Acta Tropica 140 (2014) 10–18

Contents lists available at ScienceDirect

Acta Tropica

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

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

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ARTICLE INFO

Article history:
Received 7 May 2014
Received in revised form 7 July 2014
Accepted 23 July 2014
Available online 1 August 2014

Keywords:
Trypanosoma cruzi
Discrete typing units
Triatoma infestans
Diversity
Molecular epidemiology

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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http://dx.doi.org/10.1016/j.actatropica.2014.07.014
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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

Table 1
List of study sites where *T. cruzi* DTUs were identified from domestic *T. infestans* in endemic areas in the Argentine Chaco and other Southern Cone countries. Domiciliary infestation with *T. infestans*, prevalence of infection with *T. cruzi* for vectors and hosts, and history of vector control is presented for all the areas in the Argentine Chaco. Date of triatomines collection, the number of triatomines with identified DTU and Whittam genetic variability index (*D*) are presented for all areas.

Country	Area ID	Geographical area	Prevalence of *T. cruzi* in				Triatomine collection	Years since last insecticide spraying[†]	Type of vector control actions	Level of transmission	Number of *T. infestans* with DTU identification	Diversity index
			Domiciliary infestation (%)	Domestic *T. infestans* (%)	Humans (%)	Dogs (%)						
Argentina	1-Arg-SE-ExP	Extra-peripheral area (S.E) This study, Cecere et al. (2013)	40	18[*]	ND	ND	2004	2–8	Pulsed[a]	Medium-high	66	0.14
	2-Arg-SE-P	Peripheral area (S.E) Cardinal et al. (2006,2008)	18	13	5	12	2002–2006	1–6	Pulsed[a]	Low	37	0.25
	3-Arg-SE-C	Core area (S.E) Cardinal et al. (2006,2008)	9	4	5	7	2000–2006	<1	Sustained[a]	Depressed	17	0.22
	4-Arg-CH-Ch	Chacabuco (Ch.) Diosque et al. (2003, 2004)	14[**]	30[#]	28	15	1999–2001	3–5	Sporadic	High	38	0.65
	5-Arg-CH-PI	Pampa del Indio(Ch.) Cardinal et al., 2014, Maffey et al. (2012)	40	27[#]	36[‡]	26	2007	11	Sporadic	High	69	0.47
Bolivia	6-Bo-Syl	Andean Region Sylvatic Brenière et al. (2012)					2009				232	0.02
	7-Bo-Co	Cochabamba Bosseno et al. (1996), Brenière et al. (1995)					NA				20	0.54
	8-Bo-LP	La Paz Bosseno et al. (1996), Brenière et al. (1995)					NA				74	0.61
Chile	9-Chi-II	Region II Barnabé et al. (2001)					1985–1992				11	0.33
	10-Chi-III	Region III Venegas et al. (2011)					2005–2007				11	0.51
	11-Chi-IV	Region IV Barnabé et al. (2001)					1985–1992				12	0.59
	12-Chi-V	Region V Venegas et al. (2011)					2005–2007				25	0.61
	13-Chi-Met1	Metropolitan Region Venegas et al. (2011)					2005–2007				10	0.68
	14-Chi-Met2	Metropolitan Region Bacigalupo et al. (2012)					2006–2008				91	0.20
	15-Chi-Syl	Metropolitan Region-Sylvatic Bacigalupo et al. (2012)					2003–2004 2007–2008				89	0.56
Paraguay	16-Par	Cordillera and Paraguari Del Puerto et al. (2010)					2001–2002				28	0.19

NA: not available.
[†] Prior to triatomine collection.
[*] Cecere M.C., unpublished data.
[‡] Sartor P., unpublished data.
[**] Minimum percentage of domestic infestation calculated as the number of infested houses divided by the total number of houses inspected (Diosque et al., 2004).
[#] Minimum prevalence: Prevalence may include domestic and peridomestic triatomines.
[a] Selective spraying of infested houses.

