A HISTOPATHOLOGICAL STUDY OF VESCIRAL LARVAE MIGRANS IN LABORATORY *Balb/ c* MICE IN BASRAH CITY, IRAQ

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ABSTRACT

Visceral larvae migrans of *Toxocara canis* make a big problem in both human and non canine hosts. In the present study, laboratory mice models were evaluated for investigation of the pathological changes in stomach, intestine, spleen, lung, liver, heart. There are many changes were noticed like: ectopic degeneration associated with vacculation in glandular region of the stomach, as will as papillary hyperplasia with increase cellularity in the lamina propria of the intestine were recognized. A lymphoid hyperplasia of white pulp of spleen, aggregation of inflammatory cells, necrosis and fibrosis in liver and lung, vacculation in hepatocytes and emphysema in lung, This explain the distructive effect of the migrating larvae as a problem in the infected host.

INTRODUCTION:

Stray dogs are widely distributed in Iraq. They represent a potential source of transmission of several diseases other than parasites. The most common parasitic worms in dogs are intestinal parasites which include nematodes and tapeworms (Richards, 2002) in addition to the trematodes. The genus *Toxocara* is a common roundworm includes *Toxocara canis* and *Toxocara cati* which infect dogs and cats respectively (Glickman *et al*., 1986).

In human as in other non-canid hosts infected with *T. canis*, the parasite undergoes no growth or development beyond the 2nd stage larvae. Thus, eggs are not found in faeces (Glickman *et al*., 1986). So, the effects of *T. canis* infection in non - canine hosts offer a biological model for understanding the clinical findings of human toxocariasis (Basualdo- Farjat *et al*., 1995). Human infection with *T. canis* starts when eggs are ingested by human (accidentally) and hatched in their intestines. The larvae migrated through the blood vessels then reached liver, lungs, brain, spleen, heart, kidney and muscles, then finally reached to the eyes (Garcia, 2001). Soil contamination with embryonated eggs of *T. canis* is the main source of human infection, but in some cases eating unwashed vegetables or uncooked poultry or ingestion tissues of other paratenic hosts like rabbits, pigs and cattle infected with *T. canis* larvae also make a source of human infection (Agudelo *et al*., 1990; Kazacos and Boyce 1990; Radman *et al*., 2000). *T. canis* cause at least three syndromes in human: Toxocaral visceral larva migrans (VLM), ocular larvae migrans (OLM) and covert toxocariasis (CT) (Garcia, 2001). VLM has been reported in human less than 6 years old (Morris and Katerndahl, 1987) and

individuals following the ingestion of larvae in paratenic hosts (Inoue, 1987).

The mice as an experimental model has been successfully used by many authors since the *Toxocara* larvae were identified as a cause of the disease in children (Schantz, 1989). However, this murine model allows successfully pathological and immunological studies of toxocariasis in human since the course of infection in mice resembles most closely that one in man (Schantz, 1989). Several authors have noticed the ability of *T. canis* larvae to survive and continually migrate in the hosts' tissues inspite of the immune response (Schantz, 1989). *In vitro* observations have shown the eosinophiles attach to *T. canis* larvae in the presence of immune serum and complement (Fattah *et al*., 1986; Badley *et al*., 1987).

The aim of this study was to determine the distribution of *T. canis* larvae in different organs of mice orally infected with *T. canis* larvae, as will as the pathological changes due to the migration of those larvae inside different organs.

MATERIALS AND METHODS:

Samples Collection

Five stray dogs were killed by shooting gun or by using strychnine sulphate tablets during the study. The intestine (small and large) of killed dogs was dissected lengthwise, worms were removed and the intestine contents placed in clean crystalline dishes and examined for the presence of the parasites.

Worms Collection

Twenty five male and female *T. canis* worms (10-18 cm long) were collected from intestine of dissected stray dogs. Worms were washed several times with normal saline (0.85%). The anterior third of the female worms were dissected, and then uteri and eggs were isolated in a clean Petri dish containing normal saline.

