

Detection of *Toxocara canis* infection by ELIZA, with follow-up some Biochemical and histological changes

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ABSTRACT

This study examined 100 blood samples from people visited General Hospital of Samarra and the external medical laboratories in Samarra city whose ages ranged between 6-38 years from October 2019 to March 2020 to investigate toxocariasis. 100 fecal samples were examined for the presence of intestinal parasite infections to avoid any cross-reaction with toxocariasis in the ELISA test. The study included knowledge of changes occurring in the levels of GOT and GPT, as well as knowledge of histological findings in animals experimentally infected with *Toxocara canis* parasite for each the liver, lung, spleen, kidney, and brain. The groups of animals were divided into two groups, euthanized at 7 and 14 days postinfection and necropsied for taking tissue sections to find the differences between the effects of infection on the somatic tissues. The results showed the number of positive serum samples was 13(13%), The highest number of infected people was 9 and the highest antibody value was 14.6 units compared to the control group (10 units) and was within the age group of 6-16 years. The biochemical tests showed an increase in the concentration of GOT and GPT enzymes in infected people with *T. canis* parasite compared with control. The highest concentration was recorded in the age group 17-27 and 6-16 years for both GOT and GPT (30.67 ± 4.72 and 32.79 ± 4.18 (IU/L), respectively). The histological study showed the presence of histological changes in each of the members that were studied and for each of the two groups 7 and 14 days postinfection.

Keywords: *Toxocara canis*, IgG, Histological findings, Toxocariasis

Introduction

Parasites cause disastrous diseases [1-3] and have been torture to mankind since ancient times [4]. Toxocarais is a zoonosis and has a major social and economic effect, especially on poor communities in the world. Toxocarais is caused by nematodes parasites of the genus *Toxocara*, including *Toxocara canis*; Werner, 1782, and to a lesser extent *Toxocara cati* Schranck, 1788. They cause severe diseases in people, and associated

complications include allergic and neurological disorders [5, 6]. Dogs and cats are the natural final hosts for these parasitic nematodes, which live in their intestine and release *Toxocara* eggs with feces into the soil [7]. *Toxocara* worms are the best examples of the transmission of parasites from wild dogs to their domestic counterparts and humans [8]. Dogs or cats, especially in rural and poor areas, have an important role in the transmission of *Toxocara* spp. Contaminated circumstances transmit the infection to human beings [8]. Humans are infected accidentally with this parasite, as a result, *Toxocara* larvae do not have the ability for developing into adult worms [9]. Human infection with *Toxocara* spp. occurs via swallowing of embryonated/larvated eggs found in or contaminated soil or foods [6]. Following ingestion of embryonated eggs, the larvae hatch in the small intestine and penetrate the intestinal wall to reach the bloodstream, where they migrate throughout the body, causing an obvious inflammatory response and different clinical features, depending on the infected organ [5, 10-12].

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Although human infection may be asymptomatic, *Toxocara* has a pronounced susceptibility to cause damage extra intestine [10, 13]. Toxocarasis causes general non-specific medical symptoms. Thus, although it can be diagnosed initially depending on the symptoms of patients, a laboratory diagnosis is necessary for high precision in diagnosis [14, 15].

The present study aimed to diagnose infection with *T. canis* parasite and different age groups using the IgG TES-ELISA test and monitoring changes in the activities of the two enzymes (GPT), Glutamic pyruvic transaminase and Glutamic oxaloacetic transaminase (GOT), and histological changes of some parts of the body.

Materials and Methods

Blood samples were collected in anticoagulant-free test tubes and left for 30 minutes at room temperature for blood to clot and then centrifuged (2000 rpm) for 10 minutes to collect the serum using a micropipette. The serum was conserved in a sterile test tube and stored at -20 ° C [16], pending the following tests:

1. Estimating GOT concentration in serum by using a commercial kit, equipment from Randox English Company (Catalog No.147).
2. Estimating GPT concentration in serum by using a commercial kit, equipment from Randox English Company (Catalog No.146).
3. ELISA and IgG testing by using a commercial kit, equipment from GenWay Biotech, Inc. USA (GWB-3570CC).

Histological study

The infection was caused by *T. canis* parasite experimentally in 10 laboratory rabbits. They were orally exposed by gavage to 1000 embryonated eggs, and the laboratory animals were divided into two groups (5 rabbits per group and the average weight was 1 kg per rabbit). The animals were euthanized at 7 and 14 days post-infection and necropsied for taking tissue sections of some organs [17].

