

Original Contribution

Serologic and Molecular Evidence of Arboviruses in Nonhuman Primates in Northeast Brazil

Illaira Leydira Carvalho Bandeira,¹ Marlos Gomes Martins,¹ Alessandra da Conceição Miranda Santos,² Camille Ferreira de Oliveira,² Éder Barros dos Santos,² Franko de Arruda e Silva,² Jannifer Oliveira Chiang,² Pedro Fernando da Costa Vasconcelos,^{2,3} and Patricia Avello Nicola¹

¹Universidade Federal do Vale do São Francisco, Rodovia BR-407, KM 12, Lote 543, Sem Número, Projeto de Irrigação Nilo Coelho, Petrolina, Pernambuco 56300-000, Brazil

²Instituto Evandro Chagas, Rodovia BR-316, KM 07, Sem número, Levilandia, Ananindeua, Pará 67030-000, Brazil

³Centro de Ciências Biológicas e da Saúde, Universidade do Estado do Pará, Travessa Perebeui, 2623, Marco, Belém, Pará 66087-662, Brazil

Abstract: Arbovirus surveillance in marmosets (*Callithrix* spp.) that live close to humans helps identify viral circulation in the environment and contributes to public health. We investigated the exposure to arboviral infections in 47 captive and free-living *Callithrix* from urban and peri-urban areas in the semiarid region of northeastern Brazil (SNB) in 2018. The samples were tested for antibodies against 23 arbovirus antigens and the presence of *Orthoflavivirus zikaense* (ZIKV), *Orthoflavivirus denguei*, *Alphavirus chikungunya*, and *Orthoflavivirus flavi* using hemagglutination inhibition (HI) and RT-qPCR assays. HI highlighted three samples seropositivity for *Flavivirus* (3/47; 6.4%). One of these samples displayed monotypic antibodies to *Alphavirus eastern* (formerly known as *Eastern equine encephalitis virus*; genus *Alphavirus*). RT-qPCR revealed that one sample (1/41; 2.4%) of *C. jacchus* captured in a commercial area of Petrolina-PE was positive for ZIKV. Our results indicated that marmosets living close to humans in the SNB were exposed to arboviruses, with the identification of ZIKV, even after the largest epidemic of the virus in the country and the subsequent human cases reduction. Here, we reinforce the importance of both continuous arboviruses monitoring in wildlife and preserving natural habitats to promote public health and biodiversity conservation.

Keywords: *Callithrix*, marmosets, *Flavivirus*, *Alphavirus eastern*, *Eastern equine encephalitis virus*, *Orthoflavivirus zikaense*

INTRODUCTION

Arbovirus emergence and spread are due to increased contact among humans, arthropod vectors, and other in-

fectured wildlife groups, such as the nonhuman primates (NHPs), in both sylvan and urban environments (Travassos da Rosa et al., 1997; Wolfe et al., 1998; Zanella, 2016). The *Zika virus* (ZIKV), recently renamed *Orthoflavivirus zikaense*, is an arbovirus that was first isolated from an NHP. Similar to other arboviruses, it represents a case of spillover where the virus was initially restricted to the sylvatic cycle

and has later adapted to the urban/peri-urban cycle (Dick et al., 1952; Bueno et al., 2016).

Orthoflavivirus zikaense was identified in humans for the first time in Brazil 2015 in the Northeast region (Campos et al., 2015), causing a major epidemic in the country. In 2016, the virus was confirmed as one of the causative agents of microcephaly in newborns (WHO, 2016). During this period, most cases of ZIKV and microcephaly in Brazil occurred in the country's northeastern region (Brasil, 2016, 2023). In parallel, ZIKV was first detected in marmosets (*Callithrix jacchus*) from areas close to the human population in the same region between 2015 and 2016 in municipalities which registered cases of ZIKV-associated microcephaly (Favoretto et al., 2016). Additionally, other molecular (using RT-PCR and nucleotide sequencing assays) and serological (plaque reduction neutralization test) studies detected ZIKV in *Callithrix* spp. in northeastern, central-western, and southeastern Brazil (Moreira-Soto et al., 2018; Terzian et al., 2018; Favoretto et al., 2019).

In addition to ZIKV, *Yellow fever virus* (YFV), recently renamed *Orthoflavivirus flavi* was also detected in *Callithrix* (by RT-PCR, genome sequencing, immunohistochemistry, and indirect immunofluorescence) from urban and urban-rural interface areas during or after outbreaks of the disease in humans in northeastern and southeastern Brazil (Cunha et al., 2019; Cunha et al., 2020; Mares-Guia et al., 2020; Fernandes et al., 2020; Sacchetto et al., 2020; Silva et al., 2023; Sousa et al., 2023; Garcia-Oliveira et al., 2024). These findings suggest the circulation and maintenance of YFV in the enzootic cycle involving marmosets from anthropogenic areas, even in the absence of human cases (Silva et al., 2023). Other studies in Brazil also reported antibodies against other arboviruses in *Callithrix* spp., such as *Ilheus virus* (ILHV), *Oropouche virus* (OROV), and *Mayaro virus* (MAYV) (Hoch et al., 1981; Pereira et al., 2001; Nunes et al., 2005; Gibrail et al., 2016).

Marmosets are common in urban and peri-urban forest areas and use anthropic resources directly or indirectly (tree planting, construction of water sources, and forest fragmentation), thus maintaining proximity to the human population (Bueno et al., 2016; SEMA, 2018; Sacchetto et al., 2020). Illegal and legal pet trade and humans' food provisioning to free-living marmosets expand the interface between humans and these animals (Gonzales and Júnior, 2016; SEMA, 2018). However, such direct contact can be problematic since NHPs are highly susceptible to common human infections, including some arboviral ones

(Wolfe et al., 1998; Gonzales and Júnior, 2016). On the other hand, *Callithrix*, as a diurnal and arboreal NHP, has frequent contact with vector mosquitoes in tree canopies and can provide information on arbovirus circulation in urban environments. Therefore, monitoring these animals may be helpful in preventing and control human arboviruses (Wolfe et al., 1998; Rylands et al., 2009; Batista et al., 2012; Haisi et al., 2022).

To increase the information on the circulation or maintenance of arboviruses among *Callithrix* in urban and peri-urban areas and to contribute to the surveillance of these diseases in Brazil, we investigated the presence or previous exposure to arboviral infections in captive and free-living *Callithrix* from semiarid Northeast region of Brazil from June to September 2018, after the large outbreak of ZIKV in humans. The study was performed in two neighboring cities, Petrolina (Pernambuco State) and Juazeiro (Bahia State), which belong to the states that reported the most significant number of cases of microcephaly (representing approximately 55%) in Brazil (Brasil, 2016).

These states are part of the Northeast region, region which ranked first or second in terms of the number of probable cases of dengue, chikungunya, and Zika in 2017 in Brazil (Brasil, 2023). The high number of cases of vector-borne diseases arises from the social vulnerability and environmental, sanitary, and economic conditions present in the region, which favor the vectors proliferation (Brasil, 2017a, b). Additionally, in both cities investigated, small marmoset groups are common in commercial or residential areas with some vegetational cover, and people usually feed these animals that maintain close contact. Given the convivium of people and marmosets in this area of Brazil displaying such high arbovirus diseases prevalence, we postulate that marmosets that live nearby would also be exposed to urban arboviruses.

METHODS

Ethics Statement

The Ethics Committee on the Use of Animals of the Federal University of Vale do São Francisco (UNIVASF) approved the study (n°. 0008/180418). The research was also authorized by the Chico Mendes Institute for Biodiversity Conservation (Sisbio license n°. 62,308–1) and registered in the National System for the Management of Genetic Her-

itage and Associated Traditional Knowledge (SISGEN) (n°. AF9D635).

The research followed all applicable institutional and/or national guidelines for the care and use of animals.

Sampling of Marmosets (*Callithrix* spp.)

The Juazeiro-BA and Petrolina-PE cities are located in the mesoregion of the São Francisco River Valley in the Northeast Region of Brazil and situated in the Caatinga biome, which has a semiarid tropical climate (CPATSA, 2022). IBGE estimated each city's population respectively of 215,183 and 343,865 inhabitants (IBGE, 2018).

