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Test of Recrudescence Hypothesis for Overwintering of West Nile Virus in Gray Catbirds

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ABSTRACT West Nile virus is a pathogen of concern for both human and wildlife health. Although many aspects of the ecology of West Nile virus are well understood, the mechanisms by which this and similar mosquito-borne viruses overwinter and become reinitiated each spring in temperate regions is not known. A thorough understanding of this mechanism is crucial to risk assessment and development of control strategies. One of the hypotheses to explain the mechanism by which this virus persists from year to year is the spring recrudescence of latent virus in avian reservoir hosts. Stress-related immunosuppression is implicated in the recrudescence of latent viruses in birds. We tested the spring recrudescence hypothesis in a controlled laboratory experiment using hatching-year gray catbirds (Dumetella carolinensis) captured in northern Ohio (July–August 2006). Catbirds (n = 60) were experimentally infected (September 2006) and later examined for the effects of immunosuppression through exogenous hormones and artificially induced migratory disposition. We found no effect of either testosterone or migratory behavior on infection status in any of the treatment birds. Moreover, we detected no viral RNA in the kidney, spleen, brain, or liver upon necropsy at 24 wk postinfection.

KEY WORDS West Nile virus, overwintering, Dumetella carolinensis, testosterone, migration
field evidence of recrudescence of eastern equine encephalitis, a closely related alphavirus, in a gray catbird (Dumetella carolinensis). In the Crans et al. (1994) study, a catbird was WNV seropositive in one breeding season, but in the following year, when tested again, it was WNV positive. Such evidence from field serosurveys is circumstantial and remains controversial because of our poor understanding of antibody persistence. Controlled laboratory experiments are required to evaluate the merit of the recrudescence hypothesis.

We hypothesize that stress may induce viremia in catbirds harboring latent WNV infections. We predict that birds previously infected with virus, but lacking detectable infections, would become viremic when immunosuppressed from the following: 1) the stress of migration and/or 2) hormonal changes associated with breeding. Migration is an energetically demanding, high risk period within a bird’s annual cycle, and birds are likely to divert resources away from immune mechanisms during this period (Fänge and Silverin 1985, Gylfe et al. 2000, Muñoz and Fuente 2003, Owen and Moore 2006, 2008). Numerous studies also suggest that elevated levels of testosterone, as observed during breeding, reduce immune function (Duffy et al. 2000, Deviche and Cortez 2005, but see Hasselquist et al. 1999). We chose to study gray catbirds because of the following: 1) they achieve infectious virus titers and experience no morbidity or mortality (Owen et al. 2006); 2) they are a common host in nature (Owen and Garvin 2010); and 3) they have a wide geographic distribution across the eastern half of the United States (Cimprich and Moore 1995).

Materials and Methods

Birds. We captured catbirds in Japanese mist nets (Oberlin College Institutional Animal Care and Use Committee 04RBMG1, Oberlin, OH) in July and August 2006 at three sites in north-central Ohio: Carlisle Reservation, Lorain County Metro Parks (41°17′N, 82°38′W), in LaGrange, OH; Firelands Scout Reservation (41°16′N, 82°20′W), located in Lorain County 5 miles west of Oberlin, OH; and Killbuck Marsh Wildlife Area (40°41′N, 81°58′W), in Wayne County, OH.

Blood was collected from the brachial vein into four unheparinized hematocrit tubes for detection of WNV via polymerase chain reaction (PCR) and two heparinized capillary tubes for detection of WNV antibody. One drop of blood was diluted in 300 μl of cell lysis solution (catalog D-5002; Gentra Systems, Minneapolis, MN) to determine gender via PCR-based analysis of sex-specific chromobox-helicase-DNA-binding (CHD) genes (Griffiths et al. 1998). Samples for virus and antibody detection were stored on ice and later centrifuged at 7800 rpm for 10 min. Plasma for virus detection was immediately removed and stored in a −70°C freezer. The plasma was removed and stored at −20°C. All birds were housed in individual cages in the Oberlin College animal care facility and fed ad libitum, as described below. All birds used for the experiment tested negative for both virus, via real-time PCR, and WNV-specific antibodies, via enzyme-linked immunosorbent assay (ELISA; see “Virology and Serology” below).

