

PATHOLOGICAL AND PARASITOLOGICAL STUDIES ON SOME *EIMERIA* SPECIES IN RABBITS USING LIGHT AND ELECTRON MICROSCOPE

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ABSTRACT

The prevalence of *Eimeria* in 50 examined rabbits was 84% (42/50). The prevalence was 92.6% in male rabbits while it was 73.9% in female rabbits. According to age, the prevalence was determined 88% in rabbits less than 4 months of age while it was 80% in rabbits 4 months of age or more. No significant difference was recorded between rabbits in correlation to age and sex. Ten species of *Eimeria* infecting rabbits were isolated in Assiut by parasitological examination. The prevalence of *Eimeria* species were *E. perforans* (66.7%) followed by *E. exigua* (26.2%), *E. media* (26.2%), *E. magna* (21.4%), *E. intestinalis* (19%), *E. coecicola* (19%), *E. irrisidua* (19%), *E. piriformis* (14.3%), *E. flavescens* (7.1%) and *E. stiedae* (7.1%). Single infection of *Eimeria* spp. was found in 23.8% of the infected rabbits, where as mixed infection involved two, three or four *Eimeria* spp. was observed in 76.2% of the infected rabbits. Clinical signs were depression, anorexia, diarrhea while postmortem examination revealed hepatomegaly with presence of separate yellowish-white nodules of varying sizes spread over the surface with distended gall bladder. The intestinal lesions revealed varying degree of congestion, thickening of intestinal wall. Histopathological examination of the liver revealed dilated bile ducts and formation of papilliform projections of epithelium containing different developmental stages of *Eimeria*, associated with degeneration and pathological changes in hepatic parenchyma. The intestinal coccidiosis revealed hyperplasia of the epithelial cells and presence of *Eimeria* oocytes and gametocytes within the epithelial cells of the villi associated with lymphocytic infiltration in the lamina propria of the villi. The transmission electron microscope showed asexual and sexual developmental stages of rabbit *Eimeria* including developing schizont and macrogametocyte.

Key words: Pathology, parasitology, light microscope, electron microscope, *Eimeria* species, rabbits.

INTRODUCTION

Rabbit meat is used as a good source of animal protein with low fat and cholesterol in Egypt as well as for medical and biological purposes (Ragheb *et al.*, 1999 and Beal *et al.*, 2004). Coccidiosis is almost common and highly contagious important parasitic disease in rabbits caused by *Eimeria* species (Shi *et al.*, 2016). It occurs all over the world and is associated with high morbidity and mortality (Wessels *et al.*, 2011; El-Shahawi *et al.*, 2012 and Tao *et al.*, 2017). Most of *Eimeria* spp. affect the rabbit production and constitutes a serious problem leading to major economic losses in rabbit farms including reduced growth rate and feed conversion and increased mortality according to their level of pathogenicity and its eradication is laborious (Vancraeynest *et al.*, 2008 and Pakandl, 2009).

Clinical signs of coccidiosis in rabbits are diarrhea, loss of appetite, weight loss, dehydration, secondary sepsis and death. However, it is common that rabbits present subclinical forms of intestinal and hepatic coccidiosis, characterized by reduced feed intake and higher feed conversion ratio lead to a decline in body weight gain. All domesticated rabbit breeds can be infected by coccidia, specially the younger populations between 1 and 4 months of age (Bhat *et al.*, 1996; Pakandl, 2009; Al-Quraishy *et al.*, 2012 and Metwaly *et al.*, 2013).

Eimeria species are highly host, organ and tissue specific (Levine, 1985). Eleven species of *Eimeria* infect rabbits: *E. coecicola*, *E. flavescens*, *E. intestinalis*, *E. irrisidua*, *E. exigua*, *E. magna*, *E. media*, *E. perforans*, *E. piriformis* and *E. vejtdovskiy* are the main species that cause intestinal coccidiosis in rabbits except *E. steidae* causes hepatic coccidiosis in the liver. Among these species, *E. steidae*, *E. intestinalis* and *E. flavescens* are highly pathogenic in rabbits, especially before the age of 3 months (Kvičerová *et al.*, 2008; Pakandl, 2009; Oliveira *et al.*, 2011 and Yan *et al.*, 2013).

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Sporulated oocysts of *Eimeria spp.* invade rabbits which rupture, then sporozoites invade the intestinal, liver and bile duct epithelial cells where merogony occurs. Merozoites continue to increase until they complete four generations. At the gametogony stage, macrogametes and microgametes combine into zygotes and eventually develop into oocysts (Li and Ooi, 2009 and Oliveira *et al.*, 2011). *Eimeria species* can invade and destroy intestinal cells of the hosts, causing anemia, electrolyte imbalance and poor absorption of nutrients (Szkucik *et al.* 2014 and Metwaly *et al.* 2013). While, *Eimeria stiedae* infects only the epithelial cells of the liver and bile ducts and causes huge liver damage with a variety of extremely disordered metabolic processes, increasing the secretion of toxins, diarrhea, slow growth and weight loss, and even causing the death of a large number of rabbits with a mortality rate as high as 80% after infection (Li and Ooi, 2009; Oliveira *et al.*, 2011 and Abed and Yakoob, 2013).

There is a deficiency in information on rabbit coccidiosis in Assiut. So, this investigation was performed to recognize the natural prevalence and the species of rabbit *Eimeria* with regarding the parasitological and pathological aspects of the disease and correlate the presence of the parasite with sex and age.

MATERIALS AND METHODS

A total of 50 rabbits were collected randomly from markets from August 2017 to December 2017 from Assiut Governorate. These 30 rabbits were suffering from diarrhea and 20 were apparently healthy with age from 1-12 months. Fecal samples were collected directly from the rectum using sterile gloves and were transferred immediately to the laboratory and were kept at 4°C in a refrigerator until processing within 48 hours of arrival.

Parasitological Examination: Fecal examination was adopted for the presence of oocysts using light microscope by direct smear and concentration flotation technique: according to Soulsby (1982), using saturated salt solution for detection of *Eimeria spp.* oocysts.

Sporulation of *Eimeria species* oocysts: the positive fecal samples were sporulated using 2.5% potassium dichromate solution at room temperature with good aeration. Nonsporulated oocysts and sporulated oocysts size were measured using light microscope with a calibrated eye piece micrometer and the species determined by size, shape and morphological appearance (Soulsby, 1982 and Levine, 1985).

