An Update on the Potential of North American Mosquitoes (Diptera: Culicidae) to Transmit West Nile Virus

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ABSTRACT Since first discovered in the New York City area in 1999, West Nile virus (WNV) has become established over much of the continental United States and has been responsible for >10,000 cases of severe disease and 400 human fatalities, as well as thousands of fatal infections in horses. To develop appropriate surveillance and control strategies, the identification of which mosquito species are competent vectors and how various factors influence their ability to transmit this virus must be determined. Therefore, we evaluated numerous mosquito species for their ability to transmit WNV under laboratory conditions. This report contains data for several mosquito species not reported previously, as well as a summary of transmission data compiled from previously reported studies. Mosquitoes were allowed to feed on chickens infected with WNV isolated from a crow that died during the 1999 outbreak in New York City. These mosquitoes were tested ≈2 wk later to determine infection, dissemination, and transmission rates. All *Culex* species tested were competent vectors in the laboratory and varied from highly efficient vectors (e.g., *Culex tarsalis* Coquillett) to moderately efficient ones (e.g., *Culex nigripalpus* Theobald). Nearly all of the *Culex* species tested could serve as efficient enzootic or amplifying vectors for WNV. Several container-breeding *Aedes* and *Ochlerotatus* species were highly efficient vectors under laboratory conditions, but because of their feeding preferences, would probably not be involved in the maintenance of WNV in nature. However, they would be potential bridge vectors between the avian–*Culex* cycle and mammalian hosts. In contrast, most of the surface pool-breeding *Aedes* and *Ochlerotatus* species tested were relatively inefficient vectors under laboratory conditions and would probably not play a significant role in transmitting WNV in nature. In determining the potential for a mosquito species to become involved in transmitting WNV, it is necessary to consider not only its laboratory vector competence but also its abundance, host-feeding preference, involvement with other viruses with similar transmission cycles, and whether WNV has been isolated from this species under natural conditions.

KEY WORDS West Nile virus, transmission, mosquitoes, vector competence

SINCE FIRST DISCOVERED IN the New York City area in 1999 (CDC 1999, Lanciotti et al. 1999), West Nile virus (WNV) has become established throughout much of the continental United States and has been responsible for >10,000 cases of severe disease and 400 human fatalities as well as thousands of fatal infections in horses (CDC 2002a, b, 2003a). To develop appropriate surveillance and control strategies, it is necessary to know which mosquito species are competent vectors and the factors that influence their ability to transmit this virus. A number of recent publications have examined the potential for North American mosquitoes to transmit WNV under laboratory conditions (Turell et al. 2000, 2001, 2003; Sardelis and Turell 2001; Sardelis et al. 2001, 2002; Goddard et al. 2002). However, these have been published in a variety of journals, making it difficult to keep track of current results. Additionally, there are numerous species from which WNV has been isolated in nature, for which we have no vector competence data. Therefore, we evaluated the ability of several of these species to transmit WNV under laboratory conditions and compiled the data published previously for other species.

Materials and Methods

Mosquitoes. Mosquitoes tested during this study included *Aedes* (*Aedimorphus*) *vexans* (Meigen), *Culiseta melanura* (Coquillett), *Ochlerotatus* (*Ochlerota-*

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tus) *canadensis* (Theobald), *Ochlerotatus* (*Ochlerotatus*) *cantator* (Coquillett), *Ochlerotatus* (*Protomacleaya*) *triseriatus* (Say), and *Psorophora ferox* (Von Humboldt). Mosquito larvae and pupae (*Ae. vexans*, *Oc. canadensis*, and *Oc. cantator*) were collected in Westport and Dighton, MA, during May 2001 and 2002 and brought to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), where they were placed in an incubator maintained at 26°C and a photoperiod of 16:8 (L:D) h, provided ground catfish chow (AquaMax Pond Plus 3000, Purina Mills, Inc., St. Louis, MO) for nutrition, and allowed to pupate and emerge as adults. *Ae. vexans* and *Oc. triseriatus* larvae and pupae were collected in Frederick County, MD, in May 2001; transported to USAMRIID; and reared to the adult stage as described above. Adults derived from these specimens were used in the vector competence studies. In addition, adult *Ps. ferox* were collected on the Quantico Marine Base, VA, at a miniature light trap baited with dry ice and by aspiration as they approached a human collector in June 2001 and transported back to USAMRIID. In addition to these field-collected mosquitoes, we also tested *Cs. melanura* from a colony maintained by the Connecticut Agricultural Experiment Station (origin NJ circa 1998). At USAMRIID, all mosquitoes were held in an incubator at 26°C until tested for their susceptibility to WNV.

