Damanhour Journal of Veterinary Sciences 4(1), (2020) 20–28

Damanhour Journal of Veterinary Sciences

Journal homepage: https://djvs.journals.ekb.eg/

Clinicopathological effects of diclazuril prophylaxis and treatment on rabbits experimentally infected with *Eimeria stiedae*

Tamer Allam[1] *, Moustafa AbdelGaber[2], Nahed Thabet[1], Mahmoud AbouLaila[3], Ahmed Elkhatam[4]

[1] Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City 32511, Minoufiya, Egypt

[2]Department of Pathology, Faculty of Veterinary Medicine, Menofia University, Shebin El Kom, Minoufiya, Egypt

[3]Department of Parasitology, Faculty of Veterinary Medicine, Damanhour University, Damanhour22511, El-Behera, Egypt

[4]Department of Parasitology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City 32511, Minoufiya, Egypt

ABSTRACT

Eimeria stiedae is the most pathogenic rabbit Coccidia. This study was designed to clarify hematological, biochemical, and pathological alterations associated with hepatic coccidiosis in rabbits and to investigate the effect of diclazuril on the course of the disease for both protection and treatment. Rabbits experimentally infected with 2×10^4 sporulated oocysts of *E. stiedae.* Rabbits were equally divided into 4 groups. Group 1, non-infected non-treated, was served as control negative. Group 2 was considered a control positive. Group 3 treated on the day 10 post-infection with a dose of 5 ppm of diclazuril given orally in drinking water for 2 days. Finally, group 4 or the protected group was administered diclazuril at a dose of 1 ppm orally in drinking water for seven days before infection. Blood and serum samples were collected at 18th, 25th, 32^{th,} and 39th days post-infection. Rabbits of group 2 showed significant alterations of hematological parameters [reduced of RBCs, Hb, MCV, lymphocyte counts and increased of TLC, Neutrophil counts]; serum biochemical parameters [reduced concentrations of total protein, albumin, calcium, and increase of bilirubin, urea, enzymatic activities of ALT, AST, ALP, GGT]; and histological picture of liver indicated hepatic coccidiosis, while rabbits either treated by or protected with diclazuril showed no significant hematological, biochemical and histopathological changes from negative control. In conclusion, diclazuril appeared to be a potent anticoccidial drug against *E. stiedae* infection in rabbit either for treatment or as prophylaxis.

Keywords: *Eimeria stiedae;* Rabbit; Diclazuril; Prophylaxis; Treatment; Clinicopathological parameters; Biochemical parameters; Histopathology

1. Introduction

Rabbit production can be a substantial source of income as they can provide a prime quality protein low-fat meat for healthy eating besides skin or fiber (Al-Mathal, 2008 and Sivajothi et al., 2013a). In Egypt, the rabbit industry became well established (Lebdah and Shahn, 2011) but it has received little attention from formal institutions or domestic animal producers. However, the rabbits' stock is about nine million, and rabbit meat production is about 70 thousand kg/year (El-Raffa et al., 2005).

Parasitic infections are one of the most common health problems that hinder rabbit breeding and interfering with its production (Lebas et al., 1986). Coccidiosis is a highly contagious widespread protozoal disease in rabbits (Hauptman et al., 2001). It is caused by microorganisms known as protozoa and is a part of the *Eimeria* species which are true pathogens that are always present in rabbit farms as they are virtually impossible to eradicate (Pakandl, 2009; van Praag, 2011). Up till now, 15 *Eimeria* species have been known to infect rabbits, and all of them are enteric parasites, except for *Eimeria stiedae* (*E. stiedae*), which invades only the liver and the biliary tract (Li and Ooi, 2009). Hepatic coccidiosis is highly pathogenic to rabbits leads to high morbidity and mortality (Singla et al., 2000). The liver form of coccidiosis usually runs either as a chronic course during several weeks, or it ends in death within 10 days (Gomez-Bautista et al., 1987).

The more susceptible rabbits to the disease are young kittens between the first and four months following their birth whereas older rabbits appear to be more resistant to infections (Sivajothi et al., 2013b). The disease is transmitted once the parasitic oocysts have been passed in the feces of the infected rabbit. Once the polluted material is ingested the disease is transmitted to the unaffected animal. The contaminated feed, water, and bedding can transfer the infection. The severity of coccidiosis depends on the quantity of ingested oocytes (Bhat et al., 1996).

