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AVIAN HOSTS FOR WEST NILE VIRUS IN ST. TAMMANY PARISH, LOUISIANA, 2002

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Abstract. West Nile virus (WNV) infections in free-ranging birds were studied in Slidell, St. Tammany Parish, Louisiana, after a human encephalitis outbreak peaked there in July 2002. Seroprevalence in resident, free-ranging wild birds in one suburban site was 25% and 24% in August and October, respectively, indicating that most transmission had ceased by early August. Mortality rates, seroprevalence rates, host competence, and crude population estimates were used in mathematical models to predict actual infection rates, population impacts, and importance as amplifying hosts for several common passerine birds. Northern cardinal (*Cardinalis cardinalis*) and house sparrow (*Passer domesticus*) were the principal amplifying hosts, but blue jay (*Cyanocitta cristata*) and northern mockingbird (*Mimus polyglottos*) also contributed. The blue jay population was reduced by an estimated 47%. A variety of passerine bird species combined to play an important role as amplifying hosts in the WNV transmission cycle.

INTRODUCTION

West Nile virus (WNV) is a mosquito-borne flavivirus (Flaviviridae) that has emerged as an important human, veterinary, and wildlife health threat.[1] Certain birds are the primary WNV-amplifying hosts, with ornithophilic mosquitoes serving as the principle vectors.[1] WNV was first reported in Louisiana in the fall of 2001[2] and was the etiologic agent of a human encephalitis outbreak in July 2002 in the city of Slidell and surrounding rural communities of St. Tammany Parish.[3]

Prior to 2002, avian hosts of WNV had not been studied in Louisiana but were evaluated in New York City in 1999[4] and 2000[5] and in Florida in 2001.[6] In these studies, resident passerine birds (pertaining to the order Passeriformes) were implicated as important amplifying hosts for WNV. Because WNV was new to Louisiana, no knowledge existed regarding the identity of local avian amplifying hosts or which bird species were most appropriate sentinels for surveillance programs in Louisiana.

Summer-resident passerine bird species were hypothesized to be the most important amplifying hosts of WNV in St. Tammany Parish. To evaluate this hypothesis, we sampled a variety of birds in early August, several weeks after the peak of epidemic transmission, to determine seroprevalence rates. Because human and equine cases of West Nile neuroinvasive disease (WNND) were clustered in suburban neighborhoods of Slidell, the largest city in St. Tammany Parish, we compared infection rates of birds sampled within a suburban transmission focus around the case clusters to those of birds sampled in rural areas where human population density was low and cases of human WNND were less prevalent. We also evaluated whether WNV activity continued within the suburban transmission focus after the incidence of human WNND cases had subsided by sampling birds in October and looking for evidence of recent WNV transmission. To interpret the significance of our observed seroprevalence rates in relation to the transmission cycle, we generated host competence data for three candidate amplifying hosts, the northern cardinal (*Cardinalis cardinalis*), northern mockingbird (*Mimus polyglottos*) and house sparrow (*Passer domesticus*). Finally, we derived mathematical models using our measured parameters to predict actual WNV infection rates, WNV-attributed population reductions, and relative number of infected mosquitoes deriving from these three species of birds and blue jay (*Cyanocitta cristata*).

MATERIALS AND METHODS

Study sites. The principal study site (A, 30°16.2′ N, 89°45.1′ W) was a low-density residential neighborhood in northeast Slidell, St. Tammany Parish, Louisiana, described by a quadrangle approximately 0.5 km long and approximately 0.2 km wide, consisting of residential properties generally > 1 acre. This site was selected based on a cluster of WNV-positive dead birds received by the St. Tammany Parish Mosquito Abatement District in July and close proximity to the residences of human and horse cases (Palmisano C, pers. comm.). Most of the August collections and all of the October collections were made at this site. This site corresponds with site A that was sampled for mosquitoes in August and site 1 that was sampled for free-ranging mammals in October.[7,8]

Other collection sites in August included sites B (30°16.2′ N, 89°49.8′ W) at a suburban residence in southwest Slidell, C (30°33.4′ N, 90°02.5′ W) about 40 km northwest of Slidell at a rural horse ranch (near mosquito collection site B),[7] and D (30°13.5′ N, 89°41.2′ W) about 9 km southeast of Slidell at a bird-feeding station located on a rural residential property surrounded by dense humid forest and bog. Sites B, C, and D were not associated with WNV cases.

