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NOTA CIENTÍFICA/ RESEARCH NOTE

MIGRATION ROUTES OF *TOXOCARA CANIS* AND *TOXOCARA CATI* IN TISSUES OF EXPERIMENTALLY INFECTED *RATTUS NORVEGICUS*

RUTAS DE MIGRACIÓN DE *TOXOCARA CANIS* Y *TOCARA CATI* EN LOS TEJIDOS DE *RATTUS NORVEGICUS* INFECTADOS EXPERIMENTALMENTE

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Abstract

Toxocariasis is currently considered an important zoonosis in many countries and is usually attributed to larvae of *Toxocara canis* Werner, 1782 but less frequently, *T. cat.* This study attempts to compare the migration routes followed by *T. canis* and *Toxocara cati* (Schrank, 1788) in *Rattus norvegicus* Berkenhout, 1769 and to determine the percentage of larvae recovered in each organ of experimentally infected rats. Twenty-one 8-week-old, male specimens of *R. norvegicus* (Wistar) were inoculated orally with 500 embryonated eggs of *T. canis*, while another 21 rats of the same species were inoculated orally with 300 embryonated eggs of *T. cati*. On postinfection days 3, 5, 8, 10, 15, 30 and 60, three rats from each group were sacrificed and larval recovery was performed from various organs and the carcass following digestion with 0.5% HCl method. Comparisons of the percentage of recovered larvae revealed that *T. cati* larvae migrated in greater quantities, as early as day 3 postinfection, to the lungs (23.77%, compared to 0.34% for *T. canis*), while migration of *T. cati* larvae to the carcass was observed from day 3 up to day 60 postinoculation. This experiment verified that the larvae of these two species follow distinct migration routes and have different recovery rates.

Keywords: larval recovery - migration routes - *Rattus norvegicus* - *Toxocara canis* - *Toxocara cati*.

Resumen

La toxocariasis es actualmente considerada una importante zoonosis en muchos países y es generalmente atribuida a larvas de *Toxocara canis* (Werner, 1782, entretanto, a pesar de ser menos frecuente, *T. cati* (Schrank, 1788) puede causar enfermedad. El objetivo de este estudio fue comparar las rutas de migración seguidas por *T. canis* y *T. cati* en *Rattus norvegicus* (Berkenhout, 1769) y determinar el porcentaje de larvas obtenidas en cada órgano de las ratas infectadas experimentalmente. Veintiún especímenes machos de *R. norvegicus* (Wistar), con ocho semanas de edad fueron inoculados oralmente con 500 huevos larvados de *T. canis*, en cuanto que otras 21 ratas de la misma especie fueron inoculadas oralmente con 300 huevos embrionados de *T. cati*. En los días 3, 5, 8, 10, 15, 30 y 60 pos-infección, tres ratas de cada grupo fueron sacrificadas y la recuperación de larvas fue realizada en varios órganos y la musculatura después de la digestión con el método de HCl a 0,5%. La comparación de los porcentajes de larvas recuperadas reveló que las larvas de *T. cati* migraron para los pulmones en mayor cantidad, luego en el tercer día pos-infección (23,77%, y solamente 0,34% en el caso de *T. canis*) al paso que la migración de larvas de *T. cati* para la musculatura fue observada desde el día 3 hasta el día 60 pos-inoculación. Con este experimento se verificó que las larvas de estas dos especies siguen rutas de migración distintas y tienen tasas de recuperación diferentes.

Palabras clave: recuperación de larvas - rutas de migración - *Rattus norvegicus* - *Toxocara canis* - *Toxocara cati*.

INTRODUCTION

Toxocara canis (Werner, 1782) and *Toxocara cati* (Schrank, 1788) are common intestinal nematodes of dogs and cats, respectively. Since diagnosis of human infection by embryonated eggs of *Toxocara* became available by relative safety inmunological methods, toxocariasis has been considered a public health problem in many countries. This zoonosis is usually attributed to the migrating larvae of *T. canis*; however, less frequently, *T. cati* is the cause of disease (Fisher, 2003).

Rodents are reservoirs for *Toxocara* spp., act as indicators of environmental contamination, particularly in urban areas, and are a source of infection in dogs and cats, the definitive hosts of *T. canis* and *T. cati,* respectively (Cardillo *et al.*, 2009). Together with other rodent species, *Rattus norvegicus* (Berkenhout, 1769) has been highlighted as a common paratenic host for *Toxocara* spp. (Chieffi *et al.*, 1981) and previous studies have described the distribution of larvae of *T. canis* and *T. cati* in its tissues and organs following experimental infection (Lescano *et al.*, 2004, Santos *et al.*, 2009); however, there are no reports comparing the larval migration of these species in the organs of this rodent.

