Sylvatic Triatomines (Hemiptera: Reduviidae) in Bolivia: Trends Toward Domesticity and Possible Infection with *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae)

FRANCOIS NOIREAU,[1] MARIE-FRANCE BOSSENO,[2] ROXANA CARRASCO,[3] JENNY TELLERIA,[1] FERNANDO VARGAS,[1] CLARA CAMACHO,[3] NINA YAKSIC,[3] AND SIMONE FREDERIQUE BRENIÈRE[2]

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ABSTRACT The risk of domestic transmission of *Trypanosoma cruzi* (Chagas) by sylvatic triatomines was assessed in an isolated area of the subandean region of Bolivia. None of the 390 residents examined had serological evidence of infection. Two sylvatic triatomine species, *Eratyrus mucronatus* (Stål) and *Triatoma sordida* (Stål), were found in houses and in peridomestic structures. The collection of nymphal instars of both species from some houses indicated possible domesticity. Microscopic examination of feces from 92 insects showed no parasites, and cultures from the guts of 30 insects were negative. Nevertheless, a polymerase chain reaction (PCR) test performed on the same fecal samples showed the presence of *T. cruzi* DNA in 19.1 and 12.5% of *E. mucronatus* and *T. sordida*, respectively. These 16 PCR-positive samples were hybridized with 2 *T. cruzi*-specific probes known from the domestic cycle in Bolivia (clones 20 and 39). At least 1 of these clones was identified in 7 bugs (5 *E. mucronatus* and 2 *T. sordida*). Moreover, no hybridization was observed with these probes in 8 *E. mucronatus* and 1 *T. sordida* samples that showed an amplified band by PCR. These data indicated that *T. cruzi* clones, genetically unrelated to clones 20 and 39, also were circulating in this area. Based on these results, the 2 sylvatic triatomine species encountered in Apolo should not be overlooked as possible local vectors of *T. cruzi*.

KEY WORDS *Eratyrus mucronatus, Triatoma sordida,* sylvatic triatomines, domiciliation, *Trypanosoma cruzi* characterization, Bolivia

CHAGAS DISEASE is a significant cause of morbidity and mortality in Bolivia (WHO 1992). More than 3 million people live in endemic regions, and seropositivity indicative of infection reaches 75% in several areas (Pless et al. 1992). *Triatoma infestans* (Klug) is the main vector in Bolivia, although several sylvatic triatomine species have been reported to be infected with *Trypanosoma cruzi* (Lent and Wygodzinsky 1979, Bermudez and Balderama 1991). With the exception of the sylvatic focus of *T. infestans* described by Dujardin et al. (1987) and the isolation of *T. cruzi* from sylvatic mammals as *Saimiri sciureus* (L.), *Didelphis marsupialis* (L.), and *Dasypus novemcinctus* (L.) (D'Alessandro et al. 1986, Valette et al. 1988), little is known about the sylvatic cycle and vectors in Bolivia. Several sylvatic triatomines may become vectors of *T. cruzi* after *T. infestans* control is achieved or after artificial environmental change (WHO 1992, Lainson et al. 1979). Thus, a more thorough knowledge of the ecology of the sylvatic triatomine species and the *T. cruzi* strains infecting them is essential to assess the risk of transmission.

Trypanosoma cruzi is present in nature as natural clones as demonstrated by population genetic studies (Tibayrenc and Ayala 1988). Among the large number of *T. cruzi* clones, some major clones appear to be ubiquitous and are encountered frequently in endemic areas. Among these, clones 20 and 39 are dominant in Bolivia (Tibayrenc and Ayala 1988, Brenière et al. 1989).

The current research was undertaken in an isolated area of Bolivia where seropositivity for *T. cruzi* in humans was low and where uninfected adult stages of a sylvatic triatomine species (*Eratyrus mucronatus* Stål 1859) were found occasionally in houses (Valencia Telleria 1990). To provide baseline data in preparation for the National Control Program of Chagas Disease in Bolivia, our objective was to assess the risk of domestic transmission. Specifically, we evaluated the seroprevalence of *T. cruzi* in humans, investigated a possible trend toward domiciliary infestation by sylvatic triatomines, and determined their infection rate with *T. cruzi*.

