Brazilian Journal of Microbiology
https://doi.org/10.1007/s42770-020-00393-z

VETERINARY MICROBIOLOGY - RESEARCH PAPER

Serosurvey of West Nile virus (WNV) in free-ranging raptors from Brazil

Ana Paula Morel[1] · Anelise Webster[1] · Larissa Calo Zitelli[1] · Karen Umeno[1] · Ugo Araújo Souza[1] · Fabiane Prusch[2] · Marina Anicet[2] · Gleide Marsicano[2] · Paulo Bandarra[3] · Gustavo Trainini[4] · Julian Stocker[4] · Denise Giani[4] · Flávia Borges Fortes[5] · Silvina Goenaga[6] · José Reck[1]

Received: 9 May 2020 / Accepted: 17 October 2020

Abstract

West Nile virus (WNV) is a mosquito-borne Flavivirus that can affect birds, horses, and humans, and is the only zoonotic Flavivirus that has been identified in six continents. In Brazil, until 2010, there was no evidence of WNV circulation. Recently, the virus was isolated from a horse with encephalitis, and the first human cases were registered in Brazil. Despite that, there is still no information on the enzootic cycle of this virus in birds or wildlife. This study aimed to investigate whether there is evidence of WNV circulation among wild birds from Southern Brazil. For this, we used free-living wild raptors (live-trapped or rescued) as potential sentinels to investigate the presence of WNV antibodies using ELISA and plaque reduction neutralization test (PRNT) assay. In addition, the presence of nucleic acids from Flavivirus family members was investigated. None of the birds sampled presented clinical findings compatible with WNV. Of the 200 serum samples from birds of prey belonging to 21 species, ten (5%) were positive for the presence of WNV antibodies on ELISA testing. The PRNT test did not confirm the ELISA results, but indicated that three birds had possibly been exposed to Saint Louis encephalitis virus (SLEV). All samples were negative for Flavivirus RNA. The results presented here evince the need for permanent surveillance for emerging flaviviruses in Brazil, as well as for a contingency policy in the case of human/animal outbreaks, particularly in high-risk areas.

Keywords West Nile virus (WNV) · Saint Louis encephalitis virus (SLEV) · Raptors · Birds of prey · Brazil · Flavivirus

Introduction

Flavivirus are a genus of the *Flaviviridae* family that include mosquito-transmitted pathogens such as dengue viruses (DENV), yellow fever virus (YFV), West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), Japanese encephalitis virus (JEV), Usutu virus (USUV), and Zika virus (ZIKV) [1]. Between these viruses, birds play an important role serving as reservoirs in WNV, JEV, SLEV, and USUV [1, 2].

West Nile virus was first discovered in Africa in 1937 [3]. After that, many countries have found evidence of its circulation in horses, birds, and humans [1]. In 1999, an outbreak of the disease first occurred in the Americas, in New York City [4]. At that time, humans and many birds, including wild and captive ones, died due to the disease [5–8]. The virus was further detected in Mexico (2000) in a hooded warbler (*Setophaga citrina*), a migratory Parulidae bird. In South America, the first evidence of virus circulation was in 2004, with detection of antibodies in sera of horses from Colombia

Editorial Responsibility: Fernando R. Spilki

✉ Ana Paula Morel
apmvet@gmail.com

✉ José Reck
jose.reck@gmail.com

[1] Centro de Pesquisa em Saúde Animal, Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF), Eldorado do Sul, RS, Brazil

[2] Clinica Veterinária Toca dos Bichos, Porto Alegre, RS, Brazil

[3] Nucleo de Reabilitação da Fauna Silvestre-UFPel, Pelotas, RS, Brazil

[4] Hayabusa Consultoria Ambiental, São Francisco de Paula, RS, Brazil

[5] Programa Estadual de Sanidade Avícola (PESA), Secretaria da Agricultura Pecuária e Desenvolvimento Rural (SEAPDR), Porto Alegre, RS, Brazil

[6] Instituto Nacional de Enfermedades Virales Humanas (INEVH), Pergamino, Argentina

Published online: 27 October 2020

[9, 10]. As WNV reached South America in 2004, it became the only zoonotic Flavivirus that was identified in six continents [11]. Later, in 2006, the first case of WNV was reported in Argentina; two horses died of the disease 48–72 h after presenting ataxia, circling, hypersensitivity to noise, hyperexcitability, and recumbency [12]. In Uruguay, a serosurvey showed that 1.88% (8/207) of equine sera tested positive in 2013 [13].

In Brazil, until 2010, there was no evidence of the presence of WNV, as serosurveys conducted with migratory birds between 2002 and 2010 resulted only in seronegative samples [14–16]. In 2011, a serosurvey in horses from Brazilian Pantanal wetland showed animals with the presence of antibodies but no evidence of disease, indicating that the virus had probably occurred in wild enzootic cycles [17, 18]. Recently, the virus was isolated from a horse's brain in Espirito Santo state [19]. In 2015, the first documented human case of WNV in Brazil, in Piauí state, northeast Brazil, in a rural worker [20] was reported. Recently, the seventh human case was notified by health authorities in Piauí [21].

