West Nile Virus in American White Pelican Chicks: Transmission, Immunity, and Survival

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Abstract. West Nile virus (WNV) causes significant mortality of American White Pelican chicks at northern plains colonies. We tested oropharyngeal/cloacal swabs from moribund chicks for shed WNV. Such shedding could enable chick-to-chick transmission and help explain why WNV spreads rapidly in colonies. WNV was detected on swabs from 11% of chicks in 2006 and 52% of chicks in 2007; however, viral titers were low. Before onset of WNV mortality, we tested blood from < 3-week-old chicks for antibodies to WNV; 5% of chicks were seropositive, suggesting passive transfer of maternal antibodies. Among near-fledged chicks, 41% tested positive for anti-WNV antibodies, indicating that they survived infection. Among years and colonies, cumulative incidence of WNV in chicks varied from 28% to 81%, whereas the proportion of chicks surviving WNV (i.e., seropositive) was 64–75%. Our data revealed that WNV kills chicks that likely would fledge in the absence of WNV, that infection of chicks is pervasive, and that significant numbers of chicks survive infection.

INTRODUCTION

West Nile virus (WNV) was first documented in the northern plains of North America in 2002.¹ Each year since 2002, WNV has been a source of unusually high mortality of American White Pelican (*Pelecanus erythrorhynchos*) chicks at major breeding colonies in the northern plains.² The impact of WNV is a serious concern because nearly one-half of American White Pelicans breed at these colonies³ and, during the years that we monitored the colonies, there was no evidence that the disease was abating.² Studies conducted before 2002 revealed 3–4% chick mortality from mid-July to the time of fledging (hereafter, late breeding season); however, since WNV arrived in the region, chick mortality during the late breeding season rose dramatically to as high as 44%.² WNV is an additive mortality factor for chicks in the late breeding season, when they are less vulnerable to other common causes of mortality (e.g., severe weather and predation) and normally, would have survived to fledge.

Records of morbidity and mortality in American White Pelican chicks since 2002 indicate that they are particularly vulnerable to WNV, whereas rare confirmations of WNV in adults suggest that they are not highly susceptible² (also see the US Geological Survey [USGS] National Wildlife Health Center Epizootic Data Base). American White Pelicans (hereafter, pelicans) were the first colonial species documented to have extraordinarily high rates of chick mortality attributed to WNV (USGS National Wildlife Health Center Epizootic Data Base). The *Culex tarsalis* mosquito has been identified as the primary vector of WNV in the northern plains of the United States⁴ and, specifically in the region of our study, no other mosquito species has proven to be a major vector.^{5–7} The high incidence of WNV in these colonies, its rapid spread within the colonies, and the behaviors of pelican chicks² warrant exploring the potential for bird-to-bird transmission rather than just mosquito-to-bird transmission. Direct bird-to-bird transmission has been documented in captive populations of chickens,⁸ crows,⁹ and geese,^{10,11} and

there is compelling evidence of bird-to-bird transmission in wild populations of American Crows (*Corvus brachyrhynchos*).¹² In pelican colonies, nests are in close proximity, chicks congregate in crèches about 17 days after hatch, chicks eat regurgitated food (often off the ground), and indirect cannibalism has been observed (Sovada MA and others, unpublished data); these attributes could facilitate transmission of the virus from bird to bird. Specifically, a chick could ingest regurgitated foods scavenged from the ground where the food was in contact with infected feces or, in the close quarters of a crèche, a chick could directly contact mucous or other secretions from an infected chick. Mosquitoes may start the WNV epizootic by exposing the colony, but perhaps, chick behaviors contribute to its spread within the colony.

