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WEST NILE VIRUS ANTIBODY DECAY RATE IN FREE-RANGING BIRDS

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ABSTRACT: Antibody duration, following a humoral immune response to West Nile virus (WNV) infection, is poorly understood in free-ranging avian hosts. Quantifying antibody decay rate is important for interpreting serologic results and for understanding the potential for birds to serorevert and become susceptible again. We sampled free-ranging birds in Chicago, Illinois, US, from 2005 to 2011 and Atlanta, Georgia, US, from 2010 to 2012 to examine the dynamics of antibody decay following natural WNV infection. Using serial dilutions in a blocking enzyme-linked immunosorbent assay, we quantified WNV antibody titer in repeated blood samples from individual birds over time. We quantified a rate of antibody decay for 23 Northern Cardinals (*Cardinalis cardinalis*) of 0.198 natural log units per month and 24 individuals of other bird species of 0.178 natural log units per month. Our results suggest that juveniles had a higher rate of antibody decay than adults, which is consistent with nonlinear antibody decay at different times postexposure. Overall, most birds had undetectable titers 2 yr postexposure. Nonuniform WNV antibody decay rates in free-ranging birds underscore the need for cautious interpretation of avian serology results in the context of arbovirus surveillance and epidemiology.

Key words: Antibody decay, *Culex pipiens*, Northern Cardinal, serology, West Nile virus, wild birds.

INTRODUCTION

West Nile virus (WNV; genus *Flavivirus* and family *Flaviviridae*), a member of the Japanese encephalitis virus (JEV) antigenic complex, is maintained primarily in an enzootic cycle between ornithophilic *Culex* spp. mosquito vectors and avian reservoir hosts (Gubler 2007). Birds that survive WNV infection develop antibodies that help protect against subsequent infection (Komar et al. 2003; Nemeth et al. 2009). However, there appears to be considerable variation in the nature and duration of avian immune responses to arboviruses among individuals and populations (Main et al. 1988; Kuno 2001; Reisen et al. 2001; Reisen et al. 2004; Davison et al. 2008).

Serosurveillance is used to measure disease burden and risk in a population and can provide useful data for disease prevention and epidemiologic models. Therefore, a thorough understanding of immune dynamics is essential for accurate serologic interpretation. The half-lives of detectable arboviral antibody responses in birds appear to be shorter than those of mammals (Stamm 1966; Kuno 2001). In avian hosts, following primary infection, antibody levels peak for several weeks, plateau for several months, and then

gradually decline (Stamm 1966; Kuno 2001; Ringia et al. 2004). Experimental infections in House Sparrows (*Passer domesticus*) and House Finches (*Carpodacus mexicanus*) have demonstrated the rapid decay of neutralizing antibodies to St. Louis encephalitis virus (SLEV), a flavivirus in the JEV complex, although birds lacking detectable antibodies up to 24 mo postexposure were protected against challenge infection (McLean et al. 1983; Reisen et al. 2001). In WNV experiments, detectable antibodies were observed for 12 mo in captive Fish Crows (*Corvus ossifragus*; Wilcox et al. 2007), 15 mo in naturally infected Rock Pigeons (*Columbia livia*; Gibbs et al. 2005), and 36 mo in House Sparrows (Nemeth et al. 2009), which was the duration of these studies.

These results are based upon captive and experimental inoculation studies and may not reflect avian antibody profiles in natural environments. Studies of wild birds suggest transient antibody persistence to SLEV (Gruwell et al. 2000) and Usutu virus (Meister et al. 2008), also in the JEV complex. Nemeth et al. (2008) documented the persistence of WNV antibodies in naturally exposed raptors held captive in outdoor aviaries for 4 yr, the duration of the study. Kwan et al. (2012) recaptured free-ranging House Finches and House Sparrows and detected persistent WNV antibodies for up to 5 yr; however, all but one individual demonstrated intermittent seroreversions.

In this study, we assessed WNV antibody persistence in naturally infected free-ranging birds in a region of epidemic WNV transmission.