Statistical analysis

The present results were statistically analyzed by using Duncan's multiple range test; the means of the groups were measured with $P \leq 0.05$ and by using the Minitab program [18].

Results and Discussion

Seropositive for ELISA test in the present study groups

The present results showed that the total number of serum samples positive in the ELISA test exceeded the cut-off control

average (10 units) was 13 out of 100 of the people examined from different age groups (6-38 years), i.e. infection rate was 13%. Based on the results of the distribution of number of seropositive cases according to age groups, the highest percentage was recorded in the 6-16 age group, which is 18.4%, followed by 11.1% in the 28-38 age group, then 6.0% in the age group 17-27 years (**Table 1**). Based on the results of the distribution of seropositive cases in different age groups by value in units in the ELISA test, the highest rate (14.6 units) was within the age group of 6-16 years, followed by the age group of 17-27 years (13.2 units), and 28-38 years (12.8 units) (**Table 2**).

Toxocarasis is a disease of animal origin and it is widespread, but it is still a disease unknown to health and society in general. The current study showed that 13 of a total of 100, i.e. 13% of people of different age groups (6-38 years) of both sexes were seropositive by using IgG TES-ELISA, indicating contact with the *T. canis* worm. Although the ELISA test is considered the best and most used in the diagnosis of toxocarasis, the antibody titer remains unknown. Many studies show that antibodies remain for long periods in the serum due to repeated antigen stimulation resulting from the remnants of dead larvae in the body [19], or re-exposure to infection [20]. Comparing the infection rate (13%) among people of different ages in the city of Samarra with other studies similar to climate conditions (hot and dry) is an approach, and we notice that it was 7.3% in adults in Iraq [21], 6.6% in adults in Egypt [22], 4.1% in children in northern Greece [23], 8.8% in western Iran [24], and 6.81% in adults in Iraq [25].

However, it has high prevalence rates in moderate countries (93%), La Reunion (86.8%), Marshall Island, (81%), Nepal (53.2%), and Malaysia [10, 26]. The current results are not in line with it, perhaps due to the environmental conditions with a hot and dry climate that does not allow the growth of eggs of *T. canis* into the infectious phase [27]. Interpretation of serological proliferation information is still difficult because of the use of various cut-off titers by researchers, and the difficulty in assessing the relationship between the titer level, infection and clinical signs of the disease [6, 28].

Comparing the average value of units to the ELISA results shows that the concentration of TES-IgG antibodies against *T. canis* was highest in the 6–16 age group compared to age groups 17–27, 28–38; perhaps this indicates that this age group continues to be exposed to *T. canis*. Thus, there is a greater presence of live larvae secreting their antigens in human body [25].

Table 1. The number and percentage of seropositive according to age groups, by using IgG TES-ELISA technique to detect infection with the *T. canis* in people in Samarra city.

Age groups (year)	No. of samples	No. of seropositive (Pearson)	Seropositive (%)
6-16	49	9	18.4
17-27	33	2	6

28-38	18	2	11.1
Total	100	13	13

Table 2. Absorption average and the equivalent units of control solutions and seropositive of IgG TES-ELISA test in people infected with *T. canis* in Samarra city.

Groups	Average of absorption	Value of units
Negative Control	0.205	4.7 E
Cutt-off	0.435	10 D
Positive Control	0.848	19.5 A
6-16	0.636	14.6 B
17-27	0.576	13.2 C
28-38	0.558	12.8 C

The various letters indicate the presence of significant differences $P \leq 0.05$ (a vertical comparison between groups).

Biochemical parameters

The current results showed (**Table 3**) that there was an increase in the average concentration of GOT and GPT enzymes in serum positive age groups compared to the control group. The results recorded the highest concentration of GOT within the age group 17-27 years, while the highest concentration of GPT within the age group 6-16 years was 30.67 and 32.79, respectively, compared to the control group (10.5 and 7.32, respectively).

An increase in the concentration of both GPT and GOT enzymes compared to control indicates a breakdown in the cells of organs that contain the two enzymes. There is the GPT enzyme in various tissues, which is more concentrated in the liver. Liver injury and other pathological conditions lead to the release of this enzyme to blood, thereby increasing its level [29]. The GOT enzyme is found in the heart, liver, skeletal muscles, and in smaller amounts in other tissues. The high level of this enzyme results from the death or destruction of the cells of the aforementioned organs (damaged liver, myocardial infarction, and skeletal muscle breakdown). The increase in both GPT and GOT corresponds to the results of Rokni *et al.* (2000) and Hammad *et al.*, 2012 [25, 30].

