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Infection rates and genotypes of *Trypanosoma rangeli* and *T. cruzi* infecting free-ranging *Saguinus bicolor* (Callitrichidae), a critically endangered primate of the Amazon Rainforest

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ABSTRACT

Parasites of wild primates are important for conservation biology and human health due to their high potential to infect humans. In the Amazon region, non-human primates are commonly infected by *Trypanosoma cruzi* and *T. rangeli*, which are also infective to man and several mammals. This is the first survey of trypanosomiasis in a critically endangered species of tamarin, *Saguinus bicolor* (Callitrichidae), from the Brazilian Amazon Rainforest. Of the 96 free-ranging specimens of *S. bicolor* examined 45 (46.8%) yielded blood smears positive for trypanosomes. *T. rangeli* was detected in blood smears of 38 monkeys (39.6%) whereas *T. cruzi* was never detected. Seven animals (7.3%) presented trypanosomes of the subgenus *Megatrypanum*. Hemocultures detected 84 positive tamarins (87.5%). Seventy-two of 84 (85.7%) were morphologically diagnosed as *T. rangeli* and 3 (3.1%) as *T. cruzi*. Nine tamarins (9.4%) yielded mixed cultures of these two species, which after successive passages generated six cultures exclusively of *T. cruzi* and two of *T. rangeli*, with only one culture remaining mixed. Of the 72 cultures positive for *T. rangeli*, 62 remained as established cultures and were genotyped: 8 were assigned to phylogenetic lineage A (12.9%) and 54 to lineage B (87.1%). Ten established cultures of *T. cruzi* were genotyped as TCI lineage (100%). Transmission of both trypanosome species, their potential risk to this endangered species and the role of wild primates as reservoirs for trypanosomes infective to humans are discussed.

1. Introduction

Some vector-borne zoonoses such as trypanosomiasis and leishmaniasis pose considerable risk to humans and wildlife and may have deleterious effects that trigger or accelerate the decline of threatened species. *Saguinus bicolor* (bare-faced tamarin) belongs to the family Callitrichidae with a geographical distribution restricted to the vicinities of the cities of Manaus, Rio Preto da Eva, and Itacoatiara, State of Amazonas (Egler, 1983). This species was included in the Red List of Threatened Species of the International Union for the Conservation of Nature and Natural Resources (IUCN), and is considered, with *Cebus kaapori*, the most endangered primate species of the Amazonian region (Rylands et al., 2003).

S. bicolor is a diurnal arboreal monkey that uses a mosaic of vegetation, including primary and secondary forests, "campinarana" forests, and forest edges. Tamarins are primarily insectivores and frugivorous but also feed on nectar, exudates and small vertebrates (Egler, 1993; Rylands and Faria, 1993; Vidal and Cintra, 2006). As other tamarins, this species lives in groups and uses tangles of twigs and lianas in trees, holes in trunks, and palms as nocturnal resting sites. The areas inhabited by *S. bicolor* have abundant palm trees, which provide the nocturnal refuges for the tamarins and are the preferential ecotopes of hematophagous triatomine bugs, notably *R. pictipes* and *R. robustus*, which are the most important vectors of *Trypanosoma cruzi* and *T. rangeli* all over Amazonia (Miles et al., 1983a; Coura et al., 2002; Maia da Silva et al., 2007; Aguilar et al., 2007).

Some species of trypanosomes are infective to human and non-human primates around the world (Marinkelle, 1976). Neotropical monkeys in addition to harbouring *T. cruzi* and *T. rangeli* are also

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infected by *T. saimiri* and *T. mycetae*, species allied to *T. rangeli*, and by *T. devei*, *T. minasense* and *T. lambrechti*, which belong to the subgenus *Megatrypanum*. Except for *T. cruzi* and *T. rangeli*, no other species infecting monkeys have been reported as infective to humans. Trypanosomes were commonly reported in monkeys of the families Callitrichidae (tamarins and marmosets), Cebidae (squirrel monkeys and capuchins), Aotidae (night monkeys) and Atelidae (spider monkeys and howler monkeys) (Deane et al., 1970; Sousa et al., 1974; Marinkelle, 1976; Lanham et al., 1984; D'Alessandro et al., 1986; Ziccardi and Lourenço-de-Oliveira, 1997; Ziccardi et al., 2000; Lisboa et al., 2004a,b, 2006, 2007; Maia da Silva et al., 2004a,b, 2007).

Although the available data indicate that trypanosomiasis should be very common in some Amazonian primates in the wild, few species of wild primates have so far been examined. The largest survey on Amazonian primates was done using blood smear microscopy, hemoculture and xenodiagnosis and revealed a high prevalence of trypanosomes (67.9%) in 165 squirrel monkeys (*Saimiri sciureus* and *Saimiri ustus*), with the following trypanosome species: *T. rangeli* (35.2%), *T. saimirii* (32.1%), *T. minasense* (33.3%) and *T. cruzi* (10.3%). Mixed infections were present in 52.7% of the infected animals (Ziccardi and Lourenço-de-Oliveira, 1997). High rates of *T. rangeli* were also reported for *Saguinus midas* (51%), whereas a lower prevalence was found in *Alouatta macconelli* (16%) in French Guiana (De Thoisy et al., 2001). *T. rangeli* was also the most common species (30.4%) infecting tamarins and marmosets captured in Amazonia and maintained in the National Centre of Primates, Pará, Brazil (Ziccardi et al., 2000). *T. rangeli* has also been recovered from several monkey species from the Amazonian States of Rondônia, Acre, Amazonas and Pará (Miles et al., 1983b; Maia da Silva et al., 2004a,b, 2007). This trypanosome was also highly prevalent in squirrel monkeys, tamarins and marmosets from Colombia, Bolivia and Panama (Sousa et al., 1974; Marinkelle, 1976; D'Alessandro et al., 1986). In Brazil, there are no reports of *T. rangeli* in wild primates outside Amazonia, although this species was reported in sylvatic rodent, the common opossum, *R. neglectus* and *R. nasutus* (Steindel et al., 1991; Ramirez et al., 2002; Dias et al., 2007).

