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Research paper

Is the infectiousness of dogs naturally infected with *Trypanosoma cruzi* associated with poly-parasitism?

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ABSTRACT

Interactions among different species of parasites co-infecting the same host could be synergistic or antagonistic. These interactions may modify both the frequency of infected hosts and their infectiousness, and therefore impact on transmission dynamics. This study determined the infectiousness of *Trypanosoma cruzi*-seropositive dogs (using xenodiagnosis) and their parasite load (quantified by qPCR), and tested the association between both variables and the presence of concomitant endoparasites. A cross-sectional serosurvey conducted in eight rural villages from Pampa del Indio and neighboring municipalities (north-eastern Argentina) detected 32 *T. cruzi*-seropositive dogs out of 217 individuals examined for infection. Both the infectiousness to the vector *Triatoma infestans* and parasite load of *T. cruzi*-seropositive dogs examined were heterogeneous. A statistically significant, nine-fold higher mean infectiousness was registered in *T. cruzi*-seropositive dogs co-infected with *Ancylostoma caninum* and a trematode than in *T. cruzi*-seropositive dogs without these infections. The median parasite load of *T. cruzi* was also significantly higher in dogs co-infected with these helminths. An opposite trend was observed in *T. cruzi*-seropositive dogs that were serologically positive to *Toxoplasma gondii* or *Neospora caninum* relative to dogs seronegative for these parasites. Using multiple logistic regression analysis with random effects, we found a positive and significant association between the infectiousness of *T. cruzi*-seropositive dogs and co-infections with *A. caninum* and a trematode. Our results suggest that co-infections may be a modifier of host infectiousness in dogs naturally infected with *T. cruzi*.

1. Introduction

When a parasite trespasses the physical barriers of a host (i.e., the resistance exerted by the skin to parasite internalization), it finds an "immunoenvironment" that is determined by previous and current parasitic infections and by intrinsic factors such as host age, sex, nutritional status and genotype (Telfer et al., 2008). Therefore, the susceptibility to and duration of a parasitic infection, its intensity, transmission dynamics and pathology may be altered by infections with other parasites (Telfer et al., 2008; Graham, 2002). These alterations may change the number of infected individuals or their infectiousness thus impacting on transmission dynamics (Graham et al., 2007). A mechanism suggested for this interaction among parasite species, or genotypes, is by down-regulation of the immune system (Lafferty, 2010; Telfer et al., 2010). Other factors probably involved could be competition for nutrients or place of accommodation in the host, and cross-reactivity (i.e., components of the immune response developed by a host against a parasite species or genotype recognize other species or genotypes), among others.

In general, intracellular parasites (e.g., *Trypanosoma cruzi*, *Toxoplasma gondii*) stimulate a Th1 type immune response producing IFN-γ, IL-2 and macrophage activation (Hoft et al., 2000), in opposition to extracellular parasites (e.g., helminths) which stimulate a Th2 immune response producing IL-4, IL-5, IL-6 and IL-10

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(Mosmann and Coffman, 1989). The polarization towards a certain type of immune response reduces the degree of the opposite immune response that a host can exert (Rodríguez et al., 1999), in a mechanism called "immunomodulation" (Graham, 2008; Telfer et al., 2010).

Trypanosoma cruzi, the etiological agent of Chagas disease, is characterized by a large genetic diversity and has been classified into six Discrete Typing Units (DTUs), TcI-VI (Zingales et al., 2009). DTUs are distributed differentially among vectors, mammalian hosts and geographical regions (Miles et al., 2009). In Argentina, TcVI is the most frequent DTU found in peripheral blood of dogs, whereas TcV and mixed infections have frequently been reported in humans (Cardinal et al., 2014; Enriquez et al., 2013a; Monje-Rumi et al., 2015).

Interspecies interactions between *T. cruzi* and other pathogens in humans and mammalian hosts suggest that infectious agents and parasites may affect the transmission and the pathology of *T. cruzi*. de Freitas et al. (2011) found a higher *T. cruzi* load and infectiousness to the vector *Triatoma infestans* in HIV-infected humans. In a murine model of *T. cruzi* with a helminth co-infection, the intensity of *T. cruzi* parasitemia was associated with both the time since the primary infection and the sequence of infection (Galán-Puchades and Osuna, 2012). The intensity of parasitemia by *T. cruzi* was higher in *Leontopithecus rosalia* and *L. chrysomelas* monkeys naturally co-infected with nematode worms (Trichostrongylidae) than in monkeys not co-infected with nematodes (Monteiro et al., 2007). *Trypanosoma cruzi*-seropositive dogs, naturally co-infected with *Dirofilaria immitis*, showed less intense heart inflammatory response than those free of *D. immitis* suggesting immunomodulation by the worm (Cruz-Chan et al., 2010).

