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Trypanosoma cruzi (Kinetoplastida Trypanosomatidae): Ecology of the transmission cycle in the wild environment of the Andean valley of Cochabamba, Bolivia

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Abstract

An active *Trypanosoma cruzi* transmission cycle maintained by wild rodents in the Andean valleys of Cochabamba Bolivia is described. Wild and domestic *Triatoma infestans* with 60% infection with *T. cruzi* were found and was evidenced in 47.5% (rodents) and 26.7% (marsupial) by parasitological and/or serologycal methods. *Phyllotis ocilae* and the marsupial species *Thylamys elegans*, are the most important reservoirs followed by *Bolomys lactens* and *Akodon boliviensis*. In spite of both genotypes (TCI and TCII) being prevalent in Bolivia, in our study area only *T. cruzi* I is being transmitted. Our data suggest that wild *T. infestans* and wild small mammals play an important role in the maintenance of the transmission cycle of *T. cruzi*. Furthermore, the finding of high prevalence of *T. cruzi* infection in wild *T. infestans* point to the risk of the dispersion of Chagas' disease.

Index Descirptors and Abbreviations: Chagas disease; Wild reservoirs; Andean valley Bolivia; *Trypanosoma cruzi* (Kinetoplastida Trypanosomatidae); *Triatoma infestans* (Reduviidae: Triatominae); Rodentia: *Phyllotis ocilae*; *Bolomys lactens*; *Akodon boliviensis*; *Akodon* sp.; *Graomis* sp.; *Galea musteloides*; Marsupialia: *Thylamys elegans*; DNA, deoxyribonucleic acid; dntp, deoxyribonucleotide triphosphate; pcr, polymerase chain reaction; lit, liver infusin tryptose; IFA, immunofluorescent antibody; edta, ethylenediaminetetraacetic acid; mlee, multi locus enzyme electrophoresis; NADP, nicotinamide adenine dinucleotide phosphate; TBE, Tris-borate 89 mM, 2 mM pH 8; UV, ultraviolet

1. Introduction

American trypanosomiasis or Chagas disease is well recognized as the most serious human parasitic disease of the Americas in terms of its social and economic impact (WHO, 2002). When in the early 1990s more than 16 million people were infected, the control interventions successfully pursued in wide areas of Latin America, are estimated to have reduced incidence of Chagas disease in Southern Cone countries by 60% in Paraguay and by up to 99% in Uruguay and Chile (WHO, 1991; WHO/CTD, 2004). In Bolivia, recent Chagas disease control activities have made substantial progress in control interventions (Noireau et al., 2005). The life cycle of *Trypanosoma cruzi* (Kinetoplastide: Trypanosomatidae), the agent of Chagas disease, alternates between vertebrates and insects, with

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different major developmental stages involved in each host: replicative epimastigotes and infective metacyclic trypomastigotes in the blood-sucking vector and intracellular replicative amastigotes and bloodstream trypomastigotes in the mammal host (Chagas, 1911; Brener, 1979).

T. cruzi is a widely distributed trypanosomatid. It has been detected in more than 100 mammal species, belonging to eight mammal orders, dispersed through all phytogeographic regions of the Neotropics (Barretto, 1979). In spite of the early recognition of the broad range of mammals hosts of *T. cruzi*, the role played by each species in the dispersion and/or maintenance of the parasite in the distinct areas of its occurrence is as yet not completely known. This is particularly the case of Bolivia, where only few studies on this topic have been undertaken (Torrico, 1946). Indeed, the epidemiology of the transmission cycle of *T. cruzi* in the wild is a complex issue given that *T. cruzi* is a multi host and extremely heterogeneous parasite that may display distinct interaction patterns with its hosts in distinct space and time scales depending on the genetic background of both parasite and host (Roque et al., 2005). This question is exemplified by the unique cycle of the parasite in the lumen of the scent glands of *Didelphis marsupialis*, where the protozoan multiplies as epimastigotes and differentiates into metacyclic forms (Deane et al., 1984a; Jansen et al., 1999).

Domestic, peridomestic and sylvatic transmission cycles of *T. cruzi* may be isolated or connected (Miles et al., 1977). Nevertheless, the so-called sylvatic transmission cycle is far more complex than was formerly understood, since distinct transmission cycles may occur simultaneously in the same forest fragment independently of the forest strata or behavior pattern of the hosts (Fernandes et al., 1999; Jansen et al., 2000; Pinho et al., 2000; Lisboa et al., 2000). Additionally, the importance of a given species as a reservoir of *T. cruzi* varies according to the ecotope of its occurrence (Lisboa et al., 2000).

