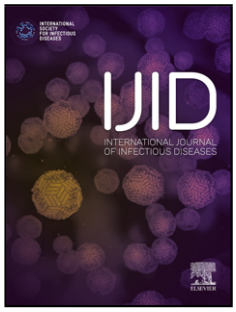

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American Trypanosomiasis and Chagas disease: sexual transmission

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Highlights

- The *Trypanosoma cruzi* infection can be sexually transmitted from males and females to naive mates.
- The *T. cruzi parasites* were detected in Chagas individual semen ejaculates by nucleic acid techniques.
- Human Chagas semen aliquot instills into the vagina of naïve female mice render *T. cruzi* infections.
- Breeding *T. cruzi*-infected males and females vertically transmitted the infections to progeny mice.

ABSTRACT

Objective: To contribute to the discussion on the research findings that indicate the American Trypanosomiasis and Chagas disease can be sexually transmitted in humans.

Methods: A review of the literature about the routes of transmission of *Trypanosoma cruzi* parasites and to evaluate the dispersion of Chagas disease now in five Continents.

Main finding: The epidemiologic profile of American Trypanosomiasis, yet considered a neglected disease of the poor people of Latin America, changed over time. A family-based study demonstrated the blood protozoan *T. cruzi* can be transmitted sexually from infected males and females to naïve mates.

Conclusion: Evidence that Chagas disease can be sexually transmitted coupled with the migration of individuals with Chagas disease to previously non-endemic countries, and as well of travelers to endemic countries, has public implication that requires improved screening of blood supplies and prenatal care to prevent congenital spread.

Key words: *Trypanosoma cruzi*; Chagas disease; Sexual transmission; Humans; Mouse model system; Vertical transmission; Diagnosis; Prevention.

The American Trypanosomiasis is endemic to people in rural areas of South America, where *T. cruzi* parasites are sympatric to the hematophagous triatomine insect-vectors (Prata 2001; Coura 2010; Teixeira et al. 2018). Migration of *T. cruzi*-infected people to the Northern Hemisphere and of travelers to endemic countries, probably, made American Trypanosomiasis a global challenge, since these infections can be transmitted from mother to offspring (Murcia et al. 2013), by blood transfusion and donated organs, and by contamination of hospital and laboratory workers (Teixeira et al. 2011a). Chagas disease is a social and economic burden, and specialized clinical centers in various countries have generated skilled personnel for provision of health care to patients and their families (Repetto 2015; Grigorenko 2014; El Ghouzi 2010). The expertise stemming from those national centers are deemed important, because the problems associated with the emergence of Chagas disease can no longer be underestimated (Hotez 2012; Teixeira et al. 2011a, 2009).

Acute *T. cruzi* infections are usually asymptomatic and unrecognized, although approximately 5% of the infected children may show fever, headache, drowsiness, tachycardia, edema, and shortness of breath (Perez-Molina, Molina 2018; Teixeira et al. 2011a, 2006). Morbidity and mortality in the acute phase of the infection are low, since an average of four deaths due to the acute disease was recorded each year in the past three decades (Andrade et al. 2014). The chronic intermediate phase of the lifelong *T. cruzi* infections ensues in the absence of clinic manifestations. However, approximately 30% of the chronically infected people develop Chagas disease. Chronic Chagas disease kills people due to megaviscera and heart failure (Coura 2010; Prata 2001);

polyneuropathy, and neuroendocrine syndromes are more rarely seen. (Perez-Molina, Molina 2018; Teixeira et al. 2011a).

Several studies showed the course of *T. cruzi* infections and pathological consequences upon inoculation of a few parasites in dogs (Marsden & Hagstrom 1968; Lana et al. 1988), primates (Falasca et al. 1990), and rabbits (Lauria-Pires 1995). Additionally, a family based study protocol was proposed to disclose chronically infected individuals with low load of parasites (Araujo et al. 2017). The nuclear DNA –PCR (nDNA-PCR), *Southern* hybridization, cloning and sequence of a specific 188-nucleotides (-nt) telomere repeat validated the diagnosis of all *T. cruzi* infections. The point of care of these techniques assures high sensitivity and specificity of the diagnosis of Chagas parasites (Almeida et al. 2018). The in-house nDNA-PCR technology detects as few as 1/10 of the total (270 fg) DNA of a single diploid *T. cruzi*, only (Araujo et al. 2013; Hecht et al. 2010, Castro 2009). In the family study the diagnosis of sexually transmitted Chagas disease was consolidated by nDNA-PCR detecting *T. cruzi* 188-nt telomere sequence in blood samples obtained at three different occasions one year apart (Araujo et al. 2017).

The research protocol approved by the Faculty of Medicine Ethical Committees in Human and Laboratory Animal Research set forth the investigations. Semen samples were obtained from nDNA-PCR positive male volunteers with the 188-nt telomere sequence present in the ejaculates: instills of aliquots of semen from Chagas positive donors into the vagina of females, and instills into the peritoneal cavity of males rendered *T. cruzi* infections into mice; the pathology revealed the parasite amastigote nests in the heart, skeletal muscle, *vas deferens* and uterine tube (Figure 1).

