

EXPERIMENTAL INFECTION WITH *Toxocara cati* IN PIGS: MIGRATORY PATTERN AND PATHOLOGICAL RESPONSE IN EARLY PHASE

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SUMMARY

Experimental inoculations of approximately 100,000 infective *Toxocara cati* larval eggs were done in twelve pigs. The *T. cati* eggs used for inoculation were collected from cat's feces. Another group of three pigs served as an uninfected control. Groups of infected pigs were euthanized at seven, 14, 21, and 28 days post-inoculation (dpi). Tissue samples were taken for digestion and histopathology changes in early phase. The number of larvae recovered from the lungs peaked at seven and 14 dpi and were also present at 21, and 28 dpi. Larvae of *T. cati* were present in the lymph nodes of the small and large intestine at seven, 14, and 28 dpi and at seven, 14, 21, and 28 dpi respectively. In other studied tissues, no larvae or less than one larva per gram was detected. The pathological response observed in the liver and lungs at seven and 14 dpi, showed white spots on the liver surface and areas of consolidation were observed in the lungs. The lungs showed an inflammatory reaction with larvae in center at 28 dpi. In the liver we observed periportal and perilobular hepatitis. The lymph nodes of the intestines displayed eosinophil lymphadenitis with reactive centers containing parasitic forms in some of them. The granulomatous reaction was not observed in any tissues. The role of the other examined tissues had less significance. The relevance of this parasite as an etiological agent that leads to disease in paratenic hosts is evident.

KEYWORDS: Animal model; Larval recovery; *Toxocara cati*; Tissue lesions; Infected pig; Toxocariasis.

INTRODUCTION

Toxocara canis and *Toxocara cati* are common parasites of dogs and cats, respectively. Both are zoonotic agents that can cause significant disease in humans as well. Human infection with larval *Toxocara* produces classical visceral larva migrans; incomplete visceral larva migrans; compartmentalized forms: ocular and neurological toxocariasis; covert toxocariasis; and asymptomatic toxocariasis¹⁴.

T. cati infects both domestic and wild felines. Undeveloped eggs deposited by adult parasites are excreted in the feces of the host but are not infective in this first stage. Infective forms develop after some time in the environment. Cats can be infected by ingesting embryonated *T. cati* eggs from the soil or larvae in paratenic hosts and play a fundamental role in the dispersion of the parasite.^{5,6,13,22} The presence of *T. cati*-infected felines in public spaces, their defecation habits, and their direct contact with humans constitute significant factors that promote the transmission of this zoonosis. A number of studies taking place worldwide have described the extent of environmental contamination with this parasite. The cat population lacks sanitary control, reproduces freely, and transforms open spaces into permanent sources of infection, thus increasing the risk of infection for the humans that spend time in these areas²¹.

Despite the use of several animal models and strategies to analyze the pathological tissue alterations and behavior of migrating of *Toxocara* spp. larvae in mammals, several controversies persist. The results of multiple studies vary based on the animal model chosen, the parasite species inoculated, and the larval dose(s) used for infection. Experimental infections with *T. canis* and *T. cati* have been described in different animal models, including mice, Mongolian gerbils, rats, and chickens^{2,3,5,8,11,12,15,18,22, 24,25}.

Pigs are paratenic hosts of the *T. cati* parasite and they have many physiological similarities to humans. Pigs have been used as an animal model for the study of the Toxocariasis due to *T. canis*. Pigs experimentally infected with *T. canis* were previously used to study the migratory pattern of the parasite as well as to analyze the pathological changes, immunological response, and hematological disturbances triggered by infection^{3,9,15,19,20,24}. *T. canis* is better recognized as the main cause of the toxocariasis. *T. cati* has been little studied as an etiological agent for human toxocariasis. STRUBE *et al.*²³ expressed that "No experimental infections of pigs with *T. cati* could be found in the literature; there for the migratory route of this species in this particular host remains to be determined". In this work we use pigs for studying toxocariasis due to this parasite. The objectives of this work were to study

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the migratory pattern of *Toxocara cati* larvae in the early phase of the infection in pigs and the pathological response of the tissues.

