DOI: 10.7589/JWD-D-21-00030

Journal of Wildlife Diseases, 58(1), 2022, pp. 205–210

West Nile Virus Infections in an Urban Colony of American White Ibises (*Eudocimus albus*) in South Florida, USA

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ABSTRACT: West Nile virus (WNV) is pathogenic in a wide range of avian hosts and is endemic in much of North America. This virus is responsible for population declines of some Passeriformes. We describe a WNV-associated mortality event in American White Ibis (*Eudocimus albus*) nestlings. This is a species, inherent to the Everglades ecosystem, which has recently begun nesting in urban areas. An urban colony in south Florida was monitored from March–July in 2020 as part of an ongoing study. Nestling carcasses were collected opportunistically and sent to the Southeastern Cooperative Wildlife Disease Study, University of Georgia within 24 h for diagnosis. Three ibis nestling deaths were confirmed to be caused by WNV infection based on histopathology, immunohistochemistry, and reverse transcription PCR. Serial plasma samples collected weekly from 36 healthy chicks of the same urban rookery were tested for WNV-neutralizing antibodies via plaque reduction neutralization test; four chicks were seropositive. Antibody titers in three seropositive chicks from which serial samples were collected waned over time, suggesting maternal antibody transfer. Ibis mortalities were consistent with a spike of WNV activity in this region of Florida. West Nile virus infection may be an important seasonal cause of mortality for wading bird nestlings.

Key words: Arboviruses, *Eudocimus albus*, maternal antibodies, nestlings, West Nile virus, White Ibis.

Since its introduction, West Nile virus (WNV; genus *Flavivirus*; family Flaviviridae) has become well established in many North American bird populations and is a significant public health concern. Transmission in temperate regions peaks in summer and early fall, due to increased mosquito vector activity (Apperson et al. 2004). The avian host range of WNV includes >300 species in the US (Centers for Disease Control and Prevention 2016), yet susceptibility to infection and clinical outcomes varies widely (Pérez-Ramírez et al. 2014). While the potential impacts of WNV in some raptor and passerine species are recognized (Dusek et al. 2010; Kilpatrick and Wheeler 2019), infection in free-ranging waterbirds has not been extensively documented. Nestling mortalities due to WNV have been reported in free-living egrets, herons, and American White Pelicans (*Pelecanus erythrorhynchos*; Reisen et al. 2009; Johnson et al. 2010). Conducting nestling mortality investigations in mixed-species colonies is difficult due to reduced accessibility and researcher-imposed limitations such as time spent on the colony (Frederick et al. 1993; Kuiken et al. 1999).

American White Ibises (*Eudocimus albus*) in South Florida, US, have increasingly colonized anthropogenic habitats as a response to natural wetland loss (Frederick and McGehee 1994; Hernandez et al. 2016). Commonly reported causes of ibis nestling mortality include predation, starvation, severe weather conditions (e.g., storms), and parental nest abandonment (Adams and Frederick 2009; Heath et al. 2009). Ibis nestling mortality was investigated as part of a project on urban rookery productivity. Continuous WNV surveillance of mixed-species colonies, particularly those close to urban areas, is useful for public health (Johnson et al. 2010). We report a WNV-associated mortality event in American White Ibis nestlings and subsequent investigation into the exposure to WNV of other American White Ibis chicks in the rookery.

The study site was an urban wading bird rookery in West Palm Beach, Florida, US, (26°49′30′′N, 80°8′59′′W) located on three

FIGURE 1. (A) Moribund American White Ibis nestling (*Eudocimus albus*; no. 359) found inside its nest on 13 July 2020 in West Palm Beach, Florida, USA. (B) Two additional nestlings from nest of no. 359 (indicated by an arrow) and an adjacent nest were found dead but with severe postmortem autolysis.