Eggs Culture

The procedure of Bowman *et al*. (1987) was used for culturing the eggs of *T. canis* The eggs were used after at least 40 days, Active infective 2nd. stage larvae were ensure inside eggs by examination under light microscope (X10) directly before infection of mice

Mice infection

A total of 10 Balb/c mice with 1.5-2 months of age were inoculated orally with 500 embryonated eggs of *T. canis* by means of a stomach tube attached to an 18 gauge needle with a 1 ml syring (Sugane and Oshima, 1982), while the control group was inoculated with one ml distilled water. The infected mice were killed after three months post-infection. The organs of infected mice were isolated after washing in formalin 10%.

Pathological sections

Infected mice were sacrified after 1, 2 and 3 months post infection. Pieces of 1X1cm from stomach, intestine, liver, lung, spleen and heart of each infected and control mice were freshelly cutted off and fixed in Bouan'"s solution for histopathological studies. A method of Annpreece (1972) were used for sectioning organs of infected mice with *T. canis* larvae.

RESULTS:

Post mortem inspection of infected mice demonstrate marked pathological changes in stomach, intestine, liver, lung spleen and heart, These organs were selected for more investigation by histo-pathological sections

The following changes were recorded

1- **Stomach:** the stomach of an infected mice showed a ectopic degeneration associated of vacculation in glandular region. (Fig. 1,2).

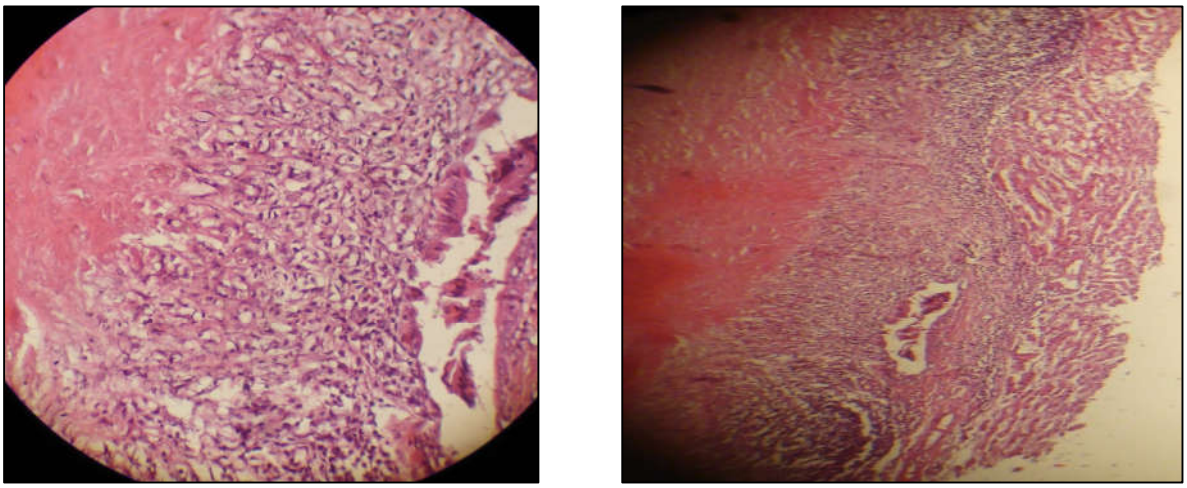

Fig. (1&2): A section in stomach of mice infected with visceral larvae migrans with (E & H). 40X and 10X.

2- **Intestine:** it can appeared a papillary hyperplasia with increase cellularity in the lamina propria, also, a passages between cells founded. (fig 3).

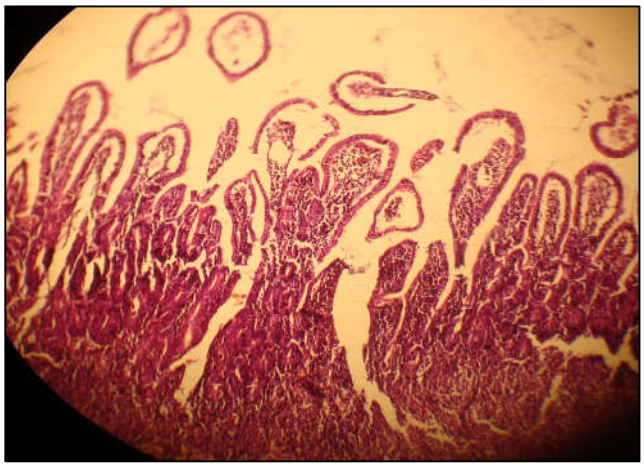

Fig (3): Section in intestine of mice infected with visceral larvae migrans with (E & H) 10X.

