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Short Communication

Detection of antibodies to Oropouche virus in non-human primates in Goiânia City, Goiás

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Abstract

Introduction: Arboviruses are associated with human disease, and non-human primates (NHPs) are important primary hosts. This study shows the detection of antibodies to Oropouche virus (OROV) in NHPs either living in urban parks or acclimatized at the Wild Animal Screening Center, Goiânia city. **Methods:** Fifty blood samples were analyzed by hemagglutination-inhibition and neutralization assays. **Results:** Two monkeys (*Alouatta caraya*) had antibodies to OROV by both techniques. **Conclusions**: This is the first report demonstrating the detection of OROV antibodies in Goiás State and may represent the introduction/ circulation of OROV in the region and a potential risk to the human population.

Keywords: Non- Human Primates. Oropouche virus. Arbovirus.

Oropouche virus (OROV) belongs to the genus *Orthobunyavirus* and the family *Bunyaviridae*, which also comprises the genera *Hantavirus, Nairovirus*, *Phlebovirus,* and *Tospovirus*. Orthobunyaviruses include many human pathogens such as Caraparu, Catu, Guaroa, Marituba, and Tacaiuma[1]. OROV, like other orthobunyaviruses, is a single stranded, negative-sense, three-segmented virus. The segments, small (S), medium (M) and large (L), encode the nucleocapsid (N) protein, the surface (Gn and Gc) glycoproteins and the viral ribonucleic acid (RNA)-dependent RNA polymerase, respectively[2] [3]. In addition, in some orthobunyaviruses, the S and M segments can also encode the non-structural proteins NSs and NSm, respectively[3]. Phylogenetic analysis of the OROV genome has identified four virus genotypes (I, II, III and IV), all of which occur in Brazil[4].

OROV is an important agent responsible for at least 30 epidemics in the Amazon region of Brazil, which resulted in approximately 500,000 human cases of infection. Oropouche fever is characterized by an acute febrile illness with sudden onset. Infected patients report headaches, chills, myalgia, arthralgia, retroocular pain, and, in a few cases, it may be accompanied by aseptic meningitis or meningoencephalitis[5].

OROV is maintained in nature in two distinct cycles: urban and sylvatic. In urban areas, the midge, *Culicoides paraensis* is considered the primary vector and humans are the vertebrate hosts, while in the sylvatic cycle its primary vector remains unknown. A single strain of OROV was recovered from a pool containing the mosquito *Aedes serratus* in the Brazilian Amazon, and in Trinidad, another isolate was obtained from the mosquito *Coquillettidia venezuelensis*. Sloths, non-human primates (NHPs), and wild birds are considered to play a role in the maintenance cycle of this virus[2] [3] [5] [6].

Worldwide, 496 species of NHPs have been described. The NHPs from Brazil belong to the suborder *Platyrrhini,* that includes five families: Atelidae, Cebidae, Callitrichidae, Aotidae and Pitheciidae. The family *Atelidae*, includes the *Alouatta caraya* species; Cebidae, includes, *Cebus libidinosus* species; and Callitrichidae, includes the *Callithrix penicillata* species[7]. These species have been associated with yellow fever epidemics in Brazil[8] and are found endemically in parks and areas of vegetation in the municipality of Goiânia, in the State of Goiás, Central Brazil (**Figure 1**).

Among the 50 NHPs studied, 27 *Cebus libidinosus* individuals were living in three urban parks in Goiânia. The other 23 animals were acclimatized in the Wild Animal Screening Center [*Centro de Triagem de Animais Silvestres/ Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* (CETAS/IBAMA)]. Of these 23 animals, 15 were *Cebus libidinosus*, five were *Alouatta caraya,* and three were *Callithrix penicillata*. The animal samples from the Goiânia urban parks were collected in 2014, while those of acclimated animals at CETAS/IBAMA were collected in 2011 and 2013.

