
Study on *Toxocara canis* in Experimentally Infected Dogs by *Toxocara canis*

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Studying on clinical and pathological characteristics in dogs of 2 months of age which were experimentally infected by *Toxocara canis* eggs: five dogs were swallowed with 10,000 eggs per dog in group 1, five dogs were swallowed with 15,000 eggs per dog in group 2 and five dogs were pertained to control group. Symptoms of experimental dogs were determined by clinical diagnostic methods. Macroscopic lesions were determined by necropsy method described by Skjrabrin (1928) in dogs of 2 months post-infection. Identifying microscopic lesions by histological template method, staining Hematoxylin - Eosin, observing under Olympus CX 221 microscope. The hematological indicators were determined on BC5800 machine. The result showed that:

Symptoms in experimental dogs manifested as emaciation, low appetite, fuzzy, vomiting, diarrhea, digestive disorder, roundworms in feces and neurological clinical signs.

In group 1, there was from 3 to 13 roundworm individuals per dog; in group 2, there was encountered from 8 to 14 roundworm individuals per dog. There was from 2 to 13 roundworm individuals in small intestine per dog, from 0 to 3 roundworm individuals in the stomach per dog.

Experimental dogs have lesions mainly concentrated in small intestine as follows: haemorrhagic, congestive, inflammatory intestinal mucosa; there was 7/14 templates (50%) which had intestinal mucosa desquamation and 42.86% of templates had cellular infiltration in inferior intestinal mucosa. Livers had abscesses and the tissue was destroyed, appeared neutrophils and eosinophils in abscesses. Lungs were inflammatory, alveolar wall was thick, inflammatory liquid condensed into alveolus.

Beside that, experimental dogs showed changes in hematological parameters were significantly different in comparison with control dogs, such as: red blood cell, hemoglobin concentration, proportion between hematocrit and platelet count in comparison with dogs of control group ($P < 0.05$). Meanwhile, leukocytes and the formula of granulocytes also were increased in comparison with dogs of control group ($P < 0.05$).

This experiment was carried out in 2015 in Phu Tho – Viet Nam.

Keywords: Dog, Clinical symptoms, Macroscopic lesions, Microscopic lesions, Hematological indicator, Nematode.

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Introduction

Actually, Toxocariasis causes by *Toxocara canis* specie in canine is a disease more gradually interested in the world. However, this disease is not only infected to animals but also to the human health. According to Habluetzel. A *et al.* (2002), studied in 295 fecal samples of dogs in Marche, Italy reported that dogs infected by *T. canis* with a prevalence of 33.6%, focusing on rural regions (48.4%), and low in the cities (26.2%).

Nevertheless, a research of Kutdang *et al.* (2010) showed that *T. canis* is the most popular helminth parasite in dogs, in the adult period *T. canis* usually secreted eggs to the environment through feces, eggs can be existed many years in soil creating a contaminated source to canine and human.

In Vietnam, some authors have mentioned this disease which causes by *T. canis* helminth parasite but mainly focused on the larval stage. Currently, most of localities in Vietnam dogs still raise freely grazing, while the prevention and control this disease have not been paid much attention leading to no effectively preventive procedure.

Objectives: To have a scientific basis in diagnose, prevention and treatment of Toxocariasis in dogs and from that can prevent in human, in 2014 – 2015, we have been studied “Study on Toxocariasis in experimentally infected dogs by *T. canis*”.

Mục tiêu: Nghiên cứu triệu chứng lâm sàng và biến đổi

Materials and methods

Materials

Dogs of 2 months of age (experimentally infected by *T. canis*), *T. canis* eggs, alcol 70⁰, electronic microcope, surgery instruments, Petri plate, templates, Hematocilin – Eosin staining substances, Olympus CX 221 machine; BC 5800 machine, chemicals and other laboratory instruments.

Methods

The experiment was divided in 3 groups: Group 1 has 5 dogs which have swallowed 10.000 *T. canis* eggs per dog; Group 2 has 5 dogs which have swallowed 15.000 *T. canis* eggs per dog; Group 3 or control group has 5 dogs which have not swallowed any eggs.

Observing daily clinical symptoms on experimental dogs according to clinical diagnostic method (Chu Đức Thang, 2007).

After 2 months of post infection, experimental dogs were processed by necropsy in accordance with the method of Skjrabin (1928) to find out *T. canis* helminth and revise macroscopic lesions.

Studying microscopic lesions by histological template method, Hematoxilin – Eosin staining and reading results under Olympus CX 221 microscope.

