ABSTRACT  Eastern equine encephalitis virus (EEEV; family Togaviridae, genus Alphavirus) epizootics are infrequent, but they can lead to high mortality in infected horses and humans. Despite the importance of EEEV to human and animal health, little is known about how the virus overwinters and reinitiates transmission each spring, particularly in temperate regions where infected adult mosquitoes are unlikely to survive through the winter. One hypothesis to explain the mechanism by which this virus persists from year to year is the spring recrudescence of latent virus in avian reservoir hosts. In this study, we tested the recrudescence hypothesis with gray catbirds (Dumetella carolinensis) captured in northern Ohio (July–August 2007). Birds were experimentally infected with EEEV on 1 October 2007. In January 2008, they were then exposed to exogenous testosterone and/or extended photoperiod to initiate reactivation of latent EEEV infection. All birds became viremic with EEEV, with mean viremia of 6.0 log10 plaque-forming units/ml serum occurring at 1 d postinoculation. One male in the testosterone, long-day treatment group had EEEV viral RNA in a cloacal swab collected on 18 January 2008. Otherwise, no other catbirds exhibited reactivated infections in cloacal swabs or blood. Antibody titers fluctuated over the course of the study, with lowest titers observed in January 2008, which corresponded with the lowest mean weight of the birds. No EEEV viral RNA was detected in the blood, kidney, spleen, brain, liver, and lower intestine upon necropsy at 19 wk postinfection.

KEY WORDS  eastern equine encephalitis virus, overwintering, Dumetella carolinensis, testosterone, recrudescence

Eastern equine encephalitis virus (EEEV), an Alphavirus in the family Togaviridae, is capable of causing acute and fatal disease in humans, nonhuman mammals, and birds in the eastern United States (CDC 2010). Although outbreaks in humans are infrequent, the 30–50% fatality rate is greater than that reported for all encephalitis viruses in North America (Feemster 1957, Przelomski et al. 1988, Letson et al. 1993). In horses, the case fatality rate is exceptionally high with ≥90–95% mortality (Waldridge et al. 2003). Likewise, other nonhuman mammalian species, such as camelids (Nolen-Walston et al. 2007), cervids (Tate et al. 2005), and swine (Pursell et al. 1972), also experience high mortality. Bird susceptibility varies with species (Holden 1955, Dein et al. 1986, Tully Jr. et al. 1992, McLean et al. 1995, Gottdenker et al. 2003). Given the high associated mortality, particularly in important species such as horses, EEEV is classified as a select or bioterrorism agent because of its potential threat to the health and security of the public (EPA 2002). Yet, despite the importance of EEEV to equine, human, and avian health, few studies have investigated overwintering and annual initiation mechanisms.

The enzootic transmission cycle of EEEV involves avian reservoir hosts and the ornithophilic mosquito, Culiseta melanura Coquillet, as the primary vector. Most EEEV activity is reported from the Atlantic and Gulf Coast states or from inland sites near freshwater wetlands where C. melanura and susceptible avian amplification hosts breed (Hayes et al. 1962, Emord and Morris 1984). Epizootics occur at these foci approximately every 9–10 yr when enzootic and bridge
vector breeding conditions are ideal and the virus is transmitted from birds to horses and humans (Hayes and Hess 1964). Prevalence of EEEV in natural avian populations is well documented (Nasci et al. 1993; Garvin et al. 2004a, 2004b), but only a handful of experimental infection studies have been conducted (Karstad et al. 1959, McLean et al. 1993, Komar et al. 1999), so knowledge on host competence and susceptibility is limited.

