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# Histopathological Changes in the Placentas and Fetuses of Mice Infected with *Trypanosoma cruzi* Isolated from the *Myotis nigricans nigricans* Bat

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**Summary**

Histopathological changes and placental transmission were studied in the late stages of pregnancy in mice infected with a strain of *Trypanosoma cruzi*, isolated from a *Myotis nigricans nigricans* bat. Large amastigote nests were observed in uterine muscles, as well as in decidual and endothelial placental cells. In addition, persistent coagulative and fibrotic vascular degeneration was observed. Large amastigote burdens were found in giant cells, spongioblasts and endothelial cells within the labyrinthine layer. Transplacental transmission was confirmed in 30% of the fetuses examined, in which amastigote nests were seen only in striated muscle. During the acute phase, intrauterine development was impaired as the result of parasitic invasion of the placenta, and fetal mortality rose to 10%.



*Keywords:* Chagas's disease; congenital infection; mouse; parasitic infection; *Trypanosoma cruzi*

## Introduction

Chagas's disease, which is caused by the protozoan *Trypanosoma cruzi*, is a major health problem in Central and South America, in which 10–14 million people are infected and 40–120 million are at risk (Report, 2002). More than 150 mammalian species are hosts. *T. cruzi* is spread in man by bloodsucking triatomine species, by blood transfusion, and from infected mothers to offspring (Dias *et al*., 2002; Report, 2002).

Congenital transmission appears to depend on factors related to the parasite and the host. Fetal infection can cause premature birth, stillbirth or neonatal death (Bittencourt, 1988). The physiopathology of congenital infection is not well known. It has been suggested that the parasite reaches the fetus via a haematogenous route, being transmitted across the placenta. However, data on placental involvement and the actual mode of congenital transmission are scarce (Bittencourt, 1976; Delgado and Santos-Buch, 1978; Andrade, 1982; Nisida *et al*., 1999).

Differences in placental parasite loads have been observed in mice infected with different strains of *T. cruzi*, suggesting that parasite strain plays an important role in congenital transmission (Andrade, 1982; Bittencourt, 1992). The present report describes histopathological changes, in late pregnancy, in the placentas and fetuses of mice infected with the Morc-1 strain of *T. cruzi* – a strain that shows high placental tropism. This strain was isolated from the *Myotis nigricans nigricans* bat, which is native to the Ribeirão Preto region in the state of São Paulo, Brazil.

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## Materials and Methods

### *Animals*

Female Swiss mice ($n = 44$), weighing 30–35 g, were obtained from the animal facility of the University of São Paulo at Ribeirão Preto. They were given free access to standard rodent food and water, and the bedding was changed three times per week to avoid high concentrations of ammonia from urine. All experiments were conducted in accordance with international standards for the care and use of laboratory animals, and the study design was approved by the University of São Paulo at Ribeirão Preto Committee for the Ethical Use of Animals in Research.

### *T. cruzi*

The Morc-1 strain, isolated from a *M. nigricans nigricans* bat and characterized as *T. cruzi* II (Dost *et al.*, 2002), was used.

### *Experimental Procedure*

Eleven non-pregnant mice were infected with *T. cruzi*, forming the NPI group; these mice were used solely for determining the parasitaemia curve.

The remaining mice were housed two to a cage, one uninfected male then being introduced into each cage and allowed to mate. The presence of a vaginal plug indicated that mating had occurred. Females showing vaginal plugs were separated from the males and considered to be at 1 day of gestation. These pregnant mice were treated as follows: 22 (PI group) were infected intraperitoneally, on day 9 of gestation, with $10^5$ blood trypomastigotes of the Morc-1 strain; and 11 (PUI group) were left uninfected. Parasitaemia levels were determined daily in all 11 non-pregnant infected mice (NPI) and 11 of the 22 pregnant infected mice (PI) by the method of Brener (1962). The other 11 pregnant infected mice were used solely for macroscopical and histological examination.

### *Histological Examination*

On day 19 of gestation, mice in the PUI group ($n = 11$) and PI group ($n = 11$) were anaesthetized with 2.5% tribromoethanol and killed by cervical dislocation. The uterus was opened by a longitudinal incision. Fetuses were rapidly extracted from the amniotic cavity to determine viability. Absence of a response to stimulation with surgical tweezers indicated that a fetus had died. Placentas and adjacent uterine tissues were also harvested. Placentas and fetuses were weighed on an analytical balance (Sartorius, Goettingen, Germany), and placental diameters and fetal lengths were measured with a digital micrometer (Mitutoyo, Kawasaki, Japan). Placental volumes were determined with a plethysmograph. All harvested tissues were immersed in a fixative solution (alcohol, acetic acid and formaldehyde) and embedded in paraffin wax. Thirty histological sections (6 μm) cut from each harvested tissue (10 placentas and 10 fetuses from the PUI group and from the PI group) were stained with haematoxylin and eosin (HE). To avoid counting the same amastigote nests more than once, parasite loads were estimated in sections separated by 70-μm intervals. For each tissue fragment, 50 microscopical fields were examined at a magnification of ×400, and the percentage of the 50 fields that contained amastigote nests was determined (Castro and Brener, 1985).

### *Statistical Analysis*

Morphological and parasitaemia data were analysed by Student's *t* test, and the level of significance was set at 5%.

## Results

### *Parasitaemia in Pregnant and Non-pregnant Mice*

Parasitaemia levels were higher in PI mice than in NPI mice over the entire course of the infection. In both groups the prepatent period was 3 days, and parasitaemia peaked on day 8 of infection (i.e., day 17 of gestation for PI mice) (Fig. 1).

### *Mortality*

Mice in the PI group began to die on day 11 of infection, mortality reaching 60% on day 14 of infection. Three PI mice died before giving birth, six died on the third or fourth day after giving birth and only

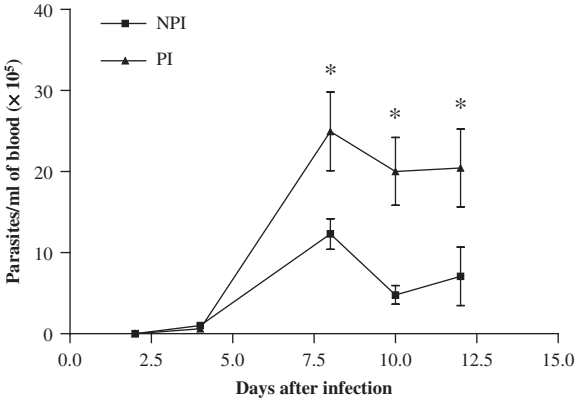


Fig. 1. Parasitaemia in pregnant and non-pregnant mice infected with the Morc-1 strain of *T. cruzi* (PI and NPI groups, respectively). Results are expressed as mean ± SD; $*P < 0.05$.