HISTOLOGICAL CHANGES IN SOME ORGANS OF THE FEMALE RATS INFECTED WITH *TOXOPLASMA GONDII PARASITE* ISOLATED FROM EMBRYO OF ABORTED SHEEP

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Abstract

The current study included identifying the lesions and the histological changes caused by the intracellular parasite *Toxoplasma gondii,* which was isolated from embryos of aborted ewes. Intraperitonially injected in 15 female albino rats (3 months old) with suspension containing 100 tissue cysts

Histological section showed after four months of injection a chronic infection characterized by autolysis in all sections, where the liver sections showed expansion in the central hepatic veins, congestion in sinusoidal curves with irregularity in hepatic cords, and the presence of parasites in the liver cells. The brain tissue showed vacuoles in the neurons with an increase in the number of Purkenji cells. Kidney sections were characterized by degenerative and necrotic changes in the endothelial cells of the proximal and distal convoluted tubules with Sloughing of necrotic and degenerated cells of the tubes which accumulated inside the cavity. The parasites appeared in the endothelial cells of the glomerular tufts. Ovary and the uterus showed increased vascular wall thickness, furthermore, the spleen showed autolytic changes, deposition of pigment with the presence of parasites within the cells.

Keywords: *Toxoplasma gondii, histopathological changes, Sheep.*

Introduction

Toxoplasmosis, caused by *Toxoplasma gondii*, is an economically important disease of livestock, especially sheep and goats, as it can cause early embryonic death, resorption, fetal death, mummification, abortion, stillbirth, and neonatal death.

Cats are the main reservoir for the toxoplasmosis and they can contaminate the environments of other animals and humans by passing oocysts with their feces (Mohammed *et al*., 2010).

Toxoplasma. gondii is an obligate intracellular parasite that infects a wide variety of hosts, including humans. Infection generally occurs through the ingestion of either sporulated oocysts shed in cat feces or viable tissue-cysts in undercooked meat (Copprin, 2003; Barakat *et al*., 2009). In addition, primary infection during pregnancy results from transplacental transmission of tachyzoites which can result in severe congenital disease in the fetus with potential abortion (Copprin *et al*., 2003), causing severe birth defects, such as hydrocephaly, calcification, neurological defects and choriorentinits (Wong, 1994).

During acute infection, tachyzoites multiply rapidly. They can invade and proliferate in all nucleated cells by active penetration and form parasitophorous vacuoles. After repeated replication, host cells are disrupted and tachyzoites disseminate via the bloodstream and can invade many tissues, including the central nervous system, eye, skeletal, heart muscles and placenta. Replication leads to cell death and rapid invasion of neighboring cells. The tachyzoite stage causes a strong inflammatory response and tissue destruction and, therefore, causes clinical manifestations of the disease (Montoya, 2004).

The cell-mediated immune response convered the tachyzoites into bradyzoites and forms tissue cyst. These tissue cysts remain viable and are capable of persisting for the life of the host (Mordue *et al.*, 2001; Montoya, 2004). The severity of toxoplasmosis varies according to the immune status of the individual, parasite strain, and host species. In mammalian species, it has been severe lesions of acute toxoplasmosis have been observed in visceral organs such as the liver, the lungs and the spleen. Some epidemiological studies have reported an association of *Toxoplasma gondii* infection with liver cirrhosis (Hasan *et al*., 2013).

The purposes of the present study were to observe the pathological changes occurring due to the experimental infection of *T.gondii* in rats.

