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Long-term reduction of *Trypanosoma cruzi* infection in sylvatic mammals following deforestation and sustained vector surveillance in northwestern Argentina

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Abstract

Long-term variations in the dynamics and intensity of sylvatic transmission of *Trypanosoma cruzi* were investigated around eight rural villages in the semiarid Argentine Chaco in 2002–2004 and compared to data collected locally in 1984–1991. Of 501 wild mammals from 13 identified species examined by xenodiagnosis, only 3 (7.9%) of 38 *Didelphis albiventris* opossums and 1 (1.1%) of 91 *Conepatus chinga* skunks were infected by *T. cruzi*. The period prevalence in opossums was four-fold lower in 2002–2004 than in 1984–1991 (32–36%). The infection prevalence of skunks also decreased five-fold from 4.1–5.6% in 1984–1991 to 1.1% in 2002–2004. Infection in opossums increased with age and from summer to spring in both study periods. The force of infection per 100 opossum-months after weaning declined more than six-fold from 8.2 in 1988–1991 to 1.2 in 2002–2004. Opossums were mainly infected by *T. cruzi* lineage I and secondarily by lineage II_d in 1984–1991, and only by *T. cruzi* I in 2002–2004; skunks were infected by *T. cruzi* II_d in 1984–1991 and by II_c in 2002–2004. The striking decline of *T. cruzi* infection in opossums and skunks occurred in parallel to community-wide insecticide spraying followed by selective sprays leading to very low densities of infected *Triatoma infestans* in domestic and peridomestic habitats since 1992; to massive deforestation around one of the villages or selective extraction of older trees, and apparent reductions in opossum abundance jointly with increases in foxes and skunks. These factors may underlie the dramatic decrease of *T. cruzi* infection in wild reservoir hosts.

Keywords

Trypanosoma cruzi; Reservoirs; Didelphis; Conepatus; Deforestation; Land use change; Skunks; Opossums; Force of infection

1. Introduction

Trypanosoma cruzi, the etiologic agent of Chagas disease, has been detected in some 180 species belonging to 25 families of mammals in the Americas, with marsupials, edentates, and rodents being the most frequent sylvatic hosts (World Health Organization, 2002). Domestic

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transmission cycles mainly include humans, dogs and cats, and several species of triatomine bugs adapted to human dwellings (Pinto Dias, 2000). Transmission cycles of *T. cruzi* display large spatial and structural heterogeneity (Diotaiuti et al., 1995).

The Gran Chaco, a natural landscape unit of about 1,000,000 km² crossing over northern Argentina, Bolivia, Paraguay and southwestern Brazil, is one of the most endemic regions for Chagas disease. Natural infection by *T. cruzi* has been reported for armadillos *Chaetophractus vellerosus*, *C. villosus*, *Cabassous unicinctus*, *Dasytus novemcinctus*, *Euphractus sexcinctus*, and *Tolypeutes matacus*; opossums *Didelphis albiventris* and *Lutreolina crassicaudata*; short-tailed opossums *Monodelphis domestica*; foxes *Lycalopex culpaeus* and *L. gymnocercus griseus*; coatis *Nasua nasua*, and mice *Calomys musculinus* and *C. laucha* (Carcavallo and Martínez, 1968; Yeo et al., 2005). In a well-defined area in the semiarid Argentine Chaco, the only wild mammals found infected with *T. cruzi* were *D. albiventris* opossums, a few *Conepatus chinga* skunks and one *Galictis cuja* ferret among 230 mammals from 20 species (Pietrokovsky et al., 1991; Wisnivesky-Colli et al., 1992). In the Bolivian Chaco, sylvatic *Triatoma infestans*, *Triatoma sordida* and *Triatoma guasayana* have been found infected with *T. cruzi* and may be the putative sylvatic vectors of *T. cruzi* (Noireau et al., 2000). In the Argentine Chaco, however, the sylvatic vector of *T. cruzi* has not been firmly established. Since the 1990's the Chaco has been undergoing accelerated deforestation and change of land use patterns which may have affected the relationship between the domestic and sylvatic transmission cycle of *T. cruzi* to an unknown extent.

T. cruzi has been classified into two major phylogenetic lineages, *T. cruzi* I (TCI) and *T. cruzi* II (TCII), and several sublineages within TCII designated as IIa, IIb, IIc, IId and IIe (Anon., 1999). These lineages appear to be distributed differentially between triatomine species and hosts throughout the Americas. TCI was originally described from sylvatic hosts and predominates in domestic transmission cycles to the north of the Amazon basin, whereas TCII predominates in domestic cycles but has sometimes been found in sylvatic mammals as well (Brisse et al., 2000; Yeo et al., 2005). In the Argentine and Paraguayan Chaco, TCI infects *D. albiventris* opossums and much less frequently humans and *T. infestans*, whereas TCII typically infects *T. infestans*, domestic dogs and cats, humans and skunks (Luca d'Oro et al., 1993; Diosque et al., 2003; Yeo et al., 2005; Marcet et al., 2006). Based on isoenzyme and molecular markers, two studies concluded that domestic and sylvatic transmission cycles of *T. cruzi* overlapped partially throughout Argentina and in two rural areas within the Chaco region (Wisnivesky-Colli et al., 1992; Luca d'Oro et al., 1993; Diosque et al., 2003). Opossums were suggested as a possible bridge between sylvatic and domestic transmission cycles (Schweigmann et al., 1999; Diosque et al., 2004). Temporal variations in the sylvatic cycle of transmission of *T. cruzi* in the presence of significant environmental changes have not been investigated.

