Rev. Inst. Med. trop. S. Paulo
46(6):323-330, November-December, 2004

ENZOOTIC TRANSMISSION OF *Trypanosoma cruzi* AND *T. rangeli* IN THE FEDERAL DISTRICT OF BRAZIL

Rodrigo GURGEL-GONÇALVES(1,2), Eduardo Dias RAMALHO(1), Marco Antônio DUARTE(1), Alexandre Ramlo Torre PALMA(2), Fernando ABAD-FRANCH(3), Julio Cesar CARRANZA(4) & César Augusto CUBA CUBA(1)

SUMMARY

The Federal District of Brazil (DF) lies within the *Cerrado* biome, where open shrubland (savannas) is interspersed with riverside gallery forests and permanent swamps (*veredas*). *Trypanosoma cruzi*-infected native triatomines occur in the area, but the enzootic transmission of trypanosomatids remains poorly characterized. A parasitological survey involving sylvatic triatomines (166 *Rhodnius neglectus* collected from *Mauritia flexuosa* palms) and small mammals (98 marsupials and 70 rodents, totaling 18 species) was conducted in 18 sites (mainly gallery forests and *veredas*) of the DF. Parasites were isolated, morphologically identified, and characterized by PCR of nuclear (mini-exon gene) and kinetoplast DNA (kDNA). Six *R. neglectus*, seven *Didelphis albiventris* and one *Akodon cursor* were infected by trypanosomes; wild reservoir infection is documented for the first time in the DF. kDNA PCR detected *T. cruzi* in five *R. neglectus* and mini-exon gene PCR revealed *T. cruzi* I in isolates from *D. albiventris*. Parasites infecting one bug yielded *T. rangeli* KP1+ kDNA amplicons. In spite of the occurrence of *T. cruzi*-infected *D. albiventris* (an important wild and peridomestic reservoir) and *R. neglectus* (a secondary vector displaying synanthropic behavior), a low-risk of human Chagas disease transmission could be expected in the DF, considering the low prevalence infection recorded in this work. The detection of *T. rangeli* KP1+ associated with *R. neglectus* in the DF widens the known range of this parasite in Brazil and reinforces the hypothesis of adaptation of *T. rangeli* populations (KP1+ and KP1-) to distinct evolutionary *Rhodnius* lineages.

KEYWORDS: *Trypanosoma cruzi*; *T. rangeli*; *Didelphis albiventris*; *Rhodnius neglectus*; Enzootic transmission; Federal District, Brazil.

INTRODUCTION

American trypanosomiasis (Chagas disease) is a parasitic zoonosis endemic to the Americas. *Trypanosoma* (*Schizotrypanum*) *cruzi*, a hemoflagellate transmitted by triatomine bugs, is the etiological agent. Over 200 species/subspecies of mammals and 120 triatomine species are known to be susceptible to infection by *T. cruzi*. Dogs, opossums, rodents, and armadillos act as major reservoirs in human-related environments[2,11].

T. cruzi is a remarkably diverse organism. Parasite populations were classified into three major groups (zymodemes Z1, Z2 and Z3) on the grounds of allozyme profiles[27,28,30]. Extensive isozymic characterization led to further subdivision into up to 43 discrete units[43]. Later on, molecular markers allowed for the distinction of two major evolutionary clades, and led to the definition of two major groups, *T. cruzi* I and II (TcI and TcII hereafter)[5,10,25]. TcI predominates in Amazonian enzootic cycles and in domestic transmission cycles north of the Amazon. A primary association of TcI with didelphid marsupials and *Rhodnius* vectors, common in Amazonian palm tree habitats, has been proposed. TcII is the main agent of human Chagas disease throughout southern South America, where *Triatoma infestans* is the primary vector. This parasite lineage may have evolved in terrestrial ecotopes (shared by armadillos, rodents, and several Triatomini), and possibly spread to human habitats when *T. infestans* became domestic[17,31]. However, TcII also appears to be prevalent among non-human primates and *Philander* opossums[33]. The extensive intraspecific diversity in *T. cruzi* probably results from a combination of predominant clonality and nuclear hybridization events[18], and has fueled hypotheses linking distinct parasite genotypes with the heterogeneous clinical epidemiology of Chagas disease[25,31].

Trypanosoma (*Herpetosoma*) *rangeli* also infects mammals and triatomines in Central and South America. Although it causes no disease to humans, it can obscure parasitological and immunological diagnosis of *T. cruzi* infection in areas where both parasites co-exist sharing hosts and vectors[19,21]. The enzootic cycles of this parasite in central Brazil remain however poorly studied[35,36].

Molecular studies based on kDNA minicircles and nuclear DNA sequence variation (mini-exon gene) revealed that two populations of

Research partially funded by CNPq and FINATEC.

(1) Laboratório de Parasitologia Médica e Biologia de Vetores, Área de Patologia, Faculdade de Medicina, Universidade de Brasília, Brasília DF, Brazil.
(2) Laboratório de Zoologia, Universidade Católica de Brasília, Brasília DF, Brazil.
(3) Centro de Pesquisa Leônidas & Maria Deane, Fiocruz/Amazônia, Manaus, Amazonas, Brazil.
(4) Laboratorio de Investigaciones en Parasitología Tropical, Universidad del Tolima, Facultad de Ciencias, Ibagué, Tolima, Colombia.
Correspondence to: César Augusto CUBA CUBA, Laboratório de Parasitologia Médica e Biologia de Vetores, Faculdade de Medicina, Universidade de Brasília, Campus Universitário Darcy Ribeiro, Asa Norte, 70910-900 Brasília, Distrito Federal, Brazil. Fax: +55-61-273.3907. E-mail: cuba@unb.br

T. rangeli with distinct genetic and biological traits are apparently transmitted by different vector species within the genus *Rhodnius*[21,44]. These populations can be distinguished by the presence or absence of a conserved, 165-basepair (bp) region in the kinetoplast minicircle, and were accordingly named KP1+ and KP1-.

T. cruzi-infected triatomines species like *Panstrongylus megistus* (BURMEISTER, 1835), *Triatoma pseudomaculata* (CORRÊA & ESPÍNOLA, 1964), *Triatoma sordida* (STAL, 1859) and *Rhodnius neglectus* (LENT, 1954) occurred in the Federal District of Brazil (DF) but the enzootic transmission of trypanosomatids remains poorly characterized. In this paper we report results from a parasitological survey showing that both *T. cruzi* and *T. rangeli* circulate in enzootic cycles in the *Cerrado* of central Brazil. Parasites were isolated and characterized using morphological and molecular techniques. The dynamics of these enzootic transmission cycles and their epidemiological implications are discussed.

