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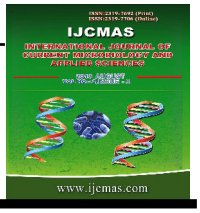

Original Research Article

Study of histological and physiological effects of *Toxocara cati* larvae infection in experimentally infected white rats

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Keywords

COD reduction; Electro-chemical; RSM; Ultra Violet.

A B S T R A C T

The current study was conducted in the period from September 2012 till September 2013 to study the histological and pathological effects of *Toxocara cati* in experimental infected rat. the laboratory animals divided to four groups (three of them groups infected by the parasites of the study and one control) treated by different concentrations **(**500,1000,2000**)** eggs/ ml , the experiment continuous for two months treated by once in one day, the result appear histological changes for some organs of infected animals by 2000 eggs / ml these were more effective compared with other groups ,the lung more effective organs was noticed destruction and increasing bronchi thickness and ulceration of lumen tracheal and destruction of B-Nemocytes (Type-2), and noticed histological changes in small intestines is showed hypertrophy and destruction microvillus and columnar cells that lined and obstruction mucosa and sub mucosa layer , while liver is show expanded in central vein of lobules congestion and bloody hemorrhage and polymorphic nucleus infiltration, furthermore heart organ was noticed absent of striation of myocardium and not appear nuclei ,finally pharynx isn't show any changes.

Introduction

Toxocara cati is a common parasite of cats and Felidae, and has a cosmopolitan distribution (Yamaguchi *et al.,* 1996) .its second stage larvae are possible causes of visceral larva migrants (VLM) or ocular larvae migrants (OLM) (Dubinsky, 1999) .Human infection can occur by accidental ingestion of embryonated eggs of Toxocara species. The embryonated *Toxocara* eggs, when fed, hatch in the gut contents of human and the larvae migrate to the soft tissue of the body such as liver, heart, lungs and other organs (Schantz, 1989; Azizi *et al.,* 2007).

Cats can become infected with *T. cati* by ingesting embryonated eggs found in contaminated soil or by predation of paratenic hosts - usually small rodents or birds containing live third-stage larvae in their tissues (2). Another means of transmission in cats is larval transmammary migration (Aza *et al.,* 2003; Coat *et al.,* 2004).

Adult feline roundworms may be brownish-yellow to cream colored to pink and may be up to 10cm in length. Adults have short, wide cervical alae giving their anterior ends the distinct appearance of an arrow (hence their name). Eggs are pitted ovals with a width of 65μm and a length of about 75μm making them invisible to the human eye. The larvae are so small that they are easily transmitted from an adult female to her nursing kittens through her milk (Bowman *et al.,* 2002).

Some treatments for infection with *T. cati* include drugs designed to cause the adult worms to become partially anaesthetized and detach from the intestinal lining, allowing them to be excreted live in the feces. Such medications include piperazine and pyrantel. These are frequently combined with the drug praziquantel which appears to cause the worm to lose its resistance to being digested by the host animal. Other effective treatments include ivermectin, milbemycin, and selamectin (Bowman *et al.,* 2002).

Due to the close association and proximity of man with his domestic animals (in particular, cats), there exist possibilities of human infection with helminthes parasites of these animals. Although the larvae of non-human acaroids, such as *T. cati*, are capable of limited development in human hosts, this may, in some circumstances, lead to serious public health problems, e.g., encephalitis and granulomatous lesions (Woodruff *et al.,* 1981).

The eggs are washed from the soil's surface into the deeper layers, and because of their resistance to climatic changes they may remain viable for several years (Buijs, 1993).

Prevention includes de-worming dogs and cats, preventing dogs and cats from defecating in public areas, and keeping children away from areas where dogs and cats may defecate. It is very important to carefully wash your hands after touching soil (Kazura, 2007).

The aim of this study is to study the pathological effects of the infection of *T. cati* in experimentally white rats an d to study the histological changes in animals tissues due to the migration of *T. cati* larvae especial the brain tissue.

Materials and Methods

During the period from 1/10/ 2012 to 25/4/ 2013, We examined 49 cats was hunt and after sedation and euthanasia with thiopental, cats were examined macroscopically to examine the infection of *T. cati* and 9 of them was infected and the larvae found in different organs of the body.