Although information is limited, experimental studies of several species have shown passive transfer of maternal antibodies to WNV to chicks through egg yolk^{13–18} or egg yolk and crop milk.¹⁹ Transferred WNV antibodies may provide some protection to chicks depending on titer and rate of decay of maternally transferred antibodies, timing of chick exposure to the virus, and whether maternally transferred antibodies interfere with the development of the juvenile immune response as shown for Newcastle disease in Black-Legged Kittiwakes (*Rissa tridactyla*).²⁰

In 2004, we initiated research at three pelican colonies in the northern plains to evaluate the impact of WNV on recruitment.² After 2 years of data collection, it was apparent that WNV caused significant mortality among chicks and had the potential to negatively impact the northern plains breeding population. We recognized the need to better understand potential mechanisms of WNV transmission in pelican colonies and to evaluate specific immunity to WNV in young chicks and nearly fledged chicks. We also had the opportunity to assess cumulative incidence of WNV in chicks during the period from first detection of the disease in the colony to fledging. During 2006–2008, we collected data to address the following objectives: (1) determine if WNV was being shed (orally and/or in feces) by infected chicks, providing a potential mechanism for bird-to-bird transmission of the disease; (2) test for evidence of passive transfer to chicks of maternal antibodies to WNV; (3) determine the proportion of chicks that survive WNV infection; and (4) assess relationships

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between the relative abundance of *Cx. tarsalis* and the prevalence of WNV in chicks.

MATERIALS AND METHODS

We collected data from three pelican breeding colonies in the northern plains: Chase Lake in central North Dakota, Bitter Lake in northeastern South Dakota, and Medicine Lake in northeastern Montana (the work by Sovada and others² has a description of study areas). Chase Lake and Bitter Lake are among the five largest colonies in North America. Chase Lake had 17,302 and 11,262 nests in 2006 and 2007, respectively; Bitter Lake had 14,762 and 14,713 nests in 2006 and 2007, respectively. Medicine Lake is among the 20 largest colonies and supported 4,589 nests in 2006.

WNV has been documented as the primary cause of late-breeding-season (mid-July to fledging) chick deaths, and the disease onset in the pelican colonies consistently occurs around the second week of July.² We collected chicks immediately after initial observations of unusual numbers of dead and moribund chicks (staggering, unable to stand, or unable to hold up head). At least three chicks were submitted to the National Wildlife Health Center for necropsy and diagnostic tests to confirm WNV infections and eliminate or identify other potential causes of deaths. We visited the colonies 3–5 days/week, and if evidence suggested other potential sources of mortality, we submitted one to three additional chicks for diagnostic testing. We estimated mortality rates of chicks during the late breeding season. To do this estimation, we banded a sample of chicks (mean = 1,551 chicks/year) in late June–early July, which is just before the seasonal onset of WNV. In each colony each year, we used recovery rate of bands to estimate the late-season mortality rate for chicks. With effectively no other significant competing cause of death identified for chicks in the late breeding season,² we assumed that the banded chicks that we recovered had died of WNV. All collections and handling of pelican chicks were conducted under federal and state permits and approved by Northern Prairie Wildlife Research Center's Animal Care and Use Committee.

Each year, at least 2 weeks after the onset of significant chick mortality in the colony, we started collecting oropharyngeal and cloacal swabs from chicks displaying severe signs of illness to determine if they were shedding WNV. Our goal was to collect samples from 50 chicks at each colony (Bitter Lake and Chase Lake) in each year (2006 and 2007). Viral presence in oral and/or cloacal secretions would support possible mechanisms for chick-to-chick transmission of WNV. Swab samples were collected with Dacron-tipped applicators, which were placed in tubes containing 1 mL BA1 medium; tubes were kept on blue ice for transport to the laboratory and frozen within 3 hours of collection. They were kept frozen at -80°C until assayed. Samples were tested for virus content by Vero cell plaque assay.²¹

To test for evidence of passive transfer of anti-WNV antibody from mother to chick, we collected blood samples from chicks that were < 3 weeks old. Our annual goal was to collect samples from 50 chicks in each colony (Bitter Lake and Chase Lake) in each year (2006, 2007, and 2008). We also had the opportunity to collect samples at Medicine Lake in 2006. From observations in previous years, we knew that mid-July consistently marked the onset of WNV infections in these colonies. To sample chicks before onset of WNV infections,

we collected blood samples during the first 2 weeks of June, which was at least 3 weeks before WNV was ever detected.² Therefore, exposure of chicks to WNV-infected mosquitoes at this time was considered unlikely.

