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Short Communication

West Nile Virus in the British Virgin Islands

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Abstract: West Nile virus (WNV) first emerged in the US in 1999 and has since spread across the Americas. Here, we report the continued expansion of WNV to the British Virgin Islands following its emergence in a flock of free-roaming flamingos. Histologic review of a single chick revealed lesions consistent with WNV infection, subsequently confirmed with PCR, immunohistochemistry and in situ hybridization. Full genome analysis revealed 99% sequence homology to strains circulating in the US over the past decade. This study highlights the need for rapid necropsy of wild bird carcasses to fully understand the impact of WNV on wild populations.

Keywords: West Nile virus (WNV), flamingo, British Virgin Islands

West Nile virus (WNV) is a single-stranded positive-sense RNA virus classified within the family Flaviviridae (genus: *Flavivirus*) and is transmitted between vertebrate hosts via the bites of *Culex* spp. mosquitoes. When it first arrived in the New World in 1999 (Briese et al. 2000; Lanciotti et al. 1999; Steele et al. 2000), the virus spread rapidly across North America (Kilpatrick 2011) and caused large mortality events in many wild bird species (Nemeth et al. 2007; Bernard et al. 2001), leading to significant long-term population impacts in a range of passerine and other species (Kilpatrick et al. 2013; LaDeau et al. 2007). WNV subsequently spread to Central and South America, including Guatemala, El Salvador, Nicaragua, Colombia, Venezuela, and Argentina; and also to the Caribbean islands (Kilpatrick 2011; Barrera et al. 2008; Bosch et al. 2007; Dupuis et al. 2003, 2005; Komar and Clark 2006; ProMED-mail 2001, 2005; Cruz et al. 2005; Fernández-Salas et al. 2003; Diaz et al. 2008; Osorio et al. 2012), however, little is known about the impact on wild bird populations in these regions. Here we demonstrate the continued expansion of WNV in the Caribbean, reporting its emergence in a flock of flamingos in the British Virgin Islands (BVI).

During the period 27th–30th March 2013, 11 Caribbean flamingo chicks (*Phoenicopterus ruber ruber*) from the BVIs were found dead (location: GPS N23 35.00819 W164 42.17903). The affected animals were part of a flock of ~150 free-roaming birds that included ~35 chicks. The death of 11 individuals (31% of chicks) in this short time period, which were all discovered in or around the nesting site, was, therefore, considered unusual, and a diagnostic investigation was initiated. Unfortunately, due to advanced autolysis in the majority of the chicks, only one individual

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was suitable for gross and histologic review. No gross lesions were apparent on necropsy, and the chick was in good nutritional status with food in the gastrointestinal tract. Sections of heart, lung, liver, kidney, spleen, bursa, small intestine, and skeletal muscle were fixed in 10% neutral buffered formalin, processed routinely and stained with hematoxylin and eosin (HE). Histologic findings included a marked lymphocytic and necrotizing myocarditis (Fig. 1) and mild nonsuppurative encephalitis. Mild splenitis, periportal to random hepatitis and interstitial nephritis were also seen. Collectively, the lesions in the one chick evaluated, especially those in the heart and brain, were suggestive of disseminated WNV infection.

Formalin fixed paraffin-embedded (FFPE) heart was subsequently sent for molecular analysis at the Center for Infection and Immunity (Columbia University) where total nucleic acids were extracted using the Recover All™ extraction kit (Ambion®) and cDNA prepared using Superscript III™ (Invitrogen); all according to the manufacturer's instructions. Two consensus PCR assays were then performed for the broad detection of all known flaviviruses (Moureau et al. 2007; Sanchez-Seco et al. 2005), and both confirmed the presence of WNV in the affected tissue. A diffuse cytoplasmic infection was subsequently confirmed with immunohistochemistry (IHC (Anthony et al. 2012)) using mono-specific antisera that target the viral matrix (M), envelope (E), or non-structural 3 (NS3) proteins (Fig. 2); and then secondarily confirmed with in situ hybridization (Anthony et al. 2012) using RNA probes targeting the viral NS3 gene (data not shown). All together these data strongly suggest that WNV is the underlying cause of death for this individual. It is not known whether WNV was also responsible for the deaths of the remaining 10 flamingo chicks as no tissues were available for analysis; however, the unusually high level of mortality in temporal and geographical proximity to the WNV-confirmed individual suggest that it might indeed be the cause of most, if not all, of the chick mortalities.