adjacent islands (ranging in area from 478–1,312 m^2) situated in a canal running through a golf course community (see Supplementary Material Figure S1). These islands, which attract approximately 300 breeding pairs of ardeids and phalacrocorcids, were monitored from April to July 2020. Each island was visited only weekly to minimize disturbance. On 19 June 2020, two nestlings (10–13 d old) were found dead (bird 314A) or moribund (314B; died while handling) in one nest. On 13 July, an additional nestling (18 d old; 359) was found moribund and alone inside its nest (Fig. 1). It was transferred to a wildlife hospital and was euthanized. Carcasses were shipped overnight on ice packs in $\leq$24 h to the Southeastern Cooperative Wildlife Disease Study (University of Georgia, Athens, Georgia, USA) for diagnostic evaluation. Necropsy was performed, and samples from all major organ systems except skeletal (i.e., respiratory, digestive, circulatory, urinary, integumentary, muscular, endocrine, and lymphatic such as spleen and proventriculus, nervous, reproductive) were collected into 10% neutral buffered formalin for histopathology. Heart from all three ibis was evaluated by immunohistochemistry (IHC), along with additional tissues (314A: ventriculus, small and large intestines, spleen, adipose, skeletal muscle, trachea, brain, lung, liver, kidney; 314B: lung, adrenal gland, testes, liver, spleen, adipose, skeletal muscle, kidney; 359: proventriculus, ventriculus, crop, trachea, skeletal muscle). Standard histologic processing, H&E staining and IHC were performed at the Athens Veterinary Diagnostic Laboratory (Athens, Georgia). Select tissues (pooled brain, kidney, and/or heart) were placed into virus media and tested by virus isolation with subsequent reverse transcription PCR of virus isolates. The cause of death of the three nestlings was determined, based on histopathology, IHC, and ancillary testing (virus isolation or reverse transcription PCR; Table 1), to be WNV infection. All three nestlings had variable

TABLE 1. Diagnostic results from the three American White Ibis (*Eudocimus albus*) nestlings that were positive for West Nile virus infection at an urban colony in West Palm Beach, Florida, USA.

Bird ID	Age (days)	Body condition	Virus isolation	RT-PCR[a]	Immunohistochemistry	Histologic lesions
314A	10	Poor	Negative	Positive	Intense	Lymphoplasmacytic myocarditis and pneumonia
314B	13	Good	Positive	Positive	Intense	Lymphoplasmacytic myocarditis
359	18	Good	Negative	Negative	Mild	Lymphoplasmacytic myocarditis, proventriculitis, and perineuritis; perivascular nephritis, hepatocellular lipidosis

[a] RT-PCR = reverse transcription PCR.

lymphoplasmacytic myocarditis with myocardial necrosis; 359 also had fibrinoid necrosis (Fig. 2). Immunolabeling for WNV antigen was most prominent and abundant in the cytoplasm of cardiomyocytes of 314A and 314B (Fig. 3); these nestlings also had occasional labeling in the renal tubular epithelium and in the adrenal gland chromaffin cells of 314B. Cardiomyocytes rarely labeled in 359, where occasional macrophages in circulation exhibited cytoplasmic labeling and also occasionally in ventricular cytoplasmic epithelium (Fig. 4). Another American White Ibis (dead) and one Tricolored Heron (*Egretta tricolor*; moribund, later euthanized) found on the islands on 20 May tested negative for WNV infection.

To better assess WNV dynamics in the rookery, we performed serologic testing on the monitored ibis nestlings. We handled 36 nestlings (oldest chick of each nest) weekly from hatch to about 20 d posthatch to collect standard morphometric measurements, blood, and feces. Blood (≤1% body weight) was collected from the jugular vein and maintained cold in an insulated container with ice packs while in the field. Plasma was separated via centrifugation within 4 h of collection, then stored at –80 C. Serial plasma samples were tested for WNV-neutralizing antibodies via the plaque reduction neutralization test (PRNT; Beaty et al. 1995), as described by Allison et al. (2004), except that cultures were inactivated on day 5 postadsorption with 10% buffered formalin and stained with 0.25–1% crystal violet for plaque visualization. The starting dilution was 1:10; titers were expressed as the reciprocal of the highest plasma dilution neutralizing ≥90% WNV plaque-forming units ($PRNT_{90}$) when compared with

