Parasitol Res (2007) 101:1689–1698
DOI 10.1007/s00436-007-0652-2

ORIGINAL PAPER

Parasite community interactions: *Trypanosoma cruzi* and intestinal helminths infecting wild golden lion tamarins *Leontopithecus rosalia* and golden-headed lion tamarins *L. chrysomelas* (Callitrichidae, L., 1766)

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Received: 11 April 2007 / Revised: 20 June 2007 / Accepted: 25 June 2007 / Published online: 4 August 2007

Abstract The parasite prevalence and infection intensity in primate wild populations can be affected by many variables linked to host and/or parasite ecology or either to interparasite competition/mutualism. In this study, we tested how host sex, age, and place of origin, as well parasitic concomitant infections affect the structure of golden lion and golden-headed lion tamarins parasite community, considering *Trypanosoma cruzi* and intestinal helminths infection in these primates. A total of 206 tamarins from two Atlantic Coastal rain forest areas in Brazil were tested during 4 years for prevalence of *T. cruzi* infection and helminth prevalence. Three intestinal helminth groups showed high prevalences in both tamarin species: *Prosthenorchis* sp., Spiruridae, and Trichostrongylidae. An association between presence of *T. cruzi* infection and higher intestinal helminth prevalence was found in both tamarin species. Two explanations for this association seem to be plausible: (1) lower helminth-linked mortality rates in *T. cruzi*-infected tamarins and (2) lower elimination rates of helminths in such tamarins. A higher frequency of *T. cruzi*-positive blood cultures was significantly correlated to female tamarins and to the presence of Trichostrongylidae infection. The possibility of an increase in the transmissibility of *T. cruzi* and the three analyzed helminths in lion tamarins with concomitant infections is discussed.

Introduction

Brazilian Atlantic rainforest is a region considered a conservation hotspot (Myers et al. 2000). There, two endemic primate species, the golden lion tamarin *Leontopithecus rosalia*

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(GLT) and the golden-headed lion tamarin *Leontopithecus chrysomelas* (GHLT), were under threat of extinction in the wild in the 1970s; populations were severely depleted because of hunting and habitat loss (Coimbra-Filho 1969, 1970). Since that time, collaborative efforts of Brazilian and foreign governmental and nongovernmental organizations (Mallinson 2001; Ryland et al. 2002) resulted not only in stabilization of their extinction risk but also in lowering it for the GLT (IUCN 2004). Recent findings (Monteiro et al. 2003, 2006, 2007) suggest that these advances may be compromised by parasitic diseases capable of reducing the size and alter structure of populations of GLTs, as has been described for other wild animals (Hudson et al. 1998). This is especially true if they are in patchy environments (McCallum and Dobson 2002), genetically depleted, or exposed to new emerging infectious agents by deforestation (Patz et al. 2004; Horwitz and Wilcox 2005; Travis et al. 2006).

T. cruzi, the etiological agent of Chagas disease, is a multihost trypanosomatid capable of parasitizing all mammalian orders studied to date; thus, transmission remains common in American forests. The recent cases of human disease because of accidental parasite ingestion in formerly nonendemic areas as well as in the Amazon basin describe a new epidemiological profile of the disease (Barbosa 2006). In light of this, a better understanding of the epidemiology of *T. cruzi* in the wild can improve knowledge of the risk of new cases of Chagas disease in humans and other mammal species. Studies of the epidemiology of *T. cruzi* in free-ranging mammals have been mainly restricted to cross-sectional studies (Bar et al. 1999; de Thoisy et al. 2000; Dereure et al. 2001). A better understanding of the transmission cycle of parasites in the wild is only possible through longitudinal studies, which typically are expensive, difficult to carry out, and time consuming.

After the identification of *T. cruzi* infection in GLTs in a biological reserve in Rio de Janeiro State (Lourenço-de-Oliveira 1990; Lisboa et al. 2000), further research showed that depending on population location in the region, *T. cruzi* seroprevalence in GLTs could be low (<20%), as it is in other Neotropical primate species, or as high as 52% (Lisboa et al. 2004). In addition, parasitemia in tamarins (assessed by positive blood cultures) also varied significantly among the conservation units because of unexplained factors. A clinical and laboratory evaluation of wild GLTs showed only slight effects of *T. cruzi* infection on tamarin health; life-threatening cardiac abnormalities detectable on electrocardiograms were estimated to occur in only 4–7% of the infected tamarins (Monteiro et al. 2006). The genetic constitution of GLT hosts and of *T. cruzi* parasites were not probable factors explaining the differences in seroprevalence and profile of infection, as both taxa were described as displaying low genetic diversity (Grativol et al. 2001; Lisboa et al. 2004).

