BRIEF COMMUNICATION

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

Paula de Lima TELMO(1), Luciana Farias da Costa de AVILA(2), Cristina Araújo dos SANTOS(3), Patrícia de Souza de AGUIAR(1), Lourdes Helena Rodrigues MARTINS(3), Maria Elisabeth Aires BERNE(2) & Carlos James SCAINI(1)

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. Infection in paratenic hosts, including humans, occurs mainly through the ingestion of embryonated Toxocara eggs present in contaminated soil and may also occur through the ingestion of larvae present in undercooked meats or viscera of birds and mammals. Although most cases are attributed to T. canis, there is evidence that T. cati might cause the disease in humans.

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 and confirmed the trans-mammary transmission in ICR mice. Almost two decades ago, ANDERSON (1996) had already warned about the possibility of T. canis larvae being 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. Although less frequent, vertical transmission in paratenic host was also reported by T. cani. Due to occurrence of trans-mammary transmission in ICR mice 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 °C with daily airings for a period of 30 days. Simultaneously, three female and three male mice were mated. After giving birth, the females were intragastrically inoculated with 1200 embryonated T. canis eggs. 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 °C ± 2 °C with a light-dark cycle of 12 h and food and water available ad libitum. The 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 HAVASOVÁ-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 °C 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...
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\(^1\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\). 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\(^1^2\). The increase of T reg cells during pregnancy appears to play an important role in blocking maternal effector T cells\(^1^3\). Moreover, the hormonal fluctuation of progesterone and prolactin promotes attenuation of the inflammatory responses during lactation\(^1^4\). 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\(^1^3\), 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.

### ACKNOWLEDGMENTS

Thanks to the Coordenação de Apefeiçoamento de Pessoal de Nível Superior (CAPES) for their financial support.

### REFERENCES

1. Alijotas-Reig J, Llurba E, Gris JM. Potentiating maternal immune tolerance in pregnancy: a new challenging role for regulatory T cells. Placenta. 2014;35:241-8.
2. Anderson BC. Warning about potential for congenital neural larva migrans. J Am Vet Med Assoc. 1996;208:185.
3. Burke TM, Roberson EL. Prenatal and lactational transmission of Toxocara canis and Ancylostoma caninum: experimental infection of the bitch at midpregnancy and at parturition. Int J Parasitol. 1985;15:485-90.
4. Da Costa de Avila LF, da Fonseca JS, Dutra GF, de Lima Telmo P, Silva AM, Berne ME, et al. Evaluation of the immunosuppressive effect of cyclophosphamide and dexamethasone in mice with visceral toxocariasis. Parasitol Res. 2012;110:443-7.
5. Dunsmore JD, Thompson RCA, Bates IA. The accumulation of Toxocara canis larvae in the brains of mice. Int J Parasitol. 1983;13:517-21.
6. Fisher M. Toxocara cati: an underestimated zoonotic agent. Trends Parasitol. 2003;19:167-70.
7. Glickman LT, Schantz PM. Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol Rev. 1981;3:230-50.
8. Havasiová-Reiterová K, Tomasovicová O, Dubinsky P. Effect of various doses of infective Toxocara canis and Toxocara cati eggs on the humoral response and distribution of larvae in mice. Parasitol Res. 1995;81:13-7.
9. Hoffmeister B, Glaser S, Flick H, Pornschiegel S, Suttrop N, Bergmann F. Cerebral toxocariasis after consumption of raw duck liver. Am J Trop Med Hyg. 2007;76:600-2.
10. Jin Z, Akao N, Ohta N. Prolactin evokes lactational transmission of larvae in mice infected with *Toxocara canis*. Parasitol Int. 2008;57:495-8.

11. Lee K, Min HK, Soh CT. Transplacental migration of *Toxocara canis* larvae in experimentally infected mice. J Parasitol. 1976;62:460-5.

12. Luppi P. How immune mechanisms are affected by pregnancy. Vaccine. 2003;21:3352-7.

13. Maffrand R, Avila-Vázquez M, Princich D, Alasia P. Toxocariasis ocular congénita en un recién nacido prematuro. An Pediatr (Barc). 2006;64:595-604.

14. Monasterio N, Vergara E, Morales T. Hormonal influences on neuroimmune responses in the CNS of females. Front Integr Neurosci. 2014;7:110.

15. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. Am J Reprod Immunol. 2010;63:425-33.

16. Moura JVLM, Santos SV, Castro JM, Chieffi PP. Estudo experimental acerca da transmissão vertical de *Toxocara cati* em *Mus musculus*. Arq Med Hosp Fac Ci Med Santa Casa Sao Paulo. 2011;56:138-40.

17. Oshima T. Influence of pregnancy and lactation on migration of the larvae of *Toxocara canis* in mice. J Parasitol. 1961;47:657-60.

18. Reiterová K, Tomasovicová O, Dubinský P. Influence of maternal infection on offspring immune response in murine larval toxocariasis. Parasite Immunol. 2003;25:361-8.

19. Schoenardie ER, Scaini CJ, Pepe MS, Borsuk S, de Avila LF, Villela M, et al. Vertical transmission of *Toxocara canis* in successive generations of mice. Rev Bras Parasitol Vet. 2013;22:623-6.

20. Yoshikawa M, Nishiofuku M, Moriya K, Ouji Y, Ishizaka S, Kasahara K, et al. A familial case of visceral toxocariasis due to consumption of raw bovine liver. Parasitol Int. 2008;57:525-9.

Received: 20 February 2014
Accepted: 28 May 2014