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Host-Feeding Sources and Infection With *Trypanosoma cruzi* of *Triatoma infestans* and *Triatoma eratyrusiformis* (Hemiptera: Reduviidae) From the Calchaqui Valleys in Northwestern Argentina

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Abstract

We assessed the prevalence of infection with *Trypanosoma cruzi*, parasite genotypes (discrete typing units, DTUs), and the host-feeding sources of domestic and peridomestic *Triatoma infestans* Klug and *Triatoma eratyrusiformis* Del Ponte in eight rural communities of the subandean Calchaqui valleys in northwestern Argentina. We sought to analyze their epidemiological role in the context of routine vector surveillance and control actions. Infection with *T. cruzi* was determined by optic microscopy or polymerase chain reaction (PCR) amplification of the hypervariable region of kinetoplast DNA minicircles. Parasite genotypes were identified through a multi PCR-based strategy. Bloodmeal contents were tested with a direct ELISA assay against nine antisera. Human sleeping quarters (domiciles) and peridomestic dry-shrub fences concentrated most of the *T. infestans* and *T. eratyrusiformis* infected with *T. cruzi*, respectively. The most frequent host-feeding sources of *T. infestans* were chickens (73.1%) in peridomiciles and humans (73.3%) in domiciles, whereas *T. eratyrusiformis* fed more often on cavid rodents (92.6%), which thrived in the dry-shrub fences. The main *T. cruzi* DTU identified in both vectors was *T. cruzi* I (TcI). *Triatoma eratyrusiformis* was implicated in the local circulation of *T*cI among cavies and perhaps mice, but infection with other typically domestic DTUs (TcVI and TcII/TcV/TcVI) indicated overlap between (peri)domestic transmission cycles in both vector species. Because dry-shrub fences were not targeted for routine insecticide spraying, they may act as sources of (peri)domestic reinfestation. *Triatoma eratyrusiformis* is an emergent secondary vector of *T. cruzi* and plays a significant role in the local transmission of *T. cruzi*.

Key words: infection, feeding source, Chagas disease, triatomine, transmission

Triatoma infestans (Klug, 1834) is the main vector of Chagas disease in the southern cone countries, where the vector-borne transmission of *Trypanosoma cruzi* has been successfully eliminated from Uruguay, Chile, and Brazil but not from large sections of Paraguay, Bolivia, and Argentina (Dias et al. 2002, Schofield et al. 2006). The latter three countries include a 1.3 million km^2 ecoregion (the Gran Chaco) that is hyperendemic for Chagas disease (Gürtler et al. 2007a, 2009). Tucuman province is partially included in the Argentine Chaco, and it has yet to achieve the interruption of vector-borne transmission of *T. cruzi* despite a long history of insecticide-spraying campaigns initiated in the 1950s (Zaidenberg et al. 2004, Spillmann et al. 2013). In addition to the occurrence of *T. infestans*, the domestic invasion of *Triatoma eratyrusiformis* (Del Ponte, 1929) has recently been noticed by the local Chagas disease vector control program (CDVCP) in Tafi del Valle department in Tucuman (unpublished observations). *Triatoma eratyrusiformis* is usually found in mountainous areas along western Argentina in association with edentates and rodents (Martinez et al. 1985,

Carcavallo et al. 1998). Current knowledge on *T. eratyrusiformis* is quite limited (Sosa 1997, Carcavallo et al. 1998, Abraham et al. 2011).

The distribution of blood-feeding sources and *T. cruzi*-infection patterns of triatomines provide insights into their epidemiological roles in different habitats. In the Argentine Chaco, domestic dogs, cats, chickens, and humans are the most common bloodmeal hosts of *T. infestans* (Gürtler et al. 1996, 2014; López et al. 1999), and dogs and cats are the most important domestic reservoir hosts of *T. cruzi* (Cardinal et al. 2007, Gürtler et al. 2007b, Enriquez et al. 2014). Different genotypes (discrete typing units, DTUs) of *T. cruzi* (TcI, TcIII, TcV, and TcVI) have been identified, and both TcV and TcVI were the most frequent parasite DTUs in *T. infestans*, humans, dogs, and cats (Fernandez et al. 2014, Monje-Rumi et al. 2015). There is little evidence on the role of other triatomine species adapted to peridomestic habitats of the Argentine Chaco (Alvarado-Otegui et al. 2012, Maffey et al. 2012, Macchiaverna et al. 2015), more so in the mountainous valleys of northwestern Argentina. In rural communities from Tafí del Valle, *T. eratyrusiformis* was found in dry-shrub fences associated with peridomestic colonies of *Microcavia australis* (Caviidae, Rodentia), also known as southern mountain, desert, or small cavy. Cavies are highly competent reservoir hosts of *T. cruzi* I in these habitats (Cecere et al. 2015), and *T. eratyrusiformis* appears to be the most likely putative vector of TcI among cavies.