Our objectives were to evaluate the role of *R. norvegicus* as a paratenic host of *T. canis* and *T. cati,* compare the migration routes followed by these two ascarids and determine the percentage of larvae recovered in each organ of *R. norvegicus* up to 60 days following experimental infection.

MATERIALS AND METHODS

Toxocara canis and *T. cati* females were dissected in Petri dishes containing acidified water (pH 3), their uteruses were removed and cut open to release the eggs. The eggs recovered were then concentrated by centrifugation at 1500 rpm for 5 min. The pellet containing the eggs was transferred to Erlenmeyer flasks containing approximately 200 ml of 2% formalin each, sealed with a hydrophobic cotton

lid. The flasks were incubated at 28°C for approximately 30 days, with daily manual agitation to ensure oxygenation of the eggs and promote the development of third stage larvae.

Twenty-one 8-week-old, male *R. norvegicus* Wistar rats were orally infected by stomach tube, with approximately 500 embryonated eggs of *T. canis* each. Another 21 8-week-old, male rats of the same species were orally infected with approximately 300 embryonated eggs of *T. cati*. Animals were provided by the Centro de Bioterismo da Faculdade de Medicina da Universidade de São Paulo, were group-housed and water and pellet commercial food were supplied *ad libitum* the cages being cleaned at regular periods.

The rats were divided into seven groups euthanized on different postinfection days (PIDs), i.e. on PIDs 3, 5, 8, 10, 15, 30 and 60. The euthanized rats were processed for larvae recovery from the liver, brain, lungs, kidneys and carcass, using the method of digestion with 0.5% HCl for 24 h at 37°C. The resulting liquid was centrifuged for 2 min at 1500 RPM and all the sediment was examined under a microscope to count the larvae (Xi & Jin, 1998).

The experimental protocol was approved by the Research Ethics Committee of the São Paulo Institute of Tropical Medicine (protocol CPE-IMT010/06).

Statistical analysis were performed using One Way Anova test , followed by the Kruskal-Wallis test using Prisma program, 5.0 version. Only probability values of p< 0,001 were considered statistically significant.

RESULTS

Table 1 presents the results obtained following larval recovery from the various organs of the infected rats. *T. cati* larvae migrated in greater quantities to the muscles and were detected from PID 3 (22.4% compared with 0% for *T. canis*). Both species of the ascarid were recovered from

the viscera as early as PID 3 (42.9% for *T. canis* and 32.7% for *T. cati*) and marked reduction of larvae in these organs was observed on PID 60. Fig. 1 represents the larval recovery in different tissues and organs and significant difference is only observed in the number of larvae in carcass of the two groups of rodents, with *T. cati* larvae being the most abundant (p<0,001).

DISCUSSION

In this study, we analyzed and compared the migration patterns of *T. canis* and *T. cati* larvae in *R. norvegicus* in the visceral and myotropic-neurotropic phases. Tests using different

experimental models have described infection in animals showing varying results, in some cases, different from those obtained in our experiment.

Studies concerning the migration of *T. canis* larvae in NIH mice (Abo-Shehada & Herbert, 1984) determined that the visceral phase peaked on PIDs 2 and 3 in the liver and lungs, respectively, whereas the myotropic-neurotropic phase peaked on PID 7. Cardillo *et al*. (2009) observed that migration of *T. cati* larvae in BALB/c mice showed a peak for the visceral phase on PID 2, while the peak for the myotropic-neurotropic phase occurred on PID 28.

Table 1. Mean number / percentage of *T. canis* and *T. cati* larvae recovered from tissues and organs of *Rattus norvegicus* – comparison of the migration routes from the two nematoda species.

