

Letter to Editor

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/apjtm.apjtm_524_25

Experimental model of a *Trypanosoma cruzi* strain *via* transplacental and breastfeeding transmission

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It is estimated that at least two million women of childbearing age in Latin America are chronically infected with *Trypanosoma (T.) cruzi*, representing a persistent risk of congenital transmission[1]. Vertical transmission has been associated with maternal immune dysfunction, which may compromise foetal development and increase the risk of adverse outcomes[2]. According to the Brazilian consensus on Chagas disease (CD), breastfeeding represents a potential route of transmission when the parasite is present in milk, especially during the acute phase or in cases of nipple lesions with bleeding[3,4]. However, experimental evidence in lactating females during the acute phase is scarce, and reports of transmission *via* breastfeeding remain inconclusive. Despite global efforts to control CD, the disease continues to pose a public health challenge, particularly due to the asymptomatic nature of chronic infections and the lack of definitive evidence regarding transmission dynamics during pregnancy and lactation.

In this context, we conducted an experimental study using BALB/c mice to investigate the potential for *T. cruzi* transmission *via* the placenta and breastfeeding. Eighteen neonates were evaluated 21 days after birth and divided into two groups: Group 1, comprising neonates born to infected females and nursed by uninfected females, to assess vertical transmission; and Group 2, comprising neonates born to uninfected females and nursed by infected females, to evaluate transmission through breastfeeding. The infected females received an intraperitoneal inoculation of 10^4 trypomastigotes/mL of the ZMC [ZMC refers exclusively to the initials of the patient from whom the isolate was obtained, serving only as a clinical identifier. The strain was kindly provided by Prof. Dr. Vicente Amato Neto, from the Institute of Tropical Medicine at USP (University of São Paulo).] strain of *T. cruzi* three weeks before delivery. After mating, parturition was induced with oxytocin (5 μL per animal). Neonates were immediately separated from their dams and allocated to groups as described above.

The animals were housed in plastic cages (28 cm × 19 cm × 12 cm) containing autoclaved pine shavings and environmental enrichment with cardboard cones. Conditions were controlled: temperature (25 ± 2) ℃, standard diet, and water *ad libitum*.

After three weeks, neonates were euthanised with Thiopental® (Cristália, Itapira, SP, Brazil) at a dose of 0.03 g per 100 g body weight, diluted in saline and administered intraperitoneally. Following euthanasia, cardiac puncture was performed to obtain approximately 1 mL of blood per animal. Blood samples were pooled from every four animals within the same group for analysis. Additionally, the heart, liver, spleen, brain, intestine, and tongue were collected for imprint preparations and subsequent staining.

Blood samples were processed using the Strout technique. Samples were centrifuged at ~250 × *g* for 5 min to remove red blood cells, followed by centrifugation at ~1 800 × *g* for 10 min. The sediment was examined on histological slides under light microscopy (400×), with 50 microscopic fields analysed per sample.

Tissue fragments (heart, liver, spleen, brain, intestine, and tongue) were pressed onto glass slides, fixed in methanol for 5 min, and

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How to cite this article: Carvalho BC, Kurokawa CS, Santos RM, Lucheis SB. Experimental model of a *Trypanosoma cruzi* strain *via* transplacental and breastfeeding transmission. Asian Pac J Trop Med 2026; 19(5): 231-233.

Article history: Received 18 September 2025 Revision 22 March 2026
Accepted 30 March 2026 Available online 8 April 2026

stained with Giemsa for 30 min. The slides were examined under light microscopy with immersion oil (1 000×) to detect intracellular amastigote forms. Data were analysed using the *Chi*-squared test, with statistical significance set at $P < 0.05$.

All procedures were performed in accordance with ethical principles for animal experimentation and were approved by the Animal Ethics Committee of São Paulo State University (CEUA–UNESP), protocol No. 1417/2022 on August 30, 2022.

A total of 27 animals were included in the study, comprising 10 females infected with the low-virulence ZMC strain and 10 uninfected controls. Near parturition, litters were cross-fostered so that neonates were nursed by females from the opposite experimental condition. After three weeks, blood was collected by cardiac puncture, and organs were processed for imprint analysis. All infected dams were confirmed positive for *T. cruzi* by imprint, with cardiac tissue showing the highest positivity rate (3/5, 60.0%). Although the Strout technique yielded negative results for circulating trypomastigotes, the presence of intracellular amastigotes in tissues indicated a transition from the acute to the chronic phase of infection.