Treatment of hepatic coccidiosis is difficult and the disease may remain present for life. The anti-coccidiosis treatment is effective for rabbits from 5 to 6 days only. Mortality and diarrhea can occur in the following days even though the treatment is successful (Karaer, 2001). Diclazuril is a potent anticoccidial benzene-aceto-nitrile derivative, a synthetic compound of the triazinone family which was tested in poultry against *Eimeria* species either in single or mixed species infections (Çam et al., 2008).

Although coccidiosis remains an important disease of rabbits that might be responsible for considerable loss in the rabbit industry, the information in certain aspects of the disease in rabbits such as hematological, biochemical, and histopathological changes are still unclear. Therefore, this study aimed to study the clinical, hematological, biochemical, and pathological alterations associated with hepatic coccidiosis in rabbits that may allow better understanding and thus controlling the disease. Also, investigate the effect of diclazuril on the course of the disease for both prophylactic prevention and treatment.

2. Material and methods

2.1. Animals

Twenty-eight New Zealand white rabbits, aged 7 weeks and weighing about 1.25 kg, were kept in metal battery cages with a metallic grid on the bottom keeping rabbits from contacting their feces. They had administrated a prophylactic dose of Ivomec® (Merial Ltd., Georgia, U. S. A) at a dose of 0.5 ml/rabbit S/C. They were vaccinated with Toxipra plus (Hipra Co, Girona, Spain) at a dose of 1 ml/rabbit. The rabbits were fed commercial pellet feed. Water was provided *adlibitum*. The nonexistence of *E. stiedae* and other coccidian oocysts preceding the trial was affirmed by the fecal examination.

2.2. Experimental design

After 7 days of adaptation, 4 groups of rabbits were prepared each of seven. Group 1 (G1), non-infected non-treated was served as a control negative group. Rabbits in the remaining groups were infected orally via a stomach catheter with *E. stiedae* 20,000 sporulated oocysts /ml, which were collected from Ashmoun district and prepared at the Department of Parasitology, Faculty of Veterinary medicine, the University of Sadat City

*Corresponding author: Dr. Tamer Allam

E-mail address: Tamer_sma@yahoo.com

Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City 32511, Minoufiya, Egypt

PISSN: 2636-3003 EISSN: 2636-2996

Received: May 20, 2020; Received in revised form: May 27, 2020; accepted: May 29, 2020.

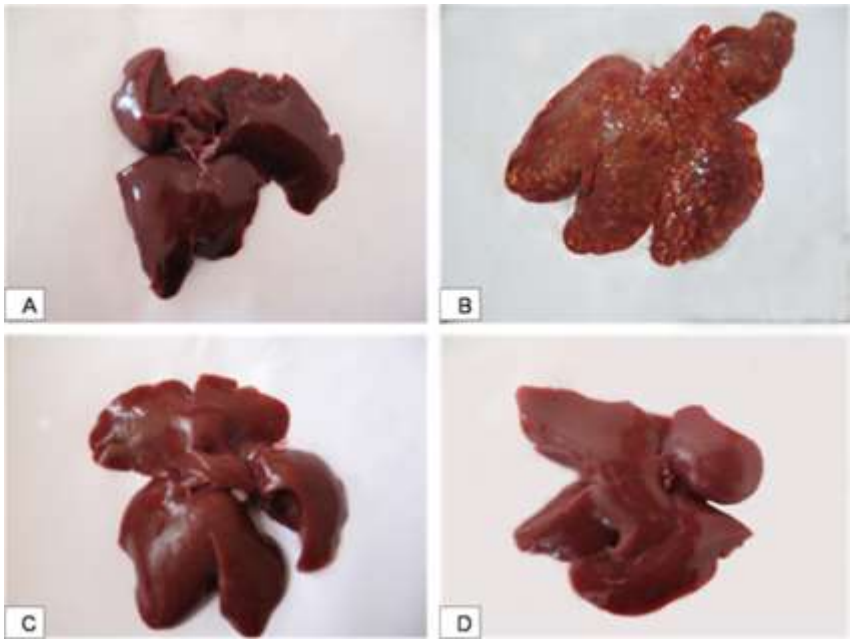

Figure (1): Macroscopic appearance of the liver of rabbits. A. Group 1 Control negative showing normal liver. B. Group 2 experimentally infected with *E. stiedae* showing abnormal liver appearance. C. Group 3 experimentally infected with *E. stiedae* and treated with diclazuril showing normal liver. D. Group 4 protected by diclazuril and experimentally infected with *E. stiedae* showing normal liver.

according to Long *et al.* (1976). Group 2 (G2) was considered as the control positive group. In Group 3 (G3= treated group), rabbits were treated on the 10th day post-infection with Diclazuril (Diclosol®, Pharma Swede, Cairo, Egypt) at a dose of 5 parts per million (ppm) (Vanparijs et al., 1989b) in drinking water for 2 days. Finally, group 4 (G4) or the protected group was administrated diclazuril at a dose of 1 ppm (Vanparijs et al., 1989a) in drinking water for seven days before infection with the oocysts. One rabbit from each group was slaughtered at 18th and 39th days post-infection for postmortem examination and livers were collected for histopathological examination.