Helminth fauna in the above cited GLT populations comprised six groups; of these, three were suggested as capable of affecting the structure of GLT populations. Helminth prevalences of these three species varied with tamarin sex, age, and helminth transmission strategy, as well as GLT population provenance (Monteiro et al. 2007). Helminth infections in other mammal species were capable of modulating the immune response (Jenkins et al. 2005; Helmby and Bickle 2006) and/or lowering their environmental fitness (Gulland 1995).

Given these findings, we hypothesized that differences in *T. cruzi* seroprevalence and infection profile among GLT populations are the result of concomitant helminth infections. A second hypothesis is that GLT sex, place of origin, and age bias the *T. cruzi* prevalence and infection profile. All these are considered as risk factors in *T. cruzi* infection in humans (Corrêa-Oliveira et al. 1999; Basquiera et al. 2003; Bustamante et al. 2003; Grijalva et al. 2003). The rich biodiversity in tropical forests extends to parasites; mammals in these regions are constantly exposed to multiple parasitic infections. Immunological studies have shown that helminths and *T. cruzi* challenge the mammalian immune system in different ways, and their adaptive responses are distinct for single and concomitant infections (Abrahamsohn and Coffman 1996; Rodriguez et al. 1999; Harnett and Harnett 2006). Sex steroids were also suggested as immunomodulators because of their feedback with cortisol. Primate age and sex are factors that change secretion of such steroids (do Prado et al. 1998, 1999; French et al. 2003; Klein 2004; Bales et al. 2006). Finally, the degree of host exposure to parasite infective stages can affect the development of resistance and extent of organic injury (Wilson et al. 2002). Overall, the assemblage of species in the parasite community in the host population can increase (or decrease) the risk that host individuals will have diminished reproductive output or longevity (Krasnov et al. 2005).

We conducted a longitudinal epidemiological study on populations of GLTs and GHLTs to: (1) evaluate the effect of tamarin age on risk of *T. cruzi* infection, using the distribution of *T. cruzi* seroprevalence as an indicator, (2) compare the risk of *T. cruzi* infection between two lion tamarin species and conservation unit of origin, (3) determine if the transmissibility of *T. cruzi* (expressed by the positive blood cultures) varies with tamarin age and if it follows the distribution of seroprevalence, (4) observe if helminth prevalence is randomly distributed between the *T. cruzi*-infected and uninfected tamarins, and (5) test for a sex bias in any of these factors.

Materials and methods

All lion tamarins included in this study were free-ranging and unmanipulated except for semiannual trapping and

biomedical examination. Reproductive groups with an average size of four to six tamarins were noninjuriously captured in individual live traps (Tomahawk model) as part of ongoing monitoring projects. Individual tamarins were anesthetized with ketamine hydrochloride (20 mg/kg, Dietz and Baker 1993; Raboy and Dietz 2004), dye-marked, and tattooed, and at least one animal in each group was fitted with a radiocollar to facilitate location (Dietz et al. 1997). A total of 206 individuals were captured in the study areas; of these, 65 were sampled from two to four times, totaling 306 samples. Birth dates were recorded ±7 days for most individuals (Dietz et al. 1994; Baker and Dietz 1996). In this study, 297 samples came from tamarins for which birth date was estimated to be ±30 days or less.

Golden lion tamarin study areas

A total of 138 GLTs were captured from October 2002 to June 2005 in two biological reserves and several privately owned farms located in Rio de Janeiro state, Brazil, as follows:

1. *Poço das Antas Biological Reserve (REBIO Poço)*: consists of 6,300 ha of secondary Atlantic forest in Silva Jardim municipality. In this reserve, 83 individuals were examined (47 males, 36 females); of these, 33 individuals were sampled multiple times (14 males, 19 females), totaling 135 samples. GLTs in this reserve are native to this or adjacent forests over evolutionary time. Size of the tamarin population in the reserve ranged from 220 to 350 individuals during the study period.
2. *União Biological Reserve (REBIO União)*: consists of 2,400 ha of advanced secondary Atlantic forest and contains an estimated 300 GLTs. Eight GLTs were sampled in this reserve (seven males, one female), and two were recaptured. The GLTs in this reserve were translocated there during the previous decade from nearby small forest remnants (Kierulff and Rylands 2003).
3. *Privately owned Reintroduction farms (Reintroduction farms)*: These are relatively small forest fragments populated with wild-born tamarins descended from zoo-born individuals reintroduced from 1985–2000 (Beck et al. 2002). Few reintroduced individuals are still alive, and 95% of the GLTs in these farms are wild-born. Owners of these farms endorse conservation efforts by transforming forested areas into federal protected areas. The GLT population living in these farms is estimated at 500 individuals; 47 GLTs individuals (31 males, 16 females) were sampled once in these farms.