MATERIALS AND METHODS

The inoculums were prepared with *T. cati* eggs obtained from the feces of naturally infected cats. *T. cati* eggs were collected by brine flotation from the stools and washed in tap water. Each 2 g of tissue was incubated in 0.5% formaldehyde with 0.03 mL of iodopovidone 10% during 35 days at 28 °C. They were shaken daily for eight hours. The embryonation of eggs followed weekly by light microscopy²⁰. To evaluate the infectivity of the embryonated *T. cati* eggs, three mice were inoculated and then euthanized at one day post-inoculation (dpi). Animals were provided by the Faculty of Veterinary Sciences of the University of Buenos Aires and were housed according to the accepted standards of laboratory animal management¹. The liver and lungs were processed by the acid digestion technique as previously described⁹. Embryonated eggs were concentrated using Benbrook's flotation technique with a saturated sugar solution. The supernatant was extracted and washed three times, with each wash followed by successive sedimentation steps by centrifugation⁷. Each inoculum contained approximately 100,000 eggs in one mL of saline solution (0.9 N NaCl). The experimental protocol was approved by the Institutional Committee for the Care and Use of Laboratory Animals, Faculty of Veterinary Science, University of Buenos Aires.

Fifteen 40-day-old male Yorkshire pigs weighing 18 kg each were obtained from a swine production facility with excellent health conditions. Four groups of three animals were randomly chosen to receive the inoculums and the remaining group was designated as the uninfected control. Prior to inoculation, pigs were fasted for 12 hours. Each one mL inoculum was administered orally using a syringe. The syringe was then washed with two mL of saline solution, which was also given to the animal. Control animals received one mL of saline solution in a similar manner. All animals were examined clinically once a week. The experimental protocol was approved by the Institutional Committee for the Care and Use of Laboratory Animals, Faculty of Veterinary Science, University of Buenos Aires, and animals were maintained according to accepted standards¹.

Groups of three infected pigs were euthanized by standard methods¹ at seven, 14, 21, and 28 dpi and were subsequently analyzed by necropsy. The control group was euthanized at 28 dpi. Each organ and tissue was weighed, and 100 g samples were randomly selected and divided into two halves. Organs and tissues including liver, lungs, kidneys, heart, eyes, brain, tongue, skeletal muscles and lymph nodes of the small and large intestines were collected. Eyes of each pig were sent one for pathological observation and the other for artificial digestion.

Macroscopic analysis was performed at necropsy. Microscopic evaluation of the selected organs and tissues was performed according to the technique previously described²⁰. Each 50 g sample of tissue had been submerged in 10% formaldehyde and then processed for histopathology. One eye from each animal was entirely analyzed.

The artificial digestion method previously described⁹ was used to recover *T. cati* larvae from each 50 g sample of tissues. One eye from each animal was completely digested. A volume of 0.3 mL of concentrated

material was observed under a light microscope (10 x), and the total number of larvae was recorded. The number of larvae per gram of tissue in each sample and the total weight of each organ was used to estimate the number of larvae present in each organ.

The recovery of larvae from tissues at different time points was analyzed using a non-parametric test (Kruskal-Wallis chi-square approximation). In cases in which the time effect was significant, ANOVA (analysis of variance) and polynomial contrasts were used. The level of significance was set at 5% (p -values < 0.05). InfoStat software was used for all statistical analyses.

RESULTS

T. cati larvae were recovered from tissues of all the inoculated pigs (Table 1). An evaluation of the number of larvae recovered from the lungs showed a significant difference among seven, 14, 21, and 28 dpi ($X^2 = 8.3497$; $p = 0.0393$) that can be explained by a significant linear trend ($p = 0.002$) that describes the greater recovered at seven and 14 dpi. Larva recovery from the lymph nodes of the small intestine peaked at 14 dpi, and significant differences among seven, 14, 21, and 28 dpi were detected ($X^2 = 9.0702$, $p = 0.0284$), also explained by a significant linear trend ($p = 0.0009$). Larva recovery from the lymph nodes of the large intestine peaked at seven dpi, and statistical analysis detected significant differences among seven, 14, 21, and 28 dpi ($X^2 = 8.5556$, $p = 0.0358$) with a significant linear trend ($p = 0.0013$). An evaluation of the number of larvae recovered from the liver, brain, kidneys, heart, muscle, and eyes showed no significant differences among seven, 14, 21, and 28 dpi. No larvae were recovered from any tissue of the control animals.