Host nutritional status can affect the susceptibility and severity of the infection in a great variety of pathogens (Louria, 2007). A cross-sectional study in Panama found that the body condition of rural dogs was negatively associated with the number of infecting parasite species (Fung et al., 2014). A vicious cycle was suggested (Beldomenico et al., 2008), in which poor nutritional conditions predispose to parasite infections and these infections undermine the host's nutritional status. A general sign of malnutrition is the presence of anemia, which could also be caused by gastrointestinal helminths (Crompton and Neishem, 2002; Dias et al., 2013).

Dogs and cats are major reservoir hosts of *T. cruzi* in domestic transmission cycles throughout the Americas (Crisante et al., 2006; Gürtler and Cardinal, 2015). Therefore, they could be the target of innovative strategies developed for the interruption of domestic transmission. In this study we evaluate the association between infectiousness (as determined by xenodiagnosis), bloodstream parasite load (as assessed by qPCR) and co-infections with helminths or intestinal protozoa and intracellular protozoa in dogs naturally infected with *T. cruzi* from a highly endemic district in the Gran Chaco region. As body condition (a surrogate of nutritional state) was negatively associated with the infectiousness of *T. cruzi*-seropositive dogs (Petersen et al., 2001; Enriquez et al., 2014), we also assessed whether the body condition of dogs and/or the presence of anemia were associated with infectiousness to *T. infestans* and bloodstream parasite load.

2. Materials and methods

2.1. Study area

This study is part of a broader research project on the transmission and control of Chagas disease in the municipality of Pampa del Indio (26° 2′ 0″ S, 59° 55′ 0″ W), Chaco Province, Argentina. Field work was conducted in five villages (El Gramillar, Tacuruzal, Campo Nuevo, Lote Cuatro and Ex Parque) in the municipality of Pampa del Indio in April and October-November 2013. Three villages neighboring to Pampa del Indio municipality were included and visited: Santa Carmen (General Güemes Department, 25° 55′ 0″ S 60° 37′ 0″ W), Pampa Bandera (25 de Mayo Department, 26° 55′ 0″ S 60° 02′ 0″ W) and El Palmar (Quitilipi Department, 52° 0′ 0″ S 60° 13′ 0″ W). The rural area of Pampa del Indio (1721 km^2) has been under sustained vector surveillance including a community participation component since 2007. As a consequence of these interventions, local vector-borne transmission of *T. cruzi* was significantly reduced or interrupted; therefore, the search for infected dogs was extended to neighboring rural villages from other municipalities, where vector control actions had been sporadic or absent, to increase the likelihood of finding *T. cruzi*-infected dogs.

2.2. Study design

To maximize the probability of finding *T. cruzi*-seropositive dogs, sampling was targeted to dogs residing in households infested with *T. cruzi*-infected *T. infestans*. Therefore, some households from Pampa del Indio were selected because of prior collection of infected bugs in 2012 or because their owners reported the presence of *T. infestans* to the surveillance system. Given that few houses fulfilled the first two criteria, a third criterion for selection of dwellings was based on having mud walls which had a higher probability of harboring triatomines (Gurevitz et al., 2011). For this study we selected 7 of 208 inhabited dwellings of Tacuruzal, 4 of 9 from El Gramillar, 3 of 32 from Campo Nuevo, 2 of 122 from Lote Cuatro, 1 of 27 from Ex Parque, 24 of 70 from Santa Carmen, 17 of 91 from Pampa Bandera, and 11 of 21 from El Palmar. All dogs were examined at their household. Of 226 dogs registered 217 were bled for serological tests.

Processing of blood samples for serum separation and DNA extraction was described elsewhere (Enriquez et al., 2014). The host's body condition was used as an index of the nutritional status of dogs (Petersen et al., 2001). The body condition of each dog (good, regular or poor) was established by a single member of our team (GFE) based on the degree of development of muscles; external evidence of bone structure, state of fur coat, the existence of fat deposits, and facial expression (Petersen et al., 2001; Enriquez et al., 2014). Only dogs aged 1 year or more were classified to avoid potential confounders due to growth effects and acute infections. Handling and examination of dogs were conducted according to the protocol approved by the 'Dr. Carlos Barclay' Independent Ethical Committee for Clinical Research (Protocol No. TW-01-004).

2.3. Serodiagnosis

At the end of each workday, dog sera was separated and tested at the field laboratory for antibodies to *T. cruzi* by indirect hemagglutination assay (IHA) following the manufacturer's instructions (Wiener Laboratories S.A.I.C., Buenos Aires, Argentina) and an in-house enzyme-linked immunosorbent assay (ELISA). Serodiagnostic methods were described elsewhere (Enriquez et al., 2013b). An individual was considered seropositive when it was reactive to at least two assays. All *T. cruzi*-seropositive dogs were selected for stool collection.

Serological examination of *T. cruzi*-seropositive dogs were conducted to detect the presence of antibodies against *T. gondii* and *Neospora caninum* by indirect immunofluorescence assay test (IFAT) by the Laboratory of Immunology, Department of Epizootiology and Public Health, Faculty of Veterinary Science, National University of La Plata, Argentina. The cut-off titer employed was 100. One dog was not tested because of insufficient serum.