As part of a broader research project on the prevention and control of vector-borne transmission of *T. cruzi* in the Calchaqui valleys of northwestern Tucuman, this study sought to assess the epidemiological role of *T. infestans* and *T. eratyrusiformis* in the context of routine vector surveillance and control activities. We measured their prevalence of infection with *T. cruzi*, identified the DTUs, and host-feeding sources stratified by type of habitat. This information may contribute to improved vector control strategies and the interruption of local transmission of *T. cruzi*. Our joint analyses of natural infection with *T. cruzi* and triatomine host-feeding patterns stratified by habitat provide novel insights into sympatric transmission cycles mediated by two species of triatomine bugs.

Materials and Methods

Study Area

This study was carried out in eight neighboring rural communities from Tafí del Valle department (26° 27′ S, 65° 59′ W), Tucumán province, northwestern Argentina, between 2007 and 2009 (Fig. 1). These villages are situated in the subandean Calchaqui valleys at 1,800 m above sea level, where scrublands predominate and the steppe is formed by resinous evergreen bushes (e.g., *Larrea* sp.) and residual forests of *Prosopis* sp. and *Salix humboldtiana* Willdenow (Unidad de Manejo de Sistema de Evaluación Forestal (UMSEF) 2004). The climate is temperate arid, with 80–250 mm of annual rainfall, and the annual mean temperature is 19°C (http://worldwildlife.org/ecoregions/nt0802). The study area had three schools and three primary health-care centers; 4–50 houses per community, each with 2–100 residents. Households usually lacked piped water, sewage system, electricity, and gas.

A house compound encompassed the domicile and the associated peridomicile (separated from human sleeping quarters). Most houses were made of adobe walls and thatched roofs. The peridomicile included a patio and three to eight structures (storerooms, kitchens, corrals, etc.) that housed domestic animals; these structures were surrounded by plots of crops and native vegetation. A typical feature of the study area was the presence of extended fences of dry shrubs built by local residents to divide plots of land for specific purposes; these fences housed sylvatic cavy colonies (Fig. 2; Cecere et al. 2015). Each type of domestic or peridomestic structure with a defined purpose is called "ecotope." Seven categories of ecotopes were defined, including "Other" for bug collection sites whose function was not recorded. Peridomestic ecotopes were within 2–150 m from the domicile.

All houses in the study area were sprayed with pyrethroid insecticides by the local CDVCP in late 2005. All houses found reinfested by *T. infestans* were subsequently resprayed with pyrethroids in early 2006. House infestation with triatomines was monitored from March 2007 to 2009 by different methods as described later. Selective insecticide treatments were carried out by CDVCP in houses infested by *T. infestans*. All houses from Anjuana, El Bañado, and Quilmes de Abajo (in 2008) and from El Paso (in 2009) were sprayed with pyrethroids.

Survey Design

Cross-sectional searches of triatomine bugs were conducted in all communities in March 2007; in Anjuana, Calimonte, El Bañado, El Paraíso, Encalilla, Quilmes de Abajo, and Quilmes Centro in October–December 2007; in Encalilla, El Bañado, and Quilmes de Abajo in April 2008; and in El Bañado and Quilmes de Abajo in September 2008.

Research team members explained the objectives of the study to householders and invited them to participate on occasion of the first house visit. Each house was georeferenced, and the location and type of building material of each (peri)domestic structure were recorded. Each household received a labeled self-sealing plastic bag to keep any triatomine bug they might capture within its house compound (i.e., householders' notifications).

House infestation was assessed by householders' notifications and timed-manual collections with a dislodging spray. Briefly, residents were asked for the bugs they had collected and for information on their capture site. Two experienced field technicians from CDVCP searched for triatomine bugs assisted with a dislodging aerosol (0.2% tetramethrin, Espacial 0.2, Argentina) during 15 min in domiciles, and used the same capture effort in peridomiciles (one person-hour per house). The ecotope where each bug was collected was classified into seven types based on its function and main local host (see Results).