MATERIALS AND METHODS

Study areas: The Federal District of Brazil (DF: 15°30'-16°03' S and 47°25'-48°12' W) lies on a high plateau (1000 m above sea level) dominated by the *Cerrado* biome within the State of Goiás. The *Cerrado* is composed of seasonally dry, open shrubland (savannas) interspersed with gallery forests and permanent swamp areas (*veredas*) where *Mauritia flexuosa* palm trees (locally known as *buriti*) are dominant[37]. The study region has a mean annual rainfall of 1545 mm with a dry season (precipitation < 100 mm) from May to September; mean annual temperatures range from 20 to 21 °C. Eighteen sites (mainly *veredas* and gallery forests) were selected within the region for the study of potential vectors and reservoirs of *T. cruzi* and *T. rangeli* (Fig. 1).

Triatomine collection and parasite detection: Triatomines were collected in eight *veredas* from *M. flexuosa* palm tree crowns; manual captures and live-baited adhesive traps were combined (see GURGEL-GONÇALVES *et al.*[23]). Twenty-five *M. flexuosa* were sampled in each *vereda* performing two hundred palm trees that were grouped in three kinds of landscapes: wild, rural and periurban accordingly to the adjacent area from the *vereda* (wild - gallery forests and savannas, rural - cattle and crops, periurban - houses and streets in a perimeter of one kilometer)[22]. The criteria of inclusion were also accessibility and entrance permission by the government and farmers. Classical parasitological[7] and molecular techniques (see below) were used for detecting natural infection by trypanosomatids.

Capture and identification of small mammals: Small mammals were captured in thirteen areas between years 2000 and 2003; our survey was linked to a research project on small mammal community ecology in the *Cerrado* biome[32]. Gallery forests of different hydrographic basins were studied preferentially. One *vereda* and one area of open shrubland were also sampled (Table 1). One 290 m-long trapping line with 30 capture stations evenly spaced (10 m) was set in each area. Capture stations consisted of two *Sherman* traps, one at ground level and one on the understory (1-2 m above ground). Half of the traps were large-sized (11 x 12.5 x 37 cm) and the rest small-sized (8 x 9 x 23 cm). Both trap types were set on the ground in alternate capture stations. Two or three *Tomahawk* traps (17 x 17 x 52 cm), set on the ground in random sites, were added to each trapping line. A mixture of peanut

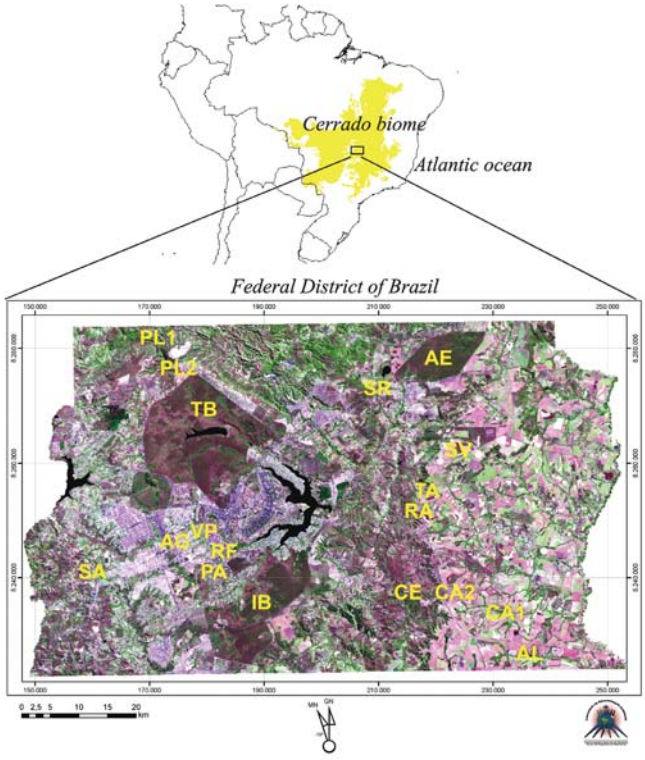

Fig. 1 - Color composition 3R4G5B – Landsat/ETM+7 image (May-23, 2003) with spatial resolution of 15 m from Federal District of Brazil with triatomine and small mammal sampling areas of this study (see Table 1 for geographical co-ordinates, location and main ecological characteristics of each area code). Processing: Laboratório de Caracterização Ambiental, Universidade Católica de Brasília (LACAMBI-UCB).

cream, corn meal, banana, and sardine was used as bait. In one area (Riacho Fundo, RF) only *Young* traps (12 x 15 x 40 cm) were used. They were regularly distributed on a line of 840 m, totaling 42 ground-level stations separated by 20 m. The small mammal captures were performed in two periods of four nights: one in dry season and the other in rainy season, performing a capture effort of 500 traps/night in each of 12 area studied. In addition, in the Riacho Fundo (RF) area the captures were done in the beginning of wet season with a continuous capture effort of 210 traps/night per week. Mammals were identified after PALMA[32].

Xenodiagnosis, *in vitro* isolation, and morphological identification of parasites: Laboratory-reared *R. neglectus* (six to ten 4th-5th instar nymphs by test) and *Dipetalogaster maxima* (1st instar nymphs) were used for xenodiagnosis on 168 mammals. Three bugs were used with mammals weighting less than 100 g. The mammals were anaesthetized using 50 mg/kg of cetamine (Ketamina®) mixed with 0.025 mg/kg of xylasine (Rompum®). The hindgut, feces, haemolymph, and salivary glands of the bugs were examined four weeks later using standard parasitological protocols[7]. Xenodiagnosis-positive triatomines were washed with White solution and the abdomen dissected[30]. Feces and hindgut were removed, submerged in Gentamycin/5-Fluorocytosine solution (100 µg/ml), homogenized, and inoculated in two or three tubes containing Blood Agar medium (DIFCO) with a liquid supernatant (medium RPMI-1640). Culture tubes

Table 1
Triatominae and small mammal sampling areas in the Federal District of Brazil: area codes, geographical co-ordinates, location and main ecological characteristics