Table 3. The relationship between the rate of some biochemical changes and age in the control group and people infected with *T. canis* parasite in Samarra city.

Groups	Age groups (year)	GOT (IU/L)	GPT (IU/L)
		Mean \pm SD.	
Infected	6-16	24.07 \pm 5.23	32.79 \pm 4.18
	17-27	30.67 \pm 4.72	31.91 \pm 6.05
	28-38	22.99 \pm 1.2	27.72 \pm 0.71
Control	-----	10.50 \pm 1.92	7.32 \pm 1.70

The various letters indicate the presence of significant differences $P \leq 0.05$ (a vertical comparison between groups).

Histological examination

Several organs were taken from animals and prepared for the microscopical examination to find out the pathological effect of larva on the texture of these organs.

Brain

The section of the brain demonstrated vacuolation around atrophied neuron cells and in between it, in 7th day postinfection (**Figure 1a**), as well as the light zone around the congested blood vessel, extensive eosinophilic in all sections and Gliosis, vacuolation around atrophied neurons, in 14th day postinfection (**Figure 1b**).

Liver

The microscopical examination revealed central vein surrounded by hepatocytes, hemorrhage in the liver parenchyma with rupture of the central vein wall, Thickened blood vessels and sinusoid, ghost hepatocytes, in 7th day postinfection (**Figure 1c**). Other sections showed degeneration of blood vessels tunics, desquamation of degenerated tunica interna of the portal vein, aggregation of lymphocytes in the portal are and degeneration of hepatocytes in 14th day postinfection (**Figure 1d**).

Lung

Histological examination showed alveoli, infiltration of inflammatory cells in between alveolar sac and degeneration of alveolar cells in 7th day postinfection (**Figure 2a**). Other sections revealed congested blood vessels with infiltration of inflammatory cell intra-alveolar sac and degeneration of alveolar cells, hemorrhage intra-air sac in 14th day postinfection (**Figure 2b**).

Spleen

This section showed necrosis in the red pulp, congested sinusoid, and infiltration by inflammatory cells, atretic in white pulp in 7th day postinfection (**Figure 2c**). Other sections showed necrosis of red pulp, degeneration of sinus epithelial cells, degeneration of connective tissue trabeculae, vacuolation in red pulp cells, Colloid in red pulp as a results of destroyed sinus epithelial cells, thickened capsular connective tissue in 14th day postinfection (**Figure 2d**).

Kidney

The microscopical examination showed degeneration in proximal tubules, amyloidosis in the lumen of distal tubules, infiltration of inflammatory cells in glomeruli in 7th day postinfection (**Figure 3a**), degeneration in proximal tubules, congested blood vessels, ghost cells in proximal tubules, karyorrhexis of proximal tubules cells in 14th day postinfection (**Figure 3b**).

The microscopical examination results were in agreement with Azizi *et al.* (2007) related to some histopathological findings in the liver of the paratenic hosts infected with *T. cati* parasite, which showed the occurrence of hemorrhage in the liver tissue as well as inflammatory infiltration [31].

The current results are consistent with da Silva *et al.* (2014) regarding the incidence of inflammatory infiltration of immune cells and the occurrence of infections in the liver tissue of the paratenic host infected with *T. canis* parasite [9]. It also indicated the occurrence of inflammatory cells infiltration between intra-alveolar cells and the occurrence of hemorrhage in the lung tissue.

The formation of the ectopic portal lymphoid was observed, where they aggregate during the late phase of infection. Inflammatory processes start with diffuse mononuclear infiltrates and progresses toward the development of lymphocytes into nodular secondary lymphoid follicles. Portal lymphoid follicle-like structures are nonspecific but have been in contact with inflammatory diseases of the chronic phase with an immunologic background. This status may be in experimental infection under study and could be related to antigens of *Toxocara* [9].

In the paratenic host infected with *Toxocara*, most of the circulating antigens are excreted from larvae and are present in

different fluids of the host. Kidneys of the host play an important role in the elimination of circulating antigens from the peripheral blood, but the detailed pathogenic mechanism is not understood.

The immune response reacts in host to various antigens produced is during the various phases of the life cycle of the parasite in chronic parasitic diseases [32]. The related glomerulopathy contact with a parasitic infection requires persistent antigenic excreted to the kidneys, usually for years, as in schistosomiasis [33-35].