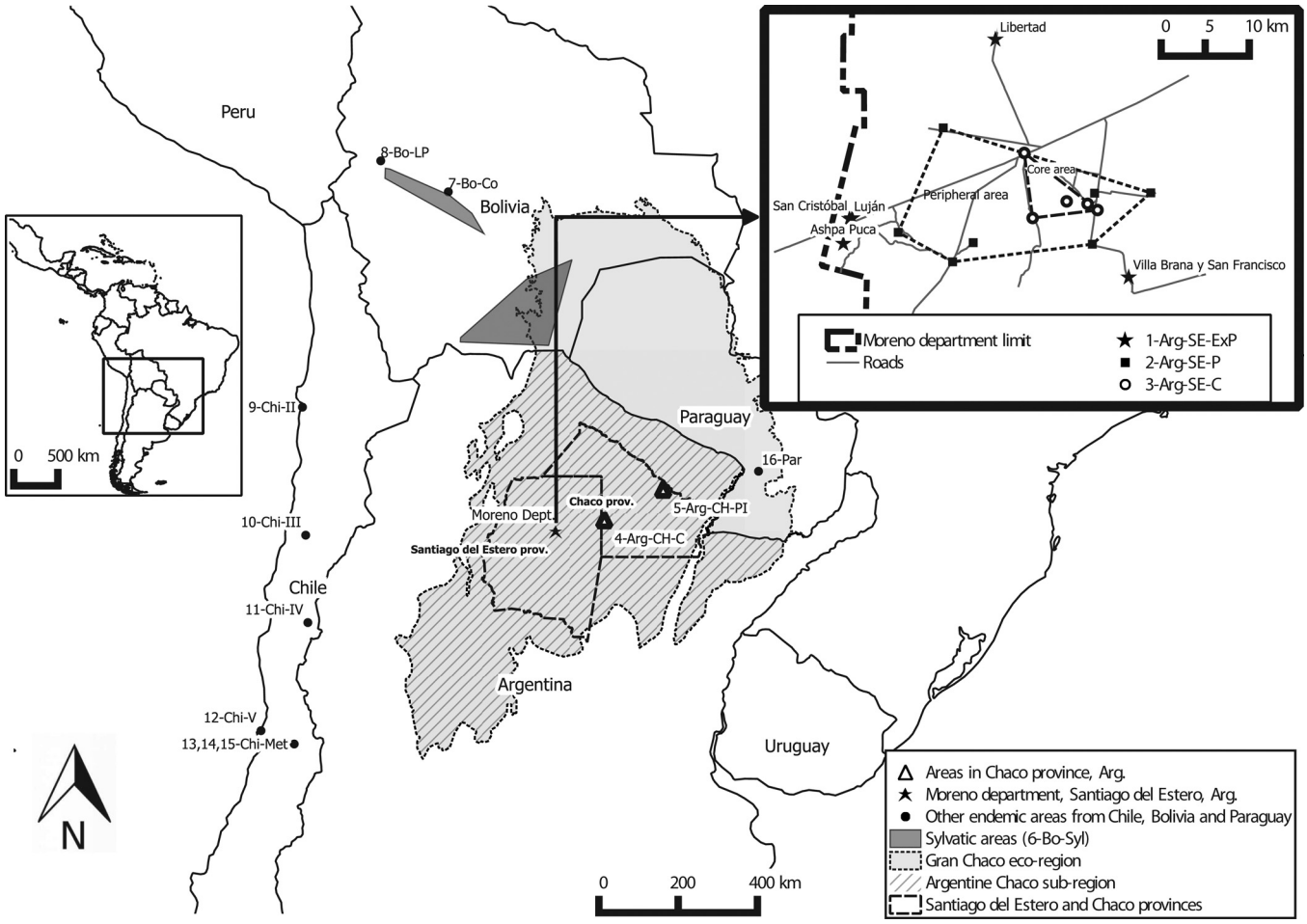


Fig. 1. Map of the study areas included in the meta-analysis. The striped area indicates the Gran Chaco region and dark dots, study areas in Paraguay, Bolivia and Chile. Within the Argentine Chaco, Moreno department in Santiago del Estero province (star) and study areas in Chaco province (triangles), are shown. Within the Moreno department (magnified area), stars identify the extra-peripheral area (1-Arg-SE-ExP), dark squares the peripheral area (2-Arg-SE-P) and white dots the core area (3-Arg-SE-C). See Table 1 for more details.

most frequent (peri) domestic hosts of *T. infestans* throughout its distribution.