In September 2007, catbirds (n = 77) were transported to the University of Southern Mississippi (Hattiesburg, Forrest County, MS), housed in the university’s Animal Research Facility on 28 August 2007, and randomly assigned to one of the two animal biosafety level (ABSL)-3 rooms (n = 30 per room) or one non-ABSL-3 room (n = 17). Birds were caged individually and fed ad libitum a semisynthetic diet consisting of meal worms, blackberries, blueberries, wheat and malted barley cereal, moistened Zupreem monkey biscuits, cottage cheese, freeze-dried crickets, and a vitamin supplement. Body condition of the birds was monitored bimonthly by taking mass and assessing extent of fat (Helms and Drury 1960) and muscle (Bairlein et al. 1995) stores. Birds were maintained on 12:12 L:D photoperiod until January (see “Recrudescence Experimental Design” below). All blood samples were collected via the brachial vein using a 26-gauge needle and heparinized microhematocrit capillary tubes.

First Experimental Infection. On 21 September 2006, catbirds (n = 60) were injected subcutaneously with 10,000 plaque-forming units (pfu) of WNV strain NY385-99 using methods described by Owen et al. (2006). Blood (0.05 ml) was drawn to monitor viremia for the first 5 d postinoculation (dpi). Whole blood was placed in cyrovials, immediately placed on dry ice, and stored at −80°C. Blood (0.05 ml) was diluted with BA-1 (0.45 ml; composed of Hank’s M-199 salts, 1% bovine serum albumin, 350 mg/L sodium bicarbonate, 100 U/ml penicillin, 100 mg/L streptomycin, 1 mg/L Fungizone in 0.05 M Tris, pH 7.6) for determination of viremia titers. Titerers were determined in duplicate using Vero cell plaque assay in six-well plates with a double 1.0% agarose overlay (Beaty et al. 1995).

Second Experimental Infection. Of the 60 birds initially inoculated, 12 did not exhibit 30% inhibition values for both antibodies used in the ELISA (see below) by 14 dpi. Furthermore, an additional 25 individuals had lower than 30% inhibition for one of the two antibodies tested. Therefore, we supplemented the Ohio catbirds with hatching year catbirds (n = 20) captured at a fall banding station (FTM) on the Fort Morgan Peninsula in Alabama (30°13′N, 85°10′W) during October 2006 (federal permit 21221, Institutional Animal Care and Use Committee protocol 21-022). Birds (n = 20) were bled to determine antibody status via ELISA (result: 100% seronegative). The Alabama birds were acclimated to captivity for 4 wk, at which time nine of them and seven Ohio (negative controls from previous experiment) birds were inoculated on 11 November 2006 (see “First Experimental Infection” for method).

Recrudescence Experimental Design. In December 2006, catbirds (6 FTM and 53 Ohio; 1 female died in December) were randomly assigned to four treatment groups, as follows: 1) testosterone implants and non-migratory (males, n = 10); 2) migratory with no in-
plants (females, n = 10) or placebo implants (males, n = 10); 3) migratory with testosterone implants (males, n = 10); and 4) nonmigratory with no implants (females, n = 9) or placebo implants (males, n = 10). On 10 January 2007, the birds in the migratory room were photoadvanced 30 min each day for 8 d until they were at a 16:8 LD photoperiod. The nonmigratory room was kept at a 12:12 LD photoperiod. Each cage was equipped with an infrared motion detector, which records activity via a data logger (JoAC Electronic, Lund, Sweden) and activity analysis software (NI LabVIEW, National Instruments, Austin, TX). On 17 January 2007, the testosterone males were implanted with silicat tubing (interior diameter 1.47 mm, outer diameter 1.96 mm, length 25 mm) packed with crystalline testosterone (Androsten-17β-ol-3-one; Sigma-Aldrich, St. Louis, MO), the ends sealed with silicon gel. Implants were placed high on the breast between the skin and muscle. The amount of testosterone we used is known to cause elevated plasma testosterone with levels comparable to free-ranging, breeding male cattails (Ketterson et al. 1991). The remaining 10 placebo males in each group received an empty implant that was otherwise identical to those described above. All implants were conducted under sterile conditions.

Two days after testosterone implant, we began sampling birds for presence of virus. Birds were bled every other day for 2 wk postimplant. Blood (0.05-0.075 ml) was collected and diluted with BA-1 diluent for a 1/10 dilution in a 1.5-ml vial. The blood was allowed to clot for 30 min at 2°C and centrifuged for 3 min at 13,000 rpm. Fifty to 75 μl of dilution was used for viral RNA extraction using a QIAamp viral RNA kit, as described below.

