Plasmodium simium/*Plasmodium vivax* infections in southern brown howler monkeys from the Atlantic Forest

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Blood infection by the simian parasite, Plasmodium simium, *was identified in captive (n = 45, 4.4%) and in wild* Alouatta clamitans *monkeys (n = 20, 35%) from the Atlantic Forest of southern Brazil. A single malaria infection was symptomatic and the monkey presented clinical and haematological alterations. A high frequency of* Plasmodium vivax*-specific antibodies was detected among these monkeys, with 87% of the monkeys testing positive against* P. vivax *antigens. These findings highlight the possibility of malaria as a zoonosis in the remaining Atlantic Forest and its impact on the epidemiology of the disease.*

Key words: simian malaria - *Plasmodium simium* - New World monkey

Plasmodium infections caused by *Plasmodium brasilianum* or *Plasmodium simium* have been identified in New World monkeys. *P. brasilianum* naturally infects several species of monkeys from a large area in Latin America and seems to be identical to *Plasmodium malariae*, a human malaria parasite (Coatney 1971, Cochrane et al. 1985, Leclerc et al. 2004). Likewise, *P. simium*, restricted to the Atlantic Forest regions, is indistinguishable from the human parasite *Plasmodium vivax* (Collins et al. 1969, Deane 1988). *P. simium* was first identified by da Fonseca (1951) in a monkey from the state of São Paulo (SP), Brazil and was described to naturally infect only three species: *Alouatta caraya* (black howler monkey), *Alouatta clamitans* (southern brown howler monkey) and *Brachytelles arachnoides* (woolly spider monkey) (Deane et al. 1966, 1968). Malaria in monkeys has been reported in the remaining Atlantic Forest in southern and southeastern Brazil, where autochthonous human malaria cases were described (Deane 1992, Wanderley et al. 1994, Curado et al. 1997, 2006, Cerutti Jr et al. 2007, Yamasaki et al. 2011). In these regions, *Anopheles (Kerteszia) cruzii* and *Anopheles (Kerteszia) bellator* are the local vectors (Deane et al. 1966, Marrelli et al. 2007). In this paper, we describe the prevalence of *Plasmodium* infection and levels of antibodies against *P. vivax* antigens among wild and captive monkeys from Atlantic Forest in the South Region of Brazil [municipality of Indaial, state of Santa Catarina (SC)].

Sixty-five southern brown howler monkeys were studied, 20 wild and 45 captive monkeys from the Centre for Biological Research (Brazilian Institute of Environment and Renewable Natural Resources, registration 1/42/98/000708-90, Indaial, SC). The wild animals were captured in the Geisler Mountain in Indaial or attended to in a veterinary hospital in the municipality of Blumenau as victims of electrical shock or running over. This study was approved by the Ethical Use of Animals in Research Committee at the Regional University of Blumenau (protocol 28953-1 2011). A preliminary survey identified four out of 13 monkeys with forms suggestive of *Plasmodium* (Table and Supplementary data, Figure). Molecular diagnosis using nested-polymerase chain reaction (PCR) (Snounou et al. 1993) and real-time PCR (Mangold et al. 2005) for the identification of the human species of plasmodia confirmed *P. vivax/P. simium* infection (Fig. 1) in two (4.4%) captive and seven (35%) wild monkeys (average 13.8%) (Table). The prevalence of *Plasmodium* in wild *A. clamitans* monkeys is much higher than previously reported for SP (5.6%) (Duarte et al. 2008). In SC, infection of *A. clamitans* caused by *P. brasilianum* and *P. simium w*as reported nearly the same rates, approximately 10% (Deane et al. 1992). Here, we identified a greater prevalence rate of *P. simium* infection; however, no infection by *P. brasilianum* was identified among the surveyed monkeys. The identification of *P. malariae* infection by PCR might be hampered by polymorphisms in the SSU rRNA gene, leading to an underestimation of its prevalence (Liu et al. 1998).

