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Modulating Variables of *Trypanosoma cruzi* and *Trypanosoma evansi* Transmission in Free-Ranging Coati (*Nasua nasua*) from the Brazilian Pantanal Region

Fernanda Moreira Alves,[1] Natalie Olifiers,[2,3] Rita de Cassia Bianchi,[4] Ana Claudia Duarte,[1] Paulo Marcelo Tenório Cotias,[5] Paulo Sérgio D'Andrea,[3] Matthew Edzart Gompper,[2] Guilherme de Miranda Mourão,[4] Heitor Miraglia Herrera,[1,4] and Ana Maria Jansen[1]

Abstract

This is a long-term follow-up of infection by *Trypanosoma cruzi* (TC) and *Trypanosoma evansi* (TE) in the free-ranging coatis (Procyonidae: *Nasua nasua*) from Pantanal region (Mato Grosso do Sul, Brazil). We evaluated TC and TE infection by immunofluorescence assay, hemoculture (HC), and microhematocrit centrifuge techniques (MHCT). We also examined coatis health by quantifying hematological parameters including packed cell volume (PCV), white blood cell (WBC) count, and differential leukocyte count. TC isolates thought HC were typed by miniexon gene. Mixed infections by both parasites and the two main lineages of TC (76% TCI, 3% TCII, and 14% TCI/TCII) were observed. *Trypanosoma rangeli* was also isolated (7%). Overall, seroprevalence of TC and TE infection were 53.5% and 42.0%, respectively. Positive HC (indicating high TC parasitemia) occurred in 34% of seropositive coatis for TC, and positive MHCT (high TE parasitemia) were observed in 36.4% of seropositive coatis for TE. We detected higher prevalence of positive HC in females (72%) than males (43%), and also during the dry season, indicating a seasonal potential of this host species on TC transmission. These features did not occur for TE infection. However, prevalence of TE based on serology and MHCT was higher among adults than subadults. Coatis with positive HC or MHCT displayed a slight decrease in their WBC. In contrast to the animals with positive HC, coatis with positive MHCT displayed a decrease on their PCV. Moreover, concurrent high TC and TE parasitemia caused a larger decrease of PCV values. This study corroborates the importance of coatis in the maintenance of TC and TE transmission cycles in the southern Pantanal and shows a seasonal character of TC transmissibility to its vector by the coati population from the study area.

Introduction

Trypanosoma cruzi (TC) and *Trypanosoma evansi* (TE) (Kinetoplastida: Trypanosomatidae) are spread through almost all biomes and habitats from the southern United States to southern Argentina. These protozoans are of public health and economic importance as the causative agents of Chagas disease in humans and "Mal de Cadeiras" disease (Surra) in horses, respectively. The sylvatic cycles of these *taxa* need to be better understood, as they are highly complex and involve a broad spectrum of vectors and mammalian hosts (Hoare 1972, Barretto 1979).

TC is a highly heterogeneous species and, based on several markers, two main lineages are recognized as TCI and TCII (Satellite Meeting 1999). Specimens not included in these groups remained in the Zimodeme 3 group (Z3) (Miles et al. 1981, Pedroso et al. 2007). Moreover, TCI and TCII also show intra genotype heterogeneity, so that currently six discrete typing units were admitted (Brisse et al. 2000, Herrera et al. 2007a, Llewellyn et al. 2009). The characterization of discrete typing units is still in its very beginning, especially if isolates of free living mammalian species are concerned. Thus, herein we used the traditional miniexon typing method.

[1]Laboratório de Biologia de Tripanossomatídeos, FIOCRUZ/RJ, Rio de Janeiro/RJ, Brazil.
[2]Department of Fisheries and Wildlife Sciences, University of Missouri, Columbia, Missouri.
[3]Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, FIOCRUZ/RJ, Rio de Janeiro/RJ, Brazil.
[4]Laboratório de Fauna Silvestre, Embrapa/Pantanal, Corumbá/MS, Brazil.
[5]Centro de Pesquisa Clínica Evandro Chagas-IPEC, FIOCRUZ/RJ, Rio de Janeiro/RJ, Brazil.