How EEEV and other arthropod-borne viruses overwinter is unknown, although several hypotheses have been proposed and tested (Reisen et al. 2001, 2006; Owen et al. 2010), including the hypothesis of spring recrudescence, which states that viruses overwinter as latent infections in birds and then reactivate to a viremic phase the following spring in response to a stressor. No experimental infection studies have been conducted to test the recrudescence hypothesis with EEEV. Crans et al. (1994) documented the first field-based evidence of recrudescence for EEEV, in which a gray catbird (Dumetella carolinensis) that tested positive for EEEV antibodies was EEEV virus positive in the following year, ~13 mo later. Furthermore, viremic birds have been reported in the early spring, before virus infection in the C. melanura population and well before peak EEEV virus activity (Emord and Morris 1984, Crans et al. 1994). Studies have examined this hypothesis with other arthropod-borne viruses (Reisen et al. 2001, Owen et al. 2010), but efforts to reactivate latent infections in birds have largely been unsuccessful (Reisen et al. 2001, Owen et al. 2010).

In the current study, we test the hypothesis that the treatment of elevated testosterone and/or the induction of migratory disposition cause the reactivation of a latent EEEV infection in gray catbirds. As mentioned above, catbirds are particularly well suited for this experiment because of the following: 1) they are the only species for which support of recrudescence hypothesis exists; 2) their nesting habitat includes forested wetlands where the primary enzootic vector in eastern North America, C. melanura, overwinters in the larval stages; and 3) they exhibit high seroprevalence for EEEV antibodies (Srihongse et al. 1978, Beaty et al. 1995). In addition to testing the spring recrudescence hypothesis, we also determine the EEEV viremia profile for catbirds.

Materials and Methods

Because most methods follow Owen et al. (2010), in which we conducted a similar experiment with catbirds and West Nile virus (WNV), in this work we only present variations from those methods.

Birds. Catbirds were captured using mist nets (Oberlin College Institutional Animal Use and Care Protocol, IACUC 04RBMC1; USFWs permit MB827915-1; Ohio State Collection Permit 229) in July and August 2007 at three sites in northcentral Ohio (Owen et al. 2010). Blood was collected from the brachial vein into four unheparinized hematocrit tubes for detection of EEEV via polymerase chain reaction, and three heparinized capillary tubes for detection of EEEV antibody (Owen et al. 2010). The plasma for antibody was removed and stored at 4°C and shipped to Florida Department of Health Bureau of Laboratories for antibody assays. All birds were housed in individual cages in the Oberlin College animal care facility and fed ad libitum, as described in Owen et al. (2010). All birds used for the experiment tested negative for both virus via reverse transcription-polymerase chain reaction (RT-PCR) and EEEV-specific antibodies via serum neutralization assays (see Virology and Serology below).

Gray catbirds (n = 76) were transported to the University of Southern Mississippi (Hattiesburg, Forrest County, MS), housed in the university’s animal research facility on 24 August 2007, and randomly assigned to one of the two animal biosafety level 3 (ABSL-3) rooms (n = 30/room) or one of two non-ABSL-3 rooms (n = 7–8/room). Birds were caged individually and fed ad libitum a semisynthetic diet, and body condition was assessed, as described by Owen et al. (2010). Birds were maintained on natural photoperiod until a 12:12 light-dark (L:D) photoperiod was reached on 27 September 2007, then maintained at 12:12 L:D photoperiod to simulate an approximate winter photoperiod until 25 December 2007. On 25 December, we photoadvanced treatment birds (see Recrudescence Experimental Design section below).

Experimental Infection. On 1 October 2007, catbirds (n = 60) were injected subcutaneously with 10,000 plaque-forming units (pfu) of EEEV strain NV69-7836 (obtained from Wadsworth Center, Albany, NY; New York State Department of Health) using methods described by Owen et al. (2006). The remaining birds (n = 16) were sham inoculated with BA-1 diluent of the same volume. Blood (0.05 ml) was drawn from all birds to monitor viremia for the first 5 d postinoculation (dpi). Whole blood was placed in cryovials containing 0.225 ml of BA-1, immediately placed on dry ice, and stored at ~80°C. Titters were determined in duplicate using Vero cell plaque assays in six-well plates with a double 1.0% agarose overlay (Beaty et al. 1995).