All cats were autopsied less than two hours after being euthanatized so that all adult worms were still alive prior to fixation in 10% formalin solution. *T. cati* worms were separated from other helminthes and the males and females were identified by microscopic examination. The worms were cleared, and stained using FAAL (formalin alcohol azocarmin lacto phenol). The mature male and female worms were identified according to the morphological features described by (Yamaguti, 1961).

Presence of ova was the criterion for distinguishing the mature female worms (Soulsby, 1982). The ova were assessed by simple observation at 400x magnification by examining the general appearance of the shell and its cellular contents. Only

those ova that were specifically located in the tapering portion of the uterus (near the vulva at the anterior end of the worm) were considered for this purpose. Those eggs that were both regular in shape and which possessed the characteristic pitted shell design were regarded as *T. cati* eggs of. The uniformity of the cellular content of the ova was also considered. The worm burdens in this study were recorded directly at autopsy by counting.

Also we examined some samples from soil by floatation method and contaminated water by centrifuge method and contaminated vegetables by centrifuge the washing water, to confirmation the distribution of Toxocara cati infection in the study area (Babylon province).

Experimental animals

Experimental white rats type *Ratus ratus* Wight 250± gm, age 4-6 week, used in this study divided to 3 groups each include 3 animals infected by the eggs of *T. cati* orally by plastic cannula as follows:

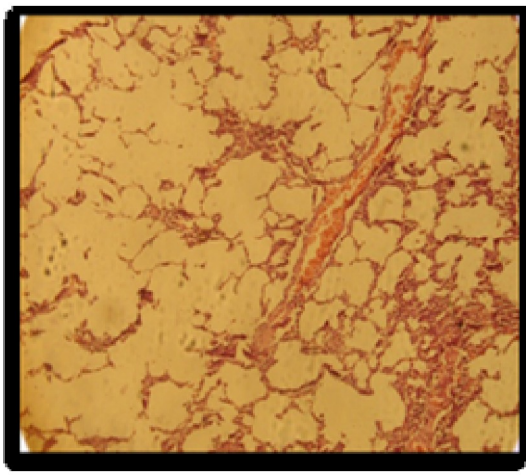
1. Group1: infected with 500 egg / ml.
2. Group2: infected with 1000 egg / ml.
3. Group3: infected with 2000 egg / ml.

Result and Discussion

Effects of *T. cati* larvae in the lungs tissue of experimentally infected animals

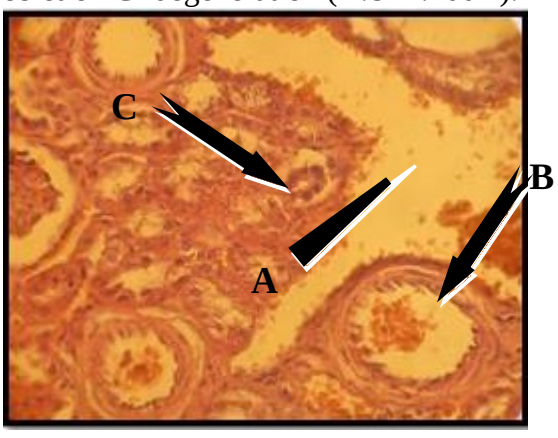
We take the third group only because the histopathological effects of the infection are more obeuvesly than other groups (1,2), The histological examination of the lung tissue for the control group shows the normal structure of the tissue without any changes, because it's not infected with *T. cati* eggs (Figure 1).

Figure.1 section in the lungs of rat (control group) shows the normal structure of the tissue without any changes (H. & E.40X).



The histological examination of lungs for animals infected with 500 egg/ml of *T. cati* after two months, shows destruction in the air sac and molting in the lumen of bronchi, inner pathogenic secretion, and degeneration (Figure 2).

Figure.2 section in the lungs of rat infected with 500 egg/ml of *T. cati* , after two months of infection shows: A- destruction in the air sac B- molting in the lumen of bronchi inner pathogenic secretion C- degeneration (H.& E.100X).