TC displays three infective forms (Brener 1973) and besides the vectorial-driven contaminative and vertical transmission routes, the oral route has been demonstrated as an effective mechanism of transmission (Coura 2006, Yoshida 2008). TE, in turn, is considered a monomorphic and biochemically homogeneous species. It is primarily transmitted mechanically through the inoculation of blood trypomastigotes by tabanid fly species (Hoare 1972); *per os* infection has not been described as an important mechanism in natural settings, although it is experimentally possible (Raina et al. 1985).

Both trypanosomatid species are inserted in complex transmission cycles that are influenced by the biology and ecology of their host species and also the environment where these hosts are inserted. Thus, the direct selective forces acting on the parasite result from overall host health, immune response, host behavior, age, gender, and concomitant infections with other parasite taxa. The indirect forces acting on the parasite are represented by abiotic factors such as climatic seasonal variation as well as climate shifts such as the El Nino Southern Oscillation (Franke et al. 2002, Botto-Mahan et al. 2010). In fact, the reproduction, life cycle dynamics, behavior, and survival of vectors and hosts may be constrained by temperature and humidity, thus leading to changes in host/vector contact rates. Thus, such climate-based seasonal variation can strongly influence the prevalence and incidence of a parasite species as well as the parasitic burden, clinical disease, and, consequently, the infectivity potential of the parasite to its vectors (McMichael 2003).

In the Pantanal region of Brazil, both TC and TE are enzootic (Herrera et al. 2004, 2007b). In some areas of Pantanal, the brown-nosed coati (Procyonidae: *Nasua nasua*) has been cited as the main reservoir for these trypanosomatid species (Nunes et al. 1993, Herrera et al. 2004, 2008). The coati is a diurnal and mostly scansorial species widely distributed across South America (Gompper and Decker 1998). The species has a complex social structure in which adult females and immature individuals form groups. Some males remain with the groups out of the breeding season, whereas others are primarily solitary, joining them only for reproduction (Gompper and Decker 1998, Resende et al. 2004, Hirsch 2007). Coatis are an omnivorous species. In the study area, they feed mainly on arthropods and fruits (Bianchi 2009).

Longitudinal surveys in free-ranging mammals are very informative, as they allow identification of (1) the effects of a given infection on individuals/populations of host species, (2) factors modulating parasite transmission rates, and (3) the effects of seasonal fluctuations on incidence and prevalence of infection (Mills and Childs 1998). However, this kind of survey is not commonly practiced, as it is highly laborious.

Herrera et al. (2008) reported a longitudinal survey of TC infection in free-ranging coatis from Nhecolândia region, Pantanal, from 2005 to 2007. We continued this longitudinal investigation in the same region evaluating the same coati groups formerly examined as well as newly captured individuals. Here, we also included an evaluation of TE infection and investigated whether seasonality, host sex, and host age influence TC and TE prevalence and pattern of infection. We also evaluated whether high TC and/or TE parasitemia influences coati health conditions. In addition, we typed new TC isolates by miniexon genes.

Materials and Methods

Study area

This study was carried out in the Nhumirim ranch (18°59′S and 56°39′W), a 4400 ha research station of the Brazilian Agricultural Research Corporation (Embrapa), located in the Nhecolândia region, Pantanal, Mato Grosso do Sul.

Pantanal is the largest Neotropical floodplain with two marked seasons: a wet season, ranging from October to March and a dry season, ranging from April to September (Moraes et al. 2000). This region is also marked by pluri-annual cycles of drought and flood, ranging from 5–7 years. Nhecolândia climate is classified as tropical, megathermic with an annual mean temperature of 25.5°C, ranging from 20.2°C and 31.6°C, and an annual average relative humidity of 80% (Soriano 1999).

