Larval recovery of *Toxocara cati* in experimentally infected *Rattus norvegicus* and analysis of the rat as potential reservoir for this ascarid

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Toxocara cati is a common feline parasite transmitted by the ingestion of embryonated eggs, by the transmammary route or by predation of paratenic hosts harbouring third-stage larvae in their bodies. In the present study, the larval distribution of T. cati in tissues and organs of Rattus norvegicus experimentally infected with 300 embryonated eggs was analysed. Third-stage larvae were recovered from livers, lungs, kidneys, eyes, brains and carcasses of infected rats, following tissue digestion with HCl 0.5% for 24 h at 37°C. Some differences from the known larval distribution of Toxocara canis in the same rodent species were found.

Key words: Toxocara cati - larval distribution - Rattus norvegicus - paratenic hosts

Visceral larva migrans (VLM) is the result of prolonged tissue migration of nematode larvae in unusual hosts (Beaver et al. 1952). In human beings, the main etiological agents for this syndrome are either *Toxocara canis* or *Toxocara cati* larvae (Schantz 1989). *T. cati* is a common helminth parasite of cats and is closely related to *T. canis*, a roundworm that frequently infects dogs.

When compared to the frequency of known human cases of infection by T. canis larvae, few records implicating *T. cati* as causative agents of VLM have been published (Fisher 2003). As a consequence, while *T. canis* is recognised as a dangerous zoonosis agent, T. cati has had its importance neglected by sanitary authorities and even sometimes by health professionals (Fisher 2003). However, there are some records of human intestinal infection by adult T. cati (Sprent 1956, Dubey 1968, Fisher 2003) and human ocular involvement by T. cati larvae causing ocular larva migrans (OLM) (Sakai et al. 1998), as well as some cases of VLM (Fisher 2003). Sprent (1956) pointed out that the larvae of T. cati can infect and migrate into tissues of some types of invertebrates (earthworms and cockroaches) as well as in birds, mice, lambs, dogs and probably human beings; the adult stage is only found in cats and, in rare cases, humans.

Cats can become infected with *T. cati* by ingesting embryonated eggs found in contaminated soil or by predation of paratenic hosts - usually small rodents or birds containing live third-stage larvae in their tissues (Sprent 1956). Another means of transmission in cats is larval transmammary migration (Sprent 1956, Coat et al. 2004). Some behavioural changes were found in paratenic hosts

harbouring third-stage *T. canis* larvae, probably favouring predation by dogs (Cox & Holland 2001), but there are no records of the occurrence of similar behavioural changes in rodents infected by *T. cati*.

Rattus norvegicus has been recognised as a usual paratenic host for *T. canis* (Chieffi et al. 1981) and probably also for third-stage *T. cati* larvae. Although the larval distribution of *T. canis* in *R. norvegicus* has already been studied (Lescano et al. 2004), the larval distribution for *T. cati* has only been analysed in mice (Dubey 1968) and has shown some significant differences when compared to that found for *T. canis* in the same rodent (Bardón et al. 1994). The aim of the present study was to recover larvae from tissues and organs of experimentally infected *R. norvegicus*, in order to characterise the migration pattern of *T. cati* larvae in this rodent species.

Twenty-one male *R. norvegicus* (Wistar strain) rats, aged eight weeks, were orally infected with 300 embryonated *T. cati* eggs containing third-stage larvae (Araújo 1972). On days 3, 5, 8, 10, 15, 30 and 60 after inoculation, three rats were killed and larvae were recovered from the liver, lungs, kidneys, eyes, brain and carcass of each rat after digestion with HCl 0.5% for 24 h at 37°C. Following sedimentation, supernatant was poured into a tube and centrifuged for 2 min at 1,500 rpm; then, 2 mL of the pelleted sediment was collected and thoroughly mixed and 0.1 cm³ samples were completely analysed under a light microscope for larval counts (Wang & Luo 1998). The final result, shown in Table, is an average of the larval numbers of all animals within each group.

The experimental protocol was approved by the Research Ethical Committee of the Instituto de Medicina Tropical, São Paulo.

Analysing *T. canis* larval migration, Lescano et al. (2004) observed a higher concentration of larvae in the liver, lungs and kidneys within the first five days after inoculation. After the 15th day of infection, an increase

Group number	Days after infection		_				
		Carcass (%)	Liver (%)	Lungs (%)	Kidneys (%)	Eyes (%)	Brain (%)
1	3	67.3 (22.43)	86.3 (28.76)	77.3 (25.76)	12.0 (4.0)	0.3 (0.1)	0 (0.0)
2	5	154.0 (51.33)	29.6 (9.86)	74.0 (24.66)	11.6 (3.86)	0.3 (0.1)	3.3 (1.1)
3	8	115.3 (38.43)	4.0 (1.33)	13.6 (4.53)	1.3 (0.43)	0.3 (0.1)	0.6 (0.2)
4	10	54.6 (18.2)	0.3 (0.1)	12.0 (4.0)	0.3 (0.1)	0 (0.0)	1.0 (0.33)
5	15	236.6 (78.86)	2.6 (0.86)	40.0 (13.33)	1.6 (0.53)	0 (0.0)	1.6 (0.53)
6	30	176.6 (58.88)	2.3 (0.76)	14.6 (4.86)	0 (0.0)	0.3 (0.1)	3.3 (1.1)
7	60	290.3 (96.76)	2.3 (0.76)	44.0 (14.66)	0.3 (0.1)	0 (0.0)	0 (0.0)

