Short Communication

Migration pattern of *Toxocara canis* larvae in experimentally infected male and female *Rattus norvegicus*

**Sergio Vieira dos Santos**[1],[2], **Felipe Henrique Yazawa Santos**[1], **Susana Angelica Zevallos Lescano**[2], **Daniel Maurício dos Santos**[1], **Érico da Silva Tiago**[1], **Gabriela Rodrigues e Fonseca**[2], **Manoel Carlos Sampaio de Almeida Ribeiro**[3] and **Pedro Paulo Chieffi**[1],[2]

Abstract

**Introduction:** Migration of *Toxocara canis* larvae was investigated in male and female *Rattus norvegicus*. **Methods:** Eighteen male and 18 female *R. norvegicus* were infected with 300 *T. canis* eggs. Three male and three female rats were euthanized at 3, 7, 10, 15, 30, and 60 days post-infection, and *T. canis* larvae were recovered by means of organ and tissue digestion. **Results:** Female rats showed a greater number of larvae in the liver than males. **Conclusions:** Paratenic host sex influences the migration pattern of *T. canis* larvae.

**Keywords:** *Toxocara canis*. *Rattus norvegicus*. Sex influence.

*Toxocara canis* is a nematode parasite whose definitive hosts are canids but can also infect other animals, such as rodents. These paratenic hosts may act as disseminators of infection through prey-predator relationships[1–2]. Consuming uncooked meat from paratenic hosts (chicken and pork) may constitute a risk factor for human infection by *T. canis*.

*Toxocara canis* infection in the definitive host occurs by the ingestion of eggs containing third-stage larvae found in the environment, by predation of infected paratenic hosts, and by transmammary and transplacental routes[4].

The larvae hatch in the intestines of the host and migrate through the blood vessels, liver, heart, and lungs, from where they are able to reach the tracheal tree and mouth. They are then swallowed and return to the intestine, where they become adults[1]. In paratenic hosts, the helminth never reach adulthood, but many larvae migrate to muscles, organs, and nervous tissue, where they can then become encysted[1,3].

Human infection with *T. canis* due to larval migration in tissues may lead to the development of visceral larva migrans (VLM), ocular larva migrans (OLM), and covert toxocariasis (CT). There is evidence that rodents experimentally infected with *Toxocara canis* display altered behavior and impairment of muscular strength[6–8].

The migration pattern of *T. canis* larvae has been observed in *Rattus norvegicus*, with a wide tissue distribution including the central nervous system[4]. The host sex has been reported to influence the development of parasitic infections, resulting in different parasite growth or pathophysiological effects in the host[10–12]. However, it is unclear whether there is any difference in the migration patterns of *T. canis* larvae according to the sex of *R. norvegicus*. In the present study, the correlation of the migration pattern of *T. canis* larvae with the sex of experimentally infected *R. norvegicus* was investigated to facilitate the choice of suitable experimental models for research into behavioral changes in infected hosts.

Adult *T. canis* were recovered from naturally infected stray dogs captured by the Center for Zoonosis Control in Guarulhos, São Paulo. Worms were placed in a glass receptacle containing saline solution and stored at 4°C until use. *Toxocara canis* females were dissected in Petri dishes containing acidified water (pH 3), and the uteri were removed and cut open to release the eggs. The recovered eggs were concentrated by centrifugation at 1,500rpm for 5 min. The pellet containing the eggs was transferred to an Erlenmeyer flask containing approximately 200mL of 2% formalin sealed with a hydrophobic cotton plug. Flasks were placed in an incubator at 28°C and manually agitated twice daily, to ensure oxygenation of the eggs to promote development of larvae up to the third stage. After
30 days (the length of time required for third-stage larval formation) the eggs were washed three times in saline solution to remove the formalin solution and prepared for infection of the rats.

A total of 36 R. norvegicus (18 male and 18 female) of 6-8 weeks of age, obtained from the Main Animal Center of the São Paulo University Medical School, were orally infected with 300 T. canis eggs. At 3, 7, 10, 15, 30, and 60 days post-infection (DPI), three male and three female rats were euthanized and T. canis larvae were recovered from the liver, lungs, kidneys, eyes, brain, and carcass after digestion with 0.5% HCl for 24h at 37°C, according to the technique described by Xi & Jin.

After infection and prior to killing the rats by overdosing with xylazine and ketamine, the rats were maintained in polypropylene cages with food and water ad libitum, in a room with a controlled temperature and 12h light/dark cycle.

The mean count of three microscope slides containing 50µL of the cultured egg solution was used to adjust the parasite dose to 300 eggs suspended in 0.2mL of water for each rat. Infection of rats was performed orally using a gavage needle.