Materials and Methods

Study Area. Field work was carried out in the small town of Apolo (14° 44′ S, 68° 30′ W; population 4,000), department of La Paz, Bolivia, which is situated ≈200 km northwest of La Paz in the subandean region of the eastern Cordillera. The

[1] Institut Français de Recherche Scientifique pour le Développement en Coopération, Casilla 9214, La Paz, Bolivia.
[2] UMR CNRS/ORSTOM, BP 5045, 34032 Montpellier Cedex, France.
[3] Instituto Boliviano de Biologia de Altura, Casilla 641, La Paz, Bolivia.

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elevation is 1,430 m, and the climate is subtropical with a mean annual temperature of 21°C and mean annual precipitation between 1,200 and 1,400 mm. The dwellings of Apolo are mainly of mud and wattle construction or made of unplastered adobe bricks. Houses are roofed with zinc sheets or straw. The walls surrounding the houses are made of adobe covered by straw. The town is situated on an isolated plain, is not accessible by major roads, and has not experienced substantial human migrations. The plain is covered by shrub savannah interspersed with a few patches of forest. A serological survey conducted in 1990 by Valencia Telleria (1990) found that 3.6% of the population was positive for *T. cruzi* infection, but cases were not questioned about previous residency in endemic areas.

Serology. In March 1993, blood from 390 residents of Apolo was collected by fingerprick on Whatman No. 3 filter paper. Overall, 53.6% (209) of the subjects were <18 yr old. Blood samples were air dried, stored in a dessicator, and tested for antibodies to *T. cruzi* using the enzyme-linked immunosorbent assay (ELISA) described by Brenière et al. (1984). For comparison, 45 blood samples were analyzed from soldiers residing in the Apolo military base. Many of these soldiers originally were residents of endemic areas in Bolivia.

Triatomine Studies. Triatomines were collected inside houses and in peridomiciliary structures during October 1992 and March 1993. Bugs were placed in plastic bottles containing filter paper and transported to the laboratory in La Paz for morphological identification according to Lent and Wygodzinsky (1979). Feces were obtained by gently squeezing the live insects. The feces were mixed with phosphate buffered saline and examined for the presence of flagellates. Feces aliquots obtained from the same triatomines were stored at −20°C for later polymerase chain reaction (PCR) amplification. The intestinal tract of some insects was placed in NNN medium in an attempt to cultivate *T. cruzi*.

Polymerase Chain Reaction Analysis of Feces and *T. cruzi* Clones Characterization. Infection of the triatomine bugs was determined by PCR amplification of the hypervariable regions of kinetoplast DNA minicircles (HVRm) of *T. cruzi* from triatomine bug feces according to Brenière et al. (1992). Specific clones were identified by subsequent hybridization using probes specific for *T. cruzi* clones (Veas et al. 1991, Brenière et al. 1992).

Samples of triatomine feces (10–20 μl) were collected individually in sterile microtubes and stored at −20°C. The forceps and gloves were rinsed with disinfectant (bleach) between each sample. This procedure was performed in a room free of *T. cruzi* manipulation. Samples were diluted in 200 μl of distilled water, boiled for 10 min, and centrifuged twice at 8,000 × *g* for 10 min. Ten microliters of the supernatant was used as a template in the PCR assay. Each protocol always included 1 positive control using total DNA as a template (DNA from a reference *T. cruzi* strain) and 2 negative controls using water, the 1st before addition of the sample and the 2nd treated the same way as the feces. The water template treated the same way as the feces was the best contamination control because it was free of possible DNA polymerase inhibiting factors. To avoid contamination, the successive steps of the amplification procedure were performed in 3 different places: the 1st one, free of *T. cruzi* manipulation, was used for repartition of amplification products and addition of *Taq* polymerase; the 2nd place, also free of *T. cruzi* manipulation, was used for the addition of the different samples to each tube; and the 3rd place was the PCR products electrophoresis laboratory. Moreover, each protocol included <10 fecal samples at the same time. Protocols with incorrect controls were discarded. The repartition of PCR reagents (buffer, primers, oligonucleotides, water, and mineral oil) was done in 30–40 sterile microtubes, and 1 tube was run previously with water template to control the amplification products. As a final PCR control, we assayed feces from known negative bugs reared in laboratory and always fed on chickens (7 *E. mucronatus* and 18 *T. sordida* Stål 1859).