The region is characterized by sandy soil with a mosaic vegetation of patches of semideciduous forest called "cordilheira," arboreal savanna ("cerradão" and "cerrado"), and grasslands with dispersed shrubs, besides a large number of temporary ponds and seasonally flooded fields. Human population density is very low ($<2/km^2$), and the dominant economic activity is extensive cattle ranching (Seidl et al. 2001).

Coatis capture

Following up the study conducted from 2005 to 2007 by Herrera et al. (2008), field excursions were carried out every 3–4 months from May 2007 to February 2009. Up to 22 traps ($1\times0.40\times0.50$ m; Zootech®) baited with bacon were placed in the study area for 15–45 days per excursion. Traps were checked in the morning, closed, and reset late in the afternoon. Total capture effort was 1400 traps×night. Captured animals were anesthetized using tiletamine hydrochloride and zolazepan hydrochloride (10 mg/kg), marked with ear-tags, measured, sexed, and weighed. Coatis were aged as adults (>2 years old) or subadults (between 6 months and 2 years old) based on teeth condition and body size measurements (Olifiers 2010). After proper asepsis, blood samples (5–10 mL) were taken from the cephalic vein and stored in Vacutainer® tubes (with and without EDTA, for hematological and serological tests, respectively). Animals were released at the site of capture after recovery from anesthesia.

We examined a total of 145 blood samples from 104 individuals (72 males and 32 females). The great majority of captured animals were adults (at least 73% per excursion). For the coati health assessment, the evaluation of TE infection, and the investigation of the effects of seasonality, host sex, and host age on TC and TE prevalences and pattern of infection, our samples were added to those collected from 2005 to 2007 by Herrera et al. (2008). Together, these summed a total of 199 samples from 132 coatis (91 males and 40 females; 19 individuals with at least 3 captures).

All animal procedures were approved by the Brazilian Government Institute for Wildlife and Natural Resources Care (IBAMA; first license No. 183/2005–CGFAU/LIC, last license No. 11772-2/2009) and University of Missouri Animal Care and Use Committee (protocol No. 4459). This study was sanctioned by the "Comitê de Ética e Utilização de Animais (CEUA) P0292-06" from IOC/FIOCRUZ, RJ. Appropriate biosafety techniques and individual protection equipment were used during animal handling and sample manipulation.

Hematological tests

Packed cell volume (PCV), measured using the standard microhematocrit method, and white blood cell (WBC) count, counted in a Neubauer chamber, were carried out within the first 12 h of blood collection. At the Laboratory of Trypanosomatid Biology/FIOCRUZ, blood smears of animals captured up to September 2007 were stained with Giemsa for differential leukocyte counts.

TC and TE detection

We used the microhematocrit centrifugue technique (MHCT) (Woo 1970) and hemoculture (HC) with subsamples of blood from the captured animals to assess for infection by trypanosomatids. MHCT searches for trypomastigote forms of trypanosomatid species and HC technique searches for epimastigote forms of TC and also *Trypanosoma rangeli*. Therefore, to detect which trypanosomatid species was on positive MHCT bloods, we evaluated the morphology of the parasites found in the blood smear Giemsa stained from positive MHCT samples. HC was conducted using two tubes containing NNN (McNeal-Novy-Nicolle) medium with a liver infusion tryptose overlay. Each was inoculated with 0.3 mL of blood from each animal under sterile conditions. The tubes were examined biweekly for up to 5 months. Positive HC and/or MHCT indicate high parasitemia, thus a high potential for parasite transmission.

Serological test

We used imunofluorescence assays to detect anti-TC and anti-TE IgG antibodies, according to Camargo (1966). TC antigens were obtained from axenic culture with parasites in the exponential phase of growth; TE antigens were obtained by inoculating mice and collecting their blood, which was then passed through an ionic exchange column to recover the parasites (Lanham and Godfrey 1970). Two-fold serial dilutions of the animal serum in the range of 1/10 to 1/320 were performed. A goat anti-raccoon IgG fluorescein conjugate (KPL®) diluted at 1/20 and two positive and negative controls were used in the imunofluorescence assays. Although some animals with positive HC or MHCT showed serological titers of 1/10, we adopted a cut-off value of 1/40 due to the possibility of serological cross-reaction with other trypanosomatid species.

