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Serological evidence of West Nile virus infection in wild birds in the area of the first confirmed human case of West Nile fever in Brazil

Evidências sorológicas de infecção por vírus do Nilo Ocidental em aves selvagens na área do primeiro caso humano confirmado de febre do Nilo Ocidental no Brasil

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ABSTRACT

OBJECTIVE: To investigate the potential role of resident and migratory wild birds in West Nile virus (WNV) epidemiology in the region of Brazil where the first human case of West Nile fever was recorded. MATERIALS AND METHODS: The study was performed across two time spans (2014 and 2015). A total of 688 birds, representing 38 species, were captured using mist nets. Serum samples of 103 birds of 27 captured species were analyzed using hemagglutination inhibition (HI) tests. RESULTS: Twenty-three specimens exhibited monotypic or heterotypic reactions to 12 of the 20 virus species screened. HI tests revealed monotypic reactions for WNV in five samples from *Paroaria dominicana*, *Mimus saturninus* (n = 3), and *Myiarchus tyrannulus*, and heterotypic cross-reaction in eight samples from *Columbina minuta*, *Columbina squammata*, *Columbina talpacoti*, *Leptotila verreauxi*, *Piaya cayana*, *Pitangus sulphuratus*, *Turdus rufiventris*, and *Paroaria dominicana*. Six samples with heterotypic reactions were further analyzed using plaque reduction neutralization tests (PRNT), resulting in three positives for WNV in non-migrant birds: *Columbina talpacoti*, *Piaya cayana*, and *Turdus rufiventris*. CONCLUSION: This study provides evidence of WNV in free-living Passeriformes in Brazil. The findings suggest that WNV dissemination may begin with migratory birds that share resting and foraging areas with resident or shorter-range migratory birds, thereby sustaining vector mosquito populations and maintaining a zoonotic risk to humans.

Keywords: Orthoflavivirus; Arbovirus; Avifauna; Caatinga.

RESUMO

OBJETIVO: Investigar o papel de aves selvagens residentes e migratórias na epidemiologia do vírus do Nilo Ocidental (*West Nile virus* – WNV) na região do Brasil onde foi registrado o primeiro caso humano de febre do Nilo Ocidental. MATERIAIS E MÉTODOS: O estudo foi realizado em dois períodos (2014 e 2015). Foram capturadas 688 aves, representando 38 espécies, usando redes de neblina. Amostras de soro de 103 aves de 27 espécies foram analisadas por testes de inibição da hemaglutinação (HI). RESULTADOS: Ao todo, 23 espécimes apresentaram reações monotípicas ou heterotípicas para 12 das 20 espécies virais testadas. Os testes HI mostraram reações monotípicas para WNV em cinco amostras de *Paroaria dominicana*, *Mimus saturninus* (n = 3) e *Myiarchus tyrannulus*, e reações cruzadas heterotípicas em oito amostras de *Columbina minuta*, *Columbina squammata*, *Columbina talpacoti*, *Leptotila*

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verreauxi, *Piaya cayana*, *Pitangus sulphuratus*, *Turdus rufiventris* e *Paroaria dominicana*. Seis amostras com reações heterotípicas foram analisadas adicionalmente com testes de neutralização por redução de placas (PRNT), resultando em três positivas para WNV em aves não migratórias: *Columbina talpacoti*, *Piaya cayana* e *Turdus rufiventris*. CONCLUSÃO: Este estudo fornece evidências de WNV em Passeriformes selvagens no Brasil. Os resultados sugerem que a disseminação de WNV pode começar com aves migratórias que compartilham áreas de descanso e alimentação com aves residentes ou migratórias de menor distância, sustentando populações de mosquitos vetores e mantendo um risco zoonótico para os humanos.

Palavras-chave: Orthoflavivírus; Arbovírus; Avifauna; Caatinga.

INTRODUCTION

The West Nile virus (*Orthoflavivirus nilense* or WNV) is an orthoflavivirus that causes an emerging zoonotic disease in several countries. WNV was first identified in 1937 from blood samples of a patient in the northwest Uganda in Central Africa[1].

