

SHORT ARTICLES

IMMUNITY TO YELLOW FEVER, OROPOUCHE AND SAINT LOUIS VIRUSES IN A WILD HOWLER MONKEY

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Introduction

Arboviruses are arthropod-borne RNA viruses that circulate in wildlife and may cause disease in people and domestic animals. Six out of about 40 arboviruses that cause disease in humans represent threats to public health in Brazil: Yellow Fever (YFV), Oropouche (OROV), Dengue, Mayaro, Saint Louis Encephalitis (SLEV) and Rocio (Vasconcelos et al., 1998). Nonhuman primates (NHP) are important hosts in the cycles of many arboviruses. An analysis of 35 NHP in central Brazil showed 10 (26%) positive for Mayaro, 5 (14%) for OROV, and 6 (17%) for more than one arbovirus (Batista et al., 2012).

The sylvatic cycle of YFV in South America includes mosquitoes, mainly *Haemagogus* spp. and *Sabethes* spp., and NHP (Vasconcelos, 2003; Cardoso et al., 2010), but the virus also circulates in several other forest mammals (de Thoisy et al. 2004). Outbreaks of this disease causing illness and/or death of free-ranging NHP are common in several Latin American countries, including Brazil (Araújo et al., 2011; Almeida et al., 2012, 2014). Whereas *Cebus* and *Sapajus* are more resistant to YF, *Saimiri*, *Ateles*, *Aotus* and, especially, *Alouatta* are particularly sensitive to the disease (Bugher, 1951).

Similar to YFV, OROV can be found in an urban cycle between humans and mosquitoes and a sylvatic cycle that involves NHP, sloths and birds as hosts, and *Culicoides* spp. mosquitoes as vectors (Vasconcelos et al., 1998). SLEV is also transmitted by mosquitoes, mainly *Culex* spp. (Vasconcelos et al., 1998). A bird-mosquito SLEV cycle is the most common in the wild. However, vectors also bite NHP, marsupials and rodents (Mondini et al., 2007).

The pathogenicity and population effects of YFV on howler monkeys are relatively well-studied (Holzmann et al., 2010; Freitas and Bicca-Marques, 2011, 2013; Almeida et al., 2012; Agostini et al. 2014; Engelmann et al., 2014), but little is known about SLEV and OROV. In this paper we report the finding of a wild adult male black-and-gold

howler monkey (*Alouatta caraya*) presenting antibodies against YFV, OROV, and SLEV.

Materials and methods

We captured the male (head-and-body length=56 cm, tail length=56 cm, weight=8 kg) in a ca. 23-ha gallery forest fragment in the municipality of Santo Antônio das Missões (28°23'27.6"S, 55°26'26.3"W), Rio Grande do Sul State, south Brazil. The animal belonged to a social group composed of, at least, two adult females with offspring and an immature individual of unknown sex. He appeared to be in good health, showing no sign of illness or any kind of weakness, and was released at the same site after recovering from the anesthesia (about 1 h later).

We anaesthetized the monkey with the help of a CO₂ propelled dart gun as part of a routine active surveillance of the circulation of YFV and other arboviruses in the state (Almeida et al., 2014). We collected blood (ca. 8 mL) from the femoral vein. We stored the serum and an additional 1 mL aliquot of blood in cryotube vials after centrifugation and frozen them in liquid nitrogen. We isolated the virus (VI) from the blood sample by inoculating it into suckling mice (Beatty et al., 1989) and C6/36 cells (Gubler et al., 1984). These trials were followed by indirect immunofluorescence assays using monoclonal antibodies. We detected antibodies in the serum by the haemagglutination inhibition (HI) and neutralization tests (NT) (Deubel et al., 1979). We performed the tests for detecting arboviruses at Instituto Evandro Chagas, a reference laboratory of the Brazilian Ministry of Health.

This study complied with ethical guidelines for the use of animals in research, the Brazilian environmental laws, and the American Society of Primatologists' guidelines for the ethical treatment of nonhuman primates. All procedures described in this report were conducted under the permit for scientific activities #13016-6 issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) of the Brazilian Ministry of the Environment in May 10, 2012.

