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Initial stage of development and migratory behavior of *Toxocara canis* larvae in BALB/c mouse experimental model

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ABSTRACT. In the present study, the initial developmental stage of *Toxocara canis* eggs and larvae, and number of recovered larvae from BALB/c mouse-infected organs are described. *In vitro* culture of *T. canis* detects the frequencies of interphasic, mitotic and embryonated eggs only within a 7-day period. Analysis by egg counting was carried out for 32 days. The results showed that at 7 days after cultivation, the frequency of larvae was 50.4% and that this frequency reached 52.8% in 32 days. In the experimental infection of BALB/c mice with *T. canis*, the number of recovered larvae statistically increased in the brain and liver, with doses of approximately 200 and 1000 eggs. After 7 days of infection, a larger number of larvae were obtained in the lung and liver, although a maximum amount was found in the brain after a 15- or 30-day post-infection period.

Key words: *Toxocara canis*; BALB/c mice; Larval recovery; Visceral larva migrans; Infection

INTRODUCTION

There is epidemic evidence that *Toxocara canis* is a parasite with the highest incidence in zoonotic zones. *Toxocara canis* and *T. cati*, common ascarids in dogs and cats, are major etiological agents of toxocariasis (Magnaval et al., 2001). Human infection by *Toxocara* sp eggs is distributed worldwide and appears in variable frequencies, depending on factors related to children's hygienic and behavioral habitats (Aguiar-Santos et al., 2004). Children are most likely to be infected, probably because of their undeveloped immune system, the amount of eggs ingested (Habluetzel et al., 2003) and the frequency of reinfections (Chorazy and Richardson, 2005). Most patients who are seropositive for *Toxocara* spp show no clinical signs. However, sometimes it can cause fever, hepatosplenomegaly, pulmonary symptoms such as asthma, acute bronchitis, dermatological disorders, myositis, lymphadenopathy, and pseudorheumatic syndromes such as arthralgia with or without eosinophilia and anti-*Toxocara* sp antibodies (Lopez et al., 2002).

A wide range of animals, including mice, rabbits, monkeys, and humans, act as paratenic hosts (Lescano et al., 2004). When ingested, the larva batch penetrates the walls of the small intestine and disseminate in a hematogenous form through the soft tissues of the body (Anderson et al., 2005). According to the literature, the most commonly affected organs are the liver, lungs and eyes. The central nervous system, heart and skeletal muscles are less often affected.

Several studies with animal models have been developed, particularly to evaluate the effects of drugs in toxocariasis treatment. An effective drug against toxocariasis is still to be developed (Caumes, 2003; Satoh et al., 2005; Lescano et al., 2005). Due to low anthelmintic efficiency against diseases caused by the migration of nematode larvae in humans, cure is difficult (Magnaval et al., 1997; Moreira-Silva et al., 2004; Satoh et al., 2005; Hamilton et al., 2006). Smith (1991) reported that the migration pathway of larvae in humans and mice is similar, and lesions in experimental mouse models and humans are comparable. In fact, the animal model has been widely used to study toxocariasis. Recently, it has been demonstrated that infection of BALB/c mice with *T. canis* results in chronic pulmonary inflammation and a dominant TH2 type of immune response, regardless of inoculum size (Pinelli et al., 2005).

The aim of the present study was to optimize the time of different phases in initial stage of development of the *T. canis* eggs and larvae for quantification in BALB/c mouse-infected organs.

MATERIAL AND METHODS

Biological material

T. canis adult worms were recovered from naturally infected dogs, after routine de-worming by anthelmintic treatment. Eggs were collected from the uteri of 10 female worms for *in vitro* culture. After processing, the eggs were maintained in 2% formalin solution for 4 weeks at 28°C, and the different phases of development were determined under light microscopy. Our experiments were adapted to oxygenate the cultures for 30 min/day. Eighteen BALB/c mice (9 males and 9 females), with 4-6 weeks of age, were

orally infected, using a syringe fitted with a blunt needle, with approximately 200 and 1000 embryonated *T. canis* eggs. On days 7, 15 and 30 after inoculation, four mice were killed by cervical dislocation and larvae were recovered from brain, lungs, liver, and kidneys, after acid digestion, as described by Xi and Jin (1998). Larval counting in fixed samples was undertaken by microscopic analysis. Eighteen control mice uninfected were submitted to the same conditions.

Statistical analysis

The analysis was carried out at the 95% confidence limit. Kruskal-Wallis ANOVA was applied for results obtained by experimental infection in BALB/c mice to evaluate the influence of dose, gender and post-infection period on the frequency of recovered larvae in brain, lungs, liver, and kidneys. The Mann-Whitney method of multiple pair-wise comparisons was also applied to determine the differences between gender and post-infection period.

