Experimental *Toxocara canis* infection in chickens

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ABSTRACT

The objective of this study was to determine the development phases of *Toxocara canis* eggs outside the host and the migration of larvae in the internal organs of chickens infected by ingestion of embryonated *T. canis* eggs. Under a microscope observation of *T. canis* eggs which were incubated in a petri dish containing 5 mL of distilled water at 30 – 33°C with regularly aerated, this study showed the development of *T. canis* egg through various stages, including one cell, two cells, three cells, four cells, early morula, late morula, blastula, gastrula, tadpole, pre-larva, embryonated larva. In addition, it took approximately 9 days for *T. canis* eggs to hatch and for infective larvae to develop at 30 - 33°C. A total of 50 chickens were randomly assigned to 3 groups including group I (10 chicks/group) was served as control group without *T. canis* eggs inoculation; two treatment groups II and III (20 chicks/group) were orally inoculated with 500 or 1000 *T. canis* eggs, respectively. On 1, 3, 6, 15 and 30 days post inoculation (dpi), two chickens/control group and 4 chickens/treatment group were necropsied. The results showed that the percentage of larvae recovered varied from 14.00 to 33.93% and 13.07 to 32.00% in treatment groups II and III, respectively. After 1, 3, 6, and 30 dpi, the significant differences about the number of larvae recovered in two treatment groups were found ($P < 0.05$). In both treatment groups, the percentage of larvae recovered from livers was higher than that in lung tissue. For 6 dpi, small white foci on the liver's surface were seen. Interstitial pneumonia, petechial hemorrhages, dark or gray inflammatory nodules in the lung tissue and the atelectatic area were observed. Histopathology examination revealed infiltrations of leukocytes and eosinophil scattered in the liver and lung tissue.

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1. Introduction

Toxocara canis and *Toxocara cati* are typically gastrointestinal helminths found in canids and felids, respectively. *T. canis* is distributed worldwide and is a major zoonotic helminth, causing human toxocarosis. Female *Toxocara* spp. worm can produce up to 200,000 eggs which are shed into the environment and caused the potential risk for human infection (Glickman & Schantz, 1981). While dogs and cats are the main hosts, larvae may also persist and even cause serious sickness in a number of paratenic hosts including other mammals, birds and earthworms. While hatching larvae behave similarly to definitive larvae in paratenic hosts, development into the adult stage does not occur, and infectious third-stage larvae survive in tissues as a developmentally arrested state (Bruňaská et al., 1995). The potential zoonotic risks of *T. canis* should not be underestimated, as toxocarosis is one of the most frequently reported zoonotic helminth infections globally, this led to an increasing interest in the biology and epidemiology of the genus *Toxocara* (Magnaval et al., 2001). Humans and other mammals are at risk of infection from paratenic

chicken hosts. Previous studies described the larval distribution, persistence, the risks of infection for humans including the consumption of undercooked chicken infected with *T. canis* (Ito et al., 1986; Nagakura et al., 1989). Furthermore, *T. canis* larvae in chicken meat were demonstrated to be exceedingly infectious even after prolonged period of times/low temperatures, hens infected with *Toxocara* spp. offer a possible health risk (Taira et al., 2011). Chickens raised in free-range environments are more likely to consume embryonated eggs or infected paratenic hosts, such as earthworms (Pahari & Sasmal, 1991). According to Taira et al. (2003), *T. canis* larvae can migrate via the chicken's hepatopulmonary pathway, reinforcing the idea that poultry harboring migratory *T. canis* larvae may pose a zoonotic risk, particularly if the liver is ingested. Moreover, Beaver (1956) discovered that *T. canis* larvae remained viable in the livers of chickens and pigeons for at least three months. In previous studies, Nakamura et al. (1991) and Taira et al. (2003) discovered considerable differences in total *T. canis* larval recoveries among hosts. In addition, Taira et al. (2003) also suggested further investigations in chickens inoculated with small doses of *T. canis* embryonated eggs, which would more nearly imitate the natural way of infection to investigate the larval migration behavior.

Therefore, the objective of this study was to determine the morphology of the developmental stages from non-embryonated egg to infective egg. Additionally, *T. canis* larval distribution in organs of chickens was also investigated within this research.