Area code	Co-ordinates (GPS)	Group*	Locality	Physiognomy	Nearest city
IB	15º55'54"S, 47º54'02"W	M/T	Reserva Ecológica IBGE(Rio Taquara)	Gallery forest and *vereda*	Paranoá
AE	15°34'27"S, 47°36'28"W	M/T	Estação Ecológica Aguas Emendadas (Rio Vereda Grande)	Gallery forest	Planaltina
SV	15º43'19"S, 47º34'23"W	M/T	Fazenda Sete Veredas	Gallery forest and *vereda*	Planaltina
TA	15º47'21"S, 47º34'37"W	T	Fazenda Tabatinga 106	Gallery forest and *vereda*	Planaltina
RA	15º46'14"S, 47º38'58"W	T	Fazenda Changrilá, Colônia Agrícola Rajadinha	*Vereda*	Planaltina
AL	16º04'05"S, 47º32'39"W	T	Alphaville, divisa DF/GO	*Vereda*	Planaltina
PA	15º54'12"S, 47º56'51"W	T	Park Way, SMPW, Quadra 16	*Vereda*	Park Way
SA	15º54'12"S, 48º09'02"W	T	Fazenda Mocambo, Br 060 (Rio Samambaia)	*Vereda*	Samambaia
SR	15°35'30"S, 47°42'20"W	M	Rio Sarandi	Gallery forest	Planaltina
CA1	15°54'02"S, 47°30'35"W	M	Rio Cariru 1	Gallery forest	Paranoá
CA2	15°52'54"S, 47°34'26"W	M	Rio Cariru 2	Gallery forest	Paranoá
CE	15°53'04"S, 47°37'46"W	M	Cerrado	*Cerrado* (open shrubland)	Paranoá
PL1	15°33'37"S, 48°03'09"W	M	Rio da Palma 1	Gallery forest	Brazlândia
PL2	15°33'58"S, 48°03'05"W	M	Rio da Palma 2	Gallery forest	Brazlândia
TB	15°35'45"S, 48°00'24"W	M	Rio Três Barras	Gallery forest	Plano Piloto
RF	15º51'26"S, 47º56'39"W	M	Santuário Vida Silvestre Riacho Fundo (Rio Riacho Fundo)	Gallery forest	Plano Piloto
VP	15º48'52"S, 48º00'14"W	M	Rio Vicente Pires	Gallery forest	Guará
AG	15º49'17"S, 48º00'40"W	M	Rio Samambaia	Gallery forest	Guará

*M: Small mammals; T: Triatominae

were maintained at 28 °C and weekly examined for two months. Isolates were cryopreserved in liquid nitrogen for future studies. Smears containing triatomine feces or material obtained from culture tubes were stained with Giemsa® buffered solution. Morphological differences between *T. cruzi* and *T. rangeli* were assessed following well-established procedures for parasite identification[7]. Field-collected triatomines were subjected to identical treatment.

DNA extraction and PCR amplification:

Opossum samples: Parasite DNA was extracted using the phenol-chloroform method and ethanol/sodium acetate precipitation from three types of samples derived from xenodiagnosis-positive triatomines: concentrated culture samples (CCS hereafter), culture samples impregnated on filter paper (CFP), and bug fecal samples impregnated on filter paper (BFP). Standard PCRs (95 ºC for five minutes plus 35 cycles of denaturation [95 ºC, one min], primer annealing [60 ºC, one min] and extension [72 ºC, one min]) were performed in a Minicycler® (MJ Research, France).

Initially a PCR using primers S35 and S36 was carried out to differentially detect kDNA from *T. cruzi* and *T. rangeli*[20] in samples from seven *D. albiventris* (see Table 2): two CCS (SV1 and RF6), five CFP (RF2, RF4, RF5, RF8, and RF11), and four BFP (RF2, RF4, RF5, and RF11). *T. cruzi*-positive samples were characterized as TcI or TcII using duplex PCR with primers TCC, TC1 and TC2[15]; four CFP (RF2, RF4, RF5, and RF8), three BFP (RF2, RF4, and RF11), and two CCS (SV1 and RF6) were tested.

Samples from sylvatic Rhodnius neglectus: DNA was extracted (as above) directly from hindgut samples of 33 bugs collected in palms of Sete Veredas (SV). Detection and characterization of *T. rangeli* (as KP1+ or KP1-) was carried out in Colombia by one of us (JC Carranza) using duplex PCR with primers S35, S36 and KP1L[44].

All PCR products were resolved by electrophoresis on 6% polyacrylamide gel stained with silver nitrate. Interpretation and analysis of banding patterns followed VALLEJO *et al*.[44].

RESULTS

Small mammals infected by *Trypanosoma cruzi* in gallery forests: 98 marsupials and 70 rodents, belonging to 17 genera and 18 species, were captured and examined by xenodiagnosis in different areas of the DF (Tables 2 and 3). Eight of these small mammals were infected by trypanosomatids: seven marsupials (*Didelphis albiventris*) and one rodent (*Akodon cursor*). Examination of salivary glands and haemolymph of triatomines fed on 74 of the mammals (rodents and marsupials) did not reveal evidence of infection by *T. rangeli*.

Seven trypanosomatid populations obtained by xenodiagnosis from *D. albiventris* (one captured in SV and six captured in RF) were isolated *in vitro*. Twenty-eight out of 37 culture tubes inoculated were positive (75.6%), while nine isolates failed to grow. Epimastigotes and metacyclic trypomastigotes with size, shape, and a large rod-shaped kinetoplast characteristic of *T. cruzi* were observed in positive culture samples. *T. cruzi*-like trypanosomes isolated from *A. cursor* by

Table 2
Natural infection by trypanosomes in small mammals (marsupials and rodents) captured in 13 *Cerrado* sites of central Brazil (Federal District, years 2000 to 2003)

Area code*	Rodents examined/infected (%)	*Didelphis* examined/infected (%)	Other marsupials examined/infected (%)	Total mammals examined/infected (%)
IB	2/0 (0)	5/0 (0)	20/0 (0)	27/0 (0)
SV	16/0 (0)	14/1** (7.1)	0/0 (0)	30/1 (3.3)
SR	4/0 (0)	0/0 (0)	1/0 (0)	5/0 (0)
AE	4/0 (0)	2/0 (0)	0/0 (0)	6/0 (0)
CA1	5/0 (0)	0/0 (0)	0/0 (0)	5/0 (0)
CA2	3/0 (0)	1/0 (0)	1/0 (0)	5/0 (0)
CE	3/0 (0)	0/0 (0)	0/0 (0)	3/0 (0)
PL1	7/0 (0)	0/0 (0)	4/0 (0)	11/0 (0)
PL2	7/1*** (14.3)	1/0 (0)	2/0 (0)	10/1 (10.0)
TB	5/0 (0)	2/0 (0)	1/0 (0)	8/0 (0)
RF	8/0 (0)	18/6**** (33.3)	1/0 (0)	27/6 (22.2)
VP	1/0 (0)	0/0 (0)	7/0 (0)	8/0 (0)
AG	5/0 (0)	6/0 (0)	12/0 (0)	23/0 (0)
Total	70/1 (1.4)	49/7 (14.3)	49/0 (0)	168*****/8 (4.8)

*Area Codes as in Table 1; ***Didelphis albiventris* (SV1); ****Akodon cursor* (AK1); *****Didelphis albiventris* (RF2, RF4, RF5, RF6, RF8, and RF11); ******275 mammals were captured and 168 were examined (61%).