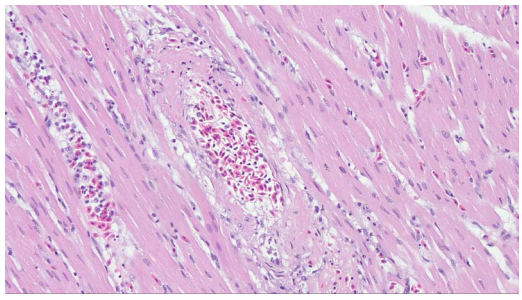

FIGURE 2. Transmural fibrinoid necrosis in an arteriole wall within the septum of American White Ibis (*Eudocimus albus*) nestling no. 359. H&E. 20×.

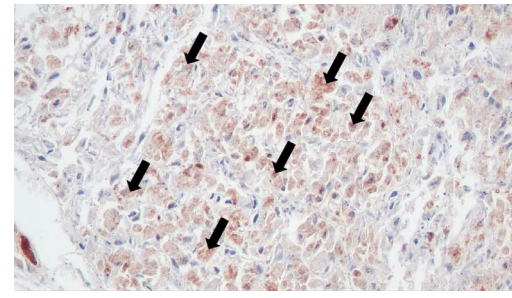

FIGURE 3. Intense cytoplasmic immunolabeling for West Nile virus antigen (arrows) within many degenerated or necrotic cardiomyocytes in the heart of American White Ibis (*Eudocimus albus*) nestling no. 314A. 40×.

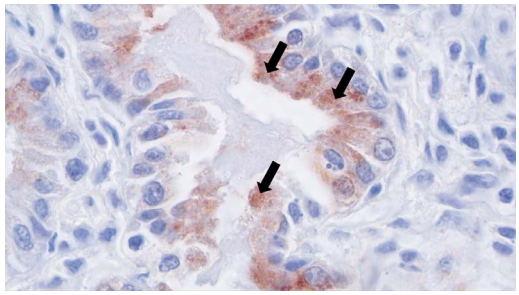

FIGURE 4. Cytoplasmic immunolabeling for West Nile virus antigen (arrows) within numerous epithelial cells along the ventricular mucosa in American White Ibis (*Eudocimus albus*) nestling no. 359. 100×.

control wells. Samples with ≥90% neutralization were co-titrated (serial 10-fold dilutions) for WNV and St. Louis encephalitis virus to determine the causative virus (with ≥fourfold higher $PRNT_{90}$). Samples with ≥10 WNV titer and <10 St. Louis encephalitis virus titer were considered seropositive for WNV (Komar et al. 2001). We found WNV-neutralizing antibodies in 36 nestlings (11% seroprevalence). With the exception of 314B (died immediately after capture), the chicks that died of WNV had not been selected for monitoring, thus had no blood collected. Nestlings neighboring 359, including its sibling (Fig. 1), were seronegative. Seronegative chicks in early samples did not test positive at later time points. Titers ranged from 10 to ≥320 and waned with age (Table 2), suggesting maternal WNV antibody transfer and decline. Maternal antibodies can be protective and persist for up to several weeks in house sparrows (*Passer domesticus*) and domestic chickens (*Gallus gallus domesticus*; Nemeth and Bowen 2007; Nemeth et al. 2008). Protective titers for American White Ibises are unknown, thus even birds with acquired antibodies might be susceptible to infection and clinical disease. Because ibis chicks are highly mobile and difficult to recapture, we were unable to document the full duration of maternal antibody persistence. One nestling (no. 36) was captured only once, thus its complete WNV serostatus is unknown.

TABLE 2. West Nile virus (WNV) neutralizing antibody titers of the four seropositive American White Ibis (*Eudocimus albus*) nestlings of 36 tested via the plaque reduction neutralization test (PRNT), and the three nestling mortalities from an urban colony in West Palm Beach, Florida, USA.