Golden-headed lion tamarin study areas

GHLTs were captured from January 2003 to July 2005 in REBIO Una, a 7,059 ha biological reserve located in southern Bahia state, Brazil. This reserve is located in Una municipality and is covered with secondary and primary Atlantic forest mixed with recovering patches of cabruca (a type of cocoa agroforest) and rubber tree plantations. Its GHLT population is estimated at 450 individuals; 68 individuals were sampled (36 males, 32 females), and 30 were resampled (15 males, 15 females), totaling 114 examinations.

T. cruzi parasitological analysis

A maximum of 2.5 ml of blood was collected during the transanesthesic period using a 3-ml syringe and a 20 × 0.55-mm (24 G, 3/4 in.) needle from the medial aspect of the inguinal vein. We used an indirect immunofluorescence assay using anti-monkey fluorescein isothiocyanate conjugate (Sygma) to detect the presence of anti-*T. cruzi* antibodies in lion tamarin serum (as in Camargo 1966). Tamarins with serologic titers above 1:10 were considered positive for *T. cruzi* infection (after Lisboa et al. 2000). We obtained *T. cruzi*-positive and *T. cruzi*-negative (control) serum samples from experimentally infected and uninfected Rhesus monkeys from FIOCRUZ Primate Center. In an attempt to isolate *T. cruzi*, we added 0.5 ml of tamarin blood to each of two sterile culture tubes containing blood agar (Novy, McNeal, and Nicolle) with on overlay of liver infusion tryptose medium, mixed with 10% of fetal calf serum (Camargo 1964). These tubes were inspected for the presence of *T. cruzi* forms at 2-week intervals during a period of at least 5 months.

Fecal analysis

Fresh feces were collected from individual tamarins when they were in live traps or during examination under anesthesia. Feces were conserved in plastic vials containing 40 ml of 4% formaldehyde until analysis was performed. Plastic vials containing feces were weighed in digital top plate scales (to the nearest 0.01 g). Vial content was suspended and filtered through a gauze mesh, and the filtrate was returned to the same flask. Feces weight was obtained from the difference between the two measures.

Fecal samples were examined using a Ritchie's modified technique (Rey 2001): Flasks containing feces filtrate were centrifuged at 1,250×*g* for 10 min. The supernatant was removed and the remaining content was resuspended with 5 ml of distilled water plus 5 ml of sulfuric ether. The content was centrifuged at 450×*g* for 2 min. The supernatant was immediately removed, and the remaining pellet was resuspended in 4% formaldehyde to a final volume of 1 ml.

The fecal concentrate was resuspended, and 80 μl of this solution was placed on a slide and covered with a 22×22-mm glass coverslip. All helminth eggs found under the glass

coverslip were counted and identified following Sloss et al. 1999. A previous assessment of helminth fauna of GLTs identified one acanthocephalan species, *Prosthenorchis* sp. (previously identified as *Oncicola* sp. in Monteiro et al. 2003), and species of five families of nematodes, Ancylostomatidae, Ascarididae, Oxyuridae, Spiruridae, and Trichostrongylidae, using the criteria of Amin 1985 and Vicente et al. 1997. Final counts of eggs per gram of feces for each helminth group in a given sample were calculated.

To avoid overpartitioning of fecal egg count (FEC) data over factor groups, we used separate analysis of variance (ANOVA) analyses to test those means against two groups of factors. First, we tested if sex, lion tamarin species, and *T. cruzi* infection significantly affected the mean FEC for helminths. Second, we evaluated if the mean FEC for each helminth group was affected by concomitant infection with *T. cruzi* and other helminths.

Statistical analysis for *T. cruzi* and helminth prevalence

Prevalence for each parasite species was calculated based on percentage of individuals that presented at least one positive result in any parasitological or serological test. The null hypothesis predicted no difference in prevalence between categories of each analyzed factor (sex, conservation unit, and lion tamarin species). We used the Fisher Exact Test, with significance level set at $\alpha=0.05$, to perform the comparisons. Helminth group prevalence was estimated separately for the three helminths with greatest prevalence (*Prosthenorchis* sp., Spiruridae, and Trichostrongylidae) and for all six species combined (overall prevalence).

Based on the null hypothesis that concomitant helminth infections occur by chance, we compared the observed and expected prevalences of each concomitant helminth infection pair (*Prosthenorchis* sp.×Spiruridae, *Prosthenorchis* sp.×Trichostrongylidae, and Spiruridae×Trichostrongylidae). To estimate expected frequencies of duos of helminths, we multiplied the prevalences for the two helminth groups. We estimated expected frequencies for triple concomitant infections in the same fashion. We used Pearson Chi-squared tests to compare observed-to-expected frequencies of helminth associations in separate tests for *T. cruzi*-infected and noninfected groups, with the significance level set at $\alpha=0.05$ and in some cases $\alpha=0.1$.

