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BRIEF COMMUNICATION

ELEVATED TRANS-MAMMARY TRANSMISSION OF *Toxocara canis* LARVAE IN BALB/c MICE

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SUMMARY

Toxocariasis is a widespread zoonosis and is considered an important worldwide public health problem. The aim of this study was to investigate the frequency of trans-mammary *Toxocara canis* infection in newborn BALB/c mice nursed by females experimentally infected with 1,200 eggs after delivery. After 50 days of age, the presence of larvae in different organs of the offspring was investigated. Trans-mammary infection was confirmed in 73.9% of the mice that had been nursed by infected females. These data show a high trans-mammary transmission of *T. canis* and confirm the significance of this transmission route in paratenic hosts.

KEYWORDS: Toxocariasis; Lactation; Breast-feed; Paratenic host.

The enzootic cycle of the nematode *Toxocara canis* in dogs, definitive hosts, is assured by congenital transmission. Quiescent larvae in pregnant female tissues are stimulated, most likely by hormonal mechanisms; the larvae then cross the placenta and migrate to the fetus. Moreover, the dogs can also become infected by ingesting larvae present in the colostrum or milk during the first weeks of life[3]. Infection in paratenic hosts, including humans, occurs mainly through the ingestion of embryonated *Toxocara* eggs present in contaminated soil[7] and may also occur through the ingestion of larvae present in undercooked meats or viscera of birds and mammals[9,20]. Although most cases are attributed to *T. canis*, there is evidence that *T. cati* might cause the disease in humans[6].

In addition to these well-documented transmission routes, in the last 50 years, several studies in experimental models have confirmed the vertical transmission of *T. canis* larvae[11,17,18,19] and one study evaluated and confirmed the trans-mammary transmission in ICR mice[10]. Almost two decades ago, ANDERSON (1996) had already warned about of the possibility of *T. canis* larvae be transmitted to the fetus when the mother acquires an infection during pregnancy; this event could lead to the development of the neurologic form in the affected child. More recently, a case of congenital newborn *T. canis* infection was recorded in Argentina[13]. Although less frequent, vertical transmission in paratenic host was also reported by *T. cati*[16]. Due to occurrence of trans-mammary transmission in ICR mice[10] and the variation between the intensity of the infection in different species of experimental models, this study aimed to investigate the frequency of trans-mammary infection of *T. canis* larvae in newborn BALB/c mice nursed by females that were experimentally infected after delivery.

T. canis eggs were collected from the uterine tubes of adult female parasites obtained after the treatment of young dogs with pyrantel pamoate (15 mg/kg). Unembryonated *T. canis* eggs were incubated in 2% formalin at 28 ºC with daily airings for a period of 30 days[4]. Simultaneously, three female and three male mice were mated. After giving birth, the females were intragastrically inoculated with 1200 embryonated *T. canis* eggs[19]. The animals were weaned after 21 days and were kept in their cages until they reached an age of 50 days. After this period, the presence of *T. canis* larvae in the organs of the dams and their offspring was investigated. Three females that were mated in the same period but not experimentally infected were used as controls. The animals were kept in an acclimatized environment at 22 ºC (± 2 ºC) with a light-dark cycle of 12 h and food and water available *ad libitum*. This study was approved by the Ethics Committee in Research at the Federal University of Rio Grande (CEPAS No. 098/2009). All the experiments were carried out following the Federal Government legislation on animal care. All of the mice were euthanized by cervical dislocation, according to animal ethics guidelines (CFMV Resolution No. 1000).

Tissue digestion was performed according to the methodology described by HAVASIOVÁ-REITEROVÁ *et al.* (1995), with modifications, for the detection of larvae in the liver, lungs, heart, kidneys, eyes, and skeletal muscles. The organs were macerated, added to a solution of 0.2% pepsin and 0.26% hydrochloric acid in Milli-Q water, and kept in an incubator shaker of 37 ºC with constant agitation overnight. The material was then centrifuged at 2000 × *g* for four minutes, and the pellet was examined under microscope at (100×) for larvae recovery from the organs and skeletal muscles of mice. To investigate the central nervous

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system infection, brain fragments from the offspring were compressed between glass slides (optical microscopy) (100×). Maternal infection was confirmed by the identification of larvae in the brain using the same methodology. The occurrence of breast transmission was calculated along with the frequency. The frequency of breast transmission and the number of larvae per fragment were calculated.

Trans-mammary transmission of *T. canis* larvae was confirmed in all litters that were nursed by the experimentally infected females. All the larvae were recovered from the brain and the parasite was not detected in other organs examined. Among all the mice nursed by the three experimentally infected females, 73.9% (17/23) had *T. canis* larvae in their brains. The transmission of *T. canis* to all three litters analyzed was confirmed; two litters exhibited 100% transmission, whereas the third litter exhibited 25% transmission (Table 1). Infection was confirmed in all the lactating animals, and no larvae were recovered from the control group.

Table 1
Number of *T. canis*-positive offspring and total larvae recovered from the brains

	Number of offspring	Positive offspring	Total larvae
Litter 1	8	8	45
Litter 2	7	7	41
Litter 3	8	2	5
Total	23	17 (73.9%)	91

In recent decades, several studies have been conducted to evaluate the vertical transmission of *T. canis* larvae in paratenic hosts[11,17,18,19]. The confirmation of *T. canis* trans-mammary transmission came only a few years ago with the observation of larvae in the brain of ICR mice nursed by females that had recently been infected with 300 eggs[10]. In the present study, trans-mammary infection was observed in mice at 50 days of age, demonstrating that the larvae are retained in the host brain during the chronic phase of the disease.

The accumulation of *T. canis* larvae in the brain favors the vertical transmission of the parasite because the larvae may remain viable in this tissue for months or even years[5]. This phenomenon is important because of the physiological immunosuppression that occurs during pregnancy and lactation[12]. The increase of T reg cells during pregnancy appears to play an important role in blocking maternal effector T cells[1]. Moreover, the hormonal fluctuation of progesterone and prolactin promotes attenuation of the inflammatory responses during lactation[14]. Thus, these factors could facilitate the transmission of larvae from the female's brain to the offspring. However, according to MOR & CARDENAS (2010), the effects of pregnancy and lactation on the female immunosuppression are misleading since the immune system is modulated, but not fully suppressed.

Because congenital *T. canis* infection is known to occur in humans[13], and high levels of trans-mammary transmission of *T. canis* larvae have been observed in experimental models, such as this study, greater attention should be paid to infection in pregnant women and to the need for serological monitoring in women before and during pregnancy. However, to better understand the importance of this transmission route, further studies should be conducted with different stages of infection and different species of paratenic hosts.

RESUMO

Elevada transmissão transmamária de larvas de *Toxocara canis* em camundongos BALB/c

A toxocaríase é zoonose amplamente difundida e considerada importante problema de saúde pública. O objetivo deste estudo foi avaliar a frequência da transmissão transmamária de *Toxocara canis* em camundongos BALB/c neonatos amamentados por fêmeas experimentalmente infectadas com 1.200 ovos logo após o parto. Após 50 dias de idade, foi avaliada a presença de larvas em diferentes órgãos dos neonatos. A infecção por via transmamária foi confirmada em 73,9% dos camundongos amamentados por fêmeas infectadas. Estes dados demonstram elevada transmissão transmamária de *T. canis* e confirmam a importância desta via de transmissão em hospedeiros paratênicos.

CONFLICT-OF-INTEREST

None.

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