Molecular characterization of TC

Genomic DNA was extracted from parasites grown in axenic medium using phenol–chloroform 1:1 (three extractions) followed by precipitation using sodium acetate 3M pH 5.2 plus ethanol (Sambrook et al. 1989). A miniexon multiplex PCR assay was carried out to type the isolates as TCI, TCII, Z3, or *T. rangeli*, following the protocols described by Fernandes et al. (2001). Amplified PCR products were analyzed in ethidium bromide-stained agarose gels (3%) and visualized under ultraviolet light.

Statistical analysis

To investigate whether high TC and TE parasitemias are influenced by seasonality or host sex, we compared the proportion of positive HC and MHCT between seasons and coati sex using chi-square tests ($\alpha = 0.05$). We also used chi-square tests to investigate whether coati age influences TC and TE parasitemia and serological results.

We evaluated the influence of high TC and/or TE parasitemia on host hematological parameters (log-transformed PCV and WBC) using two-way analyses of variance (ANOVAs; factors: wet and dry seasons, and the four combinations for TC and TE high parasitemia: TC-TE−, TC+ TE−, TC-TE+, and TC+TE+) after testing for normality (Shapiro-Wilk test). *Post hoc* Tukey tests ($\alpha = 0.10$, due to the lower power of this analysis) were used to assess pairwise results of the ANOVAs.

We assessed the influence of high TC parasitemia on the relative leukocyte type counts using Mann–Whitney tests ($\alpha = 0.05$) after data transformation (log $[n+1]$). The influence of TE parasitemia on leukocytes types was not investigated due to the small size of the samples that displayed positive MHCT.

Results

TC and TE infection

Positive serological titers for both species antigens ranged from 1/40 to 1/320. Overall, 53.5% (80.6% male and 53.1% female) of individuals were seropositive for TC, and 42.0% (64.8% male and 45% female; Tables 1 and 2) were seropositive for TE. We observed that 34% of the TC seropositive individuals showed positive HC, whereas 36.4% of the seropositive TE individuals showed positive MHCTs.

Table 1. Prevalence of *Trypanosoma cruzi* Evaluated by Hemoculture and Imunofluorescence Assay in Free-Ranging Coatis from Nhumirim Ranch, Pantanal, Brazil, Captured from 2007 to 2009

		HC		*IFA (*Trypanosoma cruzi*)*	
		% Prevalence		*% Prevalence*	
Period	*Season*	*(+/t)*	*M/F*	*(+/t)*	*M/F*
May–June 2007	Dry	64.2 (9/14)	3/6	18.1 (2/11)	1/1
August–September 2007	Dry	36.8 (7/19)	4/3	50.0 (10/20)	9/1
November 2007	Wet	47.3 (9/19)	7/2	47.3 (9/19)	8/1
January–February 2008	Wet	12.5 (4/32)	1/3	54.8 (17/31)	15/2
May–June 2008	Dry	44.4 (4/9)	2/2	100 (9/9)	7/2
September 2008	Dry	68.7 (11/16)	9/2	68.7 (11/16)	9/2
January–February 2009	Wet	29.0 (9/31)	5/4	50.0 (17/34)	9/8
Total		37.8 (53/140)	31/22	53.5 (75/140)	58/17

HC, hemoculture; IFA, imunofluorescence assay; t, total.

