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Microcavia australis (Caviidae, Rodentia), a new highly competent host of *Trypanosoma cruzi* I in rural communities of northwestern Argentina

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ABSTRACT

Rodents are well-known hosts of *Trypanosoma cruzi* but little is known on the role of some caviomorph rodents. We assessed the occurrence and prevalence of *T. cruzi* infection in *Microcavia australis* ("southern mountain, desert or small cavy") and its infectiousness to the vector *Triatoma infestans* in four rural communities of Tafí del Valle department, northwestern Argentina. Parasite detection was performed by xenodiagnosis and polymerase chain reaction amplification of the hyper-variable region of kinetoplast DNA minicircles of *T. cruzi* (kDNA-PCR) from blood samples. A total of 51 cavies was captured in traps set up along cavy paths in peridomestic dry-shrub fences located between 25 and 85 m from the nearest domicile. We document the first record of *M. australis* naturally infected by *T. cruzi*. Cavies presented a very high prevalence of infection (46.3%; 95% confidence interval, CI = 33.0–59.6%). Only one (4%) of 23 cavies negative by xenodiagnosis was found infected by kDNA-PCR. TcI was the only discrete typing unit identified in 12 cavies with a positive xenodiagnosis. The infectiousness to T. infestans of cavies positive by xenodiagnosis or kDNA-PCR was very high (mean, 55.8%; CI = 48.4–63.1%) and exceeded 80% in 44% of the hosts. Cavies are highly-competent hosts of *T. cruzi* in peridomestic habitats near human dwellings in rural communities of Tucumán province in northwestern Argentina.

1. Introduction

Chagas disease is a zoonosis caused by *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) and a neglected tropical disease in the Americas, with 9–11 million of infected persons and 15,000 annual deaths (PAHO, 2006). This flagellate parasite is mainly transmitted by hematophagous insects of the subfamily Triatominae (Hemiptera: Reduviidae). According to a modern consensus, *T. cruzi* populations have been classified into six discrete typing units (DTUs) (TcI-TcVI) (Zingales et al., 2009). A new DTU of *T. cruzi* related to lineage TCI and restricted to bat infections, called TcBat, has recently been described (Marcili et al., 2009).

Multiple DTUs circulate in three transmission cycles. The domestic cycle is maintained by humans, dogs, cats, rodents and a few species of triatomine bugs closely adapted to human dwellings, whereas the wild cycle (enzootic) involves marsupials, edentates, rodents and carnivores as the main hosts and numerous sylvatic triatomine species (WHO, 2002). The peridomestic cycle occurs around human dwellings and involves peridomestic triatomines and domestic or synanthropic mammals, with eventual parasite spillovers from wild mammals (Coura and Dias, 2009).

Wild and synanthropic rodent species are implicated in the transmission of *T. cruzi* in several regions (Jansen and Roque, 2010; Ramsey et al., 2012; Orozco et al., 2014). TcI is the most prevalent DTU detected in synanthropic and wild rodents, but there are very few DTU identifications and estimates of rodent infectiousness (Orozco et al., 2014; Galuppo et al., 2009). In Argentina, several

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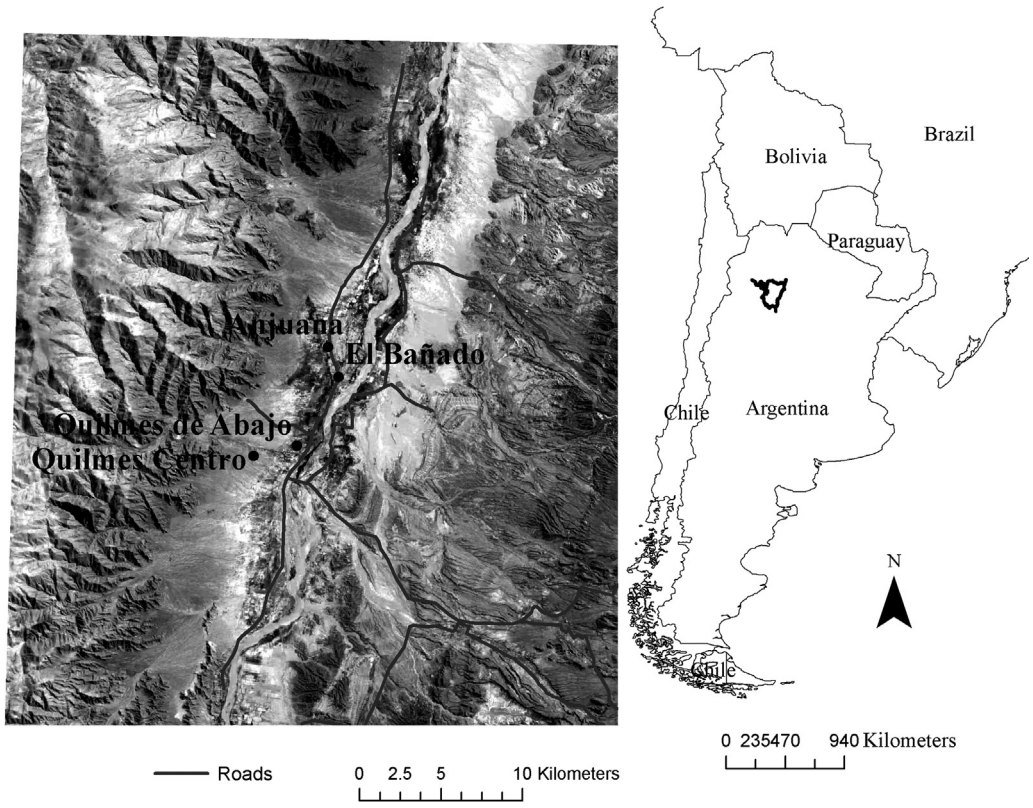

Fig. 1. Landsat image showing the study area: Quilmes de Abajo, El Bañado, Quilmes Centro, and Anjuana in Tafí del Valle department. Inset shows location of Tafí del Valle department in Tucumán province, Argentina.

species of Sigmodontinae were infected with *T. cruzi* (Orozco et al., 2014; Basso et al., 1977, 1982) whereas rodent species of the subfamily Caviinae (which include "cuises" or guinea pigs) have received little attention.