2. Materials and methods

2.1. *In vitro* hatching of *T. canis* eggs

In this study, *T. canis* eggs were collected by using sedimentation method and counted by using Mc Master method from fresh faeces of infected dog with *T. canis*. Then, these *T. canis* eggs were raised in a petri dish containing 5 mL of distilled water and incubated as described of Abou-El-Naga (2018). *T. canis* eggs were sucked into a petri dish containing 5 mL of distilled water to raise eggs, incubating at 30 – 33°C. Every day, the egg dishes were provided oxygen for 15 - 30 min and refilled with distilled water equal to the initial water level (Abou-El-Naga, 2018). Using light microscope observation and recording were performed daily until all observed embryos were in L2 larvae (infected larvae).

2.2. Experimental *T. canis* infection in chickens

A total of 50 1-day-old Luong Phuong chicks weighing from 30 to 45 g were purchased from the Cu Chi Chicken Farming Enterprise. When these chickens were 7 days old, total 50 chickens were randomly assigned to 3 groups (1) group I (10 chickens/group) was served as control group without *T. canis* embryonated eggs inoculation; (2) two treatment groups II and III (20 chickens/group) were inoculated orally under 1 mL distilled water contained 500 or 1000 *T. canis* embryonated eggs, respectively, according to instructions of Oshima (1961). All birds were housed under similar conditions. Food and water were given ad libitum. At 1, 3, 6, 15 and 30 days post-inoculation (dpi), two chickens/control group and 4 chickens/treatment groups were chosen randomly for the gross pathology findings. In each treatment group, of 4 chickens, three chickens were used for the number of larvae recovered using digestion method and one chicken was used for histopathological examination. Prior to necropsy, blood samples from two treatment groups collected from the heart were smeared and stained with Giemsa for detection of larvae in blood. Subsequently, these chickens were euthanized by cervical dislocation for gross pathology findings, recovery of larvae and histological investigations. The entire duodenum, spleen, liver, heart, lungs, right inner pectoral muscle, and brain samples from three chickens per each treatment group were collected and kept separately. These samples were separately digested in HCl-pepsin solution for counting the number of larvae recovered. Each organ was chopped up and digested with 20 mL pepsin solution (5 g pepsin, 7 mL hydrochloric acid 36%, and 1000 mL distilled water) were added in sample tube. After that, the samples were incubated at 37°C for 24 h. After the digestion, the samples were centrifuged for 2 min at 1500 rpm and the supernatant was removed. Finally, the number of larvae recovered from each organ was counted under a microscope (Santos et al., 2009).

The percentage of relative larval tissue distribution in the liver and lungs was compared using the Kruskal – Wallis test for paired samples by groups, with a significance level of 5%.

3. Results and Discussion

3.1. *In vitro* hatching of *T. canis* eggs

This study showed that early morula, late morula, blastula, gastrula, tadpole, pre-larva, embryonated larvae were among the developmental stages documented. It took 7 to 9 days of rearing *T. canis* eggs in distilled water at 30 – 33°C and regularly aerated, the eggs from the one-embryo stage have developed to infective larvae eggs (Figure 1).

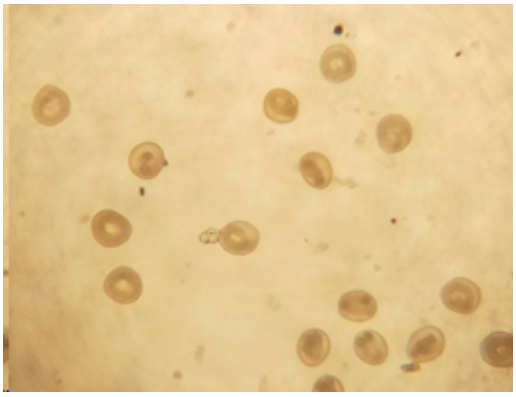

Figure 1. The morphology of T. canis infective larvae eggs (×10).

Meanwhile, if *T. canis* eggs were cultivated in distilled water at 28°C, it took 14 days for *T. canis* eggs to develop into first-stage larvae and 19 days for second-stage larvae (Abou-El-Naga, 2018). At the temperature range of 25°C - 30°C and humidity of 85 – 90%, it took 9 - 15 days for *T. canis* eggs to develop into infective larvae (Schacher, 1957; Okoshi & Usui, 1968). According to Cruz et al. (2012) and Abou-El-Naga (2018), temperature is the main factor that affects the development of roundworm eggs, the average daily growth rate increases significantly with raising temperature at 33°C. The average length and width of 50 *T. canis* larvae in this study were 383 μm and 19 μm, respectively.