2.5. Comparison of DTU distribution in the Argentine Chaco

In order to compare our results with findings from other areas of the Argentine Chaco sub-region, we performed a literature search and identified four other studies that assessed the distribution of DTUs in *T. infestans* captured in human dwellings (Cardinal et al., 2008; Diosque et al., 2004, 2003; Maffey et al., 2012). These studies reported house infestation levels and prevalence of infection in the vector (Table 1). Two of the study areas (the "core area" and the "peripheral area") were located within 30 km from the extra-peripheral area in the Moreno department, Santiago del Estero province (magnified area in Fig. 1). Extra-peripheral, peripheral and core areas differed mainly in vector-borne transmission levels (Table 1). Two other studies took place in the neighboring Chaco province (east), where intense vector-borne transmission occurred at the time of the surveys (Table 1): these included two groups of rural communities that we identified as Chacabuco (Diosque et al., 2004, 2003) and Pampa del Indio (Maffey et al., 2012) for comparison purposes.

The Argentine Chaco sub-region presents environmental gradients increasing from East to West (e.g., seasonality, woody plants density) or from West to East (e.g., rainfall, grassland) (Naumann, 2006). Herein the geopolitical division is used to identify the study areas and their relative location. The areas located in Santiago del Estero also differed from those in the Chaco province in the vector control measures carried out (Table 1). Because vector control measures apparently exerted heterogeneous impacts among areas, we defined four broad categories of vector-borne transmission (high, medium-high, low and depressed) based on local house infestation and prevalence of infection in *T. infestans* (Table 1).

2.6. Meta-analysis of DTU distribution

In order to assess geographic variations across a broader region, a literature search was carried out. Only reports with DTU, zymodeme or clonet identification in at least 10 naturally-infected *T. infestans* were included in the meta-analysis. We excluded papers without a defined geographical area of triatomine collection; those that included *T. infestans* reported previously or which had a high frequency of inconclusive DTU identifications. In total, we selected 6 papers encompassing 15 study areas across the Southern Cone countries: 7 from different regions in Chile (Bacigalupo et al., 2012; Barnabé et al., 2001; Venegas et al., 2011), 1 from Paraguay (Del Puerto et al., 2010), 3 from Bolivia (Bosseno et al., 1996; Brenière et al., 2012, 1995) and 5 areas in the Argentine Chaco sub-region (Table 1, Fig. 1). Triatomines considered in this meta-analysis had been captured either at domiciles or peridomestic sites, with the exception of one study in Bolivia and one in Chile in which infected bugs were captured in sylvatic habitats. We retrospectively

assigned DTUs to the studies reviewed when required, based on a comparison of *T. cruzi* nomenclature scheme (Lewis et al., 2009). Inconclusive DTU identifications were not included in the analyses.

2.7. Data analysis

In order to investigate whether there was an association between bloodmeal source and DTU, we considered only human and dog blood meals (i.e., potential sources of *T. cruzi* infection). In the case of mixed blood meals we assumed that each blood meal and each identified DTU were independent and counted separately.

To compare the frequency of DTUs among communities and study areas, two-tailed Fisher's exact test and χ^2 tests were used depending on sample size and validity of test assumptions. We decided to compare only relative frequencies of TcV and TcVI because they were the most prevalent DTUs across areas, which also avoided over-dispersed data. In addition, because Fisher's exact test becomes very conservative when marginal totals are not fixed and sample sizes are small, we also compared DTU distribution with a non-parametric, exploratory correspondence analysis (Diotaiuti et al., 1995; Quinn and Keough, 2012) implemented in Stata (Stata Corp, 2011). For comparisons within the extra-peripheral area, communities were grouped in three clusters according to distance between them to increase the number of infected insects in each group, assuming that neighboring communities were more similar in terms of environmental and social characteristics than more distant communities. Clusters were separated by approximately 30 km, and the maximum distance between communities within each group was approximately 7 km. One group was formed by only one community (Libertad) whereas the remainder encompassed two (Villa Brana and San Francisco) and three (Luján, Ashpa Puca and San Cristóbal) communities.

Genetic diversity was quantified using the Whittam index (D) which considers the number of genotypes found in the population (i.e., richness) and their relative frequency (i.e., equitability) (Barnabé et al., 2000). D values vary between 0 and 1, and represent the probability of finding different genotypes in two randomly selected insects.

