The Infection Rates of Trypanosomes in Squirrel Monkeys at Two Sites in the Brazilian Amazon

Mariangela Ziccardi+, Ricardo Lourenço-de-Oliveira

Laboratório de Transmissores de Hematozoários, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

A study was conducted to determine the prevalence of natural infections by trypanosome species in squirrel monkeys: Saimiri sciureus *(Linnaeus) and* Saimiri ustus *(Geoffroy) caught respectively near 2 hydroelectric plants: Balbina, in the State of Amazonas, and Samuel, in the State of Rondônia, Brazil. A total of 165 squirrel monkeys were examined by thick and thin blood smears (BS), haemocultures and xenodiagnosis: 112 monkeys, 67.9%,(being 52.7% with mix infections) were positive to trypanosomes. Four species of trypanosomes were found in monkeys from the 2 areas:* Trypanosoma (Tejeraia) rangeli *Tejera or* T. rangeli*-like parasites in 58 squirrel monkeys (35.2%),* Trypanosoma (Megatrypanum) minasense *Chagas in 55 (33.3%),* Trypanosoma (Herpetosoma) saimirii *Rodhain or* T. saimirii*-like parasites in 53 (32.1%) and* Trypanosoma (Schizotrypanum) cruzi *Chagas in 17 (10.3%). As* T. saimirii *resembles* T. minasense *in blood-stream trypomastigotes and* T. rangeli *in cultural forms and in this survey almost all monkeys presenting trypanosomes morphologically indistinguishable from* T. saimirii *and/or* T. minasense *in BS were found through xenodiagnosis and/or haemoculture to be infected by* T. rangeli, *we suggest that the validity of* T. saimirii *needs to be evaluated.*

Key words: *Trypanosoma minasense - Trypanosoma saimirii - Trypanosoma rangeli - Trypanosoma cruzi* - neotropical primates - trypanosomes - trypanosomatid flagellates - Brazil

In certain tropical areas numerous species of monkeys are infected with trypanosomes, some of which are potentially infective to man, such as *Trypanosoma cruzi*, the agent of Chagas's disease and *Trypanosoma rangeli*, considered non-pathogenic (Chagas 1924, Deane 1962, Albuquerque & Barretto 1969, 1970, Deane et al. 1970, Funayama & Barretto 1970, Hoare 1972, D'Alessandro 1976, Marinkelle 1976, Rocha & Barretto 1977, Miles 1979).

At least 7 other trypanosome species, whose infectivity for man is unknown, have been reported in neotropical monkeys and marmosets: *T.* (*Schizotrypanum*) *prowazecki* Berenberg-Gossler, *T.* (*Megatrypanum*) *minasense* Chagas, *T.* (*Herpetosoma*) *mycetae* Brumpt, *T.* (*Megatrypanum*) *devei* Leger & Porry, *T.* (*Herpetosoma*) *saimirii* Rodhain, *T.* (*Herpetosoma*) *diasi* Deane & Martins and *T.* (*Megatrypanum*) *lambrechti* Marinkelle (Deane 1961, 1964, Deane & Damasceno 1961, Dunn et al. 1963, Marinkelle 1966, 1976, Deane et al. 1970, 1974, 1989, Hoare 1972, Lourenço-de-Oliveira 1988, Lourenço-de-Oliveira et al. 1991, Resende et al. 1994, Ziccardi et al. 1994).

Squirrel monkeys of the genus *Saimiri* have been widely used in biomedical research. *Saimiri*, as well as *Aotus* monkeys, are successful experimental models (WHO 1988) for human malaria parasites *P. falciparum* and *P. vivax* (Deane et al. 1966, Deane 1967, Geiman & Meagher 1967, Young & Rossan 1969, Young et al. 1971, 1975, Rossan et al. 1972 a, b, Schmidt 1978, Gysin et al. 1980, Campbell et al. 1980, Gysin & Nussenzweig 1982, Gysin & Fandeur 1983, Collins et al. 1983, Gysin 1991). Squirrel monkeys have also been used in aerospace (Beischer 1968) and pharmacological (Hanson 1968) research. *S. sciureus* is the most widely used New World monkey in cardiovascular research (Strickland & Clarkson 1985).

