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Temporal Variation of *Trypanosoma cruzi* Infection in Native Mammals in Chile

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Abstract

In the present study, we compared *Trypanosoma cruzi* infection in four native mammals from a hyperendemic area of Chagas disease in Chile for two different periods to assess the occurrence of interannual variation (1999–2000 vs. 2005–2006). Parasite detection in mammals is performed by polymerase chain reaction assays and confirmed by Southern blot analysis and hybridization test with a universal probe. Results showed significant differences in the levels of *T. cruzi* infection between the compared periods. We suggest that the major El Niño event occurred in 1997–1998, a large-scale global climatic fluctuation, could be indirectly explaining the extremely high *T. cruzi* infection in 1999–2000 by means of a time-lag response of the wild transmission cycle of Chagas disease in semiarid Chile after the irruption of small rodent populations.

Key Words: Chagas disease—ENSO—*Trypanosoma cruzi*—Wild reservoirs.

Chagas disease is a zoonosis caused by flagellated parasite *Trypanosoma cruzi* and transmitted by triatomine insects to mammal species (Panzera et al. 2004). This kinetoplastid protozoa contains a kinetoplast DNA (kDNA) composed by maxicircle (mitochondrial DNA) and abundant minicircles (10,000–20,000/cell) (Telleria et al. 2006). *T. cruzi* multiplies and differentiates in the digestive tract of the insect vector, and infection of definitive mammal hosts occurs by contamination of mucous membranes with insect-infected feces containing the metacyclic trypomastigote stage of the flagellate (Kollien and Schaub 2000). In Chile, *Mepraia spinolai* is the main sylvatic vector with infection levels reaching up to 46% in some areas (Botto-Mahan et al. 2005). In hyperendemic areas of Chagas disease in Chile, *T. cruzi* prevalence in mammals ranges from 21.4% in the native rodent *Octodon degus* (Jiménez and Lorca 1990), 61% in several native mammal species combined (Rozas et al. 2007), 50% in peridomestic goats (Rozas et al. 2007), and 32% in free-ranging introduced rabbits (Botto-Mahan et al. 2009) using serological and/or polymerase chain reaction (PCR) assays. Even though those studies show dissimilar infection levels in mammal hosts, no information is available about the persistence of *T. cruzi* prevalence in wild mammals in different years. Here, we used PCR to amplify DNA minicircles to determine *T. cruzi* infections in four species of native mammals from a hyperendemic area of Chagas disease to assess the occurrence of interannual variation (1999–2000 vs. 2005–2006).

Wild rodents (*Phyllotis darwini*, *O. degus*, and *Abrothrix* sp.) and the mouse-opossum (*Thylamys elegans*) were collected at Las Chinchillas National Reserve (Coquimbo region; 31°30′ S, 71°06′ W), a hyperendemic zone of Chagas disease in Chile. Several native and introduced mammal species inhabit the study sites as well as the wild kissing bug *M. spinolai*, whose populations can reach up to 46.2% of *T. cruzi* infection. Animals were collected using 150 baited Sherman traps (H.B. Sherman Trap Company, Tallahassee, FL) arranged on a grid, for a total of six field trips of seven consecutive trapping days during spring–summer of 1999–2000 and 2005–2006 (i.e., three field trips per period). Each animal was weighed and anesthetized with isoflurane at a dose of 13 mg/kg of body weight. Then, 0.5–1.0 mL of blood was withdrawn by cardiac venipuncture using heparinized tuberculin syringes. Animals were hair marked to avoid additional blood sampling and released in the capturing area. Blood extraction procedure was conducted following the recommendations of the Ethics Committee of the Faculty of Medicine, University of Chile.

Whole-genomic DNA was isolated from blood samples and stored at −20°C. The amplification reaction was

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performed in triplicate with oligonucleotides 121 and 122, which anneal to the four constant regions in minicircles of *T. cruzi* (Rozas et al. 2007). Each experiment included a blank that contained water instead of DNA, and a positive control that contained purified kDNA of *T. cruzi.* The 330-bp PCR product was analyzed by electrophoresis in a 2% agarose gel and viewed by staining with ethidium bromide. To confirm infection with *T. cruzi*, Southern blot analysis and hybridization with a universal probe of kDNA was performed with 10 μL of each PCR product (Rozas et al. 2007).

We analyzed 158 and 168 blood samples from mammals in the periods 1999–2000 (*P. darwini*, $n = 55$; *O. degus*, $n = 46$; *Abrothrix* sp., $n = 44$ and *T. elegans*, $n = 13$) and 2005–2006 (*P. darwini*, $n = 62$; *O. degus*, $n = 50$; *Abrothrix* sp., $n = 41$; and *T. elegans*, $n = 15$), respectively. Overall, *T. cruzi* infection in mammal species significantly decreased from 60.8% ($n = 96$) in 1999–2000 to 16.1% ($n = 27$) in 2005–2006 (all mammal species combined: $\chi^2 = 69.21$, df = 1, $p < 0.0001$). *T. cruzi* infection in the studied species ranged from 46.2% to 70.5% in 1999–2000 and 13.3% to 20.0% in 2005–2006 (Fig. 1), but no statistically significant differences were detected among host species in their infection levels within periods (1999–2000: $\chi^2 = 3.34$, df = 3, $p = 0.34$; 2005–2006: $\chi^2 = 2.07$, df = 3, $p = 0.56$). Separate analyses for each mammal species indicate that *T. cruzi* infection was significantly or marginally lower in 2005–2006 compared to 1999–2000 (*P. darwini*: $\chi^2 = 27.00$, df = 1, $p < 0.0001$; *O. degus*: $\chi^2 = 16.73$, df = 1, $p < 0.0001$; *Abrothrix* sp.: $\chi^2 = 22.18$, df = 1, $p < 0.0001$; *T. elegans*: $\chi^2 = 3.68$, df = 1, $p = 0.055$; Fig. 1).