Table 3
Natural infection by trypanosomes in small mammals (marsupials and rodents) captured in the *Cerrado* of central Brazil (Federal District, years 2000 to 2003)

Potential reservoirs	Number of individuals Examined	Infected (%)
Marsupials		
Didelphis albiventris	49	7* (14.3)
Gracilinanus agilis	26	0 (0)
Philander opossum	20	0 (0)
Monodelphis americana	2	0 (0)
Caluromys philander	1	0 (0)
Total marsupials	**98**	**7 (7.1)**
Rodents		
Oryzomys capito	14	0 (0)
Rhipidomys macrura	14	0 (0)
Oecomys bicolor	13	0 (0)
Nectomys squamipes	9	0 (0)
Akodon cursor	7	1** (14.3)
Oligoryzomys spp.	3	0 (0)
Bolomys lasiurus	2	0 (0)
Cavia aperea	2	0 (0)
Oecomys concolor	2	0 (0)
Calomys tener	1	0 (0)
Oxymycterus robertii	1	0 (0)
Rattus norvegicus	1	0 (0)
Trichomys apereoides	1	0 (0)
Total rodents	**70**	**1 (1.4)**
Total mammals	**168**	**8 (4.8)**

**Trypanosoma cruzi*; ***Trypanosoma cruzi* like in faeces of xenodiagnosis-positive triatomine; isolate lost after *in vitro* culture contamination.

xenodiagnosis did not grow in Blood Agar cultures, precluding further characterization.

***Rhodnius neglectus* infected by *Trypanosoma* spp in *veredas* of the DF:** Natural infection of *R. neglectus* by *Trypanosoma* spp was detected in two *veredas* sited within rural farms. An overall infection rate of 3.6% was obtained (Table 4). In SV 9.1% out of 33 triatomines examined were infected by trypanosomes. In Tabatinga-106 (TA) infection index was 8.1% (3/37). In SV two bugs presented *T. cruzi*-like trypanosomes in their hindgut contents, and one bug presented molecular evidence of *T. rangeli* infection (see below). Trypanosomes from the three bugs infected in TA were morphologically indistinguishable from *T. cruzi*.

Molecular characterization of the parasites:

Parasites from Didelphis albiventris: Three of the samples tested by PCR with primers S35-S36 were positive for *T. cruzi* kDNA, yielding a 330-bp amplicon: one BFP (RF11) and two CCS (SV1 and RF6). None of these samples were positive for *T. rangeli* (Fig. 2). Infection by TcI (presence of a 350-bp amplicon) was revealed by PCR with primers TCC, TC1 and TC2 in six samples: one BFP (RF11), two CCS (SV1 and RF6) and three CFP (RF2, RF4 and RF8) (Fig. 3).

Parasites from Rhodnius neglectus: PCR with primers S35-S36 revealed *T. cruzi* infection in five sylvatic bugs: three from TA and two from SV. *T. rangeli* (characterized as KP1+ by the presence of a 165-bp amplicon after duplex PCR with primers S35, S36 and KP1L) was detected in one bug from SV (Fig. 4).

DISCUSSION

The *Cerrado* is a complex and diverse biome of central Brazil, where several native Triatominae species are present. Some of them, including *R. neglectus*, may act as secondary vectors of human Chagas

Table 4
Trypanosoma cruzi and *T. rangeli* natural infection in *Rhodnius neglectus* collected from *Mauritia flexuosa* palm trees in the *Cerrado* of central Brazil (Federal District, years 2000 to 2003)

	Wild areas		Rural areas				Periurban areas		Total
	AE	IB	TA	SV	AL	RA	PA	SA	
Number of bugs collected	12	39	39	108	57	71	10	0	336
Number of bugs examined (%)	8 (66.7)	22 (56.4)	37 (94.9)	33 (30.6)	26 (45.6)	33 (46.5)	7 (70)	0 (0)	166 (49.4)
Number of bugs infected (%)	0 (0)	0 (0)	3* (8.1)	3** (9.1)	0 (0)	0 (0)	0 (0)	0 (0)	6 (3.6)

*All with *Trypanosoma cruzi*; **Two with *T. cruzi* and one with *T. rangeli.*

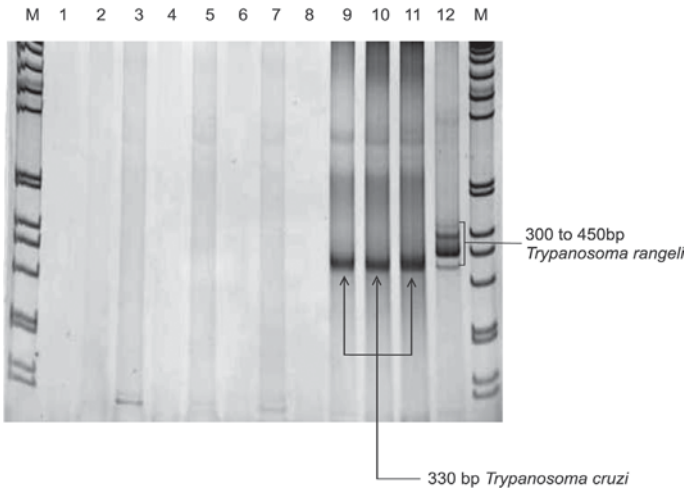

Fig. 2 - Differential detection on *Trypanosoma cruzi* and *T. rangeli*. Silver nitrate-stained polyacrylamide gel showing PCR amplicons obtained using primers S35 and S36, which amplify sequences of kDNA minicircles of *T. cruzi* and *T. rangeli*. Lane M: Molecular size marker (1kb); Lane 1: negative control; Lanes 2 to 5: culture samples of parasites isolated from *Didelphis albiventris* in Riacho Fundo, impregnated on filter paper (RF8, RF2, RF11, and RF4 respectively); Lanes 6 to 9: fecal samples from triatomines infected in *D. albiventris* xenodiagnosis, impregnated on filter paper (RF4, RF2, RF5, and RF11 respectively); Lane 10: concentrated culture sample of *T. cruzi* isolated from *D. albiventris* in Sete Veredas (SV1); Lane 11: culture sample of parasites isolated from *D. albiventris* in Riacho Fundo (RF6); Lane 12: culture sample of *T. rangeli* from Colombia (strain 1545).