Virology and Serology. With reference to the first inoculation (second inoculation in parentheses), birds were tested for WNV antibodies on 14, 30, 44, 65 (14), 81 (30), 92 (41), 111 (60), 144 (93), and 168 (117) dpi. Blood (0.30 ml) was collected from the brachial vein into centrifuge tubes and centrifuged. Plasma was drawn off and frozen at −20°C until shipped to Illinois Natural History Survey, where presence of antibodies was determined using an epitope-blocking ELISA (Blitvich et al. 2003a). Viral RNA was separated from supernatant using QIAamp viral RNA kit and stored at −80°C. ELISA analysis consisted of using two different monoclonal antibodies (MAbs): 2B2 (will react to WNV) and 3.1112G (specific for WNV). For a serum sample to be considered positive for antibodies to WNV, it had to block the binding of both MAbs by >30% relative to the negative control, normal chicken serum (Vector Laboratories, Burlingame, CA), which readily allows the binding of MAbs (Blitvich et al. 2003b, Beveroth et al. 2006). Serum from WNV-infected horses was used as a positive control (Beveroth et al. 2006).

Reverse transcription-PCR was performed with TaqMan One-Step reverse transcription-PCR assay kit (4309169; Applied Biosystems, Foster City, CA), according to Lanciotti et al. (2000). A 50-μl reaction mixture, including 5 μl of test samples and 45 μl of a master mixture, was placed in each sample reaction well. Master mix for each reaction consisted of 17.7 μl of RNase-free water, 0.5 μl each of forward and reverse primers, 0.30 μl of 6-carboxyfluorescein/5 (and 6-) carboxytetramethylrhodamine probe, 25.0 μl of TaqMan buffer, and 1.0 μl of enzyme. Thermal cycling was performed using a Bio-Rad (Hercules, CA) I-Cycler iQ real-time detection system. This procedure was done for all test samples, including positive, negative, blank, and extraction-negative controls.

Hormone Assays. Blood was drawn to measure baseline plasma corticosterone within 1-3 d of the following days: −3, 34, 69, 83, 109, 121 (3), 125 (8), 130 (13), 144 (27), and 154 (37) dpi. The numbers in parentheses refer to number of days postimplant. Every blood sampling period began at 1400, which was at least 3 h after the latest disturbance. Given the number of birds and a limited number of technicians, each sampling occurred over a 3-d period. Sampling was done within 5 min of entering the room, with most samples collected within the first 3 min. We measured the plasma levels of corticosterone using a competitive enzyme immunoassay (ELISA; Assay Designs, Ann Arbor, MI). Samples were diluted 35× in assay buffer and mixed with an equal volume of steroid displacement reagent (1:1000 concentration). All samples for an individual were assayed on the same 96-well plate, in duplicate, and in a random sequence. Six 5-fold serial dilution of 20,000 pg/ml concentration of corticosterone was added to each plate in triplicate. Each plate had negative and positive controls. The plates were read using a microplate reader and optical density of 405 nm. The intra-assay and interassay coefficients of variation were 0.11 and 0.14, respectively.

Plasma levels of testosterone in males were monitored 109 (subsampled), 121 (3), 125 (8), and 130 (13) dpi (numbers in parentheses refer to days postimplant). Samples were collected and assayed, as described above, for corticosterone, except, before conducting the assay, steroid hormones were first extracted using diethyl ether (3×). Samples were rehydrated in 350 μl of assay buffer. The intra-assay and interassay coefficients of variation were 0.14 and 0.08, respectively.