The third procedure used to reveal the presence of triatomine bugs was a live-baited sticky (Noireau) trap (Noireau et al. 1999): a small plastic container with a wire mesh top and double-sided sticky tape placed around the container's mouth. Each trap contained a live chicken as bait, bedding, and food; the traps were set in the evening and inspected ~15 h later when the chicken was replaced. The baited sticky traps were set up among the dry-shrub fences from Quilmes del Centro, Quilmes de Abajo, Anjuana, Encalilla, and El Bañado in February 2009. Additionally, peridomestic devices for detection of triatomine bugs were deployed in October 2007 only in Anjuana, El Bañado, and Quilmes Centro. These devices consist of one-liter recycled milk boxes containing pleated cardboard (Vazquez-Prokopec et al. 2002). The triatomine bugs knocked down during insecticide spraying were collected. In total, the number of houses evaluated at least once during 2007–2009 was 18 in Anjuana, 6 in Calimonte, 44 in El Bañado, 18 in Encalilla, 8 in El Paraíso, 28 in El Paso, 29 in Quilmes de Abajo, and 27 in Quilmes Centro. The outcome of the entomological surveys will be reported elsewhere.

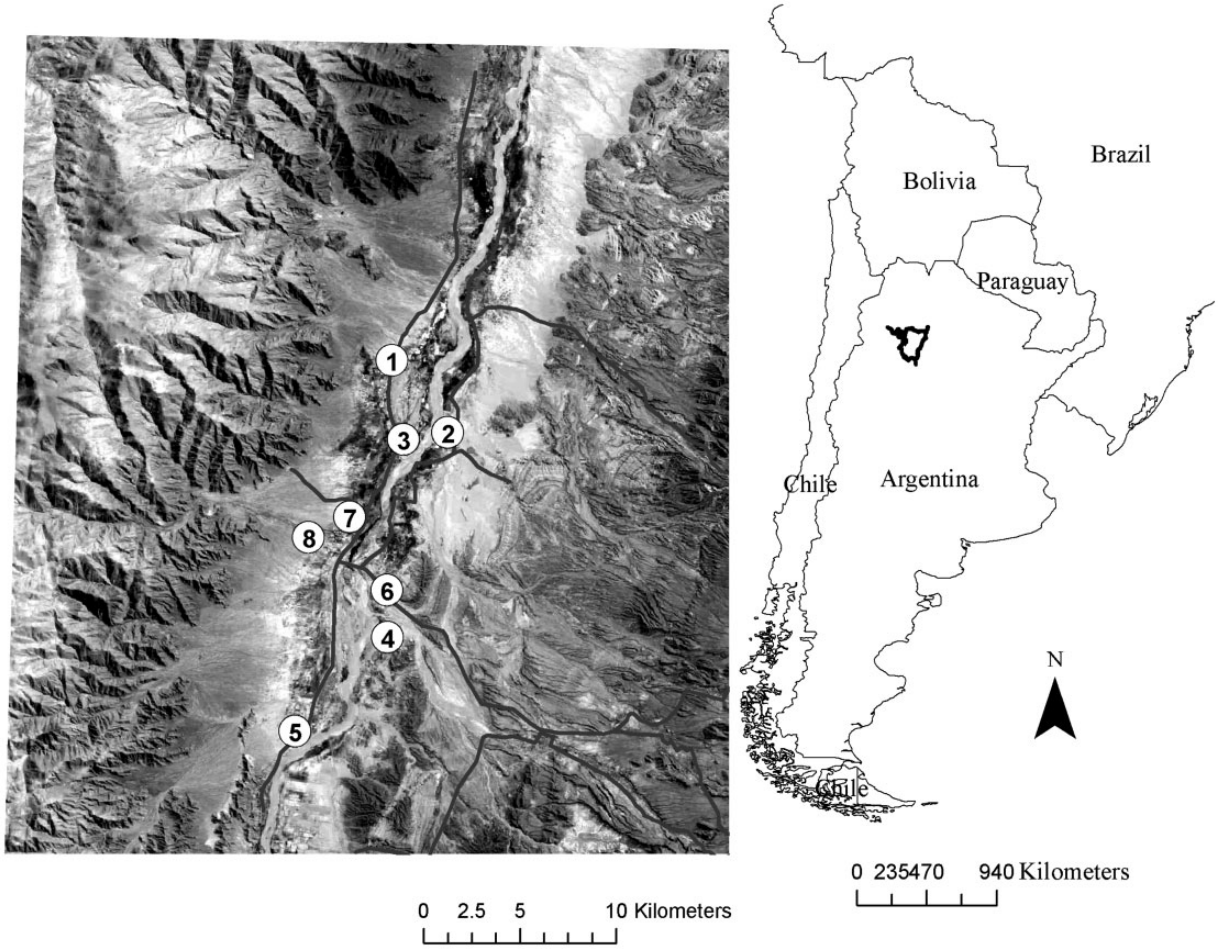

Fig. 1. Map of the study area showing the study communities (black dots) identified by a number: (**1**) Anjuana, (**2**) Calimonte, (**3**) El Bañado, (**4**) El Paraíso, (**5**) El Paso, (**6**) Encalilla, (**7**) Quilmes de Abajo, (**8**) Quilmes Centro. Inset shows Tafí del Valle Department and Tucuman Province within Argentina in South America.