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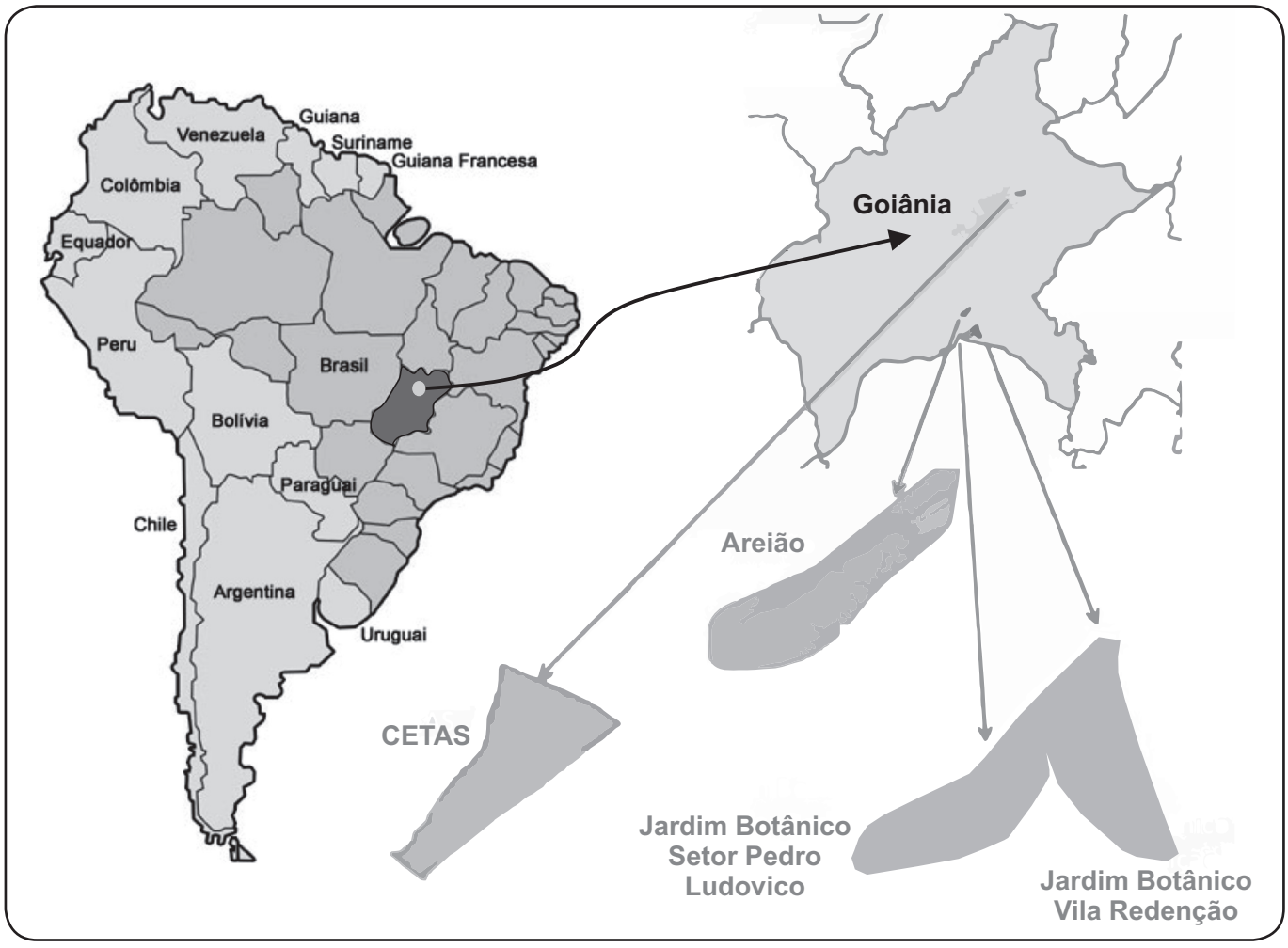

FIGURE 1 - Location of non-human primate capture and blood sampling sites. Shown are Goiânia City, Goiás: Parque Areião, Parque Jardim Botânico Setor Pedro Ludovico, Parque Jardim Botânico Vila Redenção, and CETAS-IBAMA. Adapted from SIGGO (*Sistema de Informações Geográficas de Goiânia*). **CETAS:** *Centro de Triagem de Animais Silvestres*; **IBAMA:** *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis*.

The animals were captured by attraction to a trap/cage *tomahawk* and they were chemically restrained by intramuscular administration of 2.0mg/kg tiletamine-zolazepam (Zoletil®). Physiological parameters were monitored until the end of sedation. The capture of each animal was performed only once, and confirmed on the basis of a microchip reading. From each animal, a 3-10ml blood sample was collected by femoral or brachial venipuncture depending on the species[8].

All sera samples were initially screened by an hemagglutination inhibition (HI) assay as described by Clarke and Casals et al.[9] and adapted to a microplate format by Shope[10]. Positive samples by HI, with a titer equal to or greater than 1:40, were confirmed by a separate neutralization test (NT) as described by Beaty et al.[11] Serological tests were performed in the *Laboratório de Referência para Arboviroses e Febres Hemorrágicas* of the *Instituto Evandro Chagas, Ananindeua, Pará*.