Table 1 *Trypanosoma cruzi* seroprevalence, prevalence of *T. cruzi*-positive blood cultures, and intestinal helminth prevalences in golden lion and golden-headed lion tamarins

Conservation unit	Sex	*n* sampled	*T. cruzi* prevalence (%)	Helminth prevalence (%)			
			Serology (blood culture)	Overall	Pro	Spi	Tri
Species: golden lion tamarin (*Leontopithecus rosalia*)							
REBIO União[h]	Total	8	13 (0)	75	50	50	13
REBIO Poço das	Males	47	40 (63)	49	23	28	23
Antas	Females	36	47 (77)	69	31	42	42
	Total	83	*43*[a] (69)	58	27	34	31
Reintroduction farms	Males	31	10 (100)	39	10	3	19
	Females	16	19 (100)	50	13	6	38
	Total	47	*13*[a] (100)	43	11	4	26
Total	Males	85	27 (65)	47	20	21	20
	Females	53	38 (80)	64	26	30	42
	Total	138	*31*[b] (72)	*54*[e]	*23*[f]	25	28
Species: golden-headed lion tamarin (*Leontopithecus chrysomelas*)							
REBIO Una	Males	36	81 (*57*[c])	81	50	36	28
	Females	32	75 (*83*[c])	75	47	41	38
	Total	68	*78*[b] (69)	*78*[e]	*49*[f]	38	32
Pooled prevalences							
	Males	121	43 (*61*[d])	57	29	26	*22*[g]
	Females	85	52 (*82*[d])	68	34	34	*40*[g]
	Total	206	47 (71)	62	31	29	30

For each column, percents in italics highlight significant differences between categories of factors sex and conservation unit, tested using Fisher Exact Text. Blood culture prevalence was calculated within the *T. cruzi*-seropositive individuals. Period: October, 2002 to July, 2005
n sampled The number of individuals; Helminth egg codes: *Pro Prosthenorchis* sp.; *Spi* Spiruridae; *Tri* Trichostrongylidae
Superscripts of letters in italics indicate the following statistics: [a] $p<0.001$; [b] $p<0.001$; [c] $p=0.040$; [d] $p=0.005$; [e] $p=0.001$; [f] $p<0.001$; [g] $p=0.021$
[h] Not tested against sex or conservation unit because of small sample size

Results

T. cruzi prevalence and pattern of infection

Golden lion tamarins GLTs from REBIO Poço and Reintroducion farms displayed significantly different *T. cruzi* seroprevalence, with the tamarins from Reintroduction farms displaying the lowest *T. cruzi* prevalence (Table 1). The age peak for *T. cruzi* seroprevalence occurred at 4 years of age in GLTs, (Fig. 1). The percentage of positive hemocultures was high (72%) and occurred at similar levels within and between lion tamarin species. Female GLTs had a tendency to present higher frequencies of positive blood cultures than males (Table 1). In GLTs, the presence of positive blood cultures was evenly distributed over all ages, without a peak of occurrence (41% of positive blood cultures were found in individuals more than 4 years old). *T. cruzi* transmission is active among GLTs: Seven new infections were found in REBIO Poço das Antas and Reintroduction farms (5% of GLTs sampled).

Golden-headed lion tamarins T. cruzi seroprevalence was significantly higher in GHLTs than in GLTs, no matter the conservation unit that GLTs come from. The peak of *T. cruzi* seroprevalence and positive blood cultures was coincident and occurred at 2 years of age (22% of positive blood cultures were found in GHLTs more than 4 years old; Fig. 2). Female GHLTs had a significantly higher frequency of positive blood cultures than males (Table 1). The *T. cruzi* transmission rate was higher in GHLTs: 13 individuals became infected during the study in REBIO Una, which is 19% of samples for this species. The higher *T. cruzi* transmission rate in the GHLT population resulted in a 100% *T. cruzi* infection rate in individuals more than 4 to 5 years old (Fig. 1).

Helminth prevalence in Golden lion tamarins

Helminth prevalences observed here maintained the same patterns as previously described (Monteiro et al. 2007).

Helminth prevalence in Golden-headed lion tamarins

The diversity of intestinal helminth fauna in GHLTs was the same as that for GLTs. Furthermore, as in GLTs