All bugs collected were stored in labeled plastic bags with folded filter paper and transported to Buenos Aires. Bugs were identified to species and stage and counted by stage according to Lent and Wygodzinsky (1979), and then were frozen at −20°C. Identifications of *T. eratyrusiformis* were confirmed by Delmi M. Canale (CDVCP, Cordoba).

Diagnosis of *T. cruzi* Infection and DTU Identification

Fecal smears of a sample of live second-instar nymphs and later stages were individually examined for *T. cruzi* infection by optical microscopy (OM) at 400× magnification within 20 d of collection, and then preserved at −20°C. Bugs not examined microscopically for infection were examined by polymerase chain reaction (PCR) amplification of the 330 base pair fragment of the hypervariable region of kinetoplast DNA minicircles of *T. cruzi* (kDNA-PCR) following standardized protocols (Maffey et al. 2012). *Trypanosoma cruzi* DNA was extracted from the rectal ampoule of each insect using DNAzol as described earlier (Invitrogen, Carlsbad, CA; Marcet et al. 2006).

Trypanosoma cruzi DTU identifications were performed by means of a combination of PCRs targeting the intergenic region of spliced-leader genes (SL-IRac) with primers UTCC/TCac, SL-IRII with primers UTCC/TC1, and SL-IRI with primers UTCC/TC2, with the incorporation of Platinum Taq Polimerase (Invitrogen; Burgos et al. 2007). Heminested SL-PCRs using primers TCC-TC1 or TCC-TC2 were also performed to increase sensitivity as described in Enriquez et al. (2014). The rectal ampoule contents of 15 microscope-positive bugs and from seven kDNA-positive bugs not examined by OM were processed. PCR products were visualized under ultraviolet light after electrophoresis in 3% agarose gels (Invitrogen) containing GelRed (Biotium, Inc., Hayward, CA; Maffey et al. 2012, Macchiaverna et al. 2015).

Bloodmeal Identification

All bugs were dissected and the intestinal contents extracted into a previously weighed vial, diluted with phosphate buffered saline (PBS), and subsequently frozen at −20°C. Bloodmeal contents were tested with a direct ELISA assay against human, dog, cat, chicken, pig, goat, guinea pig (cavy), rabbit, and mouse antisera with high sensitivity and specificity (Gürtler et al. 2009, 2014). Bugs with no bloodmeal contents on dissection were not tested by ELISA. We report the proportion of tested bugs that were reactive (i.e., those positive against any of the tested antisera) to each type of antiserum.

Data Analysis

Agresti-Coull binomial 95% confidence intervals (CI) were used for proportions (Brown et al. 2001). Proportions were compared using chi-square and Fisher's exact tests (StataCorp, College Station, TX).

Fig. 2. Fence of dry shrubs harboring cavy colonies.

Table 1. Distribution of bloodmeal reactivity in *T. infestans* and *T. eratyrusiformis* according to type of ecotope

Ecotope	*T. infestans*		*T. eratyrusiformis*	
	No. of houses (no. of sites)[a]	% of reactive bugs (no. of bugs examined)	No. of houses (no. of sites)[a]	% of reactive bugs (no. of bugs examined)
Domicile	13 (15)	45.5 (33)	6 (6)	33.3 (6)
Chicken coop	6 (6)	70.1 (34)	0	–
Corral	8 (11)	51.7 (29)	4 (4)	50.0 (0)
Storeroom or Kitchen	2 (2)	100 (3)	0	–
Piled material	1 (1)	62.5 (8)	0	–
Fence	1 (1)	75.0 (4)	5 (5)	33.9 (56)
Other	4 (4)	25.0 (8)	3 (3)	0.0 (3)
Total	35 (40)	56.3 (119)	18 (18)	35.1 (77)

[a] The number of houses and sites refer to those harboring the triatomines examined.

Results

In total, 206*T. infestans* and 163*T. eratyrusiformis* were captured during 2007–2009 across the study villages.

Host-Feeding Patterns

Of 196 triatomines examined for bloodmeals, 48% were reactive against one of the tested antisera: 56.3% (67/119) of *T. infestans* and 35.1% (27/77) of *T. eratyrusiformis* (Table 1). Unmixed bloodmeals prevailed in *T. infestans* (82.0%) and *T. eratyrusiformis* (85.2%). Feedings on two different host species occurred in 14.9–16.5% of the bugs. Four different blood sources were detected in one *T. infestans* bug captured in a corral.

