
**EFFECT OF SEX ON *Toxocara canis* LARVAL
MIGRATION TO THE CEREBELLUM
DURING EXPERIMENTAL INFECTION OF
*Rattus norvegicus***

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ABSTRACT

The presence of *Toxocara canis* third instar larvae in the cerebellum of *Rattus norvegicus* may alter rodent behavior and movement. In this study, we investigated whether the sex of the rodent affects the migration of larvae to the cerebellum. Thirty-six *Rattus norvegicus* specimens (18 males and 18 females) were infected with 300 *T. canis* eggs and were euthanized after 60 days. The cerebellum was removed and treated with 0.5% HCl to recover the *T. canis* larvae. The number of larvae recovered from male rodents was significantly higher than in females, suggesting that the sex of the animal influences larval migration to the cerebellum.

KEY WORDS: *Toxocara canis*; *Rattus norvegicus*; larval migration; cerebellum.

INTRODUCTION

The definitive hosts of *Toxocara canis* are canids, in which adult worms are found in the lumen of the small intestine. However, other mammals, such as rodents, may be infected by ascarid larvae; rats and mice are usually infected by *Toxocara* larvae without allowing evolution into adult worms, but acting as paratenic hosts (Strube et al., 2013).

The migration pattern of *T. canis* larvae in rodents, particularly *Rattus norvegicus*, has been studied by several authors (Burren, 1972; Abo-Shehadeh & Herbert, 1984; Bardón et al., 1994; Epe et al., 1994; Lescano et al., 2004). The migratory route of *T. canis* larvae in mice experimentally infected initially includes a hepato-pulmonary phase, in which the parasite reaches the liver and lungs in the first week post-infection; subsequently, a wide tissue distribution

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is observed (myotropic-neurotropic phase), including infection of the central nervous system (CNS) (Abo-Shehadeh & Herbert, 1984). Lescano et al. (2004) verified a similar distribution in *R. norvegicus*.

Rodents of the same sex are usually utilized for experimental infections by *T. canis* to avoid hormonal effects of the paratenic host sex in the larval migration pattern. A greater prevalence and intensity of helminth infections has been reported in male mammals as well as a greater susceptibility to infection (Harder et al., 1992; Poulin, 1996) probably due to the testosterone-impaired immunological response. However, according to Eloi-Santos et al. (1992) the development of adult *Schistosoma mansoni* is more intense in female mice than in male mice infected with the same quantity of cercariae.

The pattern of larval *Toxocara* migration in paratenic hosts, particularly to the CNS, is an important variable when evaluating rodent behavior alterations that facilitate the transmission of this ascarid to its definitive host. Santos et al. (2017) observed more intense brain migration of *T. canis* larvae in male rats, compared to females that received the same inoculum.

The purpose of the present investigation was to determine possible differences in the pattern of *T. canis* larval migration, specifically to the cerebellum, in experimentally infected male and female *R. norvegicus*.

MATERIAL AND METHODS

Dissection was performed on adult *T. canis* females, from stray dogs held at the Center for Zoonosis Control in Guarulhos, São Paulo, Brazil. The eggs recovered were transferred to 200 mL of 2% formalin solution in an Erlenmeyer flask, kept at 28°C in an incubator, and shaken twice daily. After 30 days, the time required to form third-stage larvae, the eggs were washed three times in saline solution and prepared for rat infection (Lescano et al. 2004).

Thirty-six *R. norvegicus* (18 males and 18 females; age, 6-8 weeks) were orally infected with 300 *T. canis* eggs using a gavage needle. The infected rats were placed in polypropylene cages (five rats per cage) with water and food provided *ad libitum*, in a room with controlled temperature and a 12 h light/dark cycle.

Three male and three female rats were euthanized at 3, 7, 10, 15, 30, and 60 days post-infection with an overdose of xylazine and ketamine, and the brain and cerebellum were carefully removed and digested in 0.5% HCl for 24 h at 37°C, according to the technique described by Xi and Jin, to recover and count *T. canis* larvae.

The experimental protocol was approved by the Research Ethics Committee on Animal Experiments of the Faculdade de Ciências Médicas da Santa Casa de São Paulo (Proc. 014/12).

The difference in the number of larvae found in the CNS and cerebella of both groups was analyzed by the two-way analysis of variance, where $p \leq 0.05$ was considered significant.

RESULTS

The total number of *T. canis* larvae recovered in the CNS of male rats after acid digestion was significantly higher than that found in the group of female rats. One female rat died during the experiment and only one (5.9%) among the 17 evaluated had larvae in the cerebellum whereas seven (38.9%) of the 18 male cerebella revealed the presence of *T. canis* larvae (Table).

Table. Total, mean and standard deviation of *T. canis* larvae number recovered from the cerebellum and Central Nervous System of female and male *Rattus norvegicus* experimentally infected, 3, 7, 10, 15, 30 and 60 days post-infection (dpi).

DPI	Rat	Male			Female		
		Brain	Cerebellum	Total	Brain	Cerebellum	Total
3	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	1	0	1
7	5	1	1	2	0	0	0
	6	0	1	1	0	0	0
	7	4	0	4	1	0	1
10	8	1	2	3	0	0	0
	9	3	0	3	0	0	0
15	10	0	1	1	0	0	0
	11	0	0	0	0	0	0
	12	0	2	2	2	1	3
30	13	6	1	7	0	0	0
	14	2	0	2	0	0	0
	15	0	1	1	0	0	0
60	16	1	0	1	0	0	0
	17	5	0	5	0	0	0
	18	3	0	3	NR	NR	NR
Total		26	9*	35*	4	1*	5*
Mean		1.44	0.50	1.94	0.22	0.06	0.29
Standard deviation		1.95	0.71	1.95	0.55	0.24	0.75

NR: not performed (the animal died). * $p < 0.05$ (male vs female).

DISCUSSION

Rodents are known to be paratenic hosts for *Toxocara* larvae and their predation by the definitive hosts constitutes one of the natural transmission mechanisms of *T. canis* and *Toxocara cati*. Several researchers have shown that infecting rodents with *T. canis* larvae alters their behavior, making them more susceptible to predation by canids; thus, facilitating infection (Donovick & Burright, 1987; Cox & Holland, 1998; Chieffi et al., 2010). This depends on migration of the larvae from the third stage to areas of the CNS in rodents (Lescano et al., 2004; Santos et al., 2009).

Santos et al. (2017) reported that migration of *T. canis* larvae to the CNS of *R. norvegicus* is affected by sex. Larvae accumulated in the livers of experimentally infected females, whereas no larvae were found in the brain between days 30 and 60 after infection. In contrast, a significant increase in *T. canis* larvae in the brain was observed between days 30 and 60 post-infection of *R. norvegicus* males.

In the present study, a higher frequency of cerebellar parasitism was detected in male *R. norvegicus* experimentally infected with *T. canis*, reinforcing the importance of selecting the sex when studying the effect of infection by these ascarids on rat behavior. The reasons for this difference may be related to hormonal influence as well as to the immune system, but there is a need for further studies to clarify this hypothesis.

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