Gross pathological Examination: Necropsy procedures were performed according to Hussein (2008). Observation of both intestinal and hepatic

coccidiosis was conducted through pathological examination of intestine and liver abnormalities.

Histopathology Preparation Method: The intestinal and liver lesions of each rabbit were sliced in the size of about 1 cm³ and immersed in 10% neutral formalin solution for histopathological and ultra-structural examination. Then dehydrated and embedded in paraffin wax. Serial 4-5 microns sections of paraffin embedded tissues were stained with Hematoxylin and eosin stain (H&E) for light microscope examination according to (Kiernan, 2001).

Transmission electron microscopy: Tissue specimens were excised and fixed in 5% buffered glutaraldehyde and then washed in cacodylate buffer (0.1M, PH 7.2). Tissue specimens were post fixed in 1% osmium tetroxide, dehydration in grades of ethanol alcohol series and embedded in Epon 812. Semithin sections were prepared for orientation and selection of representative tissue specimens and stained with 0.5% Toulidine blue according to Bancroft and stevans (1993). Ultrathin sections of (500-800 Å) were done using ultramicrotome then contrasted in uranyl acetate and lead citrate, and examined under a (Jeol, CX11) electron microscope in Electron Microscope unit, Assiut University.

Statistical analysis: The statistical package SPSS was used for data analyses and a value of $P < 0.05$ was considered significant difference in comparison (Jing *et al.*, 2012).

RESULTS

The prevalence of *Eimeria spp.* in the examined rabbits was 84% (42/50). The prevalence of *Eimeria spp.* was 90% (27/30) in rabbits suffering from diarrhea while it was 75% (15/20) in apparently healthy rabbits. Concerning the sex, the prevalence of *Eimeria spp.* was 92.6% (25/27) in male rabbits while it was 73.9% (17/23) in female rabbits (Table 1). The prevalence of *Eimeria spp.* according to age was determined and it was 88% (22/25) in rabbits less than 4 months of age while it was 80% (20/25) in rabbits 4 months of age or more (Table 2). No significant difference of *Eimeria* prevalence was recorded between rabbits aged less than 4 months and rabbits 4 months of age or more and also no significant difference of *Eimeria* prevalence was observed between males and females.

Parasitological examination revealed that the isolated ten species of *Eimeria* in rabbits in Assiut were *E. exigua*, *E. perforans*, *E. intestinalis*, *E. coecicola*, *E. media*, *E. flavescens*, *E. piriformis*, *E. irrasidua*, *E. magna* and *E. stiedae*. The prevalence of *Eimeria species* infecting rabbits were *E. perforans* (66.7%) followed by *E. exigua* (26.2%), *E. media* (26.2%), *E. magna* (21.4%), *E. intestinalis* (19%), *E. coecicola*

(19%), *E. irresidua* (19%), *E. piriformis* (14.3%), *E. flavescens* (7.1%) and *E. stiedae* (7.1%). Very high significant differences of prevalence was detected between different *Eimeria spp.* (Figure 1, 2 and Table 3). Single infection of *Eimeria spp.* was found in 23.8% of the infected rabbits, where as mixed infection involved two, three or four *Eimeria spp.* was observed in 76.2% of the infected rabbits. Very high significant differences of prevalence was recorded of mixed infection than single infection (Table 4).

The main clinical signs showed by the infected rabbits were depression, anorexia, diarrhea with soiling of hind quarters, emaciation, rough hair coat and distended abdomen. Jaundice was reported in one case. Postmortem examination revealed macroscopic lesions in eleven rabbit's liver which showed hepatomegaly with presence of separate yellowish-white nodules of varying sizes spread over its surface and in the parenchyma. The liver textures were extremely coarse and felt relatively hard. The gall bladders were distended with thick dark green bile or were empty with thickened tortuous wall (Figure 3). The intestinal lesions revealed varying degree of general congestion, petechial hemorrhages, thickening of intestinal wall and some distended with gases.

Histopathological examination of the liver revealed dilated bile ducts as a result of extensive proliferation and hyperplasia of bile duct epithelium leading to formation of papilliform projections of epithelium into the lumen containing a number of different developmental stages of *E. stiedae* in addition there were sloughed biliary epithelial cells in the lumen of the duct (Figures 4, 5). The surrounding portal area showed congestion and dilatation of portal vein, fibrous connective tissue reaction and inflammatory cells infiltration. Most of the hepatic parenchyma around the peripheral areas showed degenerative inflammatory changes (Figure 4). Strands of connective tissue from the periportal areas were seen invading the hepatic parenchyma giving areas of pseudolobulation (Figure 5A). Other areas showed oocyte granuloma in which coccidial oocytes were surrounded by fibrous connective tissue infiltrated

with mononuclear cells and even giant cells and hemorrhages (Figure 6).

Histopathological examination of the intestinal coccidiosis revealed different developmental stages of *Eimeria* in the intestinal epithelium including developing schizonts, macrogametyocytes, microgametyocytes and ovoid oocyst of *Eimeria* associated with lymphocytic infiltration in the lamina propria of the villi. The epithelial cells of villi showed a mild degree of hydropic degeneration and hyperplasia was observed with focal eosinophilic cellular reaction and some villi were edematous (Figure 7). Morphologically macrogametyocytes structures were oval to round and filled with large homogeneous, rounded, bright pink to bluish cytoplasmic granules (referred to as wall-forming bodies). Single large, centrally located nuclei occasionally were seen. Microgametyocytes structures were large, round, and were filled with high numbers of purple, round to comma-shaped microgametes. Oocysts were ovoid to ellipsoidal, usually bright pink wall sometimes wrinkled by fixation and drying. These stages were present within intestinal epithelial cells and extracellularly as a result of rupture of the epithelial cells (Figures 7, 8).

The use of transmission electron microscope (TEM) allowed the identification of asexual and sexual developmental stages of *Eimeria* of rabbit. Examination of semithin section of intestine showed the appearance of the various coccidian stages as developing schizont and macrogametyocyte (Figure 9). TEM showing schizogony of *Eimeria*, schizonts is multinucleated and contain wall forming bodies and veil forming bodies with amylopectin granules and lipid droplet. A cross-sectioned in developing multinucleated schizont in a parasitophorous vacuole contains mitochondria and surrounded by plasma cells (Figure 10). Also, it revealed developing macrogametyocytes (Early to mid-stage) surround by a parasitophorous vacuole in host cell. It possesses a nucleus and cytoplasm contains some areas of dilated rough endoplasmic reticulum designated as early wall forming bodies and veil forming bodies. Early parasite phagocytosis found as host phagocytic cell engulfed the parasite and surrounded by plasma cell and large number of host mitochondria indicating host defense mechanism (Figure 11).