WNV fever is considered a disease limited to one or more areas in Brazil by Brazilian animal health authorities [22]. Additionally, even in the face of two human cases and serological evidence of equine exposure to the virus, there is no information on virus transmission and the enzootic cycle in birds or wild reservoirs. This evinces the urgent need for more surveillance and epidemiological studies in the vast Brazilian territory, especially in birds, the traditional hosts of WNV. Thus, the aim of this study was to investigate whether there is evidence of WNV circulation among wild birds from Southern Brazil. For this, we used wild raptors as potential sentinels to investigate the presence of WNV antibodies.

Materials and methods

Samples of free-ranging raptors were obtained from (i) live-trapped and (ii) rescued birds. The live-trapping procedure was a part of an official bird strike control protocol of Salgado Filho International Airport at Porto Alegre municipality, Rio Grande do Sul (RS) state. The single-door Tomahawk live-traps (1.15 m × 40 × 60 cm) were baited with carcasses of domestic quail (*Coturnix japonica*) [23]. Traps were opened twice a week at 8 am, and revisited at 2-h intervals during daytime. The traps were closed at the end of the day, before sundown. The live-trapping procedure was conducted over 2 years, with most of the birds captured during autumn and winter months. The captures focused on the Southern Caracaras (*Caracara plancus*), the most abundant bird of prey in this area. The birds were captured, manually restrained, banded, and had their blood collected by venipuncture of the ulnar vein [24]. After that, animals were translocated to a releasing site previously determined by local environmental authorities. The rescued birds were sampled at three wildlife rehabilitation centers located in the municipalities of Porto Alegre, São Francisco de Paula, and Pelotas, all located in RS state. Most of the rescued birds were admitted to the rehabilitation centers after trauma. All birds were manually restrained, and had their blood collected by venipuncture of the ulnar or jugular veins. Blood collection was performed as soon as possible after the bird arrived at the center to avoid any potential influence of the captive environment. Figure 1 depicts the location of the live-trapping site and rehabilitation centers. Despite the birds being sampled in centers located in three municipalities, the exact origin of these animals is unknown, since this information is usually not recorded by environmental authorities. This means that for most of the rescued birds sampled here, we cannot determine the region where they originally lived. This study was approved by the local ethics committee on animal experimentation (CEUA-IPVDF 07/18 and 02/19) and Brazilian biodiversity authorities (SISBIO licenses 54808 and 62187).

All blood sera were obtained by centrifugation of blood without anticoagulant and then stored at − 20 °C until use. The sera were then tested using a commercial enzyme-linked immunosorbent assay (ELISA) WNV kit (ID Screen® West Nile Competition Multi-species – IDVet, France). The plate was read using a spectrophotometer at 450 nm (Biochrom EZ Read 2000). To confirm seropositivity, samples positive in the ELISA test were tested by a plaque reduction neutralization test (PRNT). For that, serum samples were ultracentrifuged and filtrated using a syringe filter with a 0.22-μm polyvinylidene membrane. The samples were diluted 1:10 in minimum essential medium (MEM) and endpoint antibody titers determined using serial

Fig. 1 Study area for investigation of West Nile virus (WNV) antibodies in wild raptors from Southern Brazil. Map shows Rio Grande do Sul state, Brazil, and neighboring countries. Light gray shading indicates the Pampa biome; dark gray shading indicates bodies of water; birds indicate the sites of sampling, as follows: (1) Porto Alegre, (2) São Francisco de Paula, and (3) Pelotas municipalities

twofold dilutions and they were incubated with 100 plaque-forming units (PFU) of WNV (strain ChimeriVax TM WNV) or SLEV (strain ChimeriVax TM SLEV) [25]. The SLEV was included in the PRNT assay since the ELISA kit's manufacturer states that there may be some cross-reaction between WNV and SLEV. Vital dye neutral red was used at 2.2% for plaque visualization after 4 days post-infection. Plaques were counted and titers were calculated and expressed as the reciprocal of the serum dilution yielding a ≥ 50% and ≥ 90% reduction in PFU on Vero cells (respectively, $PRNT_{50}$ and $PRNT_{90}$). Titers > 10 were considered positive.

Additionally, all serum samples positive in the ELISA test were further tested by RT (reverse transcription)-nested PCR (polymerase chain reaction) to verify the presence of nucleic acids from Flavivirus family members [26]. RNA was extracted from 0.2 mL using the Purelink RNA mini kit (Life Technologies, Carlsbad, CA, USA). RNA quantity and quality assessment were realized via NanoDrop equipment (ND-3300, NanoDrop Technologies, USA). The RT reaction was performed using M-MLV Reverse Transcriptase (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The primers and RT-nested PCR conditions were previously described elsewhere [26]. Briefly, for the first reaction, we used Flavi1+ (GAYYTIGGITGYGGII-GIGGIRGITGG) and Flavi1− (TCCCAICCIGCIRTR-TCRTCIGC) primers. Samples underwent an initial cycle at 38 °C for 45 min and 94 °C for 2 min, followed by a further 40 cycles at 94 °C for 30 s, 47 °C for 1 min, and 68 °C for 1 min and 15 s. A final extension step was carried out at 68 °C for 5 min. The nested reaction was performed using Flavi2+ (YGYRTIYAYAWCAYSATGGG) and Flavi2− (CCARTGITCYKYRTTIAIRAAICC). The PCR conditions were a denaturation stage (94 °C for 2 min) followed by 40 cycles similar to the conditions described above. As positive controls for RT-nested PCR, we used a reference sample of ZIKV, kindly provided by Dr. Ana Claudia Franco (Virology Laboratory, UFRGS, Porto Alegre, Brazil).