The population structure of *T. cruzi* is assumed to be basically clonal (Tybayrenc et al., 1990, 1991a), with significant high intra-specific heterogeneity that has been revealed by biological, biochemical and molecular methods (Dvorak et al., 1980; Morel et al., 1980; Tybayrenc and Ayala, 1988; Souto et al., 1996; Fernandes et al., 1998). Although some correlations between subpopulations of the parasite, outcome of infection, wild mammalian hosts and ecology have been proposed, in overall, this subject is still controversial. The pioneer biochemical characterization based on the electrophoretic profile of 6 enzymes recognizes three groups (zymodemes), respectively, Z1 and Z3, described as corresponding to the sylvatic transmission cycle and Z2, corresponding to the domestic cycle (Miles et al., 1977, 1978, 1980). Analyses of ribosomal ribonucleic acid (RNA) genes and mini-exon repeats of hundreds of isolates derived from humans, triatomines and wild mammals has led to the description of two distinct and phylogenetically distant sets (lineages) of *T. cruzi*, that were described as associated mainly with domestic (lineage 1) and sylvatic (lineage 2) transmission cycle (Souto et al., 1996; Zingales et al., 1998). In an international meeting, the main zymodemes and lineages were grouped in two genotypes termed *T. cruzi* II (TCII) and *T. cruzi* I (TCI), corresponding to Z2 (=lineage 1) and Z1 (=lineage 2) respectively; moreover, the correspondence of zymodeme Z3 to TCI or TCII is a still controversial topic (Anonymous, 1999).

Since then, several hypotheses concerning the origin, evolution and putative association of the these two main *T. cruzi* genotypes with a given mammal host, ecotope and human disease have been proposed but still need empirical support. Currently, the predominance of human infection with TCI in northern South America up to and including the Amazon region and the association of acute and chronic human chagasic cardiopathy with TCI infection is a generally accepted fact. (Ruiz-Sanchez et al., 2005; Grijalva et al., 2003). However, the set of reservoir species of TCI and TCII in the distinct biomes are still a matter of debate (Herrera et al., 2005).

In Bolivia, the two major distinct monophyletic groups of clones *T. cruzi* I and *T. cruzi* II have been identified in the domestic cycle (Brenière et al., 1998). These groups were named clonet 20 (*T. cruzi* I) and 39 (*T. cruzi* II) after characterization by hybridization with specific kDNA probes (Brenière et al., 1998). Nevertheless, their association to habitat or mammals hosts as well as of geographical distribution is unknown. All these questions led us to undertake a study of the ecology of the transmission cycle of *T. cruzi* in an Andean valley of Cochabamba Bolivia.

2. Materials and methods

2.1. Study area

Cotapachi is located in a low valley of the department of Cochabamba, in the province of Quillacollo, approximately at 15 km West of Cochabamba city, Bolivia, at 2750 m. above sea level, 17°26′S and 66°17′W. During the rainy season (October–March), the Cotapachi region presents an average temperature of 18.5 °C (highest 32.0 and lowest 5.7 °C). During the dry season (April–September), it displays an average temperature of 14.5 °C (highest 31.3 and lowest −2.2 °C). The average precipitation at the study area during the rainy season is 583.2 mm (highest 924.1 and lowest 294.5 mm), and during the dry season is 45.6 mm (highest 107.6 and lowest 1.5 mm). The values cited above were calculated over 6-month periods during 15 and 20 years, respectively, for temperature and precipitation (according to Servicio Nacional de Metereologia—SENAMIH). The study area is classified in the Bolivian–Tucuman biogeographical province. The vegetation is described and characterized in ecological level as Rio Grande lower prepunean woodland; it is included in the Xeric bioclimate semi-arid dry, of inferior mesotropical bioclimate (Navarro and Maldonado, 2002).

The wild small mammals fauna consists mainly of marsupials and rodents; that prefer the more humid microhabitats among the rocks. The local landscape is characterized by the presence of dry and rocky hills, covered by vegetation composed of scanty bushes (average size 0.5–1 m). Among the dominant plant species in the area, we may cite: Tjako (*Acácia macracantha*), Ulala (*Cereus peruvianus*), Andreshuailla (*Cestorum palqui*), Karalawa (*Nicotiana glauca*), Tunilla (*Opuntia sulphurea*), Choke-chapi (*Xanthium spinosum*), Alko tomate (*Solanum sisymbrifolium*), Hauycha (*Senecio clivicolus*), pasto (*Chloris polidactila*). Predominant arboreal elements are Molle (*Schinus molle*) and *Prosopis* cf. *alba*.

2.2. Collection of triatomines

Burrows of small mammals situated amongst rock-piles were searched for the presence of triatomines from May 2002 to July 2004. Mouse-baited traps as described by Noireau et al. (1999) were used to capture insects.

The insect sampling at the domestic habitat was performed with the aid of pieces of sticky ribbon, approximately 10 cm wide, placed over the internal walls of the houses' bedrooms, at 1.5 m from the floor. The sticky face of the ribbons was directed outside and they were fastened to the walls with the aid of nails (Cortez, 2006).

The study area is divided into five silvatic collecting sites, respectively, southern hill, westerns hill, peridomestic rocks, Inca wall and into human dwellings ($n = 4$). The Inca Wall is a 3-m high rocky wall, that offers a more mesic environment since trees (*S. molle* and *Prosopis alba*) and bushy vegetation (*Acacia macracantha*) may be found there. This more mesic habitat permits the colonization by small wild mammals.