2.3. Blood and serum samples

Blood samples were collected from the ear vein at 18th, 25th, 32^{th,} and 39th days post-infection. One ml was placed in tubes containing EDTA for hemogram and 4 ml were placed in a plain tube for separation of serum. Serum samples were divided into aliquots in Eppendorf tubes and stored at -20 ° C until assayed for the rest of the biochemical parameters.

2.4. Hematological parameters

Hematological parameters were assessed as previously described (Melillo, 2007; Weisbroth et al., 2013). They included red blood cell count (RBCs), packed cell volume (PCV), platelets count, total leukocytic counts (TLC), differential leukocytic counts (DLC), hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC).

2.5.Biochemical parameters

Serum concentrations of total protein (TP), albumin (Alb), total bilirubin (TB), direct bilirubin (DB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP), urea (U), creatinine (Cr), cholesterol (Ch), calcium (Ca), and inorganic phosphorus (IP) were calculated utilizing diagnostic kits (SPINREACT, Girona, Spain). Globulin (Glob) was estimated by subtracting serum albumin from serum total protein and then the A/G ratio was valued by dividing albumin on globulin. Sodium (Na), potassium (K), and chloride (Cl) values were measured using OPTI LION Automated Cassette-Based Electrolyte Analyzer (OPTIMEDICAL, Georgia, USA). Blood pH and bicarbonate (HCO3) were determined at 37 °C by Rapid point 340® Blood Gas Analyzer (Siemens Healthcare diagnostic Inc., Deerfield, USA). Oxidant-antioxidant status was determined by assessing serum levels of malondialdehyde (MDA) and total antioxidant capacity (TAC).

2.6.Fecal analysis

Oocysts were concentrated by the floating technique (Jarvinen, 1999) and the numbers of oocysts per gram of feces (opg) were performed by a McMaster counting technique as described by Maff (1977).

2.7.Histopathology

Liver tissues were prepared for histopathological examination according to Bancroft *et al.* (1996).

2.8.Statistical analysis procedures

All data were presented as mean ± standard error and were subjected to analysis of variance in one and two way (ANOVA) test according to Snedecore and Cochran (1980). Means were compared by Duncan test at 0.05 level of probability.

3. Results

Fecal analysis

Oocyst output was first detected in feces at 18th-day post-infection in rabbits of group 2 and attained its highest at end of the study. Oocysts were not observed in the fecal samples of other groups through the experimental period (Table 1).

Hematological parameters

Diclazuril-treated (G3) and -protected (G4) groups did not express any meaningful changes in the values of PCV, RBCs, and Hb comparing to those of negative control, except the values of RBCs in the protected group, were significantly reduced on day 39. However, the RBCS values in the protected group (G4) were significantly increased on day 32 then decreased on day 39 when compared with the treated group (G3). There was a significant rise in the values of PCV, RBCs, and Hb in treated and protected groups compared with the infected group (G2) during the whole duration of the experiment except, the values of PCV on day 32, Hb on days 25 & 32, and RBCs on day 39 post-infection were insignificant in the protected group (Table 2). There was a significant decline in the values of PCV, RBCs, and Hb in group 2 infected with *E. stiedae* throughout the experiment compared with the negative control. The maximum decline in these values was significantly verified on day 39 (Table 2).

No significant changes in MCV, MCH, and MCHC values were reported in diclazuril-treated (G3) and -protected (G4) groups throughout the experimental period (Table 2) in contrast to the negative control group. In contrast, the *E. stiedae* infected group showed a marked decrease in the average values of MCV at 25, 32, and 39 days post-infection. While the mean estimations of MCH were significantly increased at 18, 25, and 32 days post-infection with the highest values were verified at 25 days post-infection. Moreover, significant decreases in MCHC values were detected only at 39 days post-infection.