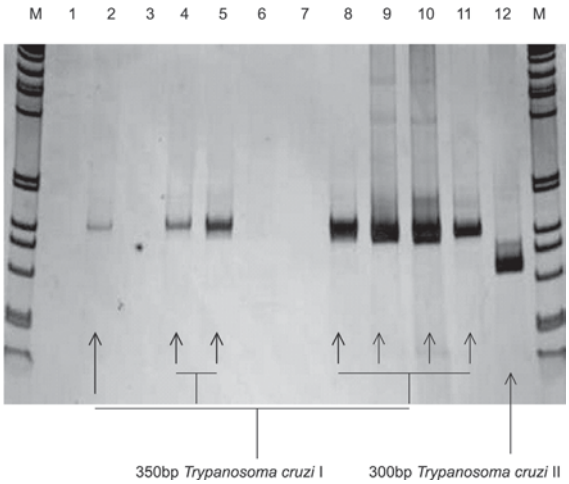

Fig. 3 - Molecular typing of *Trypanosoma cruzi*. Silver nitrate-stained polyacrylamide gel with amplicons obtained by duplex PCR with primers TCC, TC1 and TC2, which amplify the intergenic region of the mini-exon gene. Lane M: Molecular size marker (1kb); Lane 1: negative control; Lanes 2 to 5: culture samples of parasites isolated from *Didelphis albiventris* in Riacho Fundo, impregnated on filter paper (RF8, RF5, RF2, and RF4 respectively); Lanes 6 to 8: fecal samples from triatomines infected in *D. albiventris* xenodiagnosis from Riacho Fundo, impregnated on filter paper (RF4, RF2 and RF11 respectively); Lane 9: *T. cruzi* isolated from *D. albiventris* in Sete Veredas (SV1); Lane 10: concentrated culture sample of *T. cruzi* isolated from *D. albiventris* in Riacho Fundo (RF6); Lane 11: *T. cruzi* TcI isolated from *D. marsupialis* in Colombia; Lane 12: *T. cruzi* TcII (Y strain), Brazil.

disease. Control of synanthropic populations of *T. infestans* (an introduced species) is reducing Chagas disease incidence rates throughout the region, and public health strategies now concentrate on the monitoring of transmission by autochthonous vectors[11]. The circulation of trypanosomatid parasites in wild cycles in the *Cerrado* remains however poorly investigated. The characterization of the dynamics of these cycles (including parasites, vectors and mammal reservoirs) is of foremost importance for the definition of vigilance schemes; our research aims at contributing to current efforts on that direction.

The overall prevalence of *T. cruzi* infection in potential reservoirs was low (4.8%) in our study area; it was however unevenly distributed among sites. Less than 10 mammals were studied in seven out of the 10 sites where no infection was detected; only a wider sampling could allow for the hypothesis of small, contained foci of transmission being suitably tested. Taking only into account sites with larger samples (> 10 mammals) the prevalence rate rises to 6.8%, with didelphid marsupials accounting for 87.5% of infections. Regardless of infection rates, this constitutes the first documented record of the enzootic circulation of *T. cruzi* among small mammals in the DF.

As expected, the opossum *D. albiventris* played a significant role as reservoir of *T. cruzi*. Several behavioral (synanthropism, nomadic habits, use of hollow trees and palms as refuges) and biological traits (two generations/year, life-long *T. cruzi* infection with long-lasting parasitemia) of this opossum make it a good candidate link between wild and peridomestic cycles of *T. cruzi* transmission, even in areas where stable, domiciliated vector colonies are uncommon[13,14,34,36,39,40]. The relatively low specific infection rate we found (14%) rises to 33% when considering only the gallery forest where most opossums were captured (RF, 18 individuals). Rates from 19% to 92% have been reported for *Didelphis* spp from different regions of South and Central America[2,36,40].

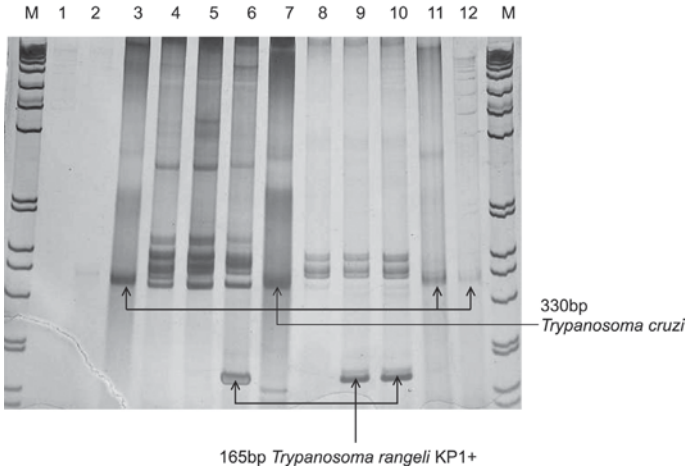

Fig. 4 - Molecular detection and typing of *Trypanosoma rangeli*. Polyacrylamide gel stained with silver nitrate showing amplicons derived from duplex PCR with primers S35, S36 and KP1L. Lane M: Molecular size marker (1kb); Lanes 1 and 2: negative controls; Lanes 3 and 7: *T. cruzi* Y strain (TcII), Brazil; Lanes 4, 5 and 8: *T. rangeli* KP1- isolated from *Rhodnius pallescens* (Colombia). Lane 6: *T. rangeli* KP1+ isolated from *R. prolixus* (Colombia); Lanes 9 to 12: fecal samples from *R. neglectus* collected in Sete Veredas, Brazil (9 and 10 are samples from the same bug).

Over 50 species of rodents have been found infected by *T. cruzi* in the Americas (22 in Brazil), but they seem to play a minor role in the maintenance of wild cycles in central Brazil. Infection rates of 0.1% (1/963) and 0.3% (2/722) have been reported from Goiás[26] and São Paulo[16], respectively. Only one rodent (*Akodon cursor*) was infected in our sample (n = 70), resulting in rates of 1.4% for all rodents and 14.3% for *A. cursor* (1/7). Contamination of culture tubes precluded further characterization of these trypanosomatid parasites. *T. cruzi* infection rates of 18.4% have been reported for *A. cursor*[2].

R. neglectus, common in *Cerrado* palm trees (mainly *Acrocomia, Attalea* and *Mauritia*), is considered as a potential secondary vector of human Chagas disease throughout its wide range in central Brazil (Bahia, DF, Goiás, Mato Grosso, Minas Gerais, Paraná, and São Paulo), where it invades and occasionally colonizes artificial ecotopes[6,11]. The presence of adult bugs in human dwellings and peridomestic habitats has been reported in the States of São Paulo, Goiás and Minas Gerais, with average *T. cruzi* infection rates of 3.4%[2]. *R. neglectus* is frequently found in houses in several districts of Goiás, where 1.5% of the bugs were infected[38], and has been sporadically captured in houses and peridomestic habitats in the DF[41], with unknown infection rates. Results presented here show that *R. neglectus* also acts as a vector in enzootic transmission cycles of *T. cruzi* in *veredas* of the *Cerrado*. The low infection rate we report (3.6%) is probably related to the preference of palm tree-living bug populations for avian blood, but illustrates a potential risk that cannot be ignored by public health officials.