2.3. Microscopic observation

Microscopic observation was considered positive if flagellate parasites were observed in the feces of a triatome specimen during 5 min examination of a drop of feces mixed with phosphate-buffered saline at a 400× magnification, and confirmation of *T. cruzi* infection was carried through by polymerase chain reaction (PCR).

2.4. Capture of small wild mammalians

In sylvatic areas, live-animal traps (Sherman) were placed in linear transects, at 20-m intervals in rock caves, natural pools and canyons, on three nights, for each site. The total capture effort was 560 trap-nights. Animals were collected on expeditions conducted in the dry seasons (260 trap-nights) and rainy seasons (300 trap-nights) from 2002 to 2005.

Blood samples for hemoculture and serum of wild mammals was collected by venepuncture under anesthesia (50 mg/kg bodyweight of ketamine by intramuscular injection). Two tubes containing NNN medium with a liver infusion triptose (LIT) overlay were each inoculated with 0.2 ml blood from each animal (Rey, 2001). The tubes were examined in the laboratory every other week up to a maximum of 4 months.

Furthermore, a total of 27 domestic animals, 05 dogs and 22 domestic guinea-pigs (*Cavia cobaya*), from Cotapachi area, were examined with the informed consent of their owners. Blood to test for anti-*T. cruzi* antibodies by IFA was drawn by puncture of the cephalic vein (dogs) or venepuncture (domestic guinea-pigs).

2.5. Microhematocrit

T. cruzi infection was also diagnosed by microscopic examination of the buffy coat from blood collected in four microhematocrit heparinized tubes (each containing 50 μL blood), as described elsewhere (Freilij et al., 1983). The presence of parasite(s) in microhematocrit tubes defined a *T. cruzi* infection.

2.6. Culture of T. cruzi isolates

Parasites from the positive hemocultures of the digestive tract of each insect and blood samples of each mammalian were amplified in LIT culture medium for a maximum of three passages. Subsequently, the medium was centrifuged (1400 g) and the cell pellet resuspended in 0.2 ml of TE buffer (10 mM Tris–HCl, pH 8.0, 10 mM EDTA) pH 8.0 for cryopreservation, isoenzyme electrophoresis and molecular characterization.

2.7. Serological tests

The immunofluorescent assay (IFA) was performed according to Camargo (1966). Briefly, serial twofold sera dilutions (1:10–1:1280) were assayed against *T. cruzi* axenic medium (LIT) epimastigote forms of strain 100/BR/00F, *T. cruzi* I (Deane et al., 1984b).

Rodent sera were tested with a commercial fluorescein isothiocyanate labeled anti-rat IgG (FITC, Sigma®). Marsupial sera were tested with specific intermediary antibody anti-opossum Ig total and antisera raised in rabbits; the reaction was visualized using a commercial FITC labeled anti-rabbit IgG serum (Sigma®). The cut-off value for the serological titers of rodents was $\geqslant$1:10, since this was the lowest serum dilution of an animal with positive hemoculture. For opossums, the cut-off value was 1:40, as described elsewhere (Jansen et al., 1985).

In addition to being assayed against *T. cruzi*, and in order to evaluate possible cross-reactions, dog sera were also assayed against *Leishmania infantum* (syn. *chagasi*) promastigotes (MHOM/BR/1996/RR050 strain); both reactions were performed with a commercial FITC labeled anti-dog IgG serum fraction (Sigma®). The cut-off value for the serological titers of dogs was $\geqslant$1:40. Two negative controls (sera from laboratory-reared animals) from each tested species were always included. Sera obtained from

experimentally infected animals from all tested species were used as positive control in all assays. Antigen was obtained from parasites harvested from axenic culture in the exponential phase. Parasites were centrifuged twice in buffer solution and maintained in formolized phosphate buffer solution (0.15 M–1%). The reaction was performed on glass slides and antigen was diluted to yield 40 parasites/field under 400× magnification. Commercial FITC conjugates were always used as recommended by the manufacturer (Sigma®).

2.8. Multilocus enzyme electrophoresis

In this study, we analysed 61 isolates of *T. cruzi* derived from triatomines ($n = 57$) and mammals ($n = 4$). The methods used to prepare samples and study eletrophoretic mobility of some enzymes in agarose gels were performed as described by Cupolillo et al. (1994). Allelic variation for the following enzymes was assessed: glucose-6-phosphate dehydrogenase (E.C.1.1.1.49), glucose phosphate isomerase (E.C.5.3.1.9.), malic enzyme (E.C.1.1.1.40), isocitrate dehydrogenase NADP (E.C.1.1.1.42), malate dehydrogenase (E.C.1.1.1.37), Leu-Gly dipeptidase (E.C.3.4.11.1), Leu-Gly dipeptidase (E.C.3.4.11), Leu-Pro dipeptidase (E.C.3.4.13.9), Leu-Leu dipeptidase (E.C.3.4.11), superóxido dismutase (SOD-E.C.1.15.1.1), fumarase (FUM-E.C.4.2.1.2), aconitate hydratase (E.C.4.2.1.3.), 6-phosphoglucomutase (E.C.1.1.1.43), mannose phosphate isomerase (E.C. 5.3.1.8). Strains MDID/BR/90/M1 (*T.cruzi I*); CANIII/BR/99 (Z3) and MHOM/BR/00/Y (*T.cruzi* II) were used as reference.