Table 2. Prevalence of *Trypanosoma evansi* Diagnosed by Microhematocrit Centrifuge Technique and Imunofluorescence Assay in Free-Ranging Coatis from Nhumirim ranch, Pantanal, Brazil, Captured from 2005 to 2009

		MHCT		*IFA (*Trypanosoma evansi*)*	
		% Prevalence		*% Prevalence*	
Period	*Season*	*(+/t)*	*M/F*	*(+/t)*	*M/F*
March 2005	Wet	0 (0/4)	0/0	50.0 (2/4)	1/1
December 2005	Wet	0 (0/6)	0/0	25.0 (1/4)	0/1
May–July 2006	Dry	0 (0/11)	0/0	0 (0/6)	0/0
October–November 2006	Wet	0 (0/10)	0/0	20.0 (2/10)	1/1
February 2007	Wet	0 (0/20)	0/0	21.0 (4/19)	2/2
May–June 2007	Dry	0 (0/11)	0/0	54.5 (6/11)	2/4
August–September 2007	Dry	20.0 (4/20)	4/0	30.0 (6/20)	6/0
November 2007	Wet	15.7 (3/19)	3/0	52.6 (10/19)	9/1
January–February 2008	Wet	31.8 (7/22)	7/0	41.9 (13/31)	13/0
May–June 2008	Dry	33.3 (3/9)	3/0	88.8 (8/9)	7/1
September 2008	Dry	37.5 (6/16)	4/2	68.7 (11/16)	9/2
January–February 2009	Wet	30.3 (10/33)	7/3	41.1 (14/34)	9/5
Total		18.2 (33/181)	28/5	42.0 (77/183)	59/18

MHCT, microhematocrit centrifuge technique; t, total.

Positive HC was detected in 37.8% of the samples, and prevalence was 1.84 times higher during the dry season than in the wet season ($\chi^2 = 10.81$, $n = 192$, df = 1, $p = 0.001$; Fig. 1), demonstrating a seasonal variation in the potential of TC transmissibility. Positive HC prevalence was higher in females (72%) than in males (43%; $\chi^2 = 7.95$, $n = 126$, df = 1, $p = 0.005$). No difference was found between subadults (42.2%) and adults based on HC (34.0%; $\chi^2 = 0.89$, $n = 142$, df = 1, $p = 0.345$) or serology ($\chi^2 = 1.81$, $n = 140$, df = 1, $p = 0.121$).

Seasonality of positive HC was observed when the whole assemblage of animals was analyzed. The examination of recaptured individuals with at least three captures ($n = 19$) showed that the majority of them did not show any pattern of seasonal transmissibility, that is, they did not display higher positive HC prevalence in the dry and negative HC during the wet season. HC positivity of those individuals captured more than once showed variable results and did not present any common pattern or tendency. Therefore, this seasonal feature occurred collectively, not individually.

Of the 18.2% of coatis with positive MHCT, all trypanosomes species found on examination were TE. Prevalence of high MHCT-based parasitemia did not vary with seasons ($\chi^2 = 0.98$, $n = 181$, df = 1, $p = 0.754$) or host sex (χ^2 with Yates correction = 2.14, $n = 124$, df = 1, $p = 0.148$), but differed between adults (27.4%) and subadults (6.7%; χ^2 with Yates correction = 6.23, $n = 134$, df = 1, $p = 0.013$). Prevalence of TE based on serological exams was also higher among adult coatis than subadults ($\chi^2 = 4.32$, $n = 140$, df = 1, $p = 0.034$).

Based on the HC and MHCT results, 10 individuals (8%) were concomitantly positive by both assessments, indicating that TC and TE can co-occur simultaneously in the same animal.

The majority of isolates ($n = 29$) were characterized as TCI (76%); TCII occurred in 3%; and mixed infection by TCI and TCII was found in 14% of individuals. Infection by *T. rangeli* was also observed (7%; Fig. 2).