As part of a longitudinal study on the ecoepidemiology of Chagas disease in a well-defined rural area in northwestern Argentina under sustained vector surveillance and selective insecticide sprays, the present study sought to assess the host range and prevalence of *T. cruzi* in a wide variety of wild mammals, and to identify the parasite sublineages circulating in them. Furthermore, to assess the existence of long-term variations in the dynamics and intensity of sylvatic transmission of *T. cruzi*, we compared our results with data collected in the same area between 1984 and 1991, before both massive deforestation around one of the villages and sustained vector surveillance in all villages was initiated in 1992.

2. Materials and methods

2.1. Study area

Field studies were carried out in the dry forest around Amamá (27°12'30"S, 63°02'30"W) and neighboring rural villages (Trinidad, Mercedes, Pampa Pozo, Villa Matilde, San Luis and La Curva) and in an isolated settlement (Lote S), situated in Moreno Department, Province of Santiago del Estero, Argentina (Fig. 1). The area is part of the semiarid southern Chaco, with a dry season from April to October. During 2002–2004 a weather station (Weather Monitor II, Davis Co., Baltimore, MD) located in Amamá measured mean annual precipitation (740 mm), relative humidity (55–68%), and mean annual temperature (22 °C), ranging from 28 °C in the warmest months (January–February) to 5 °C in the coldest month (July). The landscape is characterized by a secondary hardwood forest dominated by *Schinopsis lorentzii* and *Aspidosperma quebracho blanco*, and several *Prosopis* sp., which has been heavily exploited since the early 1920s' and especially since the 1990s'.

Amamá was the only rural village in the Moreno Department that had ever been treated with residual insecticides (in 1985), but the absence of a vector surveillance system resulted in an exponential increase of domestic reinfestation and in renewed transmission (Gürtler et al., 1991, 2005). A second insecticide treatment conducted in 1992 included other highly infested neighboring rural villages, and sustained vector surveillance during 1993–2004 resulted in very low numbers of *T. infestans* and other triatomines infected with *T. cruzi* (Cecere et al., 1999, 2002; Marcet et al., 2006).

2.2. Mammal capture and handling

Medium-sized mammals were live-trapped with National traps baited with beef, chicken or fish scraps, and rodents with Sherman traps baited with seeds, peanut butter and/or dry fruits. After occasional captures in November 2002, National traps were operated during seven surveys conducted in March (818 trap-days), July (639 trap-days), August–October (2785 trap-days) and November 2003 (821 trap-days), and April (483 trap-days), July (1286 trap-days) and November 2004 (419 trap-days), totaling 7251 trap-days. Sherman traps were operated in March (232 trap-days), July (905 trap-days), and November 2003 (1161 trap-days), and July 2004 (1169 trap-days), totaling 3,467 trap-days. Traps usually were placed in the forest, far from any human construction, but a few traps were also placed in the interphase between goat corrals and the surrounding forest to catch species that had different degree of contact with the human environment. Traps were checked daily every morning, and rebaited when necessary. Manual catches were conducted by experienced local hunters, some of which participated in previous surveys. The objectives of the study and the required mammal handling procedures until delivery of each catch to the research team were explained to all hunters. Biosafety and animal processing procedures were performed according to the Institutional Animal Care and Use Committee (IACUC) protocol No. 04223 at UIUC. An Ikonos satellite image (Space Imaging, Atlanta, GA) of the study area taken in October 2002 (spatial resolution: 1 m panchromatic and 4 m multi-spectral) was used to digitize the approximate location of 488 identified capture sites (Fig. 1).

The captured animals were transported to the field laboratory and examined clinically before and after anesthesia with tiletamine chlorhydrate and zolacepam chlorhydrate (Zelazol®, Fort Dodge Sanidad Animal, La Plata, Argentina) or ketamine chlorhydrate (Vetaset®, Fort Dodge) combined with xylacine (Ronpun®, Bayer) in doses appropriate for each species according to Carpenter et al. (2001). Rodents (mice and cavies) and mouse opossums were not anaesthetized. Opossums were assigned to a given age class based on tooth formula, eruption and tooth wear according to Schweigmann et al. (1999). Each animal was weighed, sexed and measured from snout to base of the tail. Samples of blood (except from cavies and marmosets),

hair, feces and ectoparasites were preserved for future studies. Fresh blood smears from a sample of mice were examined microscopically under 400× magnification. Whenever possible, the animals were tattooed in the ears after examination and then released at the capture site without incurring in any harm, with exception of mice which were euthanized.

