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A comparative study of *Trypanosoma cruzi* infection in sylvatic mammals from a protected and a disturbed area in the Argentine Chaco

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ABSTRACT

Understanding the complex epidemiology of *Trypanosoma cruzi* transmission cycles requires comparative studies in widely different environments. We assessed the occurrence of *T. cruzi* infection in sylvatic mammals, their infectiousness to the vector, and parasite genotypes in a protected area of the Argentine Chaco, and compared them with information obtained similarly in a nearby disturbed area. A total of 278 mammals from >23 species in the protected area were diagnosed for *T. cruzi* infection using xenodiagnosis, kDNA-PCR and nuclear satellite DNA-PCR (SAT) from blood samples. The relative abundance and species composition differed substantially between areas. *Didelphis albiventris* opossums were less abundant in the protected area; had a significantly lower body mass index, and a stage structure biased toward earlier stages. The capture of armadillos was lower in the protected area. The composite prevalence of *T. cruzi* infection across host species was significantly lower in the protected area (11.1%) than in the disturbed area (22.1%), and heterogeneous across species groups. The prevalence of infection in *D. albiventris* and *Thylamys pusilla* opossums was significantly lower in the protected area (nil for *D. albiventris*), whereas infection in sigmodontine rodents was three times higher in the protected area (17.5 versus 5.7%). Parasite isolates from the two xenodiagnosis-positive mammals (1 *Dasypus novemcinctus* and 1 *Conepatus chinga*) were typed as TcIII; both specimens were highly infectious to *Triatoma infestans*. Fat-tailed opossums, bats and rodents were kDNA-PCR-positive and xenodiagnosis-negative. *Desmodus rotundus* and *Myotis* bats were found infected with *T. cruzi* for the first time in the Gran Chaco.

1. Introduction

Protected areas play a fundamental role in the conservation of natural ecosystems and can be among the most effective tools for protecting species from extinction and from the impact of human-induced threats (Naughton-Treves et al., 2005). These areas are expected to harbor a greater number of species than disturbed, exploited natural areas. In addition, protected areas can contribute to biodiversity conservation by maintaining ecological and evolutionary processes, diverse communities, viable populations, and natural areas large enough to be resilient to large-scale disturbances and long-term changes (Noss, 1992).

The richness of ecological communities can affect the occurrence and intensity of circulation of multiple pathogens by decreasing the risk of transmission with increasing diversity of host species, giving room to a "dilution effect" (Keesing et al., 2006; Schmidt and Ostfeld, 2001). Most insect vectors feed on host species that differ in reservoir host competence. Through the dilution effect, the presence of vertebrate hosts with a lower capacity to infect the vectors that feed on them would dilute the contribution of highly competent reservoirs (Schmidt and Ostfeld, 2001). Conversely, anthropogenic landscape disturbance could increase pathogen transmission (Daszak et al., 2001; Gottdenker et al., 2012). A recent meta-analysis showed that pathogen prevalence significantly decreased with increasing biodiversity (Civitello et al., 2015).

Trypanosoma cruzi, the etiologic agent of Chagas disease, infects more than 180 mammalian species from 7 orders and 25 families in the Americas (Jansen and Roque, 2010; Noireau et al., 2009), and has been classified into six main discrete typing units (DTUs) (Zingales et al., 2012). Marsupials (e.g., *Didelphis albiventris*), edentates (e.g., *Dasypus novemcinctus*), rodents and carnivores are the

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most common sylvatic hosts in the Gran Chaco region (Alvarado-Otegui et al., 2012; Ceballos et al., 2006; Orozco et al., 2013; Yeo et al., 2005) where Chagas disease and other neglected infectious diseases are hyperendemic (Gürtler et al., 2007b; Hotez et al., 2012). Ongoing large-scale changes in land use and habitat fragmentation throughout the Gran Chaco and other affected regions may have impacted heavily on the structure and functioning of the sylvatic transmission cycles of *T. cruzi.* Evidence on the presumable relevance of anthropic disturbance on sylvatic transmission cycles still is sparse (Ceballos et al., 2006; Vaz et al., 2007).

Dasypus novemcinctus armadillos and *Didelphis* opossums (including *D. marsupialis, D. aurita* and *D. albiventris*) are the most widespread sylvatic hosts of *T. cruzi* ; they are usually infected with *T. cruzi* III (TcIII) and *T. cruzi* I (TcI), respectively, and display large infectiousness to the vector *T. infestans* in the Gran Chaco and elsewhere (Alvarado-Otegui et al., 2012; Ceballos et al., 2006; Diosque et al., 2004; Orozco et al., 2013; Yeo et al., 2005). Both TcV and TcVI are prevalent in domestic environments where *Triatoma infestans* is the primary vector of *T. cruzi* (Enriquez et al., 2012; Zingales et al., 2012). In the Argentine and Paraguayan Chaco, TcI was isolated from domestic dogs and cats, *Didelphis* opossums, and more rarely from *T. infestans* (Cardinal et al., 2008; Diosque et al., 2004; Enriquez et al., 2014; Orozco et al., 2013). TcIII was frequently found in *D. novemcinctus* and *Chaetophractus* spp. armadillos, *Conepatus chinga* skunk and in the terrestrial marsupial *Monodelphis domestica* (Yeo et al., 2005; Ceballos et al., 2006; Alvarado-Otegui et al., 2012), and rarely in domestic dogs and *T. infestans* (Cardinal et al., 2008). This pattern agrees with the general notion that arboreal transmission cycles include opossums and TcI whereas terrestrial transmission include armadillos and TcIII (Gaunt and Miles, 2000; Yeo et al., 2005; Cardinal et al., 2008; Llewellyn et al., 2009). However, it has not yet been possible to demonstrate a strict association between DTUs and mammalian host species, and mixed infections of *T. cruzi* genotypes produced by differential histotropism may occur in mammals (Burgos et al., 2010). The complexity of host-vector interactions is compounded by the fact that several species of triatomine bugs may transmit different genotypes of *T. cruzi* in enzootic cycles potentially including a wide variety of mammalian hosts.