The platelet count was the same in all groups along the whole experimental period when compared with the negative control (Table 2). Rabbits infected with *E. stiedae* demonstrated a significant rise in the total leukocytic count at 18- and 25-days post-infection when matched with the control and at day 25 post-infection compared with the protected group (Table 2). Neutrophils and eosinophils percentages were meaningfully elevated in rabbits infected with *E. stiedae* at 25- and 32-days post-infection. Similarly, lymphocytes' percentages were significantly reduced. No Significant changes in monocytes percentages were identified in all groups (Table 3).

Biochemical parameters

Comparison to negative control animals, rabbits in diclazuril-treated (G3) and -protected (G4) groups exhibited no significant changes in the amounts of total protein, albumin, and globulin (Table 4) while animals in the infected control group (G2) showed significant decreases in total protein and albumin at 25, 32, and 39 days post-infection.

There were insignificant changes in the readings of total bilirubin, direct

Table 1: Number of oocysts shed per gram of feces

Day	G 1	G 2	G 3	G 4
5	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
15	-ve	-ve	-ve	-ve
18	-ve	700	-ve	-ve
19	-ve	1340	-ve	-ve
20	-ve	3900	-ve	-ve
21	-ve	4200	-ve	-ve
22	-ve	10400	-ve	-ve
23	-ve	15390	-ve	-ve
24	-ve	19740	-ve	-ve
25	-ve	30533	-ve	-ve
26	-ve	43094	-ve	-ve
27	-ve	66840	-ve	-ve
28	-ve	90350	-ve	-ve
29	-ve	113940	-ve	-ve
30	-ve	142140	-ve	-ve
31	-ve	170455	-ve	-ve
32	-ve	223050	-ve	-ve
33	-ve	260150	-ve	-ve
34	-ve	277332	-ve	-ve
35	-ve	311423	-ve	-ve
36	-ve	342540	-ve	-ve
37	-ve	351700	-ve	-ve
38	-ve	410411	-ve	-ve
39	-ve	411012	-ve	-ve

G1: Control negative non-infected non-treated group
G2: Infected non-treated group
G3: Infected treated with diclazuril group
G4: Protected by diclazuril then infected group

Table 2: Effect of diclazuril on erythrogram and platelets in rabbits experimentally infected with *E. stiedae*