3. Results

We identified DTUs from 68 (86.1%) of 79 microscope-positive *T. infestans* collected in the extra-peripheral area of several rural communities in the Moreno department, and reached a conclusive DTU identification in 52 (76.4%) bugs. Identification from bug feces culture was most effective, with conclusive DTU identification obtained in all samples ($n = 24$). DTU identification from mice-hemoculture and triatomine fecal samples was carried out when parasite isolation from feces cultures was not possible, but proved to be less effective and reached conclusive results in 80.7% ($n = 26$) and 38.9% ($n = 18$) of the samples, respectively. Bugs with inconclusive DTU identification ($n = 16$) included 14 bugs with inconclusive TcII/TcVI. These were considered as TcVI for further analysis because other triatomines from the same house were infected with TcVI, and because in the Argentine Chaco, TcVI was highly frequent whereas TcII has not yet been identified in *T. infestans*. Inconclusive TcII/TcV/TcVI ($n = 2$) were not considered for further analysis because we could not distinguish between TcV and TcVI. Overall, including the bugs with inconclusive TcII/TcVI, TcVI was found in 92.4% (61/66) of the insects and TcV was found in 7.6% (5/66). No mixed infections were identified.

The 66 bugs with DTU identification were from 18 different houses, 66.6% (12/18) of which had more than one infected bug analyzed (Suppl. Table). However, only one DTU was found in each house compound. All five TcV-infected *T. infestans* were captured in domiciles, whereas TcVI-infected bugs were captured in domiciles or peridomiciles. When DTU distributions were compared, no significant differences were found among the three groups of communities (in all three Fisher's exact test, $p > 0.4$) (Fig. 2A). TcVI predominated in all three groups. TcV was absent in Libertad and its frequency varied from 12.5% (1/8) in one group of communities (including Luján, Ashpa Puca and San Cristóbal) to 7.5% (4/53) in another group (including Villa Brana and San Francisco) which had a greater number of insects analyzed (Fig. 2A).

Table 2
Association between the occurrence of *T. cruzi* TcV and TcVI and bloodmeal source for *T. infestans*.

Bloodmeal source	DTU	
	TcV (%)	TcVI (%)
Human	1 (20)	8 (15)
Dog	0	5 (10)
Human-dog	0	3 (6)
Chicken-dog	0	2 (4)
Goat	0	1 (2)
Non-reactive	4 (80)	33 (63)
Total	5	52

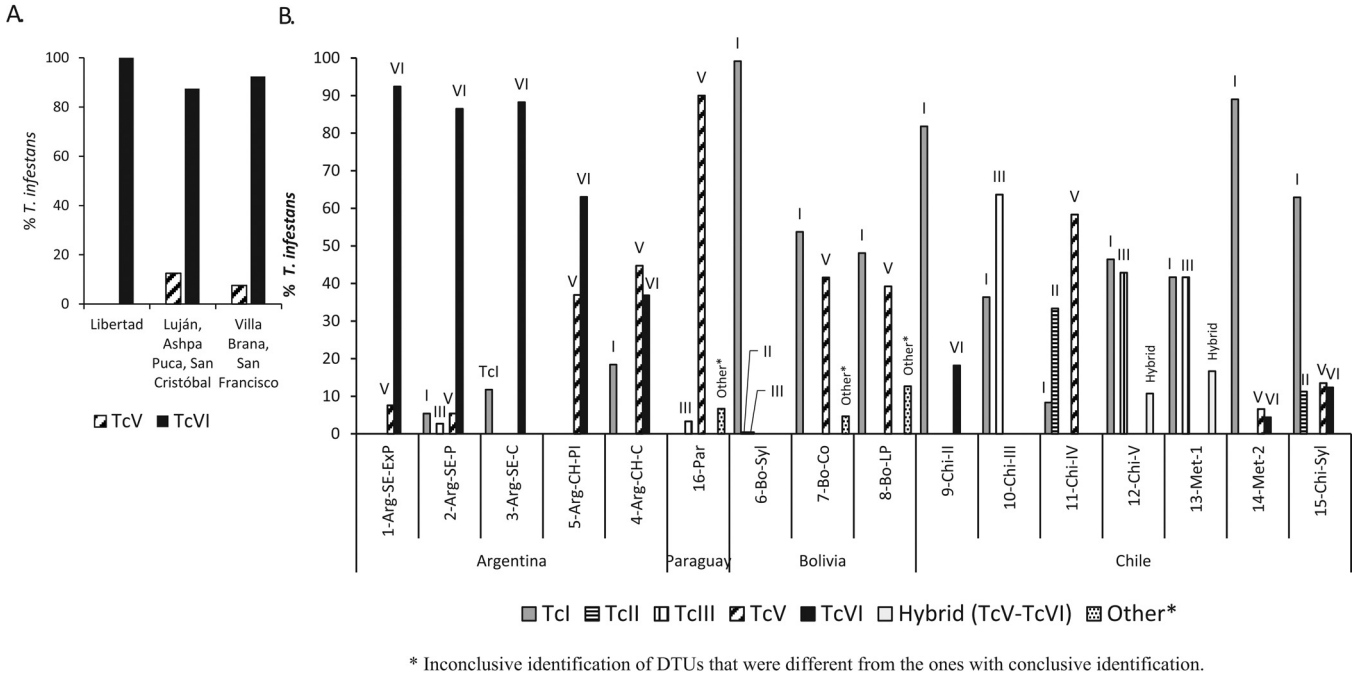
3.1. DTU distribution and bloodmeal sources