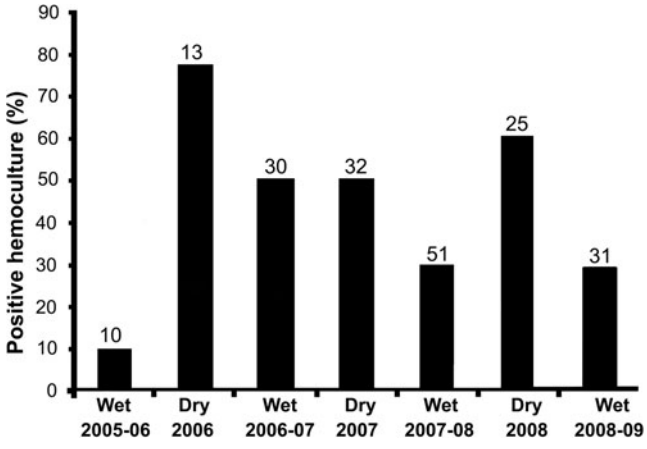

FIG. 1. Prevalence of positive hemoculture in coatis captured between 2005 and 2009 in Nhumirim ranch, Pantanal, Brazil. Total number of individuals captured is shown on top of the bars.

Hematological results

There was a significant difference in the PCV of animals with high parasitemias of TE and TC (TC+TE+) relative to those with low parasitemia or uninfected individuals (TC-TE−: $F = 2.673$, df = 3, $p = 0.049$). *Post hoc* test showed three marginally significant results ($0.05 < p < 0.10$), of which two involved comparisons between double-negative (TC-TE−) and double-positive animals (TC+TE+) from different seasons. The third marginally significant comparison was between TC+TE− and a double-positive TC+TE+ during the dry season ($p = 0.09$). Animals with high parasitemias of TE showed an average decrease of only 3% in PCV. Animals with high parasitemias of both TC and TE also had higher PCV variability (range: 22%–34%.; Fig. 3) Seasonality did not underlie this effect ($F = 0.206$, df = 3, $p = 0.892$). There was no significant difference in differential leukocyte type counts between animals with high TC parasitemias and individuals with low parasitemias or no infection (eosinophils: $U = 240$, $p = 0.598$; bands: $U = 222$, $p = 0.356$; segmented neutrophils:

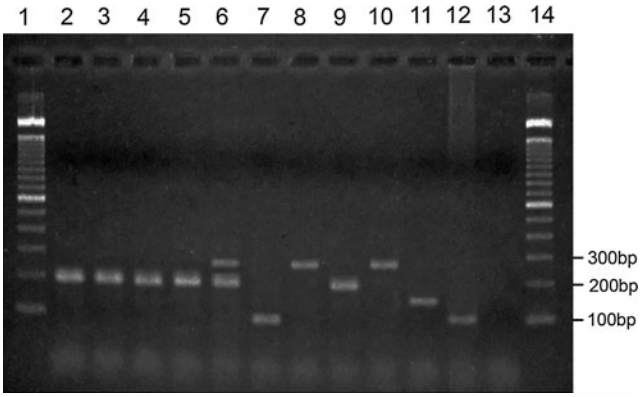

FIG. 2. Agarose gel electrophoresis of PCR-Multiplex products of miniexon gene nontranscribed spacer of *Trypanosoma cruzi* (TC) isolates in free-ranging coatis from Pantanal, Brazil. Lanes 1 and 14, molecular weight marker (100 bp DNA ladder); lanes 2–8, tested samples; lane 9, TCI control (F strain); lane 10, TCII control (Y strain); lane 11, Zimodeme 3 group control (RbIII–*Rhodnius brethesi*–Amazon, Brazil); lane 12, *Trypanosoma rangeli* control (San Augustin); and lane 13, negative control.

$U = 228$, $p = 0.429$; lymphocytes: $U = 263$, $p = 0.982$; monocytes: $U = 242$, $p = 0.628$; $n = 46$ for all cells).

Both positive HC and MCHT were also accompanied by a significant decrease of WBC counts ($F = 9.01$, $df = 3$, $p < 0.0001$). This occurred primarily during the dry season ($F_{season \times parasitemia} = 2.45$, $df = 3$, $p = 0.07$). *Post hoc* pair-wise analyses showed that approximately 45% of the comparisons between treatments were significant ($p < 0.05$) and two were marginally significant ($0.05 < p < 0.10$). Since sample size for TC+TE+ individuals is small ($n = 9$), comparisons with the other groups were usually not significant (Fig. 3). Collectively, however, these results suggest that high parasitemia infections with TC or TE influence the number of WBC counts.