Later in the season, after WNV had peaked in the colonies, we collected blood from chicks that were nearly fledged and appeared healthy. Our goal was to collect samples from 50 chicks at each colony (Bitter Lake and Chase Lake) in each year (2006, 2007, and 2008). We also had the opportunity to collect samples at Medicine Lake in 2006. We tested the blood for anti-WNV antibody to determine what proportion of chicks infected by WNV survived to fledge as evidenced by seroconversion to WNV.

Blood was collected in a Microvette[®] CB 300 capillary tube (Sarstedt Inc., Newton, NC) from a small puncture made with a lancet in the wing (cutaneous ulnar) or leg (medial metatarsal) vein. The samples were kept cool on ice packs for transport to a laboratory. In the laboratory, the chilled samples were allowed to warm for 10 minutes before centrifugation to separate serum from the cell pellet. The serum was transferred to cryovials, which were frozen and shipped to the National Wildlife Health Center, Madison, Wisconsin, for analysis.

Serum samples were screened for anti-WNV antibody with WNV epitope blocking assay enzyme-linked immunosorbent assay (ELISA).²² Slight modifications included preparation of WNV antigens in Vero cells (ATCC CCL-81), screening sera at a dilution of 1:100 to conserve the small sample volumes, and correspondingly, considering samples with a percent inhibition (PI) value of $\geq 25\%$ as provisionally positive. All provisionally positive samples were tested with the plaque reduction neutralization test (PRNT) to confirm the presence of antibody to WNV. We used standard techniques²¹ to conduct PRNT on Vero cells at a starting serum dilution of 1:20 with a 1999-NY crow isolate of WNV as the test virus. Samples were considered positive for antibodies to flavivirus if $\geq 90\%$ of the plaques in the test dose of WNV were neutralized (PRNT₉₀).

To test for differences between titers in < 3-week-old chicks and nearly fledged chicks, we conducted an analysis of variance (ANOVA; PROC GLM²³) on the geometric means of the titers. Pearson correlation analysis was used to test for an association between the annual incidence of antibodies in near-fledged chicks and late-breeding season mortality rates at each colony (PROC CORR²³).

To estimate the proportion of chicks that survived WNV infection, we first estimated the yearly cumulative incidence of WNV for chicks (i.e., percent that were infected with WNV). We made calculations starting with a hypothetical population of 100 chicks alive in mid-July, when WNV typically was first detected in the colonies. We calculated the number of the initial 100 chicks that likely died of WNV based on the mortality rate for chicks in the late breeding season (estimated from band recovery data; mean = 1,551 bands deployed/year). Based on results from prior research, we made two assumptions about mortalities in the late breeding season: (1) other causes of chick death were insignificant compared with mortality from WNV, and (2) other causes of death did not vary among years. We then multiplied the estimated number of surviving chicks among the original 100 chicks by the proportion of our sample of nearly fledged chicks that had antibodies to WNV; this result provided an estimate of the number of chicks that had been

TABLE 1

Results of Vero plaque assays conducted on oropharyngeal and cloacal swabs from moribund American White Pelican chicks that displayed symptoms of WNV

	<i>n</i>	Positive oropharyngeal only	Positive cloacal only	Positive oropharyngeal and cloacal	Chicks with evidence of shed virus (%)	95% CI
2006						
Chase Lake	50	1	2	3	12	3, 21
Bitter Lake	50	4	1	0	10	2, 18
2007						
Chase Lake	3	1	0	0	—*	—
Bitter Lake	28	11	2	2	54	1, 15

Swabs were collected at least 2 weeks after initial observation of symptomatic chicks in the colonies at Chase Lake, North Dakota, and Bitter Lake, South Dakota. 95% CI = 95% confidence interval.

*Sample size too small for calculation of meaningful proportion.

infected and survived WNV. Adding the number that died to the number that were infected and survived gave us an estimate of the cumulative incidence of WNV in chicks. Last, the number of chicks with antibodies divided by the total number infected gave us an estimate of the proportion of chicks that survived infection.