Results

Herein, we analyzed 200 serum samples from birds of prey. From these samples, 94 were from live-trapped Southern Caracaras (*C. plancus*), and 106 were from rescued birds admitted to wildlife rehabilitation centers, which belonged to 21 species of three different orders. From the 200 serum samples analyzed, ten (5%, CI 95% 2–8%) were positive for the presence of WNV antibodies by ELISA. Just one of the seropositive birds was a live-trapped *C. plancus*. The other nine seropositive birds were rescued birds, of the following species: one *C. plancus*; one *Milvago chimango* (Chimango Caracara); one *Milvago chimachima* (yellow-headed Caracara); four *Bubo virginianus* (great horned owl); one *Magascops choliba* (tropical screech owl); and one *Asio stygius* (stygian owl). None of the live-trapped *C. plancus* sampled presented any signs of disease at the moment of capture. The rescued birds were mainly admitted to rehabilitation centers due to trauma, and at the moment of sample collection, none was considered suspect for infectious encephalitis. All nine rescued birds seropositive for WNV died or were euthanized within some days after sampling, and since at that time there was no suspicion of infectious diseases, all carcasses were discarded as medical waste. The detailed results of sampled animals and serology results are shown in Table 1.

Of the ten positive samples in the ELISA test, five were further tested by PRNT assay. Due to limitation of the serum sample volume, it was not possible to test the other five positive samples. Two of the birds sampled (one *C. plancus* and one *M. choliba*) showed $PRNT_{50}$ and $PRNT_{90}$ titers above 160 against SLEV. One of these birds (*M. choliba*) presented a $PRNT_{50}$ titer of 10 for WNV and ≥ 160 for SLEV. An owl (*B. virginianus*) showed 80 as $PRNT_{50}$ titer for SLEV and < 10 for WNV, while in $PRNT_{90}$ was non-reagent for both viruses (SLEV and WNV). In Table 2, a summary of PRNT results is shown. None of the samples was positive for the RT-nested PCR for Flavivirus RNA detection.

Discussion

Since birds of prey are considered a highly sensitive avian group for some flaviviruses, such WNV, we chose these species to investigate the presence of WNV antibodies in Brazilian wild birds [27–30]. To date, despite the surveys with migratory birds performed between 2002 and 2010, there are no studies regarding this raptor's orders in Brazil. Raptors are long-life apex predators at the top of the food chain, which means they have contact with many other birds when feeding on prey or carcasses. Thus, raptors may be considered bioaccumulators and also indicators of the biomagnification of pathogens. Herein, we sampled 21 neotropical species of wild raptors to assess whether there was evidence of Flavivirus circulation among birds in Southern Brazil.

To date, antibodies against WNV have previously only been found in Brazilian horses. Herein, we found antibodies against WNV in non-migratory birds by ELISA, a screening test with high sensitivity. However, using a confirmatory and more specific test (PRNT), we did not confirm the ELISA results. Moreover, the PRNT results suggested a previous exposure to SLEV, another zoonotic mosquito-borne Flavivirus, such as WNV. Regarding the lack of detection of RNA of flaviviruses in tested samples, this could possibly be related to the short-time viremia of most flaviviruses in raptors [31]. Furthermore, we cannot rule out that these negative results could be attributed to RNA degradation due to storage conditions (− 20 °C).

Table 1 Summary of sampled animals and results of ELISA for detection of antibodies against WNV in free-ranging birds of prey from Rio Grande do Sul state, Brazil

Order	Species	Positive/ total	%	(CI 95%)	Migratory
Acciptriformes		0/21	-	-	-
	Rupornis magnirostris[b]	0/10	-	-	No
	Ictinea plumbea[b]	0/3	-	-	Yes[c]
	Heterozpizias meridionalis[b]	0/3	-	-	No
	Harpagus diodon[b]	0/2	-	-	Yes[c]
	Rostrhamus sociabilis[b]	0/1	-	-	Yes[c]
	Geranospiza caerulescens[b]	0/1	-	-	No
	Buteo brachyurus[b]	0/1	-	-	No
Falconiformes		4/132	3%	(1–7%)	-
	Caracara plancus[a]	1/94	1%	(0.2–5%)	No
	Caracara plancus[b]	1/6	16%	(3–56%)	No
	Falco sparverius[b]	0/22	-	-	No
	Milvago chimango[b]	1/4	25%	(4–69%)	No
	Milvago chimachima[b]	1/3	33%	(6–79%)	No
	Micrastur semitorquatus[b]	0/1	-	-	No
	Falco peregrinus[b]	0/1	-	-	Yes[d]
	Falco deiroleucus[b]	0/1	-	-	No
Strigiformes		6/47	12%	(5–25%)	-
	Bubo virginianus[b]	4/12	33%	(13–60%)	No
	Megascops choliba[b]	1/11	9%	(1–37%)	No
	Asio clamator[b]	0/10	-	-	No
	Tyto furcata[b]	0/6	-	-	No
	Athene cunicularia[b]	0/4	-	-	No
	Asio stygius[b]	1/3	33%	(6–79%)	No
	Pulsatix koeniswaldiana[b]	0/1	-	-	No
Total		10/200	5%	(2–8%)	-