2.3. Parasitologic methods

Each animal was examined by xenodiagnosis with 5 (rodents, caviids, marmosets), 10 (armadillos), or 20 (skunks, opossums, foxes) uninfected *T. infestans* third or fourth instar nymphs contained in one or two wooden boxes applied to the belly for 25 min, as described elsewhere (Gürtler et al., 1996). Bugs were provided by the insectary of the National Vector Control Coordination based in Córdoba, Argentina. After exposure each box was inspected to ensure that the bugs were fully engorged; in the few cases in which they were not, an additional exposure period was followed. The boxes were transported to Buenos Aires and held at ambient temperature (24–26 °C and 40–60% HR) and the bugs kept without further feeding until examination. The numbers of exuviae and dead bugs in each box were recorded as a measure of xenodiagnosis quality and to indicate that feeding had taken place. On average, 97% of the bugs survived to the first inspection and 75% to the second one; 5–41% of the nymphs molted on first inspection, and 0–20% on the second. Pools of feces from 5 bugs each that fed on a given specimen were examined for *T. cruzi* infection at 400 magnification 30 and 60 days after feeding. Bugs from each positive pool were re-examined individually and an overall proportion of infected bugs was calculated among the live bugs examined for infection at least once. Of 204 microscope-negative fecal samples from field-collected *T. infestans* screened by a polymerase chain reaction that amplified a 330 bp fragment from the variable regions of minicircles of the kinetoplastid genome, only 7 (3.4%) bugs were PCR-positive (Marcet et al., 2006). Infectiousness to the vector was defined as the number of *T. cruzi*-positive bugs divided by the total number of bugs fed on a given host individual and examined for infection at least once, excluding those bugs that did not survive to the first examination.

Feces from the infected xenodiagnosis bugs were cultured in diphasic medium at 28 °C as described by Lauricella et al. (2005). Parasite cultures were cryop-reserved and DNA extracted as described by Marcet et al. (2006). For the first two infected opossums, 11 one-month-old female Balb-c mice were inoculated intraperitoneally with feces collected from the infected xenodiagnosis bugs and then hemocultured.

Mammal infections with *T. cruzi* were confirmed by kDNA-PCR amplification of the 330 base pair (bp) fragment from the minicircle DNA of the kinetoplastid genome using the primers and cycling conditions previously published (Schijman et al., 2003). PCR tests were carried out under conditions that prevented DNA carryover contamination. Each PCR run included less than 13 samples, 100 fg of *T. cruzi* DNA as positive control, and sterile water instead of DNA as a negative control. Aliquots of 12 µl of PCR products were visualized under UV light after electrophoresis in 2.5% agarose gels containing ethidium bromide.

Culture samples of each infected mammal were typified to the sublineage level by PCR strategies targeted to spliced-leader DNA, 18s rDNA, 24s alfa rDNA and A10 genomic markers with the incorporation of Taq platinum (Invitrogen, USA) as described by Marcet et al. (2006). Parasites were also genotyped to lineage level directly from feces of infected xenodiagnosis bugs fed on an opossum.

2.4. Data analysis

The force of infection or instantaneous per capita rate of incidence (λ) was estimated retrospectively from agespecific prevalence of infection using a catalytic model with recovery rate set to 0 (Muench, 1959). This model assumes absence of parasitologic recovery; time- and

age-independent incidence of infection; individual hosts homogeneously exposed; no time lag between infection and infectiousness, and that the relation between age and prevalence is observed at equilibrium. λ was estimated using nonlinear least-squares procedures (Matlab 6.3, The MathWorks, Natick, MA), and the catalytic model $p_a = 1 - \exp(-\lambda \times a)$, where p_a is the proportion of infected opossums within the age class whose midpoint is a . The discrete age classes of opossums were translated into a continuous variable by using the approximate durations of each age class for *D. albiventris* estimated by Schweigmann et al. (1999). Because lactating *Didelphis* opossums (up to 3 months of age) appear not to be at risk of infection by congenital transmission (see below), age after weaning was deemed as a better representation of amount of exposure to bugs than absolute age. Calculations of λ for opossums between 1988 and 1991 were based on data collected by Schweigmann et al. (1999); age-prevalence data were not reported for 1984–1987.

Multiple logistic regression analysis implemented in Stata 9.0 (StatCorp, College Station, TX) was used to assess the relationship between infection prevalence and age, capture season, and year of survey. The binary response variable was infection on a given year. The predictors were survey year (categorical variable, for years 1988–1991), and age class (categorical variables with five levels, after pooling age classes 1 and 2, and age classes 6 and 7 to increase sample size). Reference levels were age classes 1 and 2 pooled, survey year 1988, and summer. Interaction terms were added to the model and dropped from the final equation if found not significant at the 5% level.

3. Results

A total of 521 mammals from at least 13 identified species was captured with an overall effort of 10,718 trap-days and additional manual captures that yielded approximately half of the total catch (Table 1). Rodents (mice, moles and cavies) comprised most of the catch ($n = 282$) followed by skunks ($n = 92$), three species of armadillos ($n = 90$), and *Didelphis* opossums ($n = 42$). Most opossums (73%) and skunks (69%) were captured around the more forested Trinidad, Mercedes and Villa Matilde villages, whereas only 22% of opossums and 18% of skunks were captured around Amamá (Fig. 2). The mean catch of mammals per unit effort using National traps in Amamá was half of that in the rest of the villages, whereas the catch with Sherman traps was similar between village clusters.