2.9. Molecular characterization of T. cruzi isolates

Genomic DNA was extracted following the protocols described by Medina-Acosta and Cross (1993). A miniexon multiplex PCR assay was carried out in order to type *Trypanosoma* isolates as TCI, TCII, Z3 or *Trypanosoma rangeli*. The strains typing was performed using the primers described by Fernandes et al. (2001): Tc I (5′-TTG CTC GCA CAC TCG GCT GCAT-3′); Tc II (5′-ACA CTT TCT GTG GCG CTG ATC G-3′); Z-3 (5′-CCG CGW ACA ACC CCT MAT AAA AAT G-3′); Tr (5′-CCT ATT GTG ATC CCC ATC TTC G-3′) and Exon (5′-TAC CAA TAT AGT ACA GAA ACT-3′). PCR was carried out with some modifications: 10 μg of DNA as template, 25 pmol of each primer, 120 μM dNTPs, 1.5 mM $MgCl_2$ and 2.5 U of Amplitaq Gold™ DNA polymerase (Perkin-Elmer). The thermal profile consisted of 1 step of 95 °C for 5 min; 35 cycles of 94 °C for 30 s; 55 °C for 30 s; 72 °C for 1 min and 1 step of 72 °C for10 min. DNA products were resolved by electrophoresis in 2% agarose gel in TBE 0.5×, stained with ethidium bromide, sized with a 100-bp ladder, visualized under UV, and documented with Polaroid film.

3. Results

3.1. Collection success and infection rates of Triatoma infestans

Table 1 shows the high prevalence of infection by *T. cruzi* observed in *T. infestans* collected in both silvatic and domiciliary environments The infection rates were similar (respectively 60 and 67.4%). In the site termed Inca Wall that is distant only 20 m. from human dwellings, the infection rate of triatomines reached 85.7%. No differences in infection rate of *T. infestans* by *T. cruzi* could be observed comparing insects collected in dormitories of houses built of plaster or mud. The abundance of insects varied in these houses according to the building type. Thus, the density of triatomines observed on bedroom walls covered only with mud was much higher than on those plastered with gypsum, respectively 89 and 11% (Table 1). After spraying procedures, as indicated by the research ethics, the houses remained free from insects during the following 20 months.

3.2. Wild mammals

Small mammals faunal composition as well as the data on *T. cruzi* infection of domestic and wild mammals in the Cotapachi area is summarized in Tables 2 and 3.

3.3. Relative abundance of small wild mammals

A total of 55 small wild mammals that included rodents and marsupials, representing seven species was sampled

Table 1
Ecology of *Trypanosoma cruzi* transmission cycle in central valley in Bolivia: *T. cruzi* infection rate in *Triatoma infestans* according to the habitat and site collected

	Sylvatic					Dwellings[a]				
	Southern hill	Western hill	Inca wall	Rocks peri-domestic	Total	1 Plaster	2 Plaster	3 Mud	4 Mud	Total
Insects examined	79	36	35	48	202	6	4	40	42	92
Positive of *T. cruzi*	44	22	30	24	121	4	3	28	27	62
% Positive of *T. cruzi*	55.7	61.1	85.7	50.0	60.0	66.7	75.0	70.0	64.3	67.4

[a] Note that after collection the houses have been sprayed and remained uninfected at second examination $1\frac{1}{2}$ year later.

Table 2
Ecology of *Trypanosoma cruzi* transmission cycle in central valley of Cochabamba Bolivia: abundance and prevalence of *T. cruzi* infection in small wild mammals according to collecting season by immunofluorescence assay (IFA)

Species captured	Abundance (%)	Dry season + *T. cruzi*/N° of animals	Rainy season + *T. cruzi*/N°of animals	Total
Phyllotis ocilae	18 (33.0)	6/12	5/6	11/18 (61.0)
Bolomys lactens	8 (14.0)	1/4	4/4	5/8 (63.0)
Akodon boliviensis	7 (13.0)	1/4	2/3	3/7 (43.0)
Akodon sp.	1 (2.0)	0/1	0/0	0/1 (0.0)
Graomys sp.	1 (2.0)	0/0	0/1	0/1 (0.0)
Thylamys elegans	15 (27.0)	1/3	3/12	4/15 (27.0)
Galea musteloides	5 (9.0)	–/–	–/–	–/–
Total	55 (100.0)	9/24 (37.5)	14/26 (54.0)	23/50 (46.0)

Table 3
Ecology of *Trypanosoma cruzi* transmission cycle in central valley of Cochabamba Bolivia: abundance of small wild mammals according to collecting season and prevalence of *T. cruzi* infection by microhematocrit method (MH), hemoculture (H) and immunofluoresence assay (IFA)