As refers to marmoset population, both municipalities are in a natural hybridization zone of *C. jacchus* and *C. penicillata*, which stretches along the São Francisco River (SFR), with cryptic hybrids have been previously found within our sampling area (Malukiewicz et al., 2015). Although none of our individuals exhibited outward phenotypic signs of being hybrids (Vivo, 1991; Malukiewicz et al., 2014), we cannot full discount the fact that these may be present.

The investigation was performed on 47 *Callithrix* spp. specimens. Of these, 18 were captive (*C. jacchus* = 07; *C. penicillata* n = 11), and were sampled at the Center for Wild Animal Screening (CETAS) of Cema fauna, located in Petrolina-PE, Brazil (Table 1). These animals were obtained from domestic captivity by the legal authorities of inspection agencies in Bahia and Pernambuco. For free-living *Callithrix*, 29 animals (*C. jacchus* = 09; *C. penicillata* = 20) were captured through Tomahawk traps (Table 1). Capture locations included university centers, vocational education center, culture center, the Petrolina airport, and business and residential areas of both Petrolina and Juazeiro municipalities. We chose these trapping places due to the coexistence and/or proximity between humans and marmosets (Fig. 1).

All sampled *Callithrix* spp. were sedated using 10% ketamine (50 mg/kg dose) to measure their temperature and weight and collect blood samples. We collected a maximum volume of 1 ml of blood from each animal (due to their weight and small size) by venipuncture (femoral vein) and centrifuged the blood to obtain the serum. When serum volume was insufficient, we used stored whole blood for molecular analysis.

Arboviruses Detection in *Callithrix* spp.

The serological and molecular diagnoses of the NHPs were performed at the Instituto Evandro Chagas (IEC), located in Ananindeua, Pará, Brazil.

Hemagglutination Inhibition (HI) Assay

The serological identification of total antibodies (IgG and IgM) was performed in serum samples (n = 47) using the hemagglutination inhibition (HI) assay, according to Clarke and Casals (1958) and adapted for microplates by Shope (1963). The samples were tested for 23 arbovirus sucrose-acetone antigens from four different genera: *Alphavirus*, *Flavivirus*, *Orthobunyavirus*, and *Phlebovirus* (Table 2). Samples displaying a reaction $\geq 1:20$ were considered positive.

Reverse Transcription–Polymerase Chain Reaction (RT–qPCR)

The molecular diagnosis was performed on blood or serum samples (n = 41) with sufficient volume for the reverse transcription–polymerase chain reaction (RT–qPCR) assay. Total RNA extraction was performed from 140 μ L of sample, enriched with 2 μ L of exogenous MS2 RNA (concentration of 0.8 ng/ μ L) for extraction control, using a PureLink® RNA mini-Kit following the manufacturer's instructions.

The RNAs were submitted to RT–qPCR using the SuperScript III Platinum One-Step q-RT–PCR System (Invitrogen®) according to the manufacturer's specifications. Previously described primers and probes were used to detect *Orthoflavivirus denguei* (DENV serotypes 1–4) (Santiago et al., 2013), *Orthoflavivirus zikaense* (ZIKV 1086, 1162c, and 1107-FAM) (Lanciotti et al., 2008), *Alphavirus chikungunya* (CHIKV 6856, 6981, and 6919-FAM) (Lanciotti et al., 2007), and *Orthoflavivirus flavi* (YFallF, YfallR, and YFallP) (Domingo et al., 2012). Primers and probes for exogenous RNA (MS2) (Menting et al., 2011) were also added to each sample to confirm the successful RNA extraction.

RT–qPCR was carried out in an ABI PRISM 7500 Fast Real-time PCR thermocycler (Thermo Fisher Scientific®) using negative controls (of extraction and qPCR) and a positive control (viral RNA extracted from lyophilized antigens isolated from IEC). The cycling conditions used for all the primers were 50 °C for 30 min and 95 °C for two

Table 1. *Callithrix* spp. from Captivity and Free-living used in the Study (2018), Presenting the Origin, Species, Sex, and Age of Individuals.

City	Origin—Collection date		Species—Samples	Sex		Age		Total
				F	M	A	S	
Petrolina, Pernambuco, Brazil	Captivity	CETAS Cemafauna (June 2018)	<i>C. penicillata</i> V177; V178; V179; V180; V181; V182; V183; V190; V191; V192; V193	03	08	11	00	11
			<i>C. jacchus</i> V184; V185; V186; V187; V188; V189; V194	03	04	06	01	07
Total individuals in captivity				06	12	17	01	18
Juazeiro, Bahia, Brazil	Free life	University Center I (August 2018)	<i>C. penicillata</i> V195; V196; V197; V198; V199; V200	03	03	04	02	06
		University Center II (September 2018)	<i>C. penicillata</i> V219; V220; V221; V222; V223	02	03	04	01	05
		Vocation Education Center (September 2018)	<i>C. penicillata</i> V215; V216; V217; V218	03	01	04	00	04
		Culture Center (September 2018)	<i>C. penicillata</i> V210; V211; V212; V213; V214	04	01	04	01	05
Subtotal				12	08	16	04	20
Petrolina, Pernambuco, Brazil	Free life	Airport (August 2018)	<i>C. jacchus</i> V201; V202; V203	02	01	03	00	03
		Business Area (September 2018)	<i>C. jacchus</i> V208; V209	00	02	02	00	02
		Peridomestic Area (August 2018)	<i>C. jacchus</i> V204; V205; V206; V207	01	03	03	01	04
Subtotal				03	06	08	01	09
Total free-living individuals				15	14	24	05	29
Total studied subjects				21	26	41	06	47

F: Female; M: Male; A: Adult; S: Subadult.

minutes, followed by 45 cycles of 95 °C for 15 s and 60 °C for one minute.

The test results were analyzed using 7500 Real-Time PCR System software (version 2.0.6; Thermo Fisher Scientific®, Waltham, Massachusetts, USA), considering the detectable values of Ct (cycle threshold) ≤ 37 for DENV 1–4 (Santiago et al., 2013), YFV (IEC protocol), and the internal control MS2 (Menting et al., 2011) and Ct ≤ 38 for ZIKV (IEC protocol) and CHIKV (Lanciotti et al., 2007).

RESULTS

HI Assay

Of the 47 samples analyzed in the HI assay, three were seropositive (6.4%), displaying heterotypic reactions (antibodies reactive for more than one virus from the same genus) among the tested viruses of the genus *Flavivirus*. These samples belonged to two captive individuals from CETAS (V178—*C. penicillata*; V188—*C. jacchus*) and one free-living individual from the Petrolina peridomestic area (V205—*C. jacchus*) (Table 3).

However, only V188 sample reacted simultaneously to viruses of two genera (*Flavivirus* and *Alphavirus*), and displayed a monotypic reaction (antibodies for a single