DPI	Carcass		Viscera*		Brain	
	T. canis	*T. cati*	*T. canis*	*T. cati*	*T. canis*	*T. cati*
3	0.0	67.2 (22.4%)	128.7 (42.9%)	98.1 (32.7%)	0.0	0.0
5	69.1 (23.2%)	153.9 (51.3%)	131.7 (43.9%)	114.9 (38.3%)	3.6 (1.2%)	3.3 (1.1%)
8	31.2 (10.5%)	115.2 (38.4%)	28.5 (9.5%)	18.6 (6.2%)	5.1 (1.7%)	0.6 (0.2%)
10	31.8 (10.6%)	54.6 (18.2%)	13.2 (4.4%)	12.3 (4.1%)	3.9 (1.3%)	0.9 (0.3%)
15	55.8 (18.6%)	236.4 (78.8%)	25.8 (8.6%)	43.8 (14.6%)	13.5 (4.5%)	1.5 (0.5%)
30	19.2 (6.4%)	176.4 (58.8%)	7.2 (2.4%)	16.5 (5.5%)	5.7 (1.9%)	3.3 (1.1%)
60	21.6 (7.2%)	240.3 (80.1%)	12.3 (4.1%)	46.2 (15.4%)	11.7 (3.9%)	0.0

* Liver, lungs and kidneys
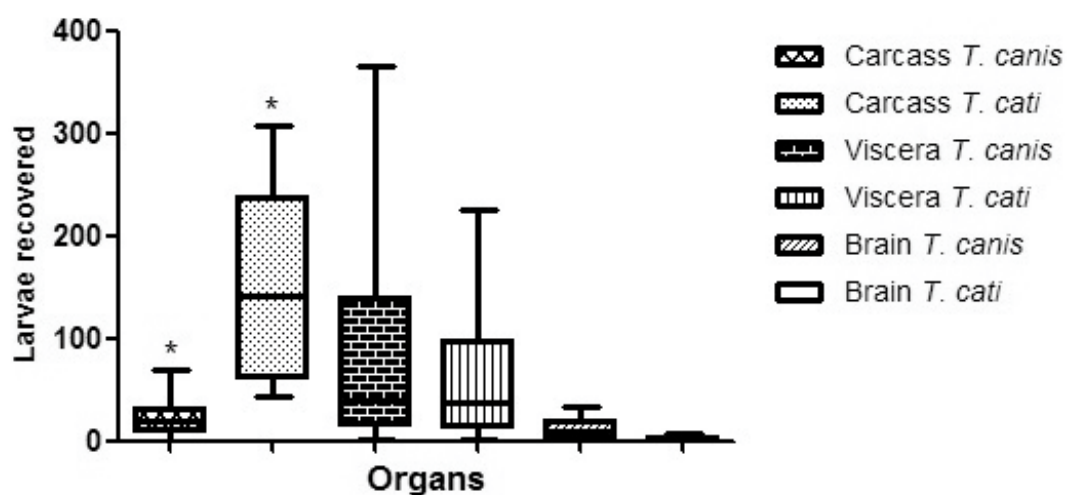
Figure 1. Larvae recovered in diverse organs of *R. norvegicus* infected with *T. canis* or *T. cati* embryonated ova.(*comparison of *T. canis* and *T. cati* larvae recovered in carcass of rats).

Hamilton *et al*. (2006) compared the migration of larval *T. canis* in seven strains of mice, including BALB/c and NIH. In these two strains, the greatest recovery of larvae in the lungs occurred on PID 7 and was greater in BALB/c mice. In contrast, liver parasite burden was very similar in both strains on PIDs 7, 35 and 42. Larval recovery in the muscles was less frequent than that recovered in the viscera; however, BALB/c mice showed increased larva load in the carcass on PID 42, with a higher frequency of larva recovery in the brain compared with other organs.

In chickens, Taira *et al*. (2003) studied the migrating larvae of *T. canis* from PIDs 1 to 6 and observed that the majority of the larvae were recovered in the lungs (PID 3) and in the liver (PID 6). Larvae were also detected in the brain and muscle on PID 6, but to a lesser extent compared with other organs. Experimental infection of chickens with *T. cati* was conducted by Taira *et al.* in (2011), who observed the initial establishment of infection in the liver and lungs between PIDs 1 and 3; later, the larvae migrated to the muscles, where almost 99% of them were recovered between PIDs 29 and 176. The migration pattern of larval *T. canis* in pigs was studied by Helwigh *et al.* (1999) and showed a similar pattern to that verified in mice (Abo-Shehada & Herbert, 1984).

The presence of larvae in the brain, detected on PID 15, was more frequent in rats infected by *T. canis* (4.5% for *T. canis* and 0.5% for *T. cati*). Havasiová-Reiterová *et al.* (1995) infected C57BL6/J mice with 500 or 1000 eggs of *T. cati* and *T. canis,* and detected higher numbers of *T. canis* larvae in the brains of these rodents too.