Squirrel monkeys are unusual among laboratory animals; many are captured in forests and not bred for research. Wild-trapped *Saimiri* monkeys are often naturally infected with pathogens. Sullivan et al. (1993) pointed out that such naturally acquired infections in experimental animals are potential sources of accidental transmission to handlers and uninfected monkeys and may introduce confounding variables into otherwise well-planned and well-carried out studies. This paper describes the high incidence and diversity of natural infections by trypanosomes in squirrel monkeys from the Brazilian Amazon basin.

+Corresponding author. Fax: +55-21-290.9339
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MATERIALS AND METHODS

The squirrel monkeys were captured in 2 areas, near hydroelectric plants built on rivers on each side of the Amazon basin: Balbina, on the Uatumã River, State of Amazonas (1°55'S 59°28'W) and Samuel, on the Jamari River, State of Rondônia (8°10'S 62°29'W), Brazil. After the construction of both dams, primates were captured in the forests of the dam reservoir flooded plains. A description of these 2 sites was given by Lourenço-de-Oliveira and Deane (1995).

From June 1988 to February 1990 blood samples were obtained by venipuncture from 165 squirrel monkeys belonging to 2 species: 70 *S. sciureus* captured in Balbina and 95 *S. ustus* from Samuel. The nomenclature and systematics of the primates are according to Hershkovitz (1984). Giemsa-stained thick and thin blood smears (BS) were examined for the presence of trypanosomes. Haemocultures were made from the blood of 24 *S. sciureus* and 14 *S. ustus* in liver infusion tryptose (LIT) over Novy, McNeal and Nicolle medium (NNN) slopes supplemented with 10% bovine serum and penicillin 200 UI/ml, maintained at 27°C for 7 days. The cultures were then microscopically examined weekly for 1 month. The isolated parasites were either subcultured into the same cultural media or cryopreserved in liquid nitrogen.

Twenty two *S. sciureus* and 14 *S. ustus*, were submitted to xenodiagnosis using 6 nymphs of 3rd and 4th instar of *Rhodnius prolixus* which were allowed to feed to repletion on each monkey. Fresh smears of faeces and hemolymph of bugs were examined around 30 days after their blood meals. Bugs harboring parasites in their hemolymph were fed on uninfected mice. After 15 days of fasting these bugs fed up on other 20 days-old albino mice. This procedure tended the triatomid's lifespan and allowed the development of the parasites for a longer period of time in the insect, increasing the probability of detecting salivary gland infections and transmission to mice. Fresh blood samples of the mice were examined from 1 to 15 days, when haemoculture (NNN + LIT) was performed.

The salivary glands and gut contents of bugs whose faeces and hemolymph were negative were examined 30 days after xenodiagnosis. Trypanosomes detected in faeces, salivary glands and gut contents of bugs were Giemsa-stained as well as inoculated intraperitoneally (IP) or subcutaneously (SC) into albino mice, whose blood was examined weekly (fresh and Giemsa-stained smears) 30 or 45 days after inoculation. Then, all mice were sacrificed and their blood haemocultured (NNN + LIT).

Samples of 0.6 µl of the haemoculture from 10 squirrel monkeys were injected in the hemocoel of adult *R. prolixus*, which were fed up on 20 days-old albino mice after 30 days and subsequently examined for the search of flagellates in their salivary glands. Blood samples (fresh and Giemsa-stained smears) of the mice were examined from 1 to 15 days, when haemoculture (NNN + LIT) was performed.

The identification of trypanosome species was done by both morphological analyses and biological parameters (Hoare 1972, D'Alessandro 1976, Miles et al. 1983, Steindel et al. 1991) that included comparisons with standard strains of *T. cruzi* and *T. rangeli* (stock R1625 - CDC) for development in arthropod vectors, in culture media and in experimental vertebrate hosts.

RESULTS

One hundred twelve (67.9%) of the squirrel monkeys were infected with trypanosomes; those from Samuel (*S. ustus*) were more frequently infected (78.9%) than those from Balbina (*S. sciureus*) (52.9%) (Table).

The same 4 *Trypanosoma* species were detected in squirrel monkeys from both areas: *T. minasense*, *T. rangeli*, *T. cruzi* and *T. saimirii*-like. In 8 animals the identification of deformed blood stream trypomastigotes was not possible.

The most common trypanosome species infecting *S. sciureus* was *T. saimirii*-like, found in 34.3% (24/70) of individuals, followed by *T. rangeli* (11/70) and *T. rangeli*-like parasites (9/70) which together accounted for 28.6% of the total examined. *T. minasense* was found in 18.6% and *T. cruzi* was detected in only 2.