Of 57 (86.4%, $n = 66$) *T. infestans* with identified DTUs and tested by ELISA, only 20 (35.1%) were reactive (i.e., had an identified bloodmeal source) (Table 2). The remaining triatomines had remnants or no bloodmeal contents. All of the ELISA-reactive bugs were captured in domiciles, except for two that were captured in kitchens. All of the infected bugs captured in domiciles fed exclusively on human and dog, with the exception of a goat-fed bug, whereas insects from kitchens had mixed blood meals on chicken and dog. The only reactive bug infected with TcV fed on human only. Bloodmeal sources (dogs and humans) were not significantly associated with DTU (TcVI and TcV) (Fisher's exact test, $p > 0.9$). The *a posteriori* power of the test was very low (0.2%).

3.2. DTU distribution and diversity within the Argentine Chaco

Although TcVI and TcV continued to be the most frequent DTUs, variation in DTU distribution was observed (Fig. 2B). The relative frequencies of TcVI and TcV in the extra-peripheral area and in the neighboring core and peripheral areas in Moreno department (Santiago del Estero province) were not significantly different (Fisher's exact tests, $p > 0.5$). Nonetheless, TcV was not found in the core area, whereas only 1 TcV-infected bug and 2 bugs with mixed TcV–TcI infections were found in the peripheral area (Fig. 2B). Conversely, when the extra-peripheral area was compared with the Chaco province areas, significant differences were observed (χ^2 tests with 1 df; $p < 0.001$), with a much higher relative frequency of TcV in Chacabuco (44.8%; 17/38) and in Pampa del Indio (36.4%; 24/66, excluding 3 mixed infections with TcV–TcVI) compared with the extra-peripheral area (7.3%; 5/66) (Fig. 2B). No significant differences were found between Chacabuco and Pampa del Indio (χ^2 test with 1 df, $p = 0.42$), whereas significant differences were found between these and the Moreno department areas (χ^2 test with 1 df, $p < 0.0001$). The correspondence analysis showed a higher similarity in DTUs among areas from the Moreno department (Santiago del Estero province) than among areas from Chaco province, and that the DTU distribution from the extra-peripheral area was more similar to the one found in Pampa del Indio than in Chacabuco (Fig. 3A).

We also quantified genetic diversity at DTU level for all study areas. The extra-peripheral area had the lowest Whittam index value ($D = 0.14$), which indicates a low probability of finding two



* Inconclusive identification of DTUs that were different from the ones with conclusive identification.

Fig. 2. Distribution of *T. cruzi* DTUs identified in *T. infestans*. (A) Within the extra-peripheral area in Santiago del Estero province, Argentina (1-Arg-SE-ExP) (B). From Argentina (areas 2–3 in Santiago del Estero (SE) and 4–5 in Chaco province (CH)), Bolivia (Sylvatic areas, La Paz and Cochabamba), Chile (Regions II, IV, V, Metropolitan and Sylvatic areas) and Paraguay. See Table 1 for more details. Numbers above bar indicate *T. cruzi* DTU and mixed infections were counted separately.

T. infestans infected with different DTUs. Conversely, the areas belonging to the Chaco province presented the highest DTU diversity in the Argentine Chaco (Table 1).

