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Histopathological Study of Different VLM Stages of *Toxocara canis* Infection in Liver of Rabbits

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Abstract: Toxocariasis is a zoonotic parasitic disease caused by *Toxocara canis* nematode emberyonated egg which is usually transmitted to humans mainly in children via the faecal–oral route, or accidently ingested larvae from uncooked liver or meat of infected ruminants and poultry. *T. canis* larvae remains a problem throughout the world because it remains on arrested stage without development to adult stage and causes multisystem disease in the paratenic hosts such as humans, ruminants, poultry and rodents, most infections are asymptomatic and manifests in humans causing the well-characterized syndrome; Visceral Larva Migrans (VLM). The result of this study indicated detection of *T. canis* larval stage in liver tissues of infected rabbits at third week post infection. In conclusion the result indicated the possibility of using histopathological examination to diagnosis if there was an infection with *T. canis* larval stage in paratenic host tissues. It was also recommend that this test could be used to ensure that meat and its products of any local or imported are free from this infection.

Keywords: histopathologcal**,** *Toxocara canis*, larvae detection, liver tissue.

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Introduction:

Toxocara canis roundworm with three different life stages; *T. canis* adult worms which were isolated from dogs, characterized by creamy to white color in fresh specimen, the male worms measure 9–13 cm but female worms measure 10–18 cm (1). *T. canis* eggs stage (approximately 85 µm x 75 µm) contain a single dense cell mass within a thick, brown outer shell which contains a distinctive finely stippled brownish-yellow proteinaceous coat and the larvae stage which are 290 to 350 µm by 18 to 21 µm (2).

Toxocariasis remains a problem throughout the world because it causes multisystem zoonotic disease in the paratenic hosts such as humans, ruminants, poultry, rabbits and rodents(3).

Human could get Toxocariasis infection accidental by the ingested contaminated soil, food, water or unwashed vegetables with *T. canis* emberyonated eggs which shed on dog feces (dirt); as well as through ingestion of *T. canis* encysted larvae present in undercooked ruminants or poultry liver or meat as a paratenic hosts (4-6). After ingested *T. canis* emberynated eggs by paratenic hosts the larvae hatching in stomach and migrate to all body organs and invade tissues like liver, lungs, eye or the nervous system causing a

symptomatic serious human Toxocariasis infection which includes many syndromes; Visceral Larva Migrans (VLM), Ocular Larva Migrans (OLM), Neurotoxocariasis (NT) and/or Covert toxocariasis (CT) (7, 8).

Epidemiological studies indicated that the prevalence of *T. canis* eggs 67.5% of *T.canis* from fecal samples of stray dogs in Baghdad (9), while 26.5% was recorded in Basrah city(10). while another study in Baghdad city indicated that lettuce polluted with *T.canis* eggs recorded 53.3% (11). In addition, positive seroprevalence Toxocariasis in Iraq was (27.27%) which is a high rate of infection at child age; some cases suffered from squint, but adult people recorded (23.33%) including seven cases, suffering from blurring of vision or unilateral disorder. While old people over 50 years age recorded 21.87%, which included seven cases suffered differing disorders in their eyes (12).

Seroimmunological diagnosis of VLM detection in suspected serum samples depend on *Toxocara* Excretory/Secretory (TES) antigen secreted from *T. canis* larvae which made problematic detection since there were antibody cross-reactivity between *T. canis* TES antigen and other TES antigen excreted by many nematodes resulting in positive immunoglobulin results, such as *Trichuris trichiura* (13), *Ascars suum* (14) and hookworm (15).

Laboratory tests that reveal the presence of larvae infection in paratenic hosts require, therefore, the aim of this study was designed to infect an experimental rabbits with *T. canis* emderyonated eggs, as an alternative to human Toxocaraisis VLM infection and use histopathological examination to diagnosis *T. canis* larvae stage infection in liver tissues.

