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Research brief

Sexual transmission of *Trypanosoma cruzi* in murine model

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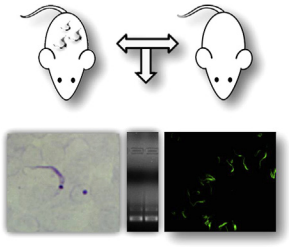
HIGHLIGHTS

- Naive mice crossbreed with infected partner show anti-*Trypanosoma cruzi* antibodies.
- *T. cruzi* DNA is in all studied couples.
- Immunohistochemistry show the parasite in testicles.
- We report *Trypanosoma cruzi* sexual transmission in murine model.

GRAPHICAL ABSTRACT



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ABSTRACT

Trypanosoma cruzi is mainly transmitted by blood-sucking triatomines, but other routes also have epidemiological importance, such as blood transfusion and congenital transmission. Although the possibility of sexual transmission of *T. cruzi* has been suggested since its discovery, few studies have been published on this subject. We investigated acquisition of *T. cruzi* by sexual intercourse in an experimental murine model. Male and female mice in the chronic phase of Chagas disease were mated with naive partners. Parasitological, serological and molecular tests demonstrated the parasites in tissues and blood of partners. These results confirm the sexual transmission of *T. cruzi* in mice.

1. Introduction

Sexually transmitted infections (STIs) place a heavy economic burden on health care systems worldwide. Several organisms are classified as causative agents of STIs, including viruses, bacteria, fungi and others parasitic pathogens. Among the pathologic protozoa, the main species transmitted through sexual intercourse is *Trichomonas vaginalis*, the etiological agent of trichomoniasis, with 248 million new cases per year (WHO, 2005). In addition, parasites such as amoebas and *Giardia* spp. can be transmitted by anal-oral intercourse (Levine, 1991). Some reports also have considered the possibility of venereal transmission of other protozoans, especially trypanosomatids. Studies in horses have demonstrated that transmission of *Trypanosoma equiperdum* occurs directly from animal to

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animal during coitus (Katz, 2008). Furthermore, other studies have demonstrated sexual transmission of *Leishmania* spp. in dogs (Silva et al., 2008; Turchetti et al., 2014), showing the possibility of venereal transmission of this flagellate without the participation of the insect vector. Thus, further research should be conducted to determine the sexual transmission of other protozoans.

Chagas disease (CD), caused by *Trypanosoma cruzi*, is a major parasitic disease in Latin America. It is estimated that about 8 million people infected with the parasite worldwide, and 100 million are at risk of acquiring infection (WHO, 2014). Initially considered a rural endemic disease, CD has spread to cities due to urban migration. Some countries free of vector transmission have also been affected by this disease due to immigration, a process that has been described in Australia, Canada, United States and Europe. Therefore, CD can be considered an emerging disease in developed countries (WHO, 2014).

CD consists of two clinical phases: acute and chronic. Acute infection is often asymptomatic and self-limited, but some symptoms may arise, such as fever, fatigue, body aches, headache, rash, loss of appetite, diarrhea and vomiting. The chronic phase lasts several years and can remain asymptomatic. In 20–30% of cases, however, clinical changes can be observed in many organ systems and tissues, including the heart and digestive system (causing the so-called "mega syndromes" of megacolossus and megaesophagus), and lead to premature death, usually due to cardiac dysfunction. Unfortunately, Chagas remains a neglected disease. There are no vaccines available; few anti parasitic drugs are effective; and, even so, can only mitigate the manifestations of the acute phase of the disease, leaving millions of individuals who have been infected for decades without appropriate care (Teixeira et al., 2011). The best way to fight CD is thus to interrupt the various routes of parasite transmission.

T. cruzi is mainly transmitted through contaminated feces of blood-sucking bugs after haematophagy. These insects belong to the family Reduviidae, subfamily Triatominae, which contains more than 150 species (Justi et al., 2014). Most are capable of transmitting the infection. The range of triatomines extends from the southern United States to southern Patagonia, and they are common throughout Latin America (WHO, 2014). *T. cruzi* endemicity is also associated to other modes of transmission, such as blood transfusions, laboratory accidents, organ transplantation, and congenital and oral transmissions. In non-endemic areas, blood transfusion is the major route of transmission in countries with substantial populations of Latin American immigrants, including the United States, Spain, France, Switzerland and Canada. Vertical transmission of *T. cruzi* is also an important route for the spread of CD, even in non-endemic countries. It is estimated that congenital transmission of *T. cruzi* occurs in 5–10% of pregnancies of patients with CD. Oral transmission has been implicated in outbreaks of acute human CD often associated with severe illness (Sánchez and Ramirez, 2013).

