Acta Tropica 111 (2009) 90–93

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Short communication

Predominance of *Trypanosoma cruzi* genotypes in two reservoirs infected by sylvatic *Triatoma infestans* of an endemic area of Chile

S. Galuppo[b], A. Bacigalupo[b], A. García[c], S. Ortiz[a], X. Coronado[a], P.E. Cattan[b], A. Solari[a,*]

[a] Program of Cellular and Molecular Biology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile
[b] Department of Biological Sciences, Faculty of Veterinary Sciences, University of Chile, Santiago, Chile
[c] Docent Unit of Parasitoloy, Faculty of Medicine, University of Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 7 November 2008
Received in revised form 23 January 2009
Accepted 23 February 2009
Available online 9 March 2009

Keywords:
Sylvatic *T. infestans*
Chagas disease
Genotypes

ABSTRACT

We report results of *Trypanosoma cruzi* infection and parasite genotypes in the wild *Octodon degus* and synantropic reservoir *Rattus rattus* from an endemic area with sylvatic *Triatoma infestans* as the only detected vector. The infection status was determined by hemi-nested PCR directed to minicircles DNA and genotyping by hybridization tests with a panel of five specific probes, including two probes for TcI subgroups (clones 19 and 20). *O. degus* was found infected with 13.3% and mainly with sublineage TcIId, and less with TcIIb and TcI. Meantime the synantropic *R. rattus* was found infected with 27.7% and mainly with TcI and much less with TcIId, TcIIb and TcIIe.

The results are discussed to explain the distribution of *T. cruzi* genotypes between these two reservoirs and the importance of sylvatic foci of *T. infestans* allowing the permanence of the wild and peridomestic cycle of Chagas disease.

Chagas disease is endemic in Chile with the presence of *Mepraia spinolai* and *Mepraia gajardoi* as sylvatic vectors, whereas *Triatoma infestans* is the only domiciliary vector. This vector so far controlled in most endemic areas of Chile (Lorca et al., 2001; WHO, 2002), has recently been found colonizing wild areas after the spraying campaign in a rural locality near the capital Santiago (Bacigalupo et al., 2006). Several species of wild rodent reservoirs circulate in that specific endemic area, including the wild endemic *Octodon degus* classically identified as reservoir in Chile (Whiting, 1946); others are *Phyllotis darwini*, *Abrothrix olivaceus* and *Abrocoma bennetti* recently found infected with *Trypanosoma cruzi* in another endemic area (Rozas et al., 2007). However the synantropic and peridomiciliary *Rattus rattus* also has been reported near Santiago (Galuppo, 2007). *T. cruzi* genotypes are complex multiclonal populations that propagate asexually (Tibayrenc, 2003). Currently, the taxon *T. cruzi* is divided into two lineages: TcI and TcII (Anonymous, 1999). Lineage TcI corresponds to the classical Zymodeme 1 and several clones, meantime the lineage TcII includes five sub lineages: TcIIa–e (Brisse et al., 2001). *T. cruzi* belongs to the kinetoplastidae family which possess a unique mitochondrion, the kinetoplast DNA (kDNA) are present the maxicircles (25–50 copies cell) and the minicircles (20,000 copies/cell) all them concatenated into a disk-shaped network. Due to the high copy numbers of the later and the presence of constant sequence regions, these aspects make minicircles suitable for sensitive parasite PCR–DNA based detection (Zulantay et al., 2004). However each minicircles also contains hypervariable regions representing different minicircle classes, some of them with significant homology with other sequences (Telleria et al., 2006). Using primers corresponding to the conserved sequence blocks (CSB) 1–3 present in the constant region, minicircle DNA are amplified by PCR for a variety of *T. cruzi* clones and amplicons are used in reciprocal hybridization experiments (Ray, 1989). Positive hybridizations were observed under specific washing stringency conditions only with amplicons from *T. cruzi* clones of the same genotype (Veas et al., 1991). This approach is frequently used for *T. cruzi* genotyping for parasites circulating in humans, vectors and wild reservoirs (Solari et al., 2001; Brenière et al., 2002; Coronado et al., 2006; Rozas et al., 2007; Campos et al., 2007).

In the present work we study prevalence of infection and the presence of *T. cruzi* genotypes circulating in reservoirs of a foci area where the sylvatic *T. infestans* is the only detected vector. Eventhough other sylvatic species as *M. spinoli* may circulate. In spite of various rodents species were collected, the results are discussed for *R. rattus* and *O. degus* which are reservoirs with different ecological distribution (Muñoz and Yañez, 2000).