Results

Isolation trials were negative, but serology was positive for arboviruses (titers of 1:40 to 1:80 for flaviviruses and 1:40 for OROV in the HI test). The logarithm of the neutralization index (LNI) in the NT test for DL50/0,02 mL was positive for viral antigens specific for YFV (LNI=4.5), SLEV (LNI=3.0) and OROV (LNI=2.9).

Discussion

The finding of antibodies against arboviruses of public health concern in this howler monkey highlights the importance of the active monitoring of NHP conducted by the State Center for Health Surveillance of the State Health

Secretariat for identifying potential areas of circulation of arboviruses in south Brazil. The surveillance of NHP in Rio Grande do Sul State was an important tool in the prioritization of target areas for vaccination during a large YF epizooty that took place between 2008 and 2009 killing >2,000 howler monkeys (*A. caraya* and *A. guariba clamitans*) (Almeida et al., 2012, 2014).

It is probable that our study subject was infected with YFV during that epizooty. Considering that *A. caraya* groups often live in home ranges <10 ha (Fortes et al., 2015) and that howlers are reluctant to cross open fields on the ground, it is likely that the adult male was infected with all three arboviruses within the forest fragment inhabited by his group. Although we cannot infer on the timing of these infections based on our single case, a previous infection with the Flaviviridae SLEV may have improved the individual's resistance to YF. This hypothesis has critical conservation implications and deserves future research because both *A. caraya* (Endangered) and *A. g. clamitans* (Vulnerable) are threatened with extinction in Rio Grande do Sul State (Decree #51797, 8 September 2014). Previous studies found antibodies against SLEV in *A. caraya* (10/19 individuals) and *A. g. clamitans* (3/7) in Rio Grande do Sul State (Santos et al., 2006) and in *A. caraya* (5/43), other primates and horses in Paraná State, Brazil (Svoboda et al., 2014).

Finally, greater surveillance efforts on broader taxonomic groups are required to inform us on the susceptibility of mammalian and avian species to these emerging infectious diseases. Long-term monitoring of the prevalence of infected and resistant individuals in populations of NHP, other mammals, and birds, as well as mosquito vectors, are critical to assess their roles in the maintenance of cycles of these viruses and the risks that they pose to primate conservation and public health.

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PRELIMINARY OBSERVATIONS ON THE SAN MARTIN TITI MONKEY *PLECTUROCEBUS OENANTHE* THOMAS, 1924 (MAMMALIA: PRIMATES: PITHECIIDAE) VOCALIZATIONS AT TARANGUE, PERU.

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Introduction

Like other members of the former *Callicebus* species group (*Callicebus*, *Cheracebus* and *Plecturocebus*, *Sensu* Byrne et al., 2016), the San Martin titi monkey (*Plecturocebus oenanthe*, *Sensu* Byrne et al., 2016) engages regularly in ritualized bouts of song, defined by Moynihan (1966) as a series of rapidly and regularly repeated notes, distinctly separated from preceding and succeeding notes by long pauses. For socially monogamous, territorial species such as titi monkeys, night monkeys and gibbons (Kawai et al., 1982; Mitani 1984; Fernandez-Duque 2011), loud calls (including song) are thought to define territorial boundaries, and may strengthen and/or maintain bonds between mates (Wickler 1980; Kinzey and Robinson 1983; Robinson et al., 1987; Müller and Anzenberger 2002; Caselli et al., 2014). The vocal behavior of titi monkeys has been the focus of several studies (for example Moynihan 1966; Robinson 1979; Kinzey and Robinson 1983; Müller and Anzenberger 2002; Kitzmann et al., 2008; Cäsar et al., 2012a; Caselli et al., 2014). However, the repertoire of only one species of titi monkey, *Plecturocebus cupreus* (formerly *Callicebus moloch*), has been well-described (Moynihan 1966; Robinson 1979; Robinson 1981; Robinson et al., 1987). More recently, researchers described in detail the acoustic properties of the syllables of which the loud calls and song of *Callicebus nigrifrons* are composed (Caselli et al., 2014).