Table 1: Effect of health status and sex on the prevalence of *Eimeria spp.* in rabbits

Health status/Sex		No. of examined rabbit	No. of positive samples	%
Health status	Diarrhea	30	27	90
	Apparently healthy	20	15	75
Sex	Male	27	25	92.6
	Female	23	17	73.9

Insignificant differences ($P > 0.05$)

Table 2: Effect of age on the prevalence of *Eimeria spp.* in rabbits

Age	No. of examined rabbit	No. of positive samples	%
<4 m	25	22	88
≥ 4m	25	20	80
Total	50	42	84

Insignificant differences (P>0.05)

Table 3: Morphological characteristics of ten *Eimeria* species isolated from infected rabbits with its prevalence rates (%)

<i>Eimeria</i> species ***	No. of infected rabbits	Total infected	%	Shape	Size (µm)	Micropyle	Residium
<i>E. perforans</i>	28	42	66.7	Ellipsiodal	27.31×17.09µ	not visible	present
<i>E. exigua</i>	11	42	26.2	Sub-spherical	18.98×16.09µ	not visible	no
<i>E. media</i>	11	42	26.2	Ellipsiodal	34.58×18.98µ	visible	Present
<i>E. magna</i>	9	42	21.4	Ovoidal	36.31×25.73µ	visible	present
<i>E. intestinalis</i>	8	42	19	Pyriiform	31.23×18.86µ	visible	present
<i>E. coecicola</i>	8	42	19	Ovoidal	30.19×19.67µ	visible	present
<i>E. irresidua</i>	8	42	19	Ovoidal	35.07×22.13µ	visible	no
<i>E. piriformis</i>	6	42	14.3	Pyriiform	32.79×22.73µ	visible	no
<i>E. flavescens</i>	3	42	7.1	Ovoidal	31.01×22.38µ	visible	no
<i>E. stiedae</i>	3	42	7.1	Ovoidal	34.17×21.71µ	visible	no

*** Very high significant differences (P<0.0001)

Table 4: Prevalence of single and mixed infection of *Eimeria species* in infected rabbits

Total positive	Single infection		Mixed infection***	
	No.	%	No.	%
42	10	23.8	32	76.2

*** Very high significant differences (P<0.0001)



Figure (1): Unsporulated oocysts of the ten *Eimeria* spp. from naturally infecting rabbits (A) *Eimeria exigua* (B) *E. perforans* (C) *E. intestinalis* (D) *E. coecicola* (E) *E. media* (F) *E. flavescens* (G) *E. piriformis* (H) *E. irresidua* (I) *E. magna* (J) *E. stiedae*

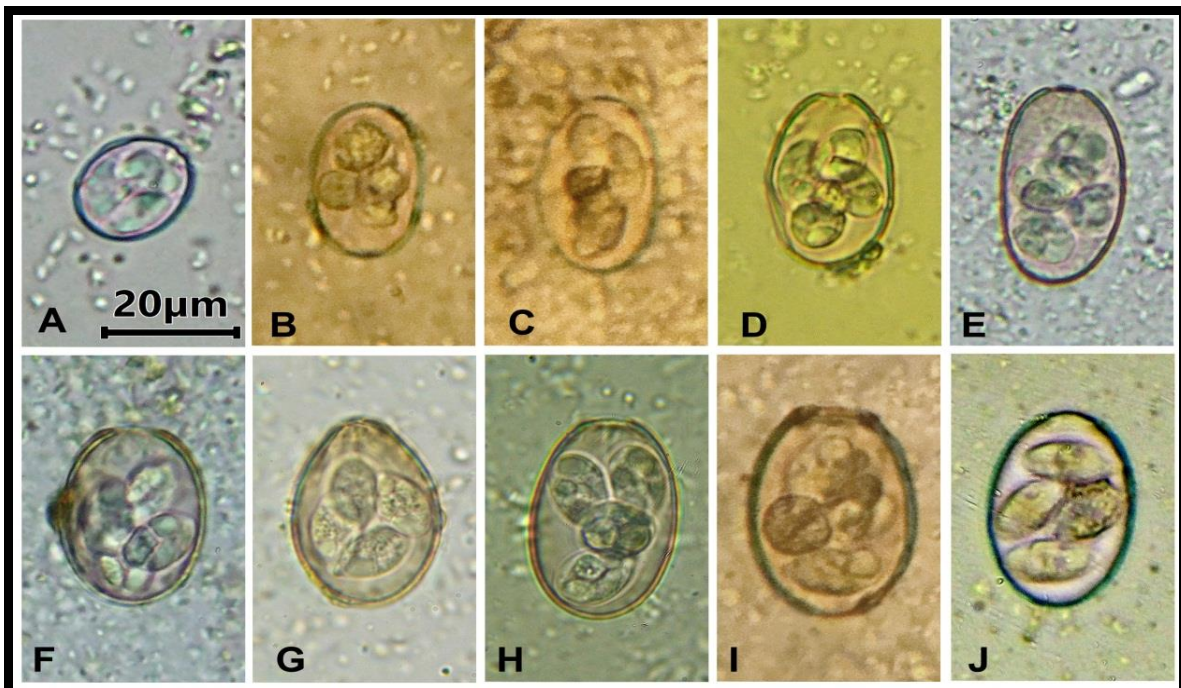


Figure (2): Sporulated oocysts of the ten *Eimeria* spp. isolated from naturally infecting rabbits (A) *Eimeria exigua* (B) *E. perforans* (C) *E. intestinalis* (D) *E. coecicola* (E) *E. media* (F) *E. flavescens* (G) *E. piriformis* (H) *E. irresidua* (I) *E. magna* (J) *E. stiedae*