Molecular characterization of parasites isolated from *D. albiventris* captured in the DF revealed TcI. Particular phylogenetic groups of *T. cruzi* seem to preferentially infect certain reservoir hosts. There is evidence suggesting that TcII (but not TcI) causes severe, parasitemic disease to rodents, while *D. marsupialis* eliminate TcII and retain TcI[24]. Field studies show that TcI is often associated with marsupials (mainly *Didelphis* spp.), while TcII is more commonly isolated from placental mammals, including humans[31]. However, enzootic cycles involving TcII and marsupials (*Philander frenata*) have also been described, even in areas where TcI was isolated from sympatric *Didelphis*[33]. The relationships among wild cycles and between these and domestic ones are extremely complex, but a common trait is that opossums seem to play a key role in transmission dynamics[13,15,24].

Studies conducted in Brazil showed that TcI was present in only a small proportion of acute chagasic patients, while TcII was isolated from the vast majority of chronic cases. TcI is believed to frequently result in asymptomatic chronic infections. However, mortality rates of acute patients from the Amazon are similar to those reported for areas where TcII prevails. Parasites characterized as TcI circulate in endemic areas of Venezuela where the major chronic manifestations of the disease seem to be present in low percentages[28]. Recent reviews[5,10,25] argue that TcI does not sustain long-lasting infections and is an opportunistic parasite generally unable to cause severe chronic disease to humans, but the authors judiciously recommend specific research to support this idea. Indeed, clinical-epidemiological data from areas where TcI prevails in domestic cycles (northern South America, Central America, and Mexico) show that the infection is a significant cause of disease and suffering[3,4]. Transmission must therefore be combated disregarding the parasite strains locally involved.

We have shown that *R. neglectus* is also involved in the transmission of *T. rangeli* KP1+, adding support to the hypothesis of adaptation (and perhaps co-evolution) of two parasite populations (KP1+ and KP1-) to distinct evolutionary *Rhodnius* lineages (the *pictipes-pallescens-colombiensis-ecuadoriensis* group with KP1- and the *prolixus-robustus-nasutus-neglectus-domesticus* group with KP1+)[21,44]. In Brazil, *T. rangeli* has been found infecting wild mammals and vectors in the Amazon[8,9,29], *R. domesticus* in Bahia[1], wild rodents (*Echimys dasythrix*) in Santa Catarina[42], sylvatic *R. neglectus* from Tocantins[12], and, recently, *D. albiventris* and *R. neglectus* in Minas Gerais[35,36]. We present here molecular evidence that *T. rangeli* infects *R. neglectus* from *M. flexuosa* palm trees in the DF, widening the known geographic range of this parasite in Brazil and warning about possible false-positive results of diagnostic tests for *T. cruzi* infection.

CONCLUSIONS

In spite of the sympatric occurrence of *D. albiventris* (an important wild and peridomestic *T. cruzi* reservoir) and *R. neglectus* (a secondary vector able of invading and sometimes colonizing human-related environments), both infected by *T. cruzi*, a low-risk of human Chagas disease transmission could be expected in the DF, considering the low level of infection prevalence amongst reservoirs and vectors observed in this work. But our results lend firm support to the idea that continuous epidemiological-entomological surveillance is crucial in areas where control of strictly domestic triatomines is yielding satisfactory results but enzootic transmission persists[11].

Inhabited rural areas (such as SV, where infected vectors and reservoirs are present) will deserve special attention, particularly whenever invasion and/or colonization of synanthropic ecotopes by native, secondary vectors are reported. The study of enzootic *T. cruzi* cycles in untamed environments (such as RF, a protected ecological

reserve) may provide researchers with a much-needed better insight on natural transmission dynamics within sylvatic foci. Ultimately, it is the elucidation of the ecological interactions between pathogens, reservoirs, vectors, humans, and their shared environment that will provide the keys for sustainable control and surveillance of anthropozoonotic infectious diseases whose eradication will remain unfeasible.

RESUMO

Transmissão enzoótica de *Trypanosoma cruzi* e *T. rangeli* no Distrito Federal, Brasil

O Distrito Federal (DF) do Brasil está localizado no bioma Cerrado, um complexo de fisionomias savânicas incluindo matas de galeria e campos úmidos permanentes (veredas). Triatomíneos silvestres infectados por *Trypanosoma cruzi* ocorrem na área, mas a transmissão enzoótica de tripanossomatídeos permanece insuficientemente caracterizada. Um estudo parasitológico envolvendo triatomíneos silvestres (166 *Rhodnius neglectus* coletados em palmeiras da espécie *Mauritia flexuosa*) e pequenos mamíferos (98 marsupiais e 70 roedores, totalizando 18 espécies) foi conduzido em 18 áreas, principalmente matas de galeria e veredas. Os parasitas foram isolados, identificados morfologicamente e caracterizados por PCR do DNA do cinetoplasto (kDNA) e núcleo (gene mini-exon). Seis *R. neglectus*, sete *Didelphis albiventris* e um *Akodon cursor* estavam infectados por tripanossomatídeos; a infecção em reservatórios silvestres é documentada pela primeira vez no DF. O PCR do kDNA detectou *T. cruzi* em cinco *R. neglectus* e o PCR do gene mini-exon revelou *T. cruzi* I nos isolados de *D. albiventris.* Um dos insetos mostrou estar infectado por *T. rangeli* KP1+. Apesar da ocorrência de *D. albiventris* (um importante reservatório silvestre e peridoméstico) e *R. neglectus* (um vetor secundário capaz de invadir domicílios) infectados por *T. cruzi,* um baixo risco de transmissão da doença de Chagas humana seria esperado no DF, considerando a baixa prevalência da infecção apresentada neste trabalho. A evidência molecular apresentada neste trabalho confirma a circulação de *T. rangeli* KP1+ com *R. neglectus* como vetor, amplia a distribuição geográfica deste parasita no Brasil e reforça a hipótese de adaptação de populações de *T. rangeli* (KP1+ e KP1-) a diferentes linhagens evolutivas de espécies de *Rhodnius*.

ACKNOWLEDGEMENTS

We are deeply grateful to Fábio, Rafael, Samuel, Rinara, Fernanda, and Wanessa for their assistance in fieldwork. Special thanks are due to Dr GA Vallejo for generously providing the primers used in molecular analyses and Marcelo Lima Reis for providing permission and technical support to work in Santuário da Vida Silvestre of Riacho Fundo. We also thanks to the anonymous reviewers of this paper.