Bird capture and classification. Bird sampling occurred August 3–11, and October 22–29, 2002. A convenience sampling was used, with the objective of sampling as many birds as possible at each study site. Wild birds were trapped in mist nets (Avinet, Inc., Dryden, NY) and captive birds were captured within their enclosures by hand. Wild birds were marked with uniquely numbered aluminum leg bands. Each bird was assigned a residence category as either "breeder" or "nonbreeder". "Breeder" is defined as a bird belonging to a population known to nest locally at or near the study site, and included all the species sampled in early August prior to most landbird migration in Louisiana. "Nonbreeder" is defined as a bird belonging to a population known to breed remotely from the study site, and would include transient birds or

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northern breeders that had arrived in the study site for the winter season. Some members of a "breeder" population may not have bred locally, and may have either entered the study site as part of postbreeding dispersal (e.g., brown-headed cowbird, *Molothrus ater*) or migration (e.g., summer tanager, *Piranga rubra*).

Bird sampling. Blood was obtained (maximum 0.6 mL) by jugular or brachial venipuncture and collected in Microtainer serum separators (Becton, Dickinson and Company, Franklin Lakes, NJ) or diluted 1:2 in BA-1 diluent (Hanks M-199 salts, 0.05 M Tris pH 7.6, 1% bovine serum albumin, 0.35 g/L sodium bicarbonate, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 μg/mL Fungizone) in cryovials for a field serum dilution of approximately 1:5. Blood samples were left at ambient temperature for up to 30 minutes and then incubated on ice until centrifuged for separation of serum or, if diluted in the field, they were frozen on dry ice for transport to the laboratory and stored at −70°C.

Testing procedures. Separated serum samples were frozen at −20°C until tested for neutralizing antibodies (at a dilution of 1:10) by plaque reduction neutralization test (PRNT) using challenge doses of approximately 100 plaque-forming units (PFU) of WNV strain NY99-4132 and Saint Louis encephalitis virus (SLEV, family Flaviviridae, genus *Flavivirus*) strain TBH-28 in 6-well plates of Vero cells, overlaid with 0.5% agarose in M199 medium containing antibiotics.[9]

Samples with ≥ 80% reduction in Vero cell plaque forming units of either virus were further titrated in duplicate serial twofold dilutions to determine end-point titers (through 1: 320) for WNV and SLEV. A fourfold or greater 90% neutralization titer (of at least 1:10) for WNV, relative to the titer for SLEV, was considered positive for neutralizing antibodies for WNV. Two specimens had weak SLEV-neutralizing antibody titers, but due to fourfold higher titers against WNV, none were scored positive for SLEV-neutralizing antibodies.

Field diluted serum samples collected in August were tested in duplicate on 6-well plates by Vero plaque assay for evidence of viremia.[9] Any plaques were harvested by standard techniques into 1 mL of BA-1 containing 20% fetal bovine serum. The harvested suspension was then identified using WNV-specific RT-PCR techniques previously published.[10]

Host competence studies. Northern cardinals ($N = 13$) and northern mockingbirds ($N = 4$) were captured using mist nets in Mississippi and Alabama and transferred to animal facilities at the University of Southern Mississippi, Hattiesburg. House sparrows ($N = 6$) were captured in baited potter traps in northern Colorado and transferred to animal facilities at CDC, Fort Collins. Birds seronegative for both WNV and SLEV (all but 4 cardinals) were inoculated subcutaneously with approximately 1,000 PFU (cardinals and mockingbirds) or 600 PFU (sparrows) of low-passage WNV-NY99-4132 and blood-sampled daily for 6 (cardinals and mockingbirds) or 5 (sparrows) days post-inoculation. Whole blood (0.05 mL) was frozen at −70°C until tested by plaque assay to determine viremia profiles. For plaque assay, the whole blood was diluted with 225 μL BA-1 for a 1:10 dilution of serum, and titrated by serial 10-fold dilution. Competence index, C_i, values were calculated as the product of three parameters: susceptibility, infectiousness and duration of infectiousness.[11,12] These values represent the relative number of infectious *Culex quinquefasciatus* (or *Cx. pipiens*) mosquitoes that may result from feeding on infected vertebrate hosts, assuming that all vertebrates are equally attractive to vector mosquitoes. To convert these needle-derived C_i values to mosquito bite-derived C_i values available for other species, a conversion factor of 3.24 was derived for house sparrows, using a published mosquito bite-derived C_i value.[12] This conversion factor was applied to the other species. The four WNV-seropositive cardinals were treated identically as the infected cardinals as a control for the effects of handling.