Both triatomine species largely differed in their main bloodmeal sources. *Triatoma infestans* fed mainly on chickens (61.2%) followed by goats (19.4%), humans (17.9%), cavies (9%), dogs (7.5%), and other hosts. In contrast, *T. eratyrusiformis* predominantly fed on cavies (92.6%; 25/27); four bugs fed on mice, and only one on pigs and one on dogs. Of 12*T. infestans* with mixed bloodmeals, 83.3% fed on chickens and other sources (cavies, dogs, and goats), whereas the four *T. eratyrusiformis* with mixed bloodmeals fed on cavies and mice.

The host-feeding patterns of both triatomine species were structured according to habitat type, which was associated closely with certain host species (Fig. 3). For *T. infestans*, nine bloodmeal sources were detected in peridomiciles, whereas only four sources occurred in domiciles (Fig. 3A). The most common sources of *T. infestans* in domiciles were humans (73.3%), dogs (26.7%), and chickens (20%), but feedings on cavies (6.7%) were also detected. In peridomiciles, however, the main sources were chickens (73.1%), goats (25.0%), and cavies (9.6%; Fig. 3A). Therefore, chickens were more frequent in the peridomicile even though they may nest in the

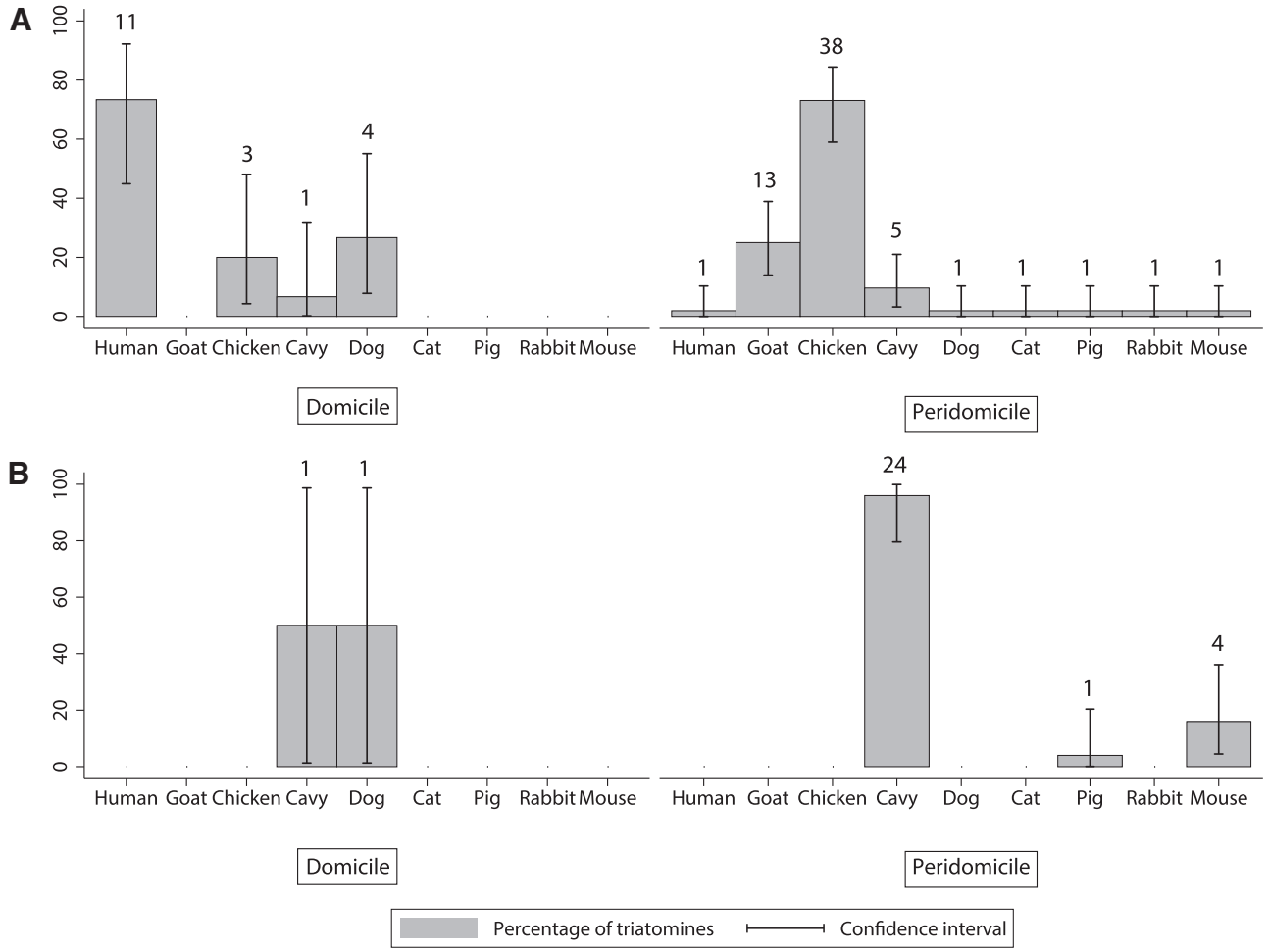

Fig. 3. Percentage of ELISA-reactive *T. infestans* (**A**) and *T. eratyrusiformis* (**B**) in domiciles and peridomiciles. Number above the bars represents the number of bugs that were reactive to each type of antiserum.