Wild and peridomestic rodents were obtained in Calera de Tango Municipality, a rural locality 16 km south from downtown Santiago, Chile. The collection sites were two areas separated each other by 4 km in a semi-arid environment. The houses of this area are under a surveillance phase of the National Vector Control Program, therefore houses with *T. infestans* are sprayed to prevent re-infestations. The area is absent of *T. infestans* colonies within

---

* Corresponding author. Tel.: +56 2 9786062; fax: +56 2 7355580.
E-mail address: asolari@med.uchile.cl (A. Solari).

0001-706X/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.actatropica.2009.02.010

houses but specimens were found outside the houses sheltered by *Puya* spp. (Bacigalupo et al., 2006). Searches for bugs were made in the outside buildings, however *M. spinolai* was not detected in this endemic area. A total of 112 animals were captured using live-traps (H.B. Sherman Trap Company, Tallahossee, FL) in diurnal and nocturnal periods during the summer of year 2006. The collected species were *O. degus*, *n* = 60; *R. rattus*, *n* = 44; *P. darwini*, *n* = 4; *A. olivaceus*, *n* = 2, and *A. bennetti*, *n* = 2. Each animal was weighed and anesthetized with isofurane at a dose of 13 mg/kg of body weight. Once anesthetized, 0.5 ml of blood was withdrawn by cardiac venipuncture using tuberculine syringes and mixed with identical volume of Guanidine HCl 6 M and EDTA 0.2 M solution. Animals were released after blood collection. Collection of blood was conducted following the recommendation of the Ethical Committee of the Faculty of Medicine, University of Chile (Santiago, Chile). Whole genomic DNA was isolated from 0.2 ml blood mixture using the E.Z.N.A. Kit (Omega Bio-tek, Inc. Doraville, GA). The DNA samples were concentrated by ethanol precipitation, resuspended in 50 μl of deionized sterile water and stored at −20 °C.

The amplification reaction was performed in triplicate with oligonucleotides 121 (5′-AAA-TAATGTACGGG (T/G) GAGATGCAT-GA-3′)) and 122 (5′-GGTTCGATTGGGGTTGGTGTAATATA-3′), which anneal to the CSB2 and CSB3 of the minicircles, respectively (Wincker et al., 1994). The DNA samples for PCR were boiled to decatenate minicircles for 10 min and 5 μl of supernatant was used as DNA template (Britto et al., 1993). Each experiment included a control that contained water instead of DNA and a positive control with purified kDNA. Finally the results were analyzed by electrophoresis on a 2% agarose gel and visualized with ethidium bromide staining. Additionally and to improve *T. cruzi* detection sensitivity a hemi-nested PCR assay was performed. 2 μl of 1/10 dilution of the PCR products were used as template and subjected to hemi-nested PCR using oligonucleotides CV1 (5′-GATTGGGGTTGGAGTACTAT-3′) and CV2 (5′-TTGAACGGCCCTCCGAAAAC-3′) which anneal to the CSB3 and CSB1, respectively to generate 290 bp DNA products (Veas et al., 1991). Further electrophoresis was done as described in the PCR assay. By doing this second PCR assay amplicons are more abundant and useful for further characterization by hybridization tests.

To genotype *T. cruzi* Southern blot analyses were performed with 10 μl of each hemi-nested PCR product. Samples subjected to electrophoresis were transferred onto Hybond N$^+$ nylon membranes (Amersham, Little Chalfond, United Kingdom), and cross-linked with ultra violet light to fix the DNA. The membranes were prehybridized for at least 2 h at 55 °C using conditions already described (Solari et al., 2001). For confirmation purposes membranes were hybridized with total *T. cruzi* kDNA as probe using conditions described (Zulantay et al., 2004). For genotyping, different *T. cruzi* clones were used as DNA template to generate probes to determine by hybridization the parasite genotype infecting each animal: [TcI (sp104cl1 or clone 19 and SO34cl4 or clone 20), TcIIa (CANIIIcl1), TcIIb (CBBcl3), TcIIc (X109cl1), TcIId (NRcl3), and TcIIe (v195cl1]. Construction of genotype specific probes was performed as described (Veas et al., 1991). These probes generated by PCR with oligonucleotides CV1 and CV2 (approximately 250 bp) do not contain the constant region that cross-hybridizes with minicircles of other *T. cruzi* genotypes. This method has been validated by hybridization tests under high stringency condition with probes constructed by PCR amplification of selected *T. cruzi* clones DNA previously genotyped with isoenzyme markers (Veas et al., 1991; Breniere et al., 1998). Statistical significance was analyzed by a χ^2 test.