Bird ID	Hatch date (2020)	Age in days (WNV, $PRNT_{90}$ titer)[b]
6	May 4	2 (80), 10 (20)
7	May 6	2 (80), 11 (10), 17 (10)
27	June 22	2 (≥320), 14 (20)
36	July 6	9 (160)
314A[a]	June 9	10 (n.d.)
314B[a]	June 6	13 (n.d.)
359[a]	June 25	18 (n.d.)

[a] Indicates nestlings that died of WNV as confirmed by histopathology, immunohistochemistry, and/or reverse transcription PCR.

[b] n.d. = not determined.

Our findings provide evidence that American White Ibis nestlings are susceptible to WNV-associated disease and mortality. During our study, 23 additional nestlings disappeared or were found dead, including the sibling of 314A and 314B and the sibling and neighbors of 359; however, carcasses found were unsuitable for diagnostic evaluation due to advanced postmortem autolysis. It is therefore possible that additional WNV-associated mortalities occurred, as supported by WNV surveillance data from the Florida Department of Health (FDOH 2020), Tallahassee, Florida, from several counties in 2020. More than 400 WNV-positive sentinel chickens were documented (25 from Palm Beach County); human case numbers spiked, with 50 symptomatic cases reported (five from Palm Beach County, June–August); and across Florida, 19 positive mosquito pools were detected. Surveillance by FDOH was suspended from March 22–June 14 due to the SARS-CoV-2 pandemic (FDOH 2020). Extended monitoring of this urban ibis colony is needed to elucidate whether WNV-associated nestling mortalities are common at this location or if they were a result of increased virus activity in 2020.

The antibodies that we detected in ibis nestlings probably reflect passive antibody

transfer from seropositive mothers, not mosquito-borne WNV infections, as titers quickly waned over time (Table 2). Nevertheless, mortalities from WNV infection occurred, which may indicate that ibis chicks are highly susceptible to developing fatal WNV disease. Additionally, these maternal antibodies suggest that adults may be less susceptible to WNV-associated disease based on survival, seroconversion, and successful breeding. Potential impacts of WNV in other rookeries are unknown. The breeding season of these ibises (April–July) overlaps with peak mosquito activity, increasing the potential for WNV mortality to late-born nestlings. Given the dense mosquito populations in wetlands, it is likely that birds breeding in natural rookeries are also exposed to WNV. As chicks are probably more susceptible to clinical disease and mortality, potential detrimental impacts of WNV on ibis populations would probably be restricted to declines in fledging rates.

American White Ibises are routinely found in urban areas across south Florida. They aggregate in parks and landfills where they are provisioned with food, readily coming into contact with mosquitoes circulating among human populations. Continuous investigation of mortalities at urban rookeries would be useful for alerting public health authorities regarding pathogens that might be circulating among local human populations.

We thank Katherine Christie and Elizabeth Kurimo-Beechuk for assistance in field captures and handling of ibis nestlings, Brian Dugovich for diagnostic support, and the histotechnicians at the Athens Veterinary Diagnostic Laboratory for technical support. We also thank the rehabilitators of Busch Wildlife Sanctuary (Jupiter, Florida) who processed the moribund birds mentioned in this study and Ricardo Zambrano of the Florida Fish and Wildlife Conservation Commission (FWC) for logistical support. This study was approved by the University of Georgia Institutional Animal Care and Use Committee (IACUC A2019 10-009-Y1-A0) and was funded by a grant from the National Science Foundation, Ecology, and Evolution of Infectious Diseases (DEB-1518611). The study held permits by the FWC (LSSC-11-00119I) and the US Fish and Wildlife Service (MB53675D-0). Diagnostic support was provided by Southeastern Cooperative Wildlife Disease Study member states, specifically the FWC.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-21-0030.

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Submitted for publication 3 March 2021.
Accepted 12 July 2021.