domicile. Chicken bloodmeals (46.1%) prevailed in *T. infestans* from chicken coops, followed by cavies (3.8%) and rabbits (1.9%), whereas goat bloodmeals predominated in goat corrals (21.2%), followed by chickens (7.7%) and cavies (5.8%). The only two *T. eratyrusiformis* from domiciles fed on cavies and dogs, whereas of those caught in peridomestic habitats 96.0% (24/25) fed on cavies, mice (16.0%), and pigs (4.0%; Fig. 3B). Most of the reactive *T. eratyrusiformis* from peridomestic habitats were captured in dry-shrub fences (76.0%) and the rest in corrals.

Bloodmeals on hosts usually not present at the capture site occurred rarely: one cavy-fed fourth-instar *T. eratyrusiformis* and one cavy-fed third-instar *T. infestans* were caught in domiciles, and one human-fed *T. infestans* was collected in a peridomestic ecotope.

Trypanosoma cruzi Infection

Table 2 shows the distribution of *T. cruzi* infection by OM or kDNA-PCR according to triatomine species and type of ecotope. *Trypanosoma cruzi* infection in *T. eratyrusiformis* (79.8%) was significantly higher than in *T. infestans* (33.3%) as determined by either method ($\chi^2 = 45.75$, df = 2, $P < 0.001$). For all peridomestic ecotopes combined, *T. cruzi* infection was significantly higher in *T. eratyrusiformis* (81.6%, 87 bugs examined) than in *T. infestans* (26.1%, 88 bugs examined; $\chi^2 = 54.15$, df = 2, $P < 0.001$).

The prevalence of *T. cruzi* infection by OM or kDNA-PCR in *T. infestans* was significantly higher in domiciles (53.1%) than in peridomiciles (26.1%; $\chi^2 = 7.69$, df = 2, $P = 0.006$), whereas the opposite trend occurred in *T. eratyrusiformis* (57.1% vs 81.6%, respectively $\chi^2 = 2.41$, df = 2, $P = 0.121$). Domiciles and fences concentrated the *T. infestans* and *T. eratyrusiformis* infected with *T. cruzi*, respectively. Of 71 infected *T. eratyrusiformis* captured in peridomiciles, 84.5% were from fences (Table 2).

The stage-specific prevalence of infection with *T. cruzi* (determined by either method) increased from second-instar nymphs to adults in both *T. eratyrusiformis* (0.0, 45.5, 81.0, 85.4, and 92.3%, respectively) and *T. infestans* (0, 7.7, 14.3, 17.4, and 63.3%, respectively). The prevalence of infection among bugs examined only by OM was significantly lower than when either OM or kDNA-PCR were used in *T. infestans* (11.9 vs 33.3%; $\chi^2 = 10.31$, df = 2, $P = 0.001$) but not in *T. eratyrusiformis* (80.3 vs 79.4%; $\chi^2 = 0.006$, df = 2, $P = 0.942$).

TcI was identified in all *T. infestans* (eight specimens) and in six (46%) of 13*T. eratyrusiformis* (Table 3). Mixed infections including TcI and inconclusive identifications of TcII or TcV or TcVI (named as TcII/TcV/TcVI) were detected; the latter were likely owing to the low concentration of parasite DNA. An unmixed infection with the domestic DTU TcVI was detected in one *T. eratyrusiformis*.

Table 2. Distribution of *T. cruzi* infection in *T. infestans* and *T. eratyrusiformis* according to type of ecotope

Ecotope	*T. infestans*		*T. eratyrusiformis*	
	No. of houses (no. of sites)[a]	% of infected bugs (no. of bugs examined)[b]	No. of houses (no. of sites)[a]	% of infected bugs (no. of bugs examined)[c]
Domicile	14 (15)	53.1 (32)	6 (6)	57.1 (7)
Chicken coop	7 (7)	25.7 (35)	0	–
Corral	11 (11)	13.3 (30)	4 (4)	81.8 (11)
Storeroom or Kitchen	2 (3)	0.0 (3)	0	–
Piled material	1 (1)	50.0 (8)	0	–
Fence	1 (1)	25.0 (4)	6 (7)	82.2 (73)
Other	1 (1)	62.5 (8)	3 (3)	66.7 (3)
Total	40 (43)	33.3 (120)	19 (20)	79.8 (94)

[a] The number of houses and sites refer to those harboring the triatomines examined.