3- **Spleen:** the infected spleen with many changes, like, lymphoid hyperplasia of white palp with lymphoid tissue as shown in Fig. (4, 5).

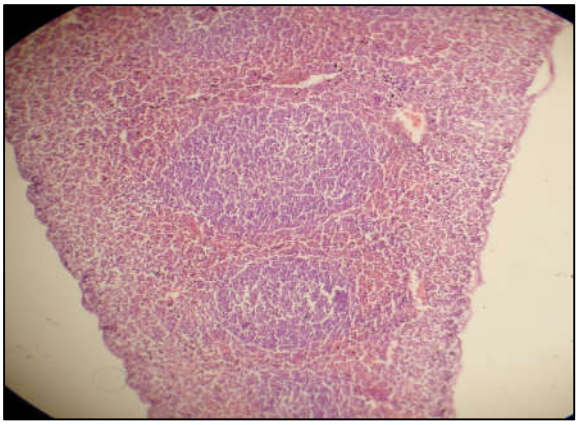
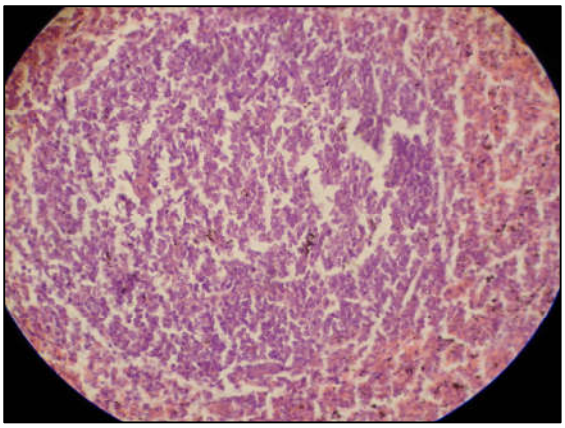

Fig. (4, 5): Section in spleen of mice infected with visceral larvae migrans with (E & H) 10X and 40X.

4- **Lungs:** the lungs of mice infected with visceral larvae migrans with many changes, like, bronchiole with dilation and intra luminal inflammatory cells with per bronchiole inflammatory cells, also, with congestion and emphysema, as in (picture, 6). While, in picture (7) can be found a congestion and emphysema, a proliferation of bronchiole epithelium associated with folding of the bronchiole epithelium. But in picture (8) a bronchiolar epithelium with proliferation associated with per bronchiolar inflammatory cells with fibrosis and congestion.

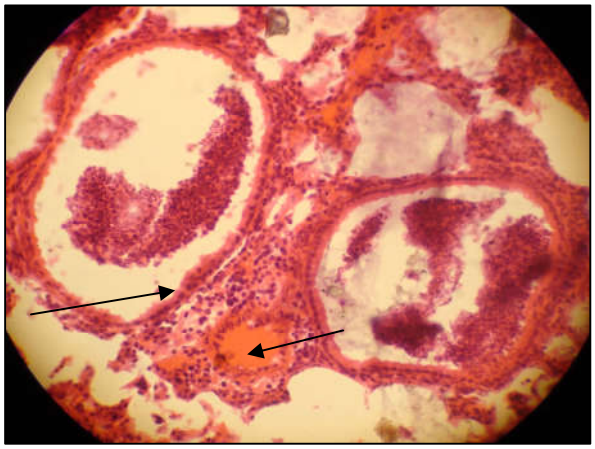

Fig (6): Section in lung of mice with visceral larvae migrans with congestion and emphysema. (E & H). 10X.