We monitored mosquitoes in coordination with a monitoring effort being conducted by the North Dakota Department of Health (<http://www.ndhealth.gov/WNV/Data/Summary.aspx>) to assess relationships between the relative abundance of *Cx. tarsalis* and the prevalence of WNV in pelican chicks. Each year, beginning the first week of June and continuing through August, samples of adult mosquitoes were collected weekly with New Jersey light traps.²⁴ Traps were placed in similar habitat adjacent to colonies. Because we had several gaps in the weekly collections of mosquitoes at colony sites, mostly caused by samples being too wet to count, we supplemented samples by including data from reporting stations nearest to the colonies (within 100 km; Bitter Lake-4, Chase Lake-5, Medicine Lake-4). Among the 16 sites, on average, data were usable from 11 sites weekly (range = 7–13), and data were never from less than three traps for a single colony. We calculated an annual index to relative abundance of *Cx. tarsalis* by averaging the weekly captures per trap.

RESULTS

In 2006 and 2007, we collected oropharyngeal and cloacal swabs from 156 moribund chicks. In 2006, collections occurred between July 27 and August 3; 50 chicks from Chase Lake and 50 chicks from Bitter Lake were sampled. In 2007, collections occurred during August 1–28; we sampled 53 chicks from Bitter Lake but were able to collect samples from only 3 chicks at Chase Lake. Regrettably, 25 of the Bitter Lake samples in 2007 were contaminated with debris that made counting and interpretation of plaques impossible; thus, these 25 samples were not included in results. WNV was cultured from swabs for 11% of the chicks in 2006 and 52% of the chicks in 2007 (Table 1); virus titers were moderately low ($10^{2.0}$ – $10^{2.8}$) compared with those titers reported in controlled experiments of other wild avian species.²⁵

We collected blood samples at all three colonies in 2006 but only at Bitter Lake and Chase Lake in 2007 and 2008. Blood was collected from 350 pelican chicks in mid-June (Table 2), which was at least 3 weeks before initial detection of WNV infections in pelican chicks. All sampled chicks were < 3 weeks old, most were within 1 week of hatch, and all appeared to be healthy. Overall, detection of anti-WNV antibodies by screening and confirmation by PRNT indicated that 5% of the serum samples from the < 3-week-old chicks were positive for WNV antibodies (Table 2), and the overall geometric mean PRNT titer was 1:30 (Table 3).

In August, we collected blood from 259 chicks that were near fledging (Table 2). Blood was collected at least 2 weeks after initial evidence of WNV (i.e., chicks tested positive for WNV) in individual colonies. All of these chicks appeared healthy and had survived the peak period of WNV outbreak in their respective colonies; 39% of the serum samples from these chicks were positive for antibodies to WNV. The highest percentages among and within colonies were recorded in 2007, and the overall calculated mean PRNT titer was 1:110 (Table 2). Seropositive chicks sampled near fledging had calculated geometric mean PRNT titers that were higher than those titers of < 3-week-old chicks (1:110 and 1:30, respectively; $F = 9.12$, $P = 0.04$). The prevalence of antibodies to WNV in near-fledged chicks was positively correlated with chick mortality rates during the late breeding season at individual colonies each year (Table 4) (Pearson correlation coefficient, $r = 0.886$, $P = 0.02$).

TABLE 2

Results of enzyme immunoassays (antibody blocking [ELISA] and PRNT) conducted on sera from American White Pelican chicks that were < 3 weeks old and chicks that were near to fledging

Year and study area	<i>n</i>	< 3-week-old chicks (June)				Near-fledged chicks (August)				
		ELISA	PRNT	Antibodies to WNV (%)	95% CI	<i>n</i>	ELISA	PRNT	Antibodies to WNV (%)	95% CI
2006										
Chase Lake	52	5	5	10	2, 18	50	11	10	20	9, 31
Bitter Lake	46	1	0	0		50	28	25	50	36, 64
Medicine Lake	50	3	0	0		50	11	11	22	10, 34
2007										
Chase Lake	52	0	0	0		28	18	18	64	45, 83
Bitter Lake	50	2	2	4	0, 10	27	17	20	74	56, 92
2008										
Chase Lake	50	9	8	16	5, 27	4	0	0	—*	—
Bitter Lake	50	3	2	4	0, 10	50	22	22	44	30, 58