The impact of WNV disease in public health has been neglected in some regions, as most infections are asymptomatic or oligosymptomatic, characterized by non-specific acute fever of low severity[2]. However, a more virulent strain of WNV was identified in the early 1990s causing neurological disorder outbreaks not only in humans, but also in equids and birds. Since then, WNV infections have been documented in different vertebrate species, such as avians, reptiles, amphibians, and other mammals. Some wild bird species act as amplifiers hosts of WNV[3].

In the early 1990s, a WNV epidemic was recorded in Europe, with high rates of neurological infections in the lower altitude areas of the Danube Valley, Bucharest, and across Romania[4]. Such events, involving humans with neurological disorders, brought attention to the potential impact of WNV on human and animal health[5,6]. In 1999, WNV was detected for the first time in the western hemisphere[7].

Since 2001, anti-WNV antibodies have been detected in Canada, Mexico, Central America, and the Caribbean[8,9,10]. Because of the high cross-reactivity among over dozens of orthoflaviviruses that circulate in South America, the detection of neutralizing antibodies by seroneutralization (SN) or plaque reduction neutralization test (PRNT) have been used as the most reliable methods to confirm WNV exposure[11].

In South America, WNV was first evidenced when specific neutralizing antibodies were detected by PRNT in equines in Colombia[12], followed by Venezuela in 2004[13]. In February 2006, WNV was finally confirmed in South America, when WNV was isolated from the brains of three horses that died from encephalitis in central Argentina[14].

In Brazil, the first evidence of WNV activity was reported in 2011, when equines sampled from 2005 to 2009 in the Pantanal, West-Central Region of the country, presented specific neutralizing antibodies for WNV confirmed by PRNT[15]. Following serosurveys indicated that WNV was perhaps more spread in Brazil than originally thought. In one study, neutralizing antibodies were detected in equids and a chicken sampled in July 2010 in Mato Grosso State[16]. Additional ELISA-based serological evidence of WNV exposure was reported in Paraíba State, suggesting that WNV could also have circulated in the Northeast Region of the country[17]. In a large-scale survey for WNV in Brazil, serological evidences of WNV infection confirmed by PRNT were found in equids sampled in 2009, once again in Mato Grosso State, West-Central Region of Brazil. The same study found ELISA-based evidence in equids from the states of Rondônia and São Paulo, and in migratory birds captured in the states of Pará, Maranhão, and Rio Grande do Sul. These data suggested potential WNV activity also in northern and southern Brazil[18].

In 2014, a ranch worker from the rural area of Piauí State, Northeast Brazil, was admitted to the hospital with clinical signs of acute encephalitis and flaccid paralysis. Serological evidence supported by clinical and epidemiological findings, confirmed the first human case of neurological disorder caused by WNV in Brazil[19,20]. In 2018, WNV was isolated from brain tissue of a dead equine during an encephalitis epizootic in Espírito Santo State. This was the first evidence that WNV was causing disease in equids and it was also circulating in Southeast Brazil, the most populated region of the country[21].

In contrast to North America, where the role of wild birds in WNV epizootiology is well documented due to increased mortality, there is a dearth of information on WNV infection in free-living wild birds in South America. Very few studies have assessed the role of wild birds in the maintenance of WNV in Brazil. In June 2019, a multi-institutional task force coordinated by the Brazilian Ministry of Health was deployed to the area where one horse tested positive for WNV in Ceará State, Northeast Brazil. Previous exposure to WNV was confirmed by seroconversion in domestic birds and by the detection of specific neutralizing antibodies in 4.7% (13/278) of free-ranging wild birds[22]. Whether wild birds have also been exposed to WNV also in the neighboring state of Piauí, when the first human case of WNV encephalitis occurred in 2014 remains unclear.

In this scenario, this study aimed to: 1) investigate the seroprevalence of anti-WNV antibodies in free-living wild birds, both resident and migratory, in a Caatinga area in Piauí; and 2) test for other arboviruses in samples from the same area where the first human case of WNV-related encephalitis in Brazil was reported.

