs. melanura</i>	12						
<i>Cx. tarsalis</i>	3						
<i>Cx. erraticus</i>	65,572	10	7/02, 8/02 (<i>n</i> = 4), 9/02 (<i>n</i> = 5)	0.15	0.12	0.07	0.25
<i>Cx. nigrapalpis</i>	11						
<i>Cx. pipiens/quinquefasciatus</i>	329	1	8/02	3.04	4.69	0.005	12.2
<i>Cx. restuans</i>	56						
<i>Cx. salinarius</i>	15,983	7	7/02 (<i>n</i> = 2), 8/02 (<i>n</i> = 2), 9/02 (<i>n</i> = 3)	0.44	0.43	0.17	0.81
<i>Cx. tarsalis</i>	32						
<i>Oc. japonicus</i>	15						
<i>Ps. columbiae</i>	8						
<i>Ps. ferox</i>	3,389	1	6/03	0.30	0.44	0.0004	1.15
<i>Ps. mathsonia</i>	103						
<i>Ur. sapphirina</i>	1,987				0	0	0.96

^aMinimum infection rate.

^bPrevalence point estimates and 95% confidence intervals were only calculated for those species for which a positive pool was identified or for which >1,000 individuals were collected.

^c*Ochlerotatus* (= *Aedes*; see Reinert 2000).

Table 2
Summary of collection and infection data from overwintering mosquitoes, Huntsville, 2005

Species	Total collected	WNV-positive pools	MIR ^a	Prevalence point estimate ^b	95% CI lower bound ^b	95% CI upper bound ^b
<i>An. punctipennis</i>	7					
<i>An. quadrimaculatus</i>	4					
<i>An. perplexans</i>	1					
<i>Cx. erraticus</i>	29					
<i>Cx. pipiens/quinq-fasciatus</i>	113	1	34.48	46.1	0.062	118
<i>Cx. restuans</i>	17					
<i>Cx. territans</i>	6					

^a Minimum infection rate.

^b Prevalence point estimates and 95% confidence intervals were only calculated for those species for which a positive pool was identified or for which >1,000 individuals were collected.

Table 3
Summary of collection and infection data from the Auburn, AL, 2005

Species	Total collected	WNV positive pools	Months positive pools found	MIR ^a	Prevalence point estimate ^b	95% CI lower bound ^b	95% CI upper bound ^b
<i>Ae. albopictus</i>	3,073						
<i>Ae. vexans</i>	3,064						
<i>An. crucians</i>	479	3	3/05, 4/05, 6/05	0.98	0	0	0.62
<i>An. perplexens</i>	4						
<i>An. punctipennis</i>	589						
<i>An. quadrimaculatus</i>	30				0	0	3.25
<i>Cq. perturbans</i>	4						
<i>Cx. restuans</i>	14						
<i>Cx. erraticus</i>	351	1	3/05	2.85	4.31	0.005	11.2
<i>Cx. nigripalpus</i>	410						
<i>Cx. peccator</i>	31						
<i>Cx. pipiens/quinqfasciatus</i>	111						
<i>Cx. restuans</i>	283						
<i>Cx. salinarius</i>	841	4	6/05, 7/05 (n = 2), 8/05	4.76	5.31	1.13	10.29
<i>Cx. territans</i>	32						
<i>Oc. atlanticus/tormentor</i> ^c	84						
<i>Oc. canadensis</i> ^c	59						
<i>Oc. dupreei</i> ^c	4						
<i>Oc. fufvus pallens</i> ^c	6						
<i>Oc. infirmatus</i> ^c	61						
<i>Oc. sticticus</i> ^c	28						
<i>Oc. triseriatus</i> ^c	214						
<i>Oc. trivittatus</i> ^c	5						
<i>Or. signifera</i>	7						
<i>Ps. ciliata</i>	1						
<i>Ps. columbiae</i>	10						
<i>Ps. ferox</i>	264						
<i>Ps. howardii</i>	57						
<i>Ur. sapphirina</i>	29						

^a Minimum infection rate.

^b Prevalence point estimates and 95% confidence intervals were only calculated for those species for which a positive pool was identified or for which >1,000 individuals were collected.

^c *Ochlerotatus* (= *Aedes*; see Reinert 2000).