Recrudescence Experimental Design. In December 2007, the 60 infected catbirds were randomly assigned to four treatment groups, as follows: 1) normal day length with testosterone implants (males, n = 10; TND); 2) normal day length with no implants (females, n = 9; FND) or placebo implants (males, n = 10; PND); 3) extended day length with testosterone implants (males, n = 10; TLD); and 4) extended day length with no implants (females, n = 10; FLD) or placebo implants (males, n = 10; PLD). The negative control birds were stratified by sex and randomly assigned to two groups, as follows: 1) normal day length (n = 8; CND), and 2) extended day length (n = 8; CLD). On 25 December 2007, the birds in the extended day length room were photoadvanced 30 min each day for 8 d until they were at a 16:8 L:D photoperiod. The normal day length room was kept at a 12:12 L:D photoperiod. Each cage was equipped...
with an infrared motion detector that recorded day and night activity via a data logger (JoAC Electronic, Lund, Sweden) and activity analysis software (NI LabVIEW; National Instruments, Austin, TX). We did not monitor migratory activity in the control bird rooms because of lack of equipment, but the photoperiod was manipulated identical to the treatment rooms. On 10 January 2008, the testosterone males received a testosterone-packed silastic tubing implant subcutaneously (see Owen et al. 2010), and the remaining placebo males received an empty implant of the same dimension. All implantation surgeries were conducted under sterile conditions.

On 12 January 2008, we began sampling both blood and cloacal swabs for presence of EEEV RNA. Birds were bled and swabbed on days 2, 4, 6, 8, 12, 14, 17, 19, 21, 23, 25, and 30 d postimplant (dpm). Blood (0.05–0.075 ml) was collected and diluted with BA-1 for a 1:10 dilution in a 1.5 ml vial. The blood was allowed to coagulate overnight at 4°C and centrifuged (Eppendorf centrifuge 5424) for 3 min at 13,000 rcf. Fifty to 75 μl of the supernatant was used for viral RNA extraction using a QIAamp viral RNA kit, as described below. Cloacal swabs were collected using a Dacron miniswab, immediately placed in 0.05 ml of BA-1, and centrifuged for 3 min at 13,000 rcf. The supernatant was drawn off and placed in a new vial for RNA extraction.

**Virology and Serology.** Blood was drawn for EEEV antibodies on days 14, 36, 70, 113, and 131 d postinculation. Blood (0.400 ml) was collected from the brachial vein into centrifuge tubes, and centrifuged (IEC Centra-CL3) for 10 min at 6,200 rcf. Plasma was kept at 4°C and then shipped on ice packs to Florida Department of Health Bureau of Laboratories, where neutralizing antibody titer was determined using a plaque reduction neutralization test. Serum dilutions were challenged with 200 pfu of EEEV strain D64-S37. This virus was first isolated from a fatal human case in 1964 through inoculation in sucking mice brain, and subsequently passed eight times for additional material in mice before inoculation into cell culture (BGM and VERO cells; Morris et al. 1994). This strain had been isolated from a fatal Florida human case in 1964 and is routinely used in laboratory serum neutralization assays. Positive and negative control antisera were obtained from previously assayed sentinel chicken sera. Serum was serially diluted 2-fold from 1:10 to 1:320. Antibody titers are reported as the reciprocal serum dilution that inhibited 90% of the virus plaque formation (PRNT90). Results that indicate titers of >320 were assigned a titer of 320 for subsequent analyses and figures, given we do not know to what extent their titer exceeded 320. If the titer was <10, it was considered EEEV antibody negative.