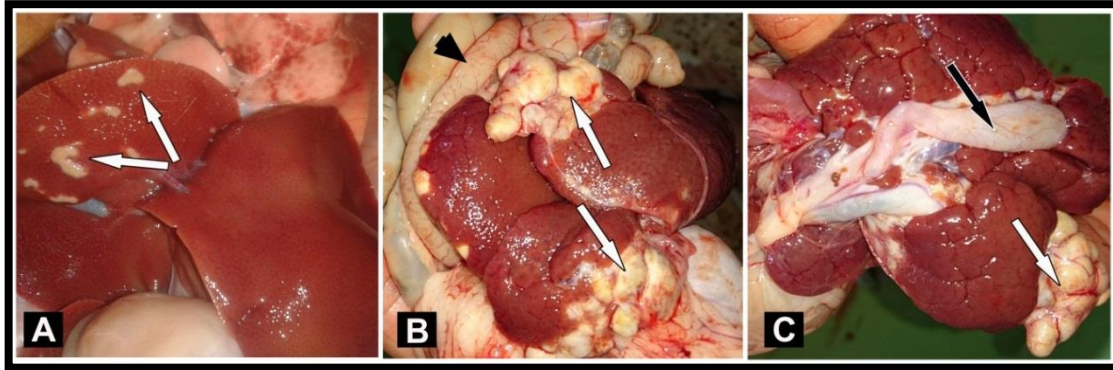


Figure (3): Gross lesion in the liver of rabbit infected with *E. stiedae* showing irregular yellowish white nodules of varying sizes on the surface (white arrow) and dilated empty gall bladder (black arrow). Thickening of intestinal wall and some distended with gases (black arrow head).

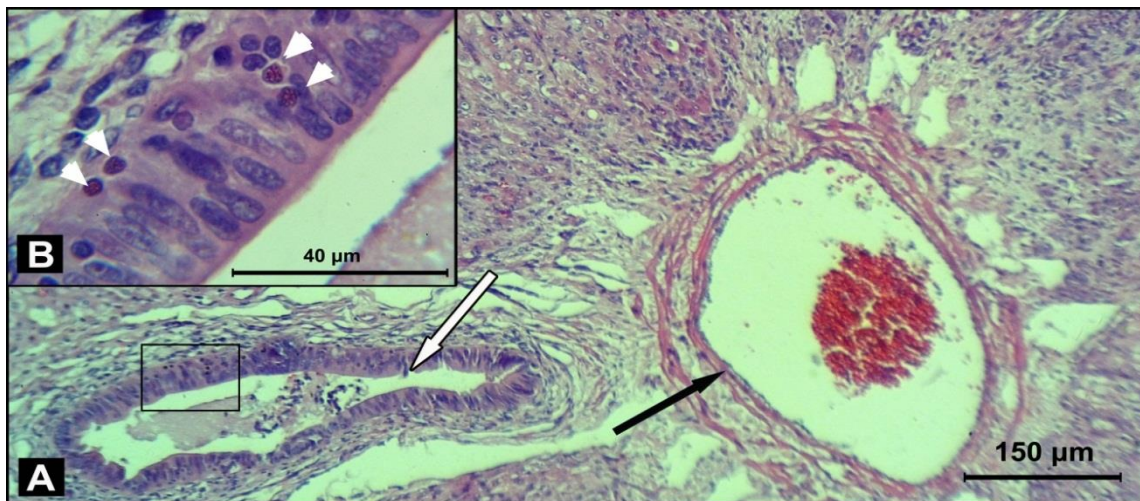


Figure (4): Histopathological section of a rabbit liver affected with coccidiosis. **A:** showing congested and dilated portal vein (black arrow) surrounded by fibrous connective tissue and infiltrated with inflammatory cells. Hyperplasia of the epithelial cells of the biliary duct (white arrow) (X 100). **B:** high magnification of the square section of the hyperplasia epithelial cells of the biliary duct showed developing schizonts of the *Eimeria stiedae* (white arrow head) H&E (X 1000).

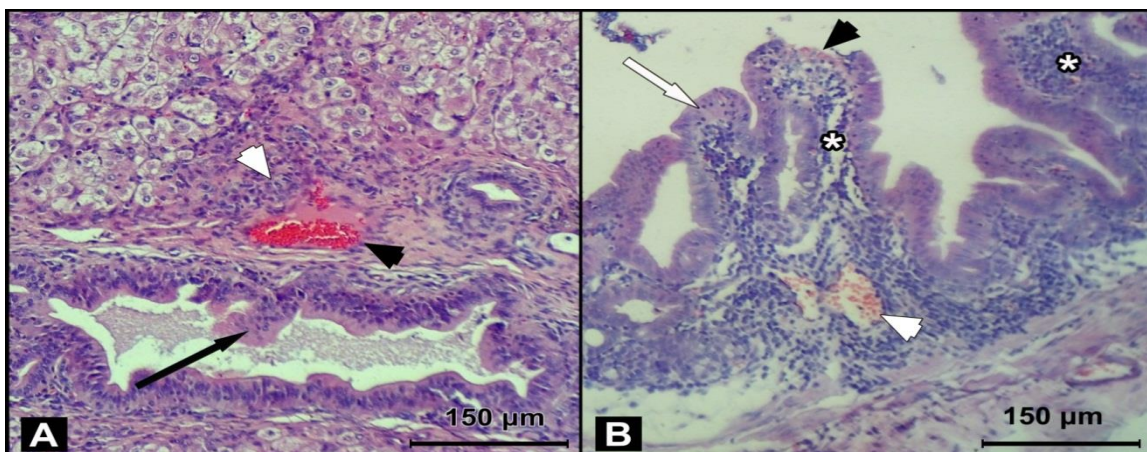


Figure (5): Histopathological section of a rabbit liver affected with coccidiosis. **A:** showing hyperplasia of the epithelial cells of the biliary duct (black arrow) congested portal vein (black arrow head) with mononuclear cells infiltration and hemorrhages (white arrow head) (X 100). **B:** showing hyperplasia of the bile duct forming papillary fronds projecting into the dilated ductal lumen (white arrow) with sloughing of epithelial cells (black arrow head) and infiltrated with inflammatory cells (astresik) with congested blood vessel (white arrow head) (H&E stain) (X 100).