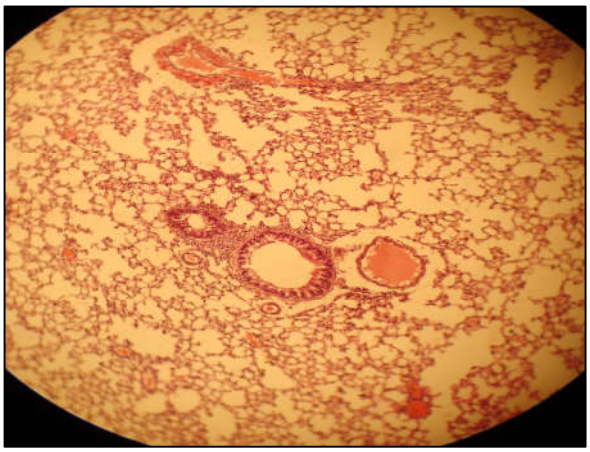

Fig (7): Section in lung of mice with visceral larvae migrans with proliferation of bronchiole epithelium. (E & H). 4X

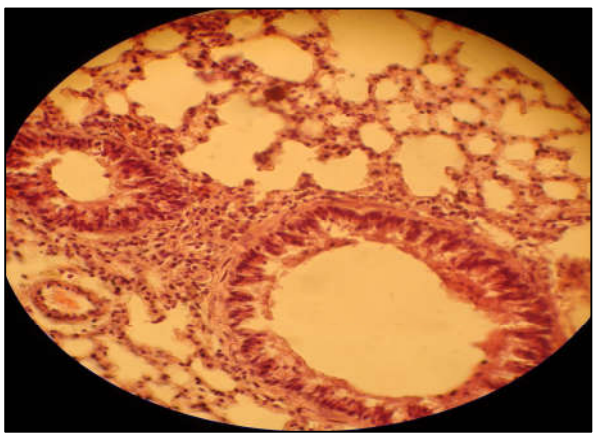

Fig. (8): Section in lung of mice with visceral larvae migrans with per bronchiolar inflammatory cells with fibrosis and congestion . (E & H). 10X.

5- Heart: it can see a vacculation of myocardial muscles in mice infected with visceral larvae migrans, as shown in Fig. (9).

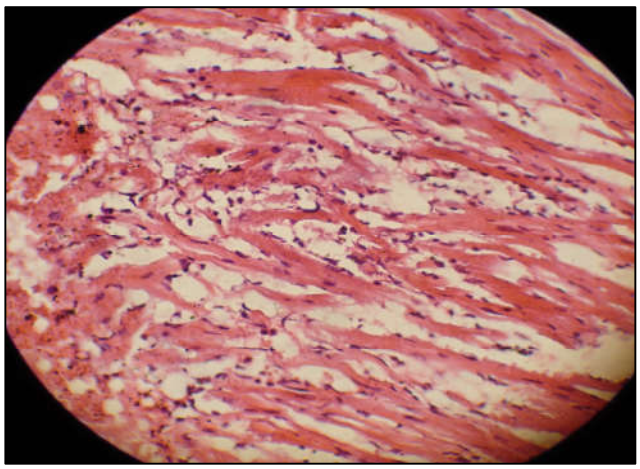

Fig . (9): Section in heart of mice with visceral larvae migrans with vacculation of myocardial muscles . (E & H). 10X.

6-Liver: it can see a chronic granulomatous inflammatory reaction consist of central necrosis and the periphery mononuclear cells with fibrosis, picture 10, also, a granulomatous inflammatory reaction with necrosis, fibrosis with Granuloma in bile duct, picture 11.

A periportal hepatocytes and central lobular hepatocytes with enlargement of center lobular hepatocytes, picture 12. In picture 13 it can see a vacculation of hepatocytes with periportal region and a mimic diffuse vacculation of hepatocytes and periportal of infiltration of inflammatory cells, furthermore, an occasional inflammatory cells with congestion of portal vein.

