Genetic characterization of Trypanosoma cruzi DTUs in wild Triatoma infestans from Bolivia: predominance of TcI

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Genetic Characterization of *Trypanosoma cruzi DTUs* in Wild *Triatoma infestans* from Bolivia: Predominance of TcI

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**Abstract**

**Background:** The current persistence of *Triatoma infestans* (one of the main vectors of Chagas disease) in some domestic areas could be related to re-colonization by wild populations which are increasingly reported. However, the infection rate and the genetic characterization of the *Trypanosoma cruzi* strains infecting these populations are very limited.

**Methodology/Principal Findings:** Of 333 wild *Triatoma infestans* specimens collected from north to south of a Chagas disease endemic area in Bolivia, we characterized 234 stocks of *Trypanosoma cruzi* using mini-exon multiplex PCR (MMPCR) and sequencing the glucose phosphate isomerase (Gpi) gene. Of the six genetic lineages (“discrete typing units”; DTU) (TcI-VI) presently recognized in *T. cruzi*, TcI (99.1%) was overdominant on TcII (0.9%) in wild Andean *T. infestans*, which presented a 71.7% infection rate as evaluated by microscopy. In the lowlands (Bolivian Chaco), 17 “dark morph” *T. infestans* were analyzed. None of them were positive for parasites after microscopic examination, although one Tc stock and one TcII stock were identified using MMPCR and sequencing.

**Conclusions/Significance:** By exploring large-scale DTUs that infect the wild populations of *T. infestans*, this study opens the discussion on the origin of TcI and TcV DTUs that are predominant in domestic Bolivian cycles.


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**Introduction**

*Trypanosoma cruzi*, the agent of Chagas disease, is a serious threat to health in the Americas, accounting for the highest disease burden in Latin American, with eight to nine million people infected and 25–90 million at risk [1–3]. This parasite, which belongs to the order Kinetoplastida, is mainly transmitted by blood-sucking bug vectors (Hemiptera, Reduviidae, Triatominae) but also by blood transfusion and oral transmission. Moreover, newborns can be infected through vertical transmission. There are infections in South Cone countries of South America. It exhibits considerable genetic diversity [9,15,16] with possible subclustering [17,18]. TcII, V, and VI are mainly associated with domestic surrounding human dwellings where vector populations associated to domestic and synanthropic animals live (peri-domestic cycle); the third one occurs in dwellings and involves triatomines living indoors, humans, and domestic animals (domestic cycle).

Population genetics analyses have shown that *T. cruzi* has a predominantly clonal mode of evolution and exhibits considerable phenotypic and genetic diversity [9]. This population genetics model refers to genetic clonality, i.e., limited or absent genetic recombination with persistence of durable multilocus associations, whatever the cytological mechanism of reproduction [10]. Six distinct genetic lineages or discrete typing units (DTUs) [11] have been described [12,13]. They have recently been validated by a committee of experts and labeled TcI to TcVI [14]. TcI is ubiquitous and prevalent in different sylvatic cycles. However, it is responsible for the large majority of human infections in the Amazon basin and more northern countries as well as part of the infections in South Cone countries of South America. It exhibits considerable genetic diversity [9,15,16] with possible subclustering [17,18]. TcII, V, and VI are mainly associated with domestic
cycles and prevalent in human infections in the Southern Cone countries; TcV and TcVI are hybrid genotypes, whose putative ancestors are TcII and TcIII [19,20]. Finally, TcIII and IV are more rarely sampled throughout the endemic area and seem to be specific to sylvatic cycles, with few reports of human infection.

In Bolivia, Triatoma infestans (Hemiptera: Reduviidae) remains the main domestic vector of T. cruzi. It is the target of the National Control Program based on house-spraying with residual insecticides. Wild populations of T. infestans are now seriously considered a problem to keep the villages free of triatomines [21–23]. Sylvatic populations of the vector have been described in different Andean valleys in Bolivia [21,22,24,25]. Moreover, the detection of wild foci of T. infestans in the Bolivian Chaco has extended the distribution of wild populations to the lowlands of Bolivia [26].

Two main genotypes belonging to TcI and TcV were previously identified in the domestic cycle in regions where T. infestans was the vector [27–33]. Moreover, these genotypes had been identified in strict sympatry in the same host [27,34]. In contrast, few data are available on DTUs circulating in sylvatic T. infestans. Dujardin et al. [35] found that wild T. infestans were infected with the same T. cruzi genotypes as domestic T. infestans (TcI and TcV), with the same frequencies. They took this as additional evidence of a lack of speciation between wild and domestic T. infestans. Another study identified TcI as the only DTU in a wild focus located in the valley of Cochabamba, Bolivia [25].