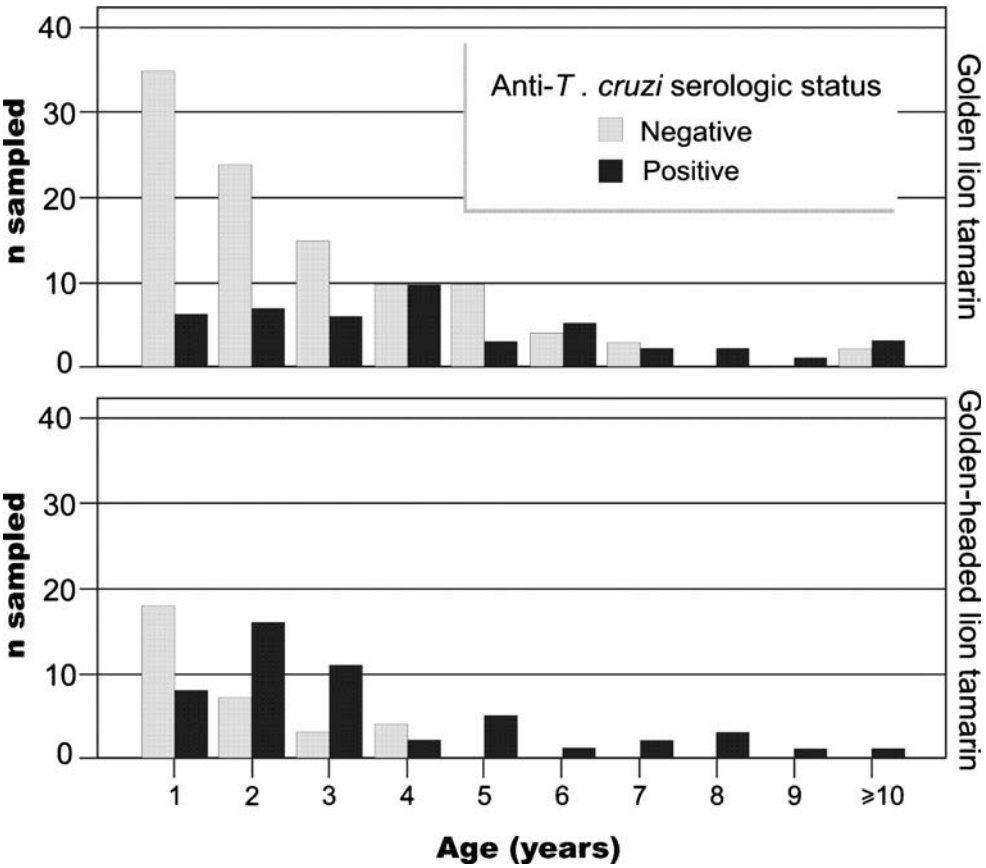

Fig. 1 Age distribution of *T. cruzi* seroprevalence in two spices of lion tamarins. Figures included 45 seropositive golden lion tamarins (148 examinations) and 50 seropositive golden-headed lion tamarins (82 examinations)

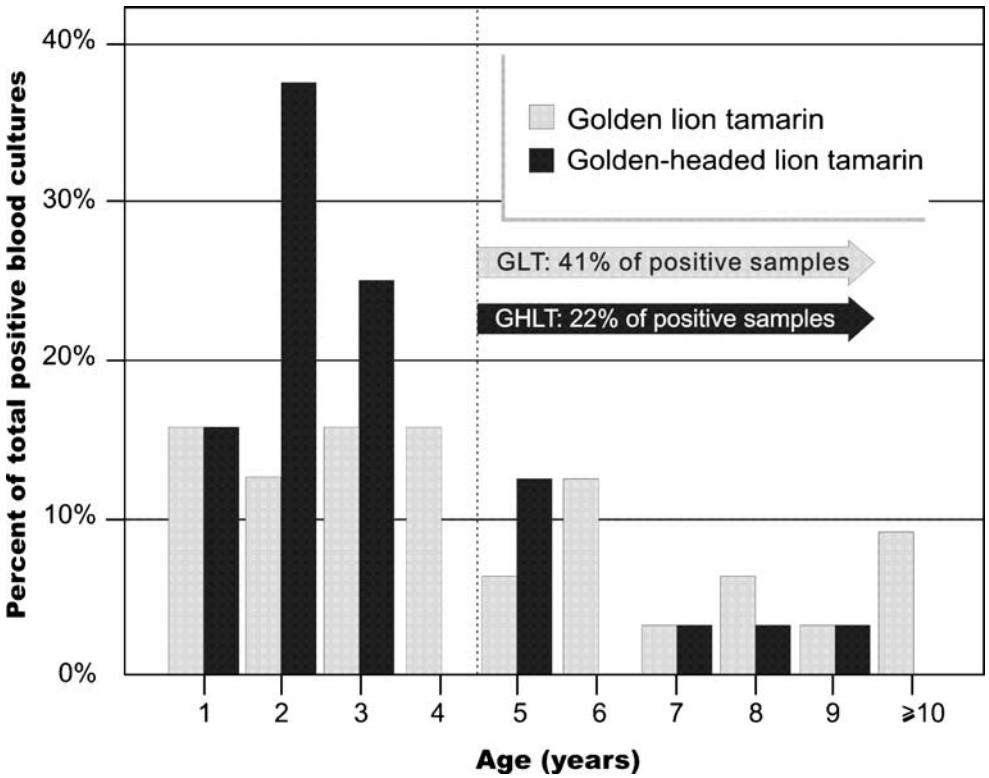

Fig. 2 Age distribution of positive blood cultures in two spices of lion tamarins. Figures included 32 positive blood cultures of 45 *T. cruzi* seropositive GLTs and 32 positive blood cultures of 50 *T. cruzi* seropositive GHLTs

(Monteiro et al. 2007), three helminth groups had low prevalences in GHLTs: Ascarididae, 3%, Oxyuridae, 3%, and Ancylostomatidae, 22%.