Material and Methods

In this study, female rats were used, which are obtained from the animal house of college of veterinary medicine, university of Mosul, after breeding until giving birth to small babies, 15 of these babies were selected for experiments, the breeding took place under laboratory conditions in the light cycle which was divided into 14-hour of darkness and 10 hours of light ,at a temperature of 22±2^{o}C), the rats were supplied with water and food daily until they became adult to be infected intraperitonially with a suspension containing 100 tissue cysts of *T. gondii* /rat. The *T. gondii* tissue cysts were obtained from sections of brain, liver, kidney, spleen and lung tissue of aborted ewes. Tissue infections were confirmed by the presence of the parasite cysts in tissue sections of infected ewes. In order to obtain the tissue cysts, these organs were cut into small pieces using scissors and forceps then digested in acid pepsin solution (2.6gm pepsin enzyme, 5gm sodium chloride, 7 ml concentrated hydrochloric acid and 500 distilled water) by adding 1gm of tissue to 10 ml of solution, then homogenized by a blender using the maximum speed (10000 r/min), the mixture was incubated at a temperature of 37 ^{o}C for a period of 90 minutes, then strained through several layers of sterile gauze, the yield was centrifuged at a speeds of 400r/min for 10 minutes, the sediment was mixed with phosphate buffer saline (pH 7.2-7.4) recentrifuged, then the sediment was suspended in phosphate buffer saline containing antibiotics (1000 IU of penicillin and 10 microgram of streptomycin /1ml) of phosphate buffer saline. About 0.4 ml of this suspension which supposed to contain 100 tissue cysts of *T.gondii* was injected intraperitonially to each rat aged 3 months (Sukthana *et al*., 2003) .The infected rats were kept in the laboratory for 4 months to develop chronic infection. Then the animals were scarified and the liver, brain, kidney, spleen and ovary were removed and embedded in paraffin wax, sectioned at 5-6µm stained with hematoxylin eosin stain (H&E) (Pearse, 1985). The sections were examined under light microscope and photographed.

Result and Discussion

The microscopical examination of the histological section of the infected liver tissues (Figures.1 and 2) showed an expansion in the central hepatic veins and disarrangement of the hepatic cords with evidence of parasites in the liver cells, Kupffer cells and sinusoids.

Brain tissue sections showed the occurrence of vacuoles in the neurons with an increase in the number of Purkenji cells(Fig.3).

Kidney sections were characterized by degenerative and necrotic changes in the endothelial cells of the proximal and distal convoluted tubules of the kidney with sloughing of necrotic and degenerated endothelial cells of the tubes which were collected inside the cavity, the parasites accumulated in the endothelial cells lining the tubes and endothelial cells of the glomerular tufts (Fig. 4 and 5). These changes in infected tissues may be attributed to the virulence of the strain, to the persistent parasite antigens or their metabolic by products which leads to the occurrence of necrosis (Laug *et al*., 2006; Djurkovic-Djakovic *et al*., 2006; Ramzam *et al*., 2009).

Ovary and uterus showed increase in the thickness of the blood vessels walls (Fig.6), while the spleen showed autolytic changes, pigment deposits, with the presence of parasites in different places of the spleen (Fig.7).

Infection acquired in embryos of aborted lamb through transplacental transmission of *T. gondii* from mothers infected during early stage of pregnancy. The mother acquire the infection through different modalities, the most important of them is the process of dissemination of infection through contaminated food with the *T.gondii* tissue cysts(Aitken, 2007;Ahmed *et al*., 2008).

The results showed that the histopathological changes of the liver tissues might be due to the fact that *T.gondii* interfere with the function of mitochondria and shifting it to anaerobic methods of energy production which is sufficient for the work of sodium pumps, causing low protein production and damaging the cell membranes as well as the mechanism of phagocytosis which occurs in the liver tissue leading to necrosis in the tissue, this might be the reason for an increase in the stimulation of free radicals leading to the severity of the histological effects in the liver (Sukhana *et al*., 2003; Riyadh, 2005).

The histopathological changes in the brain tissue which was demonstrated by occurrence of vacuoles in nervous cells and the increase in the number of Purkenji cells as the brain is the most of the body tissues affected by *T. gondii*, because the brain is rich in fatty materials and this may be the reason for the preference of *T.*

gondii, the brain tissue as they need energy for their existence and reproduction which causes the most severe congenital malformations in the brain (Buxton *et al.*, 2007), as well as, brain tissue is characterized by a lack of specific immune defenses like antibodies, but it posses non-specific immune defenses as microglial cells (Sukhana *et al*., 2003; Buxton *et al.*,2007; Williams *et al*., 2008).