Determining hematological parameters on BC 5800 machine with blood dog samples in control group and infected groups before terminate the experiment.

The obtained results were processed by statistically biological method (Nguyen Van Thien, 2008) and on Minitab 16.0 software.

Results

Cinical manifestation and body weight of experimental dogs

Table 1. Cinical manifestation and body weight of experimental dogs

| Experimental group | Dog number | Cinical symptoms | Body weight (kg) | |
|----------------------|------------|-------------------------------------------------|------------------|-------------------------------------|
| | | | Before infection | 60 th day post infection |
| Group I | 1 | Vomiting, parasites in feces | 3.5 | 3.8 |
| | 2 | Vomiting with roundworms, emaciation | 3.2 | 3.5 |
| | 3 | Less eating, low appetite, fuzzy | 3.4 | 3.6 |
| | 4 | Parasites in feces, diarrhea, fuzzy | 3.7 | 4.2 |
| | 5 | Diarrhea, feces mixed with blood | 3.0 | 3.5 |
| Group II | 1 | Emaciation, attenuation, vomiting. | 3.5 | 3.6 |
| | 2 | Emaciation, neurological signs | 3.8 | 4.0 |
| | 3 | Less eating, low appetite, parasites in feces | 4.0 | 4.4 |
| | 4 | Vomiting, fuzzy, emaciation. | 4.2 | 4.2 |
| | 5 | Depressed, fuzzy, diarrhea | 3.1 | 3.8 |
| Control group | 5 dogs | Agility, smooth hair, normal feces, eating good | 3.4 ± 0.2 | 5.3 ± 0.4 |

The result in table 1 shows that:

Experimentally infected dogs with *T. canis* helminthes in 2 groups have mainly clinical manifestations as follows: vomiting with parasites, emaciation, less eating, depressed, fuzzy, diarrhea, feces mixed with blood and parasites secreting

to environment. Body weight of dogs in the 60th day post infection did not increase or increasing very less. Clinical manifestations have encountered in experimental dogs were resulted of mechanical impacts, appropriating nutrients and impacts caused by *T. canis* toxin. This is a cause to the death of dogs if does not take a treatment in time.



Image 1. *T. canis* eggs
(100 times of amplification)



Image 2. Clinical manifestations of
T. canis infected dogs

Studying on macroscopic lesions caused by T. canis roundworms in experimental dogs

Table 2 shows that:

Applying necropsy to 5 dogs which have swallowed 10.000 *T. canis* eggs per dog, there was obtained from 3 to 13 *T. canis* roundworms per dog. *T. canis* helminthes parasite mainly in small intestine, the roundworm number varied from 2 to 10 roundworms, from 0 to 3 *T. canis* roundworms parasite in stomach per dog; there were 2/5 dogs have significantly macroscopic lesions, mainly focusing on small intestine of experimental dogs.

Also applying necropsy to 5 dogs which have swallowed 15.000 *T. canis* eggs per dog, there was obtained from 8 to 14 *T. canis* roundworms per dog. *T. canis* helminthes parasite mainly in small intestine, the roundworm number varied from 6 to 13 roundworms, from 0 to 3 *T. canis* roundworms parasite in stomach per dog; there were 2/5 dogs have significantly macroscopic lesions, mainly focusing on small intestine of experimental dogs.

Many helminthes parasite in small intestine cause significant lesions such as: haemorrhagic, hemorrhagic and intestinal cata inflammation. In addition, roundworms also secrete toxin which causes lesions in parasited localities.

Table 2. Macroscopically lesion grade of *T. canis* infected dogs

| Experimental group | Number of dogs | Number of <i>T. canis</i> | | <i>T. canis</i> Total (roundworm) | Macroscopically lesion grade in parasited localities |
|--------------------|----------------|---------------------------|-----------------|-----------------------------------|------------------------------------------------------|
| | | Stomach | Small intestine | | |
| Group I | 1 | 1 | 8 | 9 | Significant lesions |
| | 2 | 2 | 5 | 7 | No significant lesions |
| | 3 | 0 | 7 | 7 | No significant lesions |
| | 4 | 3 | 10 | 13 | Significant lesions |
| | 5 | 1 | 2 | 3 | No significant lesions |
| Group II | 1 | 2 | 11 | 13 | Significant lesions |
| | 2 | 1 | 7 | 8 | No significant lesions |
| | 3 | 1 | 13 | 14 | Significant lesions |
| | 4 | 0 | 9 | 9 | No significant lesions |
| | 5 | 3 | 6 | 9 | No significant lesions |
| Control | 5 dogs | 0 | 0 | 0 | No lesions |