	Total wild mammals				Dry season				Rainy season			
	Abundance (%)	Positive M–H (%)	Positive H (%)	Positive IFA (%)	Abundance (%)	Positive M–H (%)	Positive H (%)	Positive IFA (%)	Abundance (%)	Positive M–H (%)	Positive H (%)	Positive IFA (%)
Rodents	40 (73.0)	8 (20.0)	11 (27.5)	19 (47.5)	26 (90.0)	4 (15.4)	4 (15.4)	8 (30.8)	14 (54.0)	4 (28.5)	7 (50.0)	11 (78.5)
Marsupials	15 (27.0)	1 (6.6)	1 (6.6)	4 (26.7)	3 (10.0)	1 (33.3)	1 (33.3)	1 (33.3)	12 (46.0)	0 (0.0)	0 (0.0)	3 (25.0)
Total	55 (100.0)	9 (16.4)	12 (22.0)	23 (42.0)	29 (100.0)	5 (17.2)	5 (17.2)	9 (31.0)	26 (100.0)	4 (15.4)	7 (27.0)	14 (54.0)

Domestic mammals (domestic guinea-pigs and dogs) displayed negative resulted by M–H, H, and IFA.

and examined (Table 2). The relation between the capture effort and number of collected animals, i.e. the capture success, was 0.8%. Among rodents, *Phyllotis ocilae* was the most abundant and most widely distributed species in the study area.

The abundance of small wild mammals was similar in both the dry and rainy seasons. In this sense a total of 29 (53%) small wild mammals, 26 (90%) rodents and 3 (10%) marsupials, were collected in the dry season. A total of 26 (47%) small wild mammals, from which 14 (54%) were rodents and 12 (46%) marsupials, was collected in the rainy season Fig. 1.

3.4. *Trypanosoma cruzi* infection of small wild mammals

Among rodents, *P. ocilae* and *Bolomys lactens* were the most commonly infected species followed by the rodent *Akodon boliviensis* and marsupial *Thylamys elegans*, was the most commonly infected species as assessed by IFA (61, 63, 43 and 27%, respectively, Table 2). Five *Galea musteloides* specimens showed negative results for *T. cruzi* infection both by the microhematocrit method and by hemoculture.

T. cruzi infection showed an expressive increase in the wild mammals captured during the rainy season

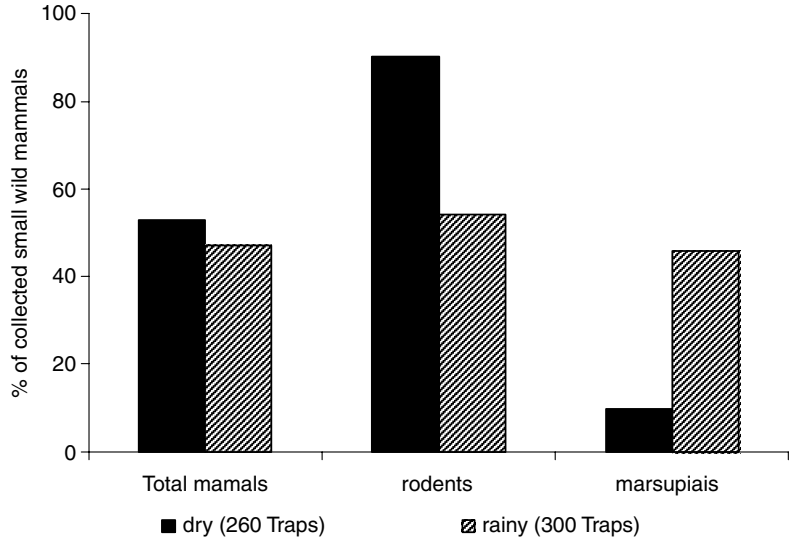

Fig. 1. Ecology of *Trypanosoma cruzi* transmission cycle in central valley of Cochabamba Bolivia: small wild mammals fauna composition.