A total of 138 slides were prepared for imprint analysis. The following animals were evaluated: five experimentally infected dams (to confirm infection status), nine neonates born to infected dams and nursed by uninfected dams, and nine neonates born to uninfected dams and nursed by infected dams (Figure 1).

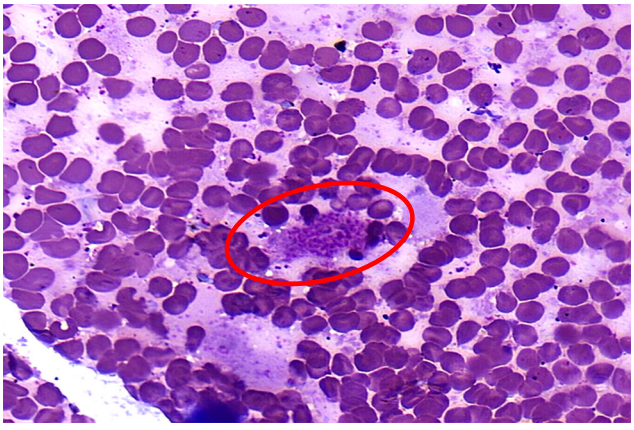

Figure 1. Imprint analysis of liver sections from BALB/c mice stained with Giemsa. The red circle indicates a cluster of amastigotes. Magnification: 100×. Source: author.

All neonates born to infected dams and nursed by uninfected females exhibited at least one infected tissue, confirming vertical transplacental transmission. The most frequently affected organs were the heart and liver(5/9, 55.6%). Similarly, all neonates born to uninfected dams and nursed by infected females also presented at least one infected tissue, suggesting transmission *via* breastfeeding, with the heart being the most frequently infected organ (100%). Table 1 presents the results of the tissues evaluated by imprint analysis across the different groups.

Table 1. Number and percentage of mice positive for the presence of amastigotes in tissues [*n* (%)].

Tissue	Amastigotes		
	Infected dams (*n*=5)	Offspring G1 (*n*=9)	Offspring G2 (*n*=9)
Heart	3 (60.0)	5 (55.6)	9 (100.0)
Spleen	2 (40.0)	3 (33.3)	6 (66.7)
Liver	2 (40.0)	5 (55.6)	6 (66.7)
Tongue	1 (20.0)	2 (22.2)	7 (77.8)
Intestine	2 (40.0)	4 (44.4)	4 (44.4)

G=group; *n*=number of animals. *Chi*-square test; χ^2=0.418; df=3; *P*=0.937. The frequencies of amastigotes in tongue and intestine were combined for the *Chi*-square analysis.

Historically, transmission of *T. cruzi* through breastfeeding has been controversial. Early studies by Nattan-Larrier and subsequent works by Disko, Krampitz, and Miles detected the parasite or antibodies in milk but failed to demonstrate successful transmission to offspring[5–7]. Later experiments by Campos *et al*. and Martins *et al*. reinforced the rarity of this route, suggesting that maternal antibodies transferred *via* milk may provide protection[8,9]. However, our findings demonstrate infection consistent with the chronic phase of CD in neonates nursed by infected dams, indicating that transmission through breastfeeding is possible under these conditions.

In conclusion, our experimental data confirm effective infection of dams by the *T. cruzi* ZMC strain and demonstrate both transplacental and breastfeeding transmission routes, each resulting in 100% infection of neonates. The heart was consistently the most affected organ, followed by the liver and intestine. These results provide novel experimental evidence supporting the hypothesis that congenital and lactational transmission of *T. cruzi* may occur even during the chronic phase of infection, underscoring the need for further investigation into maternal–foetal and postnatal dynamics of parasite transmission to inform public health policies and preventive strategies.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Funding

This work was supported by a specialization grant from the SUS/SP Human Resources Training Center, CEFORSUS/SP.

Authors' contributions

B.C.C., R.M.S., and S.B.L.: Conceptualization, experiments, and manuscript drafting; R.M.S. and C.S.K.: Data analysis; All authors approved the final version of the manuscript.

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Edited by Zhang Q, Lei Y, Pan Y