Image 3. Necropsy, collecting *T. canis* roundworms in experimental dogs



Image 4. *T. canis* roundworms

Microscopic lesions in experimentally infected dogs caused by *T.canis*

Table 3. Microscopic lesions in experimentally infected dogs caused by *T.canis*

| Number of microscopic templates | Observing results | | |
|---------------------------------|---------------------------------------------------------------------------------------------|------------------------------|----------------|
| | Mainly microscopic lesions | Number of lesional templates | Proportion (%) |
| 14 | Inflammatory intestinal mucosa, lesional, intestinal mucosa desquamation | 7 | 50.00 |
| | Necrosis on the top of villi | 5 | 35.71 |
| | <i>T. canis</i> roundworms in the transection | 3 | 21.43 |
| | Inflammatory cellular infiltration in inferior intestinal mucosa | 6 | 42.86 |
| | Intestinal mucosa cell was destroyed | 2 | 14.29 |
| | Livers have abscesses and the tissue was destroyed, infiltrated neutrophils and eosinophils | 3 | 21.43 |
| | Inflammatory lungs, infiltrated neutrophils and eosinophils | 2 | 14.29 |

The result in table 3 reports that:

*** *Microscopic lesions in small intestine***

In general, in 14 lesional templates have: 7/14 templates (50%) have lesions caused by inflammatory intestine, intestinal mucosa desquamation; 3/14 templates in the transection have *T. canis* roundworms, occupied 21,43%; Templates of destroyed intestinal tisular cells were 14,29%; templates of infiltration in inferior intestinal mucosa occupied 42,86%. Additionally, Ngoài ra, templates of necrotic villi were 35,71%. Microscopic lesions were described in following images:

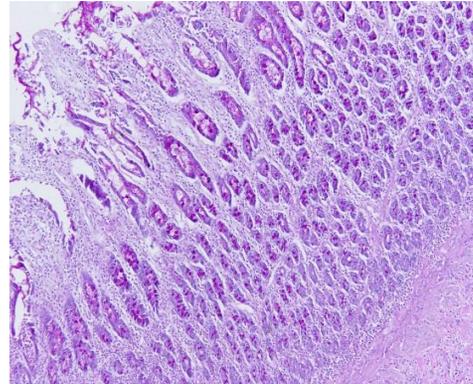
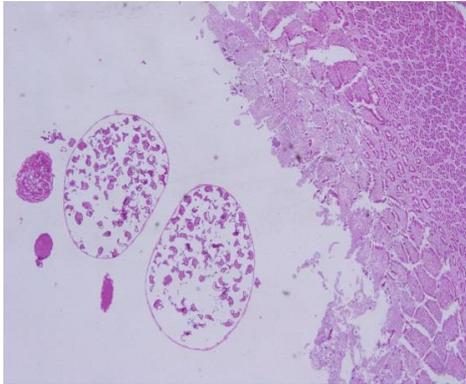


Image 5. Lesional intestinal mucosa, desquamated, mixed with roundworms (200 times of amplification) **Image 6.** Inflammatory intestinal mucosa, desquamated (400 times of amplification)

The result determined microscopic lesions in accordance with macroscopic lesions which have been observed through necropsy in experimental dogs.

T. canis roundworms parasite and influence on intestinal mucosa by mechanical impacts, secreting toxin cause lesion and hemorrhage at the same time. The body of host reacts locally, appearing inflammatory cells and infiltrated to intestinal cavity including macrophages, eosinophils and neutrophils.

*** Microscopic lesions in liver**

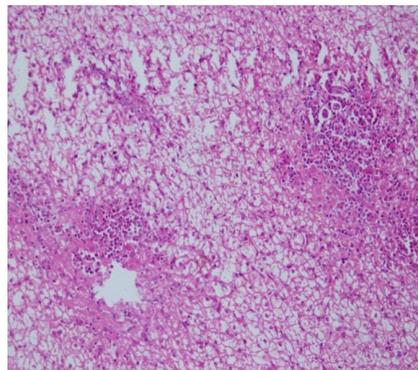
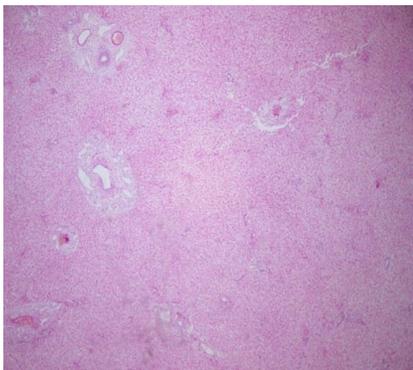


Image 7. Abscesses in liver (100 times of amplification) **Image 8.** Liver tissue was destroyed, appeared neutrophils and eosinophils (200 times of amplification)

When travelling to liver, *T. canis* larvae caused abscesses and destroyed liver tissue, appeared neutrophils and eosinophils in each abscess.