T. minasense was the most frequent parasite found in *S. ustus* (44.2%). *T. rangeli* were detected in 35 animals (36.8%) and *T. rangeli*-like parasites in 3 (3.2%), based on BS, haemoculture and xenodiagnosis. *T. saimirii*-like parasites were found in 30.5% of *S. ustus* and *T. cruzi* in 15.8% (Table).

Trypanosomes were isolated in haemoculture from 19 *S. sciureus* and all 14 *S. ustus*, which led to the detection and identification of *T. rangeli* and *T. cruzi*. *T. rangeli* or *T. rangeli*-like parasites was isolated from haemoculture from 14 *S. ustus*; *T. cruzi* was simultaneously isolated from 3 of them (Table).

T. rangeli was isolated in haemoculture from 8 *S. sciureus*, 1 of which had a mixed infection with *T. cruzi*. These *T. rangeli* stocks were identical in all pertinent morphological and biological parameters with standard strains. In the haemoculture of 7 other *S. sciureus*, flagellates morphologically indistinguishable from *T. rangeli* were also found, but these could not be compared biologically to standard stocks.

TABLE

Natural infections of trypanosomes in squirrel monkeys, *Saimiri sciureus* and *Saimiri ustus* from Balbina, State of Amazonas and Samuel, State of Rondônia, respectively, detected through thick and thin blood smears (BS), haemoculture (haemo) and xenodiagnosis (xeno). Total number of monkeys positive for trypanosomes (*T. minasense*, *T. cruzi*, *T. saimirii*-like parasites, *T. rangeli* and *T. rangeli*-like trypanosomes[a])

Species	Total exam.	Positives/ %infected	*T. minasense*/ %infected	*T. saimirii*-like/ %infected	*T.* sp.	*T. rangeli*				*T. rangeli*-like[a]			*T . cruzi*			
						BS	xeno	haemo	Total % infected	xeno	haemo	Total % infected	BS	xeno	haemo	Total % infected
S. sciureus	70	37 / 52.9	13 / 18.6	24 / 34.3	2	3	5	8	11 / 15.7	9	7	9 / 12.9	1	1	1	2 / 2.9
S. ustus	95	75 / 78.9	42 / 44.2	29 / 30.5	6	30	9	10	35 / 36.8	4	4	3 / 3.2	13	4	3	15 / 15.8
Total	165	112 / 67.9	55 / 33.3	53 / 32.1	8	33	27	29	46 / 27.9	13	11	12 / 7.3	14	5	4	17 / 10.3

T. sp.: non-identifiable *Trypanosoma* species; *a*: parasites morphologically indistinguishable from *T. rangeli*, but not compared to standard stocks.

The long and slender epimastigotes among other cultural forms, found in the haemoculture and in the gut of bugs used in xenodiagnosis from *T. saimirii*-like infected squirrel monkeys (as determined by BS) were found to resemble to those of *T. rangeli*, described by several workers (e.g. Herbig-Sandreuter 1957, Hoare 1972), but distinct from those of *T. minasense* (Ziccardi et al. 1996). Actually, the cultural forms of *T. saimirii* described by Rodhain (1937) and Deane and Damasceno (1961) also resemble those of *T. rangeli.*

Following the development in the bug of flagellates isolated from *T. saimirii*-like infected monkeys, we verified that hemolymph and salivary glands became infected in xenodiagnosis of squirrel monkeys numbered 105, 133, 137, 138, 139, 140, 147, 167 and 221. In some of these infections, the glands became packed with metatrypanosomes and the parasite was transmitted to mice through biting in half of the cases. Besides, after the injection of 0.6 µl of haemoculture from squirrel monkeys numbered 113, 119, 120, 141 and 217 in the hemocoel of adult of *R. prolixus*, the flagellates invaded their salivary glands and *T. rangeli* was transmitted to mice through their biting (except number 141).

Although the identification of parasites as *T. rangeli* by xenodiagnosis or haemoculture in squirrel monkeys numbered 108, 109, 111, 123, 125, 134 and 220 was not possible by biological criteria (such as, development up to metatrypanosomes in the salivary glands of bugs and the transmission to mice through biting) the flagellates developing in both the bugs and haemoculture were morphologically indistinguishable from *T. rangeli.*

The flagellates from faeces and/or gut contents of triatomines infected with all *T. rangeli* isolates were not infective to mice.