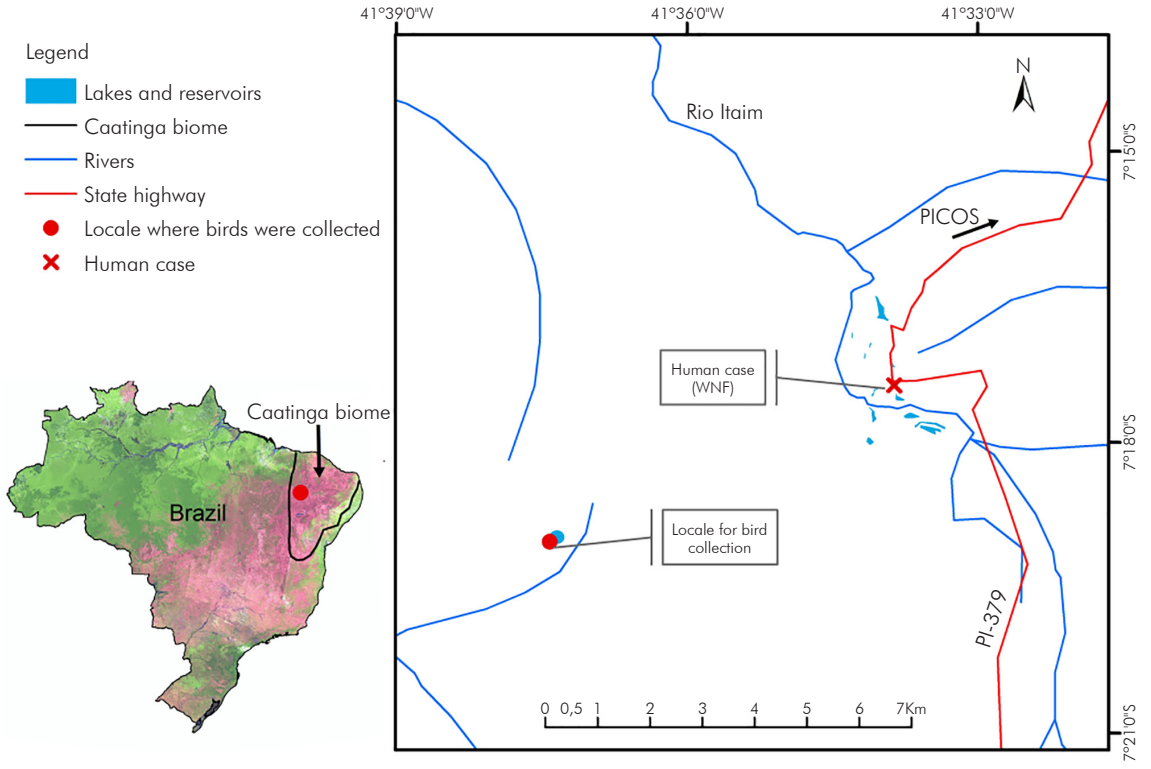
MATERIALS AND METHODS

This study was performed in Aroeiras do Itaim, Piauí State, Northeast Brazil (07°17'21"W, 41°33'50"S), within the semiarid Caatinga biome, and where the first human WNV infection in the country was detected[19]. Data collection occurred in two phases: the first from December 4th to 11th, 2014, around six months after the onset of symptoms in the index case; and the second from June 13th to 21st, 2015. Bird capture sites were selected by the presence of preserved forest fragments visited and/or inhabited by several groups of birds, around water bodies, and open field areas. Forty birds mist nets, measuring 12 x 12 m each, were used for capture. The sites were located about 7 km away from the residence of the index case (Figure 1).

Bird sampling was performed under authorization of the Brazilian National Center for Research and Conservation of Wild Birds (CEMAVE) of the Chico Mendes Institute for Biodiversity Conservation (ICMBio) and the National System for Wild Bird Banding (SNA) (authorization no. 3891/1, registered under no. 324582).

Each sampling campaign lasted three days, with nets open from 05:30 to 17:00. On two of the three days, supplementary sampling occurred late at night, between 03:00 and 05:00, to target nocturnal. Nets were checked every 30 minutes to remove captured birds. Sample collections were carried out for convenience, excluding birds of the same species when in abundance, to maximize species diversity. The birds were kept in cloth bags and transported to a field laboratory for taxonomic identification, banding, routine weight and measurement data collection. Blood sampling occurred through via venous puncture (right jugular of ulnar vein), using intradermal needles with 1 mL syringes, not exceeding 1% of the bird's body weight. After a post-handling rest period, birds were released at the capture site. Blood samples with sufficient volume were centrifuged to obtain serum; samples with insufficient volume were kept *in natura*. All samples were stored in liquid nitrogen for viral isolation and serological diagnosis. Analyses were conducted at the Instituto Evandro Chagas (IEC), Brazilian National Reference Laboratory for Arboviruses of the Health and Environmental Surveillance Secretariat of the Ministry of Health.

