3-2008

WEST NILE VIRUS ANTIBODIES IN BREEDING NORTH DAKOTA ICTERIDS

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WEST NILE VIRUS ANTIBODIES IN BREEDING NORTH DAKOTA ICTERIDS -- Exotic infectious diseases can have devastating effects on the distribution and abundance of naïve wildlife species (Friend et al. 2001). West Nile Virus (WNV) is an exotic disease that was introduced into North America in 1999 and has resulted in the deaths of tens of thousands of birds (Marra et al. 2004). The natural cycle of WNV involves *Culex* spp. mosquitoes as principle vectors and birds as principle hosts, although humans, horses, and other mammals can become incidental hosts (Lanciotti et al. 2000). Because the virus can be fatal, outbreaks have become a national health concern for the human population, an economic concern for domestic animal losses, and a conservation concern for the status of free-living wildlife populations (Campbell et al. 2002). For birds, WNV infection can be lethal, but the degree to which birds are adversely affected varies among species and even between individuals within species (Komar et al. 2003).

In light of concerns regarding the status of North American bird populations, we captured adult, juvenile, and nestling icterids in central North Dakota and tested them for WNV-specific antibodies. Specifically, we wanted to determine if antibody positive blackbirds were present during the early summer breeding season prior to the peak of mosquito populations that typically occurs later in the summer. Sampling during the icterid breeding season also allowed us to test the hypothesis that nestling blackbirds are particularly vulnerable to the virus because they are confined to the nest, lack protective feathers, and have naïve immune systems. We also trapped mosquitoes to determine if *Culex tarsalis*, a known WNV vector in North Dakota (Bell et al. 2005), was present in our study area.

This study was conducted on 10 wetlands in Stutsman County, North Dakota, from 15 May to 16 July, in 2003 and 2004. A total of 170 free-ranging icterids (132 adults, 5 juvenile, and 33 nestlings) were captured and tested for WNV antibodies. We used a food-baited Troyer V-Top trap (JWB Marketing, West Columbia, South Carolina) to capture 56 blackbirds, including the following species: yellow-headed blackbird (*Xanthocephalus xanthocephalus*, n = 13), common grackle (*Quiscalus quiscula*, n = 38), red-winged blackbird (*Agelaius phoeniceus*, n = 3), and brown-headed cowbird (*Molothrus ater*, n = 2). A nest trap (Newbrey and Reed 2008) was used to capture 81 additional yellow-headed blackbird females and we obtained blood samples from 33 of their nestlings. Nestlings were between 8 and 11 days old and were sampled from a total of 27 nests. Additional blood samples were obtained from a road kill juvenile western meadowlark (*Sturnella neglecta*) and three house sparrow (*Passer domesticus*) individuals (an adult and two juveniles), but they were not included in estimates of WNV antibody-positive icterids.

A blood sample (< 150 µl) was collected via venous puncture at the brachial vein from all captured individuals. We banded yellow-headed blackbird individuals as part of a concurrent research project and clipped the outermost tail feathers of

all other individuals to prevent re-sampling. Whole blood was kept in heparinized microcapillary tubes on ice for no longer than 6 hours before it was centrifuged for 5 minutes at 7,000 RPM in a microcentrifuge. The serum portion was removed and stored at -20°C until lab analysis.

To determine if *C. tarsalis* mosquitoes were present at our study sites, we captured mosquitoes by using two carbon dioxide-baited traps (American Biophysics Corp., North Kingstown, Rhode Island). Mosquitoes were collected adjacent to two of our study wetlands from 9 June to 15 July, 2004. Traps were run for three consecutive 24-hour periods before mosquitoes were collected and promptly frozen at -20°C (Bell et al. 2005).

We tested avian serum samples for WNV antibodies by using competitive enzyme-linked immunoabsorbent assay (ELISA). The ELISA, using WNV-specific monoclonal antibodies, provides a non-lethal, rapid, and inexpensive technique for monitoring WNV infection in wild bird populations (Blitvich et al. 2003). We used serum from an infected horse and from normal chickens as positive and negative controls. A serum sample with inhibition >30% was considered positive for WNV antibodies (Blitvich et al. 2003). Approximately 25,000 mosquitoes were collected during the summer of 2004 and 291 female *C. tarsalis* individuals were recovered. Mosquitoes were tested for WNV RNA by using Reverse Transcriptase Polymerase Chain Reaction (Lanciotti et al. 2000) to determine if the primary WNV vector in North Dakota carried the virus during the icterid breeding season.