Viral RNA was separated from supernatant using QIAamp viral RNA kit and stored at −80°C. RT-PCR for EEEV RNA was performed with TaqMan One-Step RT-PCR assay kit (Applied Biosystems 4309169, Foster City, CA; Life Technologies, Carlsbad, CA) using the primers and probe and methods described by Lambert et al. (2003) for TaqMan assay. A 50 μl reaction mixture, including 5 μl of test sample 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 diluted to 100 μM, 0.30 μl of 6-carboxyfluorescein/5-(and 6)-carboxytetramethylrhodamine probe diluted to 25 μM, 25.0 μl of TaqMan buffer, and 1.0 μl of enzyme. Thermal cycling was performed using a Bio-Rad i-Cycler iQ real-time detection system. This procedure was done for all test samples, including positive, negative, blank, and extraction negative controls. Positive control sera were from one of the captive catbirds (O264) that had been inoculated with the virus, but died prior to the recrudescence experiment. O264 was virus positive, as determined by plaque assay, and had seroconverted exhibiting PRNT95 values of 1:320. Negative controls were captive catbirds that were sham inoculated with BA-1 diluent and had no detectable virus or antibodies.

**Hormone Assays.** Blood was drawn to measure baseline plasma corticosterone within 1–3 d (Owen et al. 2010) of the following days: 24 September, 24 October, 10 December, 7 January, 16 January, and 30 January. Sampling was completed within 3 min of entering the room to ensure measurement of baseline corticosterone (Wingfield and Romero 2001). We measured the plasma levels of corticosterone using a competitive enzyme-linked immunosorbent assay (Assay Designs, Ann Arbor, MI), as described by Owen et al. (2010). The standard used for each assay was from stock of pooled serum of gray catbirds not included in the current study. The average intra-assay and interassay coefficients of variation were 0.10 and 0.21, respectively. As a result of extensive interassay variation, we corrected all values based on the deviation from the mean titer of the standard serum for each plate. Plasma levels of testosterone in males were monitored on 7 January, 16 January, and 30 January 2008, as described by Owen et al. (2010). The intra-assay and interassay coefficients of variation were 0.08 and 0.12, respectively.

**Euthanization and Necropsy.** On 9 February 2008, all catbirds were euthanized by CO2 asphyxiation and necropsied. Tissue sections of the brain, kidney, liver, spleen, and lower intestine were collected and stored at −80°C. Tissues were processed as described by Owen et al. (2010). All animal procedures at University of Southern Mississippi were approved by both Oberlin Institutional Animal Care and Use Committee and University of Southern Mississippi Institutional Animal Care and Use Committee Protocol 21-022.

**Results**

We began the experiment with 60 EEEV-infected birds and 16 negative controls. Two birds died during the experimental period. An EEEV-infected female (O264) died on 12 December 2007 and, therefore, was not included in the recrudescence experiment. An EEEV-infected PND male (Y86) died on 15 January 2008. Both birds died of unknown causes and exhibited
substantial weight loss precluding death. None of the negative control birds became infected during the study or developed EEEV antibodies.

**Avian Acute Viremia.** Our detection threshold for the plaque assays was 1.7 log_{10} pfu/ml. Peak viremia occurred 1 dpi (Table 1), with individual titers ranging from below detection to 8.4 log_{10} pfu/ml blood. Virus titers on 1 and 2 dpi did not differ between males and females (independent samples t test on log-transformed data; 1 dpi, df = 56, t = −1.07, P = 0.29; 2 dpi, df = 56, t = 0.50, P = 0.62). No clinical signs of disease or mortality were associated with EEEV infection. No relationship was observed between a bird’s starting mass on 0 dpi and its peak viremia (1 dpi, males, F = 0.42; df = 1, 39; P = 0.84; females, F = 0.33; df = 1, 18; P = 0.58). Yet, a relationship between titer and subsequent mass in males emerged, with titer on 2 dpi being a predictor of mass on 3 dpi (F = 7.67; df = 1, 38; P = 0.009). By 4 dpi, only two of the birds had detectable virus. One bird never exhibited detectable viremia, but it did exhibit EEEV-specific antibodies (see below).