Macroscopic lesions on the lungs with areas of consolidation over the entire surface were visible at seven and 14 dpi, but these lesions decreased at 21 and 28 dpi. Microscopic lesions were observed throughout the experiment. At seven dpi, the lungs displayed an inflammatory reaction consisting of a mixture of mononuclear and polymorphonuclear cells (lymphocytes, plasmocytes, and eosinophils). We detected multifocal nodular pneumonia and an accumulation of eosinophils at 14 dpi. Non-suppurative diffuse pneumonia with a lymphoid hyperplasia focus containing lymphocytes, plasmocytes, and eosinophils was observed at 21 and 28 dpi. Larvae were present at the center of the lesion at 28 dpi (Fig. 1).

At seven and 14 dpi, macroscopic lesions on the liver had small white spots 2-3 mm in diameter on its surface; very few spots were detected at 21 or 28 dpi. Microscopically we observed periportal and perilobular hepatitis with a predominance of leukocytes, lymphocytes, and plasmocytes in infected pigs, along with a smaller proportion of eosinophils at seven and 14 dpi (Fig. 2).

Macroscopically, the lymph nodes of the small and large intestines were slightly edematous at seven and 14 dpi but were normal at the other time points. Microscopically, a granulomatous reaction with larvae in the pericapsular external zone of the lymph nodes was visible at seven and 14 dpi (Fig. 3), and giant cells were also present. Such lesions were not detected at 21 or 28 dpi. The Splendore-Hoeppli phenomenon, observed in some fungal and parasitic diseases as the result of a local antigen-antibody complex formation, was detected in lymph nodes and was characterized by the deposition of amorphous eosinophilic hyaline material around the larvae (Fig. 4).

Table 1

Mean number of *T. cati* larvae recovered per gram of tissues at euthanized time points, in tissues of pigs experimentally infected with 100,000 *T. cati* eggs

Tissues	Days post-inoculation			
	7	14	21	28
Lung	18.98 ± 7.20 ^a (11-25)	19.77 ± 5.34 ^a (14.32-25)	0.91 ± 0.81 ^a (0.07-1.71)	3.17 ± 4.19 ^a (0.65-8.02)
Lymph nodes (SI)*	6.30 ± 5.08 ^a (0.78-10.8)	13.25 ± 17.28 ^a (2.76-33.2)	0	0.16 ± 0.27 ^a (0-0.48)
Lymph nodes (LI)**	15.88 ± 8.11 ^a (7-22.9)	10.25 ± 13.71 ^a (0.93-26)	0.21 ± 0.36 ^a (0-0.63)	0.29 ± 0.29 ^a (0-0.59)
Liver	0	0.05 ± 0.05 ^a (0-0.10)	0.13 ± 0.23 ^a (0-0.40)	0.01 ± 0.02 ^a (0-0.03)
Brain	0.0 ± 0.01 ^a (0-0.02)	0.02 ± 0.04 ^a (0-0.08)	0.01 ± 0.02 ^a (0-0.05)	0.02 ± 0.03 ^a (0-0.06)
Kidney	0.09 ± 0.01 ^a (0.08-0.11)	0.15 ± 0.25 ^a (0-0.45)	0	0
Heart	0.02 ± 0.02 ^a (0-0.04)	0	0	0
Muscle	0.40 ± 0.33 ^a (0.02-0.64)	0.99 ± 1.20 ^a (0.18-2.37)	0.05 ± 0.04 ^a (0.01-0.10)	0.59 ± 0.35 ^a (0.37-1.0)
Eyes	0	0	0	0.02 ± 0.02 ^a (0.01-0.05)

*: small intestine; **: large intestine. ^aRange (± S.E.).