[a] Live-trapped birds

[b] Rescued birds

[c] Migration within Brazilian territory

[d] Migration from North America

Table 2 Plaque reduction neutralization test (PRNT) for WNV and SLEV in serum samples of free-ranging birds of prey from Rio Grande do Sul state, Brazil

Bird species	$PRNT_{50}$ titer		$PRNT_{90}$ titer	
	WNV	SLEV	WNV	SLEV
Bubo virginianus	< 10	80	< 10	< 10
Caracara plancus[a]	< 10	≥ 160	< 10	≥ 160
Caracara plancus[b]	< 10	< 10	< 10	< 10
Megascops choliba	10	≥ 160	< 10	≥ 160
Milvago chimachima	< 10	< 10	< 10	< 10

[a] Live-trapped bird

[b] Rescued bird

Considering previous serosurvey studies in wild birds conducted in Brazil, only one tested a raptor, one burrowing owl (*Athene cunicularia*) that tested negative [16]. In Argentina, a few studies have tested wild and captive birds of prey, inclusively, showing some seropositive birds, such as two American kestrels (*Falco sparverius*), one crowned eagle (*Urubitinga coronata*), one Rufous-thighed hawk (*Accipiter erythronemius*), and five members of the Falconidae family (species not mentioned by the authors) [32–34]. In the USA, WNV was detected in more than 40 raptor species [35]. In Table 3, we have summarized the reports of WNV serosurveys in 27 species of birds of prey worldwide.

In North America, Corvidae family members, particularly American crows (*Corvus brachyrhynchos*) are considered the major sentinel for WNV infection [48]. Since this species is not found in Brazil, other species must be identified as potential sentinels. Considering that several raptor species seem

Table 3 Serosurvey studies of WNV in raptors worldwide

Order	Species	Country	% (+/total)	Reference
Acciptriformes	*Accipiter gentilis*	Spain	100% (1/1)	[36]
		Poland	30% (3/10)	[37]
		Germany	40% (4/10)	[38]
	Accipiter nisus	Germany	11.1% (1/9)	[38]
	Aquila adalberti	Spain	100% (1/1)	[39]
	Buteo buteo	Germany	2.7% (1/37)	[27]
		Germany	16.1% (5/31)	[38]
	Circus aeruginosus	Germany	50% (1/2)	[27]
			33.3% (1/3)	[38]
	Haliaetus albilica	Poland	33.3% (2/3)	[37]
		Germany	16.6% (1/6)	[38]
	Hieraaetus pennatus	Spain	50% (1/2)	[36]
	Milvus milvus	Germany	100% (2/2)	[38]
	Pandion haliaetus	Germany	50% (1/2)	[27]
	Pernis apivorus	Germany	33.3% (1/3)	[27]
Falconiformes	*Caracara cheriway*	USA	16.3% (13/80)	[40]
	Falco eleonorae	Spain (Canary Islands)	4.9% (4/81)	[41]
	Falco naumanni	Spain	6.6% (1/15)	[36]
	Falco peregrinus	Canada	100% (2/2)	[42]
	Falco sparverius	USA	95% (21/22)	[43]
		Canada	16.4% (25/152)	[44]
		USA	81.7% (170/208)	[45]
Strigiformes	*Asio flammeus*	Canada	100% (8/8)	[42]
	Athene cunicularia	Canada	90% (9/10)	[42]
		USA	22% (41/186)	[45]
	Bubo bubo	Spain	11.1% (2/18)	[36]
	Bubo virginianus	USA	44.4% (4/9)	[46]
		Canada	100% (12/12)	[42]
		USA	1.41% (1/71)	[47]
	Glaucidium gnoma	Canada	40% (2/5)	[42]
	Megascops asio	Canada	72.7% (24/33)	[42]
	Micrathene whitneyi	Canada	100% (1/1)	[42]
	Ottus flammeus	Canada	12.5% (1/8)	[42]
	Strix varia	Canada	100% (2/2)	[42]
	Tyto alba	Canada	80% (8/10)	[42]
	Strix aluco	Spain	8.3% (1/12)	[36]
		Germany	20% (1/5)	[38]

susceptible to WNV infection [28, 35, 49, 50], and that they occupy a great home-range area, they emerge as potential sentinels for surveillance. The six raptor species found to be seropositive by ELISA in our study are usually found at or nearby great populous cities in Southern Brazil, which reinforces the potential risk of human exposure to WNV. Since Strigiformes (owls) showed the highest seropositivity by ELISA in our study, this group could be important as potential sentinels in Brazil. The habit of preying on other birds, commonly observed for the *B. virginianus* owl [51], could be important for WNV and other Flavivirus exposure, since it was once demonstrated that some viruses could be transmitted to birds that fed on carcasses of other infected avian hosts [52]. Interestingly, Bernt reported that Passeriformes birds were generally found to be the least mosquito tolerant, in comparison to owls, such *B. virginianus*, who exhibited the fewest defensive behaviors and were most heavily mosquito-infested [53]. Considering mosquito-borne viruses, this may have some implications for infection rates. This must be considered in further epidemiological studies and surveillance in neotropical birds.