3.2. Experimental *T. canis* infection in chickens

Table 1 showed the average percentage of total *T. canis* larvae collected from two treatment groups. At the time of examination, the majority of the larvae were still alive. No larvae were found in chickens of control group. After 1 day, 3, 6, and 30 days of inoculation, the significant differences in the numbers of larvae recovered between two treatment groups were found ($P < 0.05$). In consistent with previous study, Taira et al. (2003) demonstrated that the higher infective dose of larvae was used, the higher T. canis larvae recovered would be collected from animals. However, a failure of innoculation may occur in some chickens of treatment group III, this led to the significant differences about the number of larvae recovered between two treatment groups were not found after 15 days of inoculation. Oshima (1961) stated that larval inoculation results that are inconsistent can be attributed to the eggs' stickiness, which causes an unknown number of them to adhere to the walls of containers, syringes, and residual food.

In this study, most of larvae were only found in the liver and lung. Significant differences about the number of larvae recovered in livers between two treatment groups were found at 1 day, 3, 6, and 30 dpi ($P < 0.05$). Of these, liver is the most favorite site of localization of the *T. canis* larvae. In consistent with Okoshi & Usui (1968), *T. canis* larvae were mainly collected in the livers of chickens infected with 3000 embryonated ascarid eggs. Furthermore, all blood samples were negative with *T. canis* infection in this study. Burren (1972) indicated that larvae can enter the liver from the abdominal cavity (with no injury to the neighboring liver tissue), this explained why no larvae were found in blood samples after 24 h infection. According to Taira et al. (2003), the migration of *T. canis* larvae in chickens is mainly concentrated in the liver and lungs; meanwhile, a few of *T. canis* larvae was found in different organs including the pancreas, spleen, muscle, brain. However, no fatality and clinical signs were found in chickens in this study. In agreement with Taira et al. (2003), no clinical indications or abnormal behavior occurred in chickens inoculated with 3000 *T. canis* larvae.

After 24 h of infection, the livers of infected chickens appeared in yellowish color. More than 50% of the liver was damaged with small white spots after 3 dpi (Figure 2). At 30 dpi, although there were no milky white spots on the surface, these livers had pale color and hemorrhagic spots. Despite the presence of small white foci on the liver 6 days after administration, no gross lesion was found in the other organs (Maruyama et al., 1994). The fundamental lesion caused by larval migration comprised of a central zone of necro-

Table 1. Total larval burdens and relative larval tissue distribution of *T. canis* infected chickens

Days post infection	Egg doses	Total larval burdens		Relative larval tissue distribution (%)			
				Liver		Lung	
		$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%
1	500	70 ± 2.0	14.00	65 ± 1.0	13.00	5 ± 3.0	1.00
1	1000	320 ± 9.0	32.00	311.3 ± 6.0	31.13	8.67 ± 3.1	0.87
3	500	95 ± 6.2	19.00	93.3 ± 4.7	18.67	1.67 ± 2.9	0.33
3	1000	294 ± 23.2	29.43	290.7 ± 21.2	29.07	3.67 ± 2.1	0.36
6	500	82 ± 3.0	16.40	82 ± 3.0	16.40	0.00 ± 0.0	0.00
6	1000	304 ± 14.7	30.40	301.7 ± 14.4	30.17	2.33 ± 2.52	0.23
15	500	169.7 ± 6.1	33.93	169.7 ± 6.1	33.93	0.00 ± 0.0	0.00
15	1000	167 ± 2.5	16.73	167 ± 3.0	16.70	0.333 ± 0.6	0.03
30	500	58.3 ± 4.7	11.67	58.3 ± 4.7	11.67	0.00 ± 0.0	0.00
30	1000	130.7 ± 8.5	13.07	130.7 ± 8.5	13.07	0.00 ± 0.0	0.00

$\overline{X}$: The mean of number larvae recovered.

