Journal of the American Mosquito Control Association, 21(4):474–476, 2005
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SCIENTIFIC NOTE

DETECTION OF WEST NILE VIRUS RNA FROM THE LOUSE FLY *ICOSTA AMERICANA* (DIPTERA: HIPPOBOSCIDAE)

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ABSTRACT. West Nile virus (WNV) was detected by Taqman reverse transcription-polymerase chain reaction in 4 of 85 (4.7%) blood-engorged (n = 2) and unengorged (n = 2) *Icosta americana* (Leach) hippoboscid flies that were collected from wild raptors submitted to a wildlife rehabilitation center in Mercer County, NJ, in 2003. This report represents an additional detection of WNV in a nonculicine arthropod in North America and the first documented detection of the virus in unengorged hippoboscid flies, further suggesting a possible role that this species may play in the transmission of WNV in North America.

KEY WORDS West Nile virus, *Icosta americana*, Hippoboscidae, raptors

West Nile virus (WNV) is a flavivirus that is principally maintained in an enzootic cycle between *Culex* mosquitoes and avian amplifying hosts. To date, the virus has been detected in or isolated from 59 species of mosquitoes in the United States and Canada (CDC 2004) and the importance of mosquitoes as enzootic and epidemic vectors of WNV in North America is well established (Andreadis et al. 2001, 2004; Nasci et al. 2002; Hribar 2003; Rutledge et al. 2003; Anderson et al. 2004; Mans et al. 2004).

Although WNV isolations have been occasionally reported from bird-feeding argasid and ixodid ticks in the Eastern Hemisphere (Hubalek and Halouzka 1999), the role that nonculicine hematophagous arthropods may play in the ecology of WNV in North America is unknown. Other than mosquitoes, the only other potential arthropod vectors from which WNV has been detected in nature has been a few blood-engorged hippoboscid louse flies (*Icosta* sp., Diptera: Hippoboscidae) collected from a symptomatic WNV-positive great horned owl in Pennsylvania (Komar 2003); from 16 *Icosta americana* (Leach) louse flies collected from dead or sick owls in Ontario, Canada (Gancz et al. 2004); and from 2 pools of the biting midge *Culicoides sonorensis* (Wirth and Jones) (Diptera: Ceraptogonidae) collected in Wyoming (Naugle et al. 2004).

Hippoboscid louse flies are obligate ectoparasitic blood feeders that are commonly associated with birds of prey. Both sexes readily take blood (Bequaert 1952, 1953; Maa and Peterson 1987) and females are viviparous, exhibiting a high frequency of blood feeding due to nutritional demands of developing larvae (Bequaert 1952). Host specificity varies greatly among bird-feeding species (Lloyd 2002), but most species remain and feed on the host throughout the year, thus increasing their potential to locally acquire and transmit the virus as well as harbor it through the winter months. Winged hippoboscids are also quite mobile (Lloyd 2002) and they may stray from a live or dead host (Bequaert 1952), thus increasing their vectorial capacity.

During the active mosquito-breeding season, wildlife rehabilitators regularly accept birds of prey that exhibit clinical signs of WNV infection, including lethargy, neurological abnormalities, and emaciation. However, because rehabilitation centers focus on healing animals, only a small portion of these birds are ever assayed for viral infection or examined for ectoparasites. Preliminary findings indicate that a high percentage of birds are infested with louse flies. The current investigation was undertaken to more fully assess the possible involvement of hippoboscids in the ecology of WNV in the northeastern USA.

Louse flies were collected from sick or injured raptors being held at a Wildlife Rehabilitation Center in Hopewell, NJ. Birds were inspected for ectoparasites by trained wildlife rehabilitators, and hippoboscids were removed and initially stored up to 3 wk at 4°C. Samples were later held at −70°C until identification and pooling could be conducted on a chill table by using the keys of Bequaert (1954, 1955), Maa (1969), and Maa and Peterson (1987). Individual louse flies were pooled according to date, location, species, gonotrophic status (empty or blooded), and host species. Specimens were submitted to the New Jersey Department of Health and Senior Services (NJDHSS), Trenton,

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Table 1. Distribution of West Nile virus (WNV) antibodies in wild raptors collected at a rehabilitation center in New Jersey, and summary of WNV detection by Taqman RT-PCR from the hippoboscid *Icosta americana*, 2003.