TABLE

Mean number of *Toxocara cati* larvae recovered in organs and tissues in *Rattus norvegicus* experimentally infected

in larval recovery from the brain was noted, lasting up to the 30th day of observation. Larvae were found in the muscles from the fifty day of infection up to the end of the experiment. Some significant differences were observed in our study of R. norvegicus infected with T. cati larvae and the study of Lescano et al. (2004). In our study, for example, larval migration to the brain was less expressive - similar to that previously observed in mice (Bardón et al. 1994) - and occurred mainly on the 15th and the 30th days after inoculation. On the other hand, our report of the ocular migration of *T. cati* larvae occurring principally at the beginning of the this experiment confirms the findings of Akao et al. (2000) in experimentally infected gerbils and the hypothesis regarding the importance of T. cati larvae in the etiology of OLM. Under the conditions of our present experiment (a single inoculation of T. cati eggs and only 60 days of observation period). R. norvegicus does not seem to be the best model for OLM when compared to other rodent species (Havasiová-Reiterová et al. 1995, Alba-Hurtado et al. 2009). However, it is important to highlight the raised level of R. norvegicus infection by *Toxocara* spp. in natural conditions (Chieffi et al. 1981), suggesting a role for this rat as a potential reservoir for *Toxocara* spp. in the environment.

REFERENCES

- Akao N, Takayanagi TH, Suzuki R, Tsukidate S, Fujita K 2000. Ocular larva migrans caused by *Toxocara cati* in Mongolian gerbils and a comparison of ophthalmologic findings with those produced by *T. canis. J Parasitol 86*: 1133-1135.
- Alba-Hurtado F, Muñoz-Guzmán MA, Valdivia-Anda G, Tórtora JL, Ortega-Pierres MG 2009. Toxocara canis: larval migration dynamics, detection of antibody reactivity to larval excretory-secretory antigens and clinical findings during experimental infection of gerbils (Meriones unguiculatus). Exp Parasitol 122: 1-5.
- Araújo P 1972. Observações pertinentes às primeiras ecdises de larvas de Ascaris lumbicoides, A. suum e Toxocara canis. Rev Inst Med Trop Sao Paulo 14: 89-90.

- Bardón R, Cuéllar C, Guillén JL 1994. Larval distribution of *Toxocara canis* in BALB/c mice at nine weeks and one year post-inoculation. *J Helminth* 68: 350-360.
- Beaver PC, Snyder CH, Carrera GM, Dent JH, Lafferty JW 1952. Chronic eosinophilia due to visceral larva migrans: report of three cases. *Pediatrics* 9: 7-19.
- 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). *Rev Inst Adolfo Lutz 41*: 89-91.
- Coat N, Schnieder T, Epe C 2004. Vertical transmission of *Toxocara cati* Schrank 1788 (Anisakidae) in the cat. *Parasitol Res* 92: 142-146.
- Cox DM, Holland CV 2001. Relationship between three intensity levels of *Toxocara canis* larvae in the brain and effects on exploration, anxiety, learning and memory in the murine host. *J Helminth* 75: 33-41.
- Dubey JP 1968. Migration of *Toxocara cati* larvae in mice. *Trop Geogr Med 20*: 172-176.
- Fisher M 2003. *Toxocara cati*: an underestimated zoonotic agent. *Trends Parasitol 19*: 167-170.
- Havasiová-Reiterová K, Tomasovicová O, Dubinský P 1995. Effect of various doses of infective *Toxocara canis* and *Toxocara cati* eggs on the humoral response and distribution of larvae in mice. *Parasitol Res 81*: 13-17.
- Lescano SAZ, Queiroz, ML, Chieffi PP 2004. Larval recovery of *Toxocara canis* in organs and tissues of experimentally infected *Rattus norvegicus. Mem Inst Oswaldo Cruz 99*: 627-628.
- Sakai R, Kawashima H, Shibui H, Kamata K, Kambara C, Matsuoka H 1998. Toxocara cati-induced ocular toxocariasis. Arch Ophthalmol 116: 1686-1687.
- Schantz PM 1989. *Toxocara* larva migrans now. *Am J Trop Med Hyg* 41 (Suppl. 3): 21-34.
- Sprent JFA 1956. The life history and development of *Toxocara cati* (Schrank 1788) in the domestic cat. *Parasitology 46*: 54-78.
- Wang GX, Luo ZJ 1998. A novel method for the recovery of *Toxocara* canis in mice. *J Helminthol* 72: 183-184.