Virus and Virus Assays. We used an isolate of WNV (crow 397-99) from the brain of a crow that died in the Bronx, NY, in September 1999 (Turell et al. 2000) and had been passaged twice in Vero (African green monkey kidney) cells before use in these studies.

To determine infection status, serial 10-fold dilutions of specimens were made in grinding medium (10% heat-inactivated fetal bovine serum in Medium 199 with Earle's salts, $NaHCO_3$, and antibiotics [100 U of penicillin, 100 μg of streptomycin, 5 μg of amphotericin B, and 50 μg of gentamycin/ml]) and tested for the presence of virus on Vero cell monolayers by plaque assay.

Vector Competence Studies. Based on viremia profiles in young leghorn chickens, *Gallus gallus* L., determined previously (Turell et al. 2000), mosquitoes were allowed to feed for up to 45 min on 2- to 4-d-old leghorn chickens that had been inoculated with $10^{4.3}$ plaque-forming units (PFU) of WNV 1–3 d earlier. To ensure that the various mosquito species received the same virus exposure, nonfed females of several species were combined into a single cage before feeding on the infected chicken. Immediately after mosquito feeding, 0.1 ml of blood was obtained from the jugular vein of each chicken and added to 0.9 ml of heparinized diluent. These blood suspensions were frozen at −70°C until tested for virus by plaque assay to determine the viremias at the time of mosquito feeding. After exposure to the viremic chickens, engorged mosquitoes were transferred to 3.8- or 0.9-liter screen-topped cardboard cages held at 26°C at a photoperiod of 16:8 (L:D) h. After an incubation period of >12 d, they were allowed to refeed on 1- to 2-d-old chickens, either individually or in small groups, to determine whether they could transmit virus by bite. Immediately after the transmission attempt, the mosquitoes were killed by freezing, identified to species, and their feeding status was determined. The legs and bodies of each mosquito were triturated separately in 1 ml of diluent and frozen at −70°C until assayed for WNV by plaque assay on Vero cell monolayers. Infection was determined by detecting virus in the mosquito tissue suspension. If virus was detected in its body, but not its legs, the mosquito was considered to have a nondisseminated infection limited to its midgut. In contrast, if virus was found in both the body and leg suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984). We defined the infection and dissemination rates as the percentages of mosquitoes tested that contained virus in their body or legs, respectively. Chickens used in the transmission attempts were bled from the jugular vein 1 d after mosquito feeding and the blood processed as described above. Based on previous studies (Turell et al. 2000), infected chickens would have a viremia of $>10^5$ PFU/ml at this time. Detecting virus in these blood samples indicated transmission. Because some of the mosquitoes were tested for transmission in small pools, it was not always possible to determine which mosquito in a pool actually transmitted virus by bite. Therefore, if more than one mosquito with a disseminated infection fed in a pool, data from that pool were not used to calculate the transmission rate, regardless of chicken viremia.

To more efficiently examine virus transmission, some of the unfed mosquitoes were inoculated intrathoracially (Rosen and Gubler 1974) with 0.3 μl of a virus suspension containing $10^{4.2}$ PFU of WNV/ml ($10^{0.7}$ PFU/mosquito) and allowed to feed on 1- to 2-d-old chickens 7–14 d later. Mosquitoes and blood samples from these chickens were processed as described for the orally exposed mosquitoes.

This research was conducted in compliance with the Animal Welfare Act and other Federal statues and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The facility where this research was conducted is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Infection and dissemination rates were compared by Fisher's exact tests at the 95% confidence level adjusted for multiple comparisons by using resampling techniques to reduce the probability of false significances (SAS Institute 1999).

Results

Viremias in the 20 chickens used to expose mosquitoes to WNV ranged from $10^{6.0}$ to $10^{7.5}$ PFU/ml of blood, viremias consistent with those found in many North American birds (Komar et al. 2003). To compare mosquitoes exposed to similar doses of WNV, mosquitoes were evaluated separately for those that fed on chickens with viremias $10^{6.3 \pm 0.3}$ or $10^{7.1 \pm 0.4}$.

