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Geographic variation of *Trypanosoma cruzi* discrete typing units from *Triatoma infestans* at different spatial scales

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ABSTRACT

We assessed the diversity and distribution of *Trypanosoma cruzi* discrete typing units (DTU) in *Triatoma infestans* populations and its association with local vector-borne transmission levels at various geographic scales. At a local scale, we found high predominance (92.4%) of TcVI over TcV in 68 microscope-positive *T. infestans* collected in rural communities in Santiago del Estero province in northern Argentina. TcV was more often found in communities with higher house infestation prevalence compatible with active vector-borne transmission. Humans and dogs were the main bloodmeal sources of the TcV- and TcVI-infected bugs. At a broader scale, the greatest variation in DTU diversity was found within the Argentine Chaco (227 microscope-positive bugs), mainly related to differences in equitability between TcVI and TcV among study areas. At a country-wide level, a meta-analysis of published data revealed clear geographic variations in the distribution of DTUs across countries. A correspondence analysis showed that DTU distributions in domestic *T. infestans* were more similar within Argentina (dominated by TcVI) and within Bolivia (where TcI and TcV had similar relative frequencies), whereas large heterogeneity was found within Chile. DTU diversity was lower in the western Argentine Chaco region and Paraguay ($D = 0.14–0.22$) than in the eastern Argentine Chaco, Bolivia and Chile ($D = 0.20–0.68$). Simultaneous DTU identifications of *T. cruzi*-infected hosts and triatomines across areas differing in epidemiological status are needed to shed new light on the structure and dynamics of parasite transmission cycles.

1. Introduction

Trypanosoma cruzi is transmitted by many species of triatomine bugs, with over 70 genera of mammalian hosts and a broad geographic range that extends from the United States to Argentina (Zingales et al., 2012). The human infection, named Chagas disease, is mainly acquired through vector-borne transmission in endemic regions of Central and South America (Bayer et al., 2009; Gürtler et al., 2005; Moncayo and Silveira, 2009). *Triatoma infestans* is the main vector associated with the transmission of *T. cruzi* in human sleeping quarters (i.e., domestic cycle) and peridomestic outhouses in the Southern Cone countries, including the Gran Chaco eco-region (encompassing sections of Argentina, Bolivia, Paraguay and Brazil) and southern Peru (Gürtler et al., 2007b; Noireau et al., 2009).

T. cruzi is currently subdivided in six discrete typing units (DTU) which are denominated TcI–TcVI (Brisse et al., 2000; Zingales et al., 2009). These DTUs constitute reliable units of analysis for molecular epidemiology research (Zingales et al., 2012) and exhibit differential distribution across vectors, hosts and transmission cycles (Miles et al., 2009; Noireau et al., 2009). Although all DTUs may cause human disease, TcII, TcV and TcVI are mainly associated with domestic transmission cycles in the Southern Cone region of South America whereas TcIII and TcIV are predominantly found in sylvatic cycles (Miles et al., 2009; Yeo et al., 2005). TcI is a major agent of human infection in the northern part of Latin America and the Amazon region and was also widely found in sylvatic cycles (Guhl and Ramírez, 2011; Miles et al., 2009; Zingales et al., 2012). Moreover,

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TcV predominated in the peripheral blood of humans whereas TcVI prevailed in domestic dogs and cats in the Argentine Chaco (Burgos et al., 2010; Cardinal et al., 2008; Cura et al., 2012; Diosque et al., 2003; Enriquez et al., 2013). Therefore, we hypothesized that a differential contribution of domestic host species to vector infection (e.g., due to host availability and infectiousness) would reflect in the relative frequency of DTUs identified in domestic *T. infestans*.

We also hypothesized that community-wide insecticide spraying can affect the population structure of *T. cruzi* by modifying parasite transmission links between domestic hosts and vectors, and this would modify the distribution of DTUs in triatomines. In areas under sustained vector surveillance and control (i.e., where vector-borne transmission has been severely depressed or interrupted), the proportion of houses with domestic *T. infestans* and bug infection are almost nil or absent (Cardinal et al., 2006; Gürtler et al., 2007b). Therefore, the relative contribution of humans as sources of infection would decrease, and dogs and cats would take on greater importance due to their higher infectiousness to the vector, frequent host-vector contact and association with (peri)domestic sites (Cardinal et al., 2014; Gürtler et al., 2007a). Conversely, in areas with intense vector-borne transmission the contact rates between humans and vectors would be higher and their contribution to bug infection would increase. Therefore, variations in parasite transmission intensity (indexed domestic infestation and bug infection prevalence) could explain the differential distribution of DTUs across areas within the Argentine Chaco (Cardinal et al., 2008; Maffey et al., 2012).