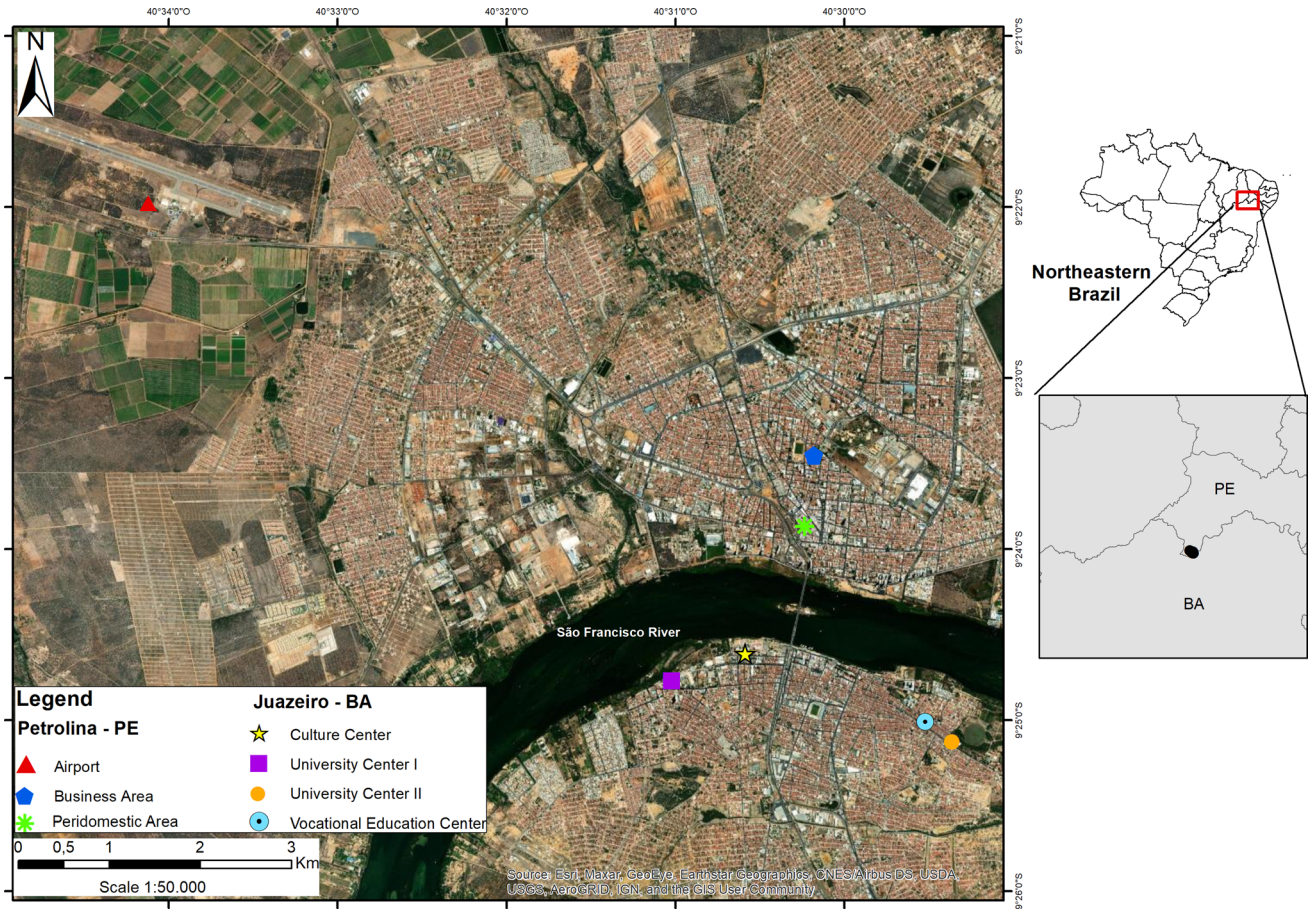


Figure 1. Capture locations for *Callithrix* spp. in anthropized areas of the municipalities of Petrolina-PE and Juazeiro-BA, 2018, Brazil.

virus in the same genus) for EEEV with a 1:40 titration (Table 3).

RT-qPCR Assay

Among the 41 samples tested via RT-qPCR, one (2.44%) was positive for the ZIKV envelope glycoprotein target gene, with an average Ct of 34.3. The positive sample belonged to a free-living *C. jacchus* individual (sample V209, male, adult, 279.8 g weight, and 37.3 °C body temperature) captured in the Petrolina-PE business area in August of 2018.

DISCUSSION

Our results suggest that *Callithrix* spp. from the Brazilian northeastern semiarid region, living in captivity or in urban and peri-urban areas, were exposed to arboviruses, especially *Flavivirus*, ZIKV, and EEEV. *Orthoflavivirus zikaense* is one of the main arboviruses that circulates and causes

epidemics in the Brazilian urban human population (Donalisio et al., 2017; Brasil, 2020). Although EEEV does not commonly circulate among humans, it has the potential to spread in Brazil and worldwide as synanthropic mosquitoes are its vectors (Aréchiga-Ceballos and Aguilar-Setién, 2015; Donalisio et al., 2017). However, additional studies would be necessary to accurately determine the exposure of the investigated marmoset to EEEV.

HI

Serological surveys in potential free-living or captive reservoirs constitute initial studies to understand viral dynamics and issues related to vertebrate host susceptibility, even though the pathogen has not been isolated (Batista et al., 2012). The HI assay is considered sensitive and ideal for seroepidemiological studies, as HI antibodies appear in the first week after infection onset and remain detectable for a long time (Travassos da Rosa et al., 1997, 1998).

Table 2. Arbovirus Sucrose-acetone Antigens Tested on Serum Aamples of *Callithrix* spp. in this Study According to the Virus Family and Genus.

Family	Genus	Virus	Abbreviation
<i>Togaviridae</i>	<i>Alphavirus</i>	<i>Alphavirus eastern</i> (Eastern equine encephalitis virus)	EEEV
		<i>Alphavirus western</i>	WEEV
		<i>Alphavirus mayaro</i>	MAYV
		<i>Alphavirus mucambo</i>	MUCV
		<i>Alphavirus chikungunya</i>	CHIKV
<i>Flaviviridae</i>	<i>Flavivirus</i>	<i>Orthoflavivirus nilense</i>	VNO
		<i>Orthoflavivirus flavi</i> (Yellow fever virus)	YFV
		<i>Orthoflavivirus denguei</i> 1	DENV-1
		<i>Orthoflavivirus denguei</i> 2	DENV-2
		<i>Orthoflavivirus denguei</i> 3	DENV-3
		<i>Orthoflavivirus denguei</i> 4	DENV-4
		<i>Orthoflavivirus ilheusense</i>	ILHV
		<i>Orthoflavivirus louisense</i>	SLEV
		<i>Orthoflavivirus cacipacoreense</i>	CPCV
		<i>Bussuquara virus</i>	BSQV
		<i>Rocio virus</i>	ROCV
		<i>Orthoflavivirus zikaense</i>	ZIKV
<i>Peribunyaviridae</i>	<i>Orthobunyavirus</i>	<i>Orthobunyavirus taciaumaense</i>	TCMV
<i>Peribunyaviridae</i>		<i>Orthobunyavirus maguariense</i>	MAGV
		<i>Orthobunyavirus caraparuense</i>	CARV
		<i>Orthobunyavirus oropoucheense</i>	OROV
		<i>Orthobunyavirus catuense</i>	CATV
<i>Phenuiviridae</i>	<i>Phlebovirus</i>	<i>Phlebovirus icoaraciense</i>	ICOV

Table 3. Serological Results of Total Antibodies (HI antibodies) in *Callithrix* spp. Captive and Free-living Captured from June to September 2018 in Brazil.

Origin	Sample	<i>Callithrix</i> Species	Virus Genus	Virus	Antibodies title
Captivity (CETAS—Cemafauna, Petrolina-PE)	V178	<i>C. penicillata</i>	<i>Flavivirus</i>	ILHV	1:20
				SLEV	1:20
	V188	<i>C. jacchus</i>	<i>Flavivirus</i>	VNO	1:20
				ILHV	1:20
				SLEV	1:20
				BSQV	1:40
				CPCV	1:40
				ROCV	1:20
Free-living (Peridomestic area, Petrolina-PE)	V205	<i>C. jacchus</i>	<i>Alphavirus</i>	EEEV	1:40
			<i>Flavivirus</i>	SLEV	1:40
				VNO	1:40
				DENV-4	1:20

ILHV: *Orthoflavivirus ilheusense*; SLEV: *Orthoflavivirus louisense*; VNO: *Orthoflavivirus nilense*; BSQV: *Bussuquara virus*; CPCV: *Orthoflavivirus cacipacoreense*; ROCV: *Rocio virus*; EEEV: *Alphavirus eastern* (Eastern equine encephalitis virus); DENV-4: *Orthoflavivirus denguei* 4.

In HI, however, cross-reactivity between arboviruses of the same genus is expected due to antigenic similarities. In these cases, it is not possible to define which virus species precisely infected the individual, only the viral genus (Travassos da Rosa et al., 1994). Based on this and being the HI employed in basic *Flavivirus* serology (Travassos da Rosa et al., 1997), our results indicate that the captive and free-living animals (Petrolina-PE) who presented heterotypic antibodies against different members of the *Flavivirus* (V178, V188, V205) were exposed to this viral genus.