Model construction. Simple mathematic models were constructed to predict actual WNV infection rates and WNV-attributed population reductions, and a mosquito inoculation index was developed to estimate the relative contributions of various bird species to WNV transmission in Slidell. The models were applied to four bird species (for which sufficient data were available), including blue jay, northern cardinal, northern mockingbird and house sparrow.

A species-specific WNV infection rate, *ir*, was predicted using the following equation:

$$ir = [(\text{No. of survivors}) + (\text{No. of deaths})]/\text{Pre-epizootic population}$$

However, none of these terms were measurable in our study system. Number of survivors is the product of the measured seroprevalence rate, *s*, and the estimated post-epizootic population sampled, P. Number of deaths is the product of the pre-epizootic population, P_0, infection rate (*ir*), and the mortality rate, *m*. P_0 can be expressed in terms of P, *ir* and *m* by the expression $P/(1 - ir*m)$. Thus *ir* can be solved for as follows:

$$ir = [(s*P) + ([(P/(1 - ir*m)]*ir*m)]/[P/(1 - ir*m)]$$

This equation simplifies to:

$$ir = s/[1 - m + (s*m)]$$

The WNV-attributed population reduction, $p_{\Delta-}$, was predicted using the following equation:

$$p_{\Delta-} = (P_0 - P)/P_0$$

This equation can be expressed in terms of P, *ir*, and *m*, as follows:

$$p_{\Delta-} = ([P/(1 - ir*m)] - P)/[P/(1 - ir*m)]$$

This equation simplifies to:

$$p_{\Delta-} = ir*m$$

For calculating a vertebrate host species-specific mosquito inoculation index, MI_i, that predicts the species' relative contribution of infected vector mosquitoes, the following equation was used:

$$MI_i = \text{population}*\text{infection rate}*\text{competence} = P'*ir*C_i$$

However, P′ used here can be either P_0 or P, depending on whether one is interested in estimating the contributions of each species for the epizootic, or potential for future contributions (which would use the current, or post-epizootic, population), respectively. We are evaluating the former situation (using P_0), and therefore we modified the equation as follows:

$$MI_i = [P/(1 - ir*m)]*ir*C_i$$

Statistical analyses. We calculated 95% confidence intervals (CI) for seroprevalence proportions using the Wilson score method (S-PLUS 6.1 Professional software, Insightful, Inc., Seattle, WA). Seroprevalence proportions were compared using the Fisher exact test or the Pearson χ^2 test. For multiple comparisons, Bonferroni adjustments were applied. Bird population sizes were estimated using the Lincoln-Petersen estimator for mark-release-recapture data,[13] with variances and CI calculated by the methods of Williams and others.[14]

RESULTS

In August 2002, we sampled serum from 264 resident free-ranging wild birds from two suburban sites in Slidell and two rural sites in St. Tammany Parish (Table 1), and detected 41 (15.5%, CI 11.7–20.4%) with WNV-neutralizing antibodies, ranging in ninety-percent neutralization titers ($PRNT_{90}$) from 1:40 to ≥ 1:320. To determine whether the seroprevalence rates between rural and suburban sites were significantly different, we restricted analysis to site A (suburban) and site C (rural) because of the larger sample collections at these sites. This difference (25% versus 7%) was statistically significant (Pearson χ^2, $P = 0.0003$). Because northern cardinals were so abundant, and easily captured in both suburban and rural settings, we compared the seroprevalence rates between rural cardinals (2.1%, $N = 47$) and suburban cardinals (52.6%, $N = 38$), and also found this difference to be significant (Fisher exact test, $P < 0.0001$).

At Site A we also sampled 9 free-ranging domestic chickens (6 adults, and 3 chicks, *Gallus gallus*), 3 adult emus (*Dromaius novaehollandiae*) in a 2-ha enclosure, and 18 caged psittacine birds including 4 monk parakeets (*Myiopsitta monachus*) and 14 cockatiels (*Nymphicus hollandicus*). All the adult chickens and emus were seropositive. The chicks and all the psittacines were seronegative. The psittacines, with the exception of one cockatiel, were held in a screened indoor aviary and therefore were subject to reduced mosquito exposure.

Within Site A, the seroprevalence rates of birds varied among species from 0 to 100%, but only chickens (67% positive, including 100% of six adults > 7 months old and 0% of three chicks < 1 month old) and northern cardinals differed significantly ($P < 0.004$, $\alpha = 0.05$, 11 comparisons including chickens and emu) from all other bird species combined.