The presence of larvae in the brain, liver, lungs, kidneys, and spleen was not detected by histological methods under study. Perhaps because of the movement of the larvae within the tissues, they are not always present in the pathological lesion [36]. Previous studies on *T. canis* have indicated the presence of larvae in the brain and other organs such as the spleen, heart, and kidneys, but the reason why the larvae are not seen in the brain, liver, lungs, kidneys, and spleen under study is not known. It may be because these larvae stayed briefly in these tissues [37], or migratory larvae left the tissue that undergoes histological fixation and preparation, which affected the histopathological results [38]. To date, there have been no studies on rabbits infected with *T. canis* as a paratenic host to answer these questions.

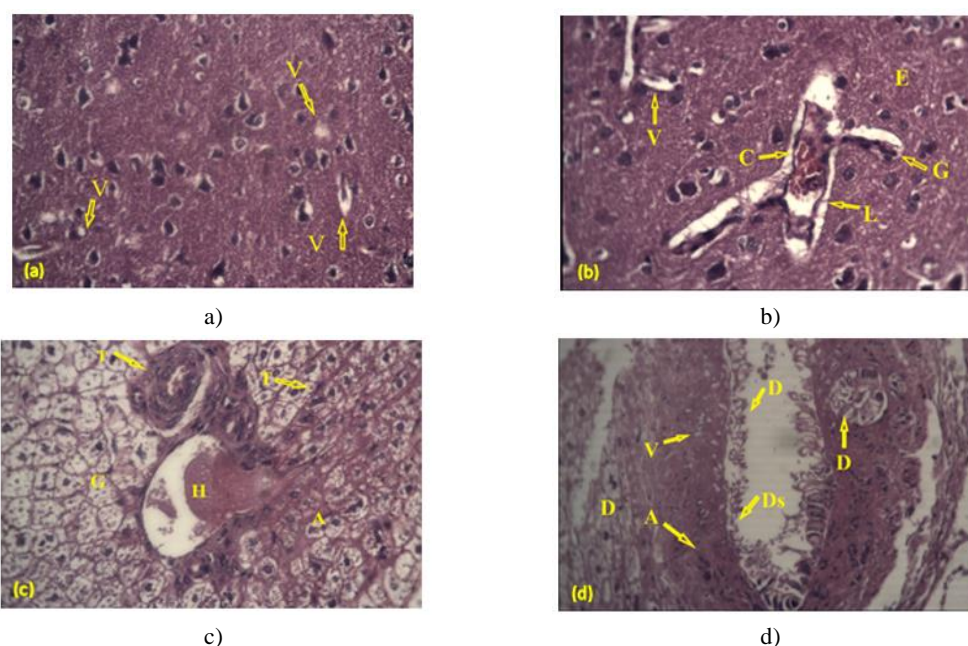


Figure 1. a) Brain section Vacuolation around atrophied neurons (V). H&E, 400X. b) Brain section. Light zone (L) around the Congested blood vessel (C). Extensive eosinophilic (E). Gliosis (G). Vacuolation around atrophied neurons (V). H&E, 400X. c) liver section, Hemorrhage in the liver parenchyma with rupture of central vein wall (H). Thickened blood vessels and sinusoid (T). Ghost hepatocytes (G). H&E, 400X. d) Liver section, Tunics Vacuolation of blood vessels wall (V). Desquamation (Ds) of degenerated tunica interna of portal vein (D), Aggregate of lymphocytes in portal area (A). Degeneration of hepatocytes (D). H&E, 400X.