Following PCR, one-tenth of the sample was loaded onto 0.8% agarose gel, separated by electrophoresis, and stained with ethidium bromide. Clone-specific identification of PCR-positive samples was performed using probes specific for major clones 20 and 39 derived from SO34 cl4 and TPK1 stocks, respectively (Tibayrenc and Ayala 1988, Brenière et al. 1992).

Results

None of 390 sera from people living in Apolo had antibodies to *T. cruzi* when tested by ELISA. However, 7 of 45 soldiers living in the military base 2 km from the town were seropositive. All of the men testing positive previously had spent several years in an area endemic for Chagas disease.

In total, 209 triatomines were captured in 11 houses and 25 peridomestic structures (chicken-coops and adobe walls surrounding houses). *E. mucronatus* was the dominant species both in domestic (84.1% of total bugs) and in peridomestic habitats (64.5%). This was the only species found in 9 houses. In 2 other houses, it was associated with *T. sordida*, the only other species collected in Apolo. In peridomestic structures, *E. mucronatus* was collected alone on 17 occasions, *T. sordida* alone on 2, and both species together on 6 occasions. Details of the infestation of the domestic habitat and peridomestic structures by *E. mucronatus* and *T. sordida* and the instar distribution are presented in Table 1. *E. mucronatus* immatures were found colonizing 4 houses, and *T. sordida* immatures in 2 houses. In 7 houses, only *E. mucronatus* adult stages were discovered.

Table 1. Age structure of triatomine bugs in domestic and peridomestic habitats at Apolo, Bolivia

Collection sites	*E. mucronatus*					*T. sordida*				
	No. positive sites	Nymph		Adult		No. positive sites	Nymph		Adult	
		Instars 1–4	Instar 5	M	F		Instars 1–4	Instar 5	M	F
Domestic habitats	11	10	14	21	29	2	4	4	3	3
Peridomestic structures	23	15	14	29	20	8	16	8	7	12
Total	34	25	28	50	49	10	20	12	10	15

Feces from 68 *E. mucronatus* and 24 *T. sordida* were examined microscopically for flagellates with negative results. Intestinal tracts from 30 bugs (20 from *E. mucronatus* and 10 from *T. sordida*) were suspended in NNN medium in an attempt to culture Trypanosomatidae. Flagellated parasites were not observed after 12 wk, and the cultures were discarded.

Fecal samples derived from the same 68 *E. mucronatus* and 24 *T. sordida* also were tested by the PCR procedure. Agarose gel electrophoretic analysis of PCR products detected 16 positive specimens, as determined by visualization of a band at 270 bp after staining the gels with ethidium bromide (Table 2; Fig. 1A). Overall, 13 (19.1%) *E. mucronatus* and 3 (12.5%) *T. sordida* were positive by PCR. Among the 30 fecal samples culture negative, 2 were PCR positive. The 25 fecal control samples from laboratory reared triatomines were PCR negative.

