


## Arboviruses in captive non-human primates from Pará state Amazon

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Marcella Kathyryne Marques Bernal<sup>1</sup>, Giselle Almeida Couceiro<sup>2</sup>, Jannifer Oliveira Chiang<sup>3</sup>, André Antônio Correa das Chagas<sup>4</sup>, Vânia Pinto Sarmiento<sup>5</sup>, Lucas Tainã Amorim Monteiro<sup>6</sup>, Larissa Pereira Rodrigues<sup>7</sup>, Ligia Souza Lima Silveira da Mota<sup>8</sup> and Washington Luiz Assunção Pereira<sup>9</sup>

### ABSTRACT

Investigations on health and the human-animal-environment interrelationship make it possible to record zoonotic etiological agents, such as arboviruses and, in relation to non-human primates (NHP), the Amazon stands out for having a rich diversity of these animals that can be potential hosts for arboviruses. In this aspect, the objective of this study was to evaluate the occurrence of arboviruses in captive NHP in the State of Pará. For this purpose, blood samples from 43 specimens from two sponsoring institutions in the State of Pará were used. The sera were analyzed at the Laboratory of Arbovirology and Hemorrhagic Fevers of the Evandro Chagas Institute, Pará State. The Hemagglutination Inhibition test and neutralization test were used. Of the analyses, 25 (58.14%) were positive for one or more of the 19 arboviruses tested, with 68% (17/25) females and 32% (8/25) males. As a result, 71.43% (20/28) of the animals that shared the same enclosure, and 33.33% (5/15) of those that lived in quarantine were positive. Also, of the 25 seropositive strains for arboviruses, 20 (80%) had monotypic reactions and 12 (48%) heterotypic reactions for flaviviruses. Only one sample presented titration  $\geq 1280$  for Oropouche arbovirus, and was submitted to the neutralization test, whose Logarithmic Index result of  $\geq 1.9$  was considered positive, suggesting that this primate has a recent infection. The presence of antibodies to the main arboviruses occurring in the Amazon in NHP, due to the expression of immunity, suggests that these animals were infected by the viruses at some stage of life, and may maintain an enzootic cycle.

**Keywords:** Arboviruses, Primates, Captivity.

<sup>1</sup> Doctor in Animal Health and Production in the Amazon, Evandro Chagas Institute.

<sup>2</sup> Doctor student in Animal Health and Production in the Amazon, Federal Rural University of the Amazon.

<sup>3</sup> Doctor in Biology of Infectious and Parasitic Agents, Evandro Chagas Institute.

<sup>4</sup> MSc in Food Science and Technology, Evandro Chagas Institute.

<sup>5</sup> Doctor student in Virology, Evandro Chagas Institute.

<sup>6</sup> Undergraduate student in Veterinary Medicine, Federal Rural University of the Amazon

<sup>7</sup> Master's Degree in Wild Animals, São Paulo State University "Júlio de Mesquita Filho".

<sup>8</sup> Doctor in Biological Sciences – Genetics, São Paulo State University "Júlio de Mesquita Filho".

<sup>9</sup> Doctor in Biological Sciences, Federal Rural University of the Amazon.



## INTRODUCTION

Arboviruses are viral microorganisms that occur in an endemic or epidemic way (LEÃO, 1997), the families that can affect both humans and animals are: Flaviviridae, Reoviridae, Rhabdoviridae, Bunyaviridae and Togaviridae (RUTS, 2012).

Its records usually involve a wild environment, being transmitted through the bite from hematophagous arthropods or from invertebrate host to invertebrate by transovarian or sexual route (LEÃO, 1997). Wild animals infected by arboviruses are usually asymptomatic, but in humans, the symptoms will depend on the nature of the virus, causing from benign fever to encephalitis, which can lead to death (CLETON et al., 2002; CASSEB et al., 2013).

These microorganisms exhibit a preferential occurrence in tropical and subtropical regions, in Brazil, there are 210 different types of arboviruses, of which 200 were isolated for the first time in the Amazon region, 34 of which are pathogenic to humans (AZEVEDO et al., 2007; VASCONCELOS et al., 2010).