The dissolution and degenerative changes in the endothelial cells of the proximal and distal convoluted tubules with the emergence of degeneration and hemolytic cells as well as the occurrence of the renal glomerular inflammation, in addition, to cellular and vascular changes, may be due to the occurrence of necrosis in the convoluted proximal and distal tubules due to the thrombosis which blocked the blood vessels causing immunological damage and degeneration, followed by necrosis and desquamation of the cells, this is called tubular necrosis. The inflammation of the renal tubules and the glomeruli may be due to the damage from the immune mechanisms, and this is the most important damage occurring to the basal membranes or glomerular deposition of the immune complexes inside the glomeruli causing inflammation associated with chronic glomerulus infection (Sukthana *et al*., 2003; Rodger *et al*., 2006).

The histological changes in the spleen may be due to the effect of some enzymes such as acid hydrolases which exudes form lysosomes and increase leaching.

In case of ischemia any shortages or lack of oxygen cause interruption of blood processing. The enzymes exuded from damaged cells change the compositions of complex organic substances to simple inorganic substances such as water, hydrogen sulphate, carbon dioxide, and nitrates. These changes in the cell are quiet similar to those occurring in necrotic cell and are more tougher leading to autolysis more rapidly in the highly effective organs as the spleen and the lymph nodes, the main function of these organs is the defense mechanism as the spleen is the largest storage of macrophagic cells and is rich in lymphatic vessels, that is why great decomposition occurs in this tissue (Sukthana *et al*., 2003; Pinheiro *et al*., 2009).

The hyperplasia and thickening of the walls of the blood vessels of the ovary caused by chronic infection of *T. gondii*, are attributed to the disruption in the architecture of the ovarian tissue due to hormonal imbalance in function of the ovary affecting the growth and maturation of the ovarian (Graafian)follicles and the disturbance in the secretion of ovarian hormones produce lesions in ovarian tissues (Husein & Kridli, 2003).

The histopathological changes occurred in rats after 4 months of experimental infection with *T. gondii* tissue cysts were very severe as compared to many studies performed by researchers in which infection periods were shorter range between 4-8 weeks and they attributed the severity of the resulted histological changes to the severity of the infection with *T. gondii* isolated from embryos, as this strain is more virulent in some animal species than in other, in addition some histological lesions start sharp and ends quickly and others start sharp and became chronic, or there may be severe lesions during the chronic phase and continue. Other researchers attributed the severity of the histopathological changes to the high ferocity of *T. gondii* isolated from embryos of aborted sheep a discouraging immunoreactive animals and suffers from stressful situations that is why *T. gondii* are more virulentand cause severe histopathological effects in the host tissue (Savio & Nieto, 1995; Riyadh, 2005; Djurkovic-Djakovic, *et al*., 2006; Laug *et al*., 2006).