Herein, we showed evidence of previous exposure to SLEV in three birds of prey, a Southern Caracara, a great

horned owl, and a tropical screech owl. The WNV ELISA kit's datasheet suggests a potential cross-reaction between WNV and SLEV antibodies, to be further confirmed by more specific assays, such as PRNT. SLEV is considered a widespread virus in the Americas. For more than 50 years, there was evidence of SLEV circulation in Brazil [54]. Few human cases, involving fever and jaundice, have been reported to date [55, 56]. SLEV infection has already been demonstrated in horses from Brazil [57], birds [17, 58], and mosquitoes [59, 60]. In spite of birds maintaining the viral life cycle and being considered the natural amplifying host, to date there is a paucity of data regarding birds of prey. Moreover, contrary to WNV, SLEV infection is usually considered asymptomatic in birds [61]. Here, we provide data indicating that raptors could be involved in the enzootic cycle of SLEV in Brazil. Usually, migratory birds are the main targets of studies concerning Flavivirus epidemiology in places where the agent was not considered established; however, the presence in non-migratory birds means that the virus is possibly established in the area. Nevertheless, as previously proposed, it is possible that WNV may have first entered Brazil through migratory birds [17, 62]. Besides migratory birds, it is important to note that imported pet birds and horses, as well as travelers coming from countries where the virus is present, could represent a risk of disease spread and infection of local potential vectors. The importation of pet birds from the USA and Europe is increasing and usually, only avian influenza and Newcastle disease viruses are considered in the health screening to allow entrance to Brazilian territory [63].

None of the sampled birds was initially considered suspect for infectious encephalitis or any other infectious disease. However, it is important to note, particularly for veterinary practitioners, that any neurological presentation and/or emaciation may be misdiagnosed as head trauma (a common presentation at wildlife rehabilitation centers) rather than any suspicion of infectious encephalitis, especially in areas where WNV and other viruses are not considered endemic. This demands a more careful physical examination and the need for availability of complementary and diagnostic tests; particularly in cases where the animal's history is unknown. WNV infection can frequently present non-specific clinical findings in birds such as disorientation, ataxia, depression, weakness, emaciation, head tremors, and posture problems (which can fit many disease's clinical signs) [28, 31]. Thus, despite the potential reservoir role of these birds, the aforementioned clinical signs (that can reduce long distance flights) and the solitary habits of most raptor species may affect virus spread.

The data concerning wild birds' exposure to Flavivirus reported here evince the need for further studies aiming at the molecular detection and isolation of the virus from wild birds in Brazil, as well as investigation of the virus in mosquitoes. Indeed, our results highlight the importance of monitoring emerging pathogens, even those considered restricted to one or more zones in the neotropical region [22]. A permanent surveillance program, especially of domestic birds and horses that live near migration points in the country, would increase the odds of early detection of virus entry. The official surveys for WNV in wild birds are no longer performed in most Brazilian states, and even when they are done, they only cover a few regions of Brazil's vast territory. The last investigation of WNV in a migratory site in RS state occurred in 2004 [15]. Unfortunately, pivotal health policies such as surveillance programs for emerging agents in potential natural reservoirs, such as WNV in wild birds, are highly dependent on government priorities and budgetary restraints. The set of results presented here demonstrates the need for permanent surveillance for WNV and other arbovirus encephalitis in Brazil, as well as for a contingency policy in the case of human/animal outbreaks, particularly in high-risk areas. Additionally, further research must contribute to public policies exploring epidemiological data of recent human cases, as well as expanding the knowledge of potential neotropical reservoirs (enzootic cycles) and vectors.

Acknowledgments We acknowledge the valuable contributions to this study from colleagues of Hayabusa Consultoria Ambiental, Clínica Veterinária Toca dos Bichos, NURFS-CETAS, IBAMA-RS, INEVH, and Instituto de Pesquisas Veterinárias Desiderio Finamor (IPVDF). Special thanks to Dr. Carla Rodenbusch (IPVDF) for helping in molecular analysis, and to Victoria Luppo (INEVH) for technical support in neutralization assays. We also thank INFRAERO, SMAM, ICMBio (SISBIO 54808 and SISBIO 62187), and CEUA-IPVDF (07/18 and 02/19) for the permits for this study.

Funding This work was supported by the Fundação Estadual de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenacão de Aperfeioamento de Pessoal de Nível Superior (CAPES), Projeto RS Biodiversidade, and INCT Entomologia Molecular.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. MacLachlan NJ, Dubovi EJ (2017) Fenner's veterinary virology, 5ed edn. Elsevier, London, p 581
2. Tisiodras S, Kelesidis T, Kelesidis I, Bauchinger U, Falagas ME (2008) Human infections associated with wild birds. J Inf Secur 56: 83–98
3. Smithburn KC, Hughes TP, Burke AW, Paul JH (1940) A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med Hyg 20:471–492
4. CDC - Centers for Disease Control and Prevention (1999) Outbreak of West Nile–like viral encephalitis: New York, 1999. Morbity Mortality Wkly Rep 48:845–849
5. Anderson JF, Andreadis TG, Vossbrinck CR, Tirrell S, Wakem EM, French RA, Garmendia AE, Van Kruiningen HJ (1999)