The Amazon has more than 100 species and subspecies of primates (KIERULFF et al., 2005) and, due to this great diversity, there is still much to be clarified about the ecology and health of these animals (MELLO, 1995; DINIZ, 1997), which contributes to the lack of knowledge that these are potential hosts for zoonotic diseases and thus establish an enzootic environment. Thus, the present study aimed to evaluate the presence of antibodies to arboviruses in non-human primates (NHP) in captivity in the State of Pará.

## METHODOLOGY

The research was approved by SISBIO No. 40309-1 and by the Ethics Committee on the Use of Animals (CEUA) of the Federal Rural University of the Amazon No. 032012-301. A total of 43 juvenile and adult animals (15 quarantine animals and 28 shared enclosure animals) belonging to the following families were examined: Cebidae, Aotidae, Atelidae and Pitheciidae, from two wildlife institutions in the State of Pará.

For blood collection, each animal was captured with the aid of puçá and then physically restrained. In some cases, it was necessary to use sedatives (Ketamine 10 mg/kg, Midazolam 0.2 mg/kg and Levomepromazine 0.2 mg/kg) intramuscularly (CUBAS, 2007). The collection was performed in the right or left femoral vein, using a sterile syringe and needle, in a volume of 2 to 5 mL of blood per animal, according to animal size/species, and then centrifuged to obtain the serum, which was stored in eppendorf at -20 °C, remaining stored until serological processing.

The processing and serological analysis of blood samples for the occurrence of Arboviruses was performed by the Laboratory of Arbovirology and Hemorrhagic Fevers of the Evandro Chagas Institute (IEC). For this procedure, the hemagglutination inhibition (HI) test was chosen, which was

performed according to the technique described by Clark and Cassals (1985) and adapted to microplates by Shope (1963), using a panel with 19 different antigens of the most common arbovirus in the Amazon (Table 1).

Table 1 - Panel of arbovirus antigens used in the Hemagglutination Inhibition test

FAMILY/GENDER			
<i>Togaviridae</i> ( <i>Alphavirus</i> )	<i>Flaviviridae</i> ( <i>Flavivirus</i> )	<i>Bunyavirity</i> ( <i>Arthbunavirus</i> ) ( <i>Phlebovirus</i> )	
VEEE	VFA	VGUA	VICO
VWEE	VILH	VTAC	-
VMAY	VSLE	VMAG	-
VMUC	VCPC	VUTI	-
-	VBUS	VCAR	-
-	VROC	VORO	-
-	-	VCAT	-
-	-	Bethlehem Virus	-

VEEE: Virus da encefalomielite equina leste; VWEE Virus da encefalite equina oeste; VMAY: Mayaro virus; VMUC: Mucambo virus; VFA: Virus da febre amarela; VILH: Ilheus virus; VSLE: Virus da encefalite Saint Louis; VCPC: Cacipacore virus; VBUS: Bussuquara virus; VROC: Rocio virus; VGUA: Guaroa virus; BIRD: Tacaiuma virus; VMAG: Maguari virus; VUTI: Utinga virus; VCAR: Carapar virus; VORO: Oropouche virus; VCAT: Platoon virus; Belém virus; VICO: Icoaraci virus.

Source: Prepared by the authors, 2023.

The sera were previously treated with PA acetone to allow the concentration of proteins and the removal of natural inhibitors, as well as adsorbed with goose red blood cells to remove nonspecific hemagglutinins. After treatment, the sera were screened (titer 1:20), and the positive samples were titrated (1:40 to  $\geq$  1:1280).

The neutralization test (NT) was used for sera that presented cross-reacting of the same family and gender in the HI test, using constant dilutions of serum and serial dilutions of ten times of the virus (CASALS, 1967). Samples with a logarithmic neutralization index (ILN) greater than or equal to 1.8 LD<sub>50</sub>/0.02 mL were considered positive.

The data obtained were tabulated and analyzed by the statistical program SAS (2000), using Fisher's Exact Test, with a significance level of 5%.

## RESULTS AND DISCUSSION

Of the animals, 58.14% (25/43) were positive for one or more of the arboviruses tested. Females were more seropositive (68%) than males (32%), with a statistically significant difference.

Regarding the type of enclosure, 33.33% (5/15) were quarantine enclosures and 71.43% (20/28) were shared enclosures (Table 2).