Material and Methods:

1) Parasite Samples Collection:

Toxocara canis adult worms were obtained from the stool of naturally 23 puppies infected after treatment with albendazole at a dose of 0.3 g/kg body weight. The samples were obtained from different geographical areas in Baghdad city from April to September in 2017.The worms were washed with physiological normal saline, then eggs were recovered from adult females uteruses were then transferred to erlenmeyer flasks containing 2.5% formalin. The flasks were incubated at 28°C for approximately 30-35 days, with daily manual agitation to ensure oxygenation of the eggs and promote the development of infected stage (larvae two- L2). After this period and after diagnosis of infective eggs which contain larval stage under optical microscope, the embryonated eggs (L2) were washed three times with distilled water to remove formalin and collected by centrifugation (3 minutes at 2,000 rpm). After the washes, 1000 embryonated eggs (egg contain moved larvae) resuspended in 5.0 mL of distilled water were counted in a Mc master slid and used to infect rabbits. An experimental infection involved two experimental groups of rabbits.The first group included (8) rabbits experimentally infected with *T. canis* eggs, and the second group, control group, included (4) rabbits that were not infected. Each rabbit in the first group was orally infected with 1000 embryonated *T. canis* eggs via a plastic pipettes. The control group was

inoculated with 5.0 mL of physiological normal saline solution.

The rabbits of all groups were anesthetized with xylazine and ketamine at concentrations of 10 mg/kg and 100 mg/kg, respectively, then divided into four groups to be sacrefied in different days post infection (DPI) as follows, sacrefying two infected rabbits at 3 and 9 DPI (group I), at 12, 19 DPI group II, at 23, 27 DPI was group III and at 31 and 35 DPI was group IV, in all four groups one rabbit was sacrefied as a control group.

2) Histopathological examinations:

The liver tissues of infected rabbits with *T. canis* emberyonated eggs were partly minced with scissors and transferred to a new a plastic cup with screw containing 10% formalin (10 ml formalin: 90 ml tap water) after 24 h the formalin was replaced then the samples were sent to the paraffin embedded tissues there were cut into 4-5 micron thick sections and stained with Haematoxylene and Eosin (H and E) to assess histopathological changes as per conventional procedure (16).

Results and Discussion:

1) Macroscopic appearance of liver tissue in infected groups:

There were no gross changes observed in liver surface of non-infected rabbit groups with *T. canis* empryonated eggs as shown in figure(1).

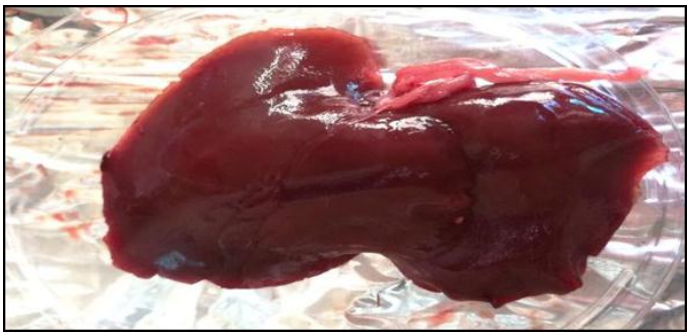

Figure (1): Photographic section of liver of Control Group showed normal surface structure.

Multifocal areas of whitish nodules with different developed stages were observed on the liver surface and within channels in cross section of four infected rabbits groups. The infections appeared earlier on group I infected rabbits which characterized by less number of whitish nodules and small size on liver surface, the infection lesion ranged from mild stage at first week to moderate stage during the second week of infection on group II which characterized by increase number of whitish nodules and still superficial small size lesion on liver. The infections were developed gradually in the (III and IV) groups that ranged from severe stage, where multifocal whitish nodules and deep large in liver parenchyma increased in size at third week after infection to extensive stages which characterized by increased number of whitish nodules and diffused up and within hepatic parenchymal surface during fourth weeks after infection as demonstrated in figure (2) and table (1).