Arbovirus isolation procedures were conducted using cell cultures (C6/36 – *Aedes albopictus* clone cells; and VERO – sabaeus monkey kidney cells), which were inoculated with the biological samples and monitored daily for cytopathogenic effects. Confirmation test was conducted by indirect immunofluorescence (IFI)[22]. Polyclonal antibodies for the main antigenic groups of arboviruses known to occur in Brazil were used. These included representatives from the six main families and genera of arboviruses: (*Togaviridae*, *Alphavirus*) group A; (*Flaviviridae*, *Orthoflavivirus*) group B; (*Peribunyaviridae*, *Orthobunyavirus*) group C, Simbu, Guama, Capim, and Bunyamwera; (*Phenuiviridae*, *Phlebovirus*); (*Sedoreoviridae*, *Orbivirus*); (*Rhabdoviridae*, *Vesiculovirus*).



Source: The cartographic base data used for the map were obtained from the open and publicly accessible databases of IBGE (Brazilian Institute of Geography and Statistics). The bird and human case collection points were georeferenced by the research team. The map was created by the authors and developed using Qgis 2.18 software.

Figure 1 – Geographical characterization of Aroeiras do Itaim City, Piauí State, bird sampling site, and residence area of the first WNV case in Brazil

Serological analyses were performed using hemagglutination inhibition (HI) tests[23], modified for microplates[24]. The following arboviruses were tested: WNV, *Orthoflavivirus louisense* (SLEV), Rocio virus (ROCV), *Orthoflavivirus cacipacoreense* (CPCV), Bussuquara virus (BSQV), *Orthoflavivirus flavi* (YFV), *Orthoflavivirus ilheusense* (ILHV), *Alphavirus madariaga* (MADV), *Alphavirus western* (WEEV), *Alphavirus mucambo* (MUCV), *Alphavirus venezuelan* (VEEV, subtype IIIA), *Alphavirus mayaro* (MAYV), *Orthobunyavirus oropoucheense* (OROV), *Orthobunyavirus maguariense* (MAGV), *Orthobunyavirus tacaiumaense* (TCMV), *Orthobunyavirus iacoense* (ICOV), *Orthobunyavirus utingaense* (UTIV), *Orthobunyavirus belemense* (BLMV), *Orthobunyavirus caraparuense* (CARV), and *Orthobunyavirus catuense* (CATUV). Samples were considered monotypic when HI titers were ≥20 for a single virus in each group and heterotypic when HI titers ≥20 were observed for more than one virus within a given genus.

To corroborate antibody detection by HI, additional serological analyses were conducted using plaque reduction neutralization tests (PRNT)[25,26]. Plates with 24 wells containing VERO cells at a concentration of 1.6 x 10^5 cells were used. Bird serum samples were tested in serial dilution (1:20 to 1:640) in duplicates and compared to an average of 100 plaque-forming units (PFU) of SLEV (ChimeraVax – SLEV), WNV (ChimeraVax – VNO), and ILHV. Neutralizing antibody titers were defined as the reciprocal value of highest serum dilution capable of reducing the number of PFU by 90%. All samples presenting neutralizing antibodies were considered seropositive if the titer was at least four times higher than the highest value obtained for the other viruses, as previously reported[11]; otherwise, they were classified as cross-reactive (CR). The chimera viruses were kindly provided by the Centers for Disease Control and Prevention (CDC, Atlanta, USA).

For data analysis, the following factors were considered: bird species, migratory or resident status, type of antibody detected by HI, type of reaction (monotypic or heterotypic), and PRNT results.