The animals which infected with 2000 egg/ml show some changes in the structure of lungs tissue after two month of infection: Polymorphic infiltration, destruction in the air sac, destruction in the B-Nemocytes (type 2) cells as in figure (4).

Figure.3 section in the lungs of rat infected with 1000 egg/ml of *T. cati* , after two months of infection shows: A-Polymorphic infiltration (H.&E.100X).

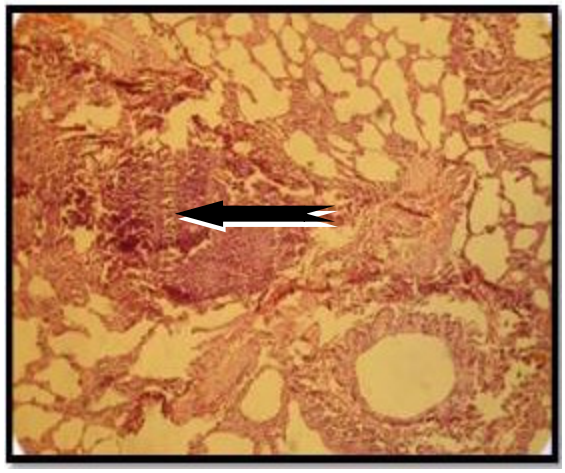
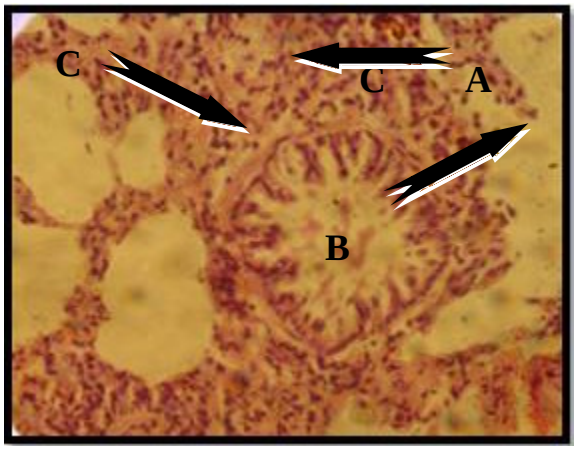


Figure. 4 section in the lungs of rat infected with 2000 egg/ml of *T. cati* , after two months of infection shows: A-Polymorphic infiltration, B-destruction in the air sac, C-destruction in the B-Nemocytes (type 2) cells (H.&E.100X)..



Effects of *T. cati* larvae in the small intestine tissue of experimentally infected animals

Figure (5) shows section in the small intestine for control group appears the normal structure of the tissue. While figure (6) appears section in the tissue of small intestine for the animals infected with 500 egg/ml and shows expansion and destruction of villi and Polymorphic infiltration, destruction epithelial cells lining the villi and debris.

Figure. 5 section in the small intestine for control group appears the normal structure of the tissue (H. & E.40X).

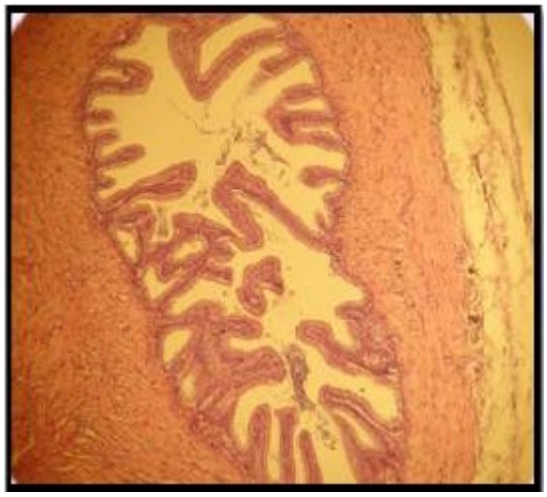


Figure (7) shows section in the tissue of small intestine for the animals infected with 1000 egg/ml of *T. cati* after two months of infection we notes the molting of epithelial cells lining the villi and Polymorphocyte infiltration.

The histological examination of small intestine tissues for animals infected with 2000 egg/ml of *T. cati* after two months of infection appears necrosis in columnar epithelial cells of villi and debris of destruction villi and necrosis epithelial cells, figure (8).