One out of 45 captive monkeys (named BL10) with positive microscopy showed symptoms suggestive of malaria, including inappetence, weakness, apathy, intermittent muscle tremors, dry and pale mucous membranes, mild dehydration and loss of muscle mass and body weight. This animal showed several haematological and biochemical alterations, mainly severe thrombocytopenia,

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TABLE

Prevalence of *Plasmodium vivax/Plasmodium simium* infection in captive and wild *Alouatta clamitans* from the municipality of Indaial, state of Santa Catarina

	Microscopy (n = 13)[a]		Nested PCR (n = 65)		Real-time PCR (n = 65)		Total (n = 65)	
Origin of samples (n)	Pos (n)	Neg (n)	Pos[b] (n)	Neg (n)	Pos[b] (n)	Neg (n)	Pos n (%)	Neg (n)
Captive (45)[a]	1	9	2	43	2	43	2 (4.4)	43
Wild (20)[a]	3	0	7	13	7	13	7 (35)	13
Total (65)[a]	4	9	9	56	9	56	9 (13.8)	56

a: the number of samples which have thin blood smears analysed by microscopy were 13 (10 captive and 3 wild monkeys); *b*: positive samples (Pos), all of them for *P. vivax/P. simium*; Neg: negative samples.

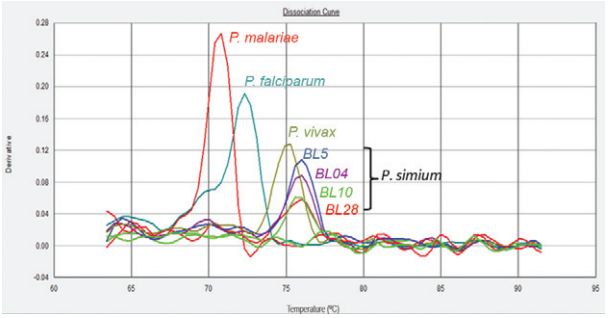

Fig. 1: real-time results (Mangold et al. 2005) showing dissociation curve of human *Plasmodium* species positive controls and samples of four *P. simium* infected monkeys: wild (BL4 and BL5) and captive (BL10) (symptomatic) and BL28.

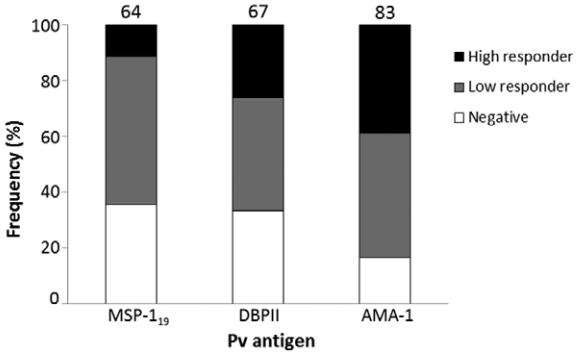

Fig. 2: frequencies of IgG antibodies among *Alouatta clamitans* monkeys against *Plasmodium vivax* antigens: 19 kDa fragment of merozoite surface antigen 1 (MSP-1_{19}), domain II of Duffy binding protein (DBPII) and apical membrane antigen 1 (AMA-1). Negative: optical density $(OD)_{492nm}$ < cut off; low responders: cut off < OD_{492nm} < 0.3; high responders: OD_{492nm} > 0.3. Numbers above the plots indicated the percentage of positive monkeys (low and high responders). Cut-off: mean OD_{492nm} of negative controls (monkeys non-exposed to infection) + 3 standard deviations.

anaemia and serum uraemia (Table, Supplementary data). *P. vivax/P. simium* infection was confirmed by PCR-based techniques (Figure, Supplementary data). This animal was treated with sulfamethoxazole/trimethoprim (23 mg/kg).

Because chronic asymptomatic infections, with very low levels of parasitaemia, could be present in that area, we evaluated the prevalence of ELISA-detected antibodies against *P. vivax* antigens (PvDBPII, PvMSP-1_{19} and PvAMA-1; the last two antigens were kindly provided by Dr Irene Soares from São Paulo University), according to Kano et al. (2010), using anti-IgG of *Macaca mulatta* as secondary antibodies (Sigma-Aldrich). The results confirmed high frequencies (ranging from 64-83% for each antigen and 87% for any antigen) of *P. vivax*-specific antibodies (Fig. 2), albeit at low levels, which confirmed chronic simian malaria infection in this area. Similar serological results were previously described in monkeys from SP by using an ELISA with *P. vivax* circumsporozoite peptides (Duarte et al. 2006).

Taken together, our results confirmed high prevalence of simian malaria in southern brown howler monkeys from the Atlantic Forest, suggesting that malaria has the potential to be a public health problem due to the close contact between humans and monkeys in these regions. These findings highlight the possibility of malaria as a zoonosis in specific geographic regions, which might impact the epidemiology of this disease.

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