The overall prevalence and *Prosthenorchis* sp. prevalence were significantly higher in GHLTs than in GLTs; Spiruridae prevalence was also higher in GHLTs. Trichostrongylidae prevalence was similar between lion tamarin species. In GHLTs, the overall prevalence was higher in males than females because of the higher male *Prosthenorchis* sp. prevalence. Prevalence in the other two helminth groups was higher in females than males.

The higher helminth prevalence in GHLTs was correlated with their higher *T. cruzi* infection prevalence. Moreover, within *T. cruzi*-seronegative and *T. cruzi*-seropositive groups, there were no significant differences in helminth prevalence between GLTs and GHLTs (data not shown); data of both species were then pooled to evaluate the effect of *T. cruzi* infection and tamarin sex on helminth prevalence.

T. cruzi and helminth concomitant infections

Infection by *T. cruzi* was significantly correlated with higher overall helminth prevalence (Table 2). We found significantly higher prevalences for *Prosthenorchis* sp. and Spiruridae in *T. cruzi*-infected tamarins than in *T. cruzi* seronegatives (12 and 16–17% higher, respectively). In male tamarins, the infection by Trichostrongylidae was two times greater in *T. cruzi*-infected individuals than in noninfected

Table 2 *T. cruzi* seroprevalence and concomitant prevalence of intestinal helminths in golden and golden-headed lion tamarins

T. cruzi serologic status	Sex	*n* sampled	Overall prevalence (%)	Pro (%)	Spi (%)	Tri (%)
Seronegative	Males	78	49	23	*18*[c]	*15*[e]
	Females	52	64	27	25	*37*[e]
	Total	130	*55*[a]	*25*[b]	*21*[d]	24
Seropositive	Males	52	65	35	*35*[c]	29
	Females	44	73	39	41	36
	Total	96	*69*[a]	*37*[b]	*38*[d]	32

For each column, percents in italics highlight prevalences statistically different between categories of factor sex or *T. cruzi* infection, using the Fisher Exact Test. The 20 tamarins that changed *T. cruzi* serologic status during the study period were included in seronegative or seropositive groups accordingly. Period: October, 2002 to July, 2005.

n sampled Number of individuals; Helminth egg codes: *Pro Prosthenorchis* sp.; *Spi* Spiruridae; *Tri* Trichostrongylidae

Superscripts of letters in italics indicate the following statistics: [a] $p=0.022$; [b] $p=0.038$; [c] $p=0.026$; [d] $p=0.004$; [e] $p=0.005$

Table 3 Prevalence of concomitant helminth infections found in fecal samples of *T. cruzi*-infected or uninfected golden lion and golden-headed lion tamarins

T. cruzi serologic status	*n* sampled	Pro×Spi	Pro×Tri	Spi×Tri	Pro×Spi×Tri
Seronegative	164	5	4	5	1
Expected frequencies		4	4	4	1
Seropositive	133	8	*4*[a]	8	5
Expected frequencies		11	*9*[a]	9	3

Expected frequencies were calculated from prevalences of individual helminths species on the total fecal samples. Percents in italics refer to observed prevalences statistically different from expected. Period: October, 2002 to July, 2005

n sampled Number of examinations; helminth egg codes: *Pro Prosthenorchis* sp.; *Spi* Spiruridae; *Tri* Trichostrongylidae

[a] Pearson Chi-squared=3.568, $\chi^2_{0.05,1} = 3.841$, $p<0.1$

individuals. In females, Trichostrongylidae prevalences were similar in infected and noninfected individuals (Table 2).

Despite the higher prevalences of *Prosthenorchis* sp. and Trichostrongylidae in *T. cruzi*-seropositive tamarins, their concomitant occurrence in tamarin samples were lower than expected by chance. The other pairs (or triplets) of concomitant helminth infections were found in the frequencies expected by chance and were independent of *T. cruzi* infection (Table 3).

High *T. cruzi* parasitemia in tamarins (positive hemocultures) was correlated with infection by Trichostrongylidae, nematodes considered highly pathogenic. Tamarins infected with Spiruridae displayed a similar trend, while tamarins infected with *Prosthenorchis* sp. showed a trend associated with negative *T. cruzi* blood cultures (Table 4).

