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# *Trypanosoma cruzi*: Distinct patterns of infection in the sibling caviomorph rodent species *Thrichomys apereoides laurentius* and *Thrichomys pachyurus* (Rodentia, Echimyidae)

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**Abstract**

*Thrichomys apereoides*, a caviomorph rodent species common in a highly endemic area for Chagas disease in Brazil, may act as reservoir of the parasite. However, no information is available concerning its sibling species *Thrichomys pachyurus*, found in the Pantanal region, where *Trypanosoma cruzi* is found only in the enzootic cycle. We followed up the cross infection of these cryptic species with two isolates derived from naturally infected *T. pachyurus* and *Thrichomys apereoides laurentius*. No regional co-adaptation between *Thrichomys* species and the regional isolates were noticed. However, significant differences in the outcome of the infection were observed. *T. a. laurentius* was more resistant than *T. pachyurus*, as expressed by lower parasitemia and less histopathological damage. The routine biochemical markers used for laboratory rodents were unsuitable for follow up of infection in *Thrichomys* spp, since they did not correlate with the histopathological findings or allowed the kinetic follow-up of tissue colonization by the parasite.
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*Index descriptors and abbreviations:* *Trypanosoma cruzi*; Caviomorphs; *Thrichomys*; Sibling species; Reservoir; Hematology, Biochemistry; Histopathology; Kinetoplastida; $CO_2$, carbonic gas; ALT, alanine aminotransferase; CK-MB, creatine kinase fraction MB; CK-NAC, total creatine kinase; DPI, days post infection; FICT, fluorescein isothiocyanate; IFA, immunofluorescence assay; IgG, immunoglobulin G; IM, intra-muscular; LIT, liver infusion tryptose medium; MCH, mean corpuscular hemoglobin; MCHV, mean corpuscular hemoglobin value; MCV, mean corpuscular value; Myr, million years; NNN, Novy-Mc Neal-Nicole medium; RBC, red blood cells; WBC, white blood cells

## 1. Introduction

*Trypanosoma cruzi* (Kinetoplastida, Trypanosomatidae), the etiological agent of Chagas disease, is a heteroxenic hemoflagellate protozoan that infects over 200 mammalian species from seven different orders in the Americas, including man. This autochthonous and ancient protozoan of wild mammals is transmitted by wild vectors (Hemiptera, Reduviidae, Triatominae) in a primary sylvatic cycle (Pinto Dias, 2000; Stevens et al., 1998).

*Trypanosoma cruzi* includes two major groups or genotypes, *T. cruzi* I (TCI) and *T. cruzi* II (TCII), described as mainly associated, in Brazil, with sylvatic and domestic transmission cycles, respectively (Fernandes et al., 1998). This dichotomy is defined, among others, by its mini-exon gene sequences and isozymic patterns, or zymodemes (Anonymous, 1999; Fernandes et al., 1998; Miles et al., 1977). The divergence between

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*T. cruzi* I and *T. cruzi* II is supposed to have occurred after caviomorphs and primates reached South America from Africa about 38 myr ago (Briones et al., 1999; Flynn and Wyss, 1998; Lavocat, 1974). Therefore, in addition to marsupials, caviomorph rodents and/or primates are recognized to be the most ancient hosts of *T. cruzi* (Briones et al., 1999; Buscaglia and Di Noia, 2003).

Caviomorph rodents from the *Thrichomys* genus have been found in various Brazilian biomes: savannah ("Cerrado"), white shrub ("Caatinga") and marshland ("Pantanal") (Alho, 1982). *Thrichomys* was considered a monospecific genus (Woods, 1993) until 2002, when Bonvicino et al. (2002) suggested a distinction into four species and subspecies. The species used in the present study were: *Thrichomys pachyurus* from west Brazil (Mato Grosso do Sul state and north of São Paulo state), where *T. cruzi* is found only in the enzootic cycle, and *Thrichomys apereoides laurentius* from northeast Brazil (mainly Piauí, Ceará and Pernambuco), an endemic area of Chagas disease. These species are morphologically indistinguishable and therefore considered cryptic and/or sibling species. Natural foci of high *T. cruzi* transmission rates, maintained by *T. a. laurentius* in the wild (Caatinga), have already been described (Herrera et al., 2005). Moreover, in experimental conditions, *T. a. laurentius* was able to maintain stable infections with both *T. cruzi* I and *T. cruzi* II subpopulations (Herrera et al., 2004).