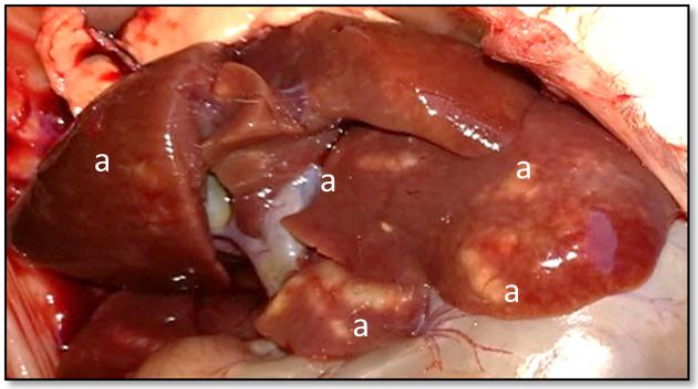

Figure (2): Photographic section of liver of Group IV showed (a) multifocal areas of whitish lesion (extensive stage).

Table (1): The Severity of Lesion occurred Post infected in rabbit.

Infected group	Stage	Explain
Group I	Mild	Rare to few whitish nodules and small size
Group II	Moderate	Increase number of whitish and superficial nodules small size lesions
Group III	Severe	High (multifocal) whitish nodules and deep, large size in liver parenchyma
Group IV	Extensive	More increased whitish nodules number, diffused like channel up and deep in liver parenchyma

2) Microscopic appearance of liver tissue in infected groups:

a) Liver of Group I (3-9 days):

Regarding histological changes observed in liver of infected rabbite group I inflammatory reactions were the hepatic section showed moderate infiltration of mononuclear cells mainly lymphocyte and macrophage as focal aggregation within hepatic parenchyma and within the dilated sinusoids also in interstitial tissue of portal areas, also the hepatocytes showed severe degenerative changes and necrosis as clarifed in figure (3).

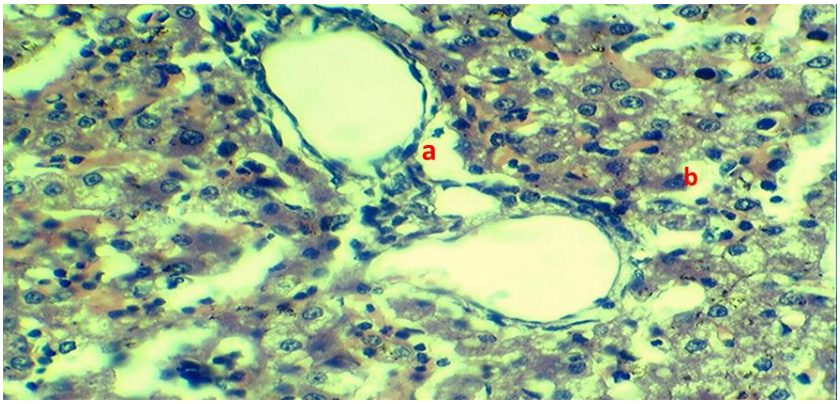

Figure (3): Histopathological section in liver in (a) portal area; mild infiltration of MNCs of infected rabbit with *T. canis* eggs (b) within dilated sinusoid (Group I) (H and E stain, 20X).

The moderate infiltration of hepatocytes MNCs post the *T. canis* infection likely acting as the first line of defense against *T. canis* larvae migration. The present results agreed with results of infected hamster that

showed an immune response against *T. canis* larvae (17), and other result that indecated moderate focal inflammatory infiltrate and were occasionally permeated with necrosis of hepatocytes and polymorphonuclear cell infiltration seen in *T. canis* infected mice (18).

b) Liver of Group II /12- 19 days:

Hepatic section showed severely congested the central veins, portal veins and sinusoidal capillary greatly dilated and filled with RBCs and contained inflammatory cells. The portal area in which the interstitial tissue showed periductal and perivascular appeared thickening due to infiltration with inflammatory cells (MNCs) in proliferated connective tissue. Portal vein and sinusoidal capillaries severe dilated which contain few edematous fluids, also perivascular infiltrations of mononuclear cells (monocytes, lymphocyte and macrophage). The formed of platelets plug obstructed the hepatic blood vessels in portal area with adhesion which cause hypoxia that lead to coagulative necrosis as clarified in figure (4).