To assess whether or not these vectors were infected with the major clones circulating in Bolivia, the 16 PCR-positive samples were hybridized with 2 clone-specific probes (Table 2). This procedure identified clone 20 (or a genetically related clone) in 3 *E. mucronatus* and in 2 *T. sordida* samples (Fig. 1B). Clone 39 (or a genetically related clone) was identified in 4 *E. mucronatus* and in 1 *T. sordida* samples (Fig. 1C). In 3 triatomine bugs (2 *E. mucronatus* and 1 *T. sordida*), a mixed infection with both clones 20 and 39 was detected. No hybridization was observed with either probe in 8 *E. mucronatus* and 1 *T. sordida* samples, all of which were positive by the PCR analysis. These triatomines presumably were infected by clones whose taxonomic status remains to be determined.

Discussion

Both *E. mucronatus* and *T. sordida* were captured from houses and peridomestic structures in Apolo. Until now very few *E. mucronatus* have been found inside human habitations in Peru and Bolivia (Torrico 1946, Lent and Wygodzinsky 1979). This species has been reported to be naturally infected by *T. cruzi*, and also was collected outside houses in urban and rural areas, particularly in hollow trees where they feed on porcupines and bats (Lumbreras 1960, D'Alessandro et al. 1971, Lent and Wygodzinsky 1979, Miles et al. 1981).

Table 2. Characterization of *T. cruzi* clones in the feces of triatomine bugs from Apolo, Bolivia, using a PCR method

Triatomine species	Bugs examined	PCR + (%)	Hybridization pattern		
			Clone 20	Clone 39	20 and 39
E. mucronatus	68	13 (19.1)	1	2	2
T. sordida	24	3 (12.5)	1	0	1

An entomological survey previously carried out at Apolo in 1990 collected only the adult stages of *E. mucronatus* in 45% of houses and in peridomestic structures (Valencia Telleria 1990). Our finding of nymphs in some houses may represent recent colonization by adult specimens attracted by electric lights. In addition the presence of nymphs in the adobe walls surrounding the property of infested houses indicated the possibility that peridomestic structures also may be refugia for domestic colonization. Nevertheless, we cannot exclude the introduction of *E. mucronatus* by straw or wattle used as construction material. However, these data demonstrate, for the 1st time, a trend toward domesticity by *E. mucronatus*. In contrast to *E. mucronatus*, *T. sordida* is a known sylvatic and peridomestic species that has become domiciliated in several areas (WHO 1992). The destruction of the natural habitats has forced this species to colonize domestic and peridomestic habitats. The absence of *T. sordida* during the 1990 survey and the finding of nymphs in our study supports the recent colonization of household environments by this species, considered 1 of the vectors of epidemiological significance in South America (WHO 1992). In addition, the absence of infected humans in Apolo supported our hypothesis of very recent domiciliation.

The PCR technique performed on fecal samples from *T. sordida* and *E. mucronatus* detected the presence of *T. cruzi* DNA in 16 insects, all of which were negative by microscopic examination. Contamination by previous amplification products, the main problem of the PCR technique, was disregarded based on the following arguments: (1) negative and positive controls were satisfactory, (2) fecal samples from triatomines reared in laboratory were PCR negative, (3) the majority of positive samples (11/16) gave weak bands but several (5/16) gave distinct ones (generally contamination