In general, the prevalence of infection by *T. cruzi* in caviomorph rodents varies among species, habitats and geographic distribution. Domesticated Andean guinea pigs (*Cavia porcellus*) are important reservoir hosts of *T. cruzi* and bloodmeal sources of *Triatoma infestans* in Peru (Levy et al., 2006) and Bolivia (Albarracin-Veizaga et al., 1999) whereas *Trichomys* spp is an important sylvatic reservoir host in Brazil (Jansen and Roque, 2010). However, *Galea musteloides* and domestic cavies have rarely been found infected with *T. cruzi* in Paraguay (Yeo et al., 2005) and Brazil (Ferriolli Filho and Barretto, 1966; Sherlock et al., 1974), and no infection has been recorded among these species and in *M. australis* in the Argentinean Chaco region (Wisnivesky-Colli et al., 1992; Ceballos et al., 2006; Alvarado-Otegui et al., 2012).

Experienced vector control personnel in Tucumán province (northern Argentina) reported to us the frequent finding of peridomestic colonies of *M. australis* (Caviidae, Rodentia; known as southern mountain, desert or small cavy; "cuis" or "coi") associated with *Triatoma eratyrusiformis* (Joaquín Zárate, personal communication). *Triatoma eratyrusiformis* is an aggressive species that inhabits mountainous areas, rocky environments and stone fences or walls ("pircas") in peridomestic areas where it is associated with edentates and rodents (Martínez et al., 1985). Besides *T. infestans* and *T. eratyrusiformis*, the following triatomine species have been also quoted for Tucumán province: *Psammolestes coreodes*, *Pastrongylus guentheri*, *Triatoma gusayana*, *Triatoma garciabesi*, *Triatoma delpontei*, *Triatoma platensis* (Carcavallo and Martinez, 1985).

M. australis is the most abundant species of Caviinae in semiarid thorn-brush habitats from southern Bolivia to southern Argentina and Chile (Tognelli et al., 2001). Early observations suggested that *M. australis* could be a host of *T. cruzi* because *Triatoma patagonica* bugs infected with *T. cruzi* were found in cavy burrows in western Argentina (Mazza, 1949). Despite the extended geographic range of cavies includes a large region endemic for Chagas disease, the role of *M. australis* as a host of *T. cruzi* in peridomestic and adjacent habitats has not been investigated.

As part of a wider research project on Chagas disease vector control in a well-defined rural area of Tucumán in northwestern Argentina, the present study sought to assess the occurrence of *T. cruzi* infection in *M. australis* residing in habitats close to human dwellings through xenodiagnosis and polymerase chain reaction amplification of the hyper-variable region of kinetoplast DNA minicircles of *T. cruzi* (kDNA-PCR) from blood samples. We also evaluated the host competence of desert cavies under field conditions and identified their DTUs.

2. Materials and methods

2.1. Study area and population

The study area included four rural communities (Quilmes de Abajo, with 24 houses; El Bañado, 30 houses; Quilmes del Centro, 34 houses, and Anjuana, 23 houses) in Tafí del Valle department (26°27′S, 65°59′W), Tucumán province, northwestern Argentina (Fig. 1). These villages in the subandean Calchaqui valleys are part of the indigenous community of Quilmes. The villages are situated within a stretch of 10 km along route 40 at 1800 m above sea level in the Monte ecoregion, where scrublands predominate and the steppe is formed by resinous evergreen bushes such as *Larrea* sp. The climate is temperate-arid, with only 80–250 mm

of annual rainfall, and the annual mean temperature is 19 °C (http://worldwildlife.org/ecoregions/nt0802).

Unpublished results provided by the local Chagas disease vector control program (CDVCP, Joaquín Zárate, personal communication) showed that peridomestic infestation by *T. eratyrusiformis* ranged from 3% to 6% during 2007–2008 and house infestation with *T. infestans* ranged from 15% to 62% in the study communities in December 2011. The occurrence of *T. cruzi* infection in both vector species was indicative of active transmission in each of the communities both in 2007–2009 and 2011 despite all houses had been sprayed with pyrethroid insecticides in 2008.

A typical feature of the study area is that houses were surrounded by fences of dry shrubs (Suppl. Fig.) that covered an extensive perimeter of hundreds of meters and housed cavy colonies. Considered part of an ancestral tradition, these fences were built by local residents to divide plots of land for specific use. Dry-shrub branches were stacked up to 1 m of height and maintained by adding dry branches on the top. The peridomestic areas (separated from human sleeping quarters) included a patio and 3–8 structures (storerooms, kitchens, corrals, etc.) which housed domestic animals and were surrounded by plots of crops and native vegetation.

2.2. Study design

A cross-sectional survey was carried out in late spring (December 12–21, 2011) and included a total of 12 houses from the four rural communities. The selection criterion was based on the frequent presence of cavy colonies in the dry-shrub fences, as reported by local householders and vector control personnel.

2.3. Capture and handling of cavies

Biosafety and animal processing procedures were performed according to protocols approved by the Ethical Committee "Dr. Carlos Barclay" (Buenos Aires). Local residents accepted to collaborate with this study and allowed the installation of traps in the yard of their houses. A team of three people (including a veterinarian, a biologist and a CDVCP field technician) set up lines of 4 to 14 traps each every 10 m along the active paths of cavies on both sides of the fences next to each house during eight days; the total capture effort was 295 trap-days. The house and trap locations were georeferenced (Garmin Legend C). Cavies were live-captured with Tomahawk-like traps baited with fruits of "algarrobo blanco" (*Prosopis alba*) and "chañar" (*Geoffroea decorticans*), as recommended by local residents. Traps were checked every 2–4 h during the afternoon and morning (to prevent any injury to cavies) and re-baited when appropriate.