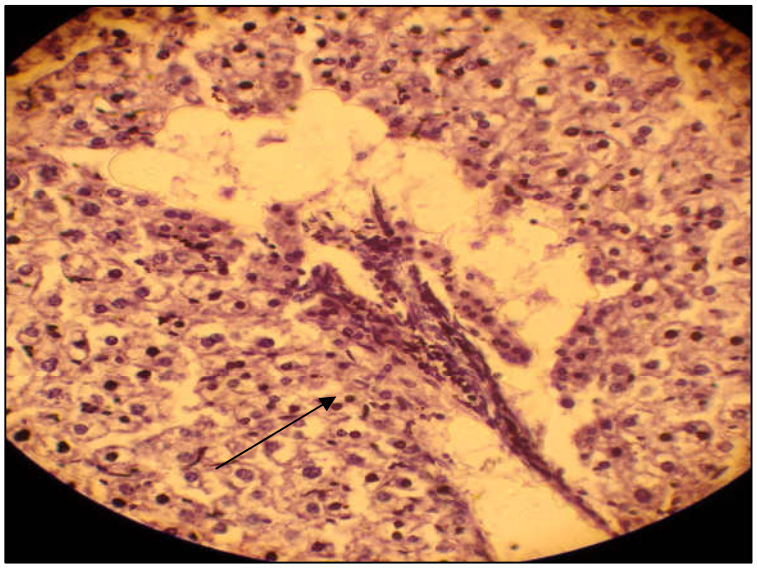

Fig. (10): Section in liver of mice with visceral larvae migrans with chronic granulomatous inflammatory . (E & H). 10X.

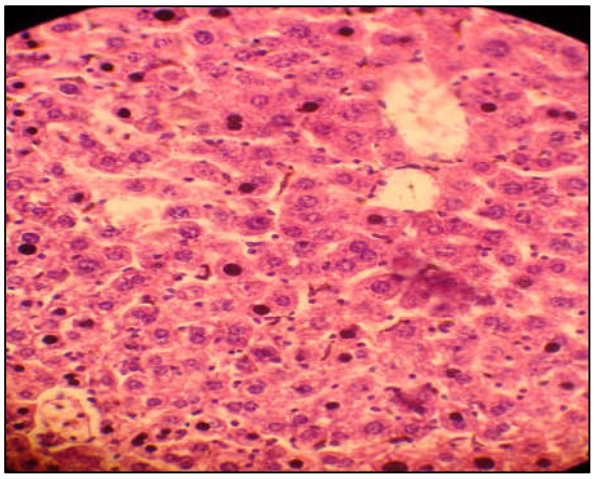

Fig. (11): Section in liver of mice with visceral larvae migrans with granulomatous inflammatory reaction with necrosis, fibrosis with Granuloma in bile duct . (E & H). 10X.

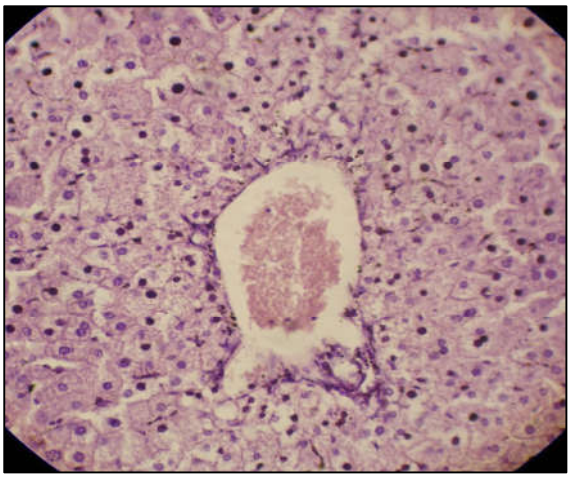

Fig. (12): Section in liver of mice with visceral larvae migrans with a periportal hepatocytes and central lobular hepatocytes with enlargement of center lobular hepatocytes . (E & H). 10X.