Only four of 501 specimens examined by xenodiagnosis (including four opossums recaptured) were positive for *T. cruzi*, with an overall prevalence of 0.8% (Table 1). Three (7.9%) *D. albiventris* opossums and one (1.1%) *C. chinga* female skunk, all adults or preadults, were found to be infected by *T. cruzi*. The three infected opossums (two females and one male, aged approximately 7–18 months old) were each caught in the secondary forest around a different village, close to (100–450 m) permanent water bodies and between 230 and 600 m from the closest house (Fig. 1). One of the infected opossums was recaptured eight months later at about 1 km from the site of first capture, and still was xenodiagnosis-positive. The infected skunk was trapped at 300 m from the closest house and very close to a canal. None of the three xenodiagnosis-negative opossums that were recaptured between 3 and 5 months apart converted parasitologically. In opossums, the annual prevalence in 2002–2003 (6.7%, 1/15) and 2004 (8.7%, 2/23) was not significantly different (Fisher's test, $P > 0.5$). The prevalence of infection increased significantly with age class from 0% (juveniles) to 17% (older adults), and from summer (0%) to spring (14%) (Fig. 3), although these prevalences are based on few infected animals. Based on the age-prevalence curve for the 2002–2004 period, the force of infection λ was 1.2 per 100 opossum-months after weaning (95% confidence interval, CI, 0.53–1.94%). The xenodiagnosis-positive opossums infected 54% of 51 xenodiagnosis bugs, whereas the skunk infected 88% of eight bugs. Among 44 mammals examined by fresh blood

films, a *T. cruzi*-like flagellate was detected in a field mouse but subsequent xenodiagnosis and anatomopathological analysis were negative.

We compared our results with data collected similarly in the study area between 1984 and 1991 (Wisnivesky-Colli et al., 1992; Pietrokovsky et al., 1991; Schweigmann et al., 1999). The overall prevalence of *T. cruzi* in opossums fell significantly from 31.9% (1984–1987, $n = 72$) and 35.9% (1988–1991, $n = 409$) to 7.9% in 2002–2004 (χ^2 -test, d.f. = 1, $P = 0.02$ and $P = 0.006$, respectively). The prevalence of *T. cruzi* infection in opossums increased significantly with age class from 3.7% in age class I to 68.7% in classes VI–VII in 1988–1991, and from 0% in classes II–IV to 16.7% in classes VI–VII in 2002–2004 (Fig. 3A). For the 1988–1991 period, the odds of infection relative to age classes I–II increased from 6.1 (95% confidence interval, CI = 2.5–14.5) in class III, 9.5 (CI = 4.2–21.4) in class IV, 14.0 (CI = 6.0–32.6) in class V, to 32.9 (CI = 10.4–104.7) in classes VI–VII. Significant differences between years were only observed for 1989 (OR = 2.3, CI = 1.2–4.2) ($\chi^2 = 83.2$, d.f. = 7, $n = 409$, $P < 0.0001$). No significant interaction between age class and year of survey was detected. The average force of infection per 100 opossum-months after weaning declined more than sixfold from 8.2 in 1988–1991 (CI, 4.66–11.70%). The annual force of infection was very similar in 1988 ($\lambda = 5.5\%$, CI, 3.6–7.4), 1989 ($\lambda = 10.2\%$, CI, 6.8–13.5), 1990 ($\lambda = 9.7\%$, CI, 5.0–14.5), and 1991 ($\lambda = 6.6\%$, CI, 0.3–13.5). Infection prevalence increased significantly from summer to spring in both study periods (Fig. 3B). All opossums captured in spring were adults or sub-adults. The infection prevalence in skunks also decreased five-fold from 5.6% (1984–1987, $n = 36$) and 4.1% (1985–1989, $n = 49$) to 1.1% in 2002–2004, although not significantly so (Fisher's test, $P = 0.29$ and $P = 0.20$, respectively).

Infection with *T. cruzi* was confirmed by kDNA-PCR in all four xenodiagnosis-positive individuals. The three opossums were infected with TCI whereas the skunk was infected with *T. cruzi* IIc, yielding the same band patterns reported by Yeo et al. (2005) (Fig. 4). Opossum parasites genotyped from feces of infected xenodiagnosis bugs (directly or from cultured feces) or after passage through mice gave the same results (lineage I) in two opossums.

4. Discussion

Our study shows a dramatic decrease in the prevalence and incidence of *T. cruzi* infection in sylvatic hosts over nearly two decades. To our knowledge, this study may be the first to assess the existence of long-term variations in the dynamics and intensity of sylvatic transmission of *T. cruzi* in a well-defined area, and to provide approximate estimates of force of infection of opossums based on age-prevalence curves. Opossums were still the main sylvatic reservoir hosts of *T. cruzi*, followed by skunks. No other mammal was found infected with *T. cruzi*, in spite of greater spatial coverage, sampling intensity, and inclusion of other common hosts (rodents) that have not been studied as extensively in the Gran Chaco. An extensive xenodiagnosis survey of four species of armadillos also gave negative results for *T. cruzi* in three provinces in northeastern Argentina (Martínez et al., 1983), unlike hemoculture surveys in the Paraguayan Chaco (Yeo et al., 2005). Interestingly, high infection prevalences in *Didelphis* opossums (36%) were also recorded in the neighboring Chaco province between 1999 and 2002 (Diosque et al., 2004), and were as high as 52% elsewhere in the Americas (Grisard et al., 2000; Telford and Tonn, 1982; Travi et al., 1994).

The prevalence of *T. cruzi* in opossums and skunks assessed by xenodiagnosis was stable between 1984 and 1991 and decreased four- or five-fold in 2002–2004. Because parasitologic and serologic methods were found to be equally sensitive for detecting *T. cruzi* infections in *Didelphis* (Fernandes et al., 1991; Grisard et al., 2000) and all of the infections we detected were confirmed by PCR, our xenodiagnosis-based estimates may be very close to the actual prevalence. The infection prevalence increased significantly with age and from summer to

spring as in the past (Schweigmann et al., 1999), suggesting that the same underlying processes occurred though at different intensities. Vertical transmission (including infections acquired congenitally and during lactation) and horizontal transmission (through urine or anal gland secretions) appear to be very unlikely routes of infection in *Didelphis* sp. (Jansen et al., 1994; Grisard et al., 2000; Telford and Tonn, 1982; Travi et al., 1994). The observed age-prevalence curves in opossums, increasing with time of exposure (age) after weaning, are consistent with vector-mediated transmission.