In our study, overall *T. cruzi* infection significantly decreased from 61% in 1999–2000 to 16% in 2005–2006. The population dynamics of native mammal hosts from Las Chinchillas National Reserve (i.e., our study site) have been followed for several years (Lima et al. 2003). Irruptions of small mammal species have been seen as a by-product of increased rainfall and primary production, associated with El Niño (El Niño Southern Oscillation [ENSO]) events (Lima et al. 2003). For example, densities of *P. darwini* show important temporal fluctuations, with population peaks closely associated with increases in annual rainfall (ENSO events 1986–1987, 1991–1992, and 1997–1998) (Lima et al. 2003). Overall, the density of small mammals (number of mammals/ha) increased from 36 to 89 in 1999–2000 and 2005–2006, respectively (Lima et al. 2003, M. Lima, personal communication). Therefore, we can estimate the number of *T. cruzi*–infected individuals per hectare in both periods. The infection prevalence in 1999–2000 is significantly higher than in 2005–2006, and this observation corresponds with the estimated number of infected mammals per unit of area (1999–2000: 20 infected mammals/ha; 2005–2006: 13 infected mammals/ha; $\chi^2 = 22.12$, df = 1, $p < 0.0001$; all four mammal species combined). Including population densities in our analysis allow us to state with more confidence that the observed reduction in infection prevalence in the period 2005–2006 is not just an abundance effect, due to an increase in the number of native mammals. We hypothesize that the high infection level in the period 1999–2000 might be the result of a time-lag response in the *T. cruzi* transmission cycle (i.e., mammal hosts-vector-parasite) from a major El Niño event occurred in 1997–1998 (Lima et al. 2003). It is likely that during El Niño events infected vectors have more chance to infect mainly defenseless newborn uninfected mammals increasing overall infection levels in the host population, considering that the pathogen can be acquired and transmitted efficiently by all the mammal hosts (i.e., similar host competence) (Keesing et al. 2006).

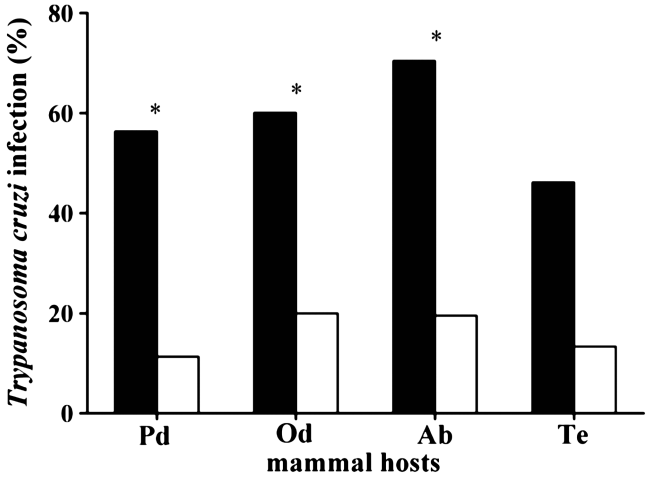

FIG. 1. Percentage of *Trypanosoma cruzi*–infected individuals for each mammal species during 1999–2000 (black bars) and 2005–2006 (white bars). Pd, *Phyllotis darwini*; Od, *Octodon degus*; Ab, *Abrothrix* sp. (including *A. longipilis* and *A. olivaceus*); Te, *Thylamys elegans*. Asterisks indiacte significant difference between periods ($p < 0.0001$).

Several studies of infectious disease outbreaks have considered ENSO event as a potential exogenous factor influencing disease cycles. For example, an 18-year time series of cholera cases in Bangladesh revealed a 3.7-year period between peaks of disease incidence, a cycle correlated with ENSO (Pascual et al. 2000). Infectious vector-borne diseases are generally sensitive to climatic conditions. Reproduction, lifecycle, and survival of several vectors are constrained by temperature and humidity (McMichael 2003). Nevertheless, variation in the incidence of vector-borne diseases is a much more complex scenario to examine (Zell 2004).

In summary, we suggest that major El Niño events may have a time-lag effect on the wild transmission cycle of Chagas disease in semiarid Chile. During El Niño events, outbreaks of mammals have been reported, implying that more potential hosts are available for Chagas disease vectors. We are aware that a 2-year comparison is not enough to make strong conclusions about the Chagas disease cycle, but information in this complex parasite-vector-host system represent an important step to understand the factors underlying the epidemiology and dynamic of this multihosts infectious disease in a changing climatic scenario.

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Disclosure Statement

No competing financial interests exist

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