RESULTS

Across two sampling campaigns, 688 birds were captured, distributed in 38 species, representing 11% (38/347) of the bird species richness recorded for the Caatinga biome[27]. From 688 captured birds, 238 samples were collected for virus isolation, and 103 samples were obtained for serological testing. The difference between the number of captured birds and the samples sent to the laboratory was due to the overrepresentation of some species, with most individuals being promptly released.

None of the 238 samples tested for WNV isolation were positive. Of the 103 serum samples from 27 bird species, 80 tested negative and 23 tested positive in the HI tests, showing mono or heterotypic reactions to 12 viruses from a 20-virus panel. Monotypic reactions for WNV were found in six samples from the following species: *Mimus saturninus* (n = 3), *Paroaria dominicana*, *Myiarchus tyrannulus*, *Tyrannus melancholicus*, and *Chrysomus ruficapillus*. Heterotypic cross-reactions were observed in eight samples from *Columbina minuta*, *Columbina squammata*, *Columbina talpacoti*, *Leptotila verreauxi* (n = 2), *Piaya cayana*, *Pitangus sulphuratus*, *Chrysomus ruficapillus*, and *Turdus rufiventris*. Monotypic reactions for other viruses were observed in *Columbina picui* (ILHV 1:20; n = 2), *Columbina minuta* (ILHV 1:20), and *Pitangus sulphuratus* (SLEV 1:20) (Table 1, Table S1).

PRNT tests on three samples from the first campaign (2014) with heterotypic reaction for WNV in the HI tests were positive for WNV. These samples were from resident (non-migratory) birds: *Columbina talpacoti*, *Piaya cayana*, and *Turdus rufiventris*. In contrast, three samples from *Myiarchus tyrannulus*, *Mimus saturninus*, and *Cyclarhis gujanensis* showed negative PRNT results (Table 1).

DISCUSSION

Despite some limitations, which includes the non-confirmation by PRNT of HI-monotypic reactions for WNV, the results presented here may indicate that free-ranging birds have been exposed to WNV in Brazil for more than a decade. The 14 bird species that exhibited mono or heterotypic reactions for WNV in the HI tests, including the three PRNT positive cases, inhabit rural environments impacted by agricultural and cattle herding activities. This finding requires further investigation for confirmation, and especially regarding the possible alterations in the ecology of ornithophilic mosquitoes (possible vectors for the tested arboviruses) due to anthropogenic activities, forest fragmentation, and the creation of open fields for agriculture and pasture[28]. These activities disrupt ecological processes and may increase the risk of contact with zoonotic pathogens[29].

Another relevant aspect is the resident (non-migratory) status of the species that showed monotypic reactions for WNV by HI tests or tested seropositive results by PRNT for WNV. These species have a broad distribution in Brazil and neighboring countries, except for *Paroaria dominicana*, an endemic species of the Caatinga biome that is well-adapted to urban environments[30]. The detection of hemagglutination-inhibition antibodies for WNV in free-living birds reported here highlights the corroborates the potential role of native avifauna as amplifying hosts of WNV and other arboviruses circulating near human settlements and domestic or synanthropic animals in both rural and urban environments.

In the present study, monotypic reactions for WNV by HI were found in three specimens of *Mimus saturninus* and one specimen of each *Paroaria dominicana*, *Myiarchus tyrannulus*, *Tyrannus melancholicus*, and *Chrysomus ruficapillus*. Interestingly, in the study conducted in 2019 in the neighboring state of Ceará, the two specimens of *Paroaria dominicana*, three specimens of *Myiarchus tyrannulus*, and one specimen of *Chrysomus ruficapillus* captured and tested by PRNT for WNV were seronegative[22]. Therefore, the results presented here bring new information regarding the potential exposure to WNV of these three species in Northeast Brazil. One sample of *Columbina talpacoti* presented heterotypic reaction by HI for orthoflaviviruses. In the study conducted in Ceará State, two out of 13 individuals

of *Columbina talpacoti* sampled in September 2019 were seropositive for WNV by $PRNT_{90}$. This species presented the second highest seroprevalence among the six species that were seropositive for WNV by $PRNT_{90}$[22]. *Columbina talpacoti* can be of particular importance for arbovirus maintenance cycles of transmission due to its widespread presence and high adaptability to large urban centers[31]. *Columbina picui strepitans* (Spix, 1825), commonly known as the Picui Ground-Dove, is widely distributed throughout Brazil. In the present study, serum samples from 22 individuals of *Columbina picui strepitans* tested negative by HI for WNV. Interestingly, these results are corroborated by the WNV-serosurvey conducted in Ceará, in 2019. In that investigation, none of the 35 individuals of *Columbina picui* tested presented neutralizing antibodies for WNV[22].