There are few studies on larval migration of *Toxocara* spp. in rats (Strube *et al*., 2013); in this report the migration routes of the larvae of two species of *Toxocara* (*T. canis and T. cati*) during the experimental infection of rats was compared. Some notable differences were observed: the migration of *T. cati* larvae to the CNS was less pronounced and occurred between PID 5 (1.1%) and PID 30 (1.1%), while *T. canis* larvae were recovered in this organ between PID 5 (1.2%)

and PID 60 (3.9%), with peak recovery occurring on PID 15 (4.5%). In the carcass, the largest count of *T. cati* larvae was obtained as early as PID 3 (22.4%), and persisted to a remarkable extent (80.1%) by the end of the experimental period, whereas *T. canis* larvae diminished progressively in the carcass from PID 15 up to the end of the experiment (7.2%).

Analysis of our results indicates that *R. norvegicus* can be considered a suitable experimental paratenic host for *T. canis* and *T. cati*, maintaining the living larvae of these roundworms in different tissues and organs at least up to 60 days postinfection. Moreover, our findings showed that the larvae of both species have distinct migration routes and different recovery rates. These rodents continue to pose a risk to public health because they are reservoirs for *Toxocara* spp. and can infect dogs and cats, the definitive hosts, thus maintaining the cycle of these ascarids in nature.

This study compares *T. canis* and *T. cati* larval migration in *R. norvegicus* however; these data must be carefully considered when other species of rodents or paratenic hosts are studied, since there are some differences in parasite behavior in diverse species.

BIBLIOGRAPHIC REFERENCES

Abo-Shehada, MN & Herbert, IV. 1984. *The migration of larval* Toxocara canis *in mice. II. Post-intestinal migration in primary infections*. Veterinary Parasitology, vol.17, pp.75-83.

Cardillo, N, Rosa, A, Ribicich, M, López, C & Sommerfelt, I. 2009. *Experimental infection with* Toxocara cati *in BALB/c mice, migratory behaviour and pathological changes*. Zoonoses Public Health, vol. 56, pp.198-205.

Chieffi, PP, Del Guercio, VM, Ueda, M & Mello, LB. 1981. *Importância de* Rattus norvegicus *capturados no município de São Paulo, SP, Brasil, como hospedeiros paratênicos de* Toxocara canis

(Ascaroidea, Nematoda). Revista do Instituto Adolfo Lutz, vol. 41, pp. 89-91.

Fisher, M. 2003. Toxocara cati: *an underestimated zoonotic agent*. Trends in Parasitology, vol. 19, pp. 167-170.

Hamilton, CM, Stafford, P, Pinelli, E & Holland, CV. 2006. *A murine model for cerebral toxocariasis: characterization of host susceptibility and behavior*. Parasitology, vol. 132, (Pt6) pp. 791-801.

Havasiová-Reiterová, K, Tomasovicová, O & Dubinsky, P. 1995. *Effect of various doses of infective* Toxocara canis *and* Toxocara cati *eggs on the humoral response and distribution of larvae in mice*. Parasitology Research, vol. 81, pp.13-17.

Helwigh, AB, Lind, P & Nansen, P. 1999. *Visceral larva migrans: migratory pattern of* Toxocara canis *in pigs*. International Journal for Parasitology, vol. 29, pp. 559-565.

Lescano, SZ, Queiroz, ML & Chieffi, PP. 2004. *Larval recovery of* Toxocara canis *in organs and tissues of experimentally infected* Rattus norvegicus. Memórias do Instituto Oswaldo Cruz, vol.99, pp. 627-628.

Santos, SV, Lescano, SZ, Castro, JM & Chieffi, PP. 2009. *Larval recovery of* Toxocara cati *in experimentally infected* Rattus norvegicus *and analysis of the rat as potential reservoir for this ascarid*. Memórias do Instituto Oswaldo Cruz, vol.104, pp. 933-934.

Strube C, Heuer L, Janecek, E. 2013. Toxocara spp. i*nfections in paratenic hosts*. Veterinary Parasitology, vol.193, pp. 375-89.

Taira, K, Permin, A & Kapel, CM. 2003. *Establishment and migration pattern of* Toxocara canis *larvae in chickens*. Parasitology Research, vol. 90, pp. 521-523.

Taira, K, Saitoh, Y & Kapel, CM. 2011. Toxocara cati *larvae persist and retain high infectivity in muscles of experimentally infected chickens*. Veterinary Parasitology, vol.180, pp. 287-291.

Xi, WG & Jin, LZ. 1998. *A novel method for the recovery of* Toxocara canis *in mice*. Journal of Helminthology, vol. 72, pp. 183-184.

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