Table 1. Infection and dissemination rates for mosquitoes orally exposed to WNV

Species	No. tested	Infection rate[a]	Dissemiation rate[b]	Transmission rate[c]	Estimated transmission rate[d]
Infectious dose = $10^{6.3 \pm 0.3}$ PFU/ml					
Cs. melanura	2	0	0	N.T.	n.a.
Oc. canadensis	24	13	0	0 (3)	0
Oc. cantator	51	22	18	0 (2)	13
Ps. ferox	17	29	12	0 (9)	0
Infectious dose = $10^{7.1 \pm 0.4}$ PFU/ml					
Cs. melanura	19	26	11a,b	0 (5)[e]	n.a.
Ae. vexans	73	44	19b	11 (18)	18
Oc. canadensis	8	50	13a,b	100 (1)	11
Oc. triseriatus	29	31	17a,b	12 (17)	10
Ps. ferox	24	33	0a	0 (15)	0

N.T., not tested; n.a., not applicable.

[a] Percentage of mosquitoes containing virus in their bodies. At each virus dose, infection rates for each species were not significantly different than each other.

[b] Dissemination rate, percentage of mosquitoes containing virus in their legs. Dissemination rates followed by the same letter are not significantly different at $\alpha = 0.05$ after adjusting for multiple comparisons. All values for mosquitoes that ingested $10^{6.3 \pm 0.3}$ were not significantly different from each other.

[c] Transmission rate, the percentage of all refeeding mosquitoes that transmitted virus by bite (no. refeeding).

[d] Estimated transmission rate, the percentage of mosquitoes with a disseminated infection 12–15 d after ingesting WNV multiplied by the transmission rate for those individuals with a disseminated infection (see Table 2).

[e] None of the *Cs. melanura* tested had a disseminated infection.

Infection and dissemination rates for *Ae. vexans* collected in the two sites in Massachusetts and the one site in Maryland were similar ($\chi^2 \leq 1.7$, df = 1, $P \geq 0.19$); therefore, the data for this species were combined for further analysis. All six mosquito species were susceptible to infection with WNV after feeding on a viremic chicken. Infection rates ranged from 26 to 50% for mosquitoes that fed on a chicken with a viremia of $10^{7.1 \pm 0.4}$ (Table 1). At least one female in each species tested developed a disseminated infection after ingesting WNV. Although none of six *Ps. ferox* with a disseminated infection (two after oral exposure and four inoculated with WNV) transmitted virus by bite, nearly all of the *Aedes* and *Ochlerotatus* with a disseminated infection tested (37 of 44; 84%), transmitted this virus by bite (Table 2).

Discussion

Since its introduction into North America in 1999, WNV has been detected in at least 43 species of mosquitoes (CDC 2003b), and reviews by Hayes (1989) and Hubalek and Halouzka (1999) indicate that a variety of Old World mosquito species are competent vectors for WNV. However, to incriminate any of these species as vectors of WNV, several criteria must be met (Reeves 1957). These include repeated detection of virus from field-collected individuals of species, demonstration of the ability of the species to become infected and transmit the virus in the laboratory (i.e., vector competence), and an association in nature between the arthropod and naturally infected vertebrate hosts. Because some mosquitoes are unable to transmit virus, even if they become infected (Hardy 1988), laboratory studies are needed to determine whether that species is capable of transmitting WNV by bite, even though there may have been numerous isolations (detections) of WNV from that species. Therefore, the detection of WNV from a mosquito species does not necessarily mean that the species is a competent vector of WNV. Likewise, the mere ability to transmit a virus in the laboratory does not mean that the species will play a significant role in nature. Factors such as population density of mosquitoes and susceptible amplifying hosts, environmental temperature, feeding pref-

Table 2. Transmission rates for mosquitoes with a disseminated infection after either oral exposure to or intrathoracic inoculation with WNV

Species	Route of exposure[a]				Total no. tested	Transmission rate[b]
	Oral		Inoculation			
	No. tested	Transmission rate	No. tested	Transmission rate		
Ae. vexans	2	100	13	92	15	93a
Oc. canadensis	1	100	7	86	8	88a
Oc. cantator	1	0	15	80	16	75a
Oc. triseriatus	2	50	3	67	5	60a,b
Ps. ferox	2	0	4	0	6	0b

[a] Route by which mosquitoes were exposed to WNV. Oral, disseminated infection after feeding on a viremic chicken. Inoculation, disseminated infection after intrathoracic inoculation.

[b] All transmission rates followed by the same letter are not significantly different at $\alpha = 0.05$ after adjusting for multiple comparisons.