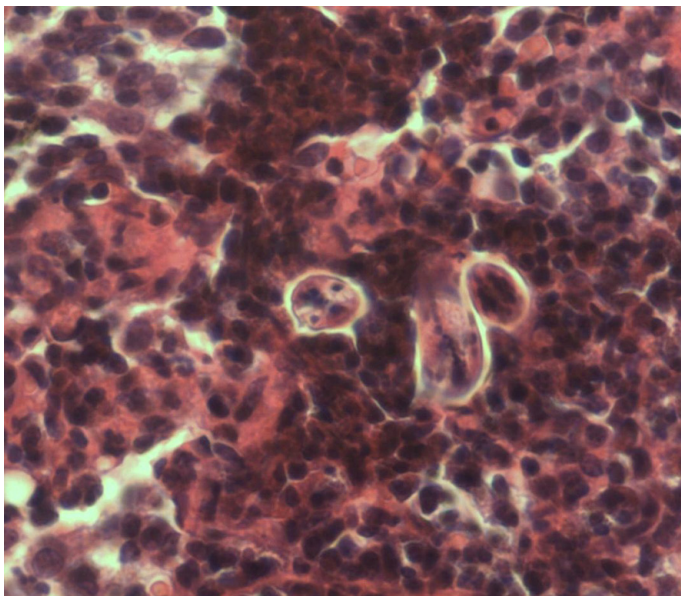


Fig. 1 - Lung tissue from a pig experimentally infected with 100,000 *T. cati* eggs, showing infiltration of eosinophils with larvae in the center (E/H 40x).

No macroscopic lesions were observed in the tongue. Tongues from infected pigs displayed microscopic lesions with non-suppurative and segmental myositis accompanied by leukocytes, lymphocytes, plasmocytes, and a small number of eosinophil cells at 14 and 28 dpi.

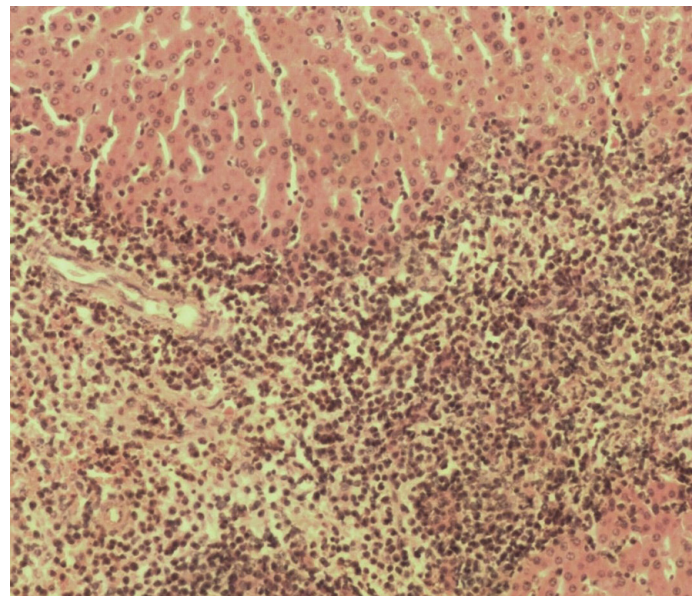


Fig. 2 - Liver tissue from a pig experimentally infected with 100,000 *T. cati* eggs, showing infiltration of lymphocytes, plasmocytes, and a small number of eosinophils (E/H 10x).

No macroscopic lesions were observed in the kidneys. Microscopic images of the kidneys showed focal non-suppurative interstitial nephritis at 14, 21 and 28 dpi.

Muscle did not present macroscopic alterations. Microscopically,

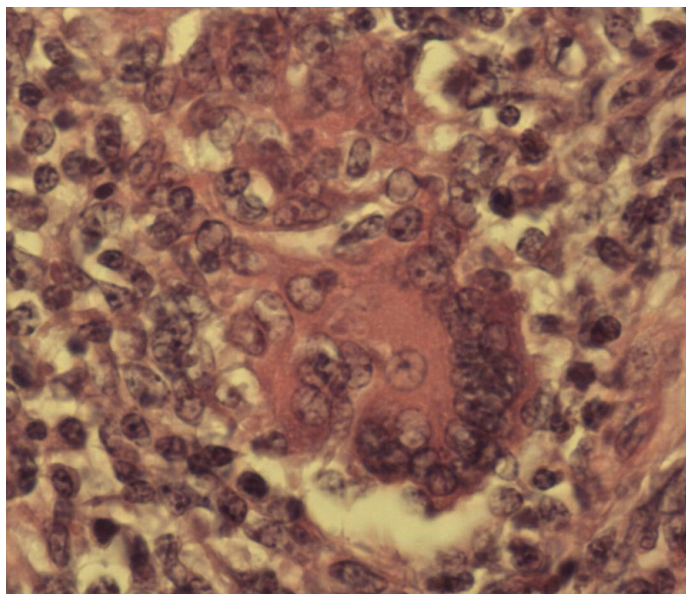


Fig. 3 -Lymph node tissue from a pig experimentally infected with 100,000 *T. cati* eggs, showing a reactive center in the follicle lymphoid with giant cells (E/H 40x).