Flaviviruses are known worldwide to cause some of the most impactful emerging diseases in terms of morbidity and mortality (Mackenzie et al., 2004). Currently, the main urban flaviviruses circulating in the Brazilian urban human population are DENV and ZIKV (Donalisio et al., 2017; Brasil, 2020). DENV is considered the most important arbovirus in humans due to the high numbers of cases, deaths, and people living in areas at risk of infection (Mackenzie et al., 2004; Lopes et al., 2014).

In Northeast Brazil, Pernambuco recorded 131,576 probable cases of dengue from 2007 to 2013, with Petrolina-PE ranking fifth in the state's "municipality infection" ranking (Brasil, 2019). It is essential to highlight that wild cycles of DENV in the Americas have not yet been identified (Degallier et al., 2001). However, experiments confirmed the *C. jacchus* and *C. penicillata* susceptibility to DENV infection (Omatsu et al., 2012; Ferreira et al., 2014), stressing the relevance of DENV monitoring in the species.

C. jacchus from CETAS (V188), which displayed EEEV seropositivity, was the only specimen that presented a reactive sample to the *Alphavirus* genus and a monotypic reaction in the study. This result suggests that the animal had been previously exposed to EEEV. However, even though the HI is sensitive, more specific tests (such as a neutralization test) would be necessary to accurately determine natural exposure to the virus, which was not possible in this study due to serum depletion. In experimental studies, already tested and confirmed this virus infection in *Callithrix* spp. (Adams et al., 2008; Porter et al., 2017).

Outbreaks of EEEV have already been observed in horses in the Brazilian states of Pernambuco, Ceará, and Paraíba (Silva et al., 2011), indicating circulation of the viral agent in the Northeast region. Moreover, two of the three mosquitoes genera transmitting EEEV to humans (*Aedes* and *Culex*) (Rust, 2012) are abundant in the Northeast region.

Alphavirus eastern is considered the most invasive and neurovirulent togavirus, with lethality in humans ranging from 30 to 70%. The infection may cause seizures, hemorrhage, coma, encephalomyelitis, and severe neurological sequelae (Rust, 2012; Aréchiga-Ceballos and Aguilar-Setién, 2015). Because it is considered a pathogen of global threat (Aréchiga-Ceballos and Aguilar-Setién, 2015), our findings in this study are of interest to public health.

The *Callithrix* spp. seropositives from CETAS might have been infected in loco or during domestic captivity before being captured by inspection agencies. Given the hypothesis of domestic exposure, the infection might have occurred outside Petrolina-PE, as the animals could have been in another municipality or state (considering animal traffic). However, for all the seropositives *Callithrix* in this study, due to the lack of more robust evidence, we cannot affirm that the exposure of the marmosets to arboviruses is related to close contact with humans, although such proximity is already a proven problem (Mätz-Rensing and Bleyer, 2018; Terzian et al., 2018; Bonfim et al., 2022; Oliveira and Santos, 2023).

The only HI-seropositive free-living individual (V205, *Flavivirus*-seropositive) in our study was in daily contact with humans who used to feed it. The animal was captured in a peridomestic area (a residence backyard) in the urban area of Petrolina-PE close to the SFR, an area where cryptic natural hybrids of *C. jacchus* versus *C. penicillata* have already been genetically identified (Malukiewicz et al., 2014, 2015).

Due to the capture location, it is possible that the V205 animal was a cryptic hybrid. In survey in southeastern Brazil, all *Callithrix* spp. positive for YFV (14.1%) were morphologically identified as hybrids (Cunha et al., 2020). Another study in the same region suggested that hybridization can influence the pathophysiology of YFV in *Callithrix* spp. (Cunha et al., 2019). Investigating these genetic aspects in *Callithrix* could reveal links between hybridization, arbovirus resistance, or susceptibility (compared to parental taxa) and implications for the genus itself and public health.

For humans, arboviruses cause, in general, socioeconomic damages (absenteeism, medical costs, transient or permanent complications, and mortality), burdening the country and affecting population quality of life (Lopes et al., 2014; Teich et al., 2017). In 2016, Pernambuco spent approximately R\$128 million on direct (associated with dengue, Zika, and chikungunya) and indirect medical costs and *Aedes aegypti* control expenses (Teich et al., 2017).

Currently, there are no effective drugs, vaccines, or treatments for most arboviruses; therefore, every effort to mitigate the impacts of infections is essential, especially for regions heavily affected by these diseases (Lopes et al., 2014; Donalisio et al., 2017).

RT-qPCR

RT-qPCR revealed ZIKV infection in free-living *C. jacchus* (V209) captured in the urban business area of Petrolina-PE during the post-epidemic period in the country. The largest ZIKV epidemic in Brazil occurred between 2015 and 2016, totaling 265,156 probable cases, 40.3% of which occurred in the Northeast region (Brasil, 2023). Despite the considerable incidence decrease in the region since 2017 (Brasil, 2023), concerns about the virus persist due to the economic and social impacts caused.

In urban areas with high occupation and disorderly urban growth, the primary ZIKV vector, *Aedes aegypti*, has a significant population density (Parra et al., 2022; Gomes et al., 2023). This situation commonly occurs in Brazilian Northeast Semiarid region, where socioeconomic vulnerabilities (infrastructure, water supply, human development, and family income) and recurrent water scarcity promote the vector proliferation (Brasil, 2017a, b).

Although the most significant number of cases of arboviruses transmitted by *A. aegypti* occur during the rainy season (January to June) (Silva et al., 2020; Sousa et al., 2021; Figueredo et al., 2023), the population begins to store water in open containers in the dry period in the region (the period in which V209 was captured), and these containers become artificial breeding grounds for mosquitoes (Brasil, 2017a, b). These containers are responsible for more than 75% of the vector reproduction sites in the region (Almeida et al., 2020; Brasil, 2017a, b). In 2018 (the year of our sampling), due to the number of properties with *A. aegypti* larvae, the city of Petrolina-PE was in outbreak alert for urban arboviruses, such as Zika (Brasil, 2018).

Based on our identification of ZIKV-positive *Callithrix* in the dry period, which is associated with a reduction in the number of human cases, we highlight concerns regarding the possible circulation or maintenance of the virus in the enzootic cycle due to conditions in the region. A Ministry of Health analysis (2015–2016) predicted the Brazilian Northeast Semiarid region would remain heavily affected by ZIKV (and other arboviral epidemics) due to maintenance of socioeconomic vulnerabilities

(Brasil, 2017a, b). This forecast has been confirmed: since 2019, the Northeast has shown the highest number of probable ZIKV cases in the country, while other regions have shown a decline (Brasil, 2023). Therefore, we warn of close human-marmoset contact, mainly in densely populated areas with competent vectors in the region, even outside epidemic periods.

Due to reported cases of ZIKV infection in *Callithrix* spp., studies have discussed the role of *Callithrix* (and other neotropical NHP species) in the urban transmission cycle of the virus and the possibility of species acting in the establishment of a ZIKV sylvatic cycle in Brazil (Favoretto et al., 2016, 2019; Moreira-Soto et al., 2018; Terzian et al., 2018).

Some evidence supports the hypothesis that these NHPs can act as reservoirs and amplification hosts that participate in the urban ZIKV maintenance and transmission (proposed by Terzian et al., 2018). One piece of evidence is the detection of ZIKV in marmosets living close to humans in urban or peri-urban areas (as seen in our findings) in the states of Ceará, Minas Gerais, and São Paulo between 2015 and 2017. The ZIKV strains detected were highly similar to those circulating in the country or related to the American lineage (Terzian et al., 2018; Favoretto et al., 2019).

Other evidence grounds on experimental ZIKV infections in *C. jacchus* and *C. penicillata* established and maintained in these animals after the viral exposure (Chiu et al., 2017; Terzian et al., 2018; Berry et al., 2019). *C. jacchus* showed high susceptibility to the strain tested and represented a possible reservoir of ZIKV as it sustained the rapidly established infection (Berry et al., 2019). Infection manifestations in marmosets were similar to those in humans, including asymptomatic infections, as seen in the ZIKV-positive *C. jacchus* in this study, which displayed no visible signs of disease (Chiu et al., 2017; Terzian et al., 2018; Berry et al., 2019). Finally, a mathematical study using NHPs (including *C. jacchus* and *C. penicillata*) suggested a high probability of establishing a sylvatic ZIKV cycle in the Americas since the NHP species that can become ZIKV hosts are abundant in South America, especially in Brazil (Althouse et al., 2016).