Figure.6 section in the small intestine of rat infected with 500 egg/ml of *T. cati* , after two months of infection shows: A- expansion and destruction of villi. B- Polymorphic infiltration. D- Destruction epithelial cells lining the villi. C- Debris (H. & E.100X).

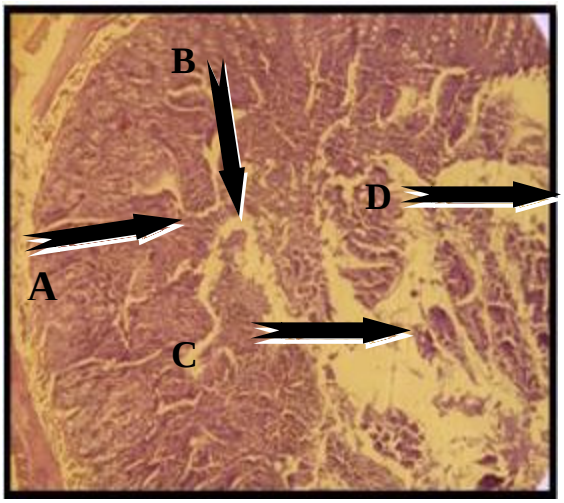


Figure. 7 section in the small intestine of rat infected with 1000 egg/ml of *T. cati* , after two months of infection shows: A- molting of epithelial cells lining the villi B-Expansion of villi and Polymorphocyte infiltration (H.& E.100X).

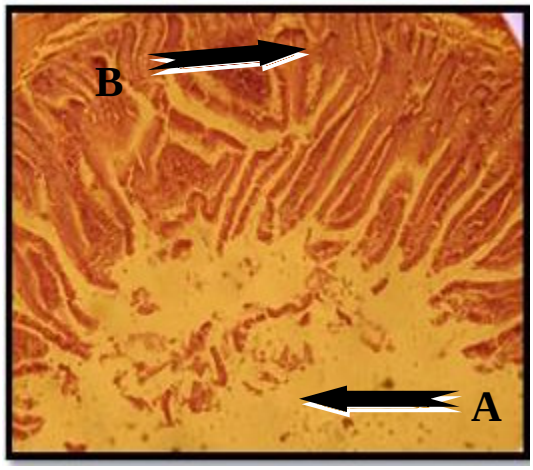
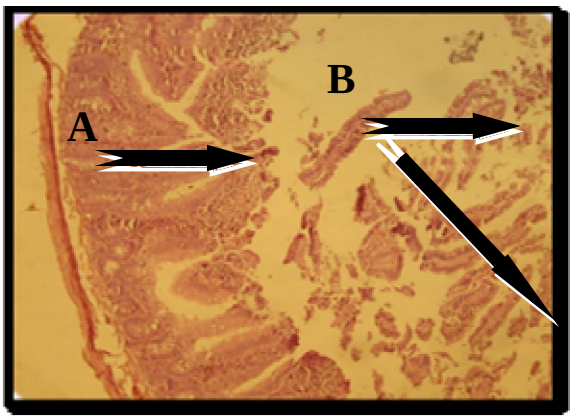


Figure.8 section in the small intestine of rat infected with 2000 egg/ml of *T. cati* , after two months of infection shows: A- necrosis in columnar epithelial cells of villi. B- Debris of destruction villi and necrosis epithelial cells (H. & E.100X).



Effects of *T. cati* larvae in the liver tissue of experimentally infected animals

Figure (9) shows section in the liver for control group appears the normal structure of the tissue. The histological section of liver for animals infected with 500 egg /ml appears Polymorphocyte infiltration, fat cells in the tissue, destruction and necrosis of nucleus figure (10).

Figure. 9 section in the liver for control group appears the normal structure of the tissue (H. & E.100X).