RESULTS AND DISCUSSION

The experiments were adapted to oxygenate the cultures for 30 min/day, and the larval developmental stage could be observed in only seven days. The data contrasted other studies that showed that the development of *T. canis* larvae needed at least one month (Rodriguez-Caballero et al., 2007).

The initial stage of *T. canis* development is shown in Figure 1 and Table 1. On the 7th day, the frequency of larvae was 50.4% and after 32 days this frequency reached 52.8%. During this period, 44.4 and 2.8% of the eggs remained in interphase and mitosis, respectively. The efficiency of this method has been recently demonstrated by Rodriguez-Caballero et al. (2007) who reported equivalent quantities of eggs in interphase and mitosis in the first day of cultivation.

Figure 1. Initial stages of *Toxocara canis* development. Eggs in interphase (A), mitosis (B and C), embryonated egg with larva inside (D), and larva reaching egg (E). Phase contrast microscopy, 200X.

Table 1. Initial stage of *Toxocara canis* development frequency.

Days	Phases of development (%)			Total cells analyzed
	I	MI	L	
1	53.2 ± 2.8	46.8 ± 2.8	0.0 ± 0.0	230
5	56.0 ± 2.3	24.8 ± 0.7	19.2 ± 2.9	643
7	49.3 ± 6.8	1.3 ± 0.6	50.4 ± 6.3	437
10	51.8 ± 9.4	1.0 ± 0.6	43.8 ± 8.8	542
15	56.2 ± 20.4	3.5 ± 1.2	40.4 ± 21.8	248
32	44.4 ± 7.5	2.8 ± 0.4	52.8 ± 1.7	529

I = interphase; MI = mitosis; L = larvae.

Our results showed that in the two tested egg doses, 200 and 1000 embryonated *T. canis* eggs, the number of recovered larvae in different organs was significant only for the brain and the liver (P = 0.0010 and 0.0004, respectively), see Figures 2 and 3. The important aspect of larval survival is that in the liver they may remain in a dormant state, triggering no response or only a weak response from the immunological system. In humans, this may cause serious ocular damage when they migrate to the retina, causing ocular larva migrans disease (Leone et al., 2006).

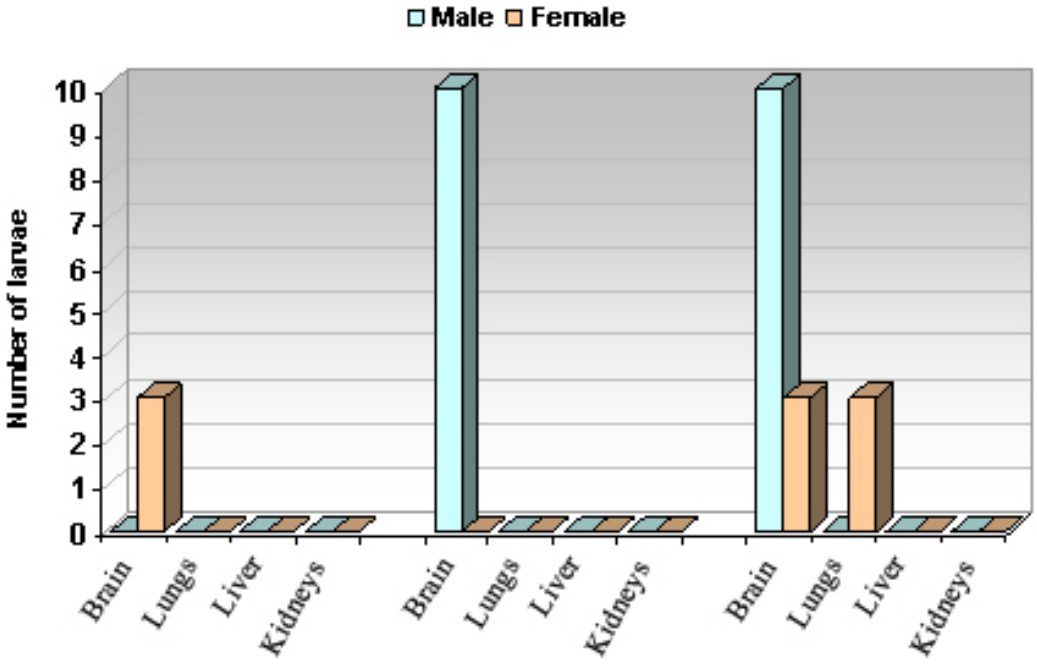

Figure 2. Mean percentage of total *Toxocara canis* larvae recovered from four organs of male and female BALB/c infected with a 200-egg dose after 7, 15 and 30 days post-infection.