MATERIALS AND METHODS

To determine antibody persistence and rate of decay, we sampled blood from birds recaptured 2005–11 in suburban Chicago, Illinois, US and 2010–12 in Atlanta, Georgia, US. Site descriptions and detailed sampling methods have been described for Chicago (Hamer et al. 2008) and Atlanta (Levine et al. 2013). Epitope blocked enzyme-linked immunosorbent assay (b-ELISA), as described by Hamer et al. (2008), was used to detect WNV antibodies. Samples from all locations and times were tested in the same laboratory under the same conditions. Two positive chicken serum controls and four negative controls were used for reference on each plate. Samples found positive during initial screening were serially diluted up to 1:640 and retested to determine end point titers. This b-ELISA protocol was adopted from a similar protocol developed for SLEV (see Supplementary Material); with the only change being that the WNV antigen replaced the SLEV antigen. However, blocking of the 6B6C-1 conjugated antibody in the ELISA protocol by using the WNV recombinant antigen could be due to SLE antibodies binding to the WNV antigen because of cross reactivity; cross reactivity was confirmed using SLEV antibody-positive chicken serum. Given the similarity between the SLEV and WNV b-ELISA, we expect both ELISA protocols to be consistent with neutralization test results (see Supplementary Material). Although some of the antibody-positive birds from the current study might have been positive for SLEV antibodies, we expect very few given the low levels of SLE transmission in Illinois in the time since the arrival of WNV. From 2005 to 2012, Illinois reported 15,371 positive mosquito pools for WNV and 23 positive mosquito pools for SLEV (Illinois Department of Public Health 2014; US Geological Survey 2014). From 2010 to 2012, the Georgia Department of Public Health (2014) reported activity for several arboviruses (WNV, eastern equine encephalitis virus, and La Crosse encephalitis virus) but not SLEV.

To estimate the rate of antibody decay, we used a linear mixed effects model with repeated measures implemented with the lme4 package in Program R (R Development Core Team 2011). The dependent variable was antibody titer (log transformed), and the fixed factor was the number of months postinitial antibody-positive sample. The individual bird identification (determined by leg band) was included as a random factor to account for repeated sampling of the same individuals, and a likelihood ratio test was used to compare models with a single random effect and two uncorrelated random effects. The fixed effect coefficient for the model with the lowest Akaike's information criterion is reported (Burnham and Anderson 2002). The first set of models included all birds: one model for Northern Cardinals (*Cardinalis cardinalis*) and one model for all other bird species. A second set of models, one model for Northern Cardinals and one model for all other bird species, excluded birds with evidence of natural

reexposure as indicated by an anamnestic response. Anamnestic response was defined as an increase in the antibody titer more than 100-fold in a serial sample. We also performed separate models for juvenile birds, with the first antibody-positive capture as a hatch year bird, and adult birds, with the first antibody-positive capture as an after hatch year. *Culex* spp. mosquitoes were collected from these studies' sites, and virus was detected using reverse transcriptase-PCR in all years.

RESULTS

In Chicago, we documented 41 antibody-positive birds that were subsequently recaptured. The species composition for the Chicago birds included Northern Cardinal ($n=21$), House Sparrow ($n=12$), Gray Catbird (*Dumetella carolinensis*; $n=5$), American Robin (*Turdus migratorius*; $n=1$), Red-winged Blackbird (*Agelaius phoeniceus*; $n=1$), and Brown-headed Cowbird (*Molothrus ater*; $n=1$). All juvenile birds were of sufficient age that antibodies detected were likely associated with recent exposure, given that maternal antibodies typically do not persist beyond a few weeks of age (Ludwig et al. 1986; Gibbs 2005). At a minimum, we obtained an antibody-positive sample followed by one subsequent sample, but some individuals had up to four serial samples. The average time between serial samples was 238 d, ranging between 13 d and 36 mo. Sample size decreased to 33 individuals when birds with suspected anamnestic response were removed. In Atlanta, there were 18 recaptured birds with initial antibody-positive samples. The Atlanta species composition included Northern Cardinal ($n=9$), American Robin ($n=1$), Northern Mockingbird (*Mimus polyglottos*; $n=3$), Eastern Towhee (*Pipilo erythrophthalmus*; $n=2$), Brown Thrasher (*Toxostoma rufum*; $n=1$), Blue Jay (*Cyanocitta cristata*; $n=1$), and Carolina Wren (*Thryothorus ludovicianus*; $n=1$). The average time between these serial samples was 213 d, ranging 22 d to 26 mo. Sample size decreased to 14 individuals when suspected anamnestic responses were removed.

The coefficient for the fixed factor months since exposure from the mixed model was -0.138 (SE$=0.05$, $t=-2.8$) for all Northern Cardinals and -0.91 (SE$=0.05$, $t=-2.0$) for all other bird species (Fig. 1). The model coefficient for birds with the initial capture event as a hatch year was -0.147 (SE$=0.08$, $t=-1.8$), and the model coefficient for birds with initial capture event as an after hatch year was -0.111 (SE$=0.04$, $t=-3.0$). Once the birds with clear evidence of reexposure were removed, the coefficient was -0.198 (SE$=0.04$, $t=-4.5$) for Northern Cardinals and -0.178 (SE$=0.05$, $t=-3.8$) for all other bird species (Fig. 2). The model coefficient when reexposed birds were removed and for birds with the initial capture event as a hatch year was -0.224 (SE$=0.08$, $t=2.7$), and the coefficient for birds with initial capture event as an after hatch year was -0.188 (SE$=0.03$, $t=-5.7$). To represent the WNV antibody titer shortly following initial exposure, we utilized the mean antibody titer for all antibody-positive juvenile birds in the entire Chicago study (not just those with repeated measures) of the species used in the analysis ($n=153$) as an initial value for plotting a mean rate of antibody decay based upon our model coefficients. In this case, the mean rate of WNV antibody titer decline was 0.198 ln (y+1) per month for Northern Cardinals and 0.178 ln (y+1) per month for all other bird species. Results were similar when analyses were stratified by location (Illinois versus Georgia; data not shown).