T. cruzi infection and helminth egg output

We found no significant effects of lion tamarin species, sex, or *T. cruzi* infection on the FECs of any of the three helminth groups. The mean FEC of *Prosthenorchis* sp., Spiruridae, and Trichostrongylidae were unaffected by the presence of *T. cruzi* or the other helminths (ANOVA, $p>0.05$).

Table 4 Helminth prevalence and *T. cruzi* blood culture results in *T. cruzi*-seropositive golden lion and golden-headed lion tamarins

T. cruzi blood culture result	*n* sampled	Pro (%)	Spi (%)	Tri (%)
Negative	28	43	29	*14*[a]
Positive	67	33	42	*40*[a]

One tamarin lacked a blood culture. Period: October, 2002 to July, 2005

Helminth egg codes: Pro: *Prosthenorchis* sp.; Spi: Spiruridae; Tri: Trichostrongylidae

[a] FET, $\chi^2 = 4.9527$, $p<0.05$

Discussion

T. cruzi transmission rates were 5% in the study period (1.25%/year) for GLTs and 19% in 4 years (4.75%/year) for GHLTs. There are no comparable estimates for these or other free-ranging nonhuman primates; these rates are comparable to those observed in humans in highly endemic areas (Dias and Coura 1997). We assume that *T. cruzi* transmission for lion tamarins is dependent, as in humans, on hemipteran invertebrates (either through the feces of blood-sucking triatomines or through tamarins eating these infected insects). If this assumption is correct, observed differences in *T. cruzi* prevalence among conservation units and lion tamarin species could be due to: (1) invertebrate vector density and accessibility to lion tamarin hosts, (2) foraging success by the invertebrate vector, and (3) prevalence and availability of *T. cruzi*-infective stages in lion tamarin blood. Items 1 and 3 are not likely to contribute to the observed differences in *T. cruzi* prevalence between lion tamarin species: Environmental temperatures are similar between the two Atlantic forest regions, as is the percentage of *T. cruzi*-positive blood cultures (Table 1). Thus, the more plausible explanation for higher *T. cruzi* seroprevalences in GHLTs seems to be the higher density of palm trees in Bahia than in Rio de Janeiro Atlantic forest. Proximity to palm trees was suggested elsewhere as a risk factor for Chagas disease (Romaña et al. 1999; Teixeira et al. 2001). GHLTs forage intensively in such trees and use them for sleeping sites (Raboy and Dietz 2004), and this could facilitate contact of triatomid invertebrates with this tamarin species.

The higher transmission rate in GHLTs explains the earlier peak of prevalence, as well as the concentration of 78% positive blood cultures in animals below 4 years old. This epidemiological scenario contrasts with that for GLTs, in which prevalence of *T. cruzi* infection is lower, the peak of prevalence is later, and presence of positive blood cultures in individuals below 4 years old is 59% (Figs. 1

and 2). The peak shift hypothesis (Woolhouse 1998) predicts that as the transmission rate of a certain parasite increases in the host population, the peak of prevalence will occur earlier; this could also lead to faster development of immunity against the parasite, with consequent reduction in prevalence in the ages after the peak. Our data are in agreement with this hypothesis: The higher transmission rate of *T. cruzi* in GHLTs resulted in an earlier and higher peak of prevalence but also led to earlier development of immunity, concentrating the *T. cruzi*-positive blood cultures in the younger tamarins. Moreover, the distinct profiles of *T. cruzi* infection (i.e., animals with and without high parasitemia as assessed by positive blood cultures) were not explained by the *T. cruzi* genotype (because all typed isolates were TCII; Lisboa et al. 2004) or presence of cardiac lesions (Monteiro et al. 2006).

The positive correlation between *T. cruzi* infection and higher helminth prevalence in all conservation units and both tamarin species sampled may be explained by three hypotheses:

1. *T. cruzi* infection could increase exposure to helminth infective stages: This seems improbable, as *Prosthenorchis* sp. and Spiruridae helminths are acquired through the ingestion of their vectors (cockroaches and crickets—insects that are part of a tamarin's normal diet). The infection by Trichostrongylidae eggs is dependent on contact with the ground, also a normal part of tamarin daily activities. Both infection routes are independent of tamarin sex and do not result in increased risk of *T. cruzi* infection.
2. *T. cruzi*-infected tamarins may be less able to defend against or rid themselves of helminths, thus increasing helminth persistence and consequently their prevalence in the *T. cruzi*-infected tamarins.
3. The helminth-linked death rate may decrease in *T. cruzi*-infected individuals, resulting in higher persistence of those individuals in the population and consequently in the increase in helminth prevalence in the *T. cruzi*-seropositive tamarins. We consider the last two hypotheses as more probable.

