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Short Communication

Survey of *Plasmodium* spp. in Free-Ranging Neotropical Primates from the Brazilian Amazon Region impacted by Anthropogenic Actions

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Abstract: This study investigated *Plasmodium* spp. infection in free-ranging neotropical primates from Brazilian Amazon regions under the impact of major anthropogenic actions. Blood samples from 19 new world primates were collected and analyzed with microscopic and molecular procedures. The prevalence of *Plasmodium* infection was 21.0% (4/19) and PCR positive samples were identified as *P. brasilianum*. Considering the social-economic changes that the Amazon is facing, the prevalence of *P. brasilianum* infection highlights the necessity to closely monitor the movement of both human and non-human primate populations, in order to mitigate pathogen exposure and the introduction of new agents into previously naïve areas.

Keywords: *Plasmodium* spp., neotropical primates, Amazon regions, Brazil

Malaria is an infectious disease caused by parasites of the genus *Plasmodium*, Class Sporozoa, and transmitted by mosquitoes belonging to the *Anopheles* genus (Bruce-Chwatt 1985). This disease is recognized as a major worldwide public health concern, with approximately 306,000 registered cases reported in Brazil in 2009 (Oliveira-Ferreira et al. 2010). More than 99% of human malaria cases in Brazil are concentrated in the endemic Amazon region. Outside of the Amazon, 92% of reported cases originate from endemic areas of Brazil and African countries (Brasil 2005a).

The identification of parasite species causing malaria has clear implications for human health, and, therefore, on the ability to treat and control malaria in affected areas (Deane 1992). Five *Plasmodium* species are responsible for human infections (*P. vivax*, *P. falciparum*, *P. malariae*, *P. ovale*, and *P. knowlesi*) (Greenwood et al. 2005, Cox-Singh et al. 2008) and are commonly targeted for malaria surveillance (Brasil 2005a). However, recent work has shown that *Plasmodium* species infecting primates, namely *P. brasilianum* and *P. simium*, are genetically similar to *P. malariae* and *P. vivax* of humans, respectively

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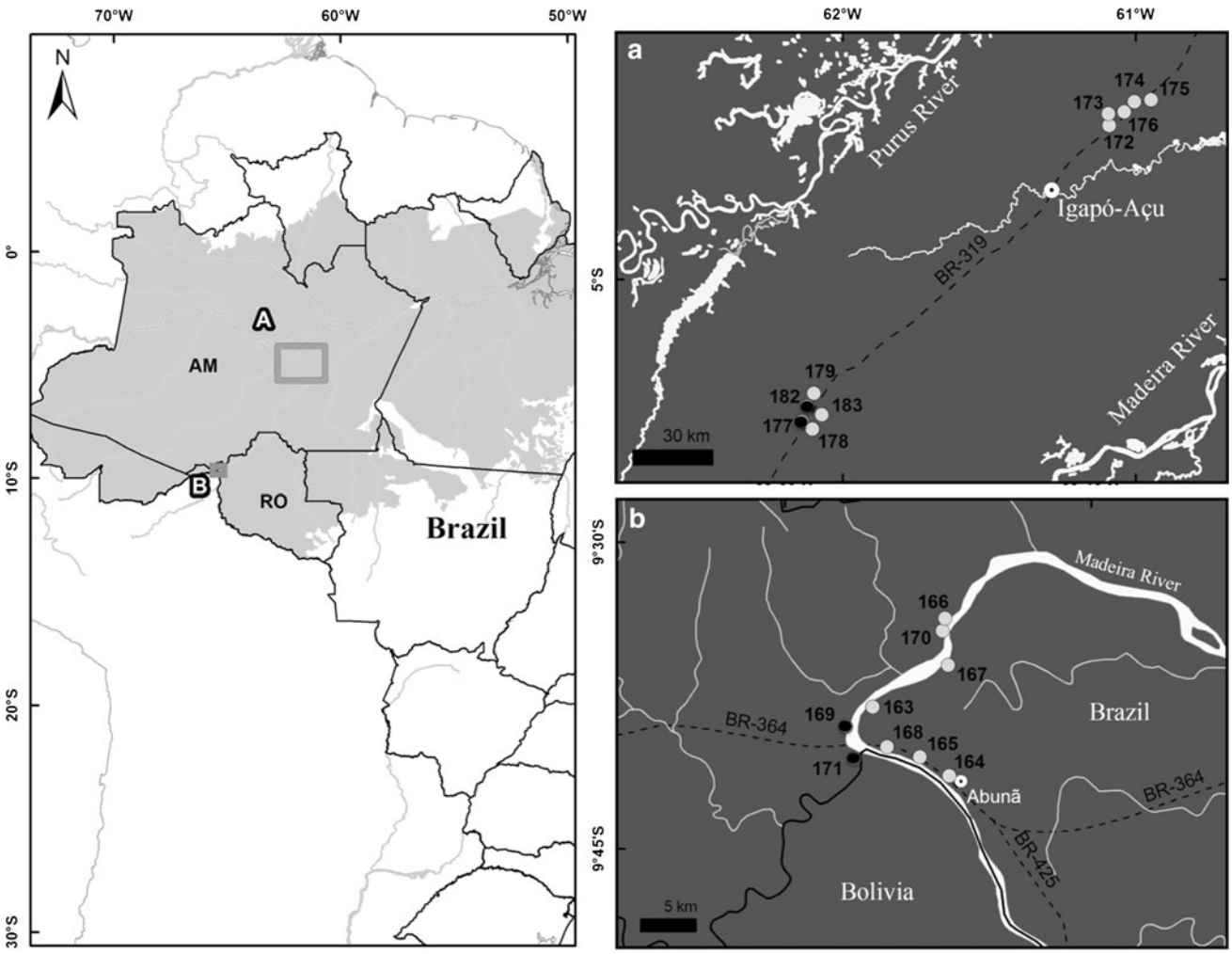