*** Microscopic lesions in lung**

When *T. canis* roundworms travelled through lung caused inflammatory lung, observing on the templates have inflammations in lung that caused thick alveolar wall, infiltration of leukocytes, increasing eosinophils, inflammatory liquid condensed in the alveoli.

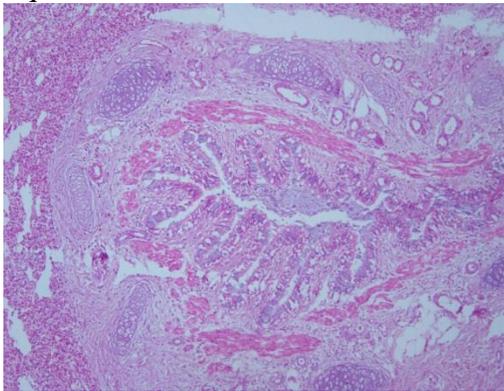


Image 9. Inflammations in lung (200 times of amplification)

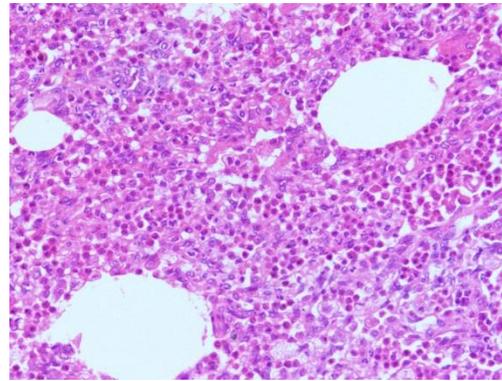


Image 10. Thick alveolar wall, infiltration of leukocytes (400 times of amplification)

Changes of hematological parameters in experimentally infected and control dogs

Table 4 reports that:

Experimental dogs have significant changes about hemological parameters, such as: Red blood cell count (RBC) and hemoglobin, hematocrit (HCT) and red distribution width (RDW-CV) decreased in comparison with control dogs ($P < 0.05$).

From our point of view, the changes of these parameters caused by *T. canis* roundworms appropriating nutrients in small intestine, dogs became anemia, mechanical lesions to lesional villi, hemorrhage. Toxin of roundworms also increased lesional capilar walls, anemic state, pale mucosa. Hematological parameters of RBC, HGB, HCT and RDW-CV in *T. canis* infected dogs decreased, but parameters of MCV and MCHC increased in comparison with control dogs.

Table 4. Hematological parameters in experimentally infected and control dogs

| Parameters | Unit | Physiological range | Experimental dog (n=10) | | Control dog (n=5) | |
|------------|---------------------|---------------------|-------------------------|------|---------------------|------|
| | | | Mean | SE | Mean | SE |
| RBC | 10 ¹² /L | 5.5-8.5 | 5.00 ^b | 0.18 | 6.07 ^a | 0.20 |
| HGB | g/l | 120-180 | 95.90 ^b | 4.42 | 132.70 ^a | 4.12 |
| HCT | % | 37-55 | 30.18 ^b | 1.23 | 40.15 ^a | 1.82 |
| MCV | fl | 60-77 | 62.29 ^a | 1.15 | 60.12 ^a | 1.77 |
| MCH | Pg | 19.5-25.5 | 19.17 ^a | 0.50 | 19.84 ^a | 0.46 |
| MCHC | g/L | 310-340 | 341.00 ^a | 7.41 | 329.20 ^a | 8.44 |
| RDW-CV | % | 14-19% | 16.74 ^a | 0.90 | 17.19 ^a | 0.32 |

* Note: The values bring different letters at the same row represent statistically significant difference ($P < 0.05$). RBC: Red blood cell count; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW - CV: Red distribution width.