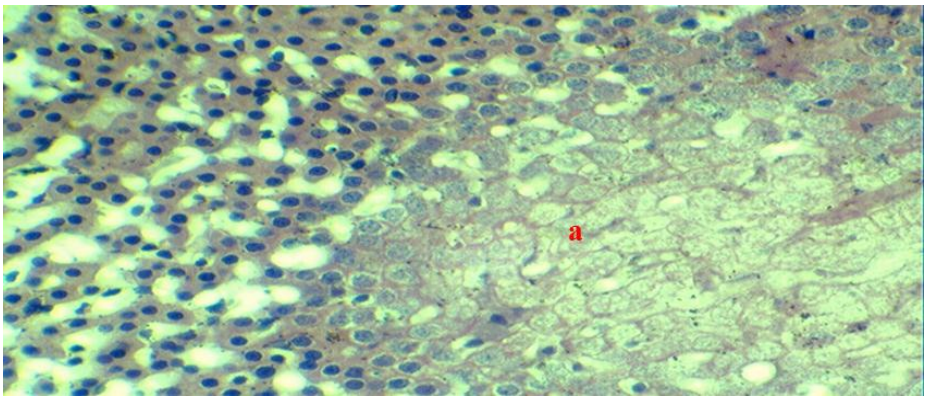

Figure (4): Histopathological section in liver of an infected rabbit with *T. canis* eggs, (Group II) with (a) coagulative necrosis (H and E stain, 40X).

The present findings in group II of infected rabbits deal with other finding which indicated accumulation of mononuclear cells in the hepatic parenchyma and fibrous connective tissue proliferation in portal areas around the biliary tubules of *T. canis* infected rats (19). Other study indicated that liver infected with *T. canis* that the larval stage was coated with fibrous connective tissue, macrophages, neutrophils, and eosinophilic granule cells since vascular alterations such as perivascular inflammatory reaction, obstructive thrombosis and coagulative necrosis, were observed. since coagulative necrosis was most commonly occur due to loss of blood supply lead to lack of oxygen (hypoxia) causes cell death in a localized area (20) and that deal with study that indicated accumulation of mononuclear cells in the hepatic parenchyma and fibrous connective tissue proliferation in portal areas around the biliary tubules of *T. canis* infected rats (19).

c) Liver of Group III /23- 27 days:

The presence of *T. canis* larvae in the liver tissues of the infected rabbits group III was the most common characteristic of the pathohistological changes among the other previous groups, as demonstrated in figure (5).

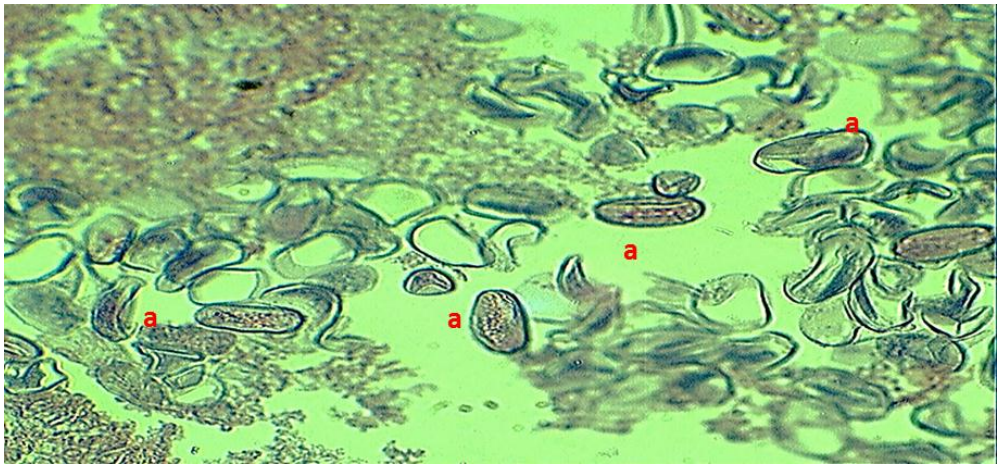

Figure (5): Histopathological section in liver of an infected rabbit with *T. canis* eggs, illustrated (a) *T. canis* larvae in liver tissue of Group III (H and E stain, 40X).