The possibility of sexual transmission of *T. cruzi* has been discussed since the discovery of CD. However, few studies on the subject have been published. The first report was made by Vianna (1911), who observed the parasite in testes and found it to cause a series of histological changes therein. Amastigotes were also identified in histopathological sections of seminiferous tubes and ovarian theca cells of children who succumbed to acute CD (Teixeira et al., 1970). The presence of *T. cruzi* in menstrual blood has been demonstrated and is evidence in favor of sexual transmission (Jörg and Oliva, 1980). Other studies reported colonization of the urogenital tract of guinea pigs with the parasite: mice infected with different strains of *T. cruzi* showed a heavy parasite burden in ovaries, testes, interstitial cells, and seminiferous tubules (Carvalho et al., 1991, 2009). Inoculation of semen from infected mice into the peritoneal cavity of healthy animals has been found to generate amastigote nests in various tissues (Carvalho et al., 2009). Additionally, molecular tests have detected the presence of *T. cruzi* nuclear DNA in the semen of infected individuals, strengthening the hypothesis of sexual transmission of the parasite (Hecht et al., 2010). Investigation for *T. cruzi* tissue parasitism of immunosuppressing female mice crossbred with acutely infected male showed a scarce probability of male-to-female *T. cruzi* sexual transmission (Martin et al., 2015). Nevertheless, sexual transmission of the *T. cruzi* has not been proven conclusively to date, especially in the chronic phase of CD.

2. Materials and methods

2.1. Animals and groups

We investigated sexual transmission of *T. cruzi* in a murine model by mating chronically infected mice with healthy partners. Ten sexually mature BALB/c mice (five males and five females) were inoculated intraperitoneally with 1×10^3 *T. cruzi* Berenice trypomastigote forms. These animals were mated with naive partners 90 days post infection (dpi) and twice more in subsequent fertile periods (120 and 190 dpi) (Fig. S1). In total, were used 28 BALB/c mice, homogeneous with respect to weight and age, which were allocated into groups as follows: A) five *T. cruzi*-infected males mated with five naive females (animals 1 to 10); B) five *T. cruzi*-infected females mated with five naive males (animals 11 to 20); C) two negative couple (non-infected); and D) two positive couple (infected by intraperitoneal route). To confirm the occurrence of sexual transmission, we also assessed 26 mice from group A progeny (Group E). All procedures were approved by the Institutional Animal Care and Use Committee (UnB Protocol 10411/2011) and conducted in accordance with international guidelines.

2.2. Parasitological and serological assays

The presence of *T. cruzi* in mice peripheral blood was evaluated by microscopic examination of blood smear. Detection of anti- *T. cruzi* antibodies was performed by enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescence (IFI), following protocols previously standardized in our laboratory (Hecht et al., 2010). Briefly, ELISA was performed with 96-well plates sensitized with 50 μL of trypomastigote soluble cell antigens (4 μg/mL). Specific antibodies were detected in diluted serum (1: 100) after incubation with *T. cruzi* antigens for 2 h at room temperature. Second antibody consisted of 1:5000 dilution of peroxidase-conjugated anti-mouse IgG (Sigma–Aldrich®). After incubation for 90 min at 37 °C, the substrate OPD (Sigma–Aldrich®) was added and optical densities (OD) were read at 490 nm. Cut-off was calculated according Nybo, 2010. Test and control sera assays were run in triplicate. IFI consisted in the incubation of serum dilutions (1:20 to 1:360) with epimastigotes formalin-killed fixed in glass slides. After 1 h incubation at 37 °C, slides were treated with 1:200 FITC-labeled IgG (Sigma–Aldrich®). A positive apple-green reaction under the epifluorescence microscope (Olympus, BX51 Model, Tokyo, Japan) indicated a positive reaction in serum dilutions 1:40 and above.

2.3. Nucleic acid analyses

For molecular experiments, 200 μL of blood from each animal was obtained by cardiac puncture and DNA extraction was conducted using Wizard Genomic DNA Purification Kit® (Promega, USA) according to manufacturer instructions. Tissues from heart and testicles/ovary were also collected and DNA extraction was