3.3. Geographic variation of DTU distribution among countries

For the meta-analysis, the total number of *T. infestans* considered was 830 bugs: 39.2% were from Bolivia, 30.0% from Chile, 27.4% from Argentina and 3.4% from Paraguay. All DTUs, except TcIV, were found throughout the region. However, an uneven distribution was recorded and not all DTUs were present in all areas (Fig. 2B). Geographic variation of DTU distribution was corroborated by the correspondence analysis (Fig. 3B) in which study areas located in the Argentine Chaco sub-region appeared to be the most different (i.e., higher χ^2 distance in the first dimension) due to their close association with TcVI, which was either absent or in lower frequency (<20%) in the rest of the areas. Given the higher prevalence of TcV in the Chaco province of Argentina, these areas were more similar to the area of Paraguay, which was closely associated with TcV. In Bolivia, TcI was the main DTU, and TcV was only found in Cochabamba and La Paz. In Chile, however, all mentioned DTUs were found and their distribution was heterogeneous among areas.

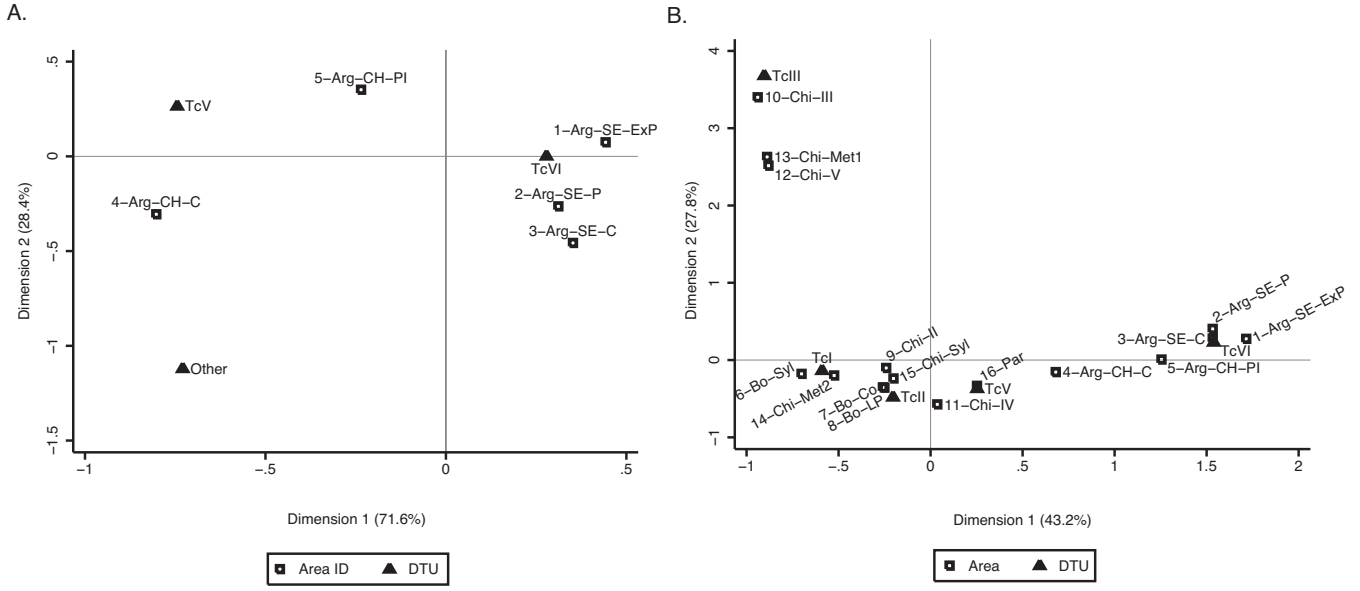


Fig. 3. "Joint plot" of the correspondence analysis of DTU (triangles) distribution among study areas (squares, see area ID in Table 1). Axes are the first and second dimensions in coordinates (in principal normalization) and the percentage of lack of independence explained is indicated. (A) Within the Argentine Chaco Region; (B) within the Southern Cone of South America (meta-analysis).

Distinct features of the areas in Chile included the widespread presence of TcI, the much higher prevalence of TcIII compared to other countries, and the presence of TcII.

Regarding DTU diversity (Table 1), the lowest diversity index was found in the sylvatic area of Bolivia ($D = 0.03$) due to the uniqueness of TcI (99.1%). The sylvatic area in Chile had greater diversity ($D = 0.56$), but TcI also prevailed (Fig. 2B, 3b). In the domestic cycle, the lowest diversity index was found in the extra-peripheral area ($D = 0.14$), followed by neighboring areas in Santiago del Estero province and the area in Paraguay ($D = 0.19$–0.22); all had one highly dominant DTU (range, 88.2–96.4%). The rest of the areas in the Argentine Chaco had higher diversity indices ($D = 0.47$–0.65), similar to the ones in Bolivia ($D = 0.43$–0.58) and Chile ($D = 0.20$–0.68) where the relative frequency of the most prevalent DTUs did not exceed 60%, except for one study in the Metropolitan region (Fig. 3B). The greatest variation in DTU diversity was found in the domestic cycles in the Argentine Chaco (range 0.14–0.65).

