

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 (Moureaux 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

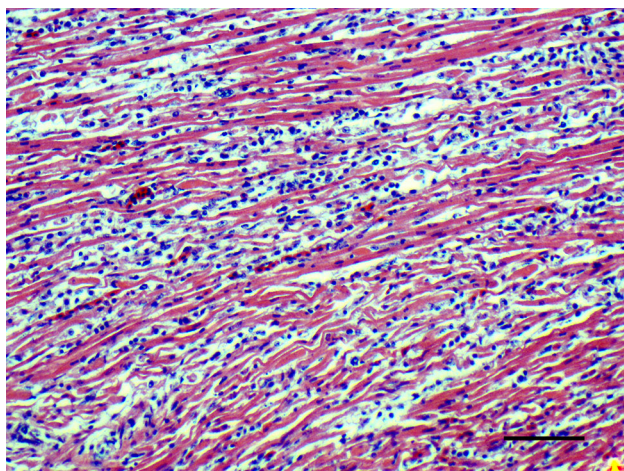


Figure 1. Heart, flamingo, and West Nile virus-associated myocarditis. Note severe lymphocytic inflammation separating myofibers in the myocardium. HE, bar = 85 μ m.

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 $\sim 200\times$. 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

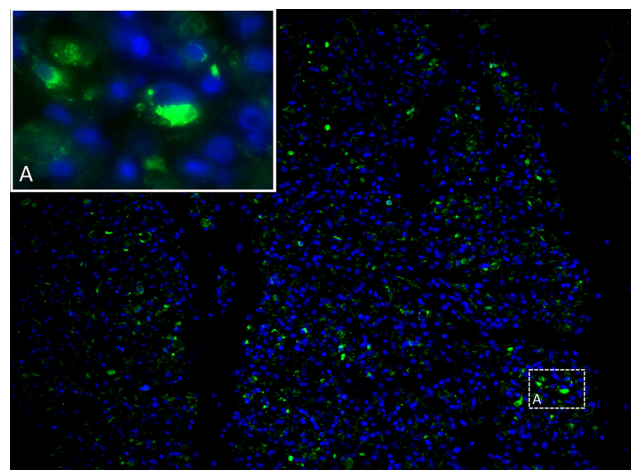


Figure 2. Immunohistochemistry (IHC) of flamingo heart showing staining for WNV (green) and DAPI (blue) at $\times 20$ magnification. IHC performed using antibodies against WNV matrix (M), envelope (E) and non-structural 3 (NS3) proteins. Only anti-M serum (dilution 1:1,500) is shown here, but staining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