REFERENCES

1. BARRETT, T.V. & OLIVEIRA, T.S. - A trypanosoma indistinguishable from *Trypanosoma rangeli* in the haemolymph of *Rhodnius domesticus* from Brazil. **Trans. roy. Soc. trop. Med. Hyg., 71:** 445-446, 1977.

2. BARRETTO, M. P. - Epidemiologia. In: BRENER, Z. & ANDRADE, Z.A., ed. ***Trypanosoma cruzi* e doença de Chagas.** Rio de Janeiro, Guanabara Koogan, 1979. p. 89-151.

3. BOSSENO, M.F.; BARNABÉ, C.; MAGALLÓN-GASTÉLUM, E. *et al.* **-** Predominance of *Trypanosoma cruzi* lineage I in Mexico. **J. clin. Microbiol., 40:** 627-632, 2002.

4. BRENIÉRE, S.F.; BOSSENO, M.F.; TELLERIA, J. *et al.* - Different behaviour of two *Trypanosoma cruzi* major clones: transmission and circulation in young Bolivian patients. **Exp. Parasit., 89:** 285-295, 1998.

5. BUSCAGLIA, C.A. & DI NOIA, J.M. - *Trypanosoma cruzi* clonal diversity and epidemiology of Chagas disease. **Microbes Infect., 5:** 419-427, 2003.

6. CARCAVALLO, R.U.; RODRÍGUEZ, M.E.F.; SALVATELLA, R. *et al*. - Habitats and related fauna. In: CARCAVALLO, R.U.; GALÍNDEZ GIRÓN, I.; JURBERG, J. & LENT, H., ed. **Atlas of Chagas disease vectors in Americas.** Rio de Janeiro, FIOCRUZ, 1998. v. 2, p. 561-600.

7. CUBA CUBA, C.A. - Revisión de los aspectos biológicos y diagnósticos del *Trypanosoma (Herpetosoma) rangeli*. **Rev. Soc. bras. Med. trop., 31:** 207-220, 1998.

8. D'ALESSANDRO, A.; EBERHARD, M.; de HINCAPIÉ, O. & HALSTEAD, S. - *Trypanosoma cruzi* and *Trypanosoma rangeli* in *Saimiri sciureus* from Bolivia and *Saguinus mistax* from Brasil. **Amer. J. trop. Med. Hyg., 35:** 285-289, 1986.

9. DEANE, L.M. - Novo hospedeiro de tripanosomas dos tipos *cruzi* e *rangeli* no Estado do Pará. O marsupial *Metachirops opossum opossum*. **Rev. bras. Malar., 10:** 531-541, 1958.

10. DEVERA, R.; FERNANDES, O. & COURA, J.R. - Should *Trypanosoma cruzi* be called "cruzi" complex? A review of the parasite diversity and the potential of selecting population after *in vitro* culturing and mice infection. **Mem. Inst. Oswaldo Cruz, 98:** 1-12, 2003.

11. DIAS, J.C.P. - Epidemiologia. In: BRENER, Z. & ANDRADE, Z.A., ed. ***Trypanosoma cruzi* e doença de Chagas**. Rio de Janeiro, Guanabara Koogan, 2000. p. 48-54.

12. DIOTAIUTI, L.; SILVEIRA, A.C.; ELIAS, M. & STEINDEL, M. - The possibility of occurrence of *Trypanosoma rangeli* in the State of Tocantins, Brazil. **Mem. Inst. Oswaldo Cruz, 87:** 451, 1992.

13. DIOTAIUTI, L.A.; PEREIRA, A.S.; LOIOLA, C.F. *et al*. - Inter-relation of sylvatic and domestic transmission of *Trypanosoma cruzi* in areas with and without vectorial transmission in Minas Gerais, Brazil. **Mem. Inst. Oswaldo Cruz, 90:** 443-448, 1995.

14. FERNANDES, A.J.; CHIARI, E.; RODRIGUES, R.R.; DIAS, J.C. & ROMANHA, A.J. - The importance of the opossum (*Didelphis albiventris*) as a reservoir for *Trypanosoma cruzi* in Bambui, Minas Gerais State. **Mem. Inst. Oswaldo Cruz, 86**: 81-85, 1991.

15. FERNANDES, O.; MANGIA, R.H.; LISBOA, C.V. *et al*. - The complexity of the sylvatic cycle of *Trypanosoma cruzi* in Rio de Janeiro State (Brazil) revealed by the non-transcribed spacer of the mini-exon gene. **Parasitology, 118:** 161-166, 1999.

16. FORATTINI, O.P.; JUÁREZ, E.; RABELLO, E.X.; PATTOLI, D. & CORREA, R.R. - Infestação domiciliar por *Triatoma infestans* e alguns aspectos epidemiológicos da tripanossomíase americana em área do Estado de São Paulo, Brasil. **Rev. Saúde públ. (S. Paulo), 3:** 159-172, 1969.

17. GAUNT, M. & MILES, M. - The ecotopes and evolution of triatomine bugs (Triatominae) and their associated trypanosomes. **Mem. Inst. Oswaldo Cruz, 95:** 557-565, 2000.

18. GAUNT, M.W.; YEO, M.; FRAME, I.A. *et al*. - Mechanism of genetic exchange in American trypanosomes. **Nature, 421**: 936-939, 2003.

19. GRISARD, E.C.; STEINDEL, M.; GUARNERI, A.A. *et al*. - Characterization of *Trypanosoma rangeli* strains isolated in Central and South America: an overview. **Mem. Inst. Oswaldo Cruz, 94:** 203-209, 1999.

20. GUHL, F.; JARAMILLO, C.; CARRANZA, J.C. & VALLEJO, G.A. - Molecular characterization and diagnosis of *Trypanosoma cruzi* and *Trypanosoma rangeli*. **Arch. med. Res., 33:** 362-370, 2002.

21. GUHL, F. & VALLEJO, G.A. - *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920: an updated review. **Mem. Inst. Oswaldo Cruz, 98:** 435-442, 2003.

22. GURGEL-GONÇALVES, R. - **Distribuição espacial de populações de Triatominae (Hemiptera: Reduviidae) em palmeiras da espécie *Mauritia flexuosa* e circulação enzoótica de *Trypanosoma cruzi* e *Trypanosoma rangeli* no Distrito Federal, Brasil**. Brasília, 2003. (Dissertação de Mestrado - Faculdade de Ciências da Saúde da Universidade de Brasília).

23. GURGEL-GONÇALVES, R.; PALMA, A.R.T.; MENEZES, M.N.A.; LEITE, R.N. & CUBA CUBA, C.A. - Sampling *Rhodnius neglectus* (Triatominae) in *Mauritia flexuosa* palm trees (Arecaceae): a field study in the Brazilian Savanna. **Med. vet. Ent., 17:** 347-349, 2003.