**Recrudescence and Evidence of Latent Infection.** We found no viral RNA in any of the blood samples collected over the experimental period. We did find one male from the testosterone and extended daylength group positive via PCR for viral RNA from a cloacal swab collected on 18 January 2008. The cycle threshold (Ct) score of this sample was 34 (positive control Ct = 31.2). We subsequently re-extracted the RNA and confirmed the positive, recording a Ct of 37 (positive control Ct = 34.7). In both cases, the slope of the sample curve followed the positive control curve. A subsequent plaque assay was conducted on this sample, but infectious virus was not detected. At the conclusion of the experiment(s), we found no EEEV RNA in the kidney, brain, spleen, liver, and lower intestine of any of the birds.

**Serology.** All the birds seroconverted by 14 dpi, and by 131 dpi 56 individuals still had detectable levels of neutralizing antibodies. Repeated measures analysis of variance performed on titer was used to test the effect of groups and time. PRNT_{90} titers fluctuated over the course of the experiment (Fig. 1; F = 8.01; df = 4, 212; P < 0.001), with lowest titers observed 14 dpi and at the last sampling on 131 dpi (Fig. 1). The interaction term time × treatment group was not significant (F = 1.24; df = 20, 212; P = 0.224), indicating that titer in each group did not vary differently at each time. The effect of treatment group on antibody titer was not statistically significant (F = 0.65; df = 5, 53; P = 0.665). Pairwise comparisons between sampling periods, with the groups combined, indicate significant differences between 14 and 70 dpi (t = −4.31, df = 58, P < 0.001), 36 dpi and 131 dpi (t = 3.54, df = 58, P = 0.001), 70 dpi and 113 dpi (t = 4.30, df = 58, P < 0.001), and 70 dpi and 131 dpi (t = 5.34, df = 58, P < 0.001). The peak antibody titers were observed on 36 and 70 dpi. A large proportion of samples had titers exceeding 320; broken down by sampling period, this number was 29, 93, 90, 67, and 54% of the samples being >320 for 14, 36, 70, 113, and 131 dpi, respectively.

**Induction of Migratory Disposition.** To assess whether birds exposed to extended daylength exhibited migratory disposition, we analyzed nocturnal activity data for significant changes over three seasonal periods, as follows: prephotoadvancing, during photoadvancement, and postphotoadvancement. We observed significant difference in activity across time (F = 3.44; df = 2, 106; P = 0.036) across all groups. However, an interaction between group and time (F = 3.53; df = 10,106; P < 0.001) was also found. TLD (F = 6.17; df = 2, 18; P = 0.009) and TND (F = 5.42; df = 2, 18; P = 0.014) were the only two groups to show increased activity across time. Thus, exposure to extended photoperiod did not induce migratory activity, but testosterone did lead to an increase in activity regardless of photoperiod treatment.

**Hormones.** Before the implant, testosterone levels did not differ between placebo and testosterone groups (df = 33; t = 1.11; P = 0.31). Testosterone concentration changed over time for both placebo and testosterone groups (F = 8.98; df = 1, 58; P < 0.001; Fig. 1. Mean (±SE) PRNT_{90} titers for EEEV-specific antibodies in gray catbirds (n = 60) on 5 sampling days after inoculation on 1 October 2007. Each line refers to a different treatment group, although treatments were not initiated until 25 December 2007.
2). There was a significant interaction detected between group and time period \((F = 9.54; \text{df} = 1, 58; P < 0.001)\). Testosterone levels decreased in the placebo group over time compared with the increase observed in the testosterone group (Fig. 2). Significant differences were observed between testosterone and placebo groups \((F = 33.27; \text{df} = 1, 29; P < 0.001)\), with testosterone-implanted males having significantly higher circulating testosterone levels than placebo-implanted males (Fig. 2).

Baseline levels of corticosterone did not change over the six sampling periods \((F = 1.19; \text{df} = 5, 205; P = 0.31)\). No significant interaction was detected between group and time period \((F = 1.12; \text{df} = 25, 205; P = 0.33)\). There were no between treatment group differences \((F = 25; 205; 1.12; P = 0.33)\). Posthoc paired \(t\) tests revealed significant differences in corticosterone concentration between males and females at the 7 January 2008 sampling, with females having significantly higher \((\text{mean} = 3.3 \text{ ng/ml})\) values than males \((\text{mean} = 1.1 \text{ ng/ml}; \text{df} = 56; t = 2.72; P = 0.009)\). No other differences were detected.