All provisionally positive samples (ELISA results) were tested with PRNT to confirm the presence of antibody to WNV. Data were collected at Chase Lake, North Dakota; Bitter Lake, South Dakota; and Medicine Lake, Montana. Each year, observation of diseased chicks first occurred in mid-July and peaked in late July. 95% CI = 95% confidence interval.

*Sample size too small for calculation of meaningful proportion.

TABLE 3

PRNT titers for anti-WNV antibody in sera collected from American White Pelican chicks sampled 3 weeks before the onset of WNV mortality, near fledging, and after the peak of WNV mortality at Chase Lake, North Dakota (2006–2008), Bitter Lake, South Dakota (2006–2008), and Medicine Lake, Montana (2006)

Titer	Year					
	2006		2007		2008	
	No. < 3-week-old chicks	No. near-fledged chicks	No. < 3-week-old chicks	No. near-fledged chicks	No. < 3-week-old chicks	No. near-fledged chicks
1:20	1	6	1	2	7	0
1:40	2	23	1	4	3	3
1:80	1	10	0	12	0	4
1:160	0	3	0	8	0	8
1:320	1	4	0	6	0	5
1:640	0	0	0	5	0	2
1:1280	0	0	0	1	0	0
Total PRNT ₉₀ -positive*	5	46	2	38	10	22
Geometric mean	5.92	5.8	4.82	7.14	4.62	7.27
Geometric mean titer	1:61	1:56	1:28	1:141	1:25	1:154

*Samples were considered positive for antibodies to flavivirus if $\geq 90\%$ of the plaques in the test dose of WNV were neutralized (PRNT₉₀).

Using the late-breeding season mortality rates combined with estimates of the number of surviving chicks that were infected with WNV (based on the presence of anti-WNV antibody in near-fledged chicks), we found that the percent of chicks alive at the onset of WNV that were subsequently infected with WNV varied from 28% to 81% among years and colonies (Table 4). Among the chicks that were infected by WNV, our data suggest that 60–75% survived infection and fledged from the colonies (Table 4).

Cx. tarsalis were first detected at colony sites the fourth week of June in 2006 and early July in 2008. At Bitter Lake in 2007, small numbers (8) of *Cx. tarsalis* were captured in early June, with a marked increase in captures in early to mid-July. The overall weekly average number of female *Cx. tarsalis* captured per trap was 2 in 2006, 63 in 2007, and 8 in 2008. Annual relative abundance of mosquitoes followed the trends in the Palmer Drought Index, with fewer mosquitoes captured in 2006 and 2008, when conditions were drier than in 2007.

DISCUSSION

Each year, detection of WNV in the pelican colonies followed the detection of the vector mosquito *Cx. tarsalis*, and the first observations of moribund chicks occurred in

mid-July. Rates of chick mortality and the cumulative incidence of WNV during the late breeding season paralleled overall abundance levels of *Cx. tarsalis* each year; all were markedly higher in 2007 than in 2006 and 2008.

A mechanism for chick-to-chick transmission of WNV is supported by our findings of shed virus, although the percent of oropharyngeal and cloacal swabs that tested positive for WNV was not as high as we expected given the rapid disease spread and magnitude of chick loss in the colonies in some years.² The lower rate of shedding in 2006 compared with 2007 may be attributed to the distinctly lower mosquito presence in 2006 relative to 2007. Notably, in 2007, record numbers of human cases of WNV were reported from the region of our study.²⁶ Overall, however, the shed virus in our samples of moribund pelican chicks had moderately low titer levels compared with titer levels in other birds studied.²⁵ Moreover, the viability of WNV shed in pelican feces or regurgitated food is unknown; thus, the potential for WNV transmission through these routes has yet to be assessed. In other species, the duration of viability is limited (e.g., nearly no infectious WNV remained in chicken feces after 24 hours⁸). Experimental study of captive pelican young would be required to assess the infectivity, infectiousness, and length of viability of chick-shed virus.