One WNV virus isolate was made from a Carolina wren (*Thryothorus ludovicianus*) sampled from site A. The titer was $10^{4.1}$ PFU/mL serum. The same specimen was negative for WNV-neutralizing antibodies.

In October, we resampled free-ranging wild birds at site A (Table 2). Of 166 resident breeder birds sampled, 40 (24.1%, CI 18.2–31.1%) tested positive, with 90% neutralization titers ranging from 1:40 to ≥ 1:320. The seroprevalence rates for different species of free-ranging breeders varied from 0 to 100%, but only the northern mockingbird and the northern cardinal differed significantly ($P < 0.006$, $\alpha = 0.05$, 9 comparisons) from all other breeder bird species combined. No positives were detected from among 7 nonbreeder birds (CI 0–35.4%).

Comparing the seropositivity among breeder birds at site A between August and October (Table 2), we noticed that the seroprevalence rate remained stable at 24–25%. A statistically significant increase in seroprevalence rates within a species was observed only for the northern mockingbird ($P = 0.01$, right-tailed Fisher exact test). No statistically significant decreases in seroprevalence rates within a species were observed. Because seroprevalence rates did not change significantly between August and October (except for the mockingbird), we combined these data (excluding the mockingbird) to look for any species-specific difference from the larger, more robust data set (Table 2). No additional species-specific differences were observed.

In October we recaptured a small number of birds that had been sampled in August at site A. Recaptured birds that remained seronegative included a chickadee, 3 titmice, 4 house sparrows, a cardinal, a red-bellied woodpecker, and a captive cockatiel. Two cardinals that tested positive in August remained positive in October, and both showed declines in 90% neutralization titers from ≥ 1:320 in August to 1:160 or 1:40 in October. One blue jay that tested negative in August (< 1:10) seroconverted to positive in October (≥ 1:320).

Mark-recapture data from site A were used to generate crude estimates of bird population sizes (Table 3). For birds with recaptures, cardinals and house sparrows were estimated to be the most abundant, with several hundred birds each utilizing the study site. Red-bellied woodpeckers, blue jays and Carolina chickadees were also common, but their populations were about an order of magnitude less than cardinals and house sparrows. Carolina wrens and northern mockingbirds were probably intermediate in abundance but no recaptures were made. Titmice were relatively uncommon.

WNV viremia profiles were derived for cardinals, mockingbirds and house sparrows (Figure 1). All inoculated birds survived infection except for two cardinals, suggesting a crude mortality rate of 25% for cardinals. Four cardinals serving as handling controls all survived. The viremia profile for fatally infected cardinals was dramatically different than for surviving cardinals (Figure 2). The fatally infected cardinals developed a mean peak viremia titer of $10^{9.4}$ PFU/mL serum (range, $10^{7.1}$–$10^{9.7}$) compared with $10^{5.6}$ PFU/mL serum (range, $10^{4.7}$–$10^{6.3}$) for the survivors. Susceptibility, *Culex quinquefasciatus* infectiousness, and duration of infectiousness parameters were estimated from the viremia profiles, and competence index values were derived from these (Table 4).

The crude mortality rate we observed for the northern cardinal and mortality rates of other species observed in previous studies[12] were combined with our crude population estimates and measured seroprevalence rates to estimate actual WNV infection rates and WNV-attributable population impacts in some of the bird species sampled (Table 5). These simple mathematical equations predicted the greatest infection rate

TABLE 1

Prevalence of West Nile virus–neutralizing antibodies in free-ranging birds sampled in August 2002 in suburban sites within Slidell, Louisiana, and rural sites nearby in St. Tammany Parish

Site	Habitat	*N*	No. positive	% (CI)
A	Surburban	130	33	25.4 (18.7–33.5)
B	Suburban	13	2	15.4 (4.3–42.2)
C	Rural	90	6	6.7 (3.1–13.8)
D	Rural	31	0	0.0 (0.0–11.0)

CI, 95% confidence interval.