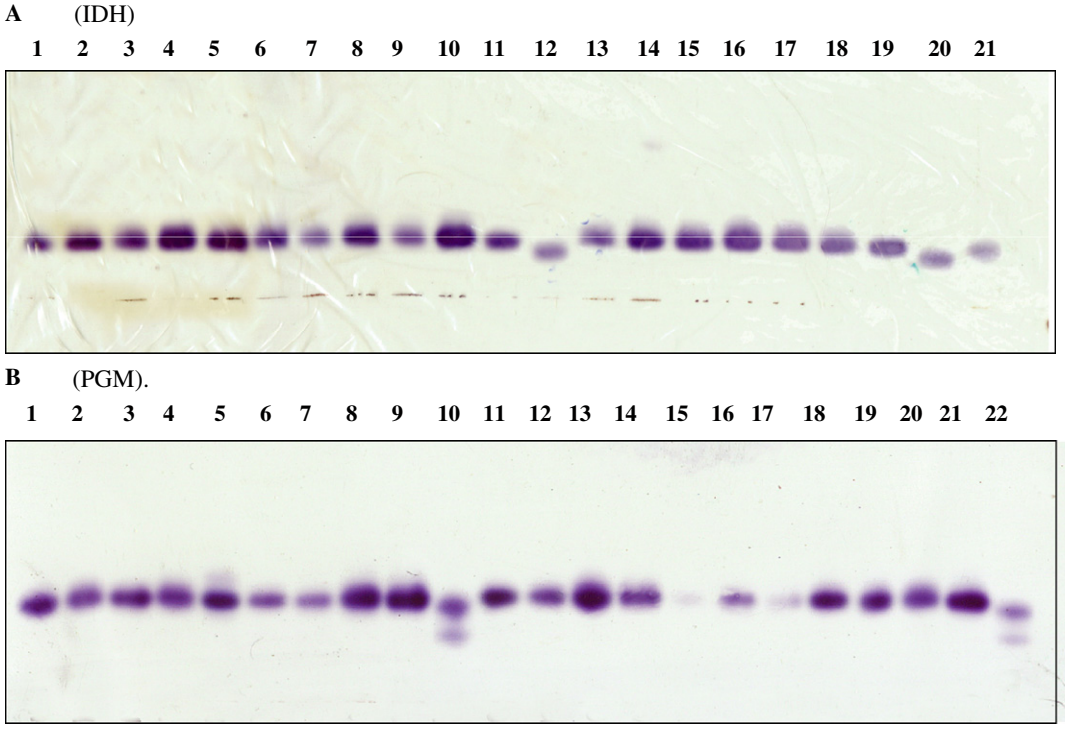

Fig. 2. Ecology of *Trypanosoma cruzi* transmission cycle in central valley of Cochabamba Bolivia: multilocus enzyme electrophoretic profiles for IDH (A) and PGM (B), products corresponding to *T. cruzi* isolates from wild *Triatoma infestans*, domestic *T. infestans* and wild small mammals of the Andean valley of Cochabamba. (A) (IDH): lanes 1 and 16, *T. cruzi* isolates from wild small rodents and marsupials (southern hill). Lanes 4, 6, 7, 8, 14 and 15, *T. cruzi* isolates from wild *T. infestans* (southern hill). Lanes 2, 3 and 18, *T. cruzi* isolates from domestic *T. infestans* (dwelling). Lanes 5, 9 and 13, *T. cruzi* isolates from wild *T. infestans* (inca wall). Lane 17, *T. cruzi* isolates from wild *T. infestans* (western hill). Lanes 10 and 21, CANIII/BR/99 (Z3). Lanes 11 and 19, MDID/BR/90/M1 (*T. cruzi* 1). Lanes 12 and 20, MHOM/BR/00/Y (*T. cruzi* 2). (B) (PGM): Lanes 1–4, *T. cruzi* isolates from wild small rodents and marsupials (southern hill). Lanes 6, 9, 13, 14, 16, 18, 19 and 20, *T. cruzi* isolates from wild *T. infestans* (southern hill). Lanes 7, 15, 17 and 21, *T. cruzi* isolates from domestic *T. infestans* (dwelling). Lanes 5 and 8, *T. cruzi* isolates from wild *T. infestans* (inca wall). Lane 12, CANIII/BR/99 (Z3). Lane 11, MDID/BR/90/M1 (*T. cruzi* I). Lanes 10 and 22, MHOM/BR/00/Y (*T. cruzi* II).

when compared to the dry season, as detected by Microhematocrit, hemoculture and/or IFA, (Table 3). Rodents were the most abundant and most widely distributed species in the area with and without human influence, and were the most commonly infected species as assessed by IFA in the rainy and dry season (78.5 and 30.8%, respectively). Among the marsupials, *T. elegans*, were also more abundant in the rainy season when compared to the dry season. However, independent of the abundance in the season, the average frequency of infection by *T. cruzi* showed relatively low values by Microhematocrit, hemoculture and/or IFA in the rainy and dry season (25.0 and 33.3%, respectively) (Table 3).

This absence of vectors in the domestic ecotope and absence of *T. cruzi* infection in domestic animals as tested by Microhematocrit, hemoculture and/or IFA, probably indicates an interruption of the transmission of *T. cruzi* infection. However, the high infection rate of sylvatic *T. infestans* and wild mammals by *T. cruzi* suggested a higher epidemiological risk, as summarized in the Tables 1 and 3.

3.5. Multilocus enzyme electrophoresis and molecular characterization of T. cruzi samples

In the current report, using a multiplex PCR assay based on the non-transcribed spacer of the mini-exon gene (Fernandes et al., 2001), the 34 *Trypanosoma* isolates from wild *T. infestans*, domestic *T. infestans* and wild small rodents and marsupials were typed as *T. cruzi* I (Fig. 3). The MLEE corroborated the characterization by mini-exon: all strains exhibited a *T. cruzi* I profile in the 14 systems analyzed, displaying, moreover, only microheterogeneity. (Fig. 2).