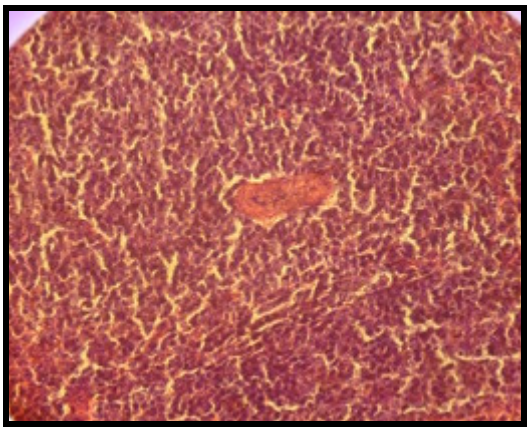


Figure.10 section in the liver of rat infected with 500 egg/ml of *T. cati* , after two months of infection shows :A- Polymorphocyte infiltration. B- Fat cells in the tissue. C- Destruction and necrosis of nucleus (H. & E.100X).

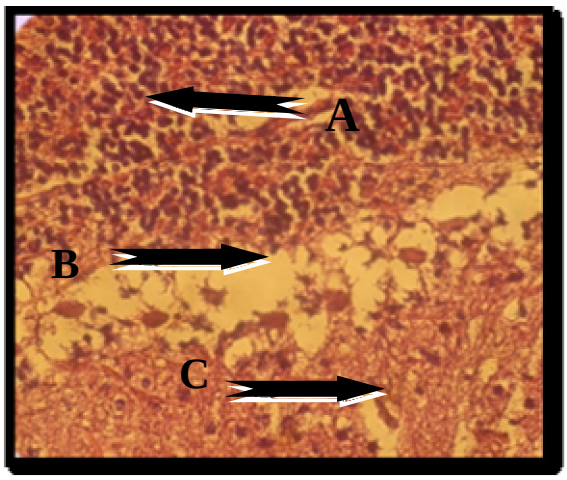


Figure.11 shows section in the tissue of liver for the animals infected with 1000 egg/ml of *T. cati* after two months of infection we notes the expansion of central vein of liver lobules and hemorrhage in liver.

Figure.11 section in the liver tissue for animals infected with 1000 egg/ml of *T. cati* after two months of infection: A- expansion of central vein of liver lobules. B- Hemorrhage (H. & E.100X).

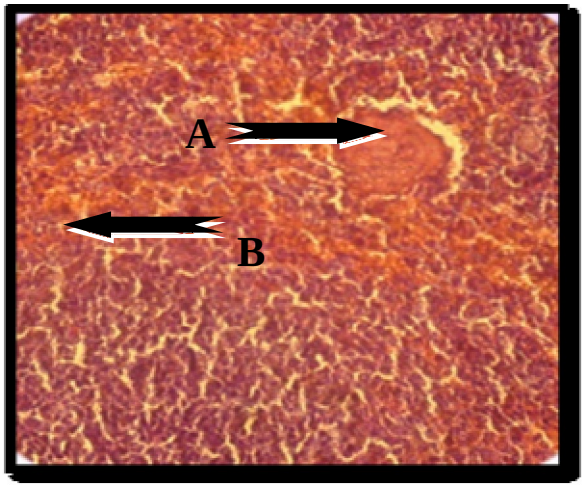
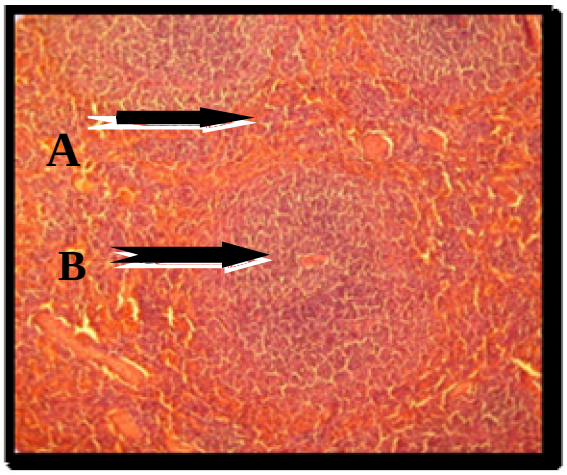


Figure (12) appears section in the tissue of liver for the animals infected with 2000 egg/ml of *T. cati* after two months of infection notes hemorrhage and necrosis in liver cords also central infiltration in liver cords.