The striking decline in the prevalence and incidence of *T. cruzi* in opossums and skunks occurred in parallel in both Amamá and Trinidad-Mercedes villages. In principle, these changes may be related to: (i) massive or selective changes in landscape over two decades; (ii) sustained vector surveillance of domestic and peridomestic habitats and selective insecticide sprays, either alone or combined with landscape changes; (iii) random fluctuations in the prevalence of infection in wild mammals. The first hypothesis is based on the major environmental changes that took place especially around Amamá since 1990, which included massive deforestation for cattle-ranching and an increase in the number of households from 1993 to 2002 (31% increase in Amamá, 25% in Trinidad and 22% in Mercedes). Around Amamá, wire fencing and selective clearing of shrubs and some tree species was followed by controlled fires and seeding pastures for cattle; some sections underwent indiscriminate deforestation with heavy machinery. Several houses were relocated along the paved and main dirt roads, and the village ended up enclosed by a wired fence and surrounded by homogeneous grassland, unlike other study villages (Fig. 1). This affected the goat-raising practices of local peasants and their subsistence economy, and probably also reduced refuge availability for opossums and other wildlife in the vicinity of houses. In theory, deforestation exerts a large negative impact on wildlife abundance, but the impact on ecologically adaptable synanthropic mammals (such as opossums) was expected to be much lower, if any at all. Deforestation may also augment the contact rate of opossums and skunks with peridomestic and domestic sites in search for food, though this increase was not perceived and reported by local villagers. Degradation of sylvatic habitats, including selective extraction of larger, older trees (more likely to have the type of tree holes used by opossums) may have also affected the abundance of triatomine bugs and contact rates between opossums and bugs through reduction of suitable habitats for such encounters. However, the local sylvatic vectors of *T. cruzi* remain to be identified conclusively, and the impacts on triatomine populations of deforestation and changes in wildlife abundance (often assumed and theoretically reasonable) are not supported by direct evidence (Walsh et al., 1993). Because domestic dogs were identified as a significant source of mortality for *D. albiventris* opossums both locally (Schweigmann, N.J., unpublished results, 1995) and in the pampas grasslands (Perez Carusi, L. unpublished results, 2006), reduced availability of suitable habitats or refuges and increased opossum mortality may explain, at least in part, the much smaller catch of opossums and skunks in Amamá.

Landscape changes were qualitatively smaller and more gradual in Trinidad and Mercedes, and therefore may not explain the marked apparent decrease in the local abundance of opossums (a decrease that occurred despite using the same capture methods, a sizable capture effort, and some of the hunters as in previous decades). Local householders reported seeing opossums and opossum tracks much less frequently than in the past, whereas the apparent abundance of skunks and foxes reportedly increased strikingly following a waning fur trade. Whether these apparent local changes in omnivorous mammals are interrelated is unclear. Opossum abundance may also have decreased through increased predation or pathogens. Increase of mesopredators (such as foxes) is predicted to decrease the equilibrium infection prevalence and abundance of infected hosts in a simple susceptible-infectious model, irrespective of whether predators tend to attack infected or healthy prey (Ostfeld and Holt, 2004). For a stable vector population, the lower abundance of opossums would reduce its rate of contact with triatomine bugs. A mathematical model of *T. cruzi* transmission (Cohen and Gürtler, 2001)

suggests that a dramatic reduction in the abundance of a primary reservoir host with high and long-lasting infectiousness to the vector (such as dogs or its putative sylvatic equivalent, opossums) would be sufficient to drive down the transmission risk and reduce the equilibrium prevalence of infection in bugs and hosts when other parameters are held constant.

The second hypothesis (effects of sustained vector surveillance and selective insecticide sprays) implies a strong link between domestic and sylvatic transmission cycles, with the domestic cycle “spilling over” *T. cruzi* and/or bugs to the sylvatic cycle. Before 1992, only Amamá experienced a transient low-risk period between 1986 and 1988 following the first residual spraying with insecticides made in the Moreno Department, while Trinidad and all other rural villages were highly infested and with ongoing active transmission until late 1992 (Gürtler et al., 1991). Interestingly, transmission of *T. cruzi* to opossums was remarkably stable during an extended period, as shown by (i) the nearly constant mean prevalence of infection in opossums in 1984–1987 and 1988–1991 (31.9–35.9%), and (ii) the very similar age-prevalence curves recorded annually between 1988 and 1991. The 1992 community-wide insecticide campaign included other neighboring villages, and sustained vector control actions during 1993–2004 reduced to marginal levels the abundance of *T. infestans* infected with *T. cruzi* in domestic and peridomestic habitats, and the transmission of *T. cruzi* to domestic dogs dropped from 65% at baseline to 8.9% and 4.7% at 7.5 and 10 years after sustained vector surveillance, respectively (Cecere et al., 1999, 2002; Cardinal et al., in press). Moreover, during 2002–2004 the mean prevalence of *T. cruzi* in opossums was again stable though at very low levels; the infected wild mammals were captured around the villages that had the lowest infestation, and only marginal prevalence of *T. cruzi* (<1%) was detected in *T. infestans* and other triatomine bugs (Cardinal et al., in press). Therefore, the likelihood of potentially infective contacts between *T. cruzi*-infected domestic or peridomestic bugs from the study villages and sylvatic mammals was probably too remote to explain the occurrence of infected mammals. The “spill-over” of domestic or peridomestic bugs into the forest and subsequent creation of sylvatic foci of transmission is an unexplored possibility in our system, but is supported by at least one example where such “spill-over” was detected in *Didelphis* opossums and *T. infestans* (Diotaiuti et al., 1995). The stable patterns observed in opossum infections between 1984 and 1991 and between 2002 and 2004 does not support that the major long-term decline in the prevalence of *T. cruzi* may be explained by random fluctuations (third hypothesis).

