

Evaluating Red-cockaded Woodpeckers for Exposure to West Nile Virus and Blood Parasites

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Abstract - A marked decline in the *Picoides borealis* (Red-cockaded Woodpecker [RCW]) population at Noxubee National Wildlife Refuge, MS, was observed in 2002. Demographic changes—including absence of hatch-year birds, decreases in size of known groups, and loss of known groups—were identified during annual fall surveys and are uncharacteristic of RCW populations. In 2003, a serosurvey of 28 adult RCWs was conducted to investigate the presence of West Nile virus (WNV) exposure in the population, possibly providing insight into whether WNV may have been responsible for this decline. Blood smears were also examined from these birds for blood parasites. We found no evidence of West Nile virus exposure or blood parasites in any of the RCWs sampled. Further monitoring of the RCW population and WNV activity in other species at Noxubee NWR is recommended to further evaluate the potential role of WNV and blood parasites in their decline.

Introduction

Picoides borealis Vieillot (Red-cockaded Woodpecker [RCW]) is an endangered species endemic to the southeastern United States (US Fish and Wildlife Service 2003). They nest and roost in tree cavities, do not migrate, and hold a defendable territory around their cavity trees (see US Fish and Wildlife Service [2003] for a complete discussion of RCW ecology). At Noxubee National Wildlife Refuge (NWR), Brooksville, MS, the RCW population had grown from 16 groups (breeding pair and helper birds) in 1989 to 37 groups in 2002 (Richardson and Stockie 1995, US Fish and Wildlife Service 2003). This population is demographically isolated and receives no detectable immigration of birds from outside the population. In 2002, the population had good reproduction, as evidenced by monitoring of nests and banding of 35 nestlings by refuge personnel following recommendations of the RCW recovery plan (US Fish and Wildlife Service, Noxubee NWR, unpubl. data). Based on this monitoring effort, refuge personnel estimated that 50 nestlings should have fledged that year. However, during scheduled fall and early winter surveys in 2002, only 2 hatch-year (HY) birds were found, 3 of the 37 RCW groups could not be located, and 8 of the remaining 34 groups had decreased in

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size. The lack of HY birds and the loss of all birds from established groups are atypical for the population dynamics for this species (R. Costa, US Fish and Wildlife Service, Clemson, SC, pers. comm.).

West Nile virus (WNV) (Flaviviridae, Flavivirus) was first discovered in the United States in the fall of 1999 (Centers for Disease Control and Prevention 2000, Lanciotti et al. 1999). Since that time, WNV has spread rapidly in the Western Hemisphere (Peterson et al. 2004). West Nile virus was first reported in Mississippi in September 2001; by fall 2002, nearly every county in Mississippi reported WNV-positive birds (Mississippi Department of Health, Jackson, MS, unpubl. data). There were also numerous reports of WNV-positive *Corvus* sp. (crows) and *Cyanocitta cristata* Linnaeus (Blue Jays) near the Noxubee NWR (Mississippi Department of Health, unpubl. data).

West Nile virus is a mosquito-borne virus that was first identified in the West Nile region of Uganda in 1937 (Smithburn et al. 1940). The natural transmission cycle of WNV is principally a mosquito-bird cycle (see Komar 2003). West Nile virus has been reported in five woodpecker species, including two members of the same genus as RCW: *P. pubescens* Linnaeus (Downy Woodpecker) and *P. villosus* Linnaeus (Hairy Woodpecker) (E. Saito, National Wildlife Health Center, Madison, WI, pers. comm.).

Due to the dramatic apparent loss of RCWs at Noxubee NWR in 2002, the possibility of a disease such as WNV being responsible for the observed decline was considered. As a part of the investigation into the disappearance of RCWs at Noxubee NWR, a survey of apparently healthy RCWs was conducted at Noxubee NWR during the spring and summer of 2003 to determine the prevalence of blood parasites and specific WNV-neutralizing antibodies.

Methods

Red-cockaded Woodpeckers were captured between 11 April 2003 and 24 July 2003 at Noxubee NWR in accordance with guidelines established by the Red-cockaded Woodpecker Recovery Team (US Fish and Wildlife Service 2003). Captures were made at dusk and dawn, as RCWs went to their roost in the evening or left their roost in the morning, using a net attached to a telescoping pole that was placed over the entrance to the roost cavity. Captured birds were held for the minimum amount of time necessary (not longer than 30 min) to obtain a blood sample and band them. Birds were then released at the site of capture.

