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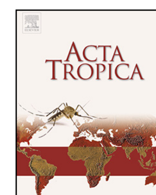

# *Trypanosoma cruzi* infection in *Triatoma sordida* before and after community-wide residual insecticide spraying in the Argentinean Chaco

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ABSTRACT

*Triatoma sordida* is a secondary vector of *Trypanosoma cruzi* in the Gran Chaco and Cerrado eco-regions where it frequently infests peridomestic and domestic habitats. In a well-defined area of the humid Argentine Chaco, very few *T. sordida* were found infected when examined by optical microscopic examination (OM). In order to further assess the role of *T. sordida* and the relative magnitude of subpatent bug infections, we examined the insects for *T. cruzi* infection, parasite Discrete Typing Units (DTUs) and bloodmeal sources using various molecular techniques. Among 205 bugs with a negative or no OM-based diagnosis, the prevalence of infection determined by kDNA-PCR was nearly the same in bugs captured before (6.3%) and 4 months after insecticide spraying (6.4%). On average, these estimates were sixfold higher than the prevalence of infection based on OM (1.1%). Only TcI was identified, a DTU typically associated with opossums and rodents. Chickens and turkeys were the only bloodmeal sources identified in the infected specimens and the main local hosts at the bugs' capture sites. As birds are refractory to *T. cruzi* infection, further studies are needed to identify the infectious bloodmeal hosts. The persistent finding of infected *T. sordida* after community-wide insecticide spraying highlights the need of sustained vector surveillance to effectively prevent *T. cruzi* transmission in the domestic and peridomestic habitats.

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## 1. Introduction

The role of various species of Triatominae as secondary vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease, remains controversial (Guhl et al., 2009). This controversy probably reflects the richness of species involved, their broad geographic range, the dynamic nature of the domestic invasion process, and the limited research efforts invested in sylvatic species of Triatominae. *Triatoma sordida* (Stäl, 1859) is a secondary vector of *T. cruzi*, exhibiting different degrees of domiciliation and colonization especially in areas where the main vector *Triatoma infestans* was suppressed (Forattini et al., 1982; Noireau et al., 1997). *T. sordida* is widely distributed across northern Argentina, eastern Bolivia, Paraguay and southeastern Brazil (Carcavallo et al., 1999). It is frequently found in tree holes, under bark, palm trees, bromeliads, small mammals' burrows, and bird nests, and usually colonizes peridomestic chicken coops or nests (Diotaiuti et al., 1993). The prevalence of *T. cruzi* infection in *T. sordida* was usually less than 2% (Oscherov et al., 2003), and given that this species shows little anthropophily, it was considered of low epidemiological significance across most of its geographic range (Silveira, 2002) except in Bolivia where it reached 21% (Noireau et al., 1997; Brenière et al., 1998).

Domestic and sylvatic transmission cycles have been characterized according to host, vector species and habitats, and may be separated or overlap to various degrees. The six parasite genotypes identified (TcI–TcVI), called discrete typing units (DTUs), usually exhibit different distributions between transmissions cycles (Zingales et al., 2012). In domestic transmission cycles of the Argentine Chaco, TcV and TcVI were the predominant DTUs identified in *T. infestans*, domestic dogs, cats and humans (Diosque et al., 2003;

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Cardinal et al., 2008; Enriquez et al., 2013; Maffey et al., 2012). The main sylvatic reservoir hosts identified were *Didelphis albiventris* opossums infected with TcI, and armadillos and *Conepatus chinga* skunks infected with TcIII (Diosque et al., 2003; Orozco et al., 2013). Several rodent species exhibited subpatent infections only revealed by kDNA-PCR (Orozco et al., 2014). The putative sylvatic vectors of *T. cruzi* have not been conclusively identified yet in the Gran Chaco eco-region. Particularly, in Pampa del Indio (Argentine Chaco), *T. sordida* and *Panstrongylus geniculatus* captured in sylvatic habitats were not found to be infected (Alvarado-Otegui et al., 2012). The predominant DTUs identified in the very few ($n = 9$) OM-positive peridomestic *T. sordida* examined were TcVI (56%) and TcI (33%), leaving unclear the role of this species in the domestic transmission cycle (Maffey et al., 2012). These findings prompted us to further examine *T. sordida* bugs for *T. cruzi* infection, DTUs and bloodmeal sources using molecular methods to elucidate its role as a secondary vector across a diversity of rural villages, seasons and bug-control contexts. We included a sizable number of *T. sordida* specimens captured in peridomestic habitats and all the *T. sordida* captured in domiciles before and after full-coverage house spraying with insecticides. Our hypotheses were that *T. sordida* harbored subpatent infections with *T. cruzi*, and that *D. albiventris* opossums were the bloodmeal source of TcI-infected bugs.

## 2. Materials and methods

### 2.1. Study area

The present study was carried out in a rural section (450 km$^2$) of Pampa del Indio Municipality (25°55′S 56°58′W), Province of Chaco, Argentina, comprising 327 inhabited households belonging to 13 communities which have already been described elsewhere (Gurevitz et al., 2011).