several myositis foci with accumulated lymphocytes and eosinophils were observed at 14 dpi.

Pathological lesions were not detected in the brain or eyes.

In any tissue of the pigs infected with *T. cati*, the typical granulomatous reaction composed of a mixture of eosinophils, lymphoid cells, and epithelial cells surrounding a central necrotic core containing visible larvae was not observed.

No pathological lesions were observed in the control animals.

DISCUSSION

This study is the first to quantify larvae recovered from *T. cati*-infected pig tissues by using digestion techniques. The histological observations previously reported by RONÉUS¹⁶ demonstrated the presence of *T. cati* larvae in inoculated pigs but did not quantify the larvae present in various tissues.

Here, the highest numbers of larvae in the early stages of infection were recovered from the lungs and the lymph nodes of the large and small intestines (seven and 14 dpi). The remarkably high persistence of larvae in the lungs throughout the study indicates a predilection of *T. cati* larvae to migrate to the lungs. The lymph nodes also appeared to be another preferred site in the migratory pattern of the parasite. We also observed the persistence of *T. cati* larvae in pig muscles. *T. cati* larvae have been shown to accumulate and persist in diverse organs, depending on the animal model used to study its migratory patterns^{2,3,5,8,11,12,15,18,22,25}.

In pigs, DONE *et al.*⁴ reported that *T. canis* larvae have been previously shown to migrate primarily to the liver and lungs; HELWIGH *et al.*¹⁰ and TAIRA *et al.*²⁴, to the lungs and lymph nodes; SOMMERFELT *et al.*²⁰ observed to the lymph nodes, liver, and lungs. Therefore, lungs

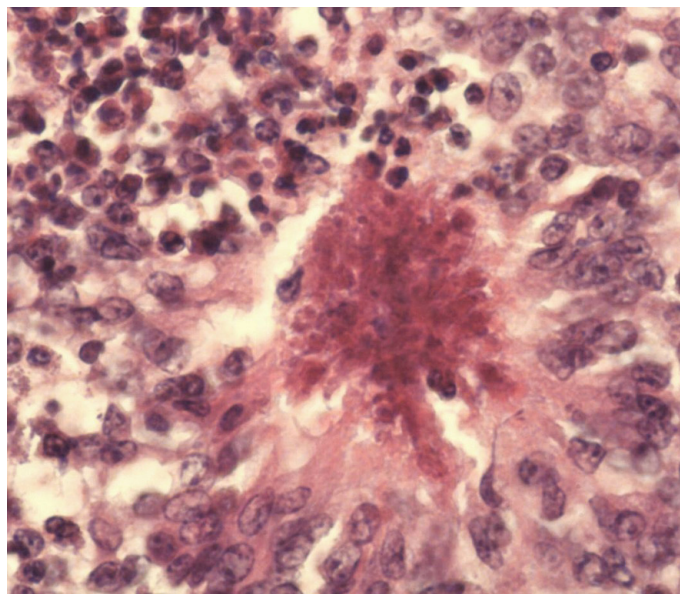


Fig. 4 - Lymph node tissue from a pig experimentally infected with 100,000 *T. cati* eggs, showing the Splendore-Hoeppli phenomenon (E/H 40x).

and lymph nodes are the most frequent sites for both *T. canis* and *T. cati* parasites during migration through the host. We found macroscopic lesions in the liver and lungs, in agreement with RONÉUS¹⁶. However, previous study also described changes in the kidneys¹⁶ that we did not observe in the present study. In the lungs, microscopic lesions were detected early in the infection and persisted throughout the study. This observation is in contrast with RONÉUS¹⁶, who reported that lung lesions appeared, later in the infection. However, the results of our study agree with those of RONÉUS¹⁶ in regard to the liver lesions; both studies showed periportal and perilobular hepatitis and demonstrated that the severity of the liver lesions depends on the time at which they were examined.