Among the genetic markers that can identify the different T. cruzi groups the non-transcribed spacer region of the mini-exon gene was previously proposed to discriminate T. cruzi I (now TcI), T. cruzi II (now TcII), T. cruzi zymodeme 5 (now TcIV), using the mini-exon multiplex PCR (MMPCR) [36]. Recently, the MMPCR analysis was applied on a large sample of stocks (previously characterized by multilocus typing) belonging to the six DTUs, showed 3 DTU tags: a 200 bp PCR product for TcI, a 250 bp for TcII, TcV and TcVI and a 150 bp for TcIII and TcIV [37]. This method was also successfully applied for rapid DTU identification in triatomine digestive tracts [5,38]. Moreover, among housekeeping genes, the glucose-6-phosphate isomerase (Gpi), a single-copy nuclear gene, presented a sequence polymorphism that is valuable for characterization of DTUs [19,39].

In this study, we applied the MMPCR and Gpi sequencing for the characterization of T. cruzi DTUs directly in the digestive tract of wild T. infestans collected in Bolivia.

Materials and Methods

Origin of T. infestans populations
The triatomines were sampled in sylvatic environments from April to November 2009 (Figure 1). Collections were carried out using mice-baited adhesive traps [40] in different ecotopes such as

Figure 1. Sampling sites of wild populations of Triatoma infestans in Bolivia. The sites were numbered from 1 to 36, Bolivian department names are indicated, for the DTU T. cruzi results see in Table 1.
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under bush and bromeliads, rocks, burrows, hollow trees, and stone walls. The bugs were transported alive to the laboratory for species confirmation using morphological taxonomic keys [41].

Table 1 summarizes the geographical and ecotope origin of the collected T. infestans according to the ecoregions defined in [42].

Briefly, the majority of the bugs were collected in Andean valleys.
where sylvatic foci have been previously described [21,24] and the others were collected from new foci in the Bolivian Chaco where the “dark morph” type of *T. infestans* was discovered [22,26]. Before dissection, feces from each bug were examined for the presence of trypansomatid by direct microscopic observation at ×400 magnification (mo). Bugs were then dissected under a safety hood, and the digestive tracts stored at −20°C.

**Mini-exon multiplex PCR (MMPCR)**

DNA was extracted from triatomin digestive tracts with the QIAamp DNA mini kit (Quiagen, Courtaboeuf, France), according to the blood sample protocol. The multiplex primer set was as previously described: three oligonucleotides derived from the hypervariable region of *T. cruzi* mini-exon repeats, and a common *T. cruzi* fragment (64 bp) matched the TcIII reference stock named M5631 (accession No AF050521.1 and AY367126.1, 98% identity). The 458 bp partial sequences (starting at site 691 and ending at 1739) of the study had been attributed to a DTU. Within the latter group captured at site 29 was sequenced and the DNA purification and direct sequencing of both strands of DNA amplicons were performed by the company MACROGEN (Seoul, South Korea). Sequences were aligned and corrected using BioEdit software v. 7.0.9 [43], and a 458 bp partial sequence was resolved for each stock from nucleotide site 691–1148.

**Sequence variability of the Gpi gene from *T. cruzi* infecting wild *T. infestans***

A partial sequence of the *Gpi* gene from 18 samples was obtained from 15 samples (13 from the set corresponding to TcI, one from the set corresponding to TcII, TcV, or TcVI, and one from the set corresponding to either TcIII or TcIV) were sequenced in order to explore the variability within TcI and to discriminate the DTUs within the other sets. The 458 bp partial sequences (starting at site 691 and ending at site 1148 of the entire CL Brener stock gene, accession no. XM815802.1) were aligned with the sequences corresponding to *T. cruzi* reference stocks belonging to the six DTUs previously deposited in GenBank (Table 2). With no ambiguity, each sequence under study had been attributed to a DTU. Within TcI, 3 sequences were observed: the most frequent (11 stocks) presented 100% identity with the two identical sequences from TcI reference stocks (OPS21 and P/209) deposited in GenBank;
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<th>Country</th>
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\(^a\)DTU, Discrete Typing Unit;
\(^b\)A for Andean, NA for Non-Andean (lowland);
\(^c\)Samples under study;
\(^d\)Ten other samples had identical sequence, they were from Northern Andean area.

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the two other sequences exhibited a single mutation and the Vis01 stock identified in a triatomine bug captured at site 27, presented a heterozygous pattern at nucleotide position 940. The sequence of the Chart09 of the second set (corresponding to TcII, TcV, or TcVI), detected in a “dark morph” (site 32), presented 100% identity with two identical TcII reference stocks (Tul18d2 and CBBc13). For the sample of the last set corresponding to either TcIII or TcIV (Toro5 from site 25), the sequence presented 100% identity with two identical TcIII reference stocks (M6241d6 and X110/8).