Every cavy was removed from the trap using a net bag for safe manipulation. Each individual was given parenteral anesthesia for induction performed with xylacine (Xilazina 10%; Ronpun®, Bayer, Leverkusen, Germany) and ketamine clorhydrate (Ketamina 5%; Vetaset®, Fort Dodge) injected intramuscularly at the minimum dose appropriate to its weight (Kreeger and Arnemo, 2007; J.P. Arrabal, unpublished data), and then released at the capture site after full recovery from anesthesia. Anesthetized animals were sexed, weighed with a spring scale (Pesola®, Switzerland), measured (body length and hind leg length), and marked with an ear punch used to take tissue samples.

Ear tissue samples were stored in 1.5 ml tubes with 0.5 ml of 1% phosphate buffered saline at −20 °C. Blood samples were drawn by cardiac puncture (1–2 ml, depending on the specimen's weight). Each blood sample was separated in two aliquots. An aliquot was diluted 1:1 in guanidine hydrochloride-EDTA buffer (GEB) for polymerase chain reaction amplification (PCR), and the other was centrifuged at 3000 rpm for 15 min for serum collection.

All live cavies (22 females and 20 males) were examined by xenodiagnosis using 6–14 uninfected fourth-instar nymphs of *T. infestans* contained in wooden boxes applied on the belly for 30–40 min, and then inspected visually to check whether they had engorged (Gürtler et al., 2007). The insects were provided by the CDVCP insectary (Córdoba, Argentina) free of any trypanosomatid and were starved for 30–40 days before using them. Xenodiagnostic bugs were transported to Buenos Aires and kept in the insectary at 24–26 °C and 40–60% relative humidity. Eight dead cavies and 23 cavies with a negative xenodiagnosis (12 females and 11 males) were tested by kDNA-PCR only.

2.4. Diagnosis and DTU identification

Xenodiagnostic bugs were individually examined by optic microscopy (Zeiss) at 400× magnification 30 and 60 days post-exposure to assess the individual host's infectiousness to the vector (i.e., number of infected bugs fed on a given individual divided by the total number of insects examined for infection at least once, excluding bugs that died prior to the first examination) (Gürtler et al., 2007). The measurement of infectiousness was restricted to cavies positive by xenodiagnosis or kDNA-PCR (i.e., infected, see below). The number of exuviae and of dead bugs in each box was used as an index of xenodiagnosis quality. Of 363 bugs used in 42 xenodiagnostic tests, 96.7% were alive on first examination and 16.2% of those alive at first inspection molted within the 60-day observation period. The survival of insects up to the first examination varied slightly among cavies (mean, 96%; SD, 9.4). Xenodiagnostic tests were therefore considered of very good quality.

Among individuals negative by xenodiagnosis, infection with *T. cruzi* was determined from GEB aliquots by PCR amplification of the 330 base pair (bp) fragment of the minicircles of the kinetoplastid genome using primers and cycling conditions described elsewhere (Burgos et al., 2007). GEB aliquots were boiled for 15 min and parasite DNA was extracted using the DNeasy Blood & Tissue Kit according to manufacturer's instructions (QIAGEN Sciences, Germantown, MD, USA) (Orozco et al., 2013).

Fecal or rectal ampoule samples of one microscope-positive bug from each of 12 xenodiagnosis-positive cavies were selected at random for DTU identification (Maffey et al., 2012). DNA from these samples was extracted with DNAzol (Invitrogen, Carlsbad, CA) (Marcet et al., 2006). Parasite DTUs were identified by PCRs targeting the intergenic region of spliced-leader genes (SL-IRac) with primers UTCC/TCac, SL-IRII with primers UTCC/TC1, SL-IRI with primers UTCC/TC2 with the incorporation of Taq Polimerase (Invitrogen) (Burgos et al., 2007). Aliquots of 12 ml of PCR products were visualized under ultraviolet light after electrophoresis in 3% agarose gels (Invitrogen, USA) containing GelRed (Biotium, Inc., Hayward, CA).

2.5. Data analysis

Logistic regression analysis implemented in Stata 10.1 (StataCorp, College Station, Texas) was used to test whether there was a significant ($P < 0.05$) effect of host attributes (sex, length, and weight) on bug infection in xenodiagnostic tests of cavies positive by xenodiagnosis or kDNA-PCR. Analyses were clustered on each individual host to account for overdispersion caused by between-host variability. Agresti-Coull binomial 95% confidence intervals (CI) were used for proportions (Brown et al., 2001) and the Shapiro–Wilk W test for checking normality (StataCorp, College Station, Texas). The weight (g) to length (cm) ratio was used as a body mass index.

Table 1
Number of *M. australis* cavies caught per unit effort (CPUE), sex ratio, and number examined for *T. cruzi* infection at each study house and community.

Community	House code	Minimum distance (m) to fence[a]	Total catch[b]	No. trap-days	Mean CPUE[c]	SD	Sex ratio (male: female)[d]
Quilmes	1	30	12	51	26.4	28.14	5:7
de Abajo	2	30	12	40	31.5	28.25	5:7
	3	25	12	45	23.1	30.08	7:5
	4	17	0	15	0	–	–
	5	40	0	10	0	–	–
Quilmes	6	37	2	31	6.7	9.12	1:1
Centro	7	35	2	7	13.3	23.09	2:0
	8	100	0	12	0	–	–
Anjuana	9	85	2	12	16.7	0	1:1
	10	80	0	10	0	–	–
El Bañado	11	50	2	27	7.7	10.87	0:2
	12	35	7	35	23.2	20.96	4:3
Total			51	295	17.4	10.69	25:26

[a] Minimum distance from house to fence where traps were set up.
[b] One female cavy recaptured in house 12 was only examined by kDNA-PCR.
[c] Catch per 100 trap-days.
[d] Only one male was juvenile; among females, 16 were non-pregnant, 8 pregnant, and 2 juveniles.

3. Results

3.1. Infection

A total of 51 cavies were captured in fences located between 25 and 85 m from the nearest domicile at eight of the 12 houses (Table 1). Only two of the cavies caught were juveniles. The sex ratio was nearly 1. One female was recaptured, and eight cavies were found dead in the traps. All live animals were clinically normal.