A word of caution is required; because the enzyme-linked immunosorbent assay (ELISA) and the indirect immunofluorescence (IIF) failed to detect *T. cruzi* antibody in a majority of chagasic subjects (Almeida et al. 2018). In the absence of specific antibody, the immune tolerance present in a majority of study family subjects is a consequence of the sexual transmission of *T. cruzi* infections in early embryo previous to development of the immune system. In this regard, the diagnosis of a majority of the progeny from Chagas parent could

not be accomplished by the immunoassays. Contrarily, the presence of *T. cruzi* antibody in a minority of chagasics indicates that *T. cruzi* parasites reached the fetus with a mature immune system (Almeida et al. 2018, Araujo et al. 2017, Guimaro et al. 2014, Teixeira et al. 2011b). Therefore, employment of immunoassays alone could underdiagnose congenital cases of Chagas disease. With this respect, the safety health facilities should rely on the nucleic acid techniques to discard blood contaminated with *T. cruzi* 188-nt telomere sequence and Chagas disease.

The investigations in the mouse model showed *T. cruzi* forms expelled through semen ejaculates (Figure 2); further experimental study suggested that males and females could transmit the Chagas parasites present either in the semen ejaculates or in the vagina fluids (Araujo et al. 2017; Teixeira et al 1970).

Moreover, *T. cruzi*-infected males and females sexually transmitted Chagas parasites to naïve mice mates in three series of independent experiments (Araujo et al. 2017, Rios et al. 2018): chagasic males and females mice bred naive females and males founders (F0) that generated F1 progeny, and further breeding generated F2 progeny with the nDNA-PCR positive 188-nt telomere repeat sequence, thus showing vertically acquired *T. cruzi* infections. The breeding experiments confirmed the absence of *T. cruzi* antibody in 78% of F1 and F2 progeny mice, and the pathology revealed *T. cruzi* amastigote nests in the reproductive system (Araujo et al. 2017; Almeida et al. 2018). Several studies corroborate sexual transmission of *T. cruzi*-infections of males and females to naive mice mates (Rios et al. 2018; Ribeiro et al. 2017; Martin et al. 2016; Araujo 2013).

Remarks:

- The sexual transmission of *T. cruzi* infections is a potential threat to public health worldwide.
- Specialized clinical centers are needed, because the emergence of Chagas disease can no longer be underestimated.
- The nDNA-PCR confirmed by *Southern* hybridization, cloning and sequence secures the diagnosis of all *T. cruzi* infections.

- A thorough-put digital platform is needed for the diagnosis of Chagas disease, epidemiologic enquires, and disclaims of contaminated blood.
- A robust education, information and communication program shall prevent sexually transmitted *T. cruzi* infections and Chagas disease.
- The perspective is that the control of Chagas disease requires international solidarity.

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Ethical Approval: Not applicable.

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Figure 1. The infectivity of the Chagas patient's ejaculates to naïve mice upon instills of nDNA-PCR positive semen aliquots into the vagina. Notice the clumps of the *T. cruzi* amastigotes (arrows) in the heart (top left), skeletal muscle (top right), into the lumen of the *vas deferens* (bottom left) and in the epithelial cells of the uterine tube (bottom right). The circle shows a dividing amastigote. Bars, 10 μm. Reprinted with the permissions from the Author and the publisher (Araujo et al. Mem Inst Oswaldo Cruz, 2017).

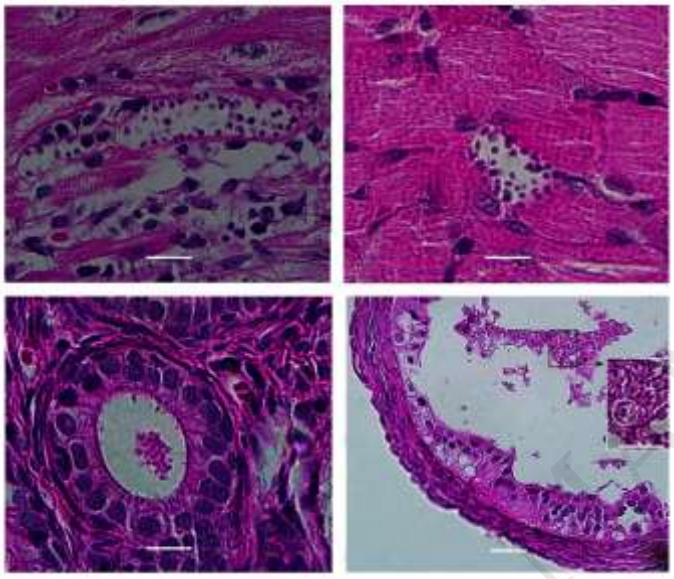

Figure 2. The *Trypanosoma cruzi* in semen ejaculates of chagasic male mouse. The arrows show: A) Amastigotes; B) Trypomastigote. The figure with modification is reprinted with the permissions from the author and the publishers (Alarcon et al. Bol Malariol Salud Amb, 2011).