Table 2 - Results of the Hemagglutination Inhibition test in non-human primates held captive in institutions in the State of Pará, according to sex and enclosure in which they lived.

NUMBER OF PRIMATES		ARBOVIRUS POSITIVE (%)
<b>Sex</b>		
Male	21	32 (8/25)
Female	22	68 (17/25)
<b>Enclosure</b>		
Quarantine	15	33,33 (5/15)
Shared enclosure	28	71,43 (20/28)

Source: Prepared by the authors, 2023.

Seropositivity was also found  $\geq 20$  in the IH test for arboviruses in seven NHP species, which are: *Atelles marginatus*, *Atelles paniscus*, *Cebus albifrons*, *Chiropotes albinasus*, *Chiropotes Santanas*, *Leontopithecus chrysomelas* and *Sapajus apella* (Table 3).

Table 3 - Frequency of arbovirus seropositivity according to non-human primate species.

Species	ARBOVIRUS(%)										
	EEE	WEE	MAY	MUC	ILH	ICO	SAO	CAC	BUS	GOLD	Flavivirus
<i>Atelles marginatus</i>	2,33						2,33	2,33		2,33	4,66
<i>Atelles paniscus</i>			2,33			2,33					6,99
<i>Cebus Albifrons</i>									4,66		2,33
<i>Chiropotes Albinasus</i>	2,33										
<i>Chiropotes santanas</i>	2,33					2,33					4,66
<i>Leontopithecus chrysomelas</i>					6,99	2,33					
<i>Saimiri sciureus</i>					2,33						
<i>Sapajus apella</i>		2,33		2,33	2,33				2,33		9,32
<b>TOTAL</b>	6,99	2,33	2,33	2,33	11,65	6,99	2,33	2,33	6,99	2,33	27,96

EEE = echelon equine encephalitis; WEE = western equine encephalitis; MAY = mayaro; MUC = mucambo; ILH = islets; icoaraci ICO; SÃO = são luiz; CAC = caciporé; BUS = bussquara; ORO = oropouche. Fisher = 0.0005648; P=0, 0.2810.

Source: Prepared by the authors, 2023.

Of the 25 arbovirus seropositive patients, 12 (48%) had heterotypic reactions to flavivirus and 20 monotypic reactions (80%) to EEE, WEE, MAY, MUC, ILH, SÃO, BUS, ORO, and ICO (Table 4).

Table 4 - Results of the titration of the HI test for arbovirus species in captive non-human primates in municipalities of the State of Pará.

ARBOVIRUS																							
HI Titration Result	Flavivirus		B And	WEE		MAY		MUC		ILH		SA O	CA C		BUS		GOLD		ICO	TO AL			
	n	%		n	%	n	%	n	%	n	%		n	%	n	%	n	%		n	%	n	%
20	-	-	2	4,66	-	-	1	2,33	-	-	-	1	2,33	1	2,33	3	6,99	-	-	-	-	8	18,64
40	-	-	1	2,33	1	2,33	-	-	1	2,33	-	-	-	-	-	-	-	-	-	2	4,66	5	11,65
80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2,33	1	2,33	-
160	-	-	-	-	-	-	-	-	-	4	9,33	-	-	-	-	-	-	-	-	-	-	4	9,33
320	-	-	-	-	-	-	-	-	-	1	2,33	-	-	-	-	-	-	-	-	-	-	1	2,33
640	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1280	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2,33	-	-	1	2,33	-

In this study, only one sample was positive for the arbovirus Oropoche in *Atelles marginatus* presented with a  $\geq 1280$  HI titration and was submitted to the Neutralization test, whose result of the logarithmic neutralization index (ILN) was  $\geq 1.9$ , and positive samples with  $ILN \geq 1.7$  were considered. Thus, it may suggest that such NHP presents a recent infection for ORO.

Arboviruses can be present in all representatives of the vertebrate orders (Murray et al., 2006). In this scenario, the Amazon is highlighted for having a great diversity of fauna, both hematoficient invertebrates and wild vertebrates, which makes the Amazon region the largest reserve of arboviruses in the world (TRAVASSOS DA ROSA et al., 1997; VASCONCELOS et al., 2001).