[b] Infection determined by OM or kDNA-PCR. The total number of *T. infestans* examined was 53 by kDNA-PCR only, 59 by OM only, and 8 by both methods.

[c] Infection determined by OM or kDNA-PCR. The total number of *T. eratyrusiformis* examined was 13 by kDNA-PCR, 80 by OM only, and one by both methods.

Table 3. *Trypanosoma cruzi* DTUs in *T. infestans* and *T. eratyrusiformis* according to ecotope and bloodmeal sources

Species	Ecotope	Instars	Bloodmeal source	DTU
T. infestans	Fence	Adult (Female)	NR	I
	Piled material	Adult (Female)	NR	I
	Corral	Adult (Female)	Goat and Chicken	I
	Chicken coop	Adult (Female)	Chicken	I and II/V/VI
	Domicile	Adult (Female)	Human	I
	Domicile	Adult (Male)	NR	I
	Domicile	Adult (Male)	NR	I and II/V/VI
	Domicile	Adult (Female)	NR	I and II/V/VI
T. eratyrusiformis	Fence	V	Cavy	II/V/VI
	Fence	V	Cavy and Mouse	II/V/VI
	Fence	IV	Cavy	II/V/VI
	Fence	IV	Cavy	I
	Fence	IV	NR	I
	Fence	III	ND	I
	Fence	III	ND	I
	Fence	III	ND	I
	Fence	III	ND	I and II/V/VI
	Corral	Adult (Female)	ND	VI
	Corral	V	Cavy	II/V/VI
	Corral	V	Cavy	II/V/VI
	Other	Male	NR	II/V/VI

NR—nonreactive by ELISA; ND—no data available (i.e., not tested by ELISA).

Relationship Between Host-Feeding Sources and *T. cruzi* Infection

Table 4 shows the relation between identified bloodmeal sources and bug infection with *T. cruzi* in both triatomine species. *Trypanosoma cruzi* infection was not significantly associated with host bloodmeals (classified as chickens, dogs or humans, and other hosts) in *T. infestans* (Fisher's exact test, $P = 0.084$), and with feedings on cavies or other hosts in *T. eratyrusiformis* ($\chi^2 = 0.04$, df = 1, $P = 0.842$).

Discussion

This study provides strong evidence of the significant role of *T. eratyrusiformis* as a peridomestic vector of *T. cruzi* in rural communities of the Calchaqui valleys that were under routine vector surveillance and control against *T. infestans*. *Triatoma eratyrusiformis* fed mostly on cavid rodents, had high *T. cruzi* infection rates, and was the most frequently infected triatomine in peridomiciles. The evidence supports that *T. eratyrusiformis* was implicated in the local circulation of *T. cruzi*, most likely of TcI among cavies and perhaps mice, but infection with other typically domestic DTUs (TcVI and TcII/TcV/TcVI) indicated some degree of overlap between transmission cycles. Our study apparently is the first to document the host-feeding sources and bug infection patterns of *T. eratyrusiformis*.

The study communities had high levels of bug infection with *T. cruzi* compatible with active transmission despite they were under routine vector surveillance and control. The local prevalence of infection in *T. infestans* was much higher than in other rural areas from the Argentine Chaco under effective vector surveillance and control (Cecere et al. 1999, Gürtler et al. 2007a). This comparison suggests that the greater intensity of transmission in the Calchaqui

Table 4. *Trypanosoma cruzi* infection in *T. infestans* and *T. eratyrusiformis* according to bloodmeal sources

Bloodmeal sources	*T. infestans*		*T. eratyrusiformis*	
	No. of bugs fed on host	% of infected bugs[a]	No. of bugs fed on host	% of infected bugs[a]
Dog	4	75.0	1	0
Human	9	33.3	0	–
Chicken	41	31.7	0	–
Cavy	6	16.2	24	70.8
Goat	13	15.4	0	–
Pig	1	0	1	100
Mouse	1	0	4	75.0
Cat	1	0	0	–
Rabbit	1	0	0	–
Total	77	28.6	30	70.0

[a] Percentage of infection was calculated over the total number of bugs examined for infection with feeding on a given host species.

valleys was most likely related with the joint presence of two highly competent vector species and infectious (peri)domestic reservoir hosts, and the limited effectiveness and coverage of vector control actions in defined ecotopes.