TABLE 2

Prevalence of West Nile virus–neutralizing antibodies in 9 species of free-ranging "breeder" birds (minimum $N = 10$) sampled in early August or late October, 2002, Slidell, Louisiana

Common name	Scientific name	August	October	Combined
		% positive (CI,* *N*)	% positive (CI, *N*)	% positive (CI, *N*)
Brown-headed cowbird†	*Molothrus ater*	0.0 (0.0–65.8, 2)	9.1 (0.5–37.7, 11)	7.7 (1.4–33.3, 13)
Blue jay	*Cyanocitta cristata*	25.0 (4.6–69.9, 4)	33.3 (9.7–70.0, 6)	30.0 (10.8–60.3, 10)
Carolina wren	*Thryothorus ludovicianus*	25.0 (8.9–53.2, 12)	60.0 (23.1–88.2, 5)	35.3 (17.3–58.7, 17)
Common grackle	*Quiscalus quiscula*	10.0 (1.8–40.4, 10)	0.0 (0.0–94.9, 1)	9.1 (0.5–37.7, 11)
House sparrow	*Passer domesticus*	29.4 (13.3–53.1, 17)	17.5 (9.8–29.4, 57)	20.2 (12.7–30.8, 74)
Mourning dove	*Zenaida macroura*	20.0 (3.6–62.4, 5)	22.2 (6.3–54.7, 9)	21.4 (7.6–47.6, 14)
Northern cardinal	*Cardinalis cardinalis*	52.6 (37.3–67.5, 38)‡	42.9 (28.0–59.1, 35)‡	48.0 (36.9–59.2, 73)‡
Northern mockingbird	*Mimus polyglottos*	0.0 (0.0–43.4, 5)	77.8 (45.3–93.7, 9)‡	50.0 (26.8–73.2, 14)
Red-bellied woodpecker	*Melanerpes carolinus*	28.6 (8.2–64.1, 7)	0.0 (0.0–43.4, 5)	16.7 (4.7–44.8, 12)
Other breeder§		0.0 (0.0–11.4, 30)	0.0 (0.0–15.5, 21)	0.0 (0.0–7.0, 51)
Other non-breeder¶		NA	0.0 (0.0–35.4, 7)	0.0 (0.0–35.4, 7)
Total breeder		25.4 (18.7–33.5, 130)	24.1 (18.2–31.1, 166)	24.7 (20.1–29.9, 296)

* CI, 95% confidence interval.
† All species are of the order Passeriformes, except mourning dove (Columbiformes) and the woodpeckers and sapsucker (Piciformes).
‡ This species has a significantly higher seroprevalence than all other species combined (within the same column), as determined by Fisher exact test with Bonferroni adjustment.
§ Other breeders included (in August) brown thrasher (*Toxostoma rufum*) 2, Carolina chickadee (*Poecile carolinensis*) 3, house finch (*Carpodacus mexicanus*) 1, prothonotary warbler (*Protonotaria citrea*) 2, red-headed woodpecker (*Melanerpes erythrocephalus*) 3, tufted titmouse (*Baeolophus bicolor*) 5, white-eyed virero (*Vireo griseus*) 1; (in October) brown thrasher 1, Carolina chickadee 4, downy woodpecker (*Picoides pubescens*) 2, eastern bluebird (*Sialia sialis*) 1, European starling (*Sturnus vulgaris*) 1, loggerhead shrike (*Lanius ludovicianus*) 1, red-winged blackbird (*Agelaius phoeniceus*) 6, summer tanager (*Piranga rubra*) 1, tufted titmouse 4.
¶ Nonbreeders included indigo bunting (*Passerina cyanea*) 5, yellow-bellied sapsucker (*Sphyrapicus varius*) 2.

(> 60%) and population reduction (about 50%) for the blue jay. The northern cardinal, the northern mockingbird and the house sparrow were infected at moderate rates (30–60%) but suffered low population impacts (0–20%).

The mosquito inoculation index value, MI_i, for each of the four species above was calculated. The relative number of mosquitoes infected for every one infected by the northern mockingbird was 5 mosquitoes by the blue jay, 11 mosquitoes by the house sparrow, and 17 mosquitoes by the northern cardinal, assuming that *Cx. quinquefasciatus* attraction to these four species is equal.

DISCUSSION

WNV transmission depends on numerous environmental factors as well as the stochastic process of introducing the virus into a transmission focus. As a result, transmission rates vary in different foci at any one point in time. Our study found different levels of seropositivity, ranging from 0 to 25%, in free-ranging resident birds ("breeders," all species combined) in different locations within St. Tammany Parish, Louisiana. This finding is consistent with other avian serosurveys after WNV outbreaks that also demonstrated the focality of WNV transmission.[4–6] Site A appeared to be a focus of intense WNV transmission based on 1) the occurrence of both human and horse disease cases; 2) the high seroprevalence rate in free-ranging breeder birds (25%); 3) the high seroprevalence rate in domestic birds (100% of adult chickens and emus); 4) the isolation of WNV from a wren; and 5) the isolation of WNV from a pool of *Culex salinarius* mosquitoes.[7]