Blood samples (0.25 ml) were obtained from the brachial vein by piercing the vein with a 27-gauge needle, then using a capillary tube to collect the blood coming from the vein. Blood smears were prepared in the field immediately after sample collection and air dried. The remaining blood sample was then placed in a cooler with blue ice until returning to the lab. In the lab, blood samples were centrifuged for 10 minutes (at approximately 100 x g) to separate the serum from the red blood cells. The serum

was then removed and dispensed into a labeled cryovial and placed into a -70 °C freezer until shipped on dry ice to the National Wildlife Health Center for testing. The sera were tested for specific WNV-neutralizing antibodies using a microneutralization test (Beaty et al. 1995). Viral titer and determination of specificity for WNV were performed according to the current CDC guidelines (Centers for Disease Control and Prevention 2003). Briefly, equal volumes (0.05 mls) of WNV (50 infectious units) and each serum, diluted 1/5 and heat inactivated at 56 °C for 30 minutes, were added to a 96-well microtiter plate in duplicate. After a 30-minute incubation at 37 °C, VERO (*Chlorocebus aethiops* L. [African green monkey] kidney) cells were added to each test well (0.15 mls of a 1.67×10^5 -cells/ml suspension). Appropriate serum, cell, and WNV test-dose controls were included in the test. Cultures were then observed for virus neutralization over a one-week period. In the lab, blood smears were fixed with methanol, stained with Giemsa, and examined for the presence of blood parasites for ≥ 10 minutes at 400x using a compound microscope. Upper 95% confidence bounds were calculated on results based on estimated populations of 90 and 100 RCWs (Lindgren 1976).

Results

Twenty-eight adult woodpeckers were captured and blood sampled. Of these, one sample did not have sufficient serum for testing, and a blood smear was not prepared for one bird. All samples tested were negative for specific WNV-neutralizing antibody (0% prevalence). No blood parasites were detected on the blood smears (0% prevalence). An upper confidence bound was calculated, resulting in a maximum prevalence of 7.8% (N = 90) and 9% (N = 100).

Discussion

We were unable to detect specific WNV-neutralizing antibodies in any of the 27 RCWs sampled in this study. Although the sample size of 27 may seem modest, it constitutes about 1/3 of the remaining RCW population on Noxubee NWR during this investigation. This sample size is sufficient for us to have statistical confidence that the seroprevalence at Noxubee, even if not exactly 0, is indeed low.

It is almost a statistical impossibility to ever ascertain that the prevalence in a population is 0, but our results show convincingly that prevalence is low, especially in light of what might be expected to produce the sorts of declines thought to have occurred. We consider four possibilities for the low prevalence: 1) RCWs at Noxubee NWR may not have had much exposure to WNV, although WNV was diagnosed in sick and dead Blue Jays from Starkville, MS, (approximately 10 miles from Noxubee NWR) and nearly all counties in Mississippi reported WNV-positive birds in 2002 (Mississippi Department of Health, unpubl. data.); 2) WNV may be unevenly distributed

across the landscape, and unknown landscape factors at Noxubee NWR may not have been favorable for the maintenance and subsequent detection of WNV; 3) Any RCWs infected with WNV may have died prior to our survey; West Nile virus is reported to cause high mortality in some species of birds, particularly crows, where > 90% of experimentally infected individuals died (Komar 2003, McLean et al. 2001); and 4) We may have been unable to detect antibody presence in RCWs because birds previously infected had lost detectable specific WNV-neutralizing antibodies prior to sampling. Currently the only data available describing long-term (ca. 1 yr) duration of detectable specific WNV-neutralizing antibody in birds is limited to *Columba livia* Gmelin (Rock Doves) (Gibbs et al. 2005). It is not clear which, if any, of these four possibilities affected our findings.

We also were unable to detect blood parasites in the RCWs sampled. Others have found unidentified microfilaria and two species of *Haemoproteus* in RCWs (Luttrell et al. 1995, Pung et al. 2000). However, Love et al. (1953) reported no blood parasites in RCW blood smears they examined. In general, reports of mortality due to blood-parasite infection are mostly confined to domesticated birds or birds held in exotic environments, or mortality caused by introduced parasites (Bennett et al. 1993).

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