Table 2 shows the prevalence of *T. cruzi* as determined by xenodiagnosis, kDNA-PCR and either method according to house and community. The overall prevalence of *T. cruzi* infection was 45.6% (95% CI, 31.2–60.1%) as determined by xenodiagnosis. Infection increased very slightly to 46.3% (23 of 50; 95% CI, 33.0–59.6%) when the four individuals positive only by kDNA-PCR were added to the total number examined for infection by either method. At least one xenodiagnosis-positive cavy infected with TcI was caught at each of the eight study houses with cavies captured and examined for infection.

TcI was the only DTU identified in the 12 xenodiagnosis-positive cavies as indicated by the 150-bp band for the SL-IRac leader sequence and the 475-bp band for the SL-IRI leader sequence. Mixed DTU infections were not detected in the rectal ampoule or fecal samples of xenodiagnostic bugs.

The prevalence of *T. cruzi* infection was significantly associated with body mass index (weight-to-length ratio) when infection was detected by either method ($\chi^2 = 8.35$, df = 1, $P = 0.004$) or by xenodiagnosis only ($\chi^2 = 6.7$, df = 1, $P = 0.009$), but not between female and male cavies (either method: $\chi^2 = 0.08$, df = 1, $P = 0.8$; xenodiagnosis: $\chi^2 = 0.0009$, df = 1, $P = 0.97$) (Table 3). The body mass index was not statistically associated with sex ($\chi^2 = 0.13$, df = 1, $P = 0.7$). The ranked body mass index of cavies with a positive xenodiagnosis (median, 14.5 g/cm) was significantly greater than in individuals with a negative xenodiagnosis (median, 12.4 g/cm) (Kruskall–Wallis test; $\chi^2 = 9.1$, df = 1, $P = 0.0025$).

3.2. Infectiousness

The mean infectiousness to the vector in the 20 infected cavies (19 positive by xenodiagnosis and one by kDNA-PCR) was 55.8% (95% CI, 48.4–63.1%), and exceeded 80% in 44% of the hosts. The distribution of infectiousness did not differ significantly from the normal distribution (Fig. 2).

The frequency of cavies with low (≤60%) or high (≥80%) infectiousness to the vector was not associated significantly with the body mass index ($\chi^2 = 0.47$, df = 1, $P = 0.5$) or sex ($\chi^2 = 0.83$, df = 1, $P = 0.4$) (Table 3). Logistic regression analysis clustered by individual detected no statistically significant differences of infectiousness by sex (odds ratio, OR = 0.65, 95% CI = 0.18–2.27), body length (OR = 0.55, 95% CI = 0.29–1.05), and weight (OR = 1.0, 95% CI = 0.99–1.0) (Wald $\chi^2 = 4.66$, df = 3, $P > 0.198$). Infectiousness was not significantly correlated with the body mass index (Spearman's $r = 0.19$, $N = 20$, $P = 0.4$).

4. Discussion

This study presents the first finding of *T. cruzi* infection in *M. australis* naturally infected with *T. cruzi* (as determined by parasitological and molecular techniques), identifies them as hosts of TcI, and shows they have high prevalence of infection and infectiousness to *T. infestans*. No record of *T. cruzi* infection in *M. australis* was found after an extensive literature search (Barreto, 1976; WHO,

Table 2
Prevalence of *T. cruzi* infection in *M. australis* cavies determined by xenodiagnosis and kDNA-PCR at each study house and community.

Community	House	% Infection prevalence (No. examined) by					
		Xenodiagnosis		kDNA-PCR[a]		Either method	
Quilmes	1	70	(10)	0	(4)	64	(11)
de Abajo	2	43	(7)	22	(9)	42	(12)
	3	42	(12)	14	(7)	50	(12)
Quilmes	6	50	(2)	0	(1)	50	(2)
Centro	7	0	(1)	0	(2)	0	(2)
Anjuana	9	100	(1)	100	(1)	100	(2)
El Bañado	11	50	(2)	0	(1)	50	(2)
	12	14	(7)	0	(6)	14	(7)
Total		45	(42)	13	(31)	46	(50)

[a] Only cavies negative or not tested by xenodiagnosis were examined by kDNA-PCR.

Table 3
Prevalence of infection with *T. cruzi* and infectiousness to the vector *T. infestans* of *M. australis* cavies according to sex and the body mass index.

Attribute	Infection						Infectiousness to the vector		
	Either method [a]			Xenodiagnosis					
	No. tested	Mean (%)	95% CI	No. tested	Mean (%)	95% CI	No. tested	Mean (%)	95% CI
Sex									
Female	25	48.3	30.0–66.5	22	46.1	26.9–65.4	10	60.7	50.3–71.2
Male	25	44.8	26.7–62.9	20	45.8	25.8–65.8	10	51.3	41.0–61.2
Body mass index (g/cm)									
5–12.6	19	21.0	27.6–54.7	18	27.1	8.5–45.7	4	51.3	35.6–67.0
13.1–17.5	30	61.8	45.5–78.2	24	60.8	42.6–78.9	16	56.8	48.6–65.1

[a] Cavies examined for infection by xenodiagnosis or kDNA-PCR.

2002; Llewellyn et al., 2009; Noireau et al., 2009; Jansen and Roque, 2010). We document that *M. australis* cavies are important hosts of *T. cruzi* in peridomestic habitats close to human dwellings in four villages, and suggest that they are most likely implicated in the transmission of TcI in habitats frequently infested by *T. eratyrusiformis* and *T. infestans*. Some of the characteristics of *M. australis* are compatible with being a reservoir host of *T. cruzi* (Noireau et al., 2009; Ashford, 1997).

Peridomestic *M. australis* cavies were as frequently infected as domesticated cavies (19–60%) were in Bolivia and Peru (Torrico, 1959; Zeledón, 1974; Minter, 1976; Albarracin-Veizaga et al., 1999), and sylvatic *Thrichomys apereoides* (45%) in Brazil (Herrera et al., 2005). Caviomorph rodents achieved high infection prevalence as *Didelphis* opossums (9–58%), one of the most important sylvatic reservoirs of TcI in different regions (Orozco et al., 2014; WHO, 2002).