Due to *T. canis* larvae continued movement that caused the thickening of the entry areas with multiple granulomatous lesions, characterized by thick layer due to fibrosis and collagen proliferation (cerotic liver) with accumulation of MNCs which cause atrophic necrosis of hepatocytes which caused by hypoxia. Cirrhosis characterized by; small-newly formed bile ductules, severe dilation of portal vein with severe necrosis of the dilated and thick infected the bile duct which filed with many *T. canis* larvae as shown in figure (6).

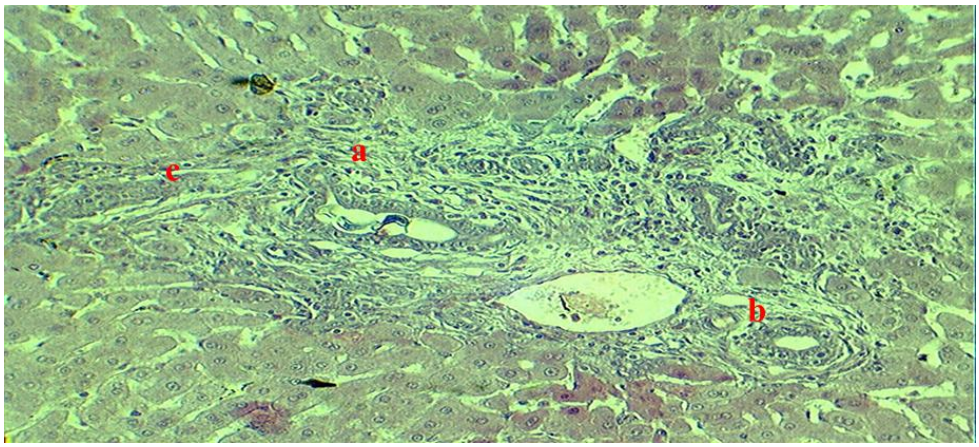

Figure (6): Histopathological section in liver of an infected rabbit with *T. canis* eggs, (Group III) illustrated (a) thickened portal area infiltrated with inflammatory cells (b) newly bile ductules (c) MNCs infiltration (H and E stain, 20X).

Severe sloughing of lining epithelial cells of mucosa and submucosa, infiltration of MNCs due to continues multi direction freely migration of *T. canis* larvae within the liver tissue which stimulate high immune response against larvae migration and causes severe necrosis due to *T. canis* larvae surface coat antigen which activate high immune response against it. Trapped *T. canis* larvae in liver tissue could be seen in infected rabbit group using histological sections were done on 23-27 DPI

compared with other result which showed larvae at 12 DPI or later (21).

d) Liver of Group IV /31-35 days:

In group IV infected rabbit liver which characterized by continuous presence of *T. canis* larvae accumulated in great dilated and necrotized bile duct and liver tissue with increase inflammatory cells like MNCs and formation of collagen from fibroblasts and fibrocytes. The lining epithelial cells with hyperplasia and long epithelial cell and increased number like papillae projections, ballooning degeneration of epithelial cells of bile duct due to multi direction for larvae migration and moved within liver tissue also, severe sloughing of lining epithelial cells in mucosa and in submucosa, infiltration of MNCs and severe necrosis in addition to severe vascular degeneration with collection of homogenous protenicious material produced from larvae surface coat and collagen formation due to cell mediated immunity as mentioned in figure (7).