Euthanization and Necropsy. On 168 (117) dpi, the majority (n = 45) of the infected birds were euthanized by CO2 asphyxiation and necropsied (the number in parentheses refers to the birds in the second experimental infection). Tissues from brain, kidney, liver, and spleen were collected and stored at −80°C. A section of each organ was combined with 400 μl of BA-1 diluent and homogenized in individual 2.0-ml Biomasher tubes with 80–145 μm pore size (9140-1350, USA Scientific, Ocala, FL), which were centrifuged for 3 min at 13,000 rpm. Because some tissue cellular debris passed through the filter, each sample was centrifuged a second time for 12 s at 13,000 rpm, and the supernatant was collected and transferred to 0.5-ml vials and stored at −80°C until RNA extraction (see “Virology and Serology” above). The remaining 10 (five males and five females) individuals from mi-
Follow-up Experiments. After the conclusion of the migration and testosterone treatments, we randomly selected 10 birds in the migratory group (five females and five placebo males) for a food deprivation trial to determine whether further stress would induce relapse. On 11 April 2007, we withheld food from the birds from 1915 until 1600 the following day, at which time we only gave them approximately half of the food to which they were accustomed. We then resumed feeding normally on the morning of 13 April 2007. The food deprivation was an attempt to mimic the amount of time a migratory bird would be without food during a long-distance flight over an ecological barrier such as the Gulf of Mexico. We measured baseline corticosterone on 12 April 2007 at 1400, immediately before receiving food. We then measured baseline corticosterone on 20 April to see whether values changed in relation to food deprivation experiment. In addition, we collected blood samples for WNV RNA on 12 April.

After the food deprivation experiment, we attempted to induce relapse by injecting the birds with cyclophosphamide, a known immunosuppressant in birds (Trust et al. 1994). On 26 April, we injected the pectoral muscle with 1.0 mg of cytoxan (45 mg/kg cyclophosphamide (Reisen et al. 2003) and repeated injections each day for a total of three injections. Blood was sampled for WNV RNA on days 0, 3, 5, and 7 postinitial cyclophosphamide injection. Three birds died during this experiment, two on day 5 and one individual on day 7.

Data Analysis. Migratory activity was monitored each night of the experimental period. We quantified amount of migratory activity by counting the number of 5-min blocks in which each bird displayed at least three hops between the hours of 2300–0500 for both rooms. Birds were considered to be migrating when they showed activity for 40% or more of the nighttime periods (Owen et al. 2006). We analyzed testosterone using mixed between-within-subjects analysis of variance. The days of sampling, preimplant, 3 dpi, 9 dpi, and females did not maintain this activity after 6 d postinoculation, with the majority doing so by 30 dpi. In the second experimental infection, all 19 birds seroconverted (had inhibition rates higher than 30%). Sixteen of the birds produced antibody levels above the 30% inhibition at some point after inoculation, with the majority doing so by 30 dpi. In the second experimental infection, all 19 birds seroconverted (had inhibition rates higher than 30%). Sixteen of the birds seroconverted by 14 dpi, and all had seroconverted by 30 dpi. Persistence of antibodies did decline, particularly for 2B2, with 75% of the birds being seropositive on 30 dpi and only 50 and 34% of the birds being seropositive at 117 and 168 dpi, respectively (Fig. 1).

Migration. In the treatment group, all but one bird, a placebo male, displayed migratory restlessness. In the control group, seven of the testosterone-implanted males and two females exhibited activity consistent with migratory restlessness. However, the females did not maintain this activity after 6 d postinoculation. The onset of migratory activity differed between the groups. T-implanted males in the migratory room initiated migration first, followed by placebo-implanted males 10 d later, and then females 12 d after placebo males (J.C.O., unpublished data).

Results

Avian Acute Viremia. The acute viremia response of gray catbirds (n = 59) was identical to that found in earlier studies with the same species (Owen et al. 2006). Our detection threshold for the plaque assays was 1.7 log pfu/ml. Peak viremia occurred 2 dpi (Table 1), with individual titers ranging from below detection to 6.94 log pfu/ml blood (mean = 4.94, SD ± 0.88). Average peak viremia did not differ between males and females (df = 47, t = 0.336, P = 0.74) or between Fort Morgan, AL and Ohio birds (df = 11, t = 0.616, P = 0.55). No morbidity or mortality was associated with the WNV infection. By 5 dpi, only one of the assayed birds (n = 50) had detectable virus. Seven of the 59 birds did not exhibit detectable viremia for the 1–3 dpi. However, these same individuals seroconverted (see below).