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Figures

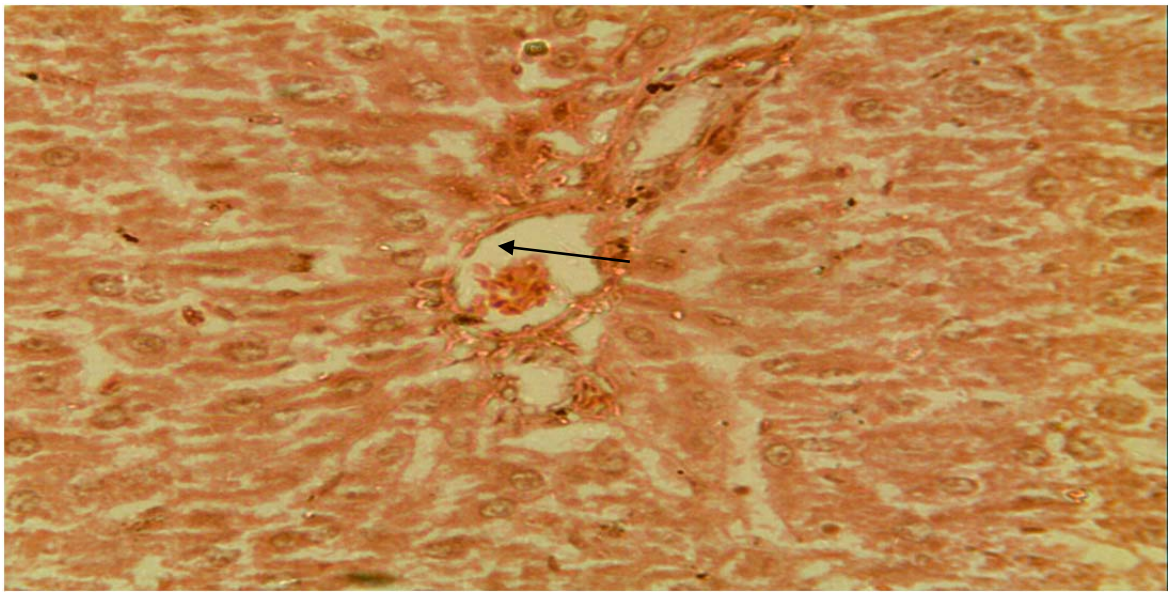

Fig. 1: Section from the liver of infected rat showing expansion in the central hepatic veins (H&E stained.400x)

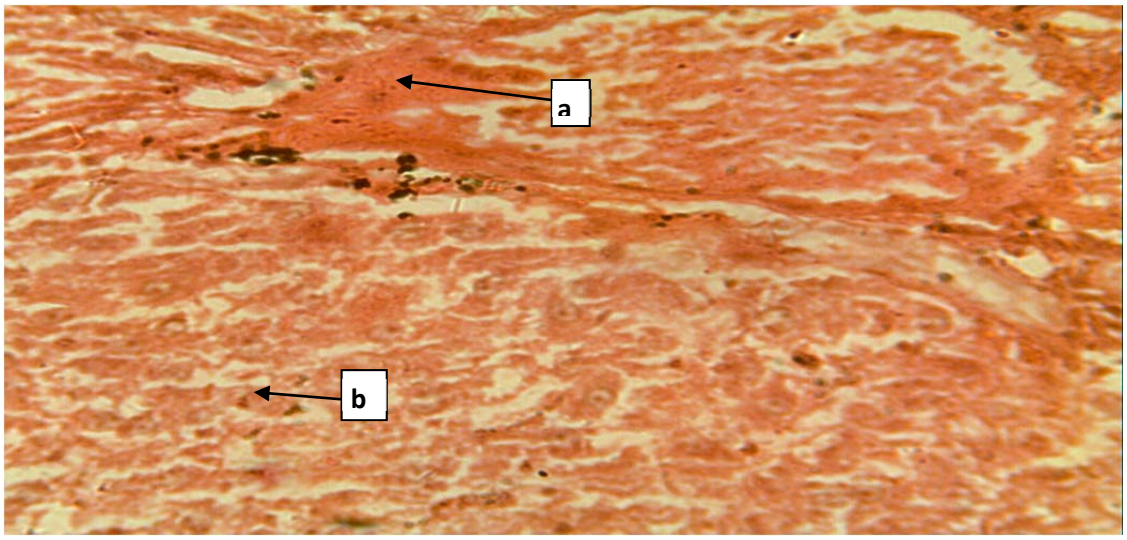

Fig. 2: Section from the liver of infected rat.(a) showing congestion, granular curves.(b) *T.gondi*i tissue cysts (H&E stained. 400x)