The high percentage (58.14%) of PNH reactive to the presence of antibodies to the main arboviruses occurring in the Amazon, demonstrates that these animals were infected at some stage of their lives. Particularly, the occurrence of Flavivirus in the analyzed samples is indicative of its circulation among NHP in the State of Pará. Where some species of these viruses exhibit neurotropism in humans. It should be noted that arboviruses can circulate between domestic animals and humans in an unapparent way, so that clinical manifestations can occur when they come into contact with wild niches in which arboviruses actively circulate (FIGUEIREDO, 2000).

Few studies involving the subject in NHP have been carried out in Brazil. Laroque et al. (2014) observed that 46 out of 100 (46%) animals were seropositive for arboviruses in *Cebus albidinosus*, while in *quarantine Cebus flavius*, there were no positive cases (0/31), suggesting that captive NHPs that are conglomerates are less susceptible to mosquito vectoring. The fact was



verified in the present study, where there was a significant difference in the results, with a positivity of 33.33% (5/15) for animals kept in quarantine, and 71.43% (20/28) for those kept in enclosures, indicating a difference in the circulation of the virus, considering that quarantine animals obtained little capture time when compared to animals in enclosures.

Batista et al. (2012), in the State of Mato Grosso do Sul, Brazil, evaluated 62 wild specimens, including *Allouata caraya*, *Callicebus donacophilus* and *Sapajus apela*, with 48.65% positivity for ORO, MAY, Alphavirus and Flavivirus in the HI test. This result is similar to that obtained in the present study. Studies on *Cebus libidinos* from northeastern Brazil showed serological positivity for arboviruses in the HI test, for the species: EEE, WEE, MAY, MUC, YF, ILH, SLE, ROC and ORO, and in an animal from the city of Belém/Pará WEE was isolated (LAROQUE et al., 2014). Experiments with NHP artificially infected by the Ilhéus virus showed the development of a high percentage of mortality in these animals (PINHEIRO; TRAVASSOS DA ROSA, 1994).

In addition, the present study verified serological positivity for arboviruses with monotypic reactions for EEE, WEE, MAY, ILH, ICO, SÃO, BUS, ORO and heterotypic for Flavivirus, in representatives of the cebidae family: *S. apella*, *Atelles marginatus*, *A. paniscus*, *Cebus albifrons*, *Saimiri sciureus*, *Leontopithecus chrysomelas*. Unprecedented results for the last five species of cebids e, for *Chiropotes satanas* and *C. albinasus* with EEE, and the last species of chiropotidae with ICO and Flavivirus. It should be noted that the Oropouche virus of the Bunyaviridae family, which in the present study had an occurrence of 2.33% and high titration in the HI test (1280), is considered the second species, among the arboviruses, that causes acute febrile systemic infection in humans in the Brazilian urban area, being an emerging viral zoonosis (MURPHY, 1998). Its cycle can be sylvatic with asymptomatic animals for this virus, highlighting PNH and cingulates, and in the urban the main vectors are *Culicoides* sp. and *Culex p.* (LEO, 1997).

One specimen studied, *A. marginatus* asymptomatic, which came from a fauna rescue apprehension and after quarantine began to live in a visitation enclosure with others of the same species, presented an ILN of  $\geq 1.9$ , suggesting that such an animal, when viremic, may serve as a potential source of infection, if there is a hematophagous invertebrate, for the other NHP. as well as for visitors and zoo workers.

## CONCLUSION

The presence of antibodies to the main arboviruses occurring in the Amazon in non-human primates, due to the expression of specific immunity, suggests that these animals were infected by the viruses at some stage of their lives, and may be maintaining an enzootic cycle.

In addition, an asymptomatic specimen of *A. marginatus* from rescue was observed during the study, which was later directed to the visitation enclosure, and due to its health condition, it was



hypothesized that if there is a hematophagous invertebrate vector, this animal, when viremic, can serve as a potential source of ingestion both for other animals and for visiting humans, evidencing the need for studies on arboviruses in other animals. captive non-human primates, in view of the zoonotic potential of several species of these viruses and the method of transmission in animals living in shared enclosures.

Also, this was the first record of evidence of the circulation of the Flavivirus for the species *Atelles marginatus* in the State of Pará, which evidences the adaptation of the virus to different species, justifying further research.



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