4. Discussion

In this study, an active *T. cruzi* transmission cycle was observed, maintained basically by wild rodents in an area of the Mesothermic Andean valleys of Cochabamba, a complex and diverse biome of central Bolivia, where wild *T. infestans* is present. High infection rates by *T. cruzi* in the populations of wild *T. infestans* seem to be not occasional in this region since its first description (Torrico,

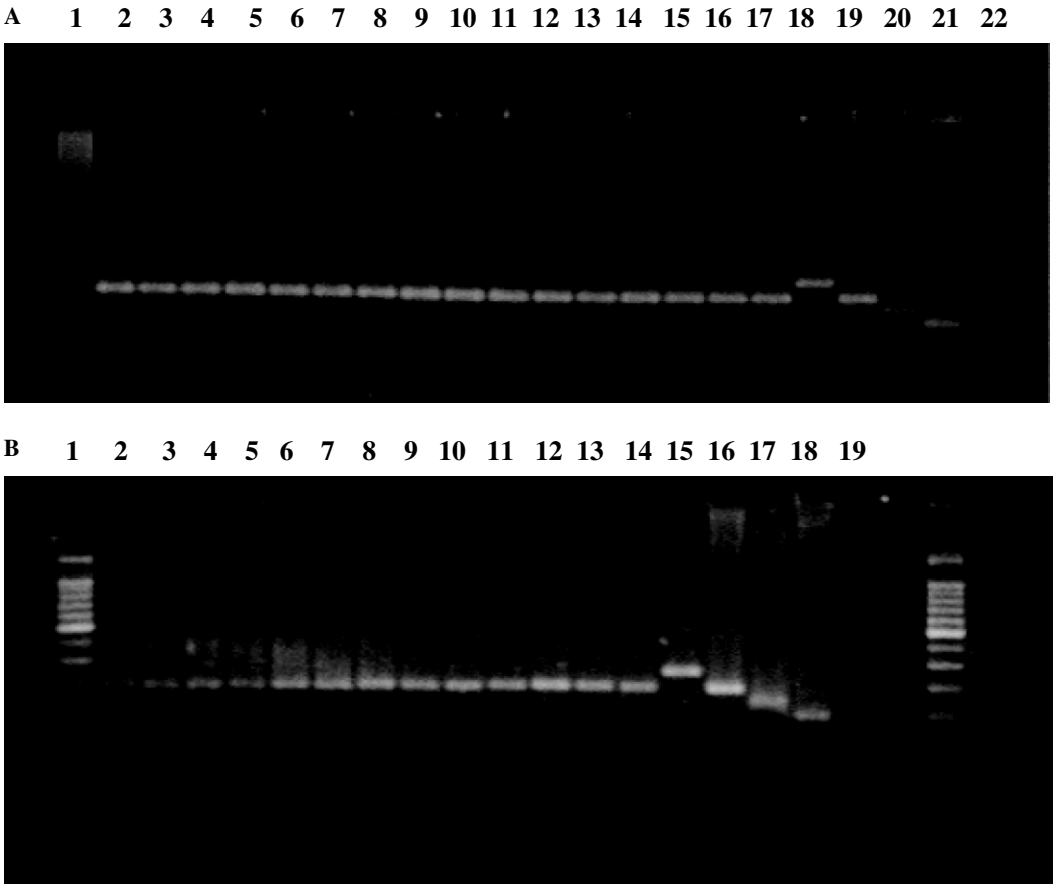

Fig. 3. Ecology of *Trypanosoma cruzi* transmission cycle in central valley of Cochabamba Bolivia: agarose electrophoresis of the amplified PCR products corresponding to the variable region of the non-transcribed spacer of mini-exon gene of *Trypanosoma cruzi* isolates from wild *Triatoma infestans*, domestic *T. infestans* and wild small mammals of the Andean valley of Cochabamba. (A) Lane 1, molecular weight marker corresponding to ϕ X DNA digested with *Hae* III. Lanes 2–7, *T. cruzi* isolates from wild *T. infestans* (southern hill). Lanes 8–10, *T. cruzi* isolates from wild *T. infestans* (inca wall). Lanes 11 and 12, *T. cruzi* isolates from wild *T. infestans* (western hill). Lanes 13–15, *T. cruzi* isolates from domestic *T. infestans* (dwelling). Lanes 16 and 17, *T. cruzi* isolates from wild small rodents and marsupials (southern hill). Lane 18, *T. cruzi* II pattern. Lane 19, *T. cruzi* I pattern. Lane 20, *T. cruzi* Z3 pattern. Lane 21, *T. rangeli* pattern. Lane 22, negative control. (B) Lane 1, molecular weight marker corresponding to ϕ X DNA digested with *Hae* III. Lanes 2 and 3, *T. cruzi* isolates from wild *T. infestans* (southern hill). Lanes 4–6, *T. cruzi* isolates from wild *T. infestans* (inca wall). Lanes 7–12, *T. cruzi* isolates from domestic *T. infestans* (dwelling). Lanes 13 and 14, *T. cruzi* isolates from wild small rodents (inca wall). Lane 15, *T. cruzi* II pattern. Lane 16, *T. cruzi* I pattern. Lane 17, *T. cruzi* Z3 pattern. Lane 18, *T. rangeli* pattern. Lane 19, negative control.