For the HI assay, serum samples were treated (100% acetone and 0.85% NaCl), hydrated with borate buffer saline (BBS; 1.5 M NaCl, 0.5M H_3BO_3, 1.0M NaOH, pH 9.0) and adsorbed with goose (*Anser cinereus*) erythrocytes. The test was performed in two steps; in the first step, each serum sample (1:20) was tested against 23 viral antigens (4 hemagglutinating units (HU) of each) prepared from intracerebral inoculation (IC) in newborn mice and centered on three different viral families, namely *Togaviridae* (eastern equine encephalitis, western equine encephalitis, Mayaro, and Mucambo viruses), *Bunyaviridae* (Caraparu, Catu, Guaroa, Maguari, Oropouche, Tacaiuma, Belem, and Icoaraci viruses) and *Flaviviridae* (yellow fever, Bussuquara, Cacipacore, Ilheus, Saint Louis encephalitis, Rocio, and dengue 1-4 viruses). After 1-hour incubation of the serum-antigen complex a suspension of goose erythrocytes was added.

In the second stage, the serially diluted samples (1:20-1:1280) were added to the specific antigen (4 HU), and subsequently, the goose erythrocyte suspension was added. The antibody titer was considered as the highest dilution that shows total inhibition of hemagglutination. For each reaction, controls were used for the red blood cells, antigens, and tested serum samples.

TABLE 1 - Seropositivity of non-human primates as tested by serum neutralizing test for arboviruses observed in Goiânia City, Goiás.

Species	Animals (n)	Neutralization index	Virus
Cebus libidinosus	11	0	Oropouche
*Alouatta caraya**	12	2,9	Oropouche
*Alouatta caraya***	20	2,8	Oropouche

*Animal with unknown provenance. **Animals from the City of Goiânia.

The NT assay was carried out using newborn mice (1-3 days). For each serum sample, 18 suckling mice were used. Three dilutions of each antigen were used. Serum samples were mixed with each of the specific antigens and incubated at 37°C for 60 minutes. Mice were then inoculated with 20µL of virus/antigen mixture by an IC route. For each reaction, negative and positive control sera were included. The assay readout was obtained by evaluating each animals survival within an inoculated group (6 animals were inoculated per dilution of virus). The antibody titer was considered as the average value of dilutions for each serum sample in which death occurred in more than 50% of inoculated animals [lethal dose (LD_{50})]. The titer was defined as the logarithmic neutralization index (LNI), using log 10, as described by Reed & Muench[12]. The sample was considered positive when its LNI was equal to or greater than 1.7.

Of the total NHPs studied, three animals had antibodies for OROV with a titer greater than 1:40 by the HI test. One of the animals was *Cebus libidinosus* and two were *Alouatta caraya*. When submitted to the neutralization test, the two samples of *Alouatta caraya* were confirmed as positive for OROV (**Table 1**).

In the period 2007-2008, a yellow fever epizootic circulation in NHPs inhabitants living in urban parks in Goiânia was registered, where the death of 61 animals from the species *Alouatta caraya*, *Cebus libidinosus* and *Callithrix penicillata* were recorded. In addition, human infections were also diagnosed[8], resulting in an outbreak of the disease.

Arboviruses have geographic distributions covering all continents. However, except for in temperate countries, maintenance cycles are interrupted owing to low temperatures[6]. Brazil has characteristics that favor the spread of these viruses: indeed, a third of the country is covered by tropical forest with is large degree of biodiversity and high density of vectors and vertebrate hosts than can easily co-circulate, enhancing the possibility of arbovirus maintenance and subsequent spread into urban and suburban areas[13].

This is the first study conducted in the State of Goiás aimed at investigating the occurrence of arboviruses in a population of NHPs living in urban conservation areas. In the present study, antibodies positive for antigens of OROV were detected in two *Alouatta caraya,* both of which had been acclimatized at the CETAS/IBAMA. The other sample, from *Cebus libidinosus*, which was first found to be positive by HI, was negative by NT. This could have been due to re-assortment, or even cross reaction, with other Orthobunyavirus species[14].