Opossums and skunks are nocturnal, omnivorous, insect-eating mammals with a limited average home range (<50 ha), short mean life expectancy (12–18 months and perhaps 2–4 years, respectively), and high fertility leading to fast population turnover. *D. albiventris* opossums are sometimes considered semi-nomads, with each individual using multiple refuges in tree holes or burrows. Both opossums and skunks may acquire *T. cruzi* infection by eating infected *T. infestans*, either when they approach houses in search for food, or when adult bugs eventually disperse by flight into sylvatic habitats, or by contamination with bug feces. The probability of experimental opossum infection with *T. cruzi* by ingestion or contamination was about 6–8% (Rabinovich et al., 2001). Regardless of the exact mechanism and habitat where vector-mediated transmission occurs, if domestic or peridomestic *T. infestans* infected with *T. cruzi* were implicated in opossum infections, the observed major decrease in the extent and abundance of infected bugs would imply an insignificant risk of infection for local opossums. The crucial piece of evidence that may cast light on the origin of wild mammal infections is the diversity of *T. cruzi* lineages in domestic and sylvatic hosts and in bugs.

Didelphis opossums have predominantly been found infected with TCI throughout the Americas (reviewed by Brisse et al., 2000; Yeo et al., 2005). In our study area, however, three of 18 opossums, two ferrets and a skunk were infected with zymodemes equivalent to *T. cruzi* II d up to the early 1990s' (Wisnivesky-Colli et al., 1992; Luca d'Oro et al., 1993), based

on current parasite divisions (Barnabé et al., 2000). Current results show opossums only infected by TCI, whereas only 3 domestic or peridomestic *T. infestans* were found infected by *T. cruzi* (out of 389 bugs from the study villages examined for infection) and had lineage I (Marcet et al., 2006). For our area therefore, it was highly unlikely that the opossums acquired TCI from domestic or peridomestic habitats. In the absence of data on the putative vector(s) implicated in transmission to sylvatic hosts, the long-term reduction of *T. cruzi* infection in opossums may be tentatively explained by environmental degradation causing a large reduction of old trees and an apparent reduction of opossum abundance, possibly through marked changes in wildlife composition and abundance. Identifying the precise mechanisms linking these processes to the decline in opossum infections requires further investigation.

Our study provides the first record of a mammal (a skunk) infected with *T. cruzi* IIc in Argentina. This lineage has also been found infecting very few *T. infestans* and dogs in the study area (Cardinal et al., unpublished data). In Paraguay, *T. cruzi* IIc has also been isolated from two species of armadillos and a short-tailed opossum, but not from *T. infestans* (Yeo et al., 2005). The co-occurrence of two species of sympatric wild mammals infected with two different lineages of *T. cruzi* may imply either (i) the simultaneous existence of two independent sylvatic transmission cycles mediated by different vector species, or (ii) the host selecting for certain parasite lineages. The former hypothesis is supported by a compendium of evidence suggesting that terrestrial mammals that excavate burrows in open terrain (such as armadillos and skunks) are closely associated with TCII, whereas mammals of arboreal habits (such as opossums) are closely associated with lineage I (Yeo et al., 2005). Persistence of TCI in opossums and the occurrence of a different sublineage in skunks (IIc up to the 1990s' and IIc more recently) also suggest that these two hosts are involved in separate transmission cycles over decades. The very low frequency of infected skunks suggests they may be incidental hosts of *T. cruzi*. Although parasite isolation methods may select for certain strains and lineages of *T. cruzi*, no lineage selection was recorded among 145 isolates from opossums in spite of using different methods (Fernandes et al., 1991). Experimental infections of *Didelphis* opossums with TCII were considered less persistent than infections with TCI (Fernandes et al., 1994). In addition, mixed infections with different lineages and sublineages contradict the hypothesis of strict sublineage selection by the host.

In conclusion, our study shows a clear-cut decline in *T. cruzi* infection in sylvatic hosts in the context of various degrees of deforestation combined with other changes in wildlife composition and apparent reduction of opossum abundance. Regardless of the underlying processes, the decline in sylvatic *T. cruzi* infections indicates a diminishing risk of introduction of sylvatic strains and lineages into the local domestic transmission cycle. Whether this is a generalized pattern in the Gran Chaco remains to be investigated.