Discussion

The present study corroborates the importance of the coati in the maintenance and transmission cycle of TC and TE in the Pantanal region (Nunes et al. 1993, Herrera et al. 2004, 2008). Our serological and parasitological results for TC infection agree with those found by Herrera et al. (2008) and demonstrate that the transmission cycle of this parasite species is enzootic among coatis and was not merely an isolate epizootic event. Further, the high TC and TE seroprevalence across the entire study period shows that coatis are continuously exposed to both trypanosomatid species. Given that coatis occupy different forest strata, and exist at high population densities in Nhecolândia region (Bianchi 2009), the species demonstrate a high potential on the maintenance and dispersion of both studied trypanosomatid species.

Decreased PCV values in coatis with positive MHCT were expected, as anemia is the main outcome of TE infection for most of the infected species, although its mechanism is not yet fully understood. Moreover, anemia has been described in coatis naturally and experimentally infected with TE (Silva et al. 1999, Herrera et al. 2001, 2004). Our study shows that concomitant high TE and TC parasitemia are causative of a higher impact on this host species health. However, most individuals were seemingly healthy during the study period (see also Herrera et al. 2001). Moreover, several individuals with high TC and/or TE parasitemia were captured many times during the study period. These findings indicate that coatis are able to support the infection with high parasitemias by both the herein studied trypanosomatid species—and potentially transmit the parasites to the vector—for quite long periods without clinical signs of disease.

Under controlled conditions, Repka et al. (1985) described neutropenia followed by neutrophilia and also eosinophilia in mice infected by TC. In addition, immunosuppression, expressed by the low lymphocyte count (Beldomenico et al. 2008), was also described in water buffaloes experimentally infected by TE (Holland et al. 2001). We did not observe changes in differential leukocyte counts of highly infected animals, although doubly infected animals had more variable WBC counts (Fig. 3). Given the many variables that influence leukocyte counts, such as captured-related stress, coinfection with other parasites, gender, seasonality, and an individual's genetic profile (Jain 1993), this finding deserves further observation.

Concerning TE infection, although the abundance of tabanids in the Nhecolândia region increases during the wet season (Barros et al. 2003), we did not find a higher proportion of coatis showing high TE parasitemia in this season. Thus, two

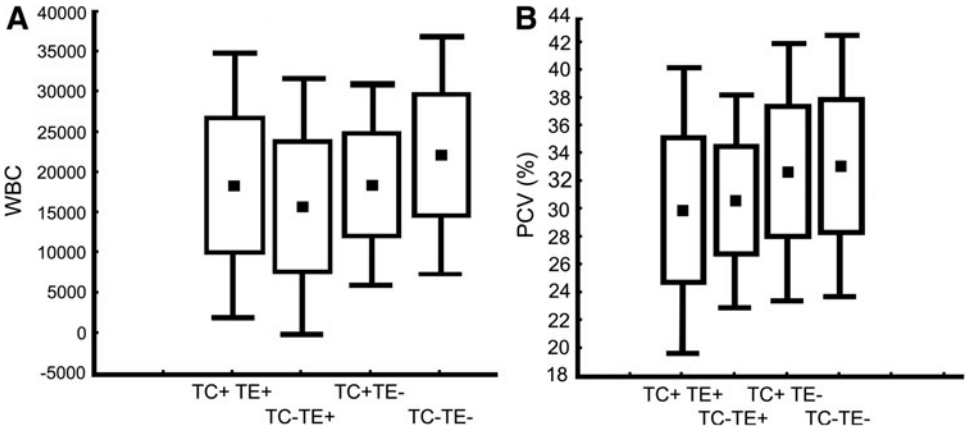

FIG. 3. Mean (square), Standard deviation (box) and 95% confidence intervals (error bars) for **(A)** white blood cell counts (WBC/mm^3) and **(B)** packed cell volume (PCV %) of free-ranging coatis showing positive (+) or negative (−) hemoculture and/or microhematocrit centrifuge technique for TC and *T. evansi* (TE) in the Nhumirim ranch, Pantanal, between 2005 and 2009.

possibilities are to be considered: (1) the relatively high life span of coatis warrant that TE infection may be maintained through both high and low tabanid density seasons; (2) the existence of a secondary vector involved in the transmission of TE during the low tabanid population season, which we consider less probable.