OROV is an arbovirus of great importance to public health owing to the increased morbidity of Oropouche fever in humans, with clinical presentations ranging from febrile illness to aseptic meningitis[5]. It has been also responsible for explosive epidemics in urban areas of large cities and small villages of the Amazon region, since its first outbreak in 1960[4]. The occurrence of this virus in humans has also been seen in Brazil and other South American countries[4], in concert with its occurrence in NHPs, as observed at Arinos, Minas Gerais State, Brazil (*Callithrix* genus)[2]. In the State of Goiás, to our knowledge, there have been no reports of the occurrence of Oropouche fever in humans. Therefore, the detection of antibodies for this virus in NHPs is important considering the possibility of dissemination among these animals, and the potential for introduction among humans living in populated areas. The presence in an NHP population may therefore present a risk to the human population. In fact, it is noteworthy that most of the animals from the CETAS/IBAMA were captured in urban parks of Goiânia, a city whose people have close contact with parks where animals live. It is not known whether *Culicoides paraensis*, the urban OROV vector, is found in the State of Goiás, but other *Culicoides* species have been described in this State that have the potential to become OROV vectors[15]. Finally, the confirmation of immunity to OROV in NHPs living in parks in Goiânia City should be considered as an important warning sign of ongoing virus circulation, and further studies should be conducted to implement continuous monitoring of these sentinel animals as an early warning of potential viral outbreaks.

ETHICAL CONSIDERATIONS

The study was approved by the Ethics Committee for Animal Use [*Comissão de Ética no Uso de Animais* (CEUA)] of the *Universidade Federal de Goiás* (Process 32/2014) and the Information System on Biodiversity [*Sistema de Informação em Biodiversidade* (SISBIO)] (Process 24629).

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Conflict of interest

The authors declare that there is no conflict of interest.

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REFERENCES

1. International Committee on Taxonomy of Viruses (Internet). Virology division: IUMS; 2013 (Cited 2015 January 28); Available at: http://ictvonline.org/virusTaxonomy.asp.
2. Nunes MRT, Martins LC, Rodrigues SG, Chiang JO, Azevedo RSS, Travassos da Rosa APA, et al. Oropouche virus isolation, Southeast Brazil. Emerg Infect Dis 2005; 11:1610-1613.
3. Shi X, Kohl A, Vincent HJ, Ping Li L, McLees A, Elliot RM. Requirement of the N-Terminal Region of Orthobunyavirus Nonstructural Protein NSm for Virus Assembly and Morphogenesis. J Virol 2006; 80:8089-8099.
4. Vasconcelos HB, Nunes MRT, Casseb LMN, Carvalho VL, Silva EVP, Silva M, et al. Molecular epidemiology of Oropouche virus, Brazil. Emerg Infect Dis 2011; 17:800-806.
5. Bastos MS, Figueiredo LTM, Naveca FG, Monte RL, Lessa M, Figueiredo RMP, et al. Identification of Oropouche Orthobunyavirus in the cerebrospinal fluid of three patients in the Amazonas, Brazil. Am J Trop Med Hyg 2012; 86:732-735.
6. Casseb AR, Casseb LMN, Silva SP, Vasconcelos PFC. Arbovírus: Importante zoonose na Amazônia brasileira. Vet e Zootec 2013; 20:9-21.
7. Primate Specialist Group. (Internet) IUCN/SSC; 2014 (Cited 2015 July 8); Available at: http://www.primate-sg.org/
8. Ministério da Saúde. Secretaria de Vigilância em Saúde. Boletim da Secretaria de Vigilância em Saúde. Situação da Febre Amarela Silvestre no Brasil, 2007 e 2008. (Internet). Brasília: MS; 2008 (Cited 2008 March 25); Available at: http://portal.saude.gov.br/saude/.
9. Clarke DH, Casals J. Technique for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. Am J Trop Med Hyg 1958; 7:561-573.
10. Shope RE. The use of a micro-hemagglutination inhibition test to follow antibody response after arthropod-borne virus infection in a community of forest animals. An Microbiol 1963; 11:167-171.
11. Beaty BJ, Calisher CH, Shope RE. Arboviruses. *In*: Schmidt NJ, Emmons EW, editors. Diagnostic procedures for viral, rickettsial and chlamydial infections. 6th edition. Washington: American Public Health Association; 1989. p. 797-855.
12. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am J Epidemiol 1938; 27: 493-497.
13. Figueiredo LTM. Emergent arboviruses in Brazil. Rev Soc Bras Med Trop 2007; 40:224-229.
14. Aguilar PV, Barrett AD, Saeed MF, Watts DM, Russell CG, Ampuero JS, et al. Iquitos Virus: a novel reassortant Orthobynyavirus associated with human illness in Peru. PLos Negl Trop Dis 2011; 5:e1315:1-10.
15. Naves HAM, Carvalho MESV, Costa JA, Oliveira RA. Frequência domiciliar de Culicidae in zona urbana de Goiânia-Goiás-Brasil. Rev Pat Trop 1996; 1:43-49.