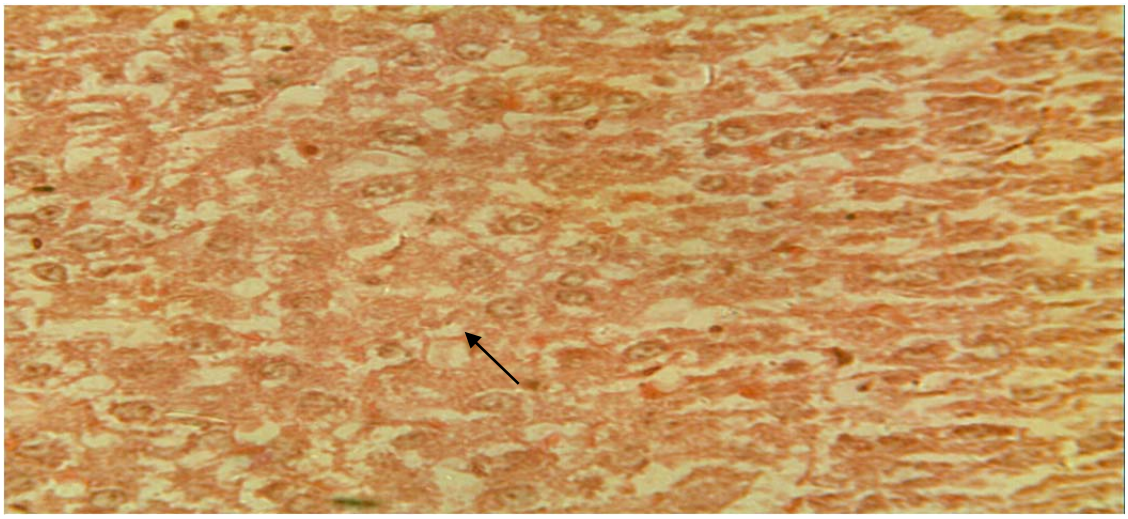

Fig. 3: Section from the brain of infected rat showing vacuoles in the neurons with an increase in Purkenji cells(H&E stained. 400x).

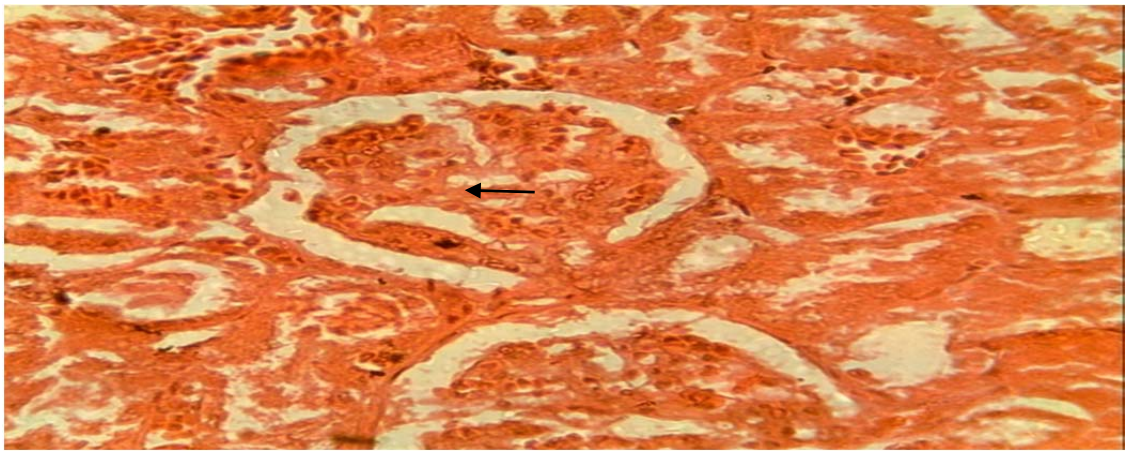

Fig. 4: Section from the kidney of infected rat showing expansion and degenerative changes of the lumen of glomeruli (H&E stained. 400x)