Presence of antibodies to WNV in the blood of pelican chicks < 3 weeks old suggests passive transfer of anti-WNV

TABLE 4

Calculations for estimates of the percent of American White Pelican chicks that were infected by WNV and survived to fledge from the breeding colonies at Chase Lake, North Dakota (2006–2007), Bitter Lake, South Dakota (2006–2008), and Medicine Lake, Montana (2006)

Year and study area	Late breeding season mortality rate (%) [*]	Chicks surviving late breeding season	Chicks with antibodies at fledging (%)	Cumulative incidence of WNV in chicks (dead + chicks with antibodies)	Of chicks before outbreak, chicks infected by WNV that survived to fledge [†]
2006					
Chase Lake	10	90	18 (20)	10 + 18 = 28	18/28 = 64%
Bitter Lake	14	86	43 (50)	14 + 43 = 57	43/57 = 75%
Medicine Lake	8	92	20 (22)	8 + 20 = 28	20/28 = 71%
2007 [‡]					
Chase Lake	30	70	45 (64)	30 + 45 = 75	45/75 = 60%
Bitter Lake	25	75	56 (74)	25 + 56 = 81	56/81 = 69%
2008 [§]					
Chase Lake [§]	8				
Bitter Lake	13	87	38 (44)	13 + 38 = 51	38/51 = 75%

For these calculations, we assume a beginning population of 100 chicks in mid-July, which is just before the start of WNV infections in each colony.

^{*}Mortality rates of American White Pelican chicks during late breeding season (mid-July to fledging).

[†]Nearly all late-breeding season mortality can be attributed to WNV.² Before the 2002 arrival of WNV in the region, reported late-breeding season mortality rate was < 4%.²

[‡]The percent of chicks with WNV neutralizing antibodies was not measured at Medicine Lake in 2007–2008.

[§]Sample size too small for meaningful calculation.

antibodies from the female adult to the chick mediated by IGY (immunoglobulin of egg yolk) antibodies. We cannot rule out the possibility that the < 3-week-old chicks that we sampled were exposed to WNV; however, it is improbable given the timing of the appearance of *Cx. tarsalis* in the colonies. Furthermore, the low PRNT titers of anti-WNV antibodies in the < 3-week-old chicks compared with the PRNT titers in chicks sampled near fledging support the hypothesis that anti-WNV antibodies in the < 3-week-old chicks were passively acquired. In most years, it is unlikely that passively acquired antibodies to WNV provide any protection against WNV by the time that chicks are first exposed to WNV-infected *Cx. tarsalis* mosquitoes. Extensive research in captive birds shows that only temporary protection is provided by maternal antibodies to WNV and other microorganisms, because passively acquired antibodies are typically catabolized in less than 3 weeks.^{15,16,18,19,27,28} During 2004–2008, WNV was detected at the pelican colonies when most of the chicks were between 4 and 10 weeks of age; the majority was from 6 to 8 weeks and well past the period when maternally acquired antibodies would provide protection against challenge.

The role of adult pelicans in the transmission and persistence of WNV in these colonies is difficult to assess. The fact that some breeding adult pelicans have antibodies to WNV (as indicated by IGY antibodies) is evidence that these adults survived exposure to WNV at some point in their lives; however, it is unknown whether the antibodies were acquired as a result of exposure when the birds were chicks or adults. In the 8 years since WNV arrived in the region, we never observed an adult pelican with clinical signs of WNV, despite our regular visits to three colonies. We must assume that adult pelicans were exposed to mosquito vectors, but there is no evidence that adult pelicans are highly susceptible to the disease. During the period of this study, the National Wildlife Health Center recorded fewer than 10 adult pelicans infected with WNV (USGS, National Wildlife Health Center Epizootic Data Base).