Serology. All the birds used for the recrudescence experiment were positive for WNV antibodies at some point postinoculation. As mentioned previously, not all the birds from the first experimental infection exhibited 30% inhibition on a blocking ELISA for WNV antibodies on 14 dpi; however, 51 of the 60 infected birds (85%) did produce antibody levels above the 30% inhibition at some point after inoculation, with the majority doing so by 30 dpi. In the second experimental infection, all 19 birds seroconverted (had inhibition rates higher than 30%). Sixteen of the birds seroconverted by 14 dpi, and all had seroconverted by 30 dpi. Persistence of antibodies did decline, particularly for 2B2, with 75% of the birds being seropositive on 30 dpi and only 50 and 34% of the birds being seropositive at 117 and 168 dpi, respectively (Fig. 1).

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Hormones. Testosterone-implanted males had significantly higher circulating testosterone levels than placebo-implanted males across the three sampling periods postinoculation (Fig. 2) (F = 182.8; df = 1, 32; P < 0.001). Individuals for which we have preimplant data demonstrated significant differences in testosterone levels between preimplantation and 3 d postimplantation for the T group (df = 7, t = −4.8, P = 0.002),

<table>
<thead>
<tr>
<th>No. successfully assayed of 59 samples</th>
<th>No. positive &gt;1.7 log pfu/ml</th>
<th>Minimum (above 1.7), maximum</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 dpi</td>
<td>58</td>
<td>37</td>
<td>2.3, 5.4</td>
</tr>
<tr>
<td>2 dpi</td>
<td>57</td>
<td>48</td>
<td>3.2, 6.7</td>
</tr>
<tr>
<td>3 dpi</td>
<td>55</td>
<td>28</td>
<td>3.0, 5.5</td>
</tr>
<tr>
<td>4 dpi</td>
<td>54</td>
<td>15</td>
<td>1.7, 4.6</td>
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<tr>
<td>5 dpi</td>
<td>50</td>
<td>1</td>
<td>4.5</td>
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but levels did not change for placebo-implanted males (df = 3, t = −1.51, P = 0.227). We found the same relationship for 8 and 13 d postimplantation.

Baseline levels of corticosterone changed between samplings (F = 4.47, 227.3 = 5.007, P < 0.001). No significant interaction was detected between group and time period (F = 1.538; df = 22.3, 227.3; P = 0.062). No differences were detected between the treatment groups (F = 0.502; df = 5, 51; P = 0.773). Of the 36 multiple post hoc comparisons (Bonferroni corrected), only two revealed significant differences. Birds had higher corticosterone levels on 69 dpi (mid-November) than when sampled in early January (before implant and initiation of migration) and 20 January (3 d postimplant). In the pilot food deprivation study, we found significant differences in average baseline corticosterone on the day of the food deprivation (mean = 49.2, SD = 101.5) compared with 8 d postdeprivation (mean = 0.66, SD = 0.33, Z = −2.51, n = 8, P = 0.01).

Recrudescence. Based on blood samples collected every 2 d, we found no WNV viral RNA in any samples. From those same samples, we then randomly selected 80 samples and tested them for infectious virus using Vero cell plaque assay. All samples tested negative for live virus. At the conclusion of the experiment(s), we found no WNV viral RNA in the kidney, brain, spleen, or liver of any of the birds.

Discussion

We found no evidence of WNV recrudescence in the experimentally infected gray catbirds in response to migratory activity, elevated testosterone levels, and/or food deprivation. Furthermore, we detected no latent infection in spleen, kidney, brain, or liver at 24 wk postinfection despite all birds being viremic at the onset of the experiment. As reported by Owen et al. (2006), we do find that catbirds are competent reservoir hosts for the virus. In addition to the fact that they achieve viral titers high enough to be infectious to mosquitoes (Turell et al. 2001, Komar et al. 2003) and are commonly exposed to WNV in nature (Owen and Garvin 2010), they also experience no morbidity or mortality when infected. Hence, if recrudescence was a mechanism for persistence, the catbird is an ideal candidate species.