Figure.12 section in the liver tissue for animals infected with 2000 egg/ml of *T. cati* after two months of infection: A- Hemorrhage between kupffer cells and necrosis in liver cords. B- Central infiltration in liver cords (H. & E.100X).



Effects of *T. cati* larvae in the heart tissue of experimentally infected animals

Figure (13) shows section in the heart for control group appears the normal structure of the tissue. Figure (14) appears section in the heart tissue animals infected with 1000 egg/ml of *T. cati* after two months of infection notes absent of cardiac streaks also unclear nucleus, and Polymorphic infiltration.

Effects of *T. cati* larvae in the pharynx and brain tissue of experimentally infected animals

Figure (15) shows section in the pharynx for control group and infected animals

Figure.13 section in the heart for control group appears the normal structure of the tissue (H. & E.100X).

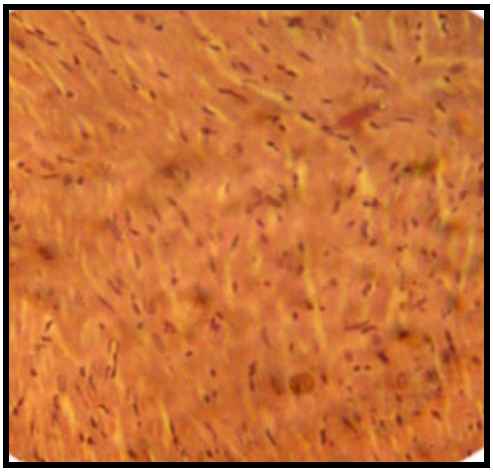
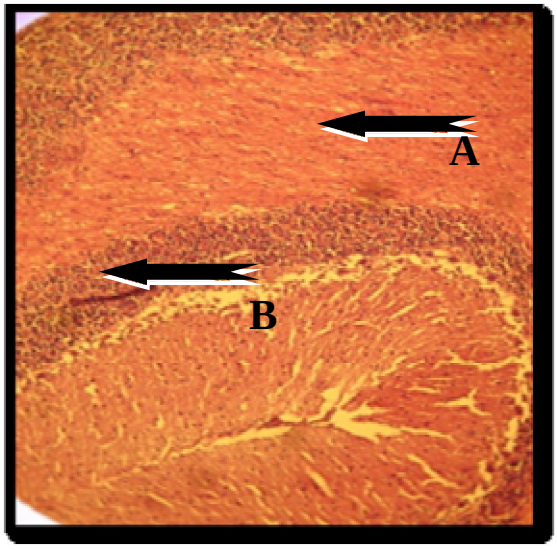


Figure.14 section in the heart tissue animals infected with 1000 egg/ml of *T. cati* after two months of infection: A- absent of cardiac streaks also unclear nucleus. B- Polymorphic infiltration (H. & E.100X).



appears the normal structure of the tissue, figure (16) shows section in rat brain infected with 2000 egg/ml after two months of infection show the larvae of *T. cati* and infiltration of lymphocytes.

Figure.15 section in the pharynx for control group appears the normal structure of the tissue (H. & E.100X).

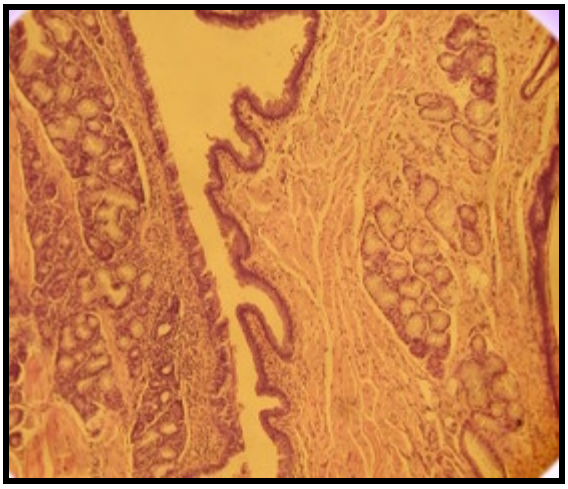
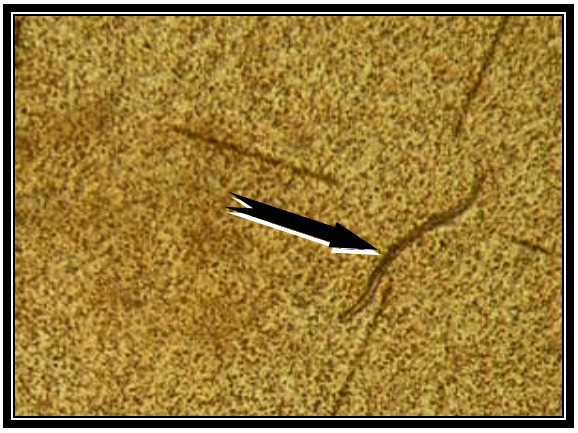