Because our data do not clearly reveal the importance of fecal–oral route or the oral–oral mechanism of viral spread between pelican chicks, other possible scenarios are worth considering. Non-mosquito arthropods might serve as competent WNV vectors. Soft ticks,^{29,30} louse flies,³¹ and biting midges (*Culicoides sonorensis*)³² are known to infect other avian species with WNV. Pelican chicks harbor a wide variety of ectoparasites; most notable are pouch lice or biting lice (*Piagetiella peralis*).^{2,33} Pouch lice taken from infected pelican chicks have tested positive for WNV²; however, in another study, pouch lice sampled from WNV-infected pelicans tested negative.³⁴

Host competence of pelicans (i.e., probability that a mosquito or other vector that bites an infected pelican will become infectious for WNV²⁵) has not been measured. LaDeau and others³⁵ reported that 20–48% of mosquitoes biting a competent amplifying host species (i.e., top 10 in host competence index; figure 2 in the work by Kilpatrick and others³⁶) would become infectious in each of the 5 days after being infected. Because pelican chicks live in crowded colonies, if they are highly infectious (i.e., have high host competence), they can quickly amplify the prevalence of WNV. Therefore, this species might be more vulnerable to infection than non-colonial species or species with low competence. Onset of the acute phase of WNV infection in individual

pelican chicks apparently comes quickly after infection; as necropsies revealed, many chicks that died of WNV were in excellent body condition.² Although not proven, this finding suggests that pelican chicks may be competent hosts, developing an elevated WNV viremia very quickly.³⁷

Non-viremic transmission of WNV is another possible mechanism that could facilitate the rapid spread of the disease observed in the colonies that we monitored. Several laboratory studies have documented uninfected mosquitoes becoming infected simply by feeding on a non-viremic host that had been fed on by an infected mosquito.^{38–40} Although most pelican chicks that die of WNV are larger with a greater blood volume than vertebrate hosts tested in laboratory studies (e.g., mice and house finches [*Carpodacus mexicanus*]), at both Chase Lake and Bitter Lake, pelicans share the nesting islands with thousands of breeding egrets and herons that have relatively small chicks in nests at the time that WNV causes pelican chick mortality. Non-viremic transmission could accelerate the spread of WNV, because additional mosquitoes can become carriers without waiting for the virus to replicate in a vertebrate host.

Starting in 1999, when WNV first arrived in North America, avian species vulnerable to WNV were greatly impacted by initial exposure to the virus. Although it is not possible to gauge the role of waning interest in surveillance and reporting of bird deaths, there generally seems to be a decrease in the prevalence of WNV infection and mortality among native bird species in North America.⁴¹ However, there are some exceptions^{41,42} to decline in prevalence, and pelican chicks may be one of those exceptions. We observed variability in both annual and colony rates of late-season chick mortality, but this variability seemed more related to water conditions needed for mosquito eggs and larvae to develop^{2,35} than a potential increase in resistance to WNV infection. The antibodies that we documented in near-fledged chicks might suggest that immunity is developing, but observations at the colonies during the years of this study did not suggest that rates of chick mortality caused by WNV were declining. It is possible that herd immunity will develop, or perhaps, WNV will undergo genetic changes with a concurrent loss of virulence.^{43,44}

The high rate of survival by infected chicks based on seroprevalence of antibodies does not support the initial impression, based on the mortality data, that WNV has a devastating effect on the chick population. The seroprevalence results reveal that infection of chicks was pervasive but that a significant number of chicks survived the infection. It is unclear whether the rarity of documented cases of WNV infection in adult pelicans indicates that adults are far less likely than chicks to die of the infection or less likely to be infected. If we assume the latter, it suggests that the adults that apparently transferred antibodies to WNV to their chicks (through egg yolk) most likely were infected as chicks themselves. In any case, the question remains: ultimately, how is recruitment to the breeding population impacted? Here, it is important to reiterate that chick losses caused by WNV are likely additive and not compensatory.² In the long term, breeding populations could be impacted by this disease. Given that the American White Pelican is a long-lived species with low reproductive potential,³³ WNV might have lasting effects that will not be detected for several years.

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