In contrast, the seasonal character of the prevalence of positive HC suggests a higher TC infectivity of coatis to its triatomine vectors in the dry season at the Nhumirim ranch. Seasonality in parasite transmission among humans and animals is a very well-known phenomenon in vector-borne as well as in directly transmitted parasitosis (Altizer et al. 2006). Long-term annual changes in TC enzootic scenarios have been previously described (Botto-Mahan et al. 2010). Thus, TC transmission apparently shows temporal trends that vary from monthly cycles to complex multiyear outbreaks, as being currently observed in the Amazon region, Brazil (Roque et al. 2008). Among the modulating factors implied in parasite transmission cycle pattern, we can point host and vector density and behavior, alterations of the biology of the parasite, and environmental alterations. However, the primary drivers of this intra- and interannual variation remain unclear, mainly if wild free-ranging mammals are considered.

At an individual perspective, seasonal variation on the prevalence of TC high parasitemia may be related to (1) worsening of a pre-existent TC infection due seasonal stressing factors and/or (2) novel infection or reinfection. Reinfection is supported by Herrera et al. (2008), in which was observed single infection by TCI, TCII, and Z3 on the same coati individual in different capture times.

The distinct proportion of TC genotypes in comparison to Herrera et al. (2008) study was unexpected. These authors observed similar prevalences of TCI and TCII (TCI, 28.0%; TCII, 32.1%; Z3, 7.1%). Herein, the prevalence of TCI was much higher (76%) and we did not isolate the Z3 genotype. This finding shows how fast an epidemiological scenario can change. Moreover, it warns against broad generalizations about such a dynamic and complex parasite as TC.

There were both age and sex differences in the prevalence of infection. The former occurred for TE only and may relate to limited exposure opportunities in subadults. However, sex differences for TC deserve further attention. Overall, females showed positive HC more frequently than males. Although higher prevalence on females was previously described in other host species (Monteiro et al. 2007, Brown et al. 2009), the basis for this sex bias is unclear. Females typically have stronger immune responses than males, as higher levels of androgens reduce immune response (Zuk and McKean 1996, Klein 2000, 2004). Indeed, for this reason, the majority of biological characterization of TC isolates is conducted on male Swiss mice. In addition, the ecology of male coatis suggests they should have higher prevalence levels. At the Nhumirim ranch, adult male fight severely during the breeding season (Bianchi 2009), which may expose them to infection. Given this scenario, four nonexclusive hypotheses may explain the lower frequency of positive HC in males: (1) males with high TC parasitemia die sooner than females, (2) males can better modulate TC infection, maintaining a lower parasitemia, (3) the nutritional stress associated with reproduction by females could reduce immune response, and (4) transmission (and eventually infection) to male coatis is lower than among females, because most adult coatis males are solitary. However, this last hypothesis is unlikely, as serological results show that males are highly susceptible to TC infection, as observed by seroprevalence (80.6%).

In conclusion, we reinforce the role played by coatis as important reservoirs for both TC and TE species in this studied region, as this species—especially females—was able to maintain high parasitemia by both TC and TE in long and stable infections. In addition, this is the first study to report seasonality of high TC parasitemia prevalence in coatis. Collectively, this reinforces that both host features and abiotic factors are likely important predictors of temporal changes in the life cycle of TC and TE.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Ana Maria Jansen
Laboratório de Biologia de Tripanossomatídeos
FIOCRUZ/RJ
Pavilhão Rocha Lima 5 andar
Av. Brasil 4365
CEP 21045-900 Rio de Janeiro/RJ
Brazil

E-mail: jansen@ioc.fiocruz.br