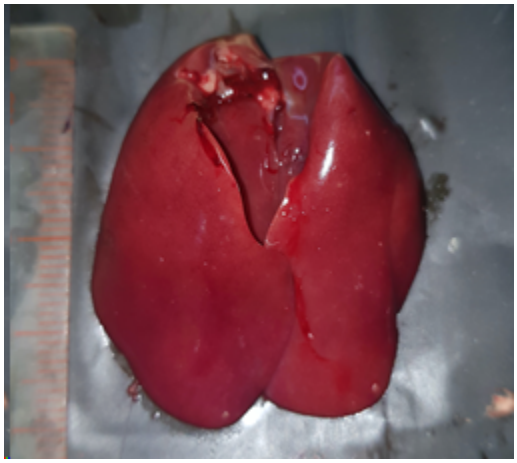

Figure 2. Gross lesions in liver (1000-egg dose on 3 day after infection: small white spots on the liver surface).

sis surrounded by a variable-intensity inflammatory zone. The necrosis usually involved the hepatic epithelium and, in some cases, the reticuloendothelial cells. The inflammatory response included varying degrees of reticuloendothelial cell hyperplasia as well as the infiltration of heterophiles and a lesser number of eosinophiles, monocytes, and lymphocytes (Galvin, 1964). No gross and histopathological changes were observed in control birds. At 15 dpi, the development of granulomas in areas surrounding the larvae was found (Figure 3A). According to Burren (1972), the majority of larvae penetrates the liver via the portal vein and is taken by the bloodstream to the "portal tracts" including a bile duct, portal vein, and artery; where they enter the lobules. However, these larvae were able to access the liver from the abdominal cavity, since no damage was observed in adjacent liver tissue, which could be attributed to internal migration, as shown in Figure 3A. The lesions were constituted of a central zone of necrosis and foreign body giant cells surrounded by a zone of reticular tissue containing a few leukocytes and ranging in size up to approximately 0.8 mm in diameter. The outermost layer was made up of fibrous connective tissue that formed a capsule containing a significant number of heterophile and eosinophils (a type of leukocyte often associated with parasites). In addition, the findings of this study are consistent with previous studies, the lungs from infected chickens appeared petechial hemorrhages on the surface. Interstitial pneumonia, the formation of dark to gray inflammatory nodules in the lung tissue as a result of migrating larvae, and the atelectatic area are also all observed lung abnormalities (Taira et al., 2003; Flecher et al., 2016). The lung injuries from infected chickens were unevenly distributed. On the 1st dpi, there was a significant quantity of eosinophils gathered and severe damage in alveolar wall structure. The gathering of eosinophils was enough evidence to prove *T. canis* larvae's presence in the lungs of inoculated chickens (Figure 3B).

4. Conclusions

This study showed the morphology changes of *T. canis* during the embryo development in the incubation *in vitro* at 30 - 33°C. The somatic migration of *T. canis* larvae was found in liver

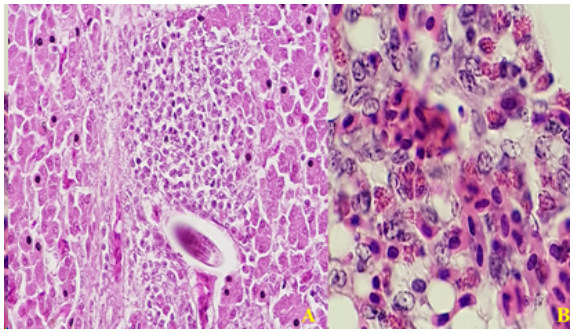

Figure 3. Microscopic lesions in the liver (A) and lung (B).

and lung of chickens. The results of this study provided the evidence that chickens can ingest *T. canis* eggs through the exposure to contaminated food/soil. Thus, the risk of eating raw or uncooked free- raising chicken especially chicken liver should be awareness.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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References

Abou-El-Naga, I. F. (2018). Development stages and viability of Toxocara canis eggs outside the host. Biom´edica: Revista del Instituto Nacional de Salud 38(2), 189-197. https://doi.org/10.7705/biomedica.v38i0.3684.

Beaver, P. C. (1956). Larva migrans. *Experimental Parasitology* 5(6), 587-621. https://doi.org/10.1016/0014-4894(56)90032-7.

Bruňaská, M., Dubinský, P., & Reiterova, K. (1995). *Toxocara canis*: ultrastructural aspects of larval moulting in the maturing eggs. *International Journal for Parasitology* 25(6), 683-690. https://doi.org/10.1016/0020-7519(94)00183-O.

Burren, C. H. (1972). The distribution of *Toxocara canis* larvae in the central nervous system of rodents. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 66, 937-942. https://doi.org/10.1016/0035-9203(72)90131-9.