Table 1 – Hemagglutination-inhibition (HI) and neutralizing (PRNT) antibodies for WNV in 103 wild birds from 27 species captured in Piauí State, Northeast Brazil, between 2014 and 2015

Species	Samples	PRNT	HI*	
			Number of seropositives (titer)	Type of reaction
Jacana jacana†	1	–	–	
Columbina minuta†	8	–	1 (1:40)	Heterotypic
Columbina picui†	22	–	–	
Columbina squammata†	10	–	1 (1:20)	Heterotypic
Columbina talpacoti†	1	(1 +) WNV	1 (1:80)	Heterotypic
Leptotila verreauxi‡	2	–	2 (1:20)	Heterotypic
Zenaida auriculata virgata†	12	–	(12 -)	
Forpus xanthopterygius‡	1	–	(1 -)	
Glaucidium brasilianum‡	1	–	(1 -)	
Piaya cayana‡	1	(1 +) WNV	1 (1:20)	Heterotypic
Nystalus maculatus†	3	–	(3 -)	
Picumnus pygmaeus‡	1	–	(1 -)	
Lepidocolaptes angustirostris‡	3	–	(3 -)	
Myiarchus tyrannulus‡	5	1 Neg.	1 (1:40)	Monotypic
Myiarchus ferox‡	1	–	(1 -)	
Tyrannus melancholicus†	1	–	1 (1:20)	Monotypic
Pitangus sulphuratus‡	4	–	1 (1:80)	Heterotypic
Pachyramphus polychopterus§	1	–	(1 -)	
Myiodynastes maculatus‡	1	–	(1 -)	
Mimus saturninus‡	3	1 Neg.	3 (1:40)	Monotypic
Turdus amaurochalinus‡	2	–	(2 -)	
Turdus rufiventris‡	3	(1 +) WNV	1 (1:80)	Heterotypic
Cyclarhis gujanensis‡	1	1 Neg.	–	
Paroaria dominicana†	3	–	1 (1:20)	Monotypic
Lanio pileatus§	1	–	–	
Chrysomus ruficapillus†	10	–	1 (1:80)	Monotypic
Cyanocorax cyanopogon‡	1	–	–	
Total	103	3 + WNV / 6 ‖	14	6 Monotypic 8 Heterotypic

* HI tests were conducted for 20 arbovirus species from the following genera: *Alphavirus*: EEEV, WEEV, VEEV (subtype IIIA), MAYV, and MUCV; *Phlebovirus*: ICOV; *Orthobunyavirus*: MAGV, TCMV, UTIV, BLMV, CARV, OROV, and CATUV; *Orthoflavivirus*: WNV, YFV, ILHV, SLEV, CPCV, BSQV, and ROCV. † Bird species commonly found in open fields (areas with anthropogenic impact, such as settlements, crops, orchards, and pastures, with limited native trees and vegetation). ‡ Bird species commonly found in all environments. § Bird species commonly found at forest edges. ‖ Of the 14 samples with monotypic WNV or heterotypic reactions in HI tests, six were further tested by PRNT, with 50% (3/6) testing positive. Conventional sign used: – Numerical data equal to zero, not resulting from rounding.

In this study, a sample of *Turdus rufiventris* presented HI tite 80 for WNV and 20 for ILHV. Despite being a heterotypic reaction, this result is corroborated by the study conducted in Ceará, in 2019. In that study, *Turdus rufiventris* presented de highest seroprevalence by PRNT for WNV[22]. The same has not been observed in other regions of the Brazil and other countries in South America. An investigation for WNV conducted from 2008 to 2010 in Brazil, 18 individuals tested negative for WNV by molecular and serological methods[18]. Between 2012 and 2013, free-ranging birds, including 13 individuals of *Turdus rufiventris* captured from green areas of the city of São Paulo, Southeastern Brazil, tested negative for flaviviruses by real-time RT-PCR[22]. In Argentina, of four individuals sampled in Buenos Aires between 2012 and 2013, none of them were seropositive for WNV[32]. Considering that *Turdus migratorius*, popularly known as American Robin, is one important amplifying host for WNV in North America[33,34], Brazilian species as *Turdus rufiventris*, *Turdus amaurochalinus*, and *Turdus leucomelas*, should also be included in future studies due to their frequent occurrence in urban centers.