Discussion

We found little support for the stress-induced EEEV recrudescence hypothesis in experimentally infected gray catbirds. We detected EEEV RNA in a cloacal swab of one male in the testosterone, long-day treatment group that can only be described as reactivation, given virus must have been present as a latent infection to recrudesce. However, we did not detect viral RNA in blood collected on the same day, and efforts to detect infectious virus from the RNA-positive sample via plaque assay were not successful. The inability to recover infectious virus may be a result of sample collection and storage or a very low viral load in the specimen. Hence, we cannot draw reliable conclusions about the infectiousness of the viral RNA found in the cloacal swab.

Catbirds responded similar to other EEEV experimentally infected passerine birds with peak virus titers on 1 dpi and most infections clearing by 3 dpi (Komar et al. 1999). Although we note that the response to the virus can vary with route of infection, the catbirds in this study exhibited titers that exceeded those reported for most of the birds experimentally infected with EEEV. These include passerines (Komar et al. 1999), with the exception of European starlings (Sturnus vulgaris), wading birds (Kissling et al. 1995, Mclean et al. 1995), and waterfowl (Aguirre et al. 1992). The threshold for infecting at least 10% of the C. melanura enzootic vector is considered to be 4.0 log\(_{10}\) dfu/ml (Komar et al. 1999). After this threshold, catbirds are considered infectious for at least 2 dpi, and, aside from starlings, which were infectious for 3 dpi, all other passerines tested by Komar et al. (1999) were considered infectious for 1 dpi. In this study, all catbirds were hatch year/juvenile birds at the time of infection, and it has been shown that first year birds are more likely to contribute to the transmission cycle of EEEV than adult birds (Unnasch et al. 2006). Overall, catbirds appear to be competent reservoirs for EEEV.

Catbirds did not suffer any apparent mortality from the EEEV infection. Two individuals died during the experiment, but both occurred after the viremic period and no EEEV viral RNA was detected in their tissues. Although EEEV is not considered especially pathogenic to birds, mortality has been documented in EEEV-infected birds, including free-ranging birds such as whooping cranes (Grus americana) (Dein et al. 1986) and a great egret (Ardea alba) (Gottdenker et al. 2005): domestic species, including turkeys (Ficken et al. 1993); and exotic, captive birds, including emus (Dromaius novaehollandiae) (Tully Jr. et al. 1992, Day and Stark 1996) and ringed-necked pheasants (Phasianus colchicus) (Williams et al. 2000). In experimental infection studies (Karstad et al. 1959, Aguirre et al. 1992, McLean et al. 1995, Komar et al. 1999), only European starlings and glossy ibis (Plegadis falcinellus) appeared to exhibit EEEV-related mortality.