The finding that all seropositive birds in the October sampling of site A were breeders (rather than "nonbreeders," i.e., transient or winter residents) is consistent with observations following the Queens 1999 and the Staten Island 2000 outbreaks.[4,5] However, insufficient numbers of non-breeders were sampled to determine the seroprevalences in these groups of free-ranging birds. Some breeders, such as cowbirds and doves, sampled in October, may in fact have been immigrants or transients, having arrived into the transmission focus after the period of intense transmission. However, one mourning dove was recaptured at the same site 1 and 4 days after sampling in October, suggesting that this bird was not migrating at the time of sampling. As with other WNV out-

TABLE 3

Abundance estimates of resident bird species in a neighborhood in Slidell, Louisiana, that served as a focus of West Nile virus transmission

Common name	No. captured, August (A)	No. captured, October (B)	No. recaptured, October (C)	Estimated population*	95% Confidence interval†
Northern cardinal	38	35	3	443	168–718
House sparrow	17	57	4	242	106–378
Carolina wren‡	12	7	0	> 84	
Northern mockingbird‡	5	9	0	> 45	
Red-bellied woodpecker	7	5	1	35	16–54
Blue jay	4	6	1	24	11–37
Carolina chickadee	3	4	1	12	6–18
Tufted titmouse	5	4	3	7	5–9

* This estimated population, calculated as A*B/C, is a crude estimate. It assumes that individuals have similar capture and recapture probabilities and that the sampling area serves a closed population with no additions or subtractions from the bird populations sampled.
† This confidence interval was calculated as follows: $\pm 1.96 \sqrt{\text{variance}}$, where variance $= [(A + 1)(B + 1)(A - C)(B - C)]/[(C + 1)^2(C + 2)]$.
‡ Carolina wren and northern mockingbird are included here because they were abundant. The October collections include one wren that was not sampled for blood. The possible explanations for the lack of recaptures are presented in the text.

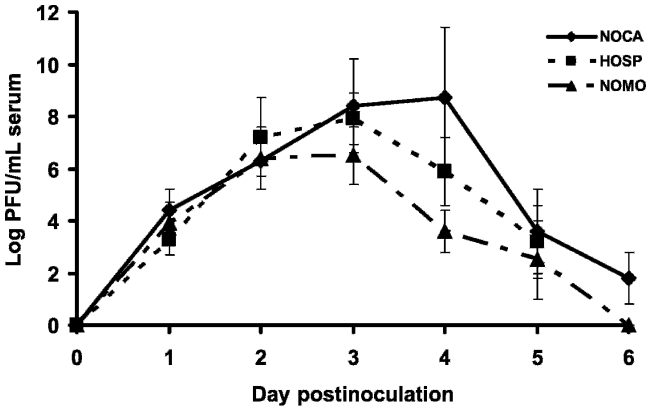

FIGURE 1. West Nile virus strain NY99-4132 viremia profiles for needle-inoculated northern cardinal (NOCA, $N = 8$), house sparrow (HOSP, $N = 6$), and northern mockingbird (NOMO, $N = 4$). Error bars ± 1 SD.

breaks in North America, summer-resident birds appear to be important amplifying hosts for WNV.[4–6]

Of the birds sampled, most were resident breeders that were common or abundant, and therefore candidate amplifying hosts for WNV.[15] The array of species sampled was biased towards those species likely to be trapped in mist nets placed at ground level, so infection rates could not be assessed in other species, such as fish crows and waterfowl. However, our sample identified certain land-dwelling species such as the northern mockingbird, the northern cardinal, the Carolina wren, the house sparrow, the blue jay, and the red-bellied woodpecker that were infected at high frequencies. The high seroprevalence rates observed for these species (ranging from 24% to 78% of captured birds) indicated that they may be useful target species in surveillance programs that use free-ranging birds as sentinels.[16] In particular, cardinals and mockingbirds were more frequently seropositive than the average bird, and thus should be targeted by free-ranging bird surveillance programs. Higher seroprevalences in adult chickens suggest that this domestic species may be even more sensitive than wild species for detecting transmission to birds in ecosystems similar to that of site A. Captive chickens have been used historically to monitor arbovirus infections, particularly