Figure 1. Capture sites and *Plasmodium* spp. survey localities. **a** Positive sites for *P. brasilianum* are shown with *black dots*. **a** Map of BR-319 (Amazonas State), showing the location of *Plasmodium* positive animals (P182 and P177). **b** Map of Rondônia State showing location of *Plasmodium* positive animals (P169 and P171). For all maps, negative animals (PCR and Smear) are shown with *white dots*.

(Ayala et al. 1999; Tazi and Ayala 2011). This finding has led some investigators to conclude that primates could serve as reservoir hosts for *Plasmodium* infection (Fandeur et al. 2000, Hayakawa et al. 2009, Duarte et al. 2008). Infection surveillance in primates, including neotropical primates, has been suggested as being important for public health malaria screening and control programs (Duarte et al. 2008, Hayakawa et al. 2009).

Previous studies have shown that simian malaria is widespread in Brazil, with 14% infection prevalence among surveyed mostly belonging to the family Cebidae (Deane 1992). This is of particular concern for human health when anthropogenic induced change disrupts the ecosystem balance, generating opportunities for zoonotic disease transmission. With the Brazilian government's recent initiative to reopen the BR-319 highway that connects the capital city of the state of Amazonas (Manaus) to the capital city of Rondônia (Porto Velho), there will be significant anthropogenic disturbance in areas that cross-protected lands. Other regions are also undergoing intense anthropogenic pressures through infrastructure development (e.g., the construction of a hydroelectric power station in Rondônia). Such anthropogenic actions have been implicated in increasing vector-borne diseases such as malaria or disseminating pathogens to new areas through the movement of wildlife (Hunter et al. 1982; Alemayehu et al. 1998; Bickel et al. 2003; Daszak et al. 2000). Therefore, *Plasmodium* infection surveillance among primates in these

Table 1. Distribution of New World Primate Species Studied According to the Capture Location, Gender, *Plasmodium* spp. Results, 2010–2011, Brazil.

Species[b]	Local of capture	♂[a]	♀[b]	Total
Callicebus dubius	Rondonia State	0	1 (1)	1 (1)
Sapajus apella	Rondonia State	0	1	1
Aotus infulatus	Rondonia State	0	1	1
Callicebus brunneus	Rondonia State	1	1	2
Pithecia sp.	Rondonia State	1 (1)	0	1 (1)
Saimiri boliviensis	Rondonia State	1	0	1
Lagothrix cana	Amazonas State (BR319) and Rondonia State	2	1	3
Sapajus macrocephalus	Amazonas State (BR319)	1	1	2
Callicebus caligatus	Amazonas State (BR319)	2 (1)	1 (1)	3 (2)
Saguinus fuscicollis mura	Amazonas State (BR319)	1	1	2
Saguinus labiatus rufiventer	Amazonas State (BR319)	0	1	1
Saimiri [ustus] madeirae	Amazonas State (BR319)	1	0	1
Total		10 (2)	9 (2)	19 (4)

[a]Number between brackets are *Plasmodium* spp. positive animals.
[b]Primate taxonomy according to Paglia et al. (2012).

regions is important to better understand the potential epidemiologic implications.

The present study aimed to investigate the occurrence of *Plasmodium* spp. infection in free-ranging primates from Amazon regions under the influence of anthropogenic action.