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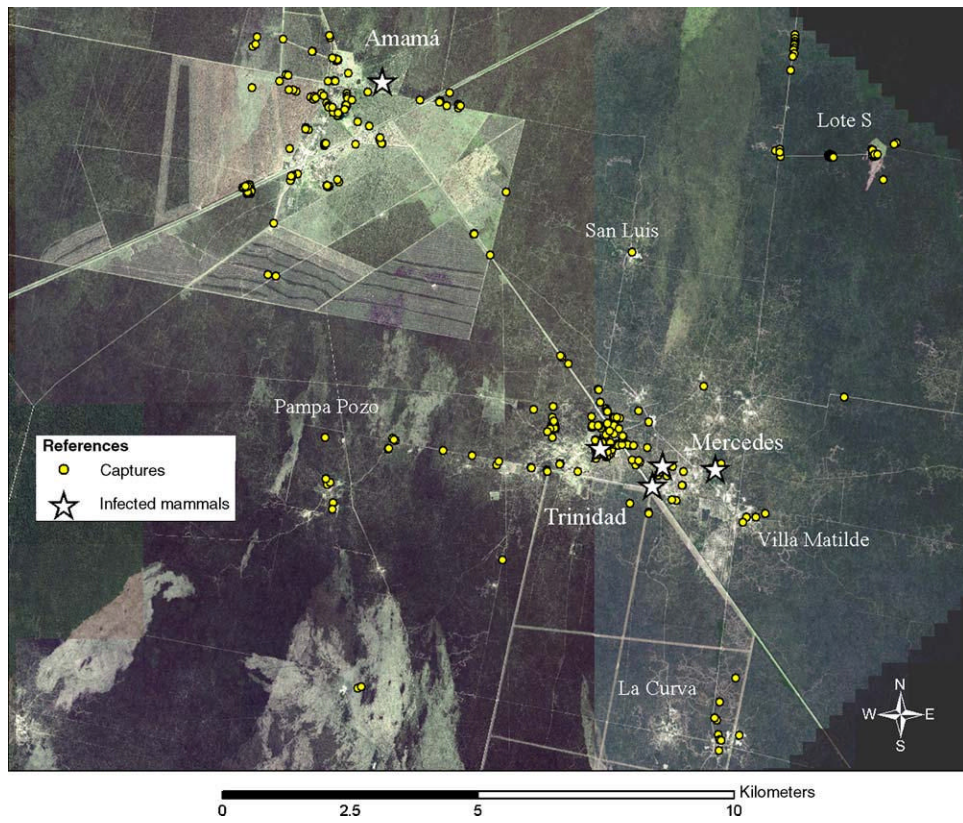


Fig. 1. Map showing capture sites of wild mammals in the Amamá area, 2002–2004.

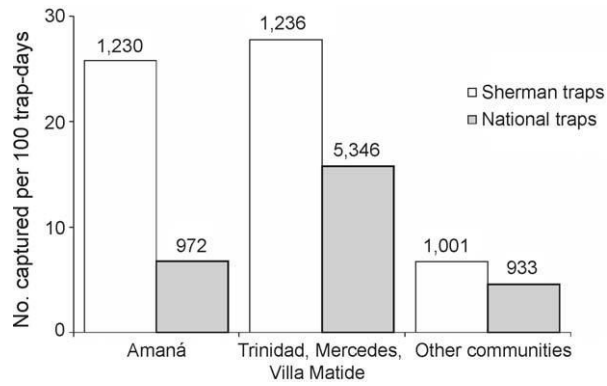


Fig. 2.

Capture of sylvatic mammals per unit effort according to type of trap (Sherman, National) in communities with different landscape. March 2003, July 2003, November 2003 and July 2004. Other communities: La Curva, San Luis, Lote S, Pampa Pozo. Numbers on top of bars represent the total number of trap-days per community.

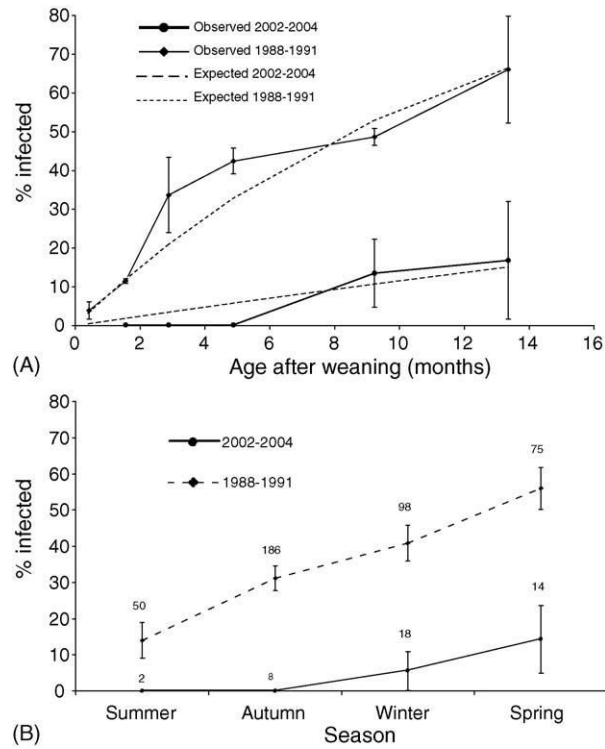


Fig. 3. Comparison of prevalence of *Trypanosoma cruzi* infection as determined by xenodiagnosis in opossums by age class (A) and seasons (B) in 1988–1991 (taken from Schweigmann et al., 1999) and 2002–2004 in the Amamá area.