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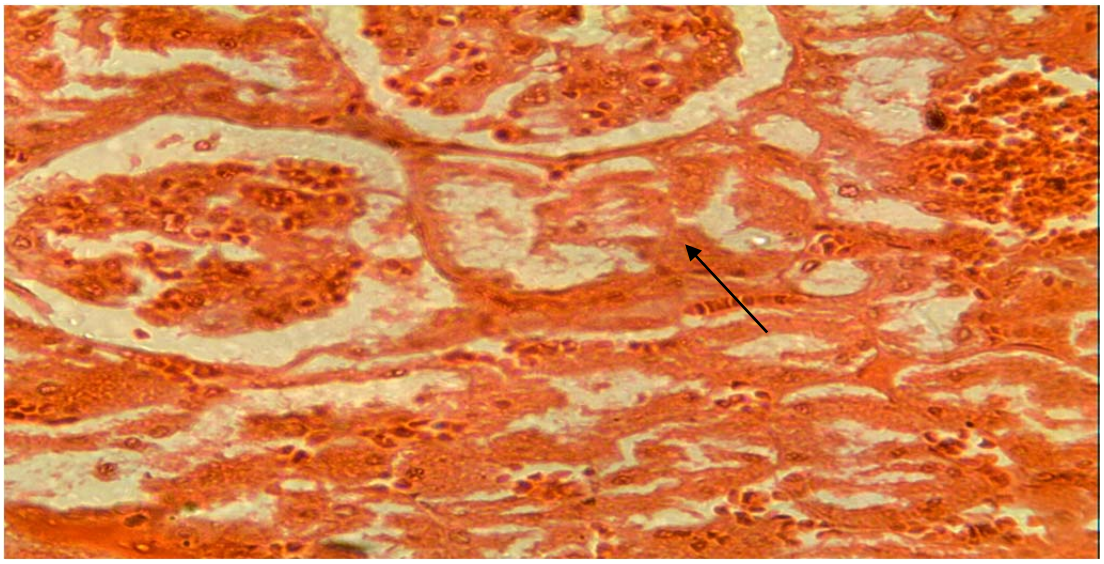

Fig. 5: Section from the kidney of infected rat showing degeneration and dissolution of the alveolar cells lining the distal tubules convoluted (H&E stained.400x)

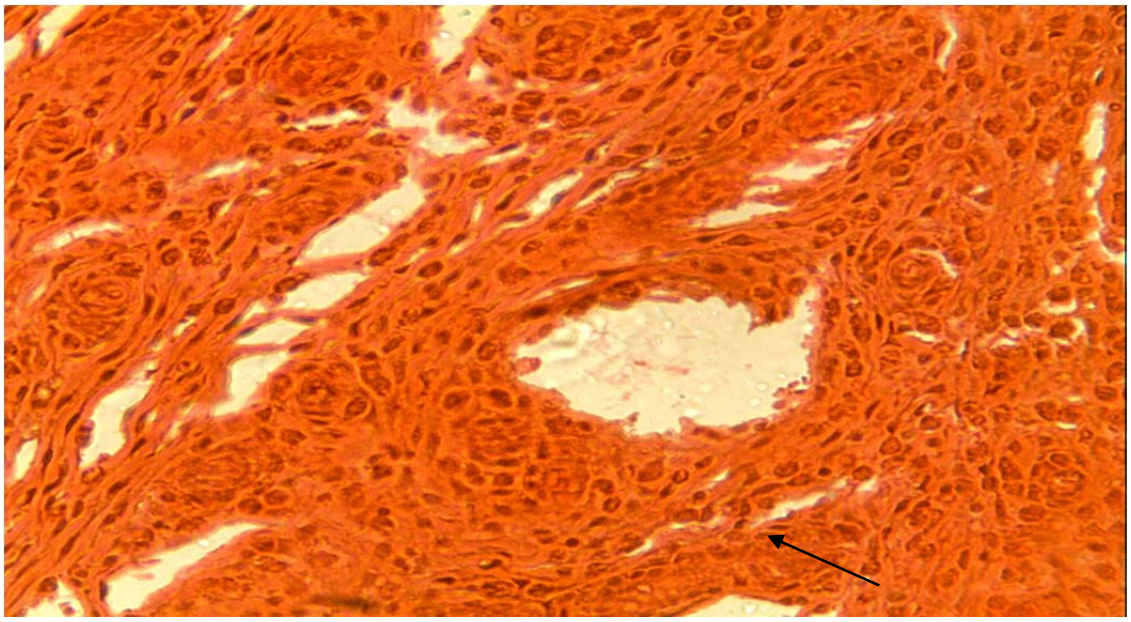

Fig. 6: Section from the ovary of infected rat showing hyperplasia in ovarian cells and increase in the thickness of blood vessels (H&E stained.400x)