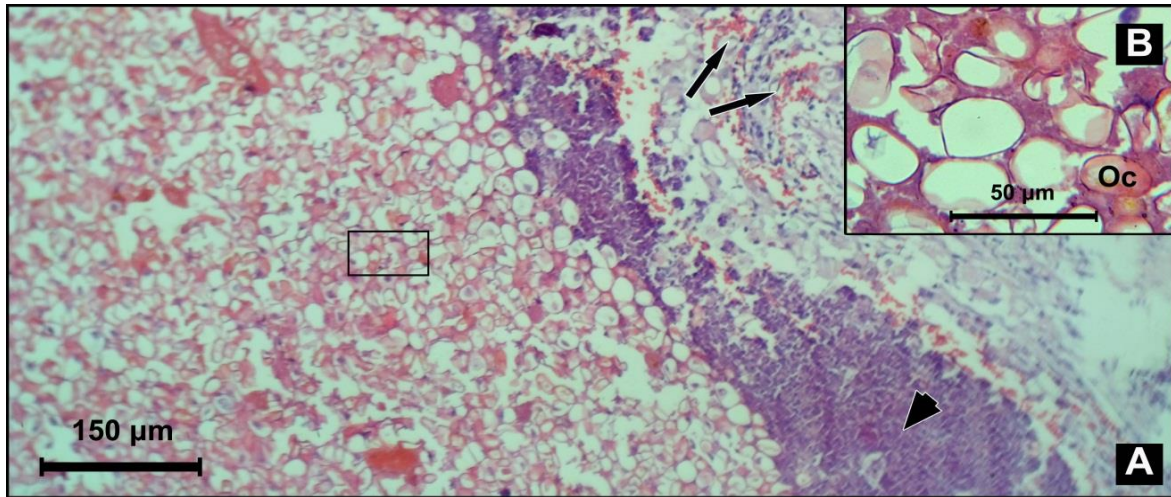


Figure (6): Liver affected with coccidiosis. **A:** showing oocyst granuloma within hepatic parenchyma surrounded by fibrous tissue and infiltrated with inflammatory cells "macrophages, lymphocytes, eosinophils and giant cell" (arrow head) and there is hemorrhage (arrows) at the periphery (X100). **B:** high magnification of the square section of the oocyst (Oc) granuloma. (H&E) (X 1000).

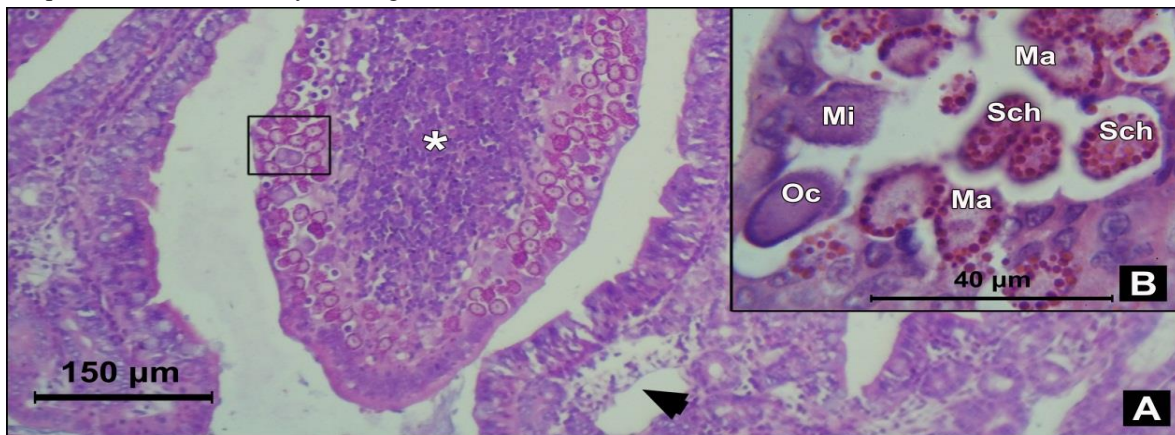


Figure (7): Intestine with coccidiosis. **A:** showing different developmental stages of *Eimeria* in the intestinal epithelium, lymphocytic infiltration in the lamina of the villi (asterisk) and edema (arrow head) (X 100). **B:** high magnification of the square section showing presence of numerous stages including developing schizonts (Sch), macrogametyocytes (Ma), microgametyocytes (Mi) and oocyst (Oc) of *Eimeria* (H&E) (X 1000).

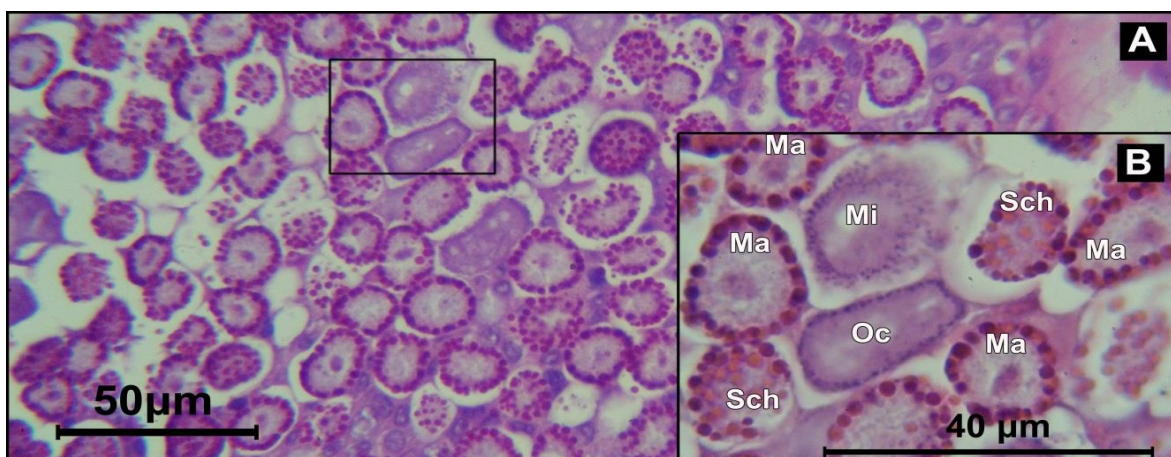


Figure (8): Intestine. **A:** showing different developmental stages of *Eimeria* in the intestinal epithelium (X 400). **B:** high magnification of the square section showing different developmental stages include developing schizont (Sch), macrogametyocytes (Ma) microgametyocytes (Mi), and oocyst (Oc) of *Eimeria* (H&E) (X 1000).