Relapse of chronic infections has been noted for other avian pathogens. For example, studies of avian malaria in passerine birds show that the phenomenon of spring relapse of latent infections in birds is likely initiated by increased levels of hormones associated with breeding or stress (Applegate and Beaudoin 1970). In addition, latent infections of the bacteria *Borrelia burgdorferi sensu lato*, the causative agent of Lyme disease, in passerine birds have been reactivated by the stress of migration (Gylfe et al. 2000). Viral recrudescence of cryptic infections has been suspected in a closely related flavivirus, SLEV (Gruwell et al. 2000). Viral recrudescence of cryptic infections has been suspected in a closely related flavivirus, SLEV (Gruwell et al. 2000). Furthermore, experimental immunosuppression by Reisen et al. (2003) provides evidence of relapse of SLEV infections after treatment with cyclophosphamide. Periodic viremias of WNV have been observed in rock pigeons infected with WNV (Semenov et al. 1973; see also Kuno 2001), and Reisen...
have data on the catbirds for as the basis for reactivation of a latent infection. Al-
titer are not uncommon (Kuno 2001) and may serve
hibition threshold. Natural ßuctuations in antibody
declare a bird seropositive. A higher percentage of our
tralization tests if only one positive MAb is required to
Notably, we followed the Blitvich et al. (2003b) pro-
duction neutralization tests (Blitvich et al. 2003b).
We observed quite a bit of variation in presence of
infected rock doves at 3, 24, and 45 wk and found little
examined anti-WNV antibody presence in naturally
reinfection (Reisen et al. 2001). Gibbs et al. (2005)
found more agreement with the plaque reduction neu-
atization tests if only one positive MAb is required to
declare a bird seropositive. A higher percentage of our
birds tested positive for one MAb using the 30% inhi-
bition for both MAbs. Gibbs et al. (2005) found that the
recovery of a latent infection is more likely
to occur when an individual has a low antibody titer. We observed quite a bit of variation in presence of antibodies within individuals across time. Some indivi-
duals tested positive, then negative, and then posi-
tive again. We believe this ßuctuation is because of the
sensitivity of the epitope-blocking ELISA in detecting
anti-WNV antibodies even when titers are very low,
and that the results correspond well with plaque re-
duction neutralization tests (Blitvich et al. 2003b). Notably, we followed the Blitvich et al. (2003b) pro-
tocol, which considers birds positive if they exhibit
>30% inhibition for both MAbs. Gibbs et al. (2005) 
found more agreement with the plaque reduction neu-
atization tests if only one positive MAb is required to
declare a bird seropositive. A higher percentage of our
birds tested positive for one MAb using the 30% inhibi-
tion threshold. Natural ßuctuations in antibody
bition rates of individuals in the 24 wk especially for
24-wk period. We did see an overall decline in inhi-
bition for both MAbs suggests either an absence of antibodies or extremely low titers. In either case, an
undetectable antibody titer may leave a bird suscep-
tible to either relapse of a latent WNV infection or
reactivation of a latent infection (Reisen et al. 2001). Gibbs et al. (2005) examined anti-WNV antibody presence in naturally
infected rock doves at 3, 24, and 45 wk and found little
variation in titer or presence within individuals. The
observed ßuctuation in our study could have resulted from stress, including repeated sampling within a
24-wk period. We did see an overall decline in inhibi-
tion rates of individuals in the 24 wk especially for
2B2, unlike Gibbs et al. (2005), who found that
Colonbela livia antibodies persisted for close to 15 mo.
Komar et al. (2003) found ßuctuations in antibody
titers of experimentally infected rock doves held for 64
dpi, with lowest titers occurring 5–6 wk postinfection.
Although gray catbirds appear to be competent
hosts for WNV, are commonly infected, and distrib-
uted across a wide geographic range, they are not
especially competent reservoir hosts relative to other
wild bird species (Kilpatrick et al. 2007). Given our
finding that they do not develop long-term, chronic
infections of WNV, other avian species most likely
serve as better candidates for harboring the virus over-
winter. Further studies should test the overwintering
hypothesis with other species of birds. House ßinches,
for example, maintain latent WNV infections more
often than other avian species (Reisen 2006). In ad-
dition, American robins, which are shown to be im-
portant in the WNV transmission cycle (Kilpatrick et
al. 2006), may be a species warranting more attention.

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catcher (Ficedula hypoleuca; Aves, Passeriformes). J.

et al. (2006) detected WNV RNA in tissues of multiple
species of birds at >6 wk postinoculation. We do not
have data on the catbirds for <17 wk (second exper-
imental group); we may have detected virus in tissue
if birds had been necropsied earlier. Regardless, for
birds to initiate a transmission cycle during the spring,
they must maintain chronic infections through the
entire winter.

American Robins, which are shown to be im-
portant in the WNV transmission cycle (Kilpatrick et
al. 2007), may be a species warranting more attention.


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