Isolation of West Nile Virus from mosquitoes, crows, and a Cooper's Hawk. Science 86:2331–2333
6. Steele KE, Linn MJ, Schoepp RJ, Komar N, Geisbert TW, Manduca RM, Calle PP, Raphael BL, Clippinger TL, Larsen T, Smith J, Lanciotti RS, Panella NA, McNamara TS (2000) Pathology of fatal West Nile Virus infections in native and exotic birds during the 1999 Outbreak in New York City, New York. Vet Pathol 37:208–224
7. Eidson M, Kramer L, Stone W, Haiwara Y, Schmit K (2001) Dead bird surveillance as an early warning system for West Nile Virus. Emerg Infect Dis 7:631–635
8. Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K, Huang A, Rosenberg A, Greenberg A (2001) The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med 344:1807–1814
9. Farfán-Ale JA, Blitvich BJ, Loroño-Pino MA, Marlenee NL, Rosado-Paredes EP, Garcia-Rejon JE, Chulim-Perera L, Lopez-Urine M, Pérez-Mendoza G, Sánchez-Hererra I, Santamaria W, Moo-Huchim J, Gubler DJ, Cropp BC, Calisher CH, Beaty BJ (2004) Longitudinal studies of West Nile Virus infection in avians, Yucatán State, México. Vector-borne and Zoonotic Diseases 4:3–14
10. Mattar S, Edwards E, Laguado J, González M, Alvarez J, Komar N (2005) West Nile Virus antibodies in Colombian horses. Emerg Infect Dis 11:1497–1498
11. Komar N, Clark GG (2006) West Nile virus activity in Latin America and the Caribbean. Rev Panam Salud Publica 19:112–117
12. Morales MA, Barrandeguy M, Fabri C, Garcia JB, Vissani A, Trono K, Gutierrez G, Pigretti S, Menchaca H, Garrido N, Taylor N, Fernandez F, Levis S, Enría D (2006) West Nile Virus isolation from equines in Argentina, 2006. Emerg Infect Dis 12:1559–1561
13. Burgueño A, Spinsanti L, Díaz A, Rivarola ME, Arbiza J, Contigiani M, Delfraro A (2013) Seroprevalence of St. Louis encephalitis virus and West Nile virus (Flavivirus, Flaviviridae) in horses, Uruguay. Biomed Res Int 2013:1–5
14. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde (2003) Primeiro inquérito sorológico em aves migratórias e nativas do Parque Nacional da Lagoa do Peixe/RS para detecção do vírus do Nilo ocidental. Boletim eletrônico epidemiológico 3:3–12
15. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde (2004) Primeiro inquérito sorológico em aves migratórias e nativas do Parque Nacional da Lagoa do Peixe/RS para detecção do vírus do Nilo ocidental. Boletim eletrônico epidemiológico 4:1–8
16. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde (2004) Inquérito sorológico em aves migratórias e residentes de Galinhos/RN para detecção do vírus do Nilo Ocidental e outros vírus. Boletim eletrônico epidemiológico 4:1–12
17. Pauvolid-Corrêa A, Morales MA, Levis S, Moraes LT, Couto-Lima D, Campos Z, Nogueira MF, Silva EE, Nogueira RMR, Schatzmayr HG (2011) Neutralising antibodies for West Nile virus in horses from Brazilian Pantanal. Mem Inst Oswaldo Cruz 106:467–474
18. Pauvolid-Corrêa A, Campos Z, Juliano R, Velez J, Nogueira RMR, Komar N (2014) Serological evidence of widespread circulation of West Nile Virus and other flaviviruses in equines of the Pantanal, Brazil. PLoS One Negl Tropical Diseases 8:1–11
19. Martins LC, Silva EVP, Casseb LMN, Silva SP, Cruz ACR, Pantoja JAS, Medeiros DBA, Martins Filho AJ, Cruz ERM, Araújo MTF, Cardoso JF, Cunha MACR, Almada GL, Romano APM, Santos MGDP, Rodrigues GAP, Chiang JO, Quaresma JAS, Carvalho VL, Vascocelos PFC (2019) First isolation of West Nile virus in Brazil. Mem Inst Oswaldo Cruz 114:e180332
20. Vieira MAC, Romano APM, Borba AS, Silva EVP, Chiang JO, Eulálio KD, Azevedo RSS, Rodrigues SG, Almeida-Neto WS, Vasconcelos PFC (2015) Case report: West Nile virus encephalitis: the first human case recorded in Brazil. Am J Trop Med Hyg 93:377–379