Due to a paucity of WNV sequence data from the Caribbean, and from Central and South America, Ion Torrent unbiased high-throughput sequencing (HTS) was performed on the FFPE heart to complete the full genome of this virus, as described previously (Anthony et al. 2013). A total of 367,000 reads (mean length 150 bp) were recovered after ambiguous nucleotides, primers, or adapter sequences were removed, and 18,289 of these reads successfully mapped to WNV (JF415928) with a coverage of ~200×. The genome sequence was additionally confirmed using PCR amplification and classical dideoxy sequencing of 500 bp overlapping fragments (Genbank accession number: KF367469). Blast analysis of the full genome (nucleotide) and polyprotein (amino acid) revealed 99% sequence homology with various avian and mosquito strains of WNV circulating in the US over the past decade, confirming the current outbreak to be the result of a geographic expansion in the distribution of the US strain.

WNV has not been previously reported in either the BVI or the US Virgin Islands (UVI), and its emergence at this time is significant not only because of the associated

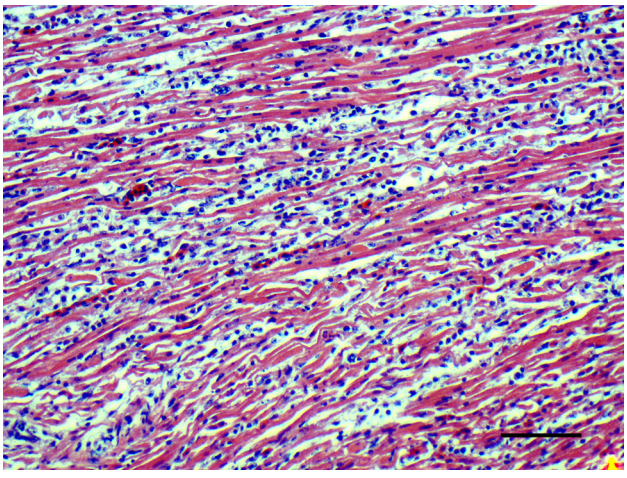

Figure 1. Heart, flamingo, and West Nile virus-associated myocarditis. Note severe lymphocytic inflammation separating myofibers in the myocardium. HE, *bar* = 85 μm.

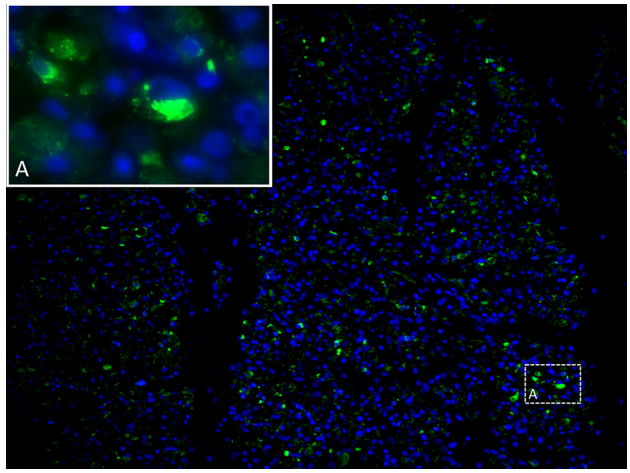

Figure 2. Immunohistochemistry (IHC) of flamingo heart showing staining for WNV (*green*) and DAPI (*blue*) at ×20 magnification. IHC performed using antibodies against WNV matrix (M), envelope (E) and non-structural 3 (NS3) proteins. Only anti-M serum (dilution 1:1,500) is shown here, but staining was consistent for all. Insert image (at ×100 magnification) demonstrates cytoplasmic localization of the virus.

mortality event in this outbreak, but also because it demonstrates the continued expansion of the virus throughout the Caribbean Islands (Barrera et al. 2008; Bosch et al. 2007; Dupuis et al. 2003, 2005; Komar and Clark 2006; ProMED-mail 2001, 2005). It is unknown by what mechanism the virus was introduced into the Virgin Islands; however, it is unlikely to have been introduced by an infected flamingo as they do not perform significant and long migrations to other areas. They do however move frequently within the Virgin Islands, and in the event of viral persistence could well facilitate the continued spread of WNV throughout the region (likewise for any of the other bird species that regularly move between islands). This study demonstrates WNV-associated avian mortality in the Caribbean, albeit in a species that is large, noticeable and under some management. Flamingos are known to be susceptible to WNV and are often among the first species to present with morbidity or mortality (Lanciotti et al. 1999; Steele et al. 2000; Osorio et al. 2012; Baitchman et al. 2007), suggesting they might act as an effective indicator species for the introduction and spread of the virus. It is currently unknown if more widespread population declines are underway in other species in the BVI (or surrounding islands) that are under less intense observation and highlights the need for rapid necropsy of wild bird carcasses within potentially affected regions.