### 2.2. Entomological surveys

A baseline (BL) survey was conducted in all households in the study area to assess triatomine infestation levels by timed manual searches (TMS) using a flushing-out agent in September–December 2007 (Gurevitz et al., 2011). Immediately after the BL survey, all households were sprayed with residual pyrethroid insecticide and house reinfestation was re-assessed at 4 months post-spraying (4 MPS). In several houses bugs were also collected by other methods (manually after timed-manual searches, i.e., post-TMS; during insecticide application, and by householders between insecticide spraying and 4 MPS). These additional bug collections were used to estimate the prevalence of house infestation at both surveys.

All collected triatomines were identified to species following Lent and Wygodzinsky (1979), stage and sex as described elsewhere (Canale et al., 2000). The taxonomy of the *T. sordida* complex has not been completely resolved (Calderón-Fernández and Juárez, 2013) and it is frequently difficult to distinguish the various member species based on external morphology only. Therefore, the study specimens may be considered as *T. sordida* "*sensu lato*".

Microscopic examination for *T. cruzi* infection (OM) was restricted to live third-instar nymphs and later stages prioritizing *T. infestans* over *T. sordida* specimens. Bugs were examined at 400× within 20 days of collection, and preserved at −20 °C.

### 2.3. Study design

Our sampling design aimed at analyzing *T. sordida* from all infested households. Adult bugs and fourth- or fifth-instars nymphs with a negative optical microscopy (OM) – diagnosis or not examined for infection were selected for kDNA-PCR. Five or less specimens of *T. sordida* were collected in most households infested by this species: 73% at BL and 72% at 4 MPS, and all of these bugs were examined for infection. In households with larger infestations, five bugs were randomly selected among adults and late nymphal stages. Households not included in the study sample usually harbored early nymphal stages or only OM-positive bugs (reported in Maffey et al., 2012). Each selected insect was dissected, and the rectal ampoule, abdomen and rest of the insect separated in labeled microtubes as described in Maffey et al. (2012).

### 2.4. PCR-based diagnosis of *T. cruzi* infection

DNA extraction from rectal ampoules' contents was performed using a commercial reagent (DNAzol, Life Technologies) to reduce the presence of inhibitors. Infection by *T. cruzi* was determined using a hot-start PCR targeting a 330 bp amplicon of the kinetoplast minicircle (kDNA-PCR) following standardized protocols (Maffey et al., 2012). In kDNA-PCR positive insects, another hot-start PCR was performed to confirm *T. cruzi* infection by targeting a *T. cruzi* satellite sequence (SAT-DNA-PCR) (Orozco et al., 2013). PCR products were analyzed in 3% agarose gels (Invitrogen, USA) and UV visualization after staining with Gel Red (GenBiotech).

### 2.5. Identification of parasite DTUs

DTUs were identified by PCR strategies directed to the spliced-leader sequence (SL, or "mini-exon"), alpha 24s rDNA, and A10 genomic marker following the protocol described by Burgos et al. (2007). Hemi-nested SL-PCRs using primers TCC-TC1 or TCC-TC2 were also performed to increase sensitivity as described in Marcet et al. (2006).

### 2.6. Bloodmeal identification

Given that most infected *T. sordida* had scarce bloodmeal contents, the abdomens were further cut into small pieces and mixed thoroughly with PBS to separate the stomach contents from abdomen walls. DNA was extracted using a commercial kit (DNeasy Blood & Tissue Kit QIAGEN Sciences, Maryland, USA) and used as a template for PCR targeting cytochrome b gene (CytB), following the protocol specified in Lee et al. (2002). To identify the bloodmeal source, amplified products were sent to an external service (Macrogen, Inc., Seoul, Korea) for automatic sequencing. Sequences were manually aligned using MEGA 5.1 software (Tamura et al., 2011). A consensus of forward and reverse sequences was created and a BLAST search was performed in GenBank database to compare sequence identity. Sequences have been submitted to GenBank (KP231499, KP231500).

### 2.7. Data analysis

Proportions were compared using Fisher's exact test. Wilson 95% confidence intervals ($CI_{95}$) were calculated for proportions. Baseline and 4 MPS entomological data were pooled together in order to perform spatial analyses. *T. sordida*-infested households and houses with infected bugs were incorporated in a GIS database of all houses of the study area using ArcGis 9.1. Infected *T. sordida* reported in Maffey et al. (2012) were also included. To test whether *T. sordida*-infested households and *T. sordida*-infected households were randomly distributed, we first performed a global spatial analysis (K-function and weighted K-function, respectively). Local spatial analysis was performed using the Getis Gi*(d) statistic to explore whether *T. sordida*-infected households were aggregated in space (Getis and Ord, 1996). Spatial analyses were performed using Point Pattern Analysis software (San Diego State University, San Diego, CA; Chen and Getis, 1998).

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