The definition of *Thrichomys* or any other mammal as a reservoir comprises more than data on prevalence of infection and ability to maintain the parasite in captive host populations. Indeed, as proposed by Ashford (1997), a reservoir should be considered as an ecological system (represented by one or more species) able to maintain a given parasite in nature. This system must be long-lived and abundant, forming a large proportion of the mammalian biomass.

Regional host–parasite co-adaptation is frequently described in invertebrate hosts, as already demonstrated for *T. cruzi* and triatomines (Ryckman, 1985); and *Shistosoma mansoni* and planorbids (Barbosa and Barreto, 1960), but this aspect is still poorly understood considering the interaction of parasites with their mammalian hosts, especially with geographically separated sibling species. To better understand at least some features of the complex interaction patterns between *T. cruzi* and its multiple hosts, our main aims were to: (i) evaluate a possible regional co-adaptation of *T. a. laurentius* and *T. pachyurus* experimentally infected with isolates derived from naturally infected *Thrichomys*; (ii) evaluate the differences of experimental infection of these two sibling species with a *T. cruzi* isolate considered as a reference strain (MHO/BR/1950/Y); (iii) establish normal hematological and biochemical parameters for *Thrichomys*; (iv) follow-up the health status of experimentally infected animals by routine hematological and biochemical markers; (v) determine the kinetics of tissue colonization by the parasite, using biochemical tissue markers of injury (ALT and CK), described as useful tools in the follow-up of *T. cruzi* infection in inbred laboratory mice (De-Souza et al., 2000; Paiva et al., 2003).

## 2. Materials and methods

### 2.1. Animals

*Thrichomys apereoides laurentius* ($n=18$) and *T. pachyurus* ($n=17$), born at animal facilities of the Department of Tropical Medicine, Oswaldo Cruz Institute, Brazil, were used. The colonies were derived from wild *T. a. laurentius* and *T. pachyurus* caught in the Piauí and Mato Grosso do Sul states, respectively. The animals were kept under conventional conditions (temperature $24\pm2$ °C, natural daylight). Single animals were housed in $41\times34\times17$-cm polycarbonate cages with sawdust substrate and were fed with NUVILAB CR1 mouse pellets (Nuvital nutrients S.A., Colombo/PR, Brazil) and water "ad libitum." The animal facilities are supported by IBAMA license 02022.002062/01-04. All procedures followed protocols approved by the FIOCRUZ Committee of Biosafety and Committee of Bioethics (license P0226/04).

### 2.2. Parasites

The following *T. cruzi* isolates were used: (i) MHO/BR/1950/Y(Y)—a human isolate considered as reference strain; (ii) MTHR/BR/1999/R4(R4)—an isolate derived from naturally infected *T. a. laurentius* (PI); (iii) MTHR/BR/2002/FRN38(FRN38)—an isolate derived from naturally infected *T. pachyurus* (MS). All of them were classified in agreement with Anonymous (1999) and maintained in liquid nitrogen.

### 2.3. Inoculation schedule

Animals from both species were divided into three batches ($n=5$–6) and inoculated subcutaneously with 200 metacyclic forms/g body weight, when 55 days old (140 g mean weight). Metacyclic forms were derived from cultures in the stationary phase in NNN medium with an LIT overlay as described by Luz et al., 1994.

### 2.4. Parasitological follow-up

Blood samples from tail tips of infected animals were microscopically examined as follows: (i) fresh blood samples were examined and parasites quantified (glass slide with $18\times18$-mm coverglass); (ii) blood samples were diluted and counted in a hemocytometer (Hoff, 1974). These procedures were performed using an optical microscope at 400× magnification, three times a week,

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