From January 2010 to February 2011, venous blood was opportunistically collected from 19 primates in two locations of the Brazilian Amazon region (Fig. 1). The animals represented seven different genus and 52.6% (10/19—6♂; 4♀) were from the state of Amazonas (along the BR-319 road, 5°12′S, 61°50′W) and 47.4% (9/19—4♂; 5♀) from the state of Rondônia (9°47′S, 65°16′W; Madeira river, Abunã municipality) (Table 1).

Sampled animals from Amazonas (Fig. 1a) were collected as part of a taxonomy and biodiversity survey conducted by Wildlife Conservation Society (WCS-Brazil) to study the impact produced by the opening of the BR-319 highway. The animals from Rondônia (Fig. 1b) were collected by the Museum of Zoology, University of São Paulo, in order to study the resulting impact on biodiversity from construction of a hydroelectric power station on the Madeira River. All animals were sampled post-mortem and in good body condition.

Thin and thick blood smears from sampled animals as well as DNA extraction were performed to screen for *Plasmodium*. Blood smears were stained according to the Walker's technique, (Brasil 2005b) and 200 microscopic fields were examined under light microscopy (1.000×). DNA was extracted from red blood cells with the GFX™ Genomic Blood DNA Purification Kit (Amersham Biosciences, GE Healthcare), following the manufacturer's instructions. In order to detect *Plasmodium*, polymerase chain reaction (PCR) was carried out according to the previously described nested, genus-specific protocol (Santos et al. 2009). A second nested PCR using methods previously described (Perkins and Schall 2002) was performed to amplify a fragment of ~1.1 kb from the mitochondrial cytochrome b gene (cytb) to identify *Plasmodium* species in positive samples. Amplified products were purified from agarose gels and sequenced using a commercial kit (BigDye TM terminator mix, Applied Biosystems, Foster City CA, USA). Sequences obtained were aligned with sequences from GenBank using BLASTN (Basic Local Alignment Search Tool) available at http://www.ncbi.nlm.nih.gov/blast/Blast.cgi (Altschul et al. 1997).

All procedures were approved by the Ethical Principles in Animal Research, of the School of Veterinary Medicine and Animal Sciences—University of São Paulo (protocol number 1617/2009), and were in full compliance with federal permits issued by the Brazilian Ministry of the Environment (SISBIO # 24319-3 and 18861-3).

The estimated prevalence of *Plasmodium* spp. was 21.1% (4/19; 95% exact CI: 6.1–45.6%), with 22.2% (2/9; 95% exact CI: 2.8–60.0%) and 20% (2/10, 95% exact CI:

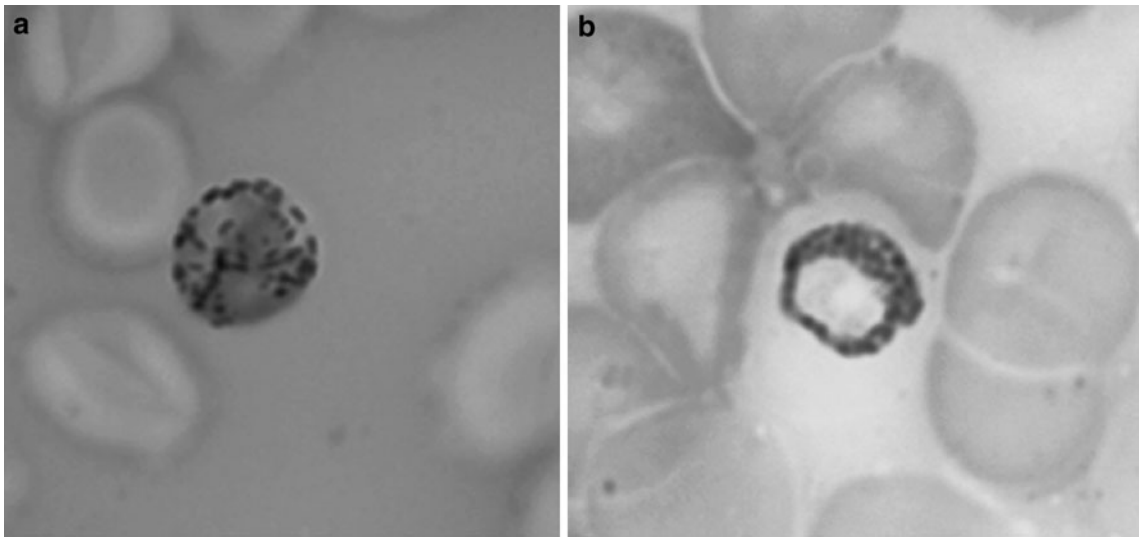

Figure 2. Photomicrographs of parasites visualized from thin blood smears obtained from neotropical primates: gametocytes (×1,000) of *P. brasilianum* in **a** *Callicebus caligatus*, Amazonas state and **b** *Callicebus dubius*, Rondônia state, 2010