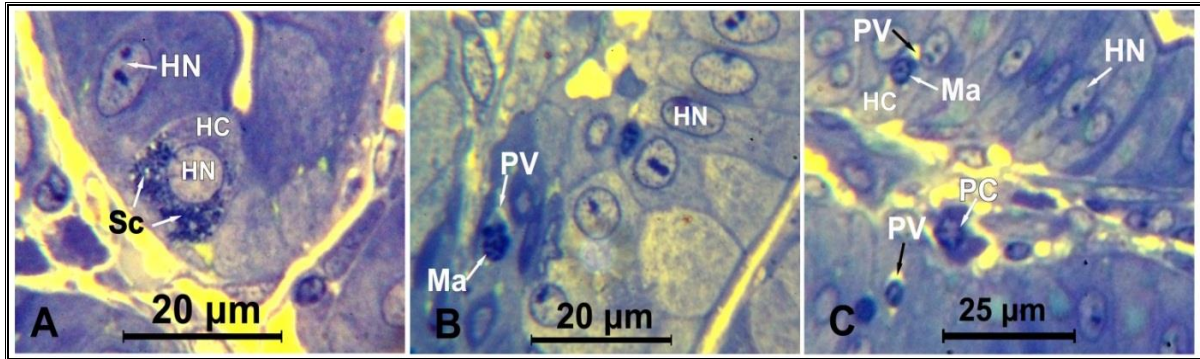


Figure (9): Semithin sections of the rabbit intestine showing the appearance of the various coccidian stages (both asexual and sexual) stained with Touildine blue. **A:** revealed developing schizont (Sc) in host cell (HC) beside host nucleus (HN) (X1000). **B** showing macrogametocyte (Ma) surrounded by parasitophorous vacuole (PV) in host cell beside host nucleus (HN) (X 1000). **C:** showing macrogametocyte (Ma) surrounded by parasitophorous vacuole (PV) in host cell (HC) beside host nucleus (HN). Note plasma cell (PC) in the lamina propria (X 400).

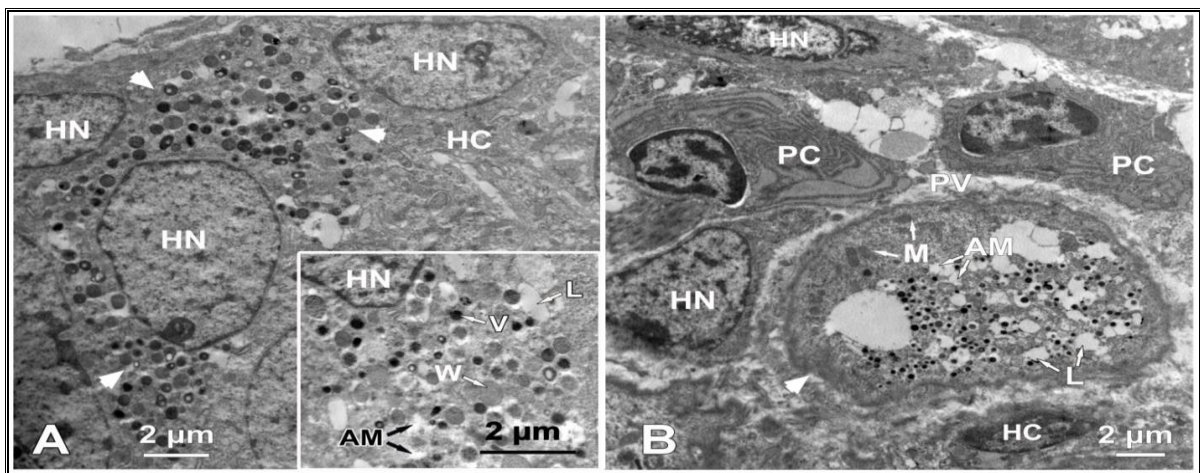


Figure (10): Transmission electron micrograph (TEM) showing schizogony of *Eimeria* in intestine of rabbit. **A:** showing the developing schizonts (arrow head) inside host cell (HC) beside host nucleus (HN). Square show high magnification of schizonts which is multinucleated and contain wall forming bodies (W) and veil forming bodies (V) with amylopectin granules (AM) and lipid droplet (L) (X4800). **B:** A cross-sectioned in developing multinucleated schizont (arrow head) in a parasitophorous vacuole (PV) and contain amylopectin granules (AM) with lipid droplet (L) and mitochondria (M). Schizont surrounded by host cell (HC) contains host nucleus (HN) and plasma cells (PC) (X3600).

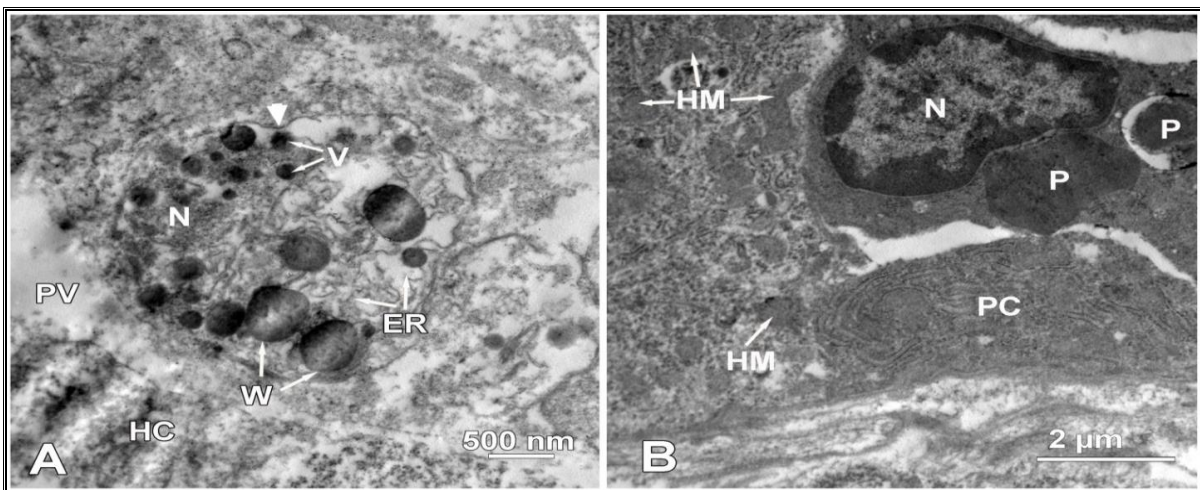


Figure (11): TEM of *Eimeria* in intestine of rabbit. **A:** showing the ultrastructural appearance of the developing macrogametocyte (Early to mid-stage) (arrow head) surround by a parasitophorous vacuole (PV) in host cell (HC). It contain a nucleus (N) and the cytoplasm which contains some areas of dilated rough endoplasmic reticulum (ER) designated as early wall forming bodies (W) and veil forming bodies (V) (X19000). **B:** showing early parasite phagocytosis, macrophage contains nucleus (N) and parasite (P) and surrounded by plasma cell (PC) and large number of host mitochondria (HM) (X10000).