The significance of viral RNA in the fecal sample of one of the birds 110 d postinfection is difficult to assess. Virus is commonly shed in the feces during an initial infection (Satriano et al. 1957, Komar et al. 2003, Reisen et al. 2004, Kipp et al. 2006), but the role of infected fecal material in the transmission of an arthropod-borne virus has not been well studied. Direct transmission of WNV through infectious fecal or oral secretions has been demonstrated in American crows (Corvus brachyrhynchos) (McLean et al. 2001) and chickens (Langevin et al. 2001), and in the closely related St. Louis encephalitis virus it has been shown in mourning doves (Zenaida macroura) (Reisen et al. 2004). With EEEV, direct transmission has not been documented via a fecal–oral route, although ring-necked pheasants became infected with EEEV through feather picking and cannibalism of infected individuals (Holden 1955). In our prior recrudescence study with WNV-infected catbirds (Owen et al. 2010), we did not look for WNV RNA in the cloacal swabs, suggesting that we may have missed a reactivation event.
No birds, including the male that recrudesced, had EEEV viral RNA-positive tissue at necropsy. ∼19 wk postinfection. As with the previous study on WNV (Owen et al. 2010), we cannot identify birds that cleared latent infections before euthanization, nor can we distinguish them from uninfected birds. The detection of recrudescing virus in a cloacal swab on 16 wk postinfection suggests that catbirds can maintain latent infections. To our knowledge, no EEEV experimental infection studies have specifically looked for latent virus in the tissues. McLean et al. (1995) isolated virus from the heart, spleen, and brain of snowy egrets (Egretta thula) and glossy ibis that died during EEEV infection; however, these isolations occurred within 3–5 d of the initial infection. Likewise, EEEV has been isolated at 14 dpi from the lung, liver, and spleen of rock pigeons (Columba livia) that were subcutaneously inoculated (Karstad et al. 1959). However, none of these were confirmed latent infections given the proximity of detection to the actual infection. In western equine encephalitis virus, another alphavirus, latent infections were detected for as long as 300 dpi (Reeves et al. 1958).

The serology data showed that all the birds inoculated with EEEV developed neutralizing antibodies during the experimental period. Low PRNT$_{90}$ titer were observed at 14 dpi and at the last sample on 131 dpi, the latter demonstrating a trend toward decreasing antibody titers over time. No significant decline in PRNT$_{90}$ titer was found during the period in which we experimentally manipulated testosterone levels and photoperiod, which would have a prerequisite for reactivation of a latent infection. However, given most of the birds had titers exceeding 320 on 30 and 76 d postinfection, our results may not be sensitive enough to detect significant differences in titers between sampling periods. The one bird that had a positive EEEV RNA cloacal swab on 18 January 2008 had a PRNT$_{90}$ titer of >320 on 22 January 2008, which did not differ from its previous and subsequent antibody titers.

Unlike previous experiments (Owen et al. 2006, 2010; Owen and Moore 2008), the catbirds in this study did not exhibit activity consistent with migration, which is heightened nighttime movement (e.g., hopping, wing fluttering) in passerine birds. There was a significant increase in nighttime activity in our testosterone-implanted males regardless of photoperiod, which is consistent with Owen et al. (2010). A few individuals in each group did exhibit migratory activity, but otherwise no other birds showed consistent elevation in nighttime activity. Our methods for inducing migratory restlessness did not differ from the previous year, except we began manipulating photoperiod in third week of December, 2 wk earlier. Furthermore, there were no differences in age or place of origin. For a migratory bird to be sensitive to photoperiod in the spring, they must go through a period of short days during the fall or winter to terminate their refractoriness (Gwinner 1987, Bairlein and Gwinner 1994). Whereas it is possible that this termination did not occur in our catbirds, it does not explain why we saw differences between the WNV (Owen et al. 2010) and the current experiment, as the photoperiod schedule did not differ between years.

In summary, gray catbirds are competent reservoirs for EEEV; this combined with their relatively high exposure rates based on seroprevalence studies (Srihongse et al. 1978, Crans et al. 1994) and the overlap of their breeding habitat with the primary vector, C. melanura, make them a potentially important maintenance host of EEEV. We show for the first time the reactivation of a latent EEEV infection in a captive bird. However, support for their role in the overwintering of EEEV is weak; the occurrence of one in 10 experimental birds exhibiting reactivation of infection in a cloacal swab is difficult to interpret. Furthermore, the one bird may not have been shedding infectious virus, further decreasing probability that they may contribute to the overwintering and reactivation of an EEEV transmission cycle. Although we are unable to draw conclusions based on positive fecal sample from one of 10 birds, this finding is notable and worth future investigation, given the ramifications for transmission. Our ability to develop effective prevention and control strategies for Eastern equine encephalitis in temperate regions will continue to be limited until we understand the mechanism by which the virus overwinters and is annually initiated.

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