Caviomorph rodents have co-evolved with *T. cruzi* since they arrived to the American continent 35–40 million years ago (Flynn and Wyss, 1998; Noireau et al., 2009), after the parasite diverged into two subgroups (TcI and TcII) between 88 and 37 million years ago (Zingales et al., 2012). This reveals the importance of caviomorph reservoirs in the evolution of trypanosomes (Guhl and Ramírez, 2011; Jansen and Roque, 2010).

TcI is the DTU most widely distributed throughout the Americas (Zingales et al., 2012; Guhl and Ramírez, 2011); in the sylvatic cycle, it is frequently found in opossums, wild primates, bats, rodents and carnivores (Marcili et al., 2009; Miles et al., 2003). In agreement with this general pattern, we only found TcI in *M. australis* despite the fact that TcI, TcII and TcIII have been reported in other species of cavies (Jansen and Roque, 2010). Associated with various species of *Triatoma* (Mazza, 1949; Martínez et al., 1985), *M. australis* cavies are herbivorous, terrestrial rodents that dig burrows under brushy vegetation and climb trees or shrubs (Tognelli et al., 2001). Therefore, our findings contradict the hypothesis that TcI is associated with arboreal hosts and habitats and TcII with terrestrial ones (Gaunt and Miles, 2000) because *M. australis* is clearly a terrestrial rodent. The evolution of different lineages of *T. cruzi* seems to be more complex than a simple association between ecotopes, hosts and vectors.

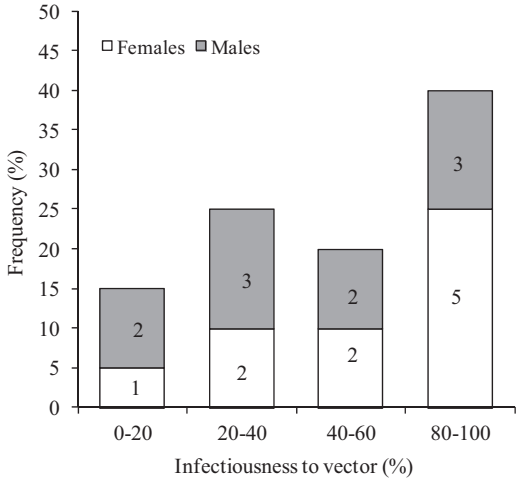

Fig. 2. Distribution of infectiousness to *T. infestans* of *M. australis* cavies by sex. The bars show the numbers of positive cavies in each category of infectiousness.

Unlike sigmodontine rodents from the Argentinean Chaco which had subpatent infectiousness to the vector (i.e., percentage of kDNA-PCR positive *T. infestans* divided by the total number of insects examined for infection by kDNA-PCR among kDNA-PCR positive rodents that had a negative xenodiagnosis), cavies were as highly infectious as *Didelphis* opossums and various species of armadillos (Orozco et al., 2013, 2014). Only one xenodiagnosis-negative cavy was positive by kDNA-PCR. The distribution of infectiousness in cavies was not overdispersed as in naturally-infected dogs (Gürtler et al., 2007); 44% of infected cavies infected more than 80% of the bugs that fed on them. Neither variations in the body mass index nor sex explained variations in infectiousness. Determinants of the infectiousness of cavies and other sylvatic mammals need to be further investigated.

The ecological and behavioral characteristics of cavies may increase the likelihood of exposure to and blood-feeding on triatomine bugs. *M. australis* has diurnal behavior, gregarious habits, strong fidelity to their burrows (Taraborelli et al., 2007), and share the same habitats with various species of triatomine bugs. Estimation of the rates of contact between cavies and vectors may shed further light on the nature of these host-vector associations.

The novel finding of *T. cruzi*-infected *M. australis* cavies thriving in bug-infested habitats close to human dwellings represents a new scenario of TcI transmission in Argentina and probably elsewhere. Cavies may play a significant role in peridomestic cycles involving the two local triatomine species. Given the ecological characteristics of *T. eratyrusifomis* and its local occurrence, it is one of the most likely candidate vectors implicated in the local circulation of TcI among cavies. *T. infestans* may also contribute to local transmission because it also occurred in domestic and peridomestic habitats and may blood-feed on cavies, as in Bolivia (Pizarro and Stevens, 2008). Unlike in Tucumán, no cavy was found infected in Santiago del Estero and TcI has rarely been found in (peri)domestic vectors or hosts despite the large number of animals examined for infection (Marcet et al., 2006; Ceballos et al., 2006; Cardinal et al., 2008).

The dry-shrub fences inhabited by *M. australis* nearby houses have occasionally been found infested by *T. infestans* and usually were not sprayed with insecticides by Chagas vector control programs (Joaquín Zárate, personal communication). Moreover, there are no clear instructions as to whether they should be included for routine vector surveillance, and treating them demands

substantial labor and expense. Dry-shrub fences may play a significant role in the reinfestation process as they are located within the range of distances over which reinfestation by *T. infestans* occurs in northwestern Argentina (Cecere et al., 2006), and within the range of dispersal of *T. infestans* and *T. eratyrusiformis* (Vazquez-Prokopec et al., 2004; Abrahan et al., 2011). Houses in close proximity to dry-shrub fences may have a higher risk of invasion by triatomine bugs that disperse either by walking or flying out of the infested fences. Therefore, environmental management measures directed to the extended fences of dry shrubs (or goat corrals, Gorla et al., 2013) may be needed for improved vector control of *T. infestans* and other secondary vectors.