DISCUSSION

Coccidiosis remains one of the most important infectious causes of digestive disorders in fattening rabbits (Vancraeynest *et al.*, 2008). The strong fecundity and prolonged resistance of *Eimeria* to the hostile environment are responsible for the wide prevalence, such that nearly all rabbits are affected (Lebas *et al.*, 1997 and Jing *et al.*, 2012).

In the present study, the overall prevalence of *Eimeria spp.* was observed in 84% (42/50) of the samples. The obtained result was in line with Qiao *et al.* (2012) and Okumu *et al.* (2014) who reported high occurrence of *Eimeria spp.* in rabbits (78.11% and 85.1%) in Northwest China and Kenya, respectively. In contrast, both Song *et al.* (2012) and Balicka-Ramisz *et al.* (2014) showed that the prevalence of infection in rabbit was 100% in China and Poland, respectively. While previous reports in Egypt showed lower prevalence rates of coccidiosis such as, EL-Masry (1983) 57.3% in Dakahlia governorate; Abdalla (1988) 72% in Assiut governorate; Ibrahim (1990) 65.8 % in Giza governorate and (El-Shahawi *et al.*, 2012) 70% in Beni-Suef governorate. This variation in infection rates is explained by the difference in environmental conditions prevailing in each region such as increase humidity, optimum temperature favoring the sporulation of oocysts, rearing conditions, the system of housing of rabbits was battery or ground breeding systems, the use of chemoprophylaxis, type of feeding as well as the number of samples examined (Al-Mathal, 2008; Abdel-Baki and Al-Quraishy 2013; Mohammed *et al.*, 2013; Laha *et al.*, 2015 and Yin *et al.*, 2016).

Our studies revealed that 10 species of *Eimeria* were identified in 50 fecal samples of domestic rabbits, namely *E. Perforans*, *E. media*, *E. magna*, *E. exigua*, *E. irresidua*, *E. intestinalis*, *E. coecicola*, *E. piriformis*, *E. flavescens* and *E. stiedae*. In previous studies undertaken in Egypt, in Assiut governorate Abdalla (1988) recognized 5 species of *Eimeria* oocysts (*E. Perforans*; *E. media*; *E. irresidua*; *E. magna* and *E. stiedae*), while Ibrahim (1990) collected 4 species of *Eimeria* (*E. stiedae*, *E. coecicola*, *E.perforans* and *E. intestinalis*) in Giza governorate. Moreover, El-Shahawi *et al.* (2012) detected 8 species of *Eimeria* (*E. stiedae*, *Eimeria media*, *E. intestinalis*, *Eimeria coecicola*, *E. magna*, *Eimeria exigua*, *Eimeria perforans* and *Eimeria flavescens*) in Beni-Suef governorate.

Postmortem examination revealed macroscopic irregular yellowish white nodules of varying sizes lesions in eleven rabbit's liver while microscopic examination of its fecal samples revealed *E. stiedae* oocyst in 3 rabbits only. Our results revealed small liver lesions with no oocysts of *E. stiedae*. This may

due to recent infection as explained by (Smetena, 1933 and Barriga and Arnoni, 1981) who recorded long prepatent period of *E. stiedae* with first oocystic shedding at the 3rd to the 4th week after infection. Also, (Hassan *et al.*, 2016) recorded peak of oocyst shedding at 25 days after infection, then began to decline.

It was noticed that, *E. perforans* was the most prevalent species (66.7%), followed in order by *E. media*, *E. exigua*, *E. magna*, *E. irresidua*, *E. coecicola* and *E. intestinalis* with prevalences of 26.2%, 26.2%, 21.4%, 19%, 19% and 19%, respectively. while prevalence of *E. piriformis*, *E. stiedae* and *E. flavescens* were 14.3%, 7.1% and 7.1%, respectively with high significant variations were noticed ($p < 0.0001$). These results agreed with Abdalla (1988) who mentioned that the prevalence of *E. Perforans*; *E. media*; *E. irresidua*; and *E. magna* was 47.3%, 41.3%, 21.6% and 18.4% in Assiut governorate, respectively. Moreover in Saudi Arabia; Toula and Ramadan (1998) found that the most common species were *E. perforans* (65%), *E. magna* (45%), *E. stiedae* (25%), *E. exigua* (20%) and *E. piriformis* (10%). In France Grès *et al.* (2003) declared that the predominant species was *E. perforans* (87%), *E. media* (55%) and *E. magna* (53%), respectively. Similarly, in China Jing *et al.* (2012) mentioned that *E. perforans* was the most prevalent species (35.2%), followed by *E. media* (31.3%), *E. magna* (28.8%), *E. irresidua* (19.4%) and *E. intestinalis* (14.8%).

Mixed infections are common and generally, more than one species of *Eimeria* often parasitize the intestinal epithelium of rabbits (Toula and Ramadan, 1998). In our study, high significant differences between mixed and single infection ($p < 0.0001$) where mixed infection with two species or more was most frequently occurring (76.2%) while infection with single species is rarely occurring (23.8%), these are agreed with the results of previous reports in Egypt and other countries (El-Shahawi *et al.*, 2012; Qiao *et al.*, 2012 and Abdel-Baki and Al-Quraishy, 2013). The consequences of these conditions could result in overwhelming diseases that may coincide with economic losses in rabbit rearing industries (Razavi *et al.*, 2010).

Higher prevalence was recorded among young individuals (< 4 months) than adults (≥ 4 months) 88% and 80%, respectively with no statistical significance ($P > 0.05$). The difference in prevalence among young and adult animals had already been reported in Taiwan, China and Brazil (Ming-Hsien *et al.*, 2010; Yin *et al.*, 2016 and Heker *et al.*, 2017) (53.6%, 31.2%); (45%, 42%) and (46.67%, 14.81%), respectively. The high level of susceptibility of infection in young rabbits may be due to lower resistance or less immunity to coccidian oocysts in

young rabbits compared to elder animals (Al-Mathal, 2008 and Yin *et al.*, 2016).

It was noticed that in males have higher prevalence (92.6%) than females (73.9%), with no significant difference ($P>0.05$). These results disagreed with Razavi *et al.* (2010) who found higher prevalence among females 63.7% than males 36.3%. However, Khider *et al.* (2015), Ming-Hsien *et al.* (2010) and Heker *et al.* (2017) found no significant difference in the prevalence of *Eimeria* between male and female rabbits.