4. Discussion

Our study shows substantial variations in the distribution and diversity of *T. cruzi* DTUs in *T. infestans* at various spatial scales throughout most of its current range. The greatest variation in DTU diversity occurred in domestic cycles from the Argentine Chaco sub-region as shown by the Whittam index, which has been previously used to quantify the clonal diversity of *T. cruzi* (Barnabé et al., 2000; Brenière et al., 1997). This index presented a wider range (0.14–0.65) in Argentina than in other countries due to differences in equitability, not richness. The lowest diversity indices were observed in domestic transmission cycles in the Moreno department (Santiago del Estero province) due to the high predominance of TcVI. In contrast, in the neighboring Chaco province, diversity indices were higher due to the increased relative frequency of TcV. These patterns may reflect a differential contribution of humans, dogs and cats to vector infection.

The high predominance of TcVI found in *T. infestans* from the Moreno department was also registered more widely in domestic dogs and cats from the Argentine Chaco sub-region, in which more than 80% were infected with TcVI (Cardinal et al., 2008; Diosque et al., 2003; Enriquez et al., 2013). In contrast, TcV was recorded only in 6–8% of the dogs in two areas within Chaco province (Diosque et al., 2003; Enriquez et al., 2013) and not in Moreno dogs (Cardinal et al., 2008). In humans, however, TcV was consistently the main DTU identified in the Argentine Chaco (>50% of patients), whereas conclusive identifications of TcVI were rarely achieved (Burgos et al., 2007; Cardinal et al., 2008; Corrales et al., 2009; Cura et al., 2012; Diez et al., 2010; Diosque et al., 2003; Monje-Rumi et al., 2013). In studies with few inconclusive samples, TcV was found in more than 70% of human patients and TcVI in less than 10% (Burgos et al., 2007; Corrales et al., 2009; Monje-Rumi et al., 2013). However, methodological limitations and low parasite DNA load may have prevented a clear differentiation between TcII, TcV and TcVI in peripheral blood samples. Therefore, the exact prevalence of TcVI infection in humans is uncertain. TcI and TcII have also been identified in human patients from the Argentine Chaco (Burgos et al., 2010; Cura et al., 2012), usually from cardiac explants or from immunosuppressed patients (bloodstream and/or chagomas) and in low frequency.

The high prevalence of TcVI found in *T. infestans* from Moreno is therefore consistent with the key role of dogs and cats as domestic reservoir hosts and infection sources for domestic triatomines, enhanced by their higher infectiousness to the vector than infected humans (Cardinal et al., 2014, 2007; Enriquez et al., 2014; Gürtler et al., 2007a, 1996a). Conversely, in the Chaco province areas, the higher frequency of TcV suggests that humans may have contributed substantially to domestic bug infection. The relative abundance of dogs to humans was approximately similar across areas in Moreno department and Chaco province (dog-human ratio, 0.52 and 0.72, respectively) (Cardinal et al., 2014, 2007), and therefore would not contribute to explain the observed differences.

The differential distribution of DTUs between domestic dogs or cats and humans, and the fact that mixed TcV–TcVI infections were only found in Pampa del Indio and in low frequency, could be interpreted as two independent transmission cycles occurring in human sleeping quarters from the same villages. However, this hypothesis is very unlikely and would require a mechanism leading to the stable segregation of DTUs that circulate in sympatry. Various lines of ecological and epidemiological evidence support the occurrence of a single domestic transmission cycle in which domestic dogs, cats and humans act as sources of *T. cruzi* for vectors and vice versa in the Moreno study areas (Gürtler et al., 2005, 2007a,). In addition, our bloodmeal identifications showed frequent contact between *T. infestans*, dogs and humans, and sizable surveys also showed a large fraction of domestic bugs with mixed blood meals on both humans and dogs (Gürtler et al., 1996b; Rabinovich et al., 2011). Thus, the differential distribution of DTUs observed among domestic host species could be related to host selection effects and technical limitations in DTU identification, and/or tissue tropism as reported by Burgos et al. (2010). Technical limitations of the protocols used for DTU isolation and/or identification could lead to differential amplification of DTUs in multiclonal infections, and less abundant DTUs might be lost. In support of this hypothesis, alternative strategies for DTU identification based on Southern blotting revealed an increasing number of mixed infections in humans (Monje-Rumi et al., 2013; Ortiz et al., 2012). Assessment of intra-DTU diversity could also help identify DTUs in lower abundance (Cura et al., 2010; Llewellyn et al., 2009) by isolating *T. cruzi* clones followed by multilocus microsatellite profiling (Llewellyn et al., 2011).