21. ProMED – International Society for Infectious Diseases (2020) https://promedmail.org/promed-post/?id=7282717 Accessed 26 June 2020
22. Brasil. Ministério da Agricultura Pecuária e Abastecimento (2018) Situação sanitária das doenças de animais terrestres. http://www.agricultura.gov.br/assuntos/sanidade-animal-e-vegetal/saude-animal/arquivos-sisa/Situacao_sanitaria_WAHID_Brasil_2018_01.pdf. Accessed 30 Apr 2020
23. Silva LTR, Silva JSA, Lima DVC, Rolim VPM, Marvulo MFV, Silva JCR, Mota RA, Oliveira AAF (2018) Anticorpos anti-*Toxoplasma gondii* em carcarás (*Caracara plancus*) procedentes da região aeroportuária do Recife, Pernambuco. Brasil Arq Bras Med Vet Zootec 70:505–510
24. Cooper JE (2002) Methods of investigation and treatment. In: _____ Birds of prey: health and disease, 3rd Edition, Oxford, pp 28-70
25. Russel PK, Nisalak A, Sukhavachana P, Vivona S (1967) A plaque reduction test for dengue virus neutralizing antibodies. J Immunol 99:291–296
26. Sánchez-Seco MP, Rosario D, Domingo C, Hernández L, Valdés K, Guzmán MG, Tenorio A (2005) Generic RT-nested-PCR for detection of flaviviruses using degenerated primers and internal control followed by sequencing for specific identification. J Virol Methods 126:101–109
27. Ziegler U, Seidowski D, Angenvoort J, Eiden M, Muller K, Nowotny N, Groschup MH (2012) Monitoring of West Nile Virus infections in Germany. Zoonoses Public Health 59:95–101
28. Alba A, Andrés MP, Ponder J, Puig P, Wunschmann A, Vander Waal K, Alvarez J, Willette M (2017) Syndromic surveillance for West Nile virus using raptors in rehabilitation. BMC Vet Res 13:368. https://doi.org/10.1186/s12917-017-1292-0
29. Kritzik KL, Kratz G, Panella NA, Burkhalter K, Clark RJ, Biggerstaff BJ, Komar N (2018) Determining raptor species and tissue sensitivity for improved West Nile Virus surveillance. J Wildl Dis 54:528–533
30. Petrović T, Sekler M, Petric D, Lazic S, Debeljak Z, Vidanovic D, Cupina AI, Lazic G, Lupulovic D, Kolarevic M, Plavsic B (2018) Methodology and results of integrated WNV surveillance programmes in Serbia. PLoS One 13(4):e0195439
31. Nemeth N, Gould D, Bowen R, Komar N (2006) Natural and experimental West Nile Virus infection in five raptor species. J Wildlife Diaseases 42:1–13
32. Diaz LA, Komar N, Visintin A, Juri MJD, Stein M, Allender RL, Spinsanti L, Konighein B, Aguilar J, Laurito M, Almirón W, Contigiani M (2008) West Nile Virus in birds, Argentina. Emerg Infect Dis 14:689–691
33. Dıaz LA, Quaglia A, Flores FS, Contigiani MS (2011) West Nile Virus in Argentina: a new emerging infectious agent raising new challenges. El Hornero 26:5–28
34. Diaz LA, Quaglia AI, Konigheim BS (2016) Activity patterns of St. Louis encephalitis and West Nile viruses in free ranging birds during a human encephalitis outbreak in Argentina. PLoS One 11(8):e0161871
35. CDC - Centers for Disease Control and Prevention (2019). https://www.cdc.gov/westnile/resources/pdfs/BirdSpecies1999-2016.pdf. Accessed 1 May 2020
36. Lopez G, Jimenez-Clavero MA, Vazquez A, Soriguer R, Gomez-Tejedor C, Tenorio A, Figuerola J (2011) Incidence of West Nile Virus in birds arriving in wildlife rehabilitation centers in southern Spain. Vector-borne and Zoonotic Diseases 11:285–290
37. Bazanow B, van Vuren PJ, Szymanski O, Stygar D, Fracka A, Twardon J, Kozdrowski R, Paweska JT (2018) A survey on West Nile and Usutu viruses in horses and birds in Poland viruses 10:87. https://doi.org/10.3390/v10020087

