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What is the role of small rodents in the transmission cycle of *Trypanosoma cruzi* and *Trypanosoma evansi* (Kinetoplastida Trypanosomatidae)? A study case in the Brazilian Pantanal

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ABSTRACT

Determining the reservoir hosts for parasites is crucial for designing control measures, but it is often difficult to identify the role that each host species plays in maintaining the cycle of infection in the wild. One way to identify potential maintenance hosts is to estimate key parameters associated with transmission and pathogenicity. Here we assess the potential for three native rodent species of the Brazilian Pantanal (*Clyomys laticeps*, *Thrichomys pachyurus* and *Oecomys mamorae*) to act as reservoir or maintenance hosts of *Trypanosoma evansi*, an important parasite of domestic livestock. By analyzing blood parameters of naturally infected wild-caught rodents of these species, we compared their levels of parasitemia and anemia due to *T. evansi* infection with literature values for other host species infected by this parasite. We also analyzed levels of these blood parameters relative to infection by *Trypanosoma cruzi*, the causative agent of Chagas disease in humans, for which wild rodents are already thought to be important reservoir species. All three species showed low impacts of the two trypanosomes on their blood parameters compared to other species, suggesting that they experience a low virulence of trypanosome infection under natural conditions in the Pantanal and might act as maintenance hosts of trypanosome infections. The low parasitemia of trypanosome infections suggests that these rodents play a secondary role in the transmission cycle compared to other species, especially compared to the capybara (*Hydrochaeris hydrochaeris*) which also experiences low pathogenicity due to infection despite much higher levels of parasitemia.

1. Introduction

The two common South American trypanosomes *Trypanosoma evansi* and *Trypanosoma cruzi* infect a range of mammalian host species, and cross-species transmission is common since vectors bite multiple host species (Hoare, 1972; Barretto, 1979). These parasites have widespread distributions and are primarily parasites of free-living mammals. *T. cruzi* causes Chagas disease in humans and is transmitted mainly via the feces of infected blood sucking triatomine bugs. *T. cruzi* transmission can also occur trophically when a predator ingests the infected blood or tissues of an infected mammal or insect. *T. evansi*, the etiologic agent of wasting disease in horses and dogs (locally called *Mal de Cadeiras*), is mechanically transmitted by tabanid flies. Since vector transmission is mechanical, it is thought to be dependent on high parasitemias in the mammalian host.

Identifying reservoir and maintenance hosts for zoonotic parasites are difficult because measuring cross-species transmission and long-term persistence within host populations requires large-scale and technically challenging studies. However, one can narrow down potential candidates by examining characteristics of host–parasite interactions which tend to differ between hosts. *T. evansi* has proven to be highly pathogenic in nearly every wild host species studied. This parasite has been shown to cause generalized loss of body condition and immune suppression coupled with anemia in both domestic and wild mammals (Silva et al., 1995, 1997; Holland et al., 2001; Aquino et al., 2002; Espinoza et al., 2002; Herrera et al., 2002; Menezes et al., 2004; Onah et al., 1996). The one clear exception is the capybara (*Hydrochaeris hydrochaeris*), which is the only mammal tested to date that has lacked evidence of anemia in natural or experimentally induced infections despite high levels of parasitemia, measured by the number of trypanosomes

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per ml of blood (Franke et al., 1994a,b; Herrera et al., 2004). Coatis and capybaras are considered as the most significant reservoirs for *T. evansi* (Franke et al., 1994b; Silva et al., 1995). For *T. cruzi* several mammalian species are considered as important sources of infection to humans. Although it is described that small wild mammals are good reservoir hosts for *T. cruzi* (Cortez et al., 2006), their role in the parasitic web of *T. evansi* is still unknown (Coura et al., 2002; Herrera et al., 2004, 2005).

The aim of this study was to determine the likely role of free-living rodents in the maintenance of *T. cruzi* and *T. evansi* of three common rodent species, two spiny rats, *Thrichomys pachyurus* and *Clyomys laticeps* (Rodentia: Echimyidae), and one arboreal rice rat, *Oecomys mamorae* (Rodentia: Sigmodontinae), in the Brazilian Pantanal. Like capybaras, these rodents are native to Brazil and probably have a long evolutionary history of infection by both *T. evansi* and *T. cruzi* (Teixeira et al., 2006). These are also the most abundant small mammal species in the Pantanal region and are frequently found around or inside human dwellings, making them potential reservoirs of infection for humans and domestic animals. To assess whether these species are potential maintenance hosts for *T. evansi* or *T. cruzi*, we examined anemia (reduction in erythrocyte numbers and hematocrit), lymphocytosis (increase in lymphocyte counts), and the change in the mean corpuscular volume in these wild-caught rodents as direct measures of pathogenicity and indirect measures of parasite induced mortality. Then we compared parasitemia levels and anemia in these rodent species to the results of previous studies of mammals naturally and experimentally infected with *T. evansi* (Franke et al., 1994a; Silva et al., 1995, 1997; Holland et al., 2001; Aquino et al., 2002; Espinoza et al., 2002; Herrera et al., 2002; Menezes et al., 2004; Onah et al., 1996; Rodrigues et al., 2005), in order to assess whether these species have the low anemia coupled with high parasitemia that would make them ideal maintenance hosts for *T. evansi*.