Limitations of this study include that results are restricted to the late spring season when the activity of triatomine bugs recommences, and to adult cavies because very few juveniles were trapped. Logistic constraints (i.e., accessibility) determined that trapping efforts were heterogeneous among houses and communities. Considering that cavies are trap-shy and reluctant to enter unfamiliar places, trapping success was relatively high and cavy infections occurred in the four study villages. However, more detailed studies on *M. australis* (including seasonality, abundance and the course of infection) should be conducted to assess more precisely its role as a reservoir host of *T. cruzi*. Another limitation to a full understanding of the role of *M. australis* is that cavies were examined once. Therefore, we only have a snapshot of the course of the *T. cruzi* infection under natural conditions in late spring; it is of relevance to confirm whether their competence to infect the vector declines over age and time. For example, parasitemia in *Cavia porcelus* (Andean guinea pigs) disappeared 115 to 165 days after experimental infection with *T. cruzi* (Castro-Sesquen et al., 2011). Nevertheless, *T. cruzi* transmission without triatomine bugs was reported for three generation of *C. porcellus* naturally infected (Sherlock and Muniz, 1976). In our study examination of adult cavies in late spring has revealed the important role *M. australis* would have in the transmission of *T. cruzi* in peridomestic environments during the warm season when triatomine bugs are more active and also invade the domiciles. Moreover, as most of the examined cavies were adults, they would be presumably in chronic stage, but were highly infectious and probably with an infectivity down hard on the rest of their life.

One of the strengths of this study was the use of parasitological and molecular methods to assess the presence of infection and the intensity of infectiousness to the vector. However, our approach only allowed us to identify the DTUs associated with peripheral blood infections. Therefore, we cannot exclude that other DTUs infecting heart or other tissues may have been present. Identification of the vector species implicated initially was beyond the scope of the current study and warrants additional research efforts that focus on the links between cavies, triatomine bug species and parasite transmission. Whether *M. australis* is implicated in the transmission of *T. cruzi* in other endemic areas of its geographical distribution deserves further enquiry.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2014.10.019.

References

Abrahan, L.B., Dujardin, J.P., Gorla, D.E., Catalá, S.S., 2011. Dispersal of *Triatoma infestans* and other Triatominae species in the arid Chaco of Argentina. Mem. Inst. Oswaldo Cruz 106, 232–239, http://dx.doi.org/10.1590/S0074-02762011000200019.

Albarracin-Veizaga, H., de Carvalho, M.E., Nascimento, E.M., Rodrigues, V.L., Casanova, C., Barata, J.M., 1999. Chagas disease in an area of recent occupation in Cochabamba. Bolivia. Rev. Saúde Pública 33, 230–236, http://dx.doi.org/10.1590/S0034-89101999000300003.

Alvarado-Otegui, J.A., Ceballos, L.A., Orozco, M.M., Enriquez, G.F., Cardinal, M.V., Cura, C., Schijman, A.G., Kitron, U., Gürtler, R.E., 2012. The sylvatic transmission cycle of *Trypanosoma cruzi* in a rural area in the humid Chaco of Argentina. Acta Trop. 124, 79–86, http://dx.doi.org/10.1016/j.actatropica.2012.06.010.

Ashford, R.W., 1997. What it takes to be a reservoir host. Belg. J. Zool. 127, 85–90.

Barreto, A.C., 1976. Possible role of wild mammals and triatomines in the transmission of Trypanosoma cruzi to man. In: Pan American Health Organization Scientific Publication No. 318. New Approaches in America Trypanosomiasis Research, Washington DC, pp. 307–316.

Basso, B., Eraso, A., Moretti, E., Albesa, I., Kravetz, F., 1977. Natural infection of *Calomys musculinus* (Rodentia, Cricetidae) by *Trypanosoma cruzi*. Rev. Asoc. Argent. Microbiol. 9, 11–16.

Basso, B., Moretti, E., Albesa, I., Eraso, A., Kravetz, F., D'Alessandro, A., 1982. Natural infection of Akodon dolores, Thomas, 1916 (Rodentia, Cricetidae) by *Trypanosoma cruzi*. Rev. Inst. Med. Trop. Sao Paulo 24, 21–26.

Brown, L.D., Cai, T.T., DasGupta, A., 2001. Interval estimation for a binomial proportion. Stat. Sci. 16, 101–117.

Burgos, J.M., Altcheh, J., Bisio, M., Duffy, T., Valadares, H.M., Seidenstein, M.E., Piccinali, R., Freitas, J.M., Levin, M.J., Macchi, L., Macedo, A.M., Freilij, H., Schijman, A.G., 2007. Direct molecular profiling of minicircle signatures and lineages of *Trypanosoma cruzi* bloodstream populations causing congenital Chagas disease. Int. J. Parasitol. 37, 1319–1327, http://dx.doi.org/10.1016/j.ijpara.2007.04.015.

Carcavallo, R.U., Martínez, A., 1985. Biología, ecología, distribución geográfica de los triatominos americanos. In: Carcavallo, R.U., Rabinovich, J.E., Tonn, R.J. (Eds.), Factores Biológicos y Ecológicos en la Enfermedad de Chagas, vol. I, CPEHS OPS/OMS, SNCh. Ministerio de Salud y Acción Social, Argentina, pp. 149–208.

Cardinal, M.V., Lauricella, M.A., Ceballos, L.A., Lanati, L., Marcet, P.L., Levin, M.J., Kitron, U., Gürtler, R.E., Schijman, A.G., 2008. Molecular epidemiology of domestic and sylvatic Trypanosoma cruzi infection in rural northwestern Argentina. Int. J. Parasitol. 38, 1533–1543.

Castro-Sesquen, Y.E., Gilman, R.H., Yauri, V., Angulo, N., Verastegui, M., Velásquez, D.E., Sterling, C.R., Martin, D., Bern, C., 2011. *Cavia porcellus* as a model for experimental infection by *Trypanosoma cruzi*. Am. J. Pathol. 179, 281–288, http://dx.doi.org/10.1016/j.ajpath.2011.03.043.

Ceballos, L.A., Cardinal, M.V., Vazquez-Prokopec, G.M., Lauricella, M.A., Orozco, M.M., Cortinas, R., Schijman, A.G., Levin, M.J., Kitron, U., Gürtler, R.E., 2006. Long-term reduction of *Trypanosoma cruzi* infection in sylvatic mammals following deforestation and sustained vector surveillance in northwestern Argentina. Acta Trop. 98, 286–296.

Cecere, M.C., Vazquez-Prokopec, G.M., Gurtler, R.E., Kitron, U., 2006. Reinfestation sources for chagas disease vector, *Triatoma infestans*, Argentina. Emerg. Infect. Dis. 12, 1096–1102, http://dx.doi.org/10.3201/eid1207.051445.