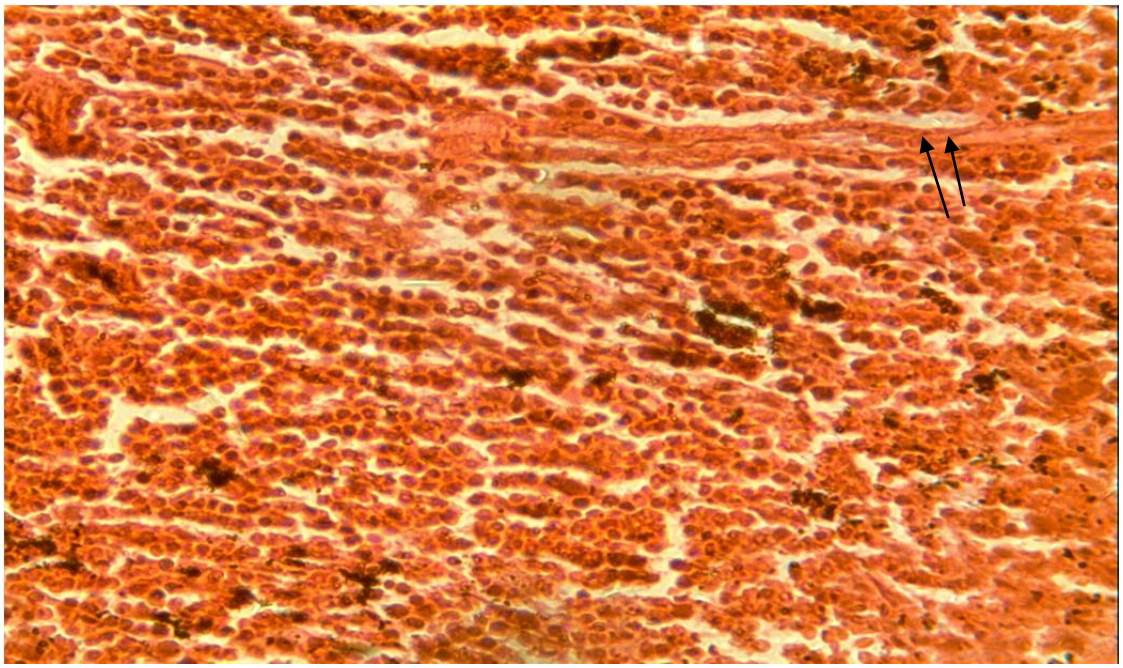

Fig. 7: Section from the spleen of infected rat showing autolytic changes in spleen cells(H&E stained.400x)

كورتى

ئەۆ ڤەكولينە هاتيە ئەنجامدان لدور دياركرنا ئەوان خانێن ژ كاركەتن و گوهورين بوين ژ لاى مشەخورى (المقوسه الكوندیه) ئەويت هاتينە كومكرن ژ ئەوان بەزنين كو تيژكيت وان ژ بەر جووين و پشتێ هنگى يێ كرين لە جردين سپێ دا لە قوناغا دريز خاين لە نەخوشيا (المقوسه الكوندیه)، يا هاتيە كرن كو نەساخيا (تجريبه) يا ڤێ مشەخورى لە١٥ جردان بە ژێ ٣ هەيڤێ يت كرين (تجويف البريتون) و ١٠٠ كيس هاتينە كومكرن ژ بەزنا ئەويّت تيژكيت وان ژ بەر جوونە ژ سببى (المقوسه الكوندیه).