On the other hand, other studies involving these species did not find such evidence. In one of them, the researchers did not detect ZIKV in any NHPs analyzed in three municipalities (Northeast, Southeast, and South Regions of Brazil) between 2018 and 2020, 30% ($n = 30/100$) of which were *Callithrix* spp. (Haisi et al., 2022).

Another study involving *C. jacchus* and *C. penicillata* ($n = 20/207$; 9,7%) from urban and peri-urban areas severely affected by ZIKV in Northeast and Midwest Brazil (2012–2017) suggested that NHPs were not easily infected by the virus, even with intense local transmission. The authors argued that human population-acquired immunity limits urban transmission cycles. However, it is possible to establish sylvatic transmission cycles involving neotropical NHPs (Moreira-Soto et al., 2018). Such cycles could maintain ZIKV in the Americas until it is possible a new human outbreak; therefore, more studies covering different geographic areas and higher diversity of neotropical NHPs are needed (Moreira-Soto et al., 2018).

If ZIKV establishes a sylvatic cycle with neotropical NHPs, as it has in Africa (Althouse et al., 2016), eradication may become impractical, with severe epidemiological impacts. This could lead to new challenges in controlling possible outbreaks of congenital ZIKV syndrome (Althouse et al., 2016; Haisi et al., 2022), along with neurological complications (such as epilepsy, seizures, and hydrocephalus), ocular and cardiovascular deficiencies, and Guillain-Barré syndrome cases associated with ZIKV (Tureta et al., 2019). Biodiversity may also be threatened by new routes of infection and transmission between animal species (Althouse et al., 2016; Haisi et al., 2022).

The preservation of forests, conservation of biodiversity, and urban vector control are measures that can mitigate the zoonoses spread and reduce contact between humans and potential ZIKV wildlife reservoirs (Borzée, et al., 2020; Hilderink and Winter, 2021). To prevent human outbreaks and monitor viral circulation in the environment, surveillance in NHPs is recommended, as these animals act as sentinels (Batista et al., 2012; Cunha et al., 2019; Fernandes et al., 2020; Sousa et al., 2023). This active surveillance requires fieldwork and challenging technical standards, involving costs, and should be supported (Brazil, 2017; Calfe and Neto, 2020). *Orthoflavivirus zikaense* studies in *Callithrix* spp. in Brazil are still recent and scarce. Therefore, efforts are needed to understand better the participation of this genus in virus maintenance in the urban transmission cycle and to evaluate the potential of *Callithrix* as a vertebrate reservoir to establish a possible sylvatic cycle of the virus in Brazil.

CONCLUSION

Our results suggest the exposure of captive or free-living marmosets (*Callithrix* spp.) which lives close to humans in the semiarid region of northeastern Brazil to arboviruses (*Flavivirus*, ZIKV, and EEEV). Moreover, our results expand the still recent information on circulating ZIKV among marmosets in Brazil, even after the large outbreak in humans. Concerning EEEV, the finding of HI antibodies in *Callithrix* in the region is relevant, not only due to its virulence but because the mosquitoes that transmit this virus are abundant in Northeast Brazil. However, additional studies would be necessary to accurately determine the exposure of the investigated marmoset to EEEV, even with previous evidence of circulation of the viral agent in the region. Here, we reinforce the importance of continuous monitoring of arboviruses in wildlife and preserving natural habitats to promote public health and biodiversity conservation. This approach is crucial, especially concerning ZIKV (positive in RT-qPCR), which is frequent in Brazilian urban areas, and as *Callithrix* spp. are neotropical NHP species susceptible to the virus and in intense contact with human populations.

ACKNOWLEDGEMENTS

We acknowledge the whole team of Cema fauna that collaborated with the study, especially the veterinaries Adriana Alves Quirino and Gabriela Felix do Nascimento Silva, who provided substantial support on the capture of the marmosets, collection of the biological samples, and in all care involved with the animals. We also acknowledge the team of the Evandro Chagas Institute for the essential support in the getting diagnostic. And we acknowledge Joanna Malukiewicz, Silvia M. M. Gutierre and Jessica Viviane Amorim Ferreira for important contributions for the development of this study.

This work was supported by a grant from the Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE) process n° IBPG 0985 2.01/16 (for ILCB). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