Coura, J.R., Dias, J.C.P., 2009. Epidemiology, control and surveillance of Chagas disease—100 years after its discovery. Mem. Inst. Oswaldo Cruz 104, 31–40, http://dx.doi.org/10.1590/S0074-02762009000900006.

Ferriolli Filho, F., Barretto, M.P., 1966. Studies on wild reservoirs and vectors of *Trypanosoma cruzi*. XIV. Natural infection of a rodent, Cavia aperea aperea Erxleben, 1777 by Trypanosoma like *Trypanosoma cruzi*. Rev. Inst. Med. Trop. Sao Paulo 8, 267–276.

Flynn, J.J., Wyss, A.R., 1998. Recent advances in South American mammalian paleontology. TREE 13, 449–454.

Galuppo, S., Bacigalupo, A., García, A., Ortiz, S., Coronado, X., Cattan, P.E., Solari, A., 2009. Predominance of *Trypanosoma cruzi* genotypes in two reservoirs infected by sylvatic *Triatoma infestans* of an endemic area of Chile. Acta Trop. 111, 90–93.

Gaunt, M., Miles, M., 2000. The ecotopes and evolution of triatomine bugs (Triatominae) and their associated trypanosomes. Mem. Inst. Oswaldo Cruz 95, 557–565, http://dx.doi.org/10.1590/S0074-02762000000400019.

Gorla, E.D., Abrahan, L., Hernández, M.L., Porcasi, X., Hrellac, H.A., Carrizo, H., Catalá, S.S., 2013. New structures for goat corrals to control peridomestic populations of *Triatoma infestans* (Hemiptera: Reduviidae) in the Gran Chaco of

Argentina. Mem. Inst. Oswaldo Cruz 108, 352–358, http://dx.doi.org/10.1590/0074-02761080320130l4.

Guhl, F., Ramírez, J.D., 2011. *Trypanosoma cruzi I* diversity: towards the need of genetic subdivision? Acta Trop. 119, 1–4, http://dx.doi.org/10.1016/j.actatropica.2011.04.002.

Gürtler, R.E., Cecere, M.C., Lauricella, M.A., Cardinal, M.V., Kitron, U., Cohen, J.E., 2007. Domestic dogs and cats as sources of *Trypanosoma cruzi* infection in rural northwestern Argentina. Parasitology 134, 69–82, http://dx.doi.org/10.1017/S0031182006001259.

Herrera, L., D'Andrea, P.S., Xavier, S.C., Mangia, R.H., Fernandes, O., Jansen, A.M., 2005. *Trypanosoma cruzi* infection in wild mammals of the National Park 'Serra da Capivara' and its surroundings (Piaui, Brazil), an area endemic for Chagas disease. Trans. R. Soc. Trop. Med. Hyg. 99, 379–388.

Jansen, A.M., Roque, A.L.R., 2010. Domestic and wild mammalian reservoirs. In: Telleria, J., Tibayrenc, M. (Eds.), American Trypanosomiasis Chagas Disease. One Hundred Years of Research. Elsevier, Amsterdam, pp. 249–276.

Kreeger, T.J., Arnemo, J.M., 2007. Handbook of Wildlife Chemical Immobilization. Sunquest, Vancouver, BC, Canada.

Levy, M.Z., Bowman, N.M., Kawai, V., Waller, L.A., Cornejo del Carpio, J.G., Cordova Benzaquen, E., Gilman, R.H., Bern, C., 2006. Periurban *Trypanosoma cruzi*—infected *Triatoma infestans*, Arequipa. Peru. Emerg. Infect. Dis. 12, 1345–1352, http://dx.doi.org/10.3201/eid1209.051662.

Llewellyn, M.S., Miles, M.A., Carrasco, H.J., Lewis, M.D., Yeo, M., Vargas, J., Torrico, F., Diosque, P., Valente, V., Valente, S.A., Gaunt, M.W., 2009. Genome-scale multilocus microsatellite typing of *Trypanosoma cruzi* discrete typing unit I reveals phylogeographic structure and specific genotypes linked to human infection. PLoS Pathog. 5, e1000410, http://dx.doi.org/10.1371/journal.ppat.1000410.

Maffey, L., Cardinal, M.V., Ordoñez-Krasnowski, P.C., Lanati, L.A., Lauricella, M.A., Schijman, A.G., 2012. Direct molecular identification of *Trypanosoma cruzi* Discrete Typing Units in domestic and peridomestic *Triatoma infestans* and *Triatoma sordida* from the Argentine Chaco. Parasitology 139, 1570–1579, http://dx.doi.org/10.1017/S0031182012000856.

Marcili, A., Lima, L., Cavazzana, M., Junqueira, A.C.V., Veludo, H.H., Maia Da Silva, F., Campaner, M., Paiva, F., 2009. A new genotype of *Trypanosoma cruzi* associated with bats evidenced by phylogenetic analyses using SSU rDNA, cytochrome b and Histone H2B genes and genotyping based on ITS1 rDNA. Parasitology 136, 641–655, http://dx.doi.org/10.1017/S0031182009005861.

Marcet, P.L., Duffy, T., Cardinal, M.V., Burgos, J.M., Lauricella, M.A., Levin, M.J., Kitron, U., Gürtler, R.E., Schijman, A.G., 2006. PCR-based identification of *Trypanosoma cruzi* lineages in feces of triatomine bugs from rural northwestern Argentina. Parasitology 132, 57–65, http://dx.doi.org/10.1017/S0031182005008772.

Martínez, A., Carcavallo, R., Cichero, J., 1985. In: Factores biológicos, ecológicos en la enfermedad de, Chagas, In: Rodolfo, U., Carcavallo, Jorge, E., Rabinovich, Robert, J., Tonn (Eds.), Capitulo XXIX Argentina. Ministerio de Salud y Acción Social, Buenos Aires, pp. 345–354.

Mazza, S., 1949. Chagas disease in the Argentine Republic. Mem. Inst. Oswaldo Cruz 47, 273–302.