Cruz, L. M., Allanson, M., Kwa, B., Azizan, A., & Izurieta, R. (2012). Morphological changes of *Ascaris* spp. eggs during their development outside the host. *Journal of Parasitology* 98(1), 63-68. https://doi.org/10.1645/GE-2821.1.

Flecher, C. M., Musso, C., Martins, I. V. F, & Pereira, F. E. L. (2016). Larval migration of the ascarid nematode *Toxocara canis* following infection and reinfection in the gerbil *Meriones unguiculatus*. *Journal of Helminthology* 90 (5), 569-576. https://doi.org/10.1017/S0022149X15000760.

Galvin, T. J. (1964). Experimental *Toxocara canis* infections in chickens and pigeons. *The Journal of Parasitology* 50 (1), 124-127. https://doi.org/10.2307/3276045.

Glickman, T. L., & Schantz, M. P. (1981). Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiologic Reviews* 3(1), 230-250. https://doi.org/10.1093/oxfordjournals.epirev.a036235.

Ito, K., Sakai, K., Okajima, T., Quchi, K., Funakoshi, A., Nishimura, J., Ibayashi, H., & Tsuji, M. (1986). Three cases of visceral larva migrans due to ingestion of raw chicken or cow liver. *Nippon Naika Gakkai Zasshi* 75, 759-766. https://doi.org/10.2169/naika.75.759.

Magnaval, J. F., Glickman, L. T., Dorchies, P., & Morassin, B. (2001). Highlights of human toxocariasis. *Korean Journal of Parasitology* 39(1), 1-11. https://doi.org/10.3347/kjp.2001.39.1.1.

Maruyama, S., Nino, T., Yamamoto, K., & Katsube, Y. (1994). Parasitism of *Toxocara canis* larvae in chickens inoculated with *Ascarid* eggs. *Journal of Veterinary Medical. Science* 56(1), 139-141. https://doi.org/10.1292/jvms.56.139.

Nagakura, K., Tachibana, H., Kaneda, Y., & Kato, Y. (1989). Toxocariasis possibly caused by ingesting raw chicken. *The Journal of Infectious Diseases* 160(4), 735-736. https://doi.org/10.1093/infdis/160.4.735.

Nakamura, S., Sotoyama, T., Hayasaka, S., Kameyama, Y., Maruyama, S., & Katsube, Y. (1991). Parasitism of *Toxocara canis* larvae in Japanese quails by inoculation of the ascarid eggs. *Journal of Veterinary Medical Science* 53(5), 865-872. https://doi.org/10.1292/jvms.53.865.

Okoshi, S., & Usui, M. (1968). Experimental studies on *Toxascaris leonina*. VI. Experimental infection of mice, chickens and earthworms with *Toxascaris leonina, Toxocara canis* and *Toxocara cati*. *The Japanese Journal of Veterinary Science* 30(3), 151-166. https://doi.org/10.1292/jvms1939.30.151.

Oshima, T. (1961). Standardization of techniques for infecting mice with *Toxocara canis* and observation on the normal migration routes of the larvae. *The Journal of Parasitology* 47(4), 652-656. https://doi.org/10.2307/3275079.

Pahari, T. K., & Sasmal, N. K. (1991). Experimental infection of Japanese quail with *Toxocara canis* larvae through earthworms. *Veterinary Parasitology* 39(3-4), 337-340. https://doi.org/10.1016/0304-4017(91)90051-V.

Santos, S. V., Lescano, S. Z., Castro, J. M., & Chieffi, P. P. (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 104(6), 933-934. https://doi.org/10.1590/S0074-02762009000600020.

Schacher, J. F. (1957). A contribution to the life history and larval morphology of Toxocara canis. *The Journal of Parasitology* 43(6), 599-612. https://doi.org/10.2307/3286548.

Taira, K., Permin, A., & Kapel, C. M. O. (2003). Establishment and migration pattern of Toxocara canis larvae in chickens. *Parasitology Research* 90(6), 521-523. https://doi.org/10.1007/s00436-003-0894-6.

Taira, K., Saitoh, Y., & Kapel, C. M. (2011). Toxocara cati larvae persist and retain high infectivity in muscles of experimentally infected chickens. *Veterinary Parasitology* 180, 287-291. https://doi.org/10.1016/j.vetpar.2011.03.020.