1946). Moreover, hitherto no data concerning mammalian reservoirs of *T. cruzi* in the studied area are available. Similarly high rates of infection of wild and domiciliary *T. infestans* (respectively 60 and 67.4%) were detected in the present study. Furthermore, after the intervention of the National Program for Control of Chagas' disease vector/ Bolivia, no *T. infestans* populations could be observed $1\frac{1}{2}$ year after spraying in domiciliary environments. This shows the importance of the maintenance of epidemiological surveillance procedures, particularly regular spraying. This is principally the case of the study area: indeed, this region located centrally in South America may act as a dispersion focus of Chagas disease as already proposed. (Noireau et al., 2005). High prevalence of infection by *T. cruzi* in wild *T. infestans* populations is a common feature not only in the presently studied area but in all Andean valleys. In spite of this, the ecology of the transmission cycle of *T. cruzi* in the Mesothermic Andean valleys of Cochabamba and similar areas remains almost not investigated. The knowledge of the ecology of these cycles (including parasite subpopulations that are involved, vectors and mammalian reservoirs) is of foremost importance for the definition of vigilance schedules (Gurgel-Goncalves et al., 2004).

Within the temporal and spatial framework of this study and considering that reservoirs are defined as one or a complex of species essential for the long-term maintenance of a given parasite in a specific ecosystem (Ashford, 1996), our results indicate that *P. ocilae* and the marsupial species z*T. elegans*, are the most important reservoirs followed by *B. lactens* and *A. boliviensis*. *P. ocilae* is indeed a very common rodent species in semi-arid climates. This basically granivorous roudent is a seasonal species. Consequently, the variations of prevalence of infection by *T. cruzi* are not surprising. The single collected marsupial species, the mouse opossum, *T. elegans*, was not abundant but displayed expressive *T. cruzi* infection prevalence and high

parasitemia as proved by the positive results of the two parasitological methods of diagnosis employed. In this scenario, *T. elegans* should also be considered as playing important role in the transmission of *T. cruzi* in this area. This Didelphid species is described as displaying a seasonal reproductive and recruitment rate. Therefore, the marked climatic seasonality, with severe drought periods and low temperatures contrasting to the rainy season with amenable temperatures of the Andean valley of Cochabamba modulates the density and survival of these two local more important *T. cruzi* host species and, consequently, the transmission cycle of the parasite. In fact, infection prevalence of *T. cruzi* in small wild mammals was higher during the rainy season (54.0%) when compared to the dry seasons (31.0%). Also, the genus Bolomys (the more prevalent rodent) is seasonal. Its higher reproductive activity occurs during the rainy seasons (Redford and Eisenberg, 1992). The higher availability of food during the rainy season favours the increase of mammalian host populations and consequently the density of sylvatic *T. infestans* populations.In contrast, during the dry season, there is a diminution of food sources in wild ecotopes especially in the winter, when very low temperatures may be recorded in the region. The high mortality among the small wild mammalian population recorded during this season probably also affects the density of the triatomine population.

In the study area, mini-exon genotyping revealed only *T. cruzi* I in both sylvatic and domestic ecotopes and no enzyme polymorphism was observed between the strains. These results contrast with available data of other regions of Bolivia where both *T. cruzi* I and *T. cruzi* II were identified in the domestic cycle, exhibiting considerable genetic heterogeneity and where frequently mixed infections have been reported (Brenière et al., 1995; Bosseno et al., 1996, 1998). This aspect probably reflects that this transmission cycle is restricted to the animals of this particular studied valley and that no interchange of animals and consequently of subpopulations of the parasite with surrounding areas is occurring. The risk of Chagas disease for local human population is obvious, mainly if it is considered that also the genotype TCI may cause severe disease in humans (Ruiz-Sanchez et al., 2005).

The absence of infection of the dogs in spite of the high prevalence of infected vectors inside human dwellings is probably the consequence of the proximity of the houses to the wild and of the feeding preference of the local *T. infestans* preference. Indeed, wild mammals may be found colonizing very close to human dwellings as is the case of the Inca wall. This area demonstrated to be a unique habitat in the studied region. Indeed, the local presence of bushy vegetation and trees, provides a more mesic environment that permit the colonization and maintenance of small wild mammal fauna and insects. Indeed, in this habitat, the density of mammals was higher than that observed in other collection sites.

The data presented suggests that wild *T. infestans* and wild small mammals play an important role in the maintenance of the transmission cycle of *T. cruzi* in the Mesothermic Andean valleys of Cochabamba Bolivia. Furthermore, the finding of a high prevalence of *T. cruzi* infection in wild *T. infestans* points to the risk of the dispersion of Chagas' disease.

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