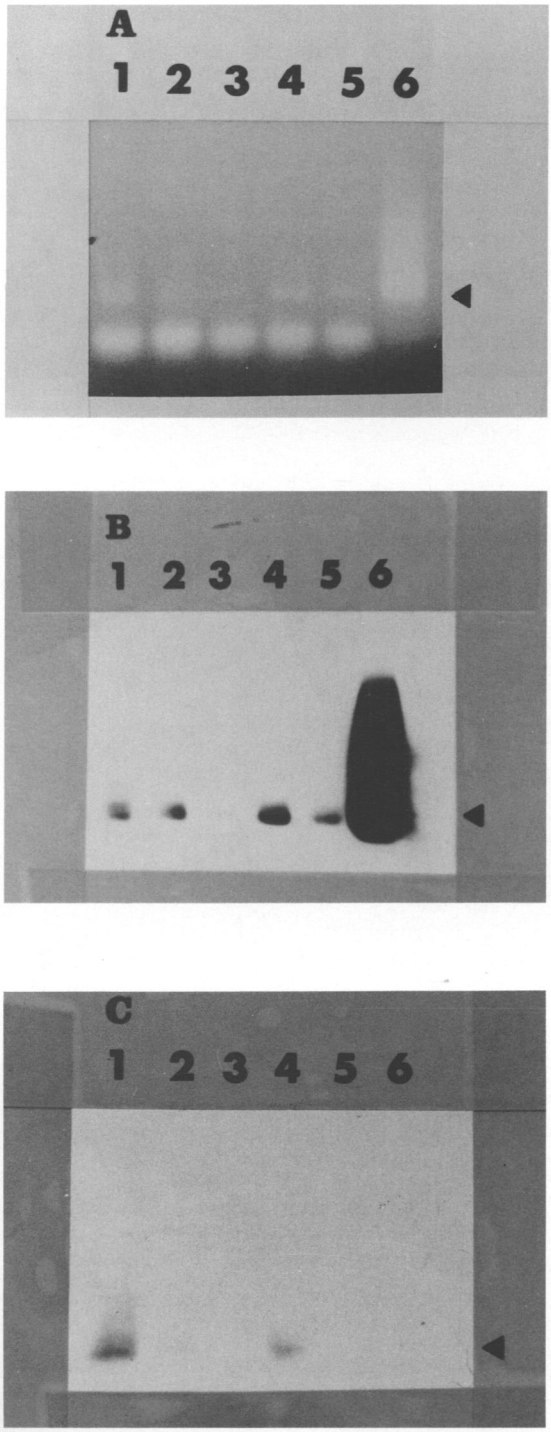

Fig. 1. (A) Ethidium bromide-stained 0.8% agarose gel containing PCR products from *T. sordida* (lanes 1 and 3) and *E. mucronatus* (lanes 2, 4, and 5) fecal samples; all 5 samples are positive. Lane 6 contains positive control (100 ng of total DNA from reference *T. cruzi* stock corresponding to natural clone 20). Arrow, major amplified band of 270 bp. (B and C) Hybridization patterns of PCR products with probes corresponding to natural clones 20 and 39; (B) positive hybridization patterns with clone 20 in lanes 1, 2, 4, 5, and 6; (C) positive hybridization patterns with clone 39 in lanes 1 and 4. Arrow, major amplified band of 270 bp.

gives weak bands), and (4) hybridization patterns indicated a heterogeneity of *T. cruzi* clones (among 16 amplified products, only 7 were recognized by probes 20 or 39).

In agreement with Shikanai-Yasuda et al. (1993), the enhanced sensitivity of PCR may explain the discrepancy between microscopic examination and PCR results. Our PCR results indicated the potential vectoral role of triatomines in our sample. The PCR technique detects only *T. cruzi* DNA, without information on whether or not the vector is carrying infectious forms of parasite. The discrepancy between microscopy and PCR supports the hypothesis of relatively poor rectal metacyclogenesis by both triatomine species.

Previous work has shown that the HVRm of the *T. cruzi* kinetoplast DNA exhibits sequences that are specific for the natural clones identified by multilocus isozyme analysis (Veas et al. 1991). This has led to the development of DNA probes for 2 *T. cruzi* major clones circulating in Bolivia: natural clones 20 and 39 (Tibayrenc and Ayala 1988). Using these 2 DNA probes, the presence of *T. cruzi* clones 20 and 39 (or genetically related ones) was observed in 7 sylvatic triatomine bugs. The domestic or sylvatic origin of these clones remains unresolved.

Both *T. sordida* and *E. mucronatus* have adapted to domestic habitat in Apolo. The phenomenon of domiciliation probably is still in progress, because Valencia Telleria (1990) detected only *E. mucronatus* adults in human dwellings. The suspicion of *T. cruzi* major clones in any triatomine bug indicates the risk of transmission. As the area is still apparently free of human *T. cruzi* infection, several ecological, biological, and behavioral parameters of these 2 triatomine species require further investigation.

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