The difference in mean values between groups was analyzed with the Mann-Whitney U test for comparison of two groups. Only the probability values (P) less than 0.05 were considered statistically significant.

The results obtained in this study (Table 1) showed that, in male R. norvegicus, the larval distribution was similar to those previously reported. However, in female R. norvegicus, the migration pattern of T. canis larvae was different, showing a clear predominance of hepatic distribution throughout the experiment. In addition, the number of larvae recovered in female specimens was greater than that recovered in male R. norvegicus.

In murine models, the migration pattern of T. canis larvae consists of two phases. The first is mainly characterized by a visceral migration that predominantly affects the liver and the lungs, known as the hepato-pulmonary phase; this is followed by a second phase that principally affects the muscles and the brain, known as the myotrophic-neurotrophic phase. Usually, animals of the same sex are used in investigations, to avoid the possible influence of paratenic host sex on the migration pattern of larvae.

There are, however, reports of differences in the pattern of parasitic infections according to the host sex, usually with a greater prevalence and intensity of infection in males, mainly in helminthic infections of mammals. According to Harder et al., rodent males would be more susceptible to parasitic infections because of the deleterious effect of testosterone, which impairs the hosts’ immunological response. However, Eloi-Santos et al. found that Schistosoma mansoni cercariae were more successful in developing into adult worms in experimentally infected male R. norvegicus than in females.

### Table 1
Mean, standard deviation, and median of Toxocara canis larvae recovered from the organs and tissues of male and female Rattus norvegicus in three stages of infection.

<table>
<thead>
<tr>
<th>Stages</th>
<th>3 to 7 days</th>
<th>10 to 15 days</th>
<th>30 to 60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (SD)</td>
<td>n</td>
<td>mean (SD)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carcass</td>
<td>3.67 (3.83)</td>
<td>3.5</td>
<td>7.33 (3.50)</td>
</tr>
<tr>
<td>liver</td>
<td>18.83 (18.96)</td>
<td>13</td>
<td>1.83* (1.17)</td>
</tr>
<tr>
<td>lungs</td>
<td>1.83 (1.16)</td>
<td>2</td>
<td>5.00 (5.21)</td>
</tr>
<tr>
<td>kidney</td>
<td>1.33 (1.50)</td>
<td>1</td>
<td>0.83 (0.98)</td>
</tr>
<tr>
<td>brain</td>
<td>0.17 (0.40)</td>
<td>0</td>
<td>1.33 (1.75)</td>
</tr>
<tr>
<td>eyes</td>
<td>0.00 (0.00)</td>
<td>0</td>
<td>0.17 (0.40)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carcass</td>
<td>1.00 (1.26)</td>
<td>0.5</td>
<td>3.17 (2.13)</td>
</tr>
<tr>
<td>liver</td>
<td>37.17 (22.66)</td>
<td>27.5</td>
<td>30.33* (13.27)</td>
</tr>
<tr>
<td>lungs</td>
<td>1.17 (0.98)</td>
<td>1.5</td>
<td>2.50 (1.64)</td>
</tr>
<tr>
<td>kidney</td>
<td>1.00 (0.89)</td>
<td>1</td>
<td>0.17 (0.40)</td>
</tr>
<tr>
<td>brain</td>
<td>0.17 (0.40)</td>
<td>0</td>
<td>0.50 (0.83)</td>
</tr>
<tr>
<td>eyes</td>
<td>0</td>
<td>0</td>
<td>0.17 (0.40)</td>
</tr>
</tbody>
</table>

SD: standard deviation. *Significant difference of recovered larval mean between male and female; P = 0.05.
infected female mice, when compared to male mice exposed to the same number of cercariae.

In the present study, a significantly greater number of *T. canis* larvae (predominantly localized to the liver) was found in female compared to male rats (*P* = 0.05) (Table 1). This was probably a consequence of hormonal influences, leading to greater larval elimination by male rats during the tissue migration process. However, this may also be due in part to the comparative ease with which *T. canis* larvae can be recovered from the liver, compared to those in other organs or tissues.

It was also observed that in male rats, *T. canis* larvae followed the pattern already reported in other studies,6,14; the female models, however, did not follow the same trend with the infection mainly restricted to the liver. In this study, we saw no significant numbers of larvae in other organs or tissues, particularly in the central nervous system (Table 1). The reasons for this difference are unclear, but may be due to hormonal influences.

Our results, which reveal clear differences in migrations pattern of *T. canis* larvae according to the sex of *R. norvegicus*, will be useful in the planning of future research into behavioral changes in ascarid-infected rodents.

**Ethical considerations**

All procedures were performed according to the guidelines for animal experimentation, as stipulated in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication number 86-23, Bethesda, Maryland, USA). 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 (process no. 014/12).

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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**REFERENCES**