38. Michel F, Fischer D, Eiden M, Fast C, Reuschel M, Müller K, Rinder M, Urbaniak S, Brandes F, Schwehn R, Lühken R, Groschup MH, Ziegler U (2018) West Nile virus and Usutu virus monitoring of wild birds in Germany. Int J Environ Res Public Health, 15171. https://doi.org/10.3390/ijerph15010171
39. Garcia-Bocanegra I, Busquets N, Napp S, Alba A, Zorrilla I, Villalba R, Arenas A (2011) Serosurvey of West Nile Virus and other flaviviruses of the Japanese encephalitis antigenic complex in birds from Andalusia, Southern Spain. Vector-borne and Zoonotic Diseases 11:1107–1113
40. Nemeth NM, Dwyer JF, Morrison JL, Fraser JD (2009) Prevalence of antibodies to West Nile Virus and other arboviruses among Crested Caracaras (*Caracara cheriway*) in Florida. J Wildl Dis 45:817–822
41. Gangoso L, Grande JM, Llorente F, Clavero MA-J, Perez M, Figuerola J (2010) Prevalence of neutralizing antibodies to West Nile Virus in Eleonora's Falcons in the Canary Islands. J Wildl Dis 46:1321–1324
42. Gancz AY, Barker IK, Lindsay R, Dibernardo A, McKeever K, Hunter B (2004) West Nile Virus outbreak in North American owls, Ontario, 2002. Emerg Infect Dis 10:2135–2142
43. Medica DL, Clauser R, Bildstein K (2007) Prevalence of West Nile Virus antibodies in a breeding population of American kestrels (*Falco sparverius*) in Pennsylvania. J Wildl Dis 43:538–541
44. Dube MC, Bird DM, Dibernardo A, Lindsay R, Charest H (2010) Prevalence of West Nile Virus in wild american kestrels (*Falco sparverius*) of Southern Quebec, Canada. J Wildl Dis 46:603–607
45. Dusek RJ, Iko WM, Hofmeister EK (2010) Occurrence of West Nile Virus infection in raptors at the Salton Sea, California. J Wildl Dis 46:889–895
46. Ringia AM, Blitvich BJ, Koo H-Y, Van de Wyngaerde M, Brawn JD, Novak RJ (2004) Antibody prevalence of West Nile Virus in birds, Illinois, 2002. Emerg Infect Dis 10:1120–1124
47. Captanian N, Hawkins MG, Diorello C, Thurber M, Reisen WK (2017) Low prevalence of West Nile Virus antibodies in select northern California owl species (2007–2014). J Zoo Wildlife Med 48:1239–1241
48. Eidson M, Komar N, Sorhage F, Nelson R, Talbot T (2001) Crow deaths as a sentinel surveillance system for West Nile Virus in the northeastern United States, 1999. Emerg Infect Dis 7:615–620
49. Randall NJ, Blitvich BJ, Blanchong JA (2012) Efficacy of wildlife rehabilitation centers in surveillance and monitoring of pathogen activity: a case study with West Nile Virus. J Wildl Dis 48:646–653
50. Nemeth N, Kratz G, Edwards E, Scherpelz J, Bowen R, Komar N (2007) Surveillance for West Nile Virus in clinic-admitted raptors, Colorado. Emerg Infect Dis 13:305–307
51. Tomazzoni AC, Pedó E, Hartz SM (2004) Food habits of great horned owls (*Bubo virginianus*) in the breeding season in Lami Biological Reserve, Southern Brazil. Ornitol Neotropical 15:279–282
52. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning M (2003) Experimental infection of north American birds with the New York 1999 strain of West Nile Virus. Emerg Infect Dis 9:3
53. Bernt MJ (2013) Blood-feeding behavior of *Culex tarsalis* at medicine Lake National Wildlife Refuge, Montana. Undergraduate Theses. Carroll College Natural Science Department, Helena, Montana
54. Pinheiro FP, LeDuc JW, Rosa APAT, Leite OF (1981) Isolation from St. Louis encephalitis virus from a patient in Belem, Brazil. Am J Trop Med Hyg 30:145–148
55. Mondini A, Bronzoni RVM, Cardeal ILS, Santos TMIL, Lázaro E, Nunes SHP, Silva GCD, Madrid MCFS, Rahal P, Figueiredo LT, Chiaravalloti Neto F, Nogueira ML (2007) Simultaneous infection by DENV-3 and SLEV in Brazil. J Clin Virol 40:84–86. https://doi.org/10.1016/j.jcv.2007.06.007
56. Rodrigues SG, Oliva OP, Araujo FAA, Martins LC, Chiang JO, Henriques DF, Silva EVP, Rodrigues DSG, Prazeres ASC, Tavares-Neto J, Vasconcelos PFC (2010) Epidemiologia do vírus da Encefalite de Saint Louis na Amazônia brasileira e no Estado do Mato Grosso do Sul, Brasil: elevada prevalência de anticorpos em equinos. Revista Pan-Amazônica de Saúde 1:81–86 https://doi.org/10.5123/S2176-62232010000100012
57. Pauvolid-Corrêa A, Tavares FN, Costa EV, Burlandy FM, Murta M, Pellegrin AO, Nogueira MF, Silva EE (2010) Serologic evidence of the recent circulation of Saint Louis encephalitis virus and high prevalence of equine encephalitis viruses in horses in the Nhecolândia sub-region in South Pantanal, Central-West Brazil. Mem Inst Oswaldo Cruz 105:829–833
58. Melandri V, Guimaraes AE, Komar N, Nogueira ML, Mondini A, Fernandez-Sesma A, Alencar J, Bosch I (2012) Serological detection of West Nile in horses and chicken from Pantanal, Brazil. Memorias do Instituto Oswaldo Cruz, Rio de Janeiro 107:1073–1075
59. Travassos da Rosa JFS, Freitas EM, Travassos da Rosa APA, Pinheiro FP (1980) Epidemiologia do vírus da encefalite de São Luis na Amazônia. Revista da FSESP 25:73–80
60. Vasconcelos PFC, Travassos da Rosa APA, Pinheiro FP, Shope RE, Travassos da Rosa JFS, Rodrigues SG, Travassos da Rosa ES, Dias, LB, Cruz, ACR (1998) Arboviruses isolated in the Evandro Chagas Institute, including some described for the first time in the Brazilian Amazon region, their known hosts, and their pathology for man. In: Travassos da Rosa APA, Vasconcelos PFC, Travassos da Rosa JFS. An overview of arbovirology in Brazil and neighboring countries. Belém (Brazil): Instituto Evandro Chagas p19–31
61. Reisen WK (2003) Epidemiology of St. Louis encephalitis virus. Adv Virus Res 61:139–183
62. Ometto TL (2013) Monitoramento do Oeste do Nilo no Brasil. PhD thesis. Universidade de São Paulo, São Paulo
63. Brasil. Ministério da Agricultura Pecuária e Abastecimento. (2018). Instrução Normativa n49 de 29 de Outubro de 2018