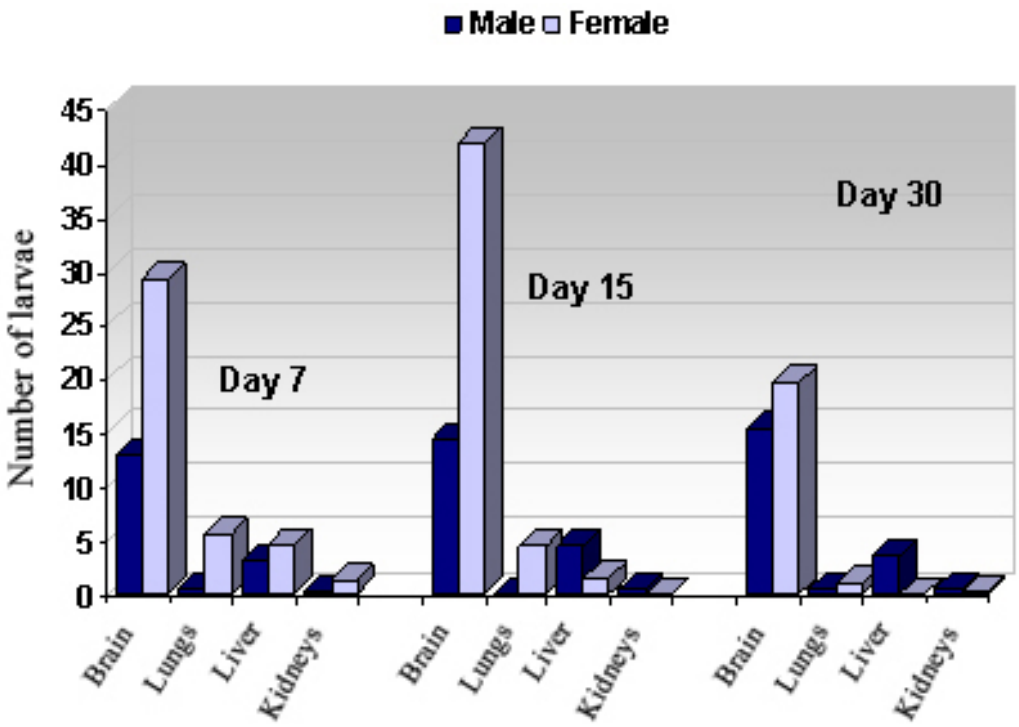

Figure 3. Mean percentage of total *Toxocara canis* larvae recovered from four organs of male and female BALB/c infected with a 1000-egg dose after 7, 15 and 30 days post-infection.

There was a significant difference in larval recovery when gender or days of infection were taken into account. The smaller dosage (200 eggs), close to natural infection, allowed the recovery of a low percentage of larvae. In this experimental condition, larvae were found in the brain and lung of female BALB/c on the 7th day after infection, while in males the larvae were recovered only in the brain on the 15th day. These data agree with those described by Xi and Jin (1998) and Lescano et al. (2004).

More larvae were also recovered from the brain with doses of 1000 eggs. There was a significant effect at 7 days post-infection on larval recovery from lungs ($P = 0.033896$) or liver ($P = 0.043115$). Higher larval recovery from the brain of BALB/c occurred on the 15th day in the female and on the 30th day in the male ($P = 0.049535$). Pinelli et al. (2005) showed that, within the same experimental conditions, recovered *T. canis* larvae increased in the lung on the 7th day and decreased thereafter, with an accumulation in the brain up to the 56th day. Studies on cerebral toxocariasis have shown that the infective dose influences not only the larval burden in the brain but also the behavior of *Toxocara*-infected mice (Cox and Holland, 1998). According to Abo-Shehada and Herbert (1989), when the larvae reach the liver and the lungs (hepato-pulmonary phases), the larvae migrate throughout the body and accumulate mainly in the carcass and in the brain (myotropic-neurotropic phases). Pinelli et al. (2007) showed that *T. canis* larvae migrated to the lungs of infected BALB/c mice and caused tissue damage and pulmonary inflammation.

Although in experimental animals the larvae frequently migrate to the brain, clinical involvement of the nervous system in visceral larva migrans is rare (Moreira-Silva et al., 2004). Hamilton et al. (2006) studied cerebral toxocariasis in seven strains of mice and chose two strains of BALB/c (susceptible and resistant) infected with 2000 embryonated eggs of *T. cani* and observed that the larvae cannot be a reliable indicator of susceptibility or resistance to *T. canis* infection. Lescano et al. (2004) studied the recovery of *T. canis* larvae in *Rathus norvegicus* infected with approximately 500 embryonated eggs. The highest number of recovered larvae occurred in the liver, especially between the third and fifth days after infection, while in the brain the highest values were obtained between the 15th and 60th days after infection.

Current analysis suggests the influence of the animal's sex, or rather, a possible hormonal interference in the female. Our results raise new questions on *T. canis* biology as well as provide information on larva migratory behavior in experimental models.

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