The overall prevalence of *T. cruzi* infection was significantly higher in *T. eratyrusiformis* than in *T. infestans*, and this difference was owing to a threefold higher infection rate of the former in peridomestic ecotopes. *Triatoma eratyrusiformis* fed almost exclusively on cavid rodents and frequently occurred in fences that harbored large colonies of cavid rodents (*M. australis*) infected with TcI (Cecere et al. 2015). Both *T. eratyrusiformis* and *T. infestans* persisted in the dry-shrub fences and sustained parasite transmission even when the density of the main vector was severely reduced or suppressed in other (peri)domestic habitats.

These fences are not targeted for routine insecticide spraying and, therefore, may act as sources of reinfestation because they are located within the flight range of *T. infestans* and *T. eratyrusiformis* (Vazquez-Prokopec et al. 2004, Abrahan et al. 2011), and within the range of distances over which reinfestation by *T. infestans* occurs in northwestern Argentina (Cecere et al. 2006). The finding of cavy-fed *T. eratyrusiformis* in domiciles and human-fed *T. infestans* in peridomiciles points to dispersal events between habitats, and suggests a mechanism linking domestic and peridomestic transmission cycles. In addition to the TcI-infected *T. infestans* and *T. eratyrusiformis* detected in (peri)domestic habitats, the typically domestic DTUs (TcVI and TcII/TcV/TcVI) were also found in *T. eratyrusiformis* despite the absence of identified bloodmeals on domestic dogs, cats, or humans, again indicating the vector's high feeding mobility. Therefore, both *T. infestans* and *T. eratyrusiformis* were implicated in the local circulation of various *T. cruzi* DTUs.

Domiciles were key ecotopes for *T. cruzi* transmission mediated by *T. infestans* as in other endemic areas from the Argentine Chaco. The most common host-feeding sources of *T. infestans* in domiciles were chickens and humans, likewise in the dry Chaco (Cecere et al. 1997, López et al. 1999, Gürtler et al. 2014); rodent bloodmeals (including one cavy-fed bug) were rare. The few *T. eratyrusiformis* collected in domiciles were frequently infected, but most of them were starved and their bloodmeals could not be identified. The fact that there was very little overlap between the main bloodmeal sources of *T. infestans* and *T. eratyrusiformis* may be explained by their disjoint habitat distribution.

Our results are referred to rural areas that were under regular vector control. Therefore, the few bugs collected at a given site and occasion were aggregated over villages and survey periods to achieve larger sample sizes. Many of the bugs tested for bloodmeal sources were unfed or nonreactive, especially *T. eratyrusiformis*. The frequent infection of chicken-fed *T. infestans* pointed to previous infectious bloodmeals that were no longer detectable, and large feeding mobility among host species. One of the strengths of this study was the use of parasitological and molecular methods to assess the presence of *T. cruzi* infection and genotype, whereas bloodmeal identification tests covered all the local (peri)domestic hosts and habitats recorded during vector surveillance.

The epidemiological scenario portrayed in the current study differs from the more typical one in the Argentine Chaco where the prevalence of *T. cruzi* infection in *T. infestans* was much greater than among other secondary vectors (*Triatoma sordida Stal*, *Triatoma garciabesi* Carcavallo et al., and *Triatoma guasayana Wygodzinsky & Abalos*) occurring in peridomestic habitats; these species are frequently associated with birds and other noncompetent hosts (Cecere et al. 1999, Cardinal et al. 2007, Gürtler et al. 2007b,Maffey et al. 2012, Macchiaverna et al. 2015). In contrast, *T. eratyrusiformis* had much greater infection rates than *T. infestans*; the former was infected with various DTUs; it was strongly associated with cavy (and mouse) populations in peridomestic fences, and infected insects were occasionally captured in domiciles.

Our results support that *T. eratyrusiformis* plays a significant role as a secondary vector in rural communities from the Calchaqui valleys. Whether it is able to colonize human sleeping quarters and the actual degree of human-vector contact remains to be assessed through routine monitoring of house infestation, especially after the elimination of *T. infestans*. Secondary vector species that are not currently considered control targets may trigger *T. cruzi* transmission after highly domiciliated vector species have been controlled, as in the Caribbean region of Colombia (Cantillo-Barraza et al. 2014) and elsewhere (Waleckx et al. 2015). Vector control actions directed against *T. infestans* were not enough to achieve its elimination and interrupt the transmission of *T. cruzi*. Dry-shrub fences deserve specific attention for achieving the goal of vector suppression. Additional environmental management measures directed to dry-shrub fences, similar to those developed for goat corrals in the dry Chaco (Gorla et al. 2013), may be instrumental for sustainable vector elimination and interruption of parasite transmission.

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