The infection rates with *T. cruzi* in Amazonian monkeys ranged from 10.3% using parasitological methods (Ziccardi and Lourenço-de-Oliveira, 1997; Ziccardi et al., 2000) to 46% detected by serology (Lisboa et al., 2006). Seroprevalence for *T. cruzi* was high (46%) for specimens of golden lion tamarins living in a broader area of the Atlantic Forest, and also in a captive primate unit located inside the Atlantic Forest (26.5%) (Lisboa et al., 2004b, 2006). In Amazonian monkeys lower prevalences have been reported for *T. cruzi* compared to *T. rangeli*. *T. rangeli* is considered non-pathogenic for man, but its pathogenicity for non-human primates has not been investigated. In contrast, *T. cruzi* is pathogenic in humans and also in non-human primates from both the Old and New Worlds. In experimentally infected monkeys, *T. cruzi* infection is generally asymptomatic but can also present symptoms similar to those of acute and chronic human Chagas disease (Miles et al., 1979; Rosner et al., 1988; Carvalho et al., 2003). Naturally infected golden lion tamarins displayed typical signs of disease, with 45% of the animals presenting cardiac abnormalities (Monteiro et al., 2006).

T. cruzi and *T. rangeli* are both generalist parasites infective for a wide range of mammals including man and several species of wild monkeys, and both species are transmitted by the same vectors in overlapping areas of Amazonia. However, knowledge on the epidemiology of these trypanosomes in both human and non-human primates is hindered by diagnostic methods of limited sensitivity and specificity. Genotyping and phylogenetic analysis of 13 isolates of *T. rangeli* from Amazonian wild monkeys were reported in our previous studies (Maia da Silva et al., 2004b, 2007) whereas only 7 isolates of *T. cruzi* from captive Amazonian monkeys have been genotyped (Lisboa et al., 2007). The small number of isolates characterized prevents a correct appraisal of the genotypes of *T. cruzi* and *T. rangeli* circulating in free-ranging primates, their transmission dynamics and pathogenicity.

Diseases transmitted among human and wild and domestic animals, especially those caused by generalist pathogens pose risks to both wildlife preservation and human health. Parasites of non-human primates are also very relevant to human health since nearly one-third of the protozoan parasites of wild monkeys have also been reported in humans (Nunn et al., 2005; Brooks and Ferrao, 2005; Lebarbenchon et al., 2008). Knowledge of the health status of wildlife and monitoring of the parasitic infections are prerequisites for conservation strategies. The main objective of this study was to assess trypanosomiasis in the endangered tamarin *S. bicolor* aiming to (a) determine the rates of infection by *T. cruzi* and *T. rangeli* by blood smear microscopy and hemoculture; (b) diagnose cultured trypanosomes by molecular methods; (c) characterize the genotypes of the trypanosomes isolated from *S. bicolor*; (d) provide data about transmission and potential risk of trypanosome infections for tamarins and humans.

2. Materials and methods

2.1. Area of study, capture and handling of monkeys

The blood samples from *S. bicolor* examined in this study were collected during the fieldwork of "Projeto Sauim-de-Coleira" executed by the Universidade Federal do Amazonas. The animals used in this study were captured using Tomahawk traps baited with banana, between the years 2001 and 2005, in several forest fragments in Manaus, Amazonas State, Brazil, and in two neighbouring areas, the Reserve Adolfo Ducke of INPA (Instituto Nacional de Pesquisa da Amazonia), Road AM-010, km 26, and a farm on the same road, km 43. Ninety-six males and females of all ages, infants excluded, were captured, kept in a relatively cool place in covered traps in order to minimize their stress, and anesthetized (using 0.1 ml intramuscular, 1% ketamine hydrochloride) to permit minor procedures including clinical examination and blood sampling. After complete recuperation, about 4 h after capture, the tamarins were set free at the site of capture. The predominant vegetation in all localities is secondary and/or primary "terra-firme" forests, with borders around the fragments, and in some places (Reserva Ducke) there are a few hectares of "campinarana" (Fig. 1). A group composed of biologists and veterinarians was responsible for capturing and handling the tamarins, according to the permit of IBAMA (Instituto Brasileiro do Meio Ambiente).

2.2. Detection of trypanosomes by microscopy and hemoculture of tamarin blood samples

Blood of tamarins was collected (0.3–0.6 ml) from the femoral vein and examined for the presence of trypanosomes by hemoculture and by microscopy of blood smears. Two Giemsa-stained smears on glass slides were prepared from each tamarin. Morphology of blood trypomastigotes was analysed in a first attempt at species identification according to the features described by Hoare (1972). Blood samples were inoculated into hemoculture tubes (3–5 for each animal) containing NNN medium with a 0.9% saline overlay (Miles et al., 1981a) and incubated at 28 °C. Giemsa-stained epimastigotes of cultured trypanosomes were also analysed aiming at morphological identification of trypanosome species according to Hoare (1972).