Of the 12 individuals of *Zenaida auriculata virgata* (Eared Dove, family Columbidae) tested in the present study, none showed hemagglutination inhibition antibodies for WNV. It is worth to mention that the number of individuals tested was very limited, considering this species occurs in flocks of thousands in the study area during the rainy season. This species is of particular interest because thousands of these birds are captured by the local populations as part of their diet[35]. In the study conducted in the neighboring state of Ceará, the only individual of *Zenaida auriculata* captured and sampled was seronegative for WNV[22]. In a study conducted in Argentina, 157 individuals tested negative for WNV antibodies[36]. It is noteworthy that the first recorded case of WNV encephalitis in Brazil reported to capture these birds near his residence for consumption. In fact, this is a common habit in the rural areas in the Northeast Region of Brazil[35]. The non-vectorial transmission of WNV by handling or ingestion of infected birds, cannot be fully discarded. The location where the first human WNV case occurred was approximately 500 m from three lakes and a river, in an area heavily impacted by agriculture. The presence of water bodies may favor the reproduction of WNV vectors, even during the dry season[37].

In the tropics, the avifauna diversity is high and includes several species with low capacity to serve as WNV amplifying hosts, reducing the likelihood of infecting vector insects and thereby decreasing the net transmission of WNV to birds and humans[38]. This may explain the smaller number of cases of WNV disease in South America when compared to North America. Based on this hypothesis, the occurrence and movement of WNV and other viruses in the Caatinga could be associated with the biome's lower avian diversity compared to other Brazilian biomes such as the Atlantic Forest and Amazonia, facilitating transmission between birds capable of amplifying and dispersing WNV and other arboviruses.

The serological evidence by HI of activity of other orthoflaviviruses (Table 1) reported here supports the hypothesis that other orthoflaviviruses circulate in the region. The monotypic reactions observed for ILHV in *Columbina minuta* (HI titer 20) e *Columbina picui* (HI titer 20) and for SLEV in *Pitangus sulphuratus* (HI titer 20) suggests that besides WNV, other zoonotic orthoflaviviruses of public health importance may circulate in the region. The high diversity of flavivirus species circulating in wild and domestic animals, as well as human populations[3,39], may result in antibody interactions from coinfections or prior infections, modulating clinical manifestations and viremia associated with WNV infection. No monotypic reactions were observed for EEEV, WEEV, VEEV, MAYV, MUCV, ICOV, MAGV, TCMV, UTIV, BLMV, CARV, OROV, CATUV, YFV, CPCV, BSQV, and ROCV.

CONCLUSION

This study brings additional serological evidence of WNV exposure in wild birds sampled over a decade ago in Northeast Brazil. These findings may indicate WNV activity in wild birds in Brazil for longer than originally thought. More studies are needed to confirm the exposure of these wild birds to WNV in Brazil and to determinate what species could act as amplifying hosts of WNV in the different biomes in Brazil.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this publication.

AUTHOR'S CONTRIBUTION

AEBAS: field sampling, handling, banding, and releasing individuals; APMR: study design, fieldwork design, field sampling, data analysis, and manuscript writing; CGZ: data analysis, protocol revision, and

manuscript writing; CRF: data analysis, study design, field sampling design, and manuscript writing; DGR, EVPS, FABM, KRLJC, LCM, MAS, and PHOP: data analysis, and manuscript writing; MABA: field sampling, sample processing, data analysis, and manuscript writing; MAV: study design, data analysis, and manuscript writing; PCA: field sampling design, field sampling, sample processing, and manuscript writing; PCL: field sampling, sample processing, data analysis, and cartography; RL: cartography, data analysis, and manuscript writing; VSM: field sampling, sample processing, and manuscript writing.

SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article online:

Table 1S – Serological results for West Nile virus and other arboviruses using the hemagglutination inhibition (HI) test for 103 wild birds from 27 species captured in Piauí State, Brazil, in 2014 and 2015.

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SUPPLEMENTARY MATERIAL

Table S1 – Serological results for West Nile virus and other arboviruses using the hemagglutination inhibition (HI) test for 103 wild birds from 27 species captured in Piauí State, Brazil, in 2014 and 2015

Species	Samples	HI*,†	Type of reaction
Jacana jacana	1	(1 -)	
Columbina minuta	8	(1 +) ILHV 1:20 (1 +) WNV 1:40, ILHV 1:80, SLEV 1:80	Monotypic Heterotypic
Columbina picui	22	(2 +) ILHV 1:20	Monotypic
Columbina squammata	10	(1 +) WNV 1:20, ILHV 1:20 (1 +) YFV 1:40; ILHV 1:160, SLEV 1:160, BSQV 1:40, ROCV 1:40	Heterotypic Heterotypic
Columbina talpacoti	1	(1 +) WNV 1:80, YFV 1:20, ILHV 1:80, SLEV 1:80, BSQV 1:20	Heterotypic
Leptotila verreauxi	2	(1 +) WNV 1:20, ILHV 1:20 (1 +) WNV 1:20, ILHV 1:20, SLEV 1:40	Heterotypic Heterotypic
Zenaida auriculata virgata	12	(12 -)	
Forpus xanthopterygius	1	(1 -)	
Glaucidium brasilianum	1	(1 -)	
Piaya cayana	1	(1 +) WNV 1:20, SLEV 1:40	Heterotypic
Nystalus maculatus	3	(3 -)	
Picumnus pygmaeus	1	(1 -)	
Lepidocolaptes angustirostris	3	(3 -)	
Myiarchus tyrannulus	5	(1 +) WNV 1:40	Monotypic
Myiarchus ferox	1	(1 -)	
Tyrannus melancholicus	1	(1 +) WNV 1:20	Monotypic
Pitangus sulphuratus	4	(1 +) WNV 1:80, EEEV 1:80, MUCV 1:40, SLEV 1:160, BSQV 1:20, SLEV 1:20 (1 +) SLEV 1:20	Heterotypic Monotypic
Pachyramphus polychopterus	1	(1 -)	
Myiodynastes maculatus	1	(1 -)	
Mimus saturninus	3	(3 +) WNV 1:40	Monotypic
Turdus amaurochalinus	2	(2 -)	
Turdus rufiventris	3	(1 +) WNV 1:80, ILHV 1:20	Heterotypic
Cyclarhis gujanensis	1	(1 +) ILHV 1:40, SLEV 1:40	Heterotypic
Paroaria dominicana	3	(1 +) WNV 1:20 (1 +) TCMV 1:20, UTIV 1:20, CARV 1:20, CATUV 1:20	Monotypic Heterotypic
Lanio pileatus	1	(1 -)	
Chrysomus ruficapillus	10	(1 +) WNV 1:80	Monotypic
Cyanocorax cyanopogon	1	(1 +) ILHV 1:20, SLEV 1:20, BSQV 1:20	Heterotypic
Total	103	6 WNV samples 8 WNV samples	Monotypic Heterotypic

* HI tests were conducted for 20 arbovirus species from the following genera: *Alphavirus*: Eastern Equine Encephalitis virus (EEEV), Western Equine Encephalitis virus (WEEV), Venezuelan Equine Encephalitis virus (VEEV, subtype IIIA), Mayaro virus (MAYV), and Mucambo virus (MUCV); *Phlebovirus*: Icoaraci virus (ICOV); *Orthobunyavirus*: Maguari virus (MAGV), Tacaiuma virus (TCMV), Utinga virus (UTIV), Belem virus (BLMV), Caraparu virus (CARV), Oropouche virus (OROV), and Catu virus (CATUV); *Flavivirus*: West Nile virus (WNV), Yellow Fever virus (YFV), Ilheus virus (ILHV), Saint Louis Encephalitis virus (SLEV), Cacipacore virus (CPCV), Bussuquara virus (BSQV), and Rocio virus (ROCV). † Results are presented as: "(Number of samples, result [+/-]) virus of reaction, titer".

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