This study sought to assess the prevalence of *T. cruzi* infection and infectiousness to the vector *T. infestans* in a large sample of sylvatic mammals from a protected area, and compare these results with information obtained similarly in a nearby disturbed area within the same municipality (Orozco et al., 2013; Orozco et al., 2014). In the disturbed area, the composite prevalence of *T. cruzi* infection was high both in *D. novemcinctus* (57.7%) and *D. albiventris* opossums (38.1%) which were much more infectious to *T. infestans* than other host species. Among other 18 mammalian species examined, some had lower prevalence (the armadillos *Euphractus sexcinctus*, *Tolypeutes matacus* and *Chaetophractus vellerosus*, and the marsupial *Thylamys pusilla*) (Orozco et al., 2013). In both areas, 24 rodents from eight species were positive for *T. cruzi* infection by molecular methods, but the intensity of bug rectal infection was below the detection limit of xenodiagnosis (Orozco et al., 2014).

There is some evidence indicating that habitat fragmentation may increase the contact rates between sylvatic or domestic hosts of *T. cruzi* and triatomine bugs (Vaz et al., 2007; Xavier et al., 2012), and disturbed areas have more abundant populations of some highly competent reservoir hosts (e.g., opossums). Therefore, based on the putative influence of the "dilution effect", habitat fragmentation and disturbance, we expected that i) the prevalence of *T. cruzi* infection in the protected area would be lower than in the nearby disturbed area, and ii) additional mammalian host species may participate in the sylvatic transmission of *T. cruzi* in the protected area.

2. Materials and methods

The field work was conducted in the protected area "Pampa del Indio Provincial Park" (26° 13′ S; 60° 00′ W), Pampa del Indio municipality (Chaco province, northeastern Argentina). The disturbed area (a rural section of 450 km^2) was described elsewhere (Orozco et al., 2013); small patches of native forest were close to the houses, usually subjected to intense human pressure. The prevalence of house infestation with *T. infestans* was 46% (Gurevitz et al., 2013) and the infection with *T. cruzi* was high (22–29%) in domestic bugs, dogs and cats (Cardinal et al., 2014) before a community-wide residual insecticide spraying conducted in November 2007.

Both areas are located in an ecotone between the wet and dry Chaco regions. The protected area (8,633 ha) is located about 30 km from the disturbed area; it had no human settlements within its limits, and was entirely surrounded by rural communities. The protected area included a primary forest of *Schinopsis balansae, Schinopsis lorentzii, Aspidosperma quebracho-blanco, Prosopis alba, Prosopis nigra* and *Tabebuia* spp.; gallery forests with bromeliads, savanna, marshes and small ponds, and is home to a diverse fauna (Bodrati et al., 2000).

In the protected area, wild mammals were caught during three-week surveys conducted in July 2009, November 2009 and July 2010. Two local collaborators assisted us in the captures. Medium-sized mammals were live-captured with Tomahawk traps set every 50 m along line transects. Armadillos also were live-captured with camouflaged home-made traps placed in the entrance of burrows. Local collaborators located burrows within the area specified by us, set up the traps, and inspected them several times at dusk and dawn. The home-made traps were built with iron mesh; are funnel-shaped and narrow (close to the average width of armadillo shell). The backdoor falls after when the armadillo enters, and it cannot turn or walk in reverse. Rodents and small marsupials were caught with Sherman traps arranged in pairs (one on the ground and the other at 1–2 m high on the trees) every 5 m along transect lines. All traps were checked and baited every morning using beef or chicken scraps for Tomahawk traps, and seeds, fruits and peanut butter pellets for Sherman traps. Bats were caught using mist nets (6.0 m wide, 2.6 m high, 38.0 mm black mesh, AFO Banding Supplies) placed in a zigzag pattern, opened at dusk and monitored every 30–40 min. for 6 h.

Capture sites were georeferenced (Garmin Legend C) and mammals were transported to the field laboratory. Capture and handling procedures were described in detail elsewhere (Orozco et al., 2013). Biosafety and animal processing procedures were performed according to protocols approved by the Dr. Carlos Barclay Ethical Committee. Wildlife permits (including transit permits for biological samples) were obtained from the provincial government through "Natural Resources Agency of Chaco".

Parenteral and/or inhalatory anesthetics were used for induction and maintenance of general anesthesia. For initial immobilization, parenteral anesthesia was performed with tiletamine clorhydrate and zolacepam clorhydrate (Zelazol; Fort Dodge, Buenos Aires, Argentina) at the minimum dose appropriate to species and weight (Kreeger and Arnemo, 2007). For maintenance, animals were given inhalatory anesthesia with Isoflurane delivered with a vaporizer (IsoTec; Datex-Ohmeda GE Healthcare, Little Chalfont, United Kingdom) and medicinal O2 (0.25–3 L/min). Anesthetized animals were maintained on thermic cushioned surfaces in a quiet and comfortable environment, and their eyes were protected with ophthalmic lubricant solutions and covered with home-made eyecups.

Animals were sexed, measured from snout to base of tail, tail length, weighed with Pesola®, and marked with numeric metal tags (National Band & Tag co.). *Didelphis* opossums were assigned to stage class (I to VII) based on tooth eruption (Schweigmann et al.,