Miles, M.A., Feliciangeli, M.D., de Arias, A.R., 2003. American trypanosomiasis (Chagas' disease) and the role of molecular epidemiology in guiding control strategies. BMJ 326, 1444–1448, http://dx.doi.org/10.1136/bmj.326.7404.1444.

Minter, D.M., 1976. Effects on transmission to man of the presence of domestic animals in infected households. In: Pan American Health Organization Scientific Publication No. 318. New Approaches in American Trypanosomiasis Research, Washington DC, pp. 330–337.

Noireau, F., Diosque, P., Jansen, A.M., 2009. *Trypanosoma cruzi*: adaptation to its vectors and its hosts. Vet. Res. 40, 26, http://dx.doi.org/10.1051/vetres/2009009.

Orozco, M.M., Enriquez, G.F., Alvarado-Otegui, J.A., Cardinal, M.V., Schijman, A.G., Kitron, U., Gürtler, R.E., 2013. New sylvatic hosts of *Trypanosoma cruzi* and their reservoir competence in the humid Chaco of Argentina: a longitudinal study. Am. J. Trop. Med. Hyg. 88, 872–882, http://dx.doi.org/10.4269/ajtmh.12-0519.

Orozco, M.M., Piccinali, R.V., Mora, M.S., Cardinal, M.V., Enriquez, G.F., Gürtler, R.E., 2014. The role of sigmodontine rodents as sylvatic hosts of *Trypanosoma cruzi* in the Argentinean Chaco. Inf. Gen. Evol. 22, 12–22, http://dx.doi.org/10.1016/j.meegid.2013.12.020.

Pizarro, J.C., Stevens, L., 2008. A new method for forensic DNA analysis of the blood meal in Chagas disease vectors demonstrated using *Triatoma infestans* from Chuquisaca, Bolivia. PLoS One 3, e3585, http://dx.doi.org/10.1371/journal.pone.0003585.

Ramsey, J.M., Gutierrez-Cabrera, A.E., Salgado-Ramirez, L., Peterson, A.T., Sanchez-Cordero, V., Ibarra-Cerdena, C.N., 2012. Ecological connectivity of *Trypanosoma cruzi* reservoirs and *Triatoma pallidipennis* hosts in an anthropogenic landscape with endemic Chagas disease. PLoS One 7, e46013, http://dx.doi.org/10.1371/journal.pone.0046013.

Sherlock, I.A., Muniz, T.M., Almeida, S.F., 1974. Encontro da *Cavia porcellus* naturalmente infectada pelo *Trypanosoma cruzi* em domicílio infestado por *Panstrongylus megistus* no estado da Bahia, Brasil. Rev. Soc. Bras. Med. Trop. 8, 267–269.

Sherlock, I.A., Muniz, T.M., 1976. Transmissao do *Trypanosoma cruzi* em tres gerações de *Cavia porcellus* sem a participação de triatomineos. Rev. Soc. Bras. Med. Trop. 1, 27–29.

Taraborelli, P., Sassi, P., Giannoni, S.M., 2007. Registro morfo-ecológico de *Microcavia australis* (Caviidae, Rodentia) en la Puna de la provincia de San Juan, Argentina. Mastozool. Neotrop. 14, 107–112.

Torrico, R.A., 1959. Enfermedad de Chagas en Bolivia. Rev. Goina Med. 5, 375–387.

Tognelli, M.F., Campos, C.M., Ojeda, R.A., 2001. *Microcavia australis*. Mamm. Species 648, 1–4.

Yeo, M., Acosta, N., Llewellyn, M., Sánchez, H., Adamson, S., Miles, G., López, E., González, N., Patterson, J., Gaunt, M., Rojas de Arias, A., Miles, M., 2005. Origins of Chagas disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of *Trypanosoma cruzi* II, including hybrids. Int. J. Parasitol. 35, 225–233, http://dx.doi.org/10.1016/j.ijpara.2004.10.024.

Wisnivesky-Colli, C., Schweigmann, N.J., Alberti, A., Pietrokovsky, S.M., Conti, O., Montoya, S., Riarte, A., Rivas, C., 1992. Sylvatic *American trypanosomiasis* in Argentina, *Trypanosoma cruzi* infection in mammals from the Chaco forest in Santiago del Estero. Trans. R. Soc. Trop. Med. Hyg. 86, 38–41.

Vazquez-Prokopec, G.M., Ceballos, L.A., Kitron, U., Gürtler, R.E., 2004. Active dispersal of natural populations of *Triatoma infestans* (Hemiptera: Reduviidae) in rural northwestern Argentina. J. Med. Entomol. 41, 614–621.

World Health Organization, 2002. Control of Chagas Disease: Second Report of the WHO Expert Committee. World Health Organization, Geneva, pp. 57–58.

Pan American Health Organization, 2006. Estimación cuantitativa de la enfermedad de Chagas en las Américas. In: Organización Panamericana de la Salud OPS/HDM/CD/425-06. Pan American Health Organization.

Zeledón, R., 1974. Epidemiology, modes of transmission and reservoir hosts of Chagas' disease. *Trypanosomiasis* and *Leishmaniasis* with special reference to Chagas Disease. In: CIBA Foundation Symposium 20 (new series), pp. 51–77.

Zingales, B., Andrade, S.G., Briones, M.R.S., Campbell, D.A., Chiaris, E., Fernandes, O., Guhl, F., Lages-Silva, E., Macedo, A.M., Machado, C.R., Miles, M.A., Romanha, A.J., Sturm, N.R., Tibayrenc, M., Schijman, A.G., 2009. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recomends TcI to TcVI. Mem. Inst. Oswaldo Cruz 104, 1051–1054, http://dx.doi.org/10.1590/S0074-02762009000700021.

Zingales, B., Miles, M., Campbell, A., Tibayrenc, D.A., Macedo, M., Teixeira, A.M., Schijman, M.M.G., Llewellyn, A.G., Lages-Silva, M.S., Machado, E., Andrade, C.R., Sturm, S.G.N.R., 2012. The revised *Trypanosoma cruzi* subspecific nomenclatura: rationale, epidemiological relevance and research applications. Infect. Genet. Evol. 12, 240–253.