Bird ID no.	Bird species[1]	Collection date	Bird status	Bird serology[2]	Hippoboscid no. (status)[3]	WNV+ no. (status)[4]	CT values NJDHSS (CAES)[5]
MC-1163	RTH	26-Jul-03	Died < 24 h	NT	4(U), 1(E)	1(E)	27.9 (29.19)
MC-1214	BWH	29-Jul-03	Died < 24 h	NT	1(U), 1(E)	0	
MC-1238	CPH	31-Jul-03	Euthanized on arrival	NT	2(U)	0	
MC-1311	GHO	11-Aug-03	Euthanized on arrival	WNV	1(U)	0	
MC-1318	GHO	11-Aug-03	Released 31-Oct-03	WNV	1(U)	0	
MC-1395	GHO	21-Aug-03	Died < 24 h	NT	1(U)	0	
MC-1494	RTH	29-Aug-03	Euthanized in care	NT	1(U)	0	
MC-1508	RTH	30-Aug-03	Died < 24 h	NT	8(U), 4(E)	1(U), 1(E)	32.87 (35.38) 29.42 (28.19)
MC-1580	RTH	8-Sep-03	Euthanized in care	NT	3(U)	0	
MC-1583	RTH	8-Sep-03	Euthanized in care	NT	2(U), 2(E)	0	
MC-1587	GHO	7-Sep-03	Died < 24 h	NT	1(E)	0	
MC-1603	GHO	9-Sep-03	Released 25-Oct-03	WNV	4(E)	0	
MC-1625	GHO	11-Sep-03	Died < 48 h	NT	4(U), 7(E)	0	
MC-1629	RTH	11-Sep-03	Died < 24 h	NT	1(U), 1(E)	0	
MC-1630	RTH	12-Sep-03	Died < 24 h	NT	1 (E)	0	
MC-1632	GHO	12-Sep-03	Released 25-Oct-03	WNV	4(E)	0	
MC-1633	GHO	12-Sep-03	Died < 24 h	NT	1(U), 1(E)	0	
MC-1641	RTH	13-Sep-03	Died < 72 h	NT	8(E)	0	
MC-1646	GHO	13-Sep-03	Died < 24 h	NT	3(U), 3(E)	0	
MC-1690	GHO	18-Sep-03	Released 29-Oct-03	NT	4(U)	0	
MC-1766	GHO	26-Sep-03	Euthanized on arrival	NT	1(U), 3(E)	0	
MC-1788	GHO	30-Sep-03	Released 26-Feb-04	WNV	2(U)[6], 4(E)	1(U)	30.97 (27.91)
MC-1892	RTH	6-Nov-03	Released 15-Dec-03	NT	1(E)	0	
Total					40(U), 46(E)	2(U), 2(E)	

[1] RTH, Red-tailed hawk; BWH, broad-winged hawk; CPH, Cooper's hawk; GHO, great horned owl.
[2] Birds serologically tested for antibodies by plaque reduction neutralization test; NT, not tested.
[3] U, unengorged; E, engorged.
[4] Hippoboscids individually tested by Taqman RT-PCR.
[5] CT, cycle threshold based on a 45-cycle amplification threshold; NJDHSS, New Jersey Department of Health and Senior Services; CAES, Connecticut Agricultural Experiment Station.
[6] Includes one *Ornithoica viccina*.

NJ, for WNV testing via Taqman reverse transcription-polymerase chain reaction (RT-PCR) procedures as outlined in Farajollahi et al. (2005). Virus isolation attempts in Vero cell culture and confirmational testing by Taqman RT-PCR was additionally conducted on all positive pools at the Connecticut Agricultural Experiment Station (CAES), New Haven, CT, using techniques described in Andreadis et al. (2004). Additionally, a portion of the birds were assayed at Cornell University, Ithaca, NY, for neutralizing antibodies to WNV by the plaque-reduction neutralization test (Beaty et al. 1989) essentially as described.

A total of 86 hippoboscids were collected from July 26, 2003 through November 6, 2003 from 23 birds comprising 4 species (Table 1). Of the 23 raptors that were sampled, 12 (52.2%) were great horned owls, 9 (39.1%) were red-tailed hawks, 1 (4.4%) was a broad-winged hawk, and 1 (4.4%) was a Cooper's hawk. Eleven (47.8%) of the admitted birds died in care within 72 h or less, 6 (26.1%) were either euthanized on arrival or during care, and 6 (26.1%) were rehabilitated and released back into the wild. Because most of the birds died within a short time after submission to the wildlife center, only a small portion was serologically tested for WNV. Of the 6 raptors that were rehabilitated and released, 4 tested positive for WNV-neutralizing antibodies, while the remaining 2 were not tested. Only 1 bird was tested of the 17 birds that either died in care or were euthanized, and that bird was also positive for WNV-neutralizing antibodies.

Of the 86 collected hippoboscids, 40 were unengorged (empty), and 46 contained visible traces of blood and were recorded as engorged (Table 1). Only a single specimen of *Ornithoica viccina* (Walker) was found, whereas all the remaining species consisted of *Icosta americana*. Infestations ranged from a single individual on a host to more than twelve, with a median of 3.7 hippoboscids per bird. Four *I. americana* tested positive for WNV viral RNA during the initial RT-PCR testing at NJDHSS. These positives were also confirmed at CAES by Taqman RT-PCR; however, live virus was not isolated by using the Vero cell culture assay. Two of the positives were unengorged, whereas the other two had traces of blood in the abdomen.

This investigation confirms the earlier observations from Pennsylvania and Ontario and demonstrates that the hippoboscid *I. americana* is likely acquiring WNV from its host during the summer season. Although we have no data on the vector competence of these arthropods, it is particularly significant that viral RNA was detected in unengorged specimens as well as those with imbibed blood. This suggests that virus may be disseminated from the gut into the hemocoel and host tissues. Therefore, vector competency studies are clearly warranted. Our inability to recover live WNV from these PCR-positive specimens may have been due to virus inactivation during our initial collection and storage procedures. The cold chain was not maintained properly, as specimens were placed in an ordinary refrigerator for up to 3 wk before pickup and appropriate storage. We also cannot rule out the possibility that the level of virus in these specimens was below the detection methods of the Vero cell assay. However, we believe that further investigations should be conducted to more fully assess the role that hippoboscids may play in the ecology of WNV in North America. If hippoboscids prove to be vector competent, then they could potentially be involved in a secondary enzootic amplification cycle or serve as an overwintering mechanism for the virus, which remains enigmatic.

We thank Mercer County Executive B. Hughes, Director A. Watson, S. Zegarski, and C. Markley for funding and support. Special thanks to J. Phelps, T. Manzke, and M. Milewsky for field assistance and technical support. This is New Jersey Agricultural Experiment Station publication D-08-08400-07-04 supported by state funds with partial support from the New Jersey State Mosquito Control Commission.

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