We analyzed the 112 blood samples from five rodent species, by PCR and hemi-nested PCR. Results with PCR revealed only 10 out of the 19 positive samples with strong DNA bands stained by ethidium bromide. The other nine samples gave minor bands only detected by repeated PCR assays and high volumes of DNA samples as template (not shown). In order to characterize the amplified DNA by hybridization tests, hemi-nested PCR was performed and higher amplicons were obtained (Fig. 1). The prevalence of *T. cruzi* infection in rodent species ranged from 13.3% for *O. degus* (*n* = 8); 25% for *P. darwini* (*n* = 1), and 27.7% for *R. rattus* (*n* = 10). Other negative samples from 2 *A. benneti* and 2 *A. olivaceus* were studied. We found single or mixed *T. cruzi* genotypes in the analyzed species. Fig. 1A shows the results of the 19 positive samples stained with ethidium bromide. Fig. 1B–F shows Southern blot analysis and hybridization with four specific minicircle probes. Probes from TcIIa and TcIIc do not show hybridization bands (not shown). These probes showed all possible hybridization patterns; samples that hybridized with zero (tracks # 6 and 13), one (tracks # 4, 7, 8, 10–12, 14–16), two (tracks # 1–3, 5, 9, 17 and 19), and three probes (track # 18). Hybridization with more than one probe indicates a mixed infection. However since PCR is not a quantitative method, these results do not determine parasitemias for each *T. cruzi* genotype but only the presence of the different *T. cruzi* genotypes. Genotype distribution is shown in Table 1. The four studied genotypes were circulating in different frequencies, to mention; TcI was more prevalent in *R. rattus* than in *O. degus*, meantime TcIId was more frequent in *O. degus* than in *R. rattus*. Statistical analyses $\chi^2 = 8.0$, df = 1, $p < 0.01$. These results indicate that TcI, TcIIb, TcIId and TcIIe genotypes are present and circulate in the same area and in both species of mammals, showing an apparent similar distribution with predominance of TcI in *R. rattus* and TcIId in *O. degus*. Single versus mixed genotypes in the studied rodent species, was nine single and eight mixed. Undetermined genotypes were found in two *O. degus* animal. Eventhough, these samples did not cross-reacted with any of the five probes, samples of *O. degus* (tracks # 6 and 13) did with total *T. cruzi* kDNA as probe. These results suggest that the infection is with an undetermined *T. cruzi* genotype rather than with other unknown trypanosomatid. We believe that undetermined *T. cruzi* genotypes are variants of TcI, TcIIb, TcIId or TcIIe, that not cross-hybridize for presence of differ-

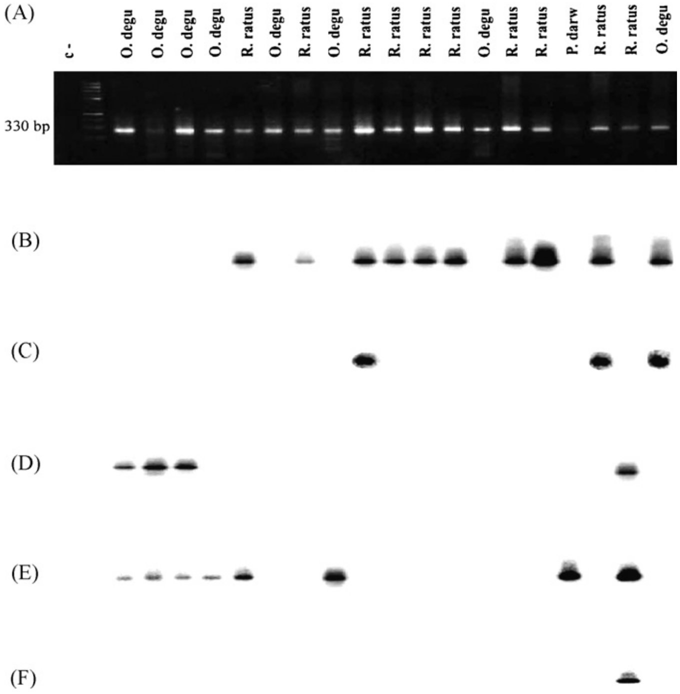

Fig. 1. *Trypanosoma cruzi* amplicons stained with ethidium bromide (A). Hybridization profiles obtained with genotype specific probes corresponding to TcI [sp104cl1 (clone 19)] (B), TcI [SO34cl4 (clone 20)] (C), TcIIb (CBBcl3) (D), TcIId (NRcl3) (E) and TcIIe (v195cl1) (F). C−: negative control, A 330 base pairs (bp) product represents a positive assay.