2.5–55.6%) of animals testing positive from Rondônia and Amazonas, respectively. All 4 animals were positive on both smears and PCR. In Rondônia, infection was detected in 01 *Pithecia* sp. (P171) and 01 *Callicebus dubius* (P169), while both animals were *Callicebus caligatus* (P177; P182) (Fig. 1) in Amazonas.

Plasmodium from all infected primates showed a 100% identity to *P. brasilianum* (Peruvian III, GenBank GQ355484) (Fig. 2). This isolate was originally obtained from a *Saimiri sciureus peruviensis* in Iquito, Peru, in 1987 (Collins et al., 1990).

Our results suggest that there is a sylvatic cycle from *P. brasilianum* in these regions with anticipated anthropogenic impacts. The importance of this finding for human health arises from the genetic similarities between *P. brasilianum* of primates and *P. malariae* of humans. Some authors hypothesize that the origin of human *Plasmodium* is related to the species that infect non-human primates due to morphological and genetic similarities (Escalante et al. 1995; Ayala et al. 1999; Fandeur et al. 2000; Tazi and Ayala 2011). The high genetic identity found between *P. malariae* from humans and *P. brasilianum* from non-human primates suggests the occurrence of recent transfers between hosts (Tazi and Ayala 2011). In South America, sometimes these parasites are described as *P. malariae/P. brasilianum*, but they can also be described based on the parasite host (non-human primate = *P. brasilianum*; human = *P. malariae*) (Escalante et al. 1995; Fandeur et al. 2000). In recent work, our group compared the genetic diversity of *Plasmodium* from these positive primates with *P. malariae* from humans (Guimarães et al. 2012) and found a greater diversity among isolates of *P. brasilianum* compared to *P. malariae* indicating that *P. malariae* might be derived from *P. brasilianum*, as has been proposed by others (Tazi and Ayala 2011). Such similarities have led some authors to conclude that these are likely the same species of *Plasmodium* and primates may serve as an important reservoir host (Fandeur et al. 2000, Duarte et al. 2008).

Additional support for new world primates as zoonotic reservoirs has been previously discussed (De Arruda et al. 1989; Duarte et al. 2008).

The opportunistic nature of animal sampling limited the number of individuals that could be evaluated for this study. Although we found a higher prevalence (21%), our findings largely corroborated Deane (1992), which reports *P. brasilianum* infection in 10% of surveyed primates from the Amazon region. Previous studies have also identified which species of *Plasmodium* infect wild primates in the Brazilian Amazon; for example, *P. brasilianum* was reported infecting *Cebus*, *Callicebus*, *Alouatta*, *Ateles*, *Lagothrix*, and *Cacajao* (Coatney et al. 1971; Deane and Neto 1969). Despite the small sample size, we detected a substantial proportion of *P. brasilianum* infection in primates in this region. Additional studies with larger sample sizes could increase the precision of prevalence estimates.

The Brazilian Amazon is facing dramatic social, economic, and cultural changes. In this scenario, public and private economic activities may potentiate the occurrence

of zoonosis. For example, after the construction of dams and highways epidemics of malaria can occur due to changes in environmental, ecological (dynamics of host-vector relationship), and social factors (Katsuragawa et al. 2008; Carvalho et al. 2009). Due to the nature of environmental disturbance around the two study sites, it is recommended that the potential for zoonotic disease transmission be considered by public health officials. Both human and primate populations should be closely monitored to mitigate risk of exposure and infection transmission and better understand the nature of the malaria risks post anthropogenic change. Along with anthropogenic changes arise wildlife rescue programs that strive to mitigate environmental impacts by releasing animals into adjacent areas or sending them to captivity in non-Amazonian states. The identification of *P. brasilianum* in primates from this region demonstrates that a substantial proportion of primates in the Amazon region are infected with simian malaria and could facilitate pathogen dissemination, as discussed by others (Daszak et al. 2000; Thoisy et al. 2001; Cunningham 1996; Woodford and Rossiter 1993). The authors recommend that all neotropical primates from the Amazon region be tested for *Plasmodium* spp. infection prior to any translocation or movement of animals in order to mitigate the risk of introducing malaria into non-endemic regions where competent invertebrate vectors are present.

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