24. JANSEN, A.M.; PINHO, A.P.S.; LISBOA, C.V. *et al*. - The sylvatic cycle of *Trypanosoma cruzi*: a still unsolved puzzle. **Mem. Inst. Oswaldo Cruz, 94:** 203-204, 1999.

25. MACEDO, A.M.; MACHADO, C.R.; OLIVEIRA, R.P. & PENA, S.D.J. - *Trypanosoma cruzi*: genetic structure of populations and relevance of genetic variability to the pathogenesis of Chagas disease. **Mem. Inst. Oswaldo Cruz, 99:** 1-12, 2004.

26. MELLO, D.A. - Aspectos do ciclo silvestre do *Trypanosoma cruzi* em regiões do Cerrado (Município de Formosa, Goiás). **Mem. Inst. Oswaldo Cruz, 76:** 227-246, 1981.

27. MILES, M.A.; SOUZA, A.; PÓVOA, M.M. *et al*. - Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochthonous patients with Chagas disease in Amazonian Brazil. **Nature, 272:** 819-821, 1978.

28. MILES, M.A.; CEDILLOS, R.A.; PÓVOA, M.H. *et al*. - Do radically dissimilar *Trypanosoma cruzi* strains (zymodemes) cause Venezuelan and Brazilian forms of Chagas disease? **Lancet, 1:** 1338-1340, 1981.

29. MILES, M.A.; ARIAS, J.R.; VALENTE, S.A.S. *et al.* - Vertebrate hosts and vectors of *Trypanosoma rangeli* in the Amazon basin of Brazil. **Amer. J. trop. Med. Hyg., 32:** 1251-1259, 1983.

30. MILES, M.A.; APT, B.W.; WIDMER, G.; PÓVOA, M.M. & SCHOFIELD, C.J. - Isoenzyme heterogeneity and numerical taxonomy of *Trypanosoma cruzi* stocks from Chile. **Trans. roy. Soc. trop. Med. Hyg., 78:** 526-535, 1984.

31. MILES, M.A.; FELICIANGELI, M.D. & ROJAS DE ARIAS, A. - American trypanosomiasis (Chagas disease) and the role of molecular epidemiology in guiding control strategies. **Brit. med. J., 326:** 1444-1448, 2003.

32. PALMA, A.R.T. - **Estrutura de comunidades de pequenos mamíferos no Cerrado.** Brasília, 2003. (Tese de Doutorado - Instituto de Biologia da Universidade de Brasília).

33. PINHO, A.P.; CUPOLILLO, E.; MANGIA, R.H.; FERNANDES, O. & JANSEN, A.M. - *Trypanosoma cruzi* in the sylvatic environment: distinct transmission cycles involving two sympatric marsupials. **Trans. roy. Soc. trop. Med. Hyg., 94:** 509-514, 2000.

34. RABINOVICH, J.; SCHWEIGMANN, N.; YOHAI, V. & WISNIVESKY-COLLI, C. - Probability of *Trypanosoma cruzi* transmission by *Triatoma infestans* (Hemiptera: Reduviidae) to the opossum *Didelphis albiventris* (Marsupialia: Didelphidae). **Amer. J. trop. Med. Hyg., 65**: 125-130, 2001.

35. RAMIREZ, L.E.; MACHADO, M.I.; MAYWALD, P.G. *et al*. - Primeira evidência de *Trypanosoma rangeli* no sudeste do Brasil, região endêmica para doença de Chagas. **Rev. Soc. bras. Med. trop., 31:** 99-102, 1998.

36. RAMIREZ, L.E.; LAGES-SILVA, E.; ALVARENGA-FRANCO, F. *et al*. - High prevalence of *Trypanosoma rangeli* and *Trypanosoma cruzi* in opossums and triatomids in a formerly-endemic area of Chagas disease in Southeast Brazil. **Acta trop. (Basel), 84:** 189-198, 2002.

37. RIBEIRO, J.F. & WALTER, B.M.T. - Fitofisionomias do bioma cerrado. In: SANO, S.M. & ALMEIDA, S.P., ed. **Cerrado ambiente e flora.** Planaltina, Embrapa, 1998. p. 89-166.

38. SANTOS, A.H.; ISAC, E.; SILVA, J.L. *et al*. - Índices de infestação domiciliar e infecção natural pelo *Trypanosoma cruzi* das espécies de triatomíneos capturadas no estado de Goiás, no período 1994-1998. **In: Congresso Brasileiro de Parasitologia, 14**, Poços de Caldas, 1999. **Resumos.** p. 150.

39. SCHWEIGMANN, N.J.; PEITROKOVSKY, S.; BOTTAZZI, V.; CONTI, O. & WISNIVESKY-COLLI, C. - Interaction between *Didelphis albiventris* and *Triatoma infestans* in relation to *Trypanosoma cruzi* transmission. **Mem. Inst. Oswaldo Cruz, 90:** 679-682, 1995.

40. SCHWEIGMANN, N.J.; PEITROKOVSKY, S.; BOTTAZZI, V. *et al.* - Prevalence of *Trypanosoma cruzi* infection in opossum (*Didelphis albiventris*) in Santiago del Estero, Argentina. **Rev. Panamer. Salud publ., 6:** 371-377, 1999.

41. SILVEIRA, A.C.; FEITOSA, V.R. & BORGES, R. - Distribuição de triatomíneos capturados no ambiente domiciliar, no período de 1975/83, Brasil. **Rev. bras. Malar., 36:** 15-312, 1984.

42. STEINDEL, M.; CARVALHO-PINTO, J.C.; TOMA, H.K. *et al*. - *Trypanosoma rangeli* (Tejera, 1920) isolated from a sylvatic rodent (*Echimys dasythrix*) in Santa Catarina Island, Santa Catarina State: first report of this trypanosoma in southern Brazil. **Mem. Inst. Oswaldo Cruz, 86:** 73-79,1991.

43. TIBAYRENC, M. & AYALA, F.J. - Isoenzyme variability of *Trypanosoma cruzi*, the agent of Chagas disease: genetic, taxonomy and epidemiological significance. **Evolution, 42:** 277-292, 1988.

44. VALLEJO, G.A.; GUHL, F.; CARRANZA, J.G. *et al*. - Parity between kinetoplast DNA and mini exon gene sequences support either clonal evolution or speciation in *Trypanosoma rangeli* strains isolated from *Rhodnius colombiensis*, *R. pallescens* and *R. prolixus* in Colombia. **Infect. Gen. Evol., 67:** 1-7, 2002.

Received: 1 June 2004
Accepted: 14 October 2004