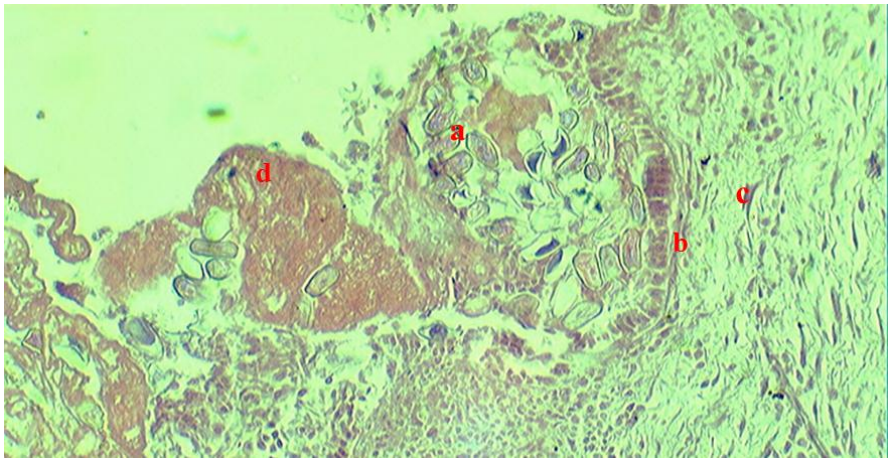

Figure (7): Histopathological section in a liver of infected rabbit with *T. canis* eggs, (Group IV) (a) Presence of *T. canis* larvae and (b) long epithelial cell like papillae projection (c) collagen formation (d) protenicious material (H and E stain, 20X).

The state in infected rabbit group IV developed to chronic stage which mainly characterized by chronic irritant due to chronic inflammatory immune response which cause hyper plastic changes with necrosis and sever vascuolar of hepatiocytes degeneration with highly collagen formation due to cell mediated immunity. Due to *T. canis* larvae interacted with host immune response, larvae appeared of different states which characterized by larvae found in lived state that indicated it was resist host immunity and freely migration inside liver tissue, degenerated state which demonstrated that a high host immune response against larvae surface cote antigen, in this state the host immune response could killed and lysis the larvae formed homogenous protenicious material of dead larvae and collagen formation, while other larvae state showed at this infected group which characterized by founded of empty capsule, and that confirmed an extraordinary larvae ability to leaved its surface cote surrounded by immune cells and escape from it as one of well characteristic larvae survival mechanism from immune system to other host tissue as clarify in figure (8).

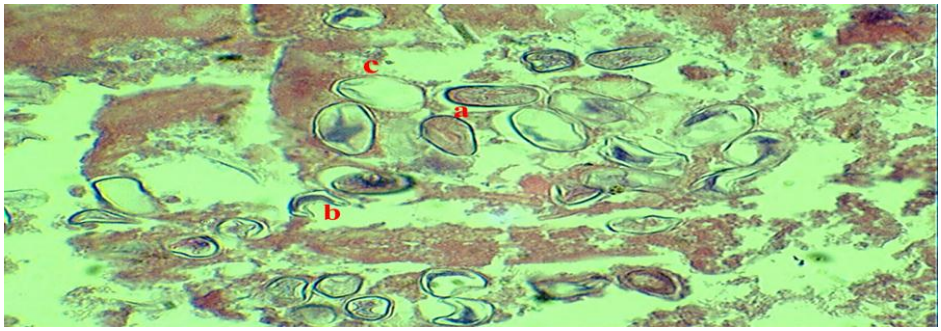

Figure (8): Histopathological section in a liver of infected rabbit with *T. canis* eggs, (Group IV) Presence of different states of *T. canis* larvae; (a) live, (b) degenerated and (c) escape (H and E stain, 40X).

The highly immune response against larvae antigen occur since *T. canis* embryonated eggs were hatched on stomach and migrate to the intestinal wall and subsequently circulate via the blood stream, after being blocked up in capillaries, larvae actively get through vascular wall and migrate into the host tissues organs (22, 23). In contrast host cells contact with the larvae epicuticule and induced its immune response, the parasite needed to develop complex and unique strategies to escape host immune reactions to survive in the host, since larvae could release large amount of *Toxocara* Excretory/Secretory (TES) antigens, such as mucin protein (TES-120), at larvae surface coat component which used as physically escape from the host immune attack (24-28) (29).

Helminth parasites as *T. canis* can invade the host immune system and stay for many years. It was concluded that the whole outer larval surface was involved in the release of ES products then hypothesized that the ability to repeatedly shed ES products on the entire surface could enable the larvae to continuously remove antibodies attached to the surface and escape from immune response.

Our finding showed that there is a clear route of migration of *T. canis* larvae in rabbit's tissues (as paratenic host) which represented VLM syndrome in infected human that elucidate unknown mechanisms of host-parasite interaction are frequently proposed accurate diagnosis method.

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