Of the 170 icterids sampled in 2003 and 2004, four individuals were positive for WNV antibodies (Table 1). In 2003, we tested serum from 53 birds and found one positive (1.9%): a male red-winged blackbird. In 2004, we tested serum from 117 blackbirds and found three positive (2.6%): two yellow-headed blackbird adult females and one adult common grackle. The juvenile western meadowlark collected in 2003 and two house sparrows (an adult and a juvenile) collected in 2004 also tested positive for WNV antibodies. All of the *C. tarsalis* mosquitoes sampled during the 2004 icterid breeding season tested negative for WNV RNA.

The occurrence of WNV antibodies in icterids in our study area was low during the early summer breeding seasons in 2003 and 2004, with 1.9 and 2.6% of blackbirds having antibodies, respectively. Our rates of antibody positive birds were slightly lower than those found in other free-living bird populations that have been monitored recently for WNV. Ringia et al. (2004) studied the prevalence of WNV antibodies in 81 species of North American birds in Illinois and found an overall rate of 5.3% (94 out of 1,784 tested) in 2002. Of 39 red-winged blackbird individuals sampled, three (7.7%) were found to be antibody positive (Ringia et al. 2004). Similarly, the North Dakota Department of Health tested 617 live birds for WNV antibodies during the summer of 2004, and found 36 positive individuals (5.8%, North Dakota Department of Health, unpublished data).

In our study we found birds with WNV antibodies despite the fact that none of the *C. tarsalis* individuals collected were infected with WNV. Possibly, adult

Table 1. Icterid species tested for West Nile Virus (WNV) antibodies in central North Dakota during the 2003 and 2004 icterid breeding seasons. The number tested and the number of WNV-antibody positive individuals are reported for each species.

	2003		2004	
Species	Tested	WNV Positive (%)	Tested	WNV Positive (%)
Yellow-headed blackbird	36	0 (0.0)	91	2 (2.2)
Common grackle	15	0 (0.0)	23	1 (4.3)
Red-winged blackbird	2	1 (50.0)	1	0 (0.0)
Brown-headed cowbird	-	-	2	0 (0.0)
Total	53	2 (1.9)	117	3 (2.6)

blackbirds were infected on their wintering grounds, but this seems unlikely. In our study, antibody positive birds were not detected until half-way through the breeding season (14 June), and one adult non-migratory house sparrow and two juvenile birds (a western meadowlark and a house sparrow) were also WNV-antibody positive, suggesting local infection. Another confounding factor could be the timing of our sampling; we sampled both birds and mosquitoes just prior to the peak of *C. tarsalis* mosquitoes. This might have contributed to the low number of birds and mosquitoes we detected with WNV.

Little is known about the influence of WNV on the reproductive success of North American bird populations. In this study, we sampled 33, 8 to 10 day-old yellow-headed blackbird nestlings, which were potentially at high risk of WNV exposure, but all were WNV-antibody negative. However, both of the WNV antibody-positive yellow-headed blackbird females experienced nest failure in 2004, suggesting potential impacts on population dynamics. Although this is anecdotal evidence, additional studies, including long-term monitoring of marked populations, are needed to help elucidate both the lethal and non-lethal impacts of WNV on North American bird populations.

We thank N. Gruber, R. Wentzel, and L. Newbrey for assistance in the field, and B. Blitvich and N. Marleen for ELISA training. J. Bell provided mosquito traps, assisted with mosquito identification, and conducted the PCR of the *C. tarsalis* specimens. We appreciate the helpful comments provided to us by the reviewers and the Associate Editor. We acknowledge the funding provided by the North Dakota Department of Health, North Dakota EPSCoR, and the North Dakota Water Resources Research Institute. This research was approved by the North Dakota

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Received: 28 December 2006 *Accepted: 27 March 2008*

Associate Editor for Ornithology: Gregory A. Smith