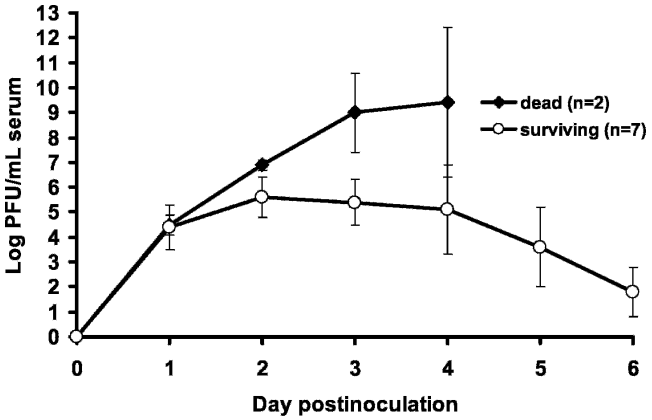

FIGURE 2. West Nile virus strain NY99-4132 viremia profiles for northern cardinals that died ($N = 2$) compared with surviving cardinals ($N = 7$). Error bars ± 1 SD.

TABLE 4

Viremia parameters and calculated competence index (C_i) values for northern cardinal, northern mockingbird, and house sparrow*

Species	Susceptibility	Mean infectiousness	Duration of infectiousness	Needle-derived C_i	Mosquito-derived C_i
Cardinal	1.0	0.18	1.50	0.27	0.87
Mockingbird	1.0	0.15	1.25	0.19	0.62
Sparrow	1.0	0.21	2.33	0.49	1.59

* The needle-derived C_i is the product of susceptibility, mean infectiousness, and duration of infectiousness (in days). Mean infectiousness is the mean proportion of *Culex quinquefasciatus* expected to develop a disseminated WNV infection per day from feeding on viremic birds. The mosquito-derived C_i was converted from the needle-derived C_i by a multiplication factor of 3.24, derived from published studies of house sparrows.

SLEV and eastern equine encephalomyelitis virus, in St. Tammany Parish, and they were useful for tracking WNV activity during the 2002 outbreak in Slidell.[17]

The seroprevalence rates reported herein may underestimate species-specific infection rates, because of WNV-attributed mortality in birds. The candidate amplifying host species named above have been reported dead with confirmed WNV infections through avian mortality surveillance.[1] Thus, a proportion of infected individual birds of each species may disappear from the sampled population due to mortality, and the result is a skewing of the measured seroprevalence in a negative direction. For example, experimental infection data for the blue jay suggests that approximately 75% of infected blue jays die of WNV infection.[12] If only 25% of infected birds survive, then our observed seroprevalence rate of 30% for blue jays grossly underestimates the true proportion of the blue jay population infected. Our model for predicting actual WNV infection rates concluded that 62% of the pre-epizootic blue jay population was infected.

High WNV mortality rates reported in laboratory studies do not necessarily portend large numbers of reported WNV-positive carcasses. Although the possible reasons for this are many, the bottom line is that few data are available on the actual reductions of bird populations due to natural transmission of WNV.[18] We used the measured seroprevalence rates in Slidell together with experimentally derived mortality rates to predict the actual effects of WNV infections on populations of four bird species in Slidell. We predicted a large reduction for the blue jay (about 50%), smaller reductions for the house sparrow and northern cardinal (10–20%), and none for the northern mockingbird. These predictions have significant implications for avian mortality surveillance, confirming the utility of blue jays in Slidell (and possibly elsewhere in the southeastern United States), but casting doubt on the utility of the other three species. We assume that a 10–20% population reduction for a common bird species like the house sparrow or the northern cardinal may go unnoticed by most people. The blue jay represented 91.5% of all WNV-positive dead birds tested in Harris County, Texas, in 2002.[19] Our derivation of a mathematical equation for predicting population reductions for birds due to WNV may have great utility beyond our small study in Slidell. The equation depends only on laboratory-derived mortality rates and field-derived seroprevalence rates. Thus, site-specific population reductions can be predicted for many species. For example, the reported WNV seroprevalence of 60% for house sparrows in Queens, New York City in 1999[4] results in a prediction of approximately 40% population reduction there. Moreover, this cal-

TABLE 5

Estimated West Nile virus infection rates and West Nile virus–attributed population impacts for four passerine bird species in Slidell, Louisiana, 2002, using observed mortality rates from experimental infections, observed seroprevalence rates from field studies, and local bird population estimates (from mark-release-recapture data) in the Slidell study site