Figure.16 section of rat brain infected with 2000 egg/ml after two months of infection show the larvae of *T. cati* and infiltration of lymphocytes (H.&E.40X).



The current study appeared histological changes in the lungs of infected animals as in figure (2) shows destruction in the air sac and molting in the lumen of bronchi, inner pathogenic secretion, and degeneration of cells, the cause of these changes may be due to the mechanical and fast movement of larvae of a parasites and the vacuolation of lung tissue make it easy for invasive by larvae of *T. cati* (Waness *et al.,* 2011).

Parasites can cause pulmonary infiltrates with peripheral blood and/or alveolar eosinophilia. This occurs as a result of the tissue inflammation caused by the presence of the parasite in the lung at a certain stage in its life cycle, and the host's immunological response which includes a local accumulation of eosinophils, and an accumulation of eosinophils in the peripheral blood. The travel history is therefore important to exclude this as a potential cause of pulmonary infiltrates with eosinophilia. In the United States, Strongyloides, Ascaris, Toxocara, and Ancylostoma are the most common parasitic causes of eosinophilic lung disease. Other parasites causing pulmonary eosinophilia around the world include Schistosoma, *Trichinella spiralis, Paragonimus westermani*, *Echinococcus granulosus,* and *Dirofi laria immitis* (Mann, 2008).

Analysing *T. canis* larval migration, observed a higher concentration of larvae in the liver, lungs and kidneys within the first five days after inoculation. After the 15th day of infection, an increase in larval recovery from the brain was noted, lasting up to the 30th day of observation (Mann, 2008).

In figure (3) shows section in the lungs of rat infected with 1000 egg/ml of *T. cati* notify the Polymorphic infiltration in the tissue. While The animals which infected with 2000 egg/ml show some changes in the structure of lungs tissue after two month of infection: Polymorphic infiltration, destruction in the air sac, destruction in the B-Nemocytes (type 2) cells as in figure (4), the results of current resemble many study as In (17) study of *T. cati* in experimentally infected chickens observed in the lungs, lesions mostly at histopathology level. Chronic peribronchiolitis with infiltration of lymphocytes and hyperplasia of bronchiolar associated lymphatic tissues (BALT) and goblet cells were evident in the lungs of the infected untreated chickens. Alveolitis with diffuse infiltration of eosinophils were also seen in these chickens. Interstitial pneumonia with infiltration of lymphocytes, plasma cells and a few eosinophils in the lungs.

Recently, using a dose of 1000 *T. cati* embryonated eggs by (Cardillo *et al.,* 2009) obtained a larger concentration of larvae in the liver by the 6th day PI. On the other hand, analyzing *T. cati* larval migration in a previous study showed a higher concentration of larvae in the lungs on day 60 after inoculation (Santos *et al.,* 2009). In an earlier report, after analyzing *T. cati* larvae migration in mice (C57BL6), it was stated that higher concentration in the liver, lungs, and kidney was found at day 5 PI, although a study noted encapsulated larvae in the liver tissue of experimentally infected chicken at day 14 PI (Hrckova *et al.,* 2001). It seems that the transmission of larvae was from the intestine to the liver and lungs then to the heart, kidneys, muscles, and brain. According to a previous study, during the first 24 hr, the hatched *T. cati* larvae in the intestine migrated to the liver and lungs and they presented in other organs, such as muscles (Dubey, 1968). Larvae were recovered from muscles of Mongolian gerbils on day 70 PI. The results suggest a likeness to that observed by (Havasiova *et al.,* 1995).