The distribution of DTUs in *T. infestans* has been documented in few geographical areas (Table 1). However, studies that quantify the diversity of parasite DTUs and assess how this distribution changes across areas and epidemiological contexts are lacking. The main objective of this study was to describe and quantify the diversity of *T. cruzi* DTUs in *T. infestans* at different scales of analysis. At a local scale, we analyzed the DTU distribution in bugs captured from human dwellings in a cross-sectional survey carried out in rural communities in the Argentine Chaco sub-region that had been under pulsed vector control measures, and evaluated its relation with bloodmeal sources and vector-borne transmission levels. At a broader scale, we analyzed if geographical variations in DTU distribution across areas in the Argentine Chaco were related to vector-borne transmission levels. We also carried out a meta-analysis to assess whether there are geographic variations in DTU distribution across the Southern Cone countries.

2. Materials and methods

2.1. Study area and bug collection

A cross-sectional survey was carried out in several rural communities in the Moreno department (27°38′46″S, 62°24′47″W), province of Santiago del Estero, Argentina, located in the dry (western) Argentine Chaco sub-region (Fig. 1). The study area was selected for an insecticide trial intervention because human dwellings were heavily infested with *T. infestans* and the last insecticide spraying had been conducted by the National Vector Control Program between 2 and 8 yr before the trial (Cecere et al., 2013). Vector-borne transmission of *T. cruzi* was confirmed by detection of recent acute human cases. Local dwellings include human sleeping quarters (domiciles) and nearby separate structures such as kitchens, storerooms, chicken coops, corrals and others (peridomicile). All the structures owned and used by one family are considered the house compound.

Timed manual collections of triatomine bugs were conducted in all house structures using 0.2% tetramethrin (Espacial 0.2, Reopen, Buenos Aires, Argentina) as a dislodging spray (Cecere et al., 2013). A house compound was considered "infested" if at least one live *T. infestans* bug was captured in at least one site.

The collected bugs were identified to species and stage at the field laboratory, and all live or moribund third- to fifth-instar nymphs and adult bugs were individually examined for *T. cruzi* infection by optic microscopy at 400× within 10 days of capture as described elsewhere (Cecere et al., 1999). Of 25 communities inspected for infestation and bug infection, only 6 had *T. cruzi*-positive *T. infestans* (Libertad, Luján, San Cristóbal, Ashpa Puca, Villa Brana and San Francisco, numbering 147 houses surveyed) and were included in the current study (Fig. 1). For comparison purposes, we denominated our study area "extra-peripheral" (see Section 2.5) by comparison to the core and peripheral areas described by Cardinal et al. (2007, 2008). Infected bugs were shipped to the Argentine National Institute of Parasitology "Dr. Mario Fatala Chabén" (Buenos Aires, Argentina) for parasite isolation.

2.2. Parasite isolation and DNA extraction

Isolation of *T. cruzi* from feces of all ($n = 79$) microscope-positive bugs and cultures in biphasic medium (Nutrient agar-rabbit blood/Brain Heart Infusion) were performed as described (Lauricella et al., 2005). Contaminated cultures were inoculated into 2–4 Balb-C mice which were euthanized 1 month post-infection and hemocultures performed. Cultures were kept at 28 °C and 50% relative humidity and microscopically monitored for parasite growth for 4 months until reaching 3×10^5 parasites/ml. Cultures were then stored in liquid nitrogen as described (Lauricella et al., 2005). Parasites used for identification of DTUs were obtained from an aliquot of cultures at the time of cryopreservation. Parasite DNA was extracted by boiling parasite pellets as described (Marcet et al., 2006). When cultures were not successful, DNA extraction was performed from identified fecal samples diluted in physiological solution and kept at 4 °C. Fecal aliquots were boiled for 10 min. and DNA extracted using a commercial reagent (DNAzol, Gibco BRL) as before (Marcet et al., 2006).

2.3. DTU identification

Parasite DTUs were identified using a combination of PCR amplifications targeted to nuclear genomic markers which had been optimized for direct identification from blood samples (Burgos et al., 2007); these procedures were successfully used for DTU identification from DNA obtained from bugs' rectal ampoules and culture (Maffey et al., 2012). The protocol targeted three different genomic markers: the intergenic region of spliced leader genes (SL-IR), the D7 domain of the 24Sα ribosomal RNA genes, and the genomic marker A10 as described (Burgos et al., 2007). PCR products were analyzed in 3% agarose gels (Invitrogen, USA) and UV visualization made after staining with Gel Red (GenBiotech). Due to the weak sensitivity of the A10 genomic marker, some samples could not be resolved as TcII or TcVI; these cases were identified as TcII/TcVI. The very low DNA concentration in two fecal samples did not allow differentiation among TcII, TcV and TcVI; these cases were identified as TcII/TcV/TcVI, and were not considered for further analysis.

2.4. Identification of bloodmeal sources

Bugs were dissected and their bloodmeal contents were extracted and stored in microtubes containing PBS buffer. A direct ELISA assay was used to test bloodmeal contents against human, dog, cat, chicken, goat and pig antisera as described (Gürtler et al., 2014). The antisera considered in the ELISA assay correspond to the