Table 3. Potential for selected North American mosquitoes to transmit WNV based on bionomics, vector competence, virus isolations, and involvement with other arboviruses

Species	Association with other viruses[a]	Host preference	Activity time	Flight range	Vector competence for WNV[b]	Field isolations of WNV[c]	Potential to serve as a	
							Enzootic vector[d]	Bridge vector[e]
Ae. aegypti		Mammals	Crepuscular/day	200 m	+++, 3	+	0	+
Ae. albopictus	EEE	Opportunistic	Crepuscular/day	200 m	++++, 3, 6	+	+	++++
Ae. vexans	EEE, WEE, SLE	Mammals	Crepuscular/night	>25 km	++ 1, 5, 8	+++	0	++
Cq. perturbans	EEE	Opportunistic	Crepuscular/night	5 km	+, 4	+	+	+
Cs. melanura	EEE	Birds	Crepuscular/night	9 km	+, 8	++	++	0
Cs. inornata	WEE	Mammals	Crepuscular/night	2 km	+++, 5	+	+	++
Cx. stigmatosoma	SLE	Birds	Night	1 km	+++, 5	0	+++	+
Cx. erythrothorax	WEE	Opportunistic	Crepuscular/day	<2 km	++++, 5	0	++	+++
Cx. nigripalpus	EEE, SLE	Opportunistic[f]	Crepuscular	5 km	++, 4	+++	+++	++
Cx. pipiens	SLE	Birds	Crepuscular/night	2 km	+++, 1, 3, 5	++++	+++++	++
Cx quinquefasciatus	SLE	Birds	Crepuscular/night	2 km	+++, 4, 5	0	++++	++
Cx. restuans	SLE	Birds	Crepuscular/night	2 km	++++, 4	+++	+++++	++
Cx. salinarius	EEE, SLE	Opportunistic	Crepuscular/night	10 km	++++, 4	+++	+++	++++
Cx. tarsalis	WEE, SLE	Opportunistic[f]	Crepuscular/night	>6 km	++++, 5, 7	++++	++++	+++
Oc. atropalpus		Mammals	Day and night	1 km	++++, 3	+	+	++
Oc. canadensis	EEE	Mammals	Day	2 km	++, 8	+	0	++
Oc. cantator	EEE	Mammals	Day	>10 km	++, 8	+	0	++
Oc. dorsalis	WEE	Mammals	Day and night	5 km	+++, 5	+	0	++
Oc. japonicus	JE?	Mammals	Crepuscular/day	unk	++++, 2, 3	+++	+	++++
Oc. melanimon	WEE	Mammals	Day and night	>10 km	+++, 5	0	0	++
Oc. sierrensis		Mammals	Crepuscular/day	1 km	+, 5	0	0	+
Oc. sollicitans	EEE	Mammals	Crepuscular/night	>25 km	++, 1, 3	+	0	+
Oc. taeniorhynchus	EEE	Mammals	Day and night	>25 km	+, 1, 3	+	0	+
Oc. triseriatus		Mammals	Day	200 m	+++, 8	++	0	+++
Ps. ferox	SLE	Mammals	Day	2 km	0, 8	+	0	0

Distribution and bionomics based on and generalized from information in Carpenter and LaCasse (1955), Darsie and Ward (1981), and Moore et al. (1993).

[a] Known association with other viruses with a similar transmission cycle. EEE, eastern equine encephalomyelitis virus; JE; Japanese encephalitis virus; SLE; St. Louis encephalitis virus; WEE; western equine encephalomyelitis virus. Based on Karabatsos (1985).

[b] Efficiency with which this species is able to transmit WNV in the laboratory. 0, incompetent; +, inefficient; ++++, extremely efficient vector. Based on 1 (Turell et al. 2000), 2 (Sardelis and Turell 2001), 3 (Turell et al. 2001), 4 (Sardelis et al. 2001), 5 (Goddard et al. 2002), 6 (Sardelis et al. 2002), 7 (Turell et al. 2003), or 8 (present study).

[c] Relative number of WNV-positive pools detected. 0, none; +, few; ++++, many.

[d] Potential for this species to be an enzootic or maintenance vector based on virus isolations from the field, vector competence, feeding behavior, etc. 0, little to no risk; +++++, this species may play a major role.

[e] Potential for this species to be an epizootic or bridge vector based on virus isolations from the field, vector competence, feeding behavior, etc. 0, little to no risk; +++++, this species may play a major role.