يا هاتيە دياركرن كو پارچيت (النسيج) پشتى بوريا ٤ هەيڤا ژ كرنا ئەڤێ مشەخورى لە ناۆ لەشى جردان دا ديتن كو حلاندنا جيێ دبيت لە هەمى پارچێن لەشێ دا، ديار بوى كو پارچيت ميلكێ يێت مەزن بوى ژبەر كو مشەخور يا لەناۆ خانى ميلكێ و خانى (كوفر) دا هەيە. هەروسا ديار بى كو پارچين مشكێ هندەك (فجوات) جيبوونە لە خانێت هەستيارى دا لەگەل زيدەكرنا ژمارا خانيت (بركنجي)، و تيتە ديتن كو لە كلوچيزكا دا هندەك حلاندن و كوهورين جيبنە لە (الخلايا الطلائية) ئەو بوريت دورى وى نيزيكى و دوير و گەل هندەك (تنكس سلخي) و حلاندن خانين (الخلايا الطلائية) دگەل كومبونا وى لە ناف زكێ دا. هەروەسا مشەخور هاتيە ديتن لە خانيت (الخلايا الطلائية المبطنة للأنابيب والخلايا البطانية). و تيبينى تيتكرن كو زيادەكا ئاشكرا لە ستيراتيّا بورين خوين لە هيلكەدانى هەيە، و گەل هندەى ديار دەبيت كو خانيت (طحال) هندەك گوهورين يت حلاندنى هاتينە كرن لەكەل كومبونا هندەك صبغى دكەل ديتنا مشەخورێ.

التغيرات النسجية في بعض أعضاء إناث الجرذان المصابة بطفيلي المقوسة الكوندية *Toxoplasma gondii* المعزولة من أجنة الأغنام المجهضة

الخلاصة

تضمنت الدراسة الحالية تحديد الآفات والتغيرات النسجية المرضية التي يحدثها طفيلي المقوسة الكوندية *Toxoplasma gondii* المعزولة من أجنة النعاج المجهضة بعد حقنها في الجرذان البيض أثناء المرحلة المزمنة والطويلة من الخمج بالمقوسات الكوندية، حيث تم إحداث إصابة تجريبية بهذا الطفيلي بحقن 15 جرذ بعمر 3 أشهر داخل تجويف البريتون بمعلق حاوي على 100 كيس نسيجي تم عزلها من أجنة النعاج المجهضة المخمجة بالمقوسات الكوندية.

أظهرت المقاطع النسجية بعد مرور أربعة أشهر من الحقن لإحداث الإصابة المزمنة حدوث درجات من التحلل الذاتي في جميع المقاطع. حيث أظهرت مقاطع الكبد توسع في الأوردة الكبدية المركزية واحتقان في المنحنيات الجيبية وعدم انتظام في الروابط الكبدية مع وجود الطفيليات في الخلايا الكبدية وخلايا كوفر. كذلك اظهر نسيج الدماغ حدوث فجوات في الخلايا العصبية مع زيادة في عدد خلايا بركنجي، وتميزت مقاطع الكلى بوجود انحلالات وتغيرات تنكسية في الخلايا الطلائية المبطنة للأنابيب الملتفة القريبة والبعيدة مع ظهور تنكس سلخي وانحلالي للخلايا الطلائية المبطنة للأنابيب مع تجمعها داخل التجويف، كما ظهرت الطفيليات في الخلايا الطلائية المبطنة للأنابيب والخلايا البطانية لعناقيد الكبيبات. كذلك لوحظ وجود زيادة واضحة في سمك جدار الأوعية الدموية في المبيض، في حين أظهرت خلايا الطحال تغيرات انحلالية ذاتية مع ترسبات صبغية رافقت وجود الطفيليات.

الكلمات المفتاحية:داء المقوسات الكوندية، التغيرات المرضية النسجية، الأغنام.