The increase in helminth prevalence associated with *T. cruzi* infection is apparently related to the type of helminth pathogenic action. The high pathogenicity of *Prosthenorchis* sp. is due to mechanical damage caused by attachment to the host intestinal wall. However, the resulting loss of metabolic resources of the host is negligible (Starling 1985; Toft 1982). *T. cruzi* infection association with *Prosthenorchis* sp. infection was, despite statistical significance, the weakest in proportion (12% higher in *T. cruzi*-seropositive tamarins) and was independent of sex.

T. cruzi infection seems to facilitate the transmissibility of Spiruridae by lowering the tamarin's ability to eliminate this helminth. Indeed, the significant difference of Spiruridae prevalence between *T. cruzi*-infected and uninfected tamarins was the highest observed in comparison to concomitant infections with other helminth groups, independent of the sex of the tamarin. Even when in concomitant infections with *Prosthenorchis* sp. or Trichostrongylidae, a spirurid infection does not increase significantly the pathogenicity to the tamarin (Table 3).

Our data also show that *T. cruzi*-infected males are better able to support Trichostrongylidae infection than uninfected ones. This helminth is known to be highly pathogenic and causes significant metabolic loss in its hosts (Toft 1982). *T. cruzi* modulation of immune response seems to be sex-biased in tamarins; survival of infected males may be increased through lowering the Trichostrongylidae-linked death rate. Concomitant infection of the highly pathogenic helminths Trichostrongylidae and *Prosthenorchis* sp. and *T. cruzi* apparently increases mortality or, less probably, augments their elimination rate by tamarins because the prevalence of this association is lower than expected in the *T. cruzi*-infected tamarin group (Table 3).

T. cruzi infection does not seem to affect helminth physiology, as the fecal egg output of all analyzed helminths remains unaffected in *T. cruzi*-infected tamarins. However, all three helminths will have longer environmental egg output because of the increased persistence of helminth–host association in presence of *T. cruzi* infection, especially Spiruridae.

Two factors were associated with high *T. cruzi* parasitemia in tamarins: tamarin sex and concomitant infection with Trichostrongylidae worms. Prevalence of positive blood cultures was significantly higher in females than in males. Two points could explain this sex difference: (1) Males are better able to control *T. cruzi* parasitemia, and (2) males are more susceptible to infection and males with higher parasitemias have a higher mortality rate. Given the similar *T. cruzi* prevalences between sexes (Table 1), the second explanation seems more plausible. Moreover, higher susceptibility of males to *T. cruzi* infection has already been described for males of other species (do Prado et al. 1998, 1999; Basquiera et al. 2003; Santos et al. 2007; Schuster and Schaub 2001a, b). A sex bias suggests that *T. cruzi* infection is modulating the immune system of males and females in different ways; sex bias in immune system capabilities have been described in other host–parasite systems (Klein 2004; Bouman et al. 2005). These data suggest a trade off between pathogenicity and transmissibility of *T. cruzi* in tamarins: Females support higher parasitemias and consequently display higher transmissibility (higher parasitemias=higher percentage of positive

hemocultures), and *T. cruzi*-related mortality is higher in males.

The second factor significantly correlated with higher prevalence of *T. cruzi*-positive blood cultures was Trichostrongylidae infection. Given the higher prevalences of the two exploitative helminths (Spiruridae and Trichostrongylidae) in tamarins with blood culture positive for *T. cruzi*, we suggest that loss of metabolic resources or immune function modulation caused by these helminths hampers the lion tamarin's ability to control *T. cruzi* parasitemia. In comparison, *Prosthenorchis* sp. is not an exploitative helminth, and its infection may improve the host's ability to control *T. cruzi* parasitemia. If we assume that there is a higher risk of exposure to Trichostrongylidae infective stages in patchy environments (because of an increased need to travel on the ground), habitat fragmentation may contribute to increase *T. cruzi* transmissibility in such areas.

Our data suggest that *T. cruzi* infection may have both beneficial and detrimental effects on lion tamarin hosts: lowering helminth-linked death rates but also lowering their ability to eliminate helminth infection. *T. cruzi* and helminths would both benefit from these effects, through increased transmission rate to new hosts, because of longer persistence of infected hosts in the population. The mutual influence between *T. cruzi* and helminths in concomitant infections is certainly shaping the population structure of host tamarins and reinforces the importance of studying community interactions rather than focusing on single-species effects, as well as taking in account region fito-fisionomic characteristics.

Acknowledgments We thank the director and staff of IBAMA in REBIO Poço das Antas and in REBIO Una for logistic support, the owners of Reintroduction farms for access to their properties, and the director and staff of the Associação Mico Leão Dourado for logistic and technical support. This work was conducted under authorizations from IBAMA, CNPq and the Animal Care and Use Committee of FIOCRUZ and the University of Maryland. This work was supported by grants from the Brazilian Conselho Nacional de Pesquisa e Desenvolvimento—CNPq, Edital Universal no. 472777/2003 and the US National Science Foundation.

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