- Adams AP, Aronson JF, Tardif SD, Patterson JL, Brasky KM, Geiger R, de la Garza M, Carrion R Jr, Weaver SC (2008) Common marmosets (*Callithrix jacchus*) as a nonhuman primate model to assess the virulence of eastern equine encephalitis virus strains. *Journal of Virology* 82:9035–9042. <https://doi.org/10.1128/JVI.00674-08>
- Almeida LS, Cota ALS, Rodrigues DF (2020) Saneamento, Arboviroses e Determinantes Ambientais: impactos na saúde urbana. *Ciência & Saúde Coletiva* 25:3857–3868. <https://doi.org/10.1590/1413-812320202510.30712018>
- Althouse BM, Vasilakis N, Sall AA, Diallo M, Weaver SC, Kathryn AH (2016) Potential for Zika Virus to Establish a Sylvatic Transmission Cycle in the Americas. *PLOS Neglected Tropical Diseases* 10:e0005055; <https://doi.org/10.1371/journal.pntd.0005055>.
- Aréchiga-Ceballos N, Aguilar-Setién A (2015) Alphaviral equine encephalomyelitis (Eastern, Western and Venezuelan). *Revue scientifique et technique* (International Office of Epizootics) 34:491–501; <https://doi.org/10.20506/rst.34.2.2374>.
- Batista PM, Andreotti R, Chiang JO, Ferreira MS, Vasconcelos PFC (2012) Seroepidemiological monitoring in sentinel animals and vectors as part of arbovirus surveillance in the state of Mato Grosso do Sul, Brazil. *Revista Da Sociedade Brasileira De Medicina Tropical* 45:168–173. <https://doi.org/10.1590/S0037-86822012000200006>
- Berry N, Ferguson D, Ham C, Hall J, Jenkins A, Giles E, Devshi D, Kempster S, Rose N, Dowall S, Fritzsche M, Bleazard T, Hewson R, Almond N (2019) High susceptibility, viral dynamics and persistence of South American Zika virus in New World monkey species. *Scientific Reports* 9(1):14495; <https://doi.org/10.1038/s41598-019-50918-2>.
- Borzée A, McNeely J, Magellan K, Miller JRB, Porter L, Dutta T, Kadinjappalli KP, Sharma S, Shahabuddin G, Aprilinayati F, Ryan GE, Hughes A, Mutalib AHA, Wahab AZA, Bista D, Chavanich SA, Chong JL, Gale GA, Ghaffari H, Ghimirey Y, Jayaraj VK, Khatiwada AP, Khatiwada M, Krishna M, Lwin N, Paudel PK, Sadykova C, Savini T, Shrestha BB, Strine CT, Sutthacheep M, Wong EP, Yeemin T, Zahirudin NZ, Zhang L (2020) COVID-19 Highlights the Need for More Effective Wildlife Trade Legislation. *Trends in Ecology & Evolution* 35:1052–1055. <https://doi.org/10.1016/j.tree.2020.10.001>
- Brasil (2016) Vírus Zika no Brasil: a resposta do SUS. Ministério da Saúde. Secretaria de Vigilância em Saúde. Available: http://bvsms.saude.gov.br/bvs/publicacoes/virus_zika_brasil_resposta_sus.pdf [accessed February 2, 2019].
- Brasil (2017) Guia de vigilância de epizootias em primatas não humanos e entomologia aplicada à vigilância da febre amarela. 2. ed. atual. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. Brasília. 100 p. Available: <http://vigilancia.saude.mg.gov.br/index.php/download/guia-de-epizootias-febre-amarela-2a-edicao-2017?wpdmdl=3736> [accessed April 03, 2018].
- Brasil (2017) Resumo executivo Saúde Brasil 2015/2016: uma análise da situação de saúde e da epidemia pelo vírus Zika e por outras doenças transmitidas pelo *Aedes aegypti* [recurso eletrônico]. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância de Doenças e Agravos Não Transmissíveis e Promoção da Saúde. Brasília. Available: http://bvsms.saude.gov.br/bvs/publicacoes/resumo_saude_brasil_2015_2016.pdf [accessed March 21, 2019].
- Brasil (2018) PE: 127 municípios em situação de alerta ou risco para dengue, zika e chikungunya. Ministério da Saúde. Available: <https://www.gov.br/saude/pt-br/assuntos/noticias/2018/dezembro/pe-127-municipios-em-situacao-de-alerta-ou-risco-para-dengue-zika-e-chikungunya> [accessed May 06, 2023].
- Brasil (2019) Dengue - Notificações registradas no sistema de informação de agravos de notificação – Pernambuco. Available: <http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinanet/cnv/denguePE.def> [accessed October 3, 2022].
- Brasil (2020) Óbito por arboviroses no Brasil, 2008 a 2019. Boletim epidemiológico 33(51). Ministério da Saúde. Available: <http://plataforma.saude.gov.br/anomalias-congenitas/boletim-epidemiologico-SVS-33-2020.pdf> [accessed February 16, 2021].
- Brasil (2023) Situação Epidemiológica. Série histórica - Casos prováveis de Zika (2015–2023); Série histórica - Casos prováveis de Dengue (2000 - 2023); Série histórica - Casos prováveis de Chikungunya (2014 a 2023). Ministério da Saúde. Available: <https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z> [accessed February 5, 2024].
- Bonfim FFO, Mares-Guia MAMM, Horta MA, Chame M, Lopes AO, Santos R, Matias CAR, Pinto MA, de Filippis AMB, de Paula VS (2022) *Callitrichine gammaherpesvirus 3* and *Human alphaherpesvirus 1* in New World Primate negative for yellow fever virus in Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz* 117:e210258; <https://doi.org/10.1590/0074-02760210258>.
- Bueno MG, Martinez N, Abdalla L, Duarte dos Santos CN, Chame M (2016) Animals in the Zika Virus Life Cycle: What to Expect from Megadiverse Latin American Countries. *PLOS Neglected Tropical Diseases* 10:e0005073; <https://doi.org/10.1371/journal.pntd.0005073>.
- Callefe JLR, Neto JSF (2020) Sistemas de Vigilância em Saúde Animal. São Paulo: Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia, 103 p. ISBN 978–65–87778–04–4.
- Campos GS, Bandeira AC, Sardi SI (2015) Zika virus outbreak, Bahia, Brazil. *Emerging Infectious Diseases* 21:1885–1886. <https://doi.org/10.3201/eid2110.150847>
- Chiu CY, Sánchez-San Martín C, Bouquet J, Li T, Yagi S, Tamhankar M, Hodara VL, Parodi LM, Somasekar S, Yu G, Giavedoni LD, Tardif S, Patterson J (2017) Experimental Zika Virus Inoculation in a New World Monkey Model Reproduces Key Features of the Human Infection. *Scientific Reports* 7:17126; <https://doi.org/10.1038/s41598-017-17067-w>.
- Clarke DH, Casals J (1958) Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *The American Journal of Tropical Medicine and Hygiene* 7:561–573. <https://doi.org/10.4269/ajtmh.1958.7.561>
- CPATSA. Centro de Pesquisa Agropecuária do Trópico Semi-Árido (2022). Empresa Brasileira de Pesquisa Agropecuária. Uma breve descrição do Semi-Árido Brasileiro e das duas cidades irmãs: PETROLINA - PE e JUAZEIRO – BA. Available: <http://www.cpatas.embrapa.br/catalogo/script/localizacao.htm> [accessed July 09, 2022].
- Cunha MS, da Costa AC, de Azevedo Fernandes NCC, Guerra JM, dos Santos FCP, Nogueira JS, D'Agostino LG, Komninakis SV, Witkin SS, Ressio RA, Maeda AY, Vasami FGS, Kaigawa UMA, de Azevedo LS, Facioli PAS, Macedo, FLL, Sabino EC, Leal É, de Souza RP (2019) Epizootics due to Yellow Fever Virus in São Paulo State, Brazil: viral dissemination to new areas (2016–2017). *Scientific Reports* 1:5474; <https://doi.org/10.1038/s41598-019-41950-3>.