A novel finding of our study is the higher frequency of TcV in areas with higher domestic bug infestation and infection in the Moreno department; within the extra-peripheral area (Table S1), and between the extra-peripheral and the neighboring peripheral or core areas (as shown by the correspondence analysis). These results support the hypothesis that with more intense vector-borne transmission, humans would make a greater contribution as sources of *T. cruzi* infection, although these comparisons were not statistically significant (the power of the tests was also <9%). Higher infestation levels may increase the absolute frequency of vector-human contacts and the probability of occurrence of TcV-infected bugs.

At a broader scale within the Argentine Chaco sub-region, the frequency of TcV was higher in Chaco province than in the Moreno areas, although house infestation levels in the extra-peripheral area (Moreno department) and Pampa del Indio (Chaco province) were similar. Nonetheless, bug infection prevalence with *T. cruzi* was higher in Pampa del Indio, suggesting higher vector-borne transmission intensity which may be related to the frequency of vector control measures (sporadic vs. pulsed), and ensuing host infection prevalence. Higher prevalence of infection in humans may also increase the probability of occurrence of TcV-infected bugs. These differences in the relative frequencies of TcV and TcVI between the Moreno and Chaco province areas could also be related to environmental and socio-cultural factors (Gurevitz et al., 2011) that modify host exposure to the vector. Stark differences in house construction materials and domestic animal management practices determine large variations in the availability of refuges for bugs, bug abundance and parasite transmission. In the Chaco province areas, dogs were seldom allowed to sleep inside human sleeping quarters, unlike in Moreno areas (Santiago del Estero), with a consequent reduced exposure to domestic bugs (Cardinal et al., 2014).

As we broadened the spatial scale to include Paraguay, Bolivia and Chile, all DTUs were identified except TcIV, which has not been yet been found in *T. infestans* (Noireau et al., 2009). However, not all DTUs were identified in all areas and variations in their distribution were found. DTU distributions in domestic *T. infestans* were more similar among areas within Argentina and Bolivia than within Chile. Variations in DTU distribution among countries most likely reflect the characteristics of local transmission cycles. However, potential sources of bias associated with DTU identification techniques and type of sample may add additional sources of variation. DTUs were sometimes identified after parasite culture in biphasic medium (this study, Cardinal et al., 2008; Maffey et al., 2012) or LIT monophasic medium (Barnabé et al., 2001; Diosque et al., 2003) whereas other studies identified DTUs directly from bug feces. The higher growth rate of TcI over TcV in LIT medium may favor the selection of TcI (Laurent et al., 1997) and may explain the increased frequency of TcI in Chacabuco compared to other areas in the Argentine Chaco. In addition, the selected studies used multiple DTU identification techniques (i.e., Multilocus Enzyme Electrophoresis, PCR and hybridization, Mini-exon Multiplex-PCR, microsatellites and PCR targeting nuclear markers). These additional sources of variation may explain the large heterogeneity observed in DTU distribution among study areas within Chile compared to other countries. 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.

Acknowledgements

The authors are grateful to the National Vector Control Program staff from Tucumán and Córdoba, Diego Glaser, Silvana Ferreyra, Mariana Carro and Cristina G. Maidana for field and laboratory assistance. Reference strains of TcI–TcVI were kindly provided by Patricio Diosque, Miguel A. Basombrío and Michel Tibayrenc to A.G.S. This study received financial support from Fundación Bunge y Born (grant number: FBB02/04) (to M.C.C.), University of Buenos Aires (grant number: 20020100100944) and Agencia Nacional de Promoción Científica y Técnica (grant numbers: PICT 2011-2072 and PICTO-GLAXO 2011-0062) (PICT and PICTO-GLAXO) to R.E.G. R.E.G., M.V.C., M.C.C. A.G.S. are members of CONICET Researcher's Career.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2014.07.014.

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