Species	Mortality rate, m*	Post-epizootic population estimate, P†	Sero-prevalence rate, s‡	Infection rate, ir§	Population reduction, $p_{\Delta-}$¶
Blue jay	0.75	24	0.300	0.62	0.47
Northern cardinal	0.22	443	0.480	0.54	0.12
Northern mockingbird	0.00	46	0.500	0.50	0.0
House sparrow	0.50	242	0.202	0.34	0.17

* Mortality rates taken from published experimental infection studies[12] and this study (for cardinal and mockingbird).
† Values taken from Table 3. We used a conservative estimate for northern mockingbird.
‡ Values taken from Table 2.
§ This model-predicted parameter is derived from the function $s/[1 - m + (s*m)]$. The derivation of the equation for WNV infection rate, ir, is provided in the text.
¶ This model-predicted parameter is derived from the function $ir*m$. The derivation of the equation for the species-specific WNV-attributed population reduction, $p_{\Delta-}$, is provided in the text.

culation would apply to any infection and any host population.

With the exception of the woodpecker, all of the sampled candidate amplifying hosts were passerine birds and thus were likely to be competent for infecting mosquitoes. Published competence data are available for the blue jay and the house sparrow.[12] Of 25 species evaluated in the laboratory, the blue jay was the most infectious to mosquitoes, and was expected to infect about 60% more *Culex quinquefasciatus* mosquitoes than the house sparrow. *Cx. quinquefasciatus* is suspected to be the vector responsible for the Slidell outbreak[7] and is susceptible to WNV infection at increasing rates with orally ingested doses above 10^5 PFU/mL.[20] Based on the competence data presented herein, the house sparrow and the cardinal would infect 156% and 40%, respectively, more mosquitoes than the mockingbird. By inference, the blue jay would infect 250% more mosquitoes than the mockingbird. Using the predicted actual WNV infection rates from our mathematical model together with the competence data and species-specific population estimates, we predicted the relative importance of the blue jay, the house sparrow, the northern cardinal and the northern mockingbird as amplifying hosts in the transmission cycle by calculating a relative mosquito inoculation index value, MI_i. Because field conditions are not standardized, species-specific MI_i values are expected to vary for each transmission focus, and will not always correlate with laboratory-derived host competence C_i values. Thus, whereas the blue jay was the most competent of the four bird species evaluated, it inoculated many fewer mosquitoes than either the house sparrow or the cardinal in Slidell.

The analysis above assumes accurate population data derived from the mark-recapture study. However, these data are crude, and may be influenced by numerous factors related to the bird species studied, including differing probabilities of capture and recapture, different probability for survival after blood sampling (possibly an issue for small birds such as Carolina wren and Carolina chickadee), and others. An inherent limitation of mark-release-recapture data is the assumption of a closed population, which would require an island situation to achieve. Although no birds were likely to have been born between early August and late October, it is likely that some individual birds entered the population as a consequence of postbreeding dispersal or even migration. For example, both cardinals and blue jays are partially migratory in some parts of their ranges. Furthermore, some birds may have emigrated from this population during this period. Certainly some birds would have died due to natural causes, although natural mortality probably would not have exceeded 20% for most passerine birds during the study period.[21] If WNV was still circulating in the study population, then mortality may have been even higher for some species. Mortality, permanent emigration or immigration would result in a negative bias of detection probability and a positive bias in population size.

By sampling at site A in both August and October, we were able to obtain evidence for ongoing transmission between these sampling periods. Although the overall seroprevalence in birds remained constant, one of 12 recaptured birds seroconverted between the two sampling periods indicating that transmission did occur during the interim. Also, WNV was isolated from mosquitoes collected at other locations near case residences in Slidell in mid-August.[7] However, most of the transmission had probably occurred prior to the August sampling, a finding consistent with the human epidemic study in Slidell (Bunning M, pers. comm.). Transmission may have been curtailed by intensive mosquito control efforts used during July and August.[17]

In summary, this study evaluated the avian hosts of WNV within a suburban transmission focus in Slidell, Louisiana. Primarily the northern cardinal and the house sparrow, but also the blue jay and the northern mockingbird, have been implicated by our model as important amplifying hosts due to high levels of exposure, moderate to high abundances, and experimentally demonstrated host competence. The northern cardinal and the northern mockingbird are likely to serve as effective target species for a surveillance program that utilizes free-ranging birds as sentinels in Slidell. The blue jay was implicated as an important target for avian mortality surveillance due to a very high predicted population reduction in Slidell. Chickens were frequently infected and will likely serve as effective captive sentinels in Slidell. This study supports the conclusion that certain summer-resident passerine birds are important amplifying hosts for MNV Slidell.

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