DISCUSSION

We report a measure of antibody decay after WNV infection in wild-caught birds. Our estimated rate of antibody decay in Northern Cardinals of 0.198 natural log units per month and 0.178 natural log units per month for all other bird species, based on the mixed model results, indicates that most individuals will lose detectable antibodies by about 2 yr following initial exposure to WNV. By removing

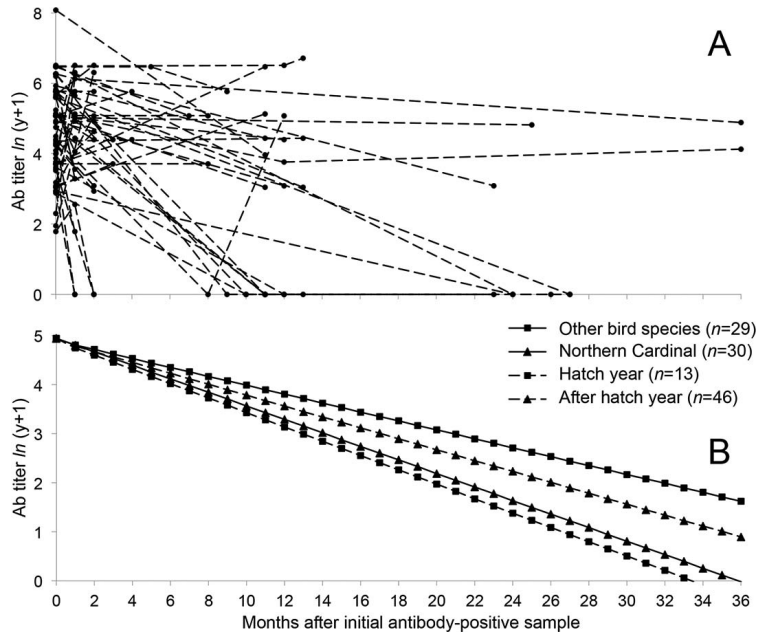

FIGURE. 1. West Nile virus antibody titer for serially sampled free ranging birds by month after initial antibody-positive sample (A). The mean linear antibody decay rate for Northern Cardinals (*Cardinalis cardinalis*) ($y=-0.138x+4.95$), for all other bird species combined ($y=-0.091x+4.95$), for birds that were captured as hatch year for the first antibody-positive sample ($y=-0.147x+4.95$), and (B) for birds that were captured after hatch year for the first antibody-positive sample ($y=-0.111x+4.95$). Ab=antibody.

individuals with overt signs of reexposure, we still present a conservative estimate of the rate of antibody decay because some reexposed birds may still have been included in our analysis but with antibody levels that may have peaked and returned to baseline. Additionally, we could not determine the temporal lag between the exposure event and the first antibody-positive sample, which complicated analysis of the precise shape of the decay curve. However, the higher antibody decay rates for juveniles compared with adults would suggest a nonlinear antibody decay (i.e., faster decay rates following recent exposure and slower decay rates at longer periods postexposure). This finding is consistent with prior studies of human antibody decay following vaccine and natural infection that demonstrated short periods of rapid antibody decline followed by prolonged periods of slower decay (Wiens et al. 1996; Desai et al. 2012) with variable decay rates among individuals (Teunis et al. 2012). Our data run counter to an experimental infection study of captive House Sparrows that showed relatively constant titers of neutralizing WNV antibodies for 36 mo (Nemeth et al. 2009) but are consistent with a long-term study recapturing free-ranging House Sparrows that showed a loss of neutralizing antibodies over time (Kwan et al. 2012), although this comparison is complicated by the ELISA results reported in our study and the plaque reduction neutralization test (PRNT) reported in the prior studies. The lack of agreement between studies of captive and free-ranging birds might reflect the different biotic and abiotic challenges facing these birds. The immune system of wild birds may be influenced by resource limitation, competition and stress and because they are generally infected with a suite of pathogens that have a range of immune pressures (Hawley and Altizer 2011; Pederson and Babayan 2011). We acknowledge that the