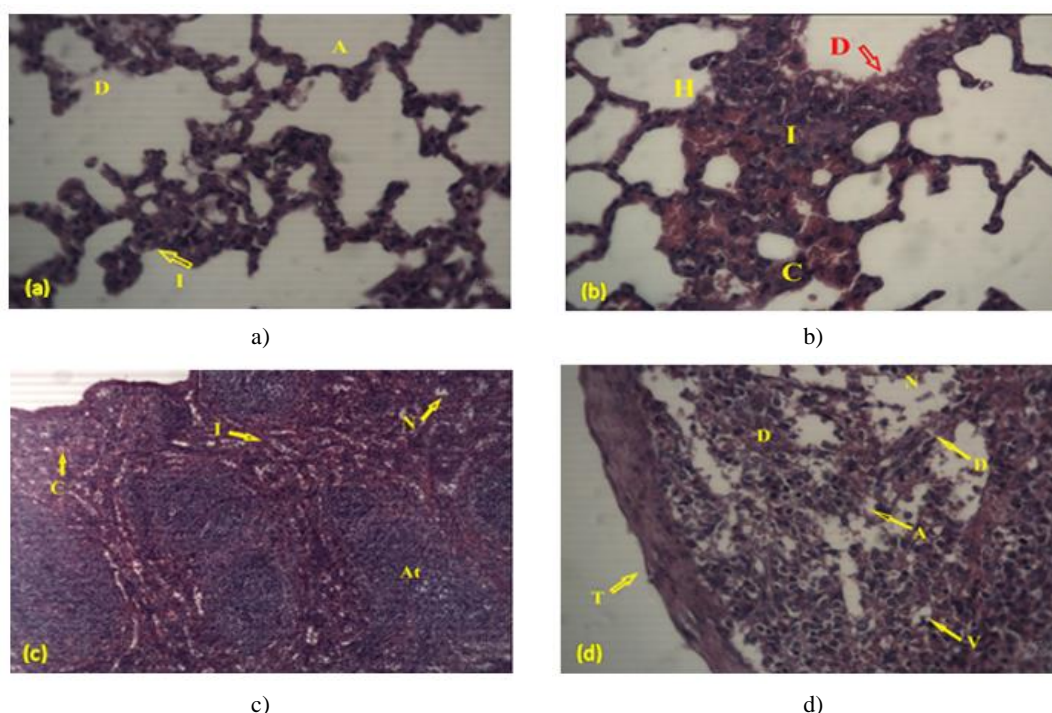


Figure 2. a) Lung section, Air sac (A). Infiltration of inflammatory cells in between alveolar (I). Degeneration of alveolar cells (D). H&E, 400 X. b) Lung section, Hemorrhage in between air sac (H). Infiltration of inflammatory cells intra-alveolar cells (I). Degeneration of epithelial layer (D). Congested blood vessels (C). H&E, 400X. c) Spleen section, Necrosis in red pulp (N). Congested sinus of red pulp (C). Infiltrated by inflammatory cells (I). Atretic in white pulp (At) H&E, 100X. d) Spleen section, Necrosis of red pulp (N), Degeneration of sinus epithelial cells (D). degeneration of connective tissue trabeculae (D). Vacuolation in red pulp cells (V). Colloid in red pulp (A). Thickened of capsular connective tissue (T). H&E, 400X .

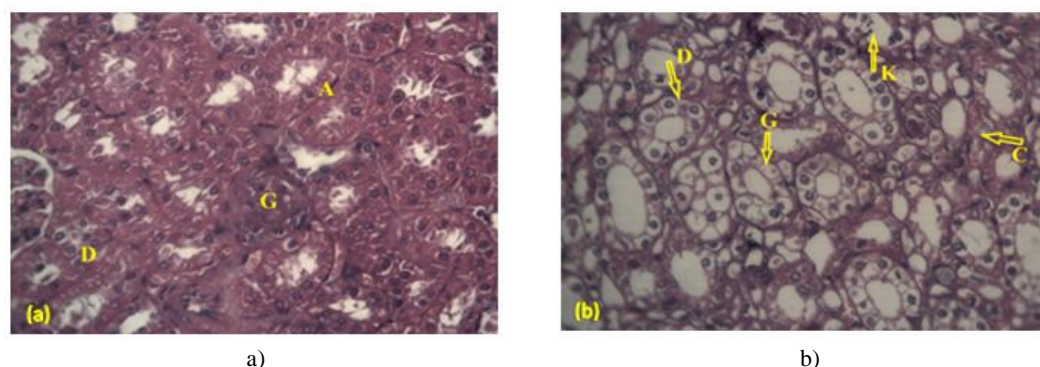


Figure 3. a) kidney section, Degeneration in proximal tubules (D). Amyloidosis was appear into the lumen of distal tubules (A). Infiltration of inflammatory cells in Glomeruli (G). H&E, 40X. b) Kidney section, Degeneration in proximal tubules (D). Congested blood vessels (C). Ghost cells in proximal tubules (G). Karyorrhexis of proximal tubules cells (K). H&E, 400X.

Conclusion

1. The highest rate of infection was recorded in the age group of 6-16 years.
2. The occurrence of an increase in the concentration of GPT and GOT enzymes in the serum positive cases with IgG TES-ELISA test, compared with the control group.
3. The presence of tissue changes in rabbits infected with the larvae of *Toxocara canis* parasite in the organs under study.

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