Discussion

Recently, an active search for new foci of wild T. infestans in Bolivia enabled us to show that their distribution was broader than initially described [21,44]. Also, few data on the genetic characterization of T. cruzi stocks infecting these vector populations were available, apart from the work by Dujardin et al [35], conducted using multilocus enzyme electrophoresis, and the detection of the only TcI at Cotapachi 15 km west of Cochabamba city (Andean area) [25]. In the present context, where wild T. infestans highly infected can enter houses and recolonize them after domestic populations have been eliminated by insecticide spraying, it is important to know which T. cruzi DTUs are carried by the vectors. In this study, 234 T. cruzi stocks isolated from wild T. infestans were characterized by MMPCR. The vectors came from several areas mainly situated in two ecoregions in Bolivia, the Inter-Andean Dry Forest and the Gran Chaco where the “dark morph” was found. Regarding the detection of parasites in bugs, the correlation between detection of infection by microscopy (mo) and by the method of MMPCR was high (82%). However, some infected bugs (mo positive) were MMPCR negative probably due to the presence of inhibitors factors of the polymerase in the DNA extracts. At the contrary, several samples mo negatives were MMPCR positive, which allowed us to detect and identify few strains in dark morph specimens. In the overall sample, the TcI DTU is widely dominant, but in the Andean and intermediate areas TcIII stocks were detected. In the lowlands, only TcI and TcII were characterized in the “dark morph” specimens.

Interestingly, the DTU distribution in wild T. infestans is very different from that reported in domestic T. infestans collected before the vector control campaigns undertaken on a large scale in Bolivia since 2003; the frequencies of TcI only, TcV only, and mixed infections (TcI and TcV) were 38.6%, 16.8% and 32.7% respectively [31]. At the same time, TcV was mostly detected in mixed infections (TcI and TcV) were 38.6%, 16.8% and 32.7% different from that reported in domestic T. cruzi DTUs are carried by the vectors. In this study, 234 T. cruzi stocks isolated from wild T. infestans were characterized by MMPCR. The vectors came from several areas mainly situated in two ecoregions in Bolivia, the Inter-Andean Dry Forest and the Gran Chaco where the “dark morph” was found. Regarding the detection of parasites in bugs, the correlation between detection of infection by microscopy (mo) and by the method of MMPCR was high (82%). However, some infected bugs (mo positive) were MMPCR negative probably due to the presence of inhibitors factors of the polymerase in the DNA extracts. At the contrary, several samples mo negatives were MMPCR positive, which allowed us to detect and identify few strains in dark morph specimens. In the overall sample, the TcI DTU is widely dominant, but in the Andean and intermediate areas TcIII stocks were detected. In the lowlands, only TcI and TcII were characterized in the “dark morph” specimens.

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Among the six T. cruzi DTUs, TcV and TcVI are composed of stocks that appear to be recent hybrids between TcII and TcIII [19]. Consequently, it is tempting to speculate that they might have arisen in an area where the putative parental DTUs coexist. Moreover, this hybridization event is still considered to have occurred much earlier than human colonization in South America [48]. Consequently, parental and hybrid DTUs are likely to coexist in the sylvatic cycle in a putative geographical area in South America. Lately, the Andean origin of T. infestans was challenged by the hypothesis of Chaquean origin [26,44,49,50]. If parental and hybrid DTUs are not found in the sylvatic cycle in the Andes, an alternative might be the Gran Chaco region. These is no information regarding the genetic characterization of T. cruzi in the sylvatic cycle at the Bolivian lowlands, except for a report of a TcVI stock isolated from a D. marshalli specimen captured on the Amazon slope [51]. In the Paraguayan Chaco, TcII, TcIII and TcV have been identified in different wild mammal species [52] and in the Argentinean Chaco TcI was identified in D. albiventris and TcVI in one C. chinga [53]. In spite of fairly scarce data, the hypothesis that hybrid DTUs may have originated in Chaco should be considered, especially considering the detection of all DTUs except for TcIV in the domestic cycle in the Bolivian Gran Chaco (unpublished data). The search for DTUs circulating in sylvatic cycles will provide more valid information on the evolution of T. cruzi than studies conducted in domestic cycles where the geographical distribution of the DTUs is skewed by passive transport of parasites (human migration, triatomine transports) and by the selection of specific DTUs by hosts, considering that host diversity is lower in the domestic cycle than in sylvatic cycles.

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Author Contributions

Conceived and designed the experiments: SFB CB FN. Performed the experiments: CA. Analyzed the data: SFB CA CB. Contributed reagents/materials/analysis tools: SFB CA EW RB RS FN. Wrote the paper: SFB CA CB MT FN.

References