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References

Anthony SJ, et al. (2012) Emergence of fatal avian influenza in New England harbor seals. *MBio* 3(4):e00166–e00212

Anthony SJ, et al. (2013) Identification of a novel cetacean polyomavirus from a common dolphin (*Delphinus delphis*) with tracheobronchitis. *Plos One* 8(7):e68239. doi:10.1371/journal.pone.0068239

Baitchman EJ, Tlusty MF, Murphy HW (2007) Passive transfer of maternal antibodies to west nile virus in flamingo chicks (*Phoenicopterus chilensis* and *Phoenicopterus ruber ruber*). *Journal of Zoo and Wildlife Medicine* 38(2):337–340

Barrera R, et al. (2008) First isolation of West Nile virus in the Caribbean. *The American Journal of Tropical Medicine and Hygiene* 78(4):666–668

Bernard KA, et al. (2001) West Nile virus infection in birds and mosquitoes, New York State, 2000. *Emerging Infectious Diseases* 7(4):679–685

Bosch I, et al. (2007) West Nile virus, Venezuela. *Emerging Infectious Diseases* 13(4):651–653

Briese T, Glass WG, Lipkin WI (2000) Detection of West Nile virus sequences in cerebrospinal fluid. *Lancet* 355(9215):1614–1615

Cruz L, et al. (2005) Short report: serological evidence of West Nile virus activity in El Salvador. *The American Journal of Tropical Medicine and Hygiene* 72:612–615

Diaz LA, et al. (2008) West Nile virus in birds, Argentina. *Emerging Infectious Diseases* 14:689–691

Dupuis AP II, Marra PP, Kramer LD (2003) Serologic evidence of West Nile virus transmission, Jamaica, West Indies. *Emerging Infectious Diseases* 9(7):860–863

Dupuis AP 2nd, et al. (2005) Serologic evidence for West Nile virus transmission in Puerto Rico and Cuba. *The American Journal of Tropical Medicine and Hygiene* 73(2):474–476

Fernández-Salas I, et al. (2003) Serologic evidence of West Nile Virus infection in birds, Tamaulipas State, Mexico. *Vector-Borne and Zoonotic Diseases* 3:209–213

Kilpatrick AM (2011) Globalization, Land Use, and the Invasion of West Nile Virus. *Science* 334(6054):323–327

Kilpatrick AM, et al. (2013) Predicted and observed mortality from vector-borne disease in wildlife: West Nile virus and small songbirds. *Biological Conservation* 165:79–85

Komar N, Clark GG (2006) West Nile virus activity in Latin America and the Caribbean. *Pan American Journal of Public Health* 19:112–117

LaDeau SL, Kilpatrick AM, Marra PP (2007) West Nile virus emergence and large-scale declines of North American bird populations. *Nature* 447(7145):710–713

Lanciotti RS, et al. (1999) Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286(5448):2333–2337

Moureau G, et al. (2007) A real-time RT-PCR method for the universal detection and identification of flaviviruses. *Vector-Borne and Zoonotic Diseases* 7:467–478

Nemeth NM, et al. (2007) Avian mortality surveillance for West Nile virus in Colorado. *American Journal of Tropical Medicine and Hygiene* 76(3):431–437

Osorio JE, et al. (2012) Characterization of West Nile viruses isolated from captive American Flamingoes (*Phoenicopterus ruber*) in Medellin, Colombia. *American Journal of Tropical Medicine and Hygiene* 87(3):565–572

ProMED-mail (2001) *West Nile Virus, Humans; Cayman Islands*

ProMED-mail (2005) *West Nile Virus, Humans, Equines; Cuba.* 2005. Archive No. 20050202.0355. http://www.promedmail.org

Sanchez-Seco MP, et al. (2005) Generic RT-nested-PCR for detection of flaviviruses using degenerated primers and internal control followed by sequencing for specific identification. *Journal of Virological Methods* 126(1–2):101–109

Steele KE, et al. (2000) Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Veterinary Pathology* 37(3):208–224