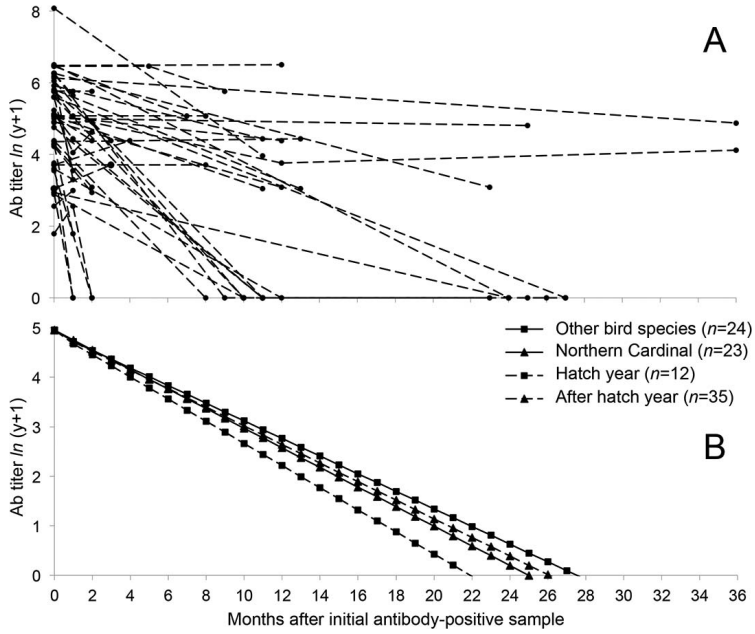

FIGURE. 2. West Nile virus antibody titer for serially sampled free ranging birds by month after initial antibody-positive sample, excluding birds with overt evidence of a natural reexposure event (A). The mean linear antibody decay rate for Northern Cardinals (*Cardinalis cardinalis*) ($y=-0.198x+4.95$), for all other bird species combined ($y=-0.178+4.95$), for birds that were captured as hatch year for the first antibody-positive sample ($y=-0.224x+4.95$), and for birds (B) that were captured after hatch year for the first antibody-positive sample ($y=-0.188x+4.95$). Ab=antibody.

antibodies detected with this b-ELISA protocol include some that have no neutralizing ability. Determining neutralizing titers with PRNT would have improved this study, but the lack of biosafety level 3 (BSL-3) facilities prevented this option, although future studies may utilize a chimeric WNV strain to allow PRNT at BSL-2 (Monath 2001; Monath et al. 2001). If neutralizing antibodies have greater longevity than non-neutralizing antibodies, our antibody decay estimates based on b-ELISA could be inflated. However, our data comparing SLEV b-ELISA titer and neutralization test titer show comparable results (see Supplementary Material Fig. S1).

The estimated antibody decay rate we report highlights the importance of interpreting serology results from free-ranging birds cautiously. Many studies have reported differences in antibody prevalence between juveniles and adult birds. In many cases, adult bird antibody prevalence is higher than in juveniles (Gibbs et al. 2006; Hamer et al. 2008; Lampman et al. 2013), which is explained by the additive effect of adult birds being exposed in prior years. However, few studies consider that antibody-negative adult birds could represent false negatives, due to waning antibodies, which could confound analyses that rely on antibody status to infer population metrics, such as mortality (Ward et al. 2010; Kilpatrick et al. 2013).

The WNV antibodies appear to be protective in birds even at low titers and may provide lifelong immunity (Nemeth et al. 2008; Nemeth et al. 2009). However, WNV shedding in bird blood can last weeks or months following exposure (Semenov et al. 1973; Reisen et al. 2006; Wheeler et al. 2012a). Although neutralizing antibodies in bird blood can prevent these viremias from infecting mosquitoes, Wheeler et al. (2012b) reported evidence that low neutralizing antibody titers in bird

blood allow an incompletely neutralized virus to infect a small proportion of susceptible mosquitoes. Although these events would be rare, it is premature to dismiss their potential biologic importance. The relevance of antibody persistence in hosts to the dynamics of infectious disease also depends on host longevity and birth rates (Dobson 2009). Passerine birds tend to be short-lived species with high levels of recruitment, which would suggest that the pool of susceptible hosts is not greatly affected by seroreversion. However, several studies have documented important WNV avian amplifier species, such as American Robin, House Sparrow, and Northern Cardinal (Hamer et al. 2011; Levine et al. 2013), with maximum life spans over 10 yr (Laskey 1944; Farner 1945; Lowther and Cink 1992). Additionally, long-lived birds, such as the American Crow (*Corvus brachyrhynchos*; Zwickel and Verbeek 1997), Ring-billed Gull (*Larus delawarensis*; Southern 1975), Red-tailed Hawk (*Buteo jamaicensis*), and Great Horned Owl (*Bubo virginianus*; Goodrich and Smith 2008) are competent for WNV (Komar et al. 2003; Nemeth et al. 2006). Epidemiologic modeling would be needed to evaluate the impact of seroreversion in avian hosts on WNV transmission dynamics in the context of birth and death rates appropriate for birds.

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SUPPLEMENTARY MATERIAL

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