DISCUSSION

In the Americas, many animals have been examined as reservoirs of microorganisms pathogenic to man. During these surveys, many species of parasites, including trypanosomes, have been found. Except for *T. cruzi* and *T. rangeli*, no other trypanosome species is considered potentially pathogenic or infective to humans. However, other trypanosomes may be as frequent as *T. cruzi* and *T. rangeli* in certain mammals, such as squirrel monkeys (Table).

Approximately 32% of the squirrel monkey from both study areas were infected with *T. saimirii*-like trypanosomes. This prevalence is much higher than that found by Deane and Damasceno (1961) in *S. sciureus* from the State of Pará, Brazil (1.38%). Lourenço-de-Oliveira (1988) and Lourenço-de-Oliveira et al. (1991) reported

respectively 66.7% and 28.8% of a few *S. sciureus* previously examined from Balbina infected with *T. saimirii*-like parasites, i.e., harboring blood trypomastigotes indistinguishable from those described by Rodhain (1937) and Deane and Damasceno (1961). Lourenço-de-Oliveira et al. (1991) observed that the flagellates developing in haemocultures and triatomine bugs fed on infected squirrel monkeys were indistinguishable from *T. rangeli*, and that in some of the insects the hemolymph and salivary glands became infected.

T. minasense has been recorded from 12% to 23.1% of squirrel monkeys from the Amazon, Brazil (Deane et al. 1989, Lourenço-de-Oliveira et al. 1991). Dunn (1968), in a survey of hemoparasites of *Saimiri* from Colombia and Peru, determined that the prevalence of *T. minasense* and similar trypanosomes was respectively 36.8% and 47.6%. However, this author considered *T. minasense* a synonym for a group of simian trypanosomes, some of which are usually considered as distinct species, such as *T. devei* and *T. mycetae* (Deane & Damasceno 1961, Hoare 1972, Lourenço-de-Oliveira 1988, Deane et al. 1989, Lourenço-de-Oliveira et al. 1991).

T. rangeli and *T. rangeli*-like parasite was the most frequent trypanosome among the squirrel monkeys from Balbina and Samuel (Table). However, if the haemoculture and xenodiagnosis were performed on all animals a higher incidence of infection by *T. rangeli* would certainly be found.

T. rangeli, or *T. rangeli*-like parasites, are the most frequently reported trypanosome in squirrel monkeys from Colombia, Peru, Panama, Bolivia and Brazil (Dunn et al. 1963, Ayala 1964, Marinkelle 1966, Baker 1972, D'Alessandro et al. 1986, Sullivan et al. 1993). Most authors usually identify the majority of the blood trypomastigotes detected in squirrel monkeys as *T. rangeli* and *T. rangeli*-like, or *T. minasense*, but almost never as *T. saimirii*, due to the fact that *T. saimirii* is poorly characterized. *T. saimirii*, as described by Rodhain (1941) and other authors (e.g. Deane & Damasceno 1961), resembles *T. minasense* in blood-stream trypomastigotes (although narrower, shorter, with its kinetoplast nearer to the posterior end of body that is gradually sharpened to a point, unlike *T. minasense*). Besides, *T. saimirii* develops in the gut of triatomine bugs, without invading the hemocoel and salivary glands, as *T. rangeli* generally does. On the other hand, *T. minasense* do not develop in triatomine bugs and its vector is still unknown (Rodhain 1941, Dias & Campos-Seabra 1943, Deane & Damasceno 1961).

In this survey almost all squirrel monkeys presenting trypanosomes morphologically indistinguishable from *T. saimirii* and/or *T. minasense* in BS were finally found through xenodiagnosis and/or haemoculture to be infected by *T. rangeli* (or *T. rangeli*-like). Only 2 animals (numbered 112 and 220) had negative haemoculture and/or xenodiagnosis.

The data presented here strengthen the hypothesis of Deane and Damasceno (1961) that the flagellates developing in the gut of bugs fed on squirrel monkeys infected with *T. saimirii* may belong to another *Trypanosoma* species undetected by BS. The probability of the squirrel monkeys examined by Rodhain (1937, 1941) and Deane and Damasceno (1961) being infected with mixed *T. rangeli* in subpatent parasitaemia must be considered and the validity of *T. saimirii* needs to be evaluated.

Other monkeys, marmosets and tamarins species from both Balbina and Samuel have also been examined for the search of trypanosomes and plasmodia (Lourenço-de-Oliveira 1988, Deane et al. 1989, Lourenço-de-Oliveira et al. 1991, Deane 1992, Lourenço-de-Oliveira & Deane 1995).

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