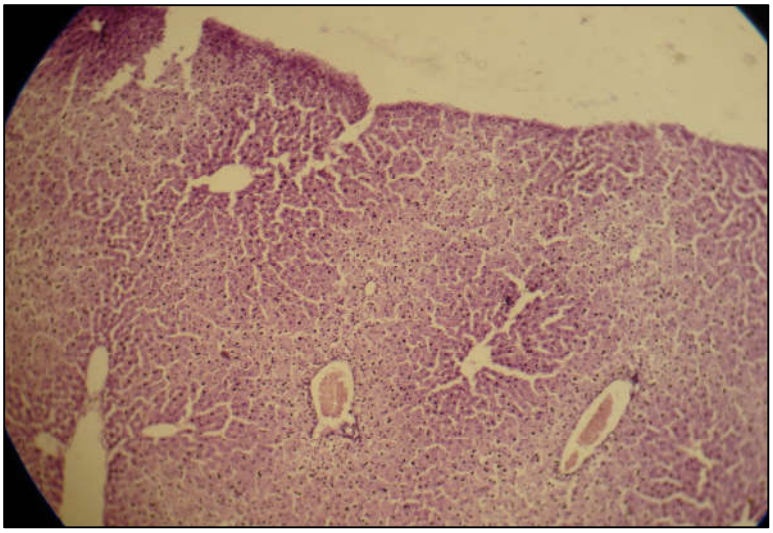

Fig. (13): Section in liver of mice with visceral larvae migrans with granulomatous inflammatory reaction with vacculation of hepatocytes with periportal region . (E & H). 10X.

DISCUSSION:

Visceral larvae migrans is a public health problem in both human and non canine hosts. Human acts as undefinitive hosts in which *Toxocara* larvae will not develop but migrate and survive for a long time.

A laboratory model mice were used under this study for detection a pathological changes of visceral larvae migrans. Cox and Holland (2001) showed that many factors may affect the infection of mice with *T. canis* larvae such as strain of mice, dose and larval intensity and period post - infection.

A many changes were founded in many organs in mice with congestion, aggregation of inflammatory cells in liver and lung, granulomatous inflammatory reaction with necrosis, a vacculation of hepatocytes with periportal region and a mimic diffuse vacculation of hepatocytes and periportal of infiltration of inflammatory cells in liver. While, in spleen, a lymphoid hyperplasia of white palp with lymphoid tissue. But in intestine a papillary hyperplasia with increase cellularity in the lamina propria, also, a passages between cells founded. All these changes can explain that the larvae of T. canis survive, surrounded and release a excretory secretory materials which make the host effect and the organs change, furthermore, the larvae penetrate the intestine tissue to run away from the digestive enzymes and enter the blood vesicles to go to the other organs.

This observation suggested that *T. canis* larvae after hatching in intestine migrated by a hepatopulmonary route in the mice. Furthermore, the differences between inoculated eggs and larvae recovered from infected mice may be due to the number of inoculated eggs passing out through the intestine without hatching. Larval recovery from mice organs in this study is evidence that larvae are not only trapped but survive within these organs.

Oshima (1961) demonstrated that approximately 98% of larvae were concentrated in the liver and lungs of mice infected with *T. canis* larvae at 44 hours post- infection. While, Kayes *et al*. (1985) and Piergili-Fioretti *et al.* (1989) showed that liver and spleen indices increased between 11-14 days and continued till 21 days post-infection, also, they observed that with a small inoculum the spleen index returned to its normal value which was quicker than high dose inoculum.

Taira *et al*. (2003) studied chickens experimentally infected with 5000, 10000 and 20000 eggs of *T. canis* and they found that larvae were perdominately (>87%) recovered from liver and lungs, while only few larvae were seen in other organs or tissues.

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دراسة أمراضية- نسيجية لليرقة الحشوية المهاجرة في الفئران المختبرية في مدينة البصرة

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الخلاصة:

اليرقة الحشوية المهاجرة متمثلة بالطور اليرقي لطفيلي التوكسوكارا كانس تسبب مشكلة مرضية خطيرة لكل من الانسان والحيوانات التي لا تنتمي للفصيلة الكلبية. في هذه الدراسة تم استخدام نموذج الفئران المختبرية للإصابة المختبرية ومعرفة ما تسببه هذه اليرقة من تغيرات نسيجية لبعض الأعضاء كالمعدة، الأمعاء، الطحال، القلب، الكبد والرئة. لقد سجلت العديد من التغيرات الامراضية النسيجية لهذه الأعضاء والتي تمثلت بـ:

تكشف وتفج خلايا الجزء الغدي من المعدة، حالة فرط تنسج الزغابات وتليف الطبقة البرانية من الأمعاء الدقيقة. حدوث حالة فرط التنسج اللمفاوي في اللب الأبيض من الطحال، تجمع الخلايا الالتهابية وتنخر وتليف في الكبد والرئة، كذلك تفج الخلايا الكبدية وحدوث النفاخ الرئوي في الرئة. وعليه فأن لهذه اليرقة قابلية إحداث مشاكل نسيجية كثيرة على المضائف التي تصيبها.