- Cunha MS, Tubaki RM, de Menezes RMT, Pereira M, Caleiro GS, Coelho E, Saad Ldel C, Fernandes NCCdeA, Guerra JM, Nogueira JS, Summa JL, Coimbra AAC, Zwarg T, Witkin SS, Mucci LF, Timenetsky MdoCST, Sabino EC, de Deus JT (2020) Possible non-sylvatic transmission of yellow fever between non-human primates in São Paulo city, Brazil, 2017–2018. *Scientific Reports* 10:15751; <https://doi.org/10.1038/s41598-020-72794-x>.
- Degallier N, Teixeira JMS, AdeJM Chaib, Barbosa HF, CarvalhoMSLde, Oliveira, Cde, Knox MB, (2001) Avaliação do risco de transmissão silvestre da dengue no Brasil. *Informe Epidemiológico Do Sus* 10:13–15. <https://doi.org/10.5123/S0104-16732001000500003>
- Dick GW, Kitchen SF, Haddow AJ (1952) Zika virus. I. Isolations and serological specificity. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 46:509–520; [https://doi.org/10.1016/0035-9203\(52\)90042-4](https://doi.org/10.1016/0035-9203(52)90042-4).
- Domingo C, Patel P, Yillah J, Weidmann M, Méndez JA, Nakouné ER, Niedrig M (2012) Advanced Yellow Fever Virus Genome Detection in Point-of-Care Facilities and Reference Laboratories. *Journal of Clinical Microbiology* 50:4054–4060. <https://doi.org/10.1128/JCM.01799-12>
- Donalísio MR, Freitas ARR, Von Zuben APBV (2017) Arboviroses emergentes no Brasil: desafios para a clínica e implicações para a saúde pública. *Revista De Saúde Pública* 51:1–6. <https://doi.org/10.1590/s1518-8787.2017051006889>
- Favoretto S, Araujo D, Oliveira D, Duarte N, Mesquita F, Zanotto P, Durigon E (2016) First detection of Zika virus in neotropical primates in Brazil: a possible new reservoir. *bioRxiv*; <https://doi.org/10.1101/049395>.
- Favoretto SR, Araujo DB, Duarte NFH, Oliveira DBL, da Crus NG, Mesquita F, Leal F, Machado RRG, Gaio F, Oliveira WF, Zanotto PMA, Durigon EL (2019) Zika Virus in Peridomestic Neotropical Primates, Northeast Brazil. *EcoHealth* 16:61–69. <https://doi.org/10.1007/s10393-019-01394-7>
- Fernandes NCC de A, Guerra JM, Cunha MS, Beraldo KRF, Ressio RA, Cirqueira C dos S, D' Agostini TL, de Camargo JP, Landi NCSF, Saad L del C, Spinola RMF, de Paula RAC, Sanches TC, Rivas L, Catão-Dias JL (2020) Yellow fever surveillance challenge: Investigation of a marmoset non-autochthonous case. *Acta Tropica* 212: 105702; <https://doi.org/10.1016/j.actatropica.2020.105702>.
- Ferreira MS, Castro PHG, Silva GA, Casseb SMM, Dias Júnior AG, Rodrigues SG, Azevedo RdoS, Costa e Silva MF, Zauli DA, Araújo MS, Béla SR, Teixeira-Carvalho A, Martins-Filho OA, Vasconcelos PF (2014) *Callithrix penicillata*: a feasible experimental model for dengue virus infection. *Immunology Letters* 158: 126–33; <https://doi.org/10.1016/j.imlet.2013.12.008>
- Figueredo SA, Melo Sousa NSdeM, Melo AFM, Sousa RN, Monroe TGR (2023) Perfil epidemiológico de arboviroses no estado do Maranhão durante os anos de 2017 a 2021. *Revista Ibero-Americana de Humanidades, Ciências e Educação* 1:504–513; <https://doi.org/10.51891/rease.v1i1.10541>
- Garcia-Oliveira GF, Guimarães ACDS, Moreira GD, Costa TA, Arruda MS, de Mello EM, Silva MC, de Almeida MG, Hanley KA, Vasilakis N, Drumond BT (2024) YELLOW ALERT: Persistent Yellow Fever Virus Circulation among Non-Human Primates in Urban Areas of Minas Gerais State, Brazil (2021–2023). *Viruses* 16(1):31; <https://doi.org/10.3390/v16010031>.
- Gibrail MM, Fiaccadori FS, Souza M, Almeida TNV, Chiang JO, Martins LC, Ferreira MS, Cardoso DdasDdeP (2016) Detection of antibodies to Oropouche virus in non-human primates in Goiânia City, Goiás. *Revista da Sociedade Brasileira de Medicina Tropical* 49(3):357–360; <https://doi.org/10.1590/0037-8682-0425-2015>.
- Gomes H, Gomes de Jesus A, Quaresma JAS (2023) Identification of risk areas for arboviruses transmitted by *Aedes aegypti* in northern Brazil: A One Health analysis. *One Health* 16:100499; <https://doi.org/10.1016/j.onehlt.2023.100499>.
- Gonzales IAA, Júnior CAOM (2016) Concepções e Práticas dos Visitantes do Parque do Ingá, Maringá-PR Acerca da Alimentação dos Saguís (*Callithrix jacchus*). *Journal of Health Sciences* 18:19–23. <https://doi.org/10.17921/2447-8938.2016v18n1p23-2>
- Haisi A, Wu S, Zini N, da Silva MLCR, Malossi CD, Cubas ZS, Cubas PH, Teixeira RHF, de Sousa MS, Lucena RB, Svoboda WK, Osaki SC, Nogueira ML, Ullmann LS, Junior JPA (2022) Lack of serological and molecular evidences of Zika virus circulation in nonhuman primates in three states from Brazil. *Memórias do Instituto Oswaldo Cruz* 117:e220012; <https://doi.org/10.1590/0074-02760220012>.
- Hoch AL, Peterson NE, Leduc JW, Pinheiro FP (1981) An outbreak of Mayaro virus disease in Belterra, Brazil. *The American Society of Tropical Medicine and Hygiene* 30(3):689–698; <https://doi.org/10.4269/ajtmh.1981.30.689>.
- IBGE. Estados e cidades do Brasil (2018) Instituto Brasileiro de Geografia e Estatística. Available: <https://cidades.ibge.gov.br> [accessed November 17, 2018].
- Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, Campbell GL, 2007 Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, Campbell GL (2007) Chikungunya Virus in US Travelers Returning from India, 2006. *Emerging Infectious Diseases* 13:764–767; <https://doi.org/10.3201/eid1305.070015>
- Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, Stanfield SM, Duffy MR (2008) Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007. *Emerging Infectious Diseases* 14:1232–1239. <https://doi.org/10.3201/eid1408.080287>
- Lopes N, Nozawa C, Linhares REC (2014) Características gerais e epidemiologia dos arbovírus emergentes no Brasil. *Revista Pan-Amazônica De Saúde* 5:55–64. <https://doi.org/10.5123/S2176-62232014000300007>
- Hilderink MH, de Winter II (2021) No need to beat around the bushmeat—The role of wildlife trade and conservation initiatives in the emergence of zoonotic diseases. *Heliyon* 7:e07692; <https://doi.org/10.1016/j.heliyon.2021.e07692>.
- Mackenzie J, Gubler D, Petersen L (2004) Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nature Medicine* 10:S98–S109. <https://doi.org/10.1038/nm1144>
- Malukiewicz J, Boere V, Fuzessy LF, Grativol AD, de Oliveira e Silva I, Pereira LCM, Ruiz-Miranda CR, Valença YM, Stone AC (2015) Natural and Anthropogenic Hybridization in Two Species of Eastern Brazilian Marmosets (*Callithrix jacchus* and *C. penicillata*). *PLoS ONE* 10:e0127268; <https://doi.org/10.1371/journal.pone.0127268>.
- Malukiewicz J, Boere V, Fuzessy LF, Grativol AD, French JA, de Oliveira e Silva I, Pereira LCM, Ruiz-Miranda CR, Valença YM, Stone AC (2014) Hybridization effects and genetic diversity of the common and black-tufted marmoset (*Callithrix jacchus* and *Callithrix penicillata*) mitochondrial control region. *American journal of physical anthropology* 155:522–536; <https://doi.org/10.1002/ajpa.22605>