The data from this study and previous reports suggest a type of larval migratory pattern in which the larvae reach the lungs and the liver by migrating through the mesenteric lymph nodes. Previous reports of macroscopic or microscopic lesions produced by *T. cati* in other animal models are somewhat in agreement with our observations of lesions in lungs, liver, kidneys, and muscle^{2,3,5,11,22}. Our results also agree with previous observations of macroscopic lesions in pigs inoculated with *T. canis*^{4,10,17,20}. White spots on the liver were also observed by TAIRA *et al.*²⁴. One notable difference between our study and previous research that inoculated *T. canis*, was the absence of the typical granulomatous reaction in our sample.

In conclusion, the results of this study show that the *T. cati* parasite is able to migrate through diverse tissues of the pig. Therefore, the relevance of this parasite as an etiological agent that leads to disease in paratenic hosts is evident. In our animal model, the pig, the parasite primarily migrated to the lungs and the lymph nodes. The migration of the larvae to the eyes observed in our research suggests its participation in the production of ocular larva migrans. The absence of the typical granulomatous reaction in tissues should be studied further to evaluate its influence on the immune response to *T. cati* infection and compare with the response of *T. canis* where the granulomatous reaction was present^{19,20}.

Our study contributes to the knowledge of the migratory behavior of *T. cati* larvae in pigs that could help for the comprehension of this parasite distribution on other paratenic host, as humans and to elucidate relevance of the host-parasite relationships especially in human toxocaríasis.

ETHICAL STANDARDS

The experimental protocol was approved by the Institutional Committee for the Care and Use of Laboratory Animals, Faculty of Veterinary Science, University of Buenos Aires. Argentina.

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

RESUMEN

Infeción experimental en cerdos con *Toxocara cati*: patrón migratorio y respuesta patológica en etapa temprana

Se realizó la infección experimental de doce cerdos con aproximadamente 100.000 huevos infectivos de *Toxocara cati*. Los huevos de *T. cati* utilizados en la inoculación fueron recolectados de heces felinas. Otro grupo de tres cerdos no infectados se utilizó como control. Grupos de cerdos infectados se eutanaciaron a los 7,14,21 y 28 días posinoculación (pi). Se tomaron muestras de tejidos para digestión y evaluación de cambios histopatológicos en la etapa temprana de la infección. El número de larvas recuperadas de los pulmones se incrementó en los días 7 y 14 pi, recuperándose también los días 21 y 28 pi. Se encontraron larvas de *T. cati* en los linfonódulos del intestino delgado y grueso los días 7,14 y 28 pi y los días 7,14,21 y 28 pi respectivamente. En los restantes tejidos estudiados o no se recuperaron larvas o los valores fueron menores a una larva por gramo de tejido. La respuesta patológica observada en el hígado y los pulmones a los 7 y 14 días posinoculación, mostró en la superficie del hígado manchas blancas y en los pulmones áreas de consolidación. Los pulmones presentaron una reacción inflamatoria con presencia de larva en el centro en el día 28 pi. En el hígado se observó una hepatitis periportal y perilobular. Los linfonódulos del intestino presentaron una linfadenitis eosinofílica con un centro reactivo conteniendo formas parasitarias en algunos de ellos. En ninguno de los tejidos se observó la típica reacción granulomatosa. El rol de los restantes tejidos examinados fue de menor significancia. Queda evidenciada la importancia de éste parásito como un agente etiológico que desarrolla la enfermedad en hospederos paraténicos.

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AUTHORS CONTRIBUTIONS

Conceived and designed the experiments: I E Sommerfelt, A J Franco. Performed the experiments: I E Sommerfelt, A Duchene, B Daprato, C M López, N Cardillo, A J Franco. Analyzed the data: C M López, I E Sommerfelt. Contributed reagents/materials/analysis tools: A Duchene,

N Cardillo, B Daprato, I E Sommerfelt, C M López, A J Franco. Wrote the paper: I E Sommerfelt.

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