The current study appeared pathohistological changes in the small intestine of infected rats, figure (6) shows the tissue of small intestine for the animals infected with 500 egg/ml appears expansion and destruction of villi and

Polymorphic infiltration, destruction epithelial cells lining the villi and debris , the reason for these changes could be due to the Pathogenicity of *T. cati* larvae because it's have virulence factors as produce many toxic material and lyso enzyme which lyses cells and villi that's lead to infection of mucosa layer of small intestine also the infection lead to hypoemia and the helminthes depend in nutrition on its host and excrete toxic materials that make auto immune to depression , and the presence of leukocytes due to infection respond of the body and the expansion and destruction of villi as a result for inadequacy of epithelial tissue in small intestine (Beisel, 1982; Kayes, 1997).

May be the damage which happened in the epithelial tissue decrease the ability of intestine to absorb the benefit nutrients that lead to weakness hypoemia because of losing a lot of necessary and important materials.

Figure.7 shows the molting of epithelial cells lining the villi and Polymorphocyte infiltration in the tissue of small intestine for the animals infected with 1000 egg/ml of *T. cati* , and the histological examination of small intestine tissues for animals infected with 2000 egg/ml of *T. cati* after two months of infection appears necrosis in columnar epithelial cells of villi and debris of destruction villi and necrosis epithelial cells, (figure 8).

Perhaps the cause of these changes is the goblet cells excrete mucin which prevent the helminthes invade deeply through intestine layers , a disorder in digestion and absorption as a result of infection respond to toxic molecules as peptides and histamine , that cause immune depression (Stephenson and Holland, 1987; Tomkins and Waston, 1989).

Figure.9 shows section in the liver for control group appears the normal structure of the tissue, the histological section of liver for animals infected with 500 egg /ml appears Polymorphocyte infiltration, fat cells in the tissue, destruction and necrosis of nucleus figure (10), may be that due to immune response to *T. cati* infection and the presence of fat cells in the tissue, destruction and necrosis of nucleus that explained Hyperplasia of the tissue and as a results the cell lose their nucleus (Lescano *et al.,* 2004).

In figure.11 we note the expansion of central vein of liver lobules and hemorrhage in liver , may be the cause for these changes due to the longest of infection and to metabolism materials which produced by the parasite also the effect of immune responds because the parasitic infection induce production of acidophil cells and factor -α and β which cause expansion of central vein of liver and could be the cause for these change is anesthetization of the animal before the anatomy (Hrckova *et al.,* 2001).

In figure (12) appears hemorrhage and necrosis in liver cords also central infiltration in liver cords and Hemorrhage between kupffer cells that due to its ability to phagocyte strange materials which produce by the parasite as waste products found in the blood and change it to soluble materials to out dismiss by the kidneys (Roberts, 2009). In addition the liver consider in third level effected by parasitic infection because it's not compact tissue also the channels which found in the liver made the passage of parasite throw the tissue easy (Hrckova *et al.,* 2001).

In the study of (Al-Rubaie , 1998) found the larva restrict in the liver and lungs because of albendazole and the extract of

A. herba-alba that inhibited the migration of larva because they concentrate in the plasma and liver.

Figure (14) appears section in the heart tissue animals infected with 1000 egg/ml of *T. cati* after two months of infection notes absent of cardiac streaks also unclear nucleus, and Polymorphic infiltration , maybe this due to actins and myosin which give streaky appearance to the tissue and this layer is more effect with parasitic infection because its connected with inflammatory cells and many chemical changes could take place due to the infection (Roberts, 2009).

This study appears there is no changes in the pharynx for control group and infected animals in figure (15) because this organ is specialized for passage of food so the larva doesn't change the structure of the pharynx (Aza *et al.*, 2003).

In figure (16) we found the larvae of *T. cati* in brain and cause infiltration of lymphocytes , this state is rare and symbol for the migration of larvae , in study of (Al-Rubaie , 1998) infected mice with 1016 egg and after 20 day of infection he didn't find any larvae in the brain in compared with other organs.

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