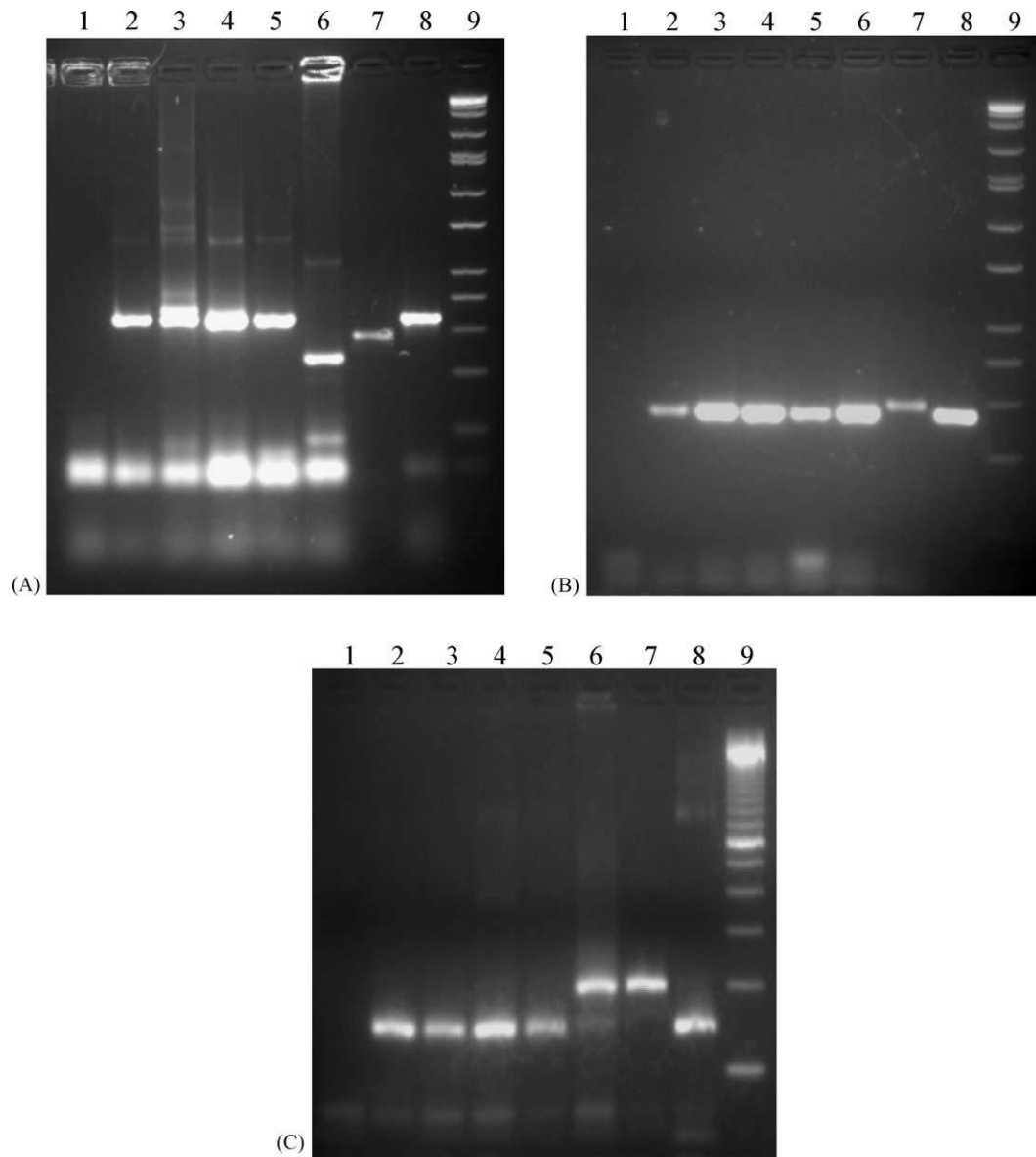


Fig. 4. Amplification of the intergenic region of the mini-exon genes (A), the D7 DNA ribosomal 24S-alfa domain (B) and A10 genomic marker (C); lanes (1) negative control; 2–5 *Didelphis albiventris T. cruzi* I (2) and (3) are the same individual on different capture occasions; (6) *Conepatus chinga skunk T. cruzi* IIc; (7) reference strain of lineage IIb (A and B) and lineage IIa (C); (8) reference strain of lineage I; (9), 100 bp ladder.

Table 1

Prevalence of *Trypanosoma cruzi* infection as determined by xenodiagnosis in wild mammals captured in the Amamá area between 2002 and 2004

Host		Number captured	Number examined by xenodiagnosis	Number infected with <i>T. cruzi</i> (%)
Common name	Scientific name			
White-eared opossum	<i>Didelphis albiventris</i>	42	38 ^a	3 (7.9) ^a
Molina's hog-nosed skunk	<i>Conepatus chinga</i>	92	91	1 (1.1)
Hairy armadillo	<i>Chaetophractus villosus</i>	6	6	0
Small armadillo	<i>Chaetophractus vellerosus</i>	21	21	0
Three-banded armadillo	<i>Tolypeutes matacus</i>	63	63	0
Mole	<i>Ctenomys sp.</i>	3	3	0
Wild guinea pig	<i>Microcavia australis</i>	116	114	0
Mice	Various species ^b	163	151	0
Small fat-tailed mouse opossum	<i>Thylamys pusilla</i>	8	7	0
Plains viscacha	<i>Lagostomus maximus</i>	2	2	0
Grey fox	<i>Lycalopex gymnocercus</i>	5	5	0
Total		521	501	4 (0.6)

^aExcludes four opossums that were recaptured, one of which was infected.

^bIncludes Cricetidae mice: *Graomys griseoflavus*, *Akodon* sp., *Calomys* sp., and an unidentified species.