Macroscopic observations in this study are in agreement with previous report, in which the infected liver showed hepatomegaly, pale color and multiple scattered yellowish white nodules of variable sizes (AL-Naimi *et al.*, 2012). Additionally, the gall bladder may also be enlarged and contain exudates (Pakes and Gerrity, 1994). Moreover, there was a distended empty gall bladder with thickened tortuous wall was observed in chronic case of hepatic coccidiosis which could be the result of granulomatous hepatitis and stasis of bile due to biliary obstruction by proliferation of bile duct epithelium along with massive number of oocysts within the bile duct lumen results in obstructive jaundice (Erdogmus and Eroksuz, 2006).

Histologically, bile ducts showed dilatation and extensive proliferation of the biliary epithelium accompanied with lymphoplasmacytic infiltration. Various developmental stages of *E. stiedae* were noticed in the biliary epithelium. Oocysts could be seen obstruct the lumen. Rupture of enlarged bile ducts results in asevere granulomatous response, while compression of adjacent liver parenchyma results in ischemic hepatic necrosis and gradually replaced by fibrous connective tissue (Erdogmus and Eroksuz, 2006 and Al-Mathal, 2008). The remarkable macroscopic observation in the intestine of infected animals was hyperemia of the infected parts of intestine and in other cases there was thickening of the wall appeared like white streaks. Microscopic result is in accordance with Abdalla (1988) who noted that intestinal coccidiosis usually occurs as mild infection where, the epithelial cells showed mild degree of hydropic degeneration with focal eosinophilic cellular reaction and some of the villi were edematous. However Baker (1998) described the changes in infected intestinal segments include epithelial necrosis, mucosal ulceration, congestion, edema and occasionally hemorrhages, villous atrophy and leukocytic exudate. Different developmental stages of *Eimeria* were noticed in the intestinal epithelium. Similar results were observed by Yakhchali and Tehrani (2007) who suggested that intestinal coccidiosis causes atrophy of the enterocytes lining the villi results in interference with intestinal function and impairs intestinal motility.

Ultrastructurally, in general, the exact number of the endogenous merogonic generations among the genus *Eimeria* is not fixed (Dai *et al.*, 2005). After a specific number of merogonic generations, the merozoites develop into either macrogamonts and/or microgamonts (Hammond, 1973). In the present study, various coccidian stages (both asexual and sexual) were recognized including developing schizont and macrogametocyte characterized by the peripherally arranged wall-forming bodies and surrounded by parasitophorous vacuole were noted within the epithelial cell, even in the lamina propria of villi. These results are in accordance with those reported for many other *Eimeria* (Abdel-Ghaffar *et al.*, 1991; Dai *et al.*, 2005; Mehlhorn, 2006 and Bashtar *et al.*, 2010). Additionally, there were plasma cells and macrophage phagocyte early parasite were observed, this may occurs as inflammatory defense reaction against *Eimeria* parasite.

CONCLUSION

In conclusion, we identified 10 species of *Eimeria* in rabbit in Assiut, with the presence of high prevalence of infections with multiple *Eimeria* species in the same animal. In addition we found that *Eimeria* in rabbits caused significant pathological lesions in liver, bile duct and intestine especially in young ages. Results of this study can help the understanding of disease occurrence to design effective control scheme for rabbit coccidiosis in the area.

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دراسات باثولوجية وطفيلية على بعض انواع الایمریا فی الارانب باستخدام المیکروسکوب الضوئی والإلکترونی

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في هذه الدراسة، قد وجد معدل انتشار طفيل الأيمريا في الأرانب التي تم فحصها ٨٤٪ (٥٠/٤٢). وكان معدل انتشاره ٩٢,٦٪ في ذكور الأرانب في حين كان ٧٣,٩٪ في إناث الأرانب. أما وفقا للعمر قد تم تحديد معدل انتشار ٨٨٪ في الأرانب التي عمرها أقل من ٤ أشهر في حين كان ٨٠٪ في الأرانب التي عمرها ٤ أشهر أو أكثر. لم يسجل أي فرق معنوي في انتشار الأيمريا في الأرانب من حيث ارتباطها بالعمر والجنس. وقد تم عزل عشرة أنواع من الأيمريا من الأرانب المصابة في أسبوط بواسطة الفحص الطفيلي. كانت الأيمريا الأكثر انتشارا هي الأيمريا بيرفورانز بنسبة (٦٦,٧٪) تليها الأيمريا إكسيغوا (٢٦,٢٪)، الأيمريا ميديا (٢٦,٢٪)، الأيمريا ماجنا (٢١,٤٪)، الأيمريا انتستيناليز (١٩٪)، الأيمريا كوسيكولا (١٩٪) والأيمريا إريسيديوا (١٩٪) والأيمريا بيريفورميس (١٤,٣٪) والأيمريا فلافيسنس (٧,١٪) والأيمريا ستيدي (٧,١٪). وجدت عدوى بنوع واحد من الأيمريا في ٢٣,٨٪ من الأرانب المصابة، في حين أن العدوى المختلطة بعدد اثنين أو ثلاثة أو أربعة أنواع من الأيمريا في ٧٦,٢٪ من الأرانب المصابة. كانت الاعراض الأكلينيكية الظاهرة على حيوانات في صورة اعراض الخمول وفقدان الشهية. أما الوصف التشريحي اوضح تضخم في الكبد مع انتشار بثرات بيضاء مصفرة اللون تبرز علي سطح الكبد متباينة في الحجم ، مصحوبة بتمدد في الحوصلة المرارية. أما الوصف التشريحي للامعاء ابرز وجود احتقان بدرجات متباينة مع وجود سماكة في جدار الامعاء. وبالفحص الهستوباثولوجي للكبد اوضح اتساع القنوات المرارية داخل الكبد الناتج عن تضاعف الخلايا الطلائية المبطنة للقنوات مكونة بروزات مخملية محتوية علي المراحل المختلفة لتطور الطفيل مسببا تغيرات انحلالية وباثولوجية في النسيج المحيط بها في الكبد. وبفحص الامعاء اوضحت نفس التغيرات حيث ظهر زيادة عددية في الخلايا المبطنة لخمالات الامعاء محتوية على البويضات والجاميتات المختلفة للطفيل مصحوبة بارتشاح للخلايا الليمفاوية في طبقة البروبريا للخمالات. أظهر المجهر الإلكتروني بعض مراحل النمو الجنسية واللاجنسية لطفيل الأيمريا في الأرنب ومنها تطور شيزونت وماكروجاميتوسيت.