# LETTERS

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## Lack of Detection of Avian Influenza, Newcastle Disease, and West Nile Viruses in Wild Birds of Northeastern Brazil

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ABSTRACT: We tested 529 wild birds captured in northeastern Brazil for infection by avian influenza, Newcastle disease, and West Nile. Viruses were not detected by real-time PCR with the exception of one Tropical Gnatcatcher (*Polioptila plumbea*) positive for influenza virus, but this could not be confirmed by viral isolation or gene sequencing.

Avian influenza viruses (AIVs), Newcastle disease virus (NDV), and West Nile virus (WNV) are significant to animal and public health and may be relevant to the conservation of wild birds worldwide. Despite their importance, few studies of these viruses in wild birds have been conducted in Brazil.

The AIV (*Orthomyxoviridae*) are a global threat to food animal production and distribution systems, as well as to human health, and have been detected in a broad variety of mammals and birds (Salomon and Webster 2009). Aquatic birds are traditionally perceived as the main reservoirs of these viruses; however, recent studies have shown that AIV maintenance is dependent upon complex multiavian systems (Caron et al. 2017). There have been relatively few studies about AIV in Brazil, and to date, only low-pathogenicity AIV strains have been isolated, including an H2N1 strain from a Semipalmated Sandpiper (*Calidris pusilla*), an H3 strain from a Ruddy Turnstone (*Arenaria interpres*), a Kelp Gull (*Larus dominicanus*), and a Semipalmated Sandpiper, and an H11N9 strain from Ruddy Turnstones (Hurtado and Vanstreels 2016).

The NDV (*Paramyxoviridae*), a variant of avian paramyxovirus 1, is classified as either lentogenic, mesogenic, or velogenic. It is one of the most important viruses of avian species globally, with outbreaks potentially leading to substantial economic losses to the poultry industry. Newcastle disease virus was first introduced to Brazil in 1953, and a series of outbreaks was recorded in Brazil during the 1970s and 1980s. It was only in 2003, after stricter control measures and extensive vaccination campaigns of poultry with attenuated strains were implemented, that the country was recognized as free of pathogenic NDV strains (Orsi et al. 2010). However, serologic studies demonstrate more recent circulation of lentogenic NDV strains in wild and domestic birds (Silva et al. 2006). Lentogenic NDV was detected by real-time PCR in a Sanderling (*Calidris alba*) and a Semipalmated Sandpiper in northeastern Brazil in 2007 (Thomazelli et al. 2012).

The WNV (*Flaviviridae*) is a mosquito-borne virus maintained in nature in an enzootic transmission cycle between birds and ornithophilic mosquitoes that infect a range of vertebrate hosts and may have a high impact on human and animal health (McLean and Ubico 2007). Serologic surveys in Brazil have identified equids and chickens seropositive for WNV, but not wild birds, and no studies have obtained positive results in equine and avian hosts through direct diagnostic methods (Ometto et al. 2013). However, the first human case of WNV encephalitis

in the country was recorded in 2014 in Piauí State, highlighting the importance of surveillance of the virus in northeastern Brazil (Vieira et al. 2015).

In this study, we investigated the occurrence of AIV, NDV, and WNV in wild birds in two morphoclimatic domains in northeastern Brazil: Caatinga and Atlantic Forest. The Caatinga is in a semiarid region with a hot and dry climate, composed of a mosaic of thorn scrub and seasonally dry forest; it harbors about 510 avian species (Silva et al. 2003). The Atlantic Forest is an extensive block of evergreen forests that extend mostly along the coast of Brazil and parts of Paraguay and Argentina, harboring about 620 avian species, of which 29% are endemic (Myers et al. 2000). Despite their remarkable biodiversity, Caatinga and the northeastern parts of Atlantic Forest have been largely neglected by the scientific community and are underprotected (Silva et al. 2003; Tabarelli et al. 2010), and there is virtually no information on the circulation of AIV, NDV, and WNV in the avian communities of these habitats.

From July 2012 to July 2013, oropharyngeal and cloacal swabs were collected from 529 wild birds (adults and juveniles) from 89 species belonging to 26 families in two protected areas (Figure 1, Table S1): Guaribas Biological Reserve, an area of coastal Atlantic Forest in Paraíba State (6°43′10″S, 35°11′6″W), and Raso da Catarina Ecological Station, an area of Caatinga in Bahia State (9°45′47″S, 38°31′26″W). Sampling and sample storage were conducted in accordance with the protocol of Hurtado et al. (2016). All RNAs were extracted using 5× MagMAX™ 96 viral isolation kit (AM1836, Applied Biosystems™, ThermoFisher Scientific, Foster City, California, USA) following the manufacturer's instructions. Methodology for viral nucleic acid detection, virus isolation, and sequencing is described by Araujo et al. (2014) for AIV, Thomazelli et al. (2012) for NDV, and Ometto et al. (2013) for WNV.