Table 5. Changes of platelet parameters

| Parameters | Unit | Physiological range | Infected dogs (n=10) | | Control dogs (n=5) | |
|------------|--------------------|---------------------|----------------------|------|---------------------|-------|
| | | | Mean | SE | Mean | SE |
| PLT | 10 ⁹ /l | 200-500 | 274.30 ^b | 34.5 | 423.00 ^a | 36.10 |
| MPV | fl | 3.9-11.1 | 9.94 ^a | 0.36 | 10.34 ^a | 0.26 |
| PDWc | % | - | 22.39 ^a | 2.17 | 28.01 ^a | 2.51 |
| PCT | % | - | 0.23 ^a | 0.08 | 0.31 ^a | 0.05 |

* Note: The values bring different letters at the same row represent statistically significant difference ($P < 0.05$). PLT: Platelet count, MPV: Mean platelet volume, PDWc: Platelet distribution width, PCT: Plateletcrit

Table 5 shows that platelet count (PLT) significantly decreased from 423.00 (10⁹/l) in control dogs to 274.3 (10⁹/l) in infected dogs. These values showed statistically significant difference ($P < 0.05$). Platelet distribution width (PDWc) also decreased from 28.01% in control dogs to 22.39% in infected dogs. Plateletcrit (PCT), mean platelet volume (MPV) decreased in comparison with control dogs. However, the difference was not significant ($P > 0.05$).

Table 6. Leukocytes and leukocyte formula

| Parameters | Unit | Physiological range | Infected dogs (n=10) | | Control group (n=5) | |
|------------|--------------------|---------------------|----------------------|------|---------------------|------|
| | | | Mean | SE | Mean | SE |
| WBC | 10 ⁹ /l | 6 -17 | 21.61 ^a | 1.87 | 12.05 ^b | 0.69 |
| LYM | 10 ⁹ /l | 1- 4.8 | 3.43 ^a | 0.20 | 3.06 ^a | 0.31 |
| MID | 10 ⁹ /l | 0.2 -1.35 | 2.08 ^a | 0.67 | 1.28 ^a | 0.37 |
| GRA | 10 ⁹ /l | 3 - 12 | 12.85 ^a | 1.10 | 6.51 ^b | 0.46 |
| LYM | % | - | 19.69 | 2.07 | 27.91 | 2.33 |
| MID | % | - | 11.04 | 3.52 | 11.61 | 3.07 |
| GRA | % | - | 69.28 | 3.28 | 60.47 | 4.03 |

* Note: The values bring different letters at the same row represent statistically significant difference ($P < 0.05$). WBC: White blood cells, LYM: Lymphocytes, MID: Monocytes, GRA: Granulocyte.

Table 6 shows that number of leukocytes in infected dogs significantly differed with control dogs, changes of granulocytes (GRA) ($P < 0.05$). White blood cells in *T. canis* infected dogs significantly increased, granulocytes were encountered increasing from $6.51 \times 10^9/l$ (in control dogs) to $12.85 \times 10^9/l$ (in infected dogs).

Monocytes (MID) have increased from 1.28 ± 0.37 (in control dogs) to 2.08 ± 0.67 (in infected dogs).

Lymphocytes (LYM) increased gradually (from $3.06 \times 10^9/l$ to $3.43 \times 10^9/l$) but this increasing did not represent statistically significant difference ($P > 0.05$).

Discussions

According to Parsons J. C. (1987), Beveridge and David Emery (2015), roundworm infected dogs are emaciated, fuzzy, less eating, low appetite, abdominal pains, diarrhea, feces mixed with parasite, neurological signs, convulsive and paralysis. Therefore, infected dogs with *T. canis* roundworms in our experiment have clinical manifestations coincide with the description of all mentioned authors.

According to Bowman (1999): *T. canis* roundworms parasite mainly in small intestine and stomach of dogs. Nguyen The Kim Lan (2012) reported

that *T. canis* adult roundworms parasite in small intestine cause intestinal obstruction, perforation and intestinal cata inflammation.

In their lifecycle process, *T. canis* roundworms moved and caused lesions in organs. According to Sally Gardiner (2007), *T. canis* larvae parasite in corporal tissues of dogs. Larvae become developed from second stage in lung, heart or stomach to the third larval developmental stage. The third larval stage develops fourth larval stage in lung and stomach.

Our results coincide with research of Nguyễn Thị Duyên (2014), dogs infected by roundworms, the number of red blood cell count had decreased from 6.66 millions/mm³ blood (no infected dogs) to 5.32 million /mm³ blood (infected dogs).

Oshima (1976) and Zimmerman (1985) reported: roundworms appropriate nutrients in host decreasing red blood cell count, hemoglobin; increasing eosinophils was a important parameter to diagnose roundworm disease. Our results coincide with these of mentioned authors.

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