[f] Feeds primarily on avian hosts in spring and early summer and mixed between avian and mammalian hosts in late summer and fall.

erences, and even time of day of feeding all affect how important a particular species will be in transmitting an arbovirus. There is no simple answer for "What is the vector of WNV?" The answer depends on which mosquitoes are present and their relative population densities in that area. A given species, e.g., *Culex pipiens* L., may seem to be the most important vector in one area but may be unimportant in other areas.

West Nile virus is maintained in nature in a bird-mosquito cycle, involving several *Culex* species and a variety of avian hosts. In this cycle, mosquitoes that feed preferentially on birds will tend to be more efficient vectors. Thus, species such as *Culex nigripalpus* Theobald, *Cx. pipiens*, *Culex quinquefasciatus* Say, and *Culex tarsalis* Coquillett would tend to be highly efficient in maintaining and amplifying WNV. However, whereas feeding almost entirely on birds makes them more efficient maintenance or amplification vectors, it would tend to decrease the risk of their transmitting WNV to humans or horses. Some species, such as *Cx. nigripalpus* and *Cx. tarsalis* are known to change their feeding preference depending on season and host availability and may change from primarily ornithophilic to general feeders (Tempelis et al. 1965, Edman and Taylor 1968). Therefore, early in the season, these species would serve as amplification vectors, whereas later in the summer they would pose a serious risk to mammals such as humans and horses. In contrast, mosquitoes that are opportunistic feeders might not be able to maintain WNV in nature (too few bird–bird feedings) but are more likely to serve as bridge vectors by becoming infected while feeding on a viremic bird and then transmitting virus to a susceptible human or horse. As the number of infected (infectious) birds and populations of competent mosquito vectors increase, the risk of transmissions of WNV from the bird-mosquito cycle to humans and equines increases.

Based on the detection of WNV from field-collected specimens, association of these species with other viruses with a similar transmission cycle to WNV (e.g., St. Louis encephalitis virus), feeding behavior, and laboratory vector competence studies, we have indicated the potential for several North American mos-

quito species to be involved as maintenance/amplifying vectors or as bridge vectors of WNV (Table 3).

Our study extends the number of species tested and consolidates the results of various studies on the potential for North American mosquito species to serve as potential vectors of WNV. Although WNV has been detected in field-collected *Ps. ferox* (CDC 2003b) and this species is susceptible to infection with WNV, relatively few individuals developed a disseminated infection after oral exposure to WNV, and none of six *Ps. ferox* with a disseminated infection transmitted this virus by bite. Therefore, despite the reported detection of WNV in this species, this species does not seem to play a significant role in the transmission of WNV in North America.

Both *Oc. canadensis* and *Oc. cantator* became infected and developed a disseminated infection after ingesting a blood meal containing WNV. Because both species transmitted WNV by bite, they should be considered potential bridge vectors; however, because of the relatively low infection and dissemination rates, they may play only a limited role in the transmission of WNV to humans or horses. The *Ae. vexans* tested in this study were moderately susceptible to WNV and individuals with a disseminated infection readily transmitted virus by bite. Because of its high population densities in many areas (Easton 1987, Janousek and Kramer 1999, Andreadis et al. 2001, Samui et al. 2003), the repeated detection of WNV in this species (Anderson et al. 1999, Kulasekera et al. 2001, CDC 2003b), and its preference for feeding on mammals (Nasci 1984), *Ae. vexans* should be considered a potentially important bridge vector for WNV.

Although the species tested varied in their susceptibility to WNV, for nearly all of the species tested, if a mosquito developed a disseminated infection, it transmitted virus by bite. This indicated that midgut infection and escape barriers (Kramer et al. 1981) seem to be the principal factors controlling vector competence with WNV. All of the specimens were tested after 12–15 d of extrinsic incubation at 26°C. Holding mosquitoes for longer periods of time increased dissemination, and thus transmission rates for *Cx. pipiens* (Dohm et al. 2002). Therefore, studies may need to be conducted that evaluate mosquitoes after a longer period of extrinsic incubation. Likewise, environmental temperatures are known to influence vector competence for arboviruses in general, and WNV in particular (Jupp 1974, Cornel et al. 1993, Dohm et al. 2002). Therefore, additional studies are needed to determine the impact of environmental temperature on the ability of North American mosquitoes to transmit WNV. With the potential spread of WNV to Central and South America, studies are needed to evaluate various mosquito species for their potential to transmit WNV and to determine the effects of environmental conditions on their ability to transmit this virus. These data are needed to develop a comprehensive global disease and vector control program.

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