2. Materials and methods

2.1. Study area

The study was undertaken from 2002 to 2003 at two locations in the southern Pantanal 120 km apart: Rio Negro farm (19°34′54″S and 56°14′62″W), with an area of 7700 ha and active conservation management, and a cattle ranching area of 20,000 ha (19°08′28″S and 56°49′23″W) where traditional cattle management practices include grass fires and selective tree cutting. The vegetation in both locations is a mosaic of Cerrado (savannah-like forest), grassland patches and semi-deciduous forest, with gallery forests alongside the rivers. The climate is sub-humid tropical, with heavy seasonal rainfall. The average annual rainfall reaches approximately 1100–1400 mm, 80% of which occurs during the wet season. During the wet season, much of the open grassland becomes flooded, whereas during the dry season only a few pools and creeks remain.

2.2. Capture methods

All animal procedures were undertaken in accordance with regulations, and licenses obtained from the Brazilian Government Institute for Wildlife and Natural Resource Care (IBAMA). Rodents were captured twice on each farm, once during the dry season and again during the wet season. Traps were set for seven consecutive nights on linear transects, each of which included 20 trapping stations spaced 10 m apart. Transect locations were chosen to obtain a representative sample of different habitat types. At each station, two traps were placed next to each other on the ground, one Tomahawk® (model 201, 16 in. × 5 in. × 5 in.) to catch large rodent species and one Sherman® (model XLK 3 in. × 3.75 in. × 12 in.) to catch small species. In forested areas, we placed one additional trap in a tree at each station, a Tomahawk® trap on odd numbered station and a Sherman® trap on even numbered stations. Traps were baited with a mixture of banana, peanut butter, oat meal and bacon and checked daily. For each animal captured, species, sex, reproductive condition, body mass, body length, hind foot length, ear length, identifying characteristics and trapping station were recorded.

2.3. Blood analyses

Captured animals were anesthetized with Zoletil®50 (Virbac) and blood collected via heart puncture prior to euthanasia. Blood samples were stored in tubes with anti-coagulant (EDTA) and were kept under refrigeration at 4 °C until analyzed within 12 h from collection. Sample sizes for the analysis were reduced when insufficient blood could be collected or the blood became contaminated.

We searched for trypanosomes using serological and parasitological methods with priority given to the latter. We considered an animal infected when it was positive in at least one of the methods used. A complete analysis of the serological results and prevalence of *T. cruzi* and *T. evansi* between farms using data from all rodent species collected has been described in another paper (Herrera et al., 2008).

Blood samples were analyzed for the presence of trypanosomes as described by Herrera et al. (2008), using two semi-quantitative methods, hemoculture and microhematocrit centrifuge technique (Woo, 1970) and serological assay. Counts of erythrocytes and leukocytes were performed using a Neubauer chamber, and hematocrit was obtained by capillary tube centrifugation of 100 μl blood samples. The latter samples were also examined for hemoparasites following the microhematocrit centrifuge technique. Mean cell volume was estimated as the hematocrit value multiplied by 10 and divided by erythrocyte counts divided by 10^6. The remaining blood was centrifuged and the plasma stored at −20 °C until serological analysis. Blood cell counts, serum collection and the microhematocrit centrifuge technique were undertaken in the field laboratory within 12 h of blood collection. Further analyses were undertaken at the Laboratório de Biologia de Tripanosomatídeos, FIOCRUZ, Rio de Janeiro. The hemoculture for *T. cruzi* was analyzed two times monthly for 5 months or until trypanosomes were detected. The immuno-fluorescence antibody test for detecting IgG was conducted according to Camargo (1966).

The immuno-fluorescence antibody test (IFAT) for detecting IgG was conducted. Briefly, serial twofold sera dilutions (1:10–1:1280) were assayed against trypanosomes antigen deriving from axenic medium (*T. cruzi*) or ionic exchange column (*T. evansi*). Control serum samples were obtained from animals born at a wildlife breeding center located in the Oswaldo Cruz Institute, before (negative controls) or after experimental infection (positive controls). Standardization of the protocol was undertaken in order to establish suitable working dilutions for each specific fluorescein antibody conjugate. We used anti-rat IgG (FITC, Sigma®) for rodents species. The cut-off values for *T. cruzi* and *T. evansi* were: 1:10 for rodents, since it was the lowest serum sample titer of rodents in which parasites could be detected by MHCT or BC, as described elsewhere (Herrera et al., 2004). Serum samples were assayed separately against *T. cruzi* and *T. evansi* antigen according to Herrera et al. (2008).

2.4. Statistical analysis

To examine the influence of *T. evansi* and *T. cruzi* on host condition, expressed by blood parameters, we used generalized linear models (GLMs). For each response variable (hematocrit, lymphocyte and erythrocyte counts, and mean cell volume) a model was built including trypanosome infection (*T. evansi* or *T. cruzi*). We also