- Mares-Guia MAMdeM, Horta MA, Romano A, Rodrigues CDS, Mendonça MCL, dos Santos CC, Torres MC, Araujo ESM, Fabri A, de Souza ER, Ribeiro ROR, Lucena FP, Junior LCA, da Cunha RV, Nogueira RMR., Sequeira PC, de Filippis AMB (2020) Yellow fever epizootics in nonhuman primates, Southeast and Northeast Brazil (2017 and 2018). *Parasites Vectors* 13:1-8; <https://doi.org/10.1186/s13071-020-3966-x>
- Mätz-Rensing K, Bleyer M (2019) Chapter 15 - Viral Diseases of Common Marmosets. In: *The Common Marmoset in Captivity and Biomedical Research*, Marini R, Wachtman L, Tardif S, Mansfield K, Fox J (editors), American College of Laboratory Animal Medicine : Academic Press, pp. 251–264; <https://doi.org/10.1016/B978-0-12-811829-0.00015-7>.
- Menting S, Thai KTD, Nga TTT, Phuong HL, Klatser P, Wolthers KC, Binh TQ, de Vries PJ, Beld M (2011) Internally Controlled, Generic Real-Time PCR for Quantification and Multiplex Real-Time PCR with Serotype-Specific Probes for Serotyping of Dengue Virus Infections. *Advances in Virology* 2011:514681; <https://doi.org/10.1155/2011/514681>.
- Moreira-Soto A, Carneiro IO, Fischer C, Feldmann M, Kümmerer BM, Silva NS, Santos UG, Souza BFC, Liborio FA, Valença-Montenegro MM, Laroque PO, da Fontoura FR, Oliveira AVD, Drosten C, de Lamballerie X, Franke CR, Drexler JF (2018) Limited Evidence for Infection of Urban and Peri-urban Non-human Primates with Zika and Chikungunya Viruses in Brazil. *mSphere* 3: e00523–17; <https://doi.org/10.1128/mSphere.00523-17>.
- Nunes MRT, Martins LC, Rodrigues SG, Chiang JO, RdoS Azevedo, Travassos da Rosa APA, Vasconcelos PFC (2005) Orpouche virus isolation, southeast Brazil. *Emerging Infectious Diseases* 11:1610–1613. <https://doi.org/10.3201/eid1110.050464>
- Oliveira AR, Santos RL (2023) Infectious diseases of neotropical primates. *Brazilian Journal of Veterinary Pathology* 16:1–34. <https://doi.org/10.24070/bjvp.1983-0246.v16i1p1-34>
- Omatsu T, Moi ML, Takasaki T, Nakamura S, Katakai Y, Tajima S, Ito M, Yoshida T, Saito A, Akari H, Kurane I (2012) Changes in hematological and serum biochemical parameters in common marmosets (*Callithrix jacchus*) after inoculation with dengue virus. *Journal of Medical Primatology* 41:289–296. <https://doi.org/10.1111/j.1600-0684.2012.00552.x>
- Parra MCP, Lorenz C, Milhim BHGdeA, Dibo MR, Guirado MM, Chiaravalloti-Neto F, Nogueira ML (2022) Detection of Zika RNA virus in *Aedes aegypti* and *Aedes albopictus* mosquitoes, São Paulo, Brazil. *Infection, Genetics and Evolution* 98:105226; <https://doi.org/10.1016/j.meegid.2022.105226>.
- Pereira LE, Suzuki A, Coimbra TLM, de Souza RP, Chamelet ELB (2001) Arbovírus Ilheus em aves silvestres (*Sporophila caerulescens* e *Molothrus bonariensis*)*. *Revista De Saúde Pública* 35:119–123. <https://doi.org/10.1590/S0034-89102001000200003>
- Porter AI, Erwin-Cohen RA, Twenhafel N, Chance T, Yee SB, Kern SJ, Norwood D, Hartman LJ, Parker MD, Glass PJ, DaSilva L (2017) Characterization and pathogenesis of aerosolized eastern equine encephalitis in the common marmoset (*Callithrix jacchus*). *Virology Journal* 14:25; <https://doi.org/10.1186/s12985-017-0687-7>.
- Rust RS (2012) Human arboviral encephalitis. *Seminars in Pediatric Neurology* 19:130–151. <https://doi.org/10.1016/j.spen.2012.03.002>
- Rylands AB, Coimbra-Filho AF, Mittermeier RA (2009) The Systematics and Distributions of the Marmosets (*Callithrix*, *Callibella*, *Cebuella*, and *Mico*) and Callimico (*Callimico*) (Callitrichidae, Primates). In: *The Smallest Anthropoids, Developments in Primatology: Progress and Prospects*, Ford SM, Porter LM, Davis LC (editors), Boston, DC: Springer, pp. 25–61. https://doi.org/10.1007/978-1-4419-0293-1_2.
- Sacchetto L, Silva NIO, de Rezende IM, Arruda MS, Costa TA, de Mello EM, Oliveira GFG, Alves PA, de Mendonça VE, Stumpp RGAV, Prado AIA, Paglia AP, Perini FA, Lacerda Nogueira M, Kroon EG, de Thoisy B, Trindade GS, Drumond BP (2020) Neighbor danger: Yellow fever virus epizootics in urban and urban-rural transition areas of Minas Gerais state, during 2017–2018 yellow fever outbreaks in Brazil. *PLOS Neglected Tropical Diseases* 14: e0008658; <https://doi.org/10.1371/journal.pntd.0008658>.
- Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, Medina F, Colón C, Margolis H, Muñoz-Jordán JL (2013) Analytical and Clinical Performance of the CDC Real Time RT-PCR Assay for Detection and Typing of Dengue Virus. *PLOS Neglected Tropical Diseases* 7:e2311; <https://doi.org/10.1371/journal.pntd.0002311>.
- SEMA. Secretaria de Estado do Meio Ambiente (2018) Resolução SMA n° 164, de 27 de novembro de 2018. Available:<https://smastr16.blob.core.windows.net/legislacao/2018/11/resolucao-sma-164-2018-processo-2029-2016-estabelece-procedimentos-para-reproducao-de-callithrix.pdf> [accessed March 14, 2018].
- Shope RE (1963) The use of a microhemagglutination-inhibition test to follow antibody response after arthropod-borne virus infection in a community of forest animals. *Anais de Microbiologia* 11:167–171; http://iah.iec.pa.gov.br/iah/fulltext/memo_iec/v7p175-178.pdf.
- Silva MLCR, Galiza GJN, Dantas AFM, Oliveira RN, Iamamoto K, Achkar SM, Riet-Correa F (2011) Outbreaks of Eastern equine encephalitis in Northeastern Brazil. *Journal of Veterinary Diagnostic Investigation* 23:570–575. <https://doi.org/10.1177/1040638711403414>
- NdeS Silva, Alves JMB, da Silva EM, Lima RR (2020) Avaliação da Relação Entre a Climatologia, as Condições Sanitárias (Lixo) e a Ocorrência de Arboviroses (Dengue e Chikungunya) em Quixadá-CE no Período Entre 2016 e 2019. *Revista Brasileira De Meteorologia* 35:485–492. <https://doi.org/10.1590/0102-7786353001>
- Silva NIO, Albery GF, Arruda MS, Oliveira GFG, Costa TA, de Mello EM, Moreira GD, Reis EV, da Silva SA, Silva MC, de Almeida MG, Becker DJ, Carlson CJ, Vasilakis N, Hanley KA, Drumond BP (2023) Ecological drivers of sustained enzootic yellow fever virus transmission in Brazil, 2017–2021. *PLOS Neglected Tropical Diseases* 17(6): e0011407; <https://doi.org/10.1371/journal.pntd.0011407>.
- Sousa DER, Wilson TM, Macêdo IL, Romano APM, Ramos DG, Passos PHO, Costa GRT, Fonseca VS, Mares-Guia MAMM, Giovanetti M, Alcantara LCJ, de Filippis AMB, Paludo GR, Melo CB, Castro MB (2023) Case report: Urbanized non-human primates as sentinels for human zoonotic diseases: a case of acute fatal toxoplasmosis in a free-ranging marmoset in coinfection with yellow fever virus. *Front Public Health* 11:1236384; <https://doi.org/10.3389/fpubh.2023.1236384>.
- Sousa SSdaS, Silva BPda, Tadei WP, Silva JSda, Bezerra JMT, Pinheiro VCS (2021) Perfil reprodutivo de *Aedes aegypti* e *Aedes albopictus* de uma área urbana endêmica para arboviroses da região Nordeste do Brasil. *Research, Society and Development* 10: e6310917631; <https://doi.org/10.33448/rsd-v10i9.17631>
- Teich V, Arinelli R, Fahham L (2017) *Aedes aegypti* e sociedade: o impacto econômico das arboviroses no Brasil / *Aedes aegypti* and society: the economic burden of arboviruses in Brazil. *Jornal Brasileiro De Economia Da Saúde (Impr.)* 9:267–276. <https://doi.org/10.21115/JBES.v9.n3.p267-76>

- Terzian ACB, Zini N, Sacchetto L, Rocha RF, Parra MCP, Del Sarto JL, Dias ACF, Coutinho F, Rayra J, da Silva RA, Costa VV, Fernandes NCC de A, Réssio R, Díaz-Delgado J, Guerra J, Cunha MS, Catão-Dias JL, Bittar C, Reis AFN, dos Santos INP, Ferreira ACM, Cruz LEAA, Rahal P, Ullmann L, Malossi C, de Araújo Jr JP, Widen S, de Rezende IM, Mello É, Pacca CC, Kroon EG, Trindade G, Drumond B, Chiaravalloti-Neto F, Vasilakis N, Teixeira MM, Nogueira ML (2018) Evidence of natural Zika vírus infection in neotropical non-human primates in Brazil. *Scientific Reports* 8:16034; <https://doi.org/10.1038/s41598-018-34423-6>.
- Travassos da Rosa APA, Travassos da Rosa JFS, Pinheiro FP, Vasconcelos PFC (1997) Arboviroses. In: *Doenças Infecciosas e Parasitárias: Enfoque Amazônico*, Freire G, Freire ARC (editors), Belém, DC: Instituto Evandro Chagas, pp. 208–225.
- Travassos da Rosa APA, Travassos da Rosa ES, Travassos da Rosa JFS, Dégallier N, Vasconcelos PFC, Rodrigues SG, Cruz ACR (1998) Os arbovírus no Brasil: Generalidades, Métodos e Técnicas de Estudo. Documento técnico n. 02, 46 p. Instituto Evandro Chagas, Belém. Available: <https://patua.iec.gov.br/handle/iec/2703?show=full> [accessed May 25, 2018].
- Tureta EF, Rosa RLda, Berger M, Santi L, Guimarães JA, Beys-da-Silva WO (2019) O impacto do vírus Zika no Brasil e no mundo. *Revista Liberato* 20:113-210. <https://doi.org/10.31514/rliberato.2019v20n34.p153>
- Vivo M (1991) *Taxonomia de Callithrix Erxleben, 1777* (Callitrichidae, Primates), Rylands AB, Bernardes AT (editors), Belo Horizonte, DC: Fundação Biodiversitas para Conservação da Diversidade Biológica, pp. 21–70.
- WHO. World Health Organization (2016) Surveillance for Zika virus infection, microcephaly and Guillain-Barré syndrome: interim guidance. Available: https://apps.who.int/iris/bitstream/handle/10665/204897/WHO_ZIKV_SUR_16.2_por.pdf [accessed January 21, 2019].
- Wolfe ND, Escalante AA, Karesh WB, Kilbourn A, Spielman A, Lal AA (1998) Wild primate populations in emerging infectious disease research: the missing link? *Emerging Infectious Diseases* 4:149–158. <https://doi.org/10.3201/eid0402.980202>
- Zanella JRC (2016) Zoonoses emergentes e reemergentes e sua importância para saúde e produção animal. *Pesquisa Agropecuária Brasileira* 51:510–519. <https://doi.org/10.1590/S0100-204X2016000500011>

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.