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Differential detection of *Blastocrithidia triatomae* and *Trypanosoma cruzi* by amplification of 24sα ribosomal RNA genes in faeces of sylvatic triatomine species from rural northwestern Argentina

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Abstract

Flagellates indistinguishable from *Trypanosoma cruzi* were detected by microscopy in faecal samples of 2/110 *Triatoma guasayana* and 2/283 *Triatoma garciabesi* captured in a rural area of northwestern Argentina. Inoculation of faecal homogenates to mice followed by xenodiagnosis, haemoculture, histopathology and culture from cardiac homogenates, and PCR based on *T. cruzi* minicircle and nuclear sequences failed to detect *T. cruzi* infection, pointing to another trypanosomatidean. A PCR strategy targeted to the D7 domain of 24sα ribosomal DNA genes amplified a 250 bp sequence from one *T. guasayana* and one *T. garciabesi* faecal lysate. Sequence analysis revealed 100% identity with 24sα rDNA amplicons from *Blastocrithidia triatomae* obtained from faeces of reared *Triatoma infestans* bugs. Phylogenetic analysis clustered this sequence with *C. fasciculata* and *L. major*, separated from the *Trypanosoma* branch (bootstrap: 968/1000), in concordance with a Neighbour-joining dendrogram based on 18s rDNA sequences. This PCR procedure provides a rapid sensitive tool for differential diagnosis of morphologically similar trypanosomatids in field surveys of Chagas disease vectors and laboratory-reared triatomines used for xenodiagnosis.
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Keywords: *Triatoma guasayana*; *Triatoma garciabesi*; *Trypanosoma cruzi*; *Blastocrithidia triatomae*; Chagas disease; 24sα ribosomal RNA genes

Several sylvatic or peridomestic triatomine species, such as *Triatoma sordida* and *Triatoma guasayana* have been found infected with *T. cruzi* and accordingly implicated as secondary vectors of Chagas disease in endemic regions of northern Argentina and Bolivia (Noireau et al., 1995; Castanera et al., 1998; Cecere et al., 1997, 1999; Canale et al., 2000; Lauricella et al., 2005). *Trypanosoma cruzi* infection in triatomine bugs is currently detected through microscopic observation (MO) of flagellated

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forms in unstained fresh faecal preparations. However, such infections were referred to as "*T. cruzi*-like trypanosomes" (Wisnivesky-Colli et al., 1993) or "flagellates" (Noireau et al., 1995) because light microscopy has limited specificity to discriminate between *T. cruzi* and other overlapping and morphologically similar trypanosomatids, such as *Blastocrithidia triatomae* or *Trypanosoma rangeli* (Cerisola et al., 1971; Chiurillo et al., 2003). Nucleic acid amplification of polymorphic regions within conserved genes of the order Kinetoplastida may provide a rapid laboratory tool to univocally identify flagellates directly from faecal samples of naturally or experimentally infected triatomines (Breniere et al., 1995; Souto et al., 1999; Chiurillo et al., 2003). As part of a wider eco-epidemiological project conducted in a well-defined rural area of northwestern Argentina (Marcet et al., 2006), we sought to assess the distribution of *T. cruzi* infection in sylvatic triatomine species. Accordingly, 124 *T. guasayana* and 317 *T. garciabesi* specimens were captured in domiciles and peridomestic and sylvatic sites in October 2002. Diluted faecal drops obtained by abdominal compression were thoroughly examined for active trypanosomes with a microscope at 220–400×. Flagellates indistinguishable from *T. cruzi* were microscopically observed in only two *T. guasayana* and two *T. garciabesi* specimens. To confirm *T. cruzi* infection, the rectal ampoule from each MO-positive bug was obtained to prepare homogenates for: (1) bi-phasic culture into four to six tubes (50–100 μl homogenate/tube) containing 3 ml nutrient agar Difco (31 g/l)–0.5 ml defibrinated rabbit blood (penicillin 200 U/ml–streptomicin 200 U/ml) and 2 ml brain heart infusion-10% fetal calf serum (BHI-FCS) (Bioser, Buenos Aires) and (2) intraperitoneal inoculation in groups of Balb-C suckling mice (20–30 days of age, 8 g) with 0.2 ml of faecal homogenates in BHI-FCS 10% (Lauricella et al., 2005). One mouse from each group was studied by xenodiagnosis and examined 30 and 60 days post-feeding, as described (Cerisola et al., 1974); after xenodiagnosis and under anesthesia, heparinized blood was extracted by cardiac puncture and inoculated into two to three tubes per mouse and into other mice. The remaining mice of each group were tested by haemoculture and histopathological analysis, 30–45 days after inoculation, as described elsewhere (Lauricella et al., 2005). All these attempts at culturing and isolating *T. cruzi* or detecting histologic evidence of chagasic infection were negative (Table 1). PCR strategies targeted to *T. cruzi* minicircle DNA (kDNA), satellite DNA (Sat-DNA) and the intergenic regions of miniexon genes

Table 1
Studies in mice and PCR-based identification of flagellates from microscopically positive faecal samples of sylvatic triatomines

	Triatomine species			
	T. garciabesi		*T. guasayana*	
	LA-9-1[a]	CD-13-3[a]	6-11[a]	PE-6-2[a]
Developmental stage	Adult male	Fifth instar nym.	Adult male	Adult female
Capture site	Tree	Pig corral	Cemetry (LT)	Goat Corral
Microscopic examination	Positive	Positive	Positive	Positive
Culture	Negative	Positive	Not done	Negative
Studies in mice				
Xenodiagnosis[b]	Negative	Not done[c]	Not done	Negative
Haemoculture	Negative	Negative	Not done	Negative
Histology[d]	Negative	Negative	Not done	Negative[e]
Heart culture	Negative	Negative	Not done	Not done
PCR studies				
k-DNA	Negative	Negative	Negative	Negative
Sat-DNA	Negative	Negative	Negative	Negative
SL-DNA	Negative	Negative	Negative	Negative
24S alpha rDNA	Negative	250 bp (F + C)	250 bp (F)	Negative
Inhibition[f]	Yes	No	No	Yes

F: faeces; C: culture; LT: light trap; Nym.: nymph. k-DNA: kinetoplastid DNA; Sat-DNA: satellite DNA; SL-DNA: spliced leader DNA.
[a] Specimen identification.
[b] Four boxes containing four *T. infestans* third instar stage/box.
[c] Two mice died 14 dpi; heart homogenates were inoculated into other two mice, with negative findings.
[d] Intestine, skeletal muscle, lymph nodes, lung, liver and kidney.
[e] Histologic examination performed from tissues obtained after a second passage to mice.
[f] PCR inhibition was checked in all negative PCR DNA samples, as described in Schijman et al. (2003).

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