All samples were negative for NDV and WNV, and all but one sample were negative for AIV. The only AIV-positive result was obtained from an adult female of Tropical Gnatcatcher (*Polioptila plumbea*) captured at Raso da Catarina Ecological Station in April 2013 that appeared healthy. The cycle threshold (CT) value for this sample was 38, which indicates a relatively low concentration of viral RNA. The positive sample was inoculated into 9-d-old specific-pathogen-free embryonated chicken eggs; Sanger sequencing of a conserved region of 192 base pairs of genomic nonstructural segment was attempted for the PCR-positive sample (Araujo et al. 2014). We could not retrieve AIV by these methods and it was concluded that the sample was negative. This was not surprising, as it is well established that these techniques have limited success when applied to samples with high CT values due to the low quantity of viable virions or the partial degradation of viral RNA (Stallknecht et al. 2012; Hurtado et al. 2016).

We were therefore unable to confirm active shedding of AIV, NDV, or WNV in the birds sampled. This does not exclude the circulation of these viruses in the region, as the prevalence of these viruses may vary temporally. Hurtado et al. (2016) reported that, with few exceptions, real-time PCR-positive results were obtained only for species with >100 sampled individuals, possibly an indication that the species sampled in this study may also have been infected but the sample size was too small to allow for detection.

In conclusion, Brazil harbors highly diverse avian communities that remain poorly studied for these viruses. Further surveillance efforts to detect these and other avian-borne viruses are therefore necessary, particularly in areas of high avian diversity and endemism.

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## SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2017-09-218.

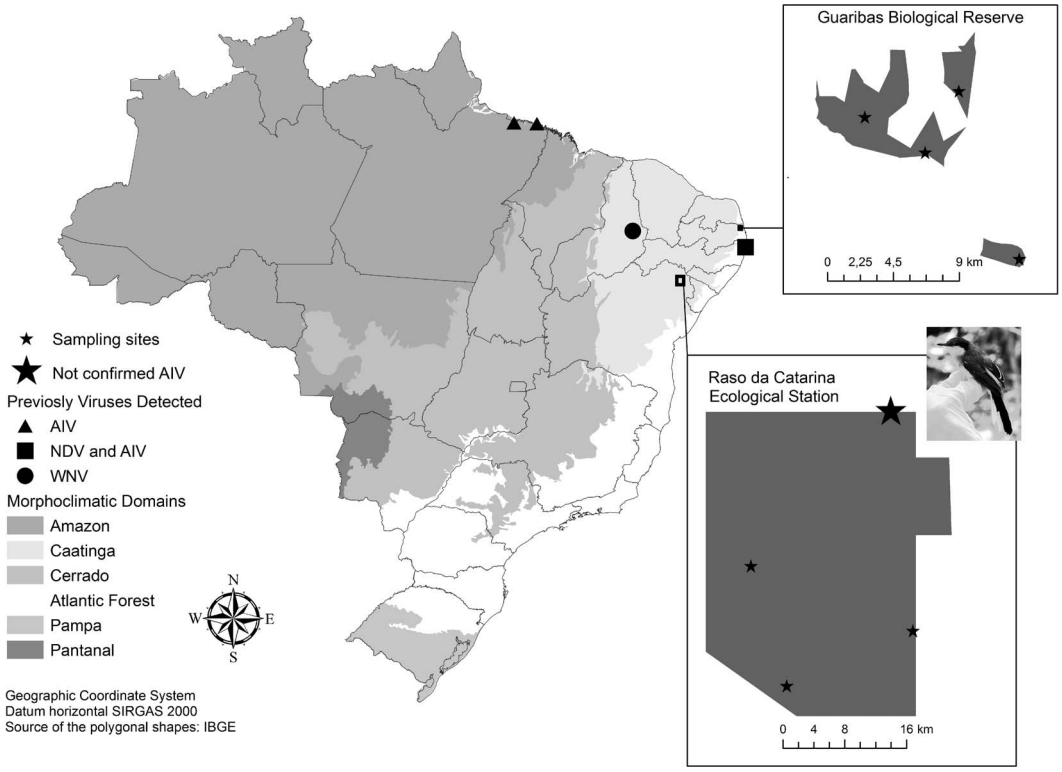


FIGURE 1. Map of the study areas: Guaribas Biological Reserve in the northeastern Brazilian Atlantic Forest and Raso da Catarina Ecological Reserve in Caatinga. Sampling sites are indicated with small stars. The positive avian influenza virus (AIV) sample from a Tropical Gnatcatcher (*Polioptila plumbea*) was detected at Raso da Catarina Ecological Station (large star). Previously reported virus detections (Obenauer et al. 2006, Senne et al. 2010, Thomazelli et al. 2012, Araújo et al. 2014, Vieira et al. 2015) are indicated and include: AIV in wild birds (triangles), AIV and lentogenic Newcastle disease virus (NDV) in wild birds (square), and the first human case of West Nile virus (WNV) encephalitis in Brazil (circle).

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