Parameters	Groups (n=5)	Days Post-Infection			
		18	**25**	**32**	**39**
PCV (%)	G1	42.60 ± 1.12 $^{a\ A}$	38.00 ± 0.63 $^{a\ B}$	38.40 ± 0.75 $^{a\ B}$	39.60 ± 0.75 $^{a\ B}$
	G2	36.60 ± 0.51 $^{b\ A}$	34.20 ± 0.86 $^{b\ AB}$	31.80 ± 1.53 $^{b\ BC}$	29.20 ± 0.73 $^{b\ C}$
	G3	43.60 ± 1.86 $^{a\ A}$	37.80 ± 0.58 $^{a\ AB}$	38.40 ± 1.89 $^{a\ AB}$	38.40 ± 0.60 $^{a\ AB}$
	G4	40.20 ± 0.58 $^{a\ A}$	38.20 ± 1.93 $^{a\ AB}$	36.80 ± 2.42 $^{ab\ AB}$	37.20 ± 2.13 $^{a\ B}$
Hb (g/dl)	G1	13.85 ± 0.11 $^{a\ A}$	12.22 ± 0.47 $^{a\ BC}$	11.39 ± 0.86 $^{a\ C}$	13.35 ± 0.47 $^{a\ AB}$
	G2	12.11 ± 0.30 $^{b\ A}$	10.55 ± 0.30 $^{b\ B}$	8.88 ± 0.37 $^{b\ C}$	8.34 ± 0.17 $^{b\ C}$
	G3	13.76 ± 0.34 $^{a\ A}$	12.05 ± 0.27 $^{a\ B}$	11.20 ± 1.07 $^{a\ C}$	13.47 ± 0.63 $^{a\ A}$
	G4	13.31 ± 0.20 $^{a\ A}$	11.11 ± 0.54 ab ^{AB}	9.82 ± 0.95 $^{ab\ B}$	13.18 ± 1.17 $^{a\ A}$
RBCs (X 10^6)	G1	7.64 ± 0.23 $^{a\ A}$	7.84 ± 0.37 $^{a\ A}$	6.95 ± 0.49 $^{ab\ A}$	7.67 ± 0.21 $^{a\ A}$
	G2	6.30 ± 0.10 $^{b\ A}$	5.15 ± 0.10 $^{b\ B}$	4.77 ± 0.23 $^{c\ BC}$	4.52 ± 0.10 $^{b\ C}$
	G3	8.08 ± 0.26 $^{a\ A}$	7.14 ± 0.56 $^{a\ B}$	6.36 ± 0.22 $^{b\ C}$	6.96 ± 0.60 $^{a\ BC}$
	G4	7.76 ± 0.24 $^{a\ A}$	6.82 ± 0.41 $^{a\ B}$	7.83 ± 0.32 $^{a\ A}$	5.06 ± 0.45 $^{b\ C}$
MCV (fl)	G1	55.88 ± 1.69 $^{ab\ C}$	67.35 ± 1.14 $^{a\ A}$	65.88 ± 1.05 $^{a\ B}$	64.58 ± 0.75 $^{ab\ B}$
	G2	51.99 ± 1.69 $^{b\ A}$	48.86 ± 2.15 $^{b\ A}$	49.36 ± 2.03 $^{b\ A}$	51.72 ± 0.86 $^{c\ A}$
	G3	54.06 ± 2.31 $^{ab\ B}$	54.18 ± 4.04 $^{ab\ B}$	62.47 ± 0.97 $^{a\ A}$	56.76 ± 4.70 $^{bc\ B}$
	G4	58.11 ± 0.60 $^{a\ B}$	61.56 ± 6.94 $^{ab\ B}$	62.12 ± 2.49 $^{a\ B}$	66.59 ± 2.44 $^{a\ A}$
MCH (Pg)	G1	17.22 ± 0.62 $^{bc\ AB}$	15.72 ± 0.88 $^{b\ AB}$	14.61 ± 1.11 $^{bc\ B}$	18.46 ± 0.19 $^{ab\ A}$
	G2	19.23 ± 0.43 $^{a\ B}$	21.24 ± 1.00 $^{a\ A}$	18.66 ± 0.31 $^{a\ B}$	17.18 ± 0.68 $^{b\ B}$
	G3	17.09 ± 0.68 $^{c\ B}$	17.23 ± 1.20 $^{ab\ B}$	17.60 ± 1.55 $^{ab\ B}$	20.04 ± 2.05 $^{ab\ A}$
	G4	18.98 ± 0.58 $^{ab\ B}$	17.84 ± 1.83 $^{ab\ BC}$	14.07 ± 0.72 $^{c\ C}$	21.89 ± 1.39 $^{a\ A}$
MCHC (%)	G1	34.00 ± 0.87 $^{a\ A}$	32.11 ± 0.72 $^{a\ AB}$	29.58 ± 1.87 $^{a\ B}$	33.71 ± 0.98 $^{a\ A}$
	G2	33.08 ± 0.44 $^{a\ A}$	31.51 ± 1.15 $^{a\ AB}$	28.00 ± 0.51 $^{a\ B}$	28.58 ± 0.20 $^{b\ B}$
	G3	31.68 ± 0.88 $^{a\ AB}$	31.88 ± 0.72 $^{a\ AB}$	28.97 ± 1.55 $^{a\ B}$	35.12 ± 1.71 $^{a\ A}$
	G4	33.14 ± 0.72 $^{a\ A}$	29.91 ± 0.45 $^{a\ B}$	26.51 ± 1.21 $^{a\ B}$	35.18 ± 1.42 $^{a\ A}$
Platelets (X 10^3)	G1	540.80 ± 2.22 $^{a\ A}$	543.00 ± 1.79 $^{a\ A}$	542.40 ± 2.14 $^{a\ A}$	545.60 ± 1.57 $^{a\ A}$
	G2	541.60 ± 3.23 $^{a\ A}$	540.20 ± 2.84 $^{a\ A}$	540.60 ± 1.81 $^{a\ A}$	547.80 ± 2.15 $^{a\ A}$
	G3	542.60 ± 1.08 $^{a\ A}$	543.00 ± 1.79 $^{a\ A}$	540.60 ± 2.79 $^{a\ A}$	546.20 ± 0.97 $^{a\ A}$
	G4	540.80 ± 2.60 $^{a\ AB}$	539.00 ± 2.51 $^{a\ B}$	540.80 ± 3.06 $^{a\ AB}$	547.80 ± 2.40 $^{a\ A}$

Values are means ± SE. Means in the same column without a common small letter differ significantly at (P<0.05).
Means in the row without a common capital letter differ significantly at (P<0.05). **G1**: Control negative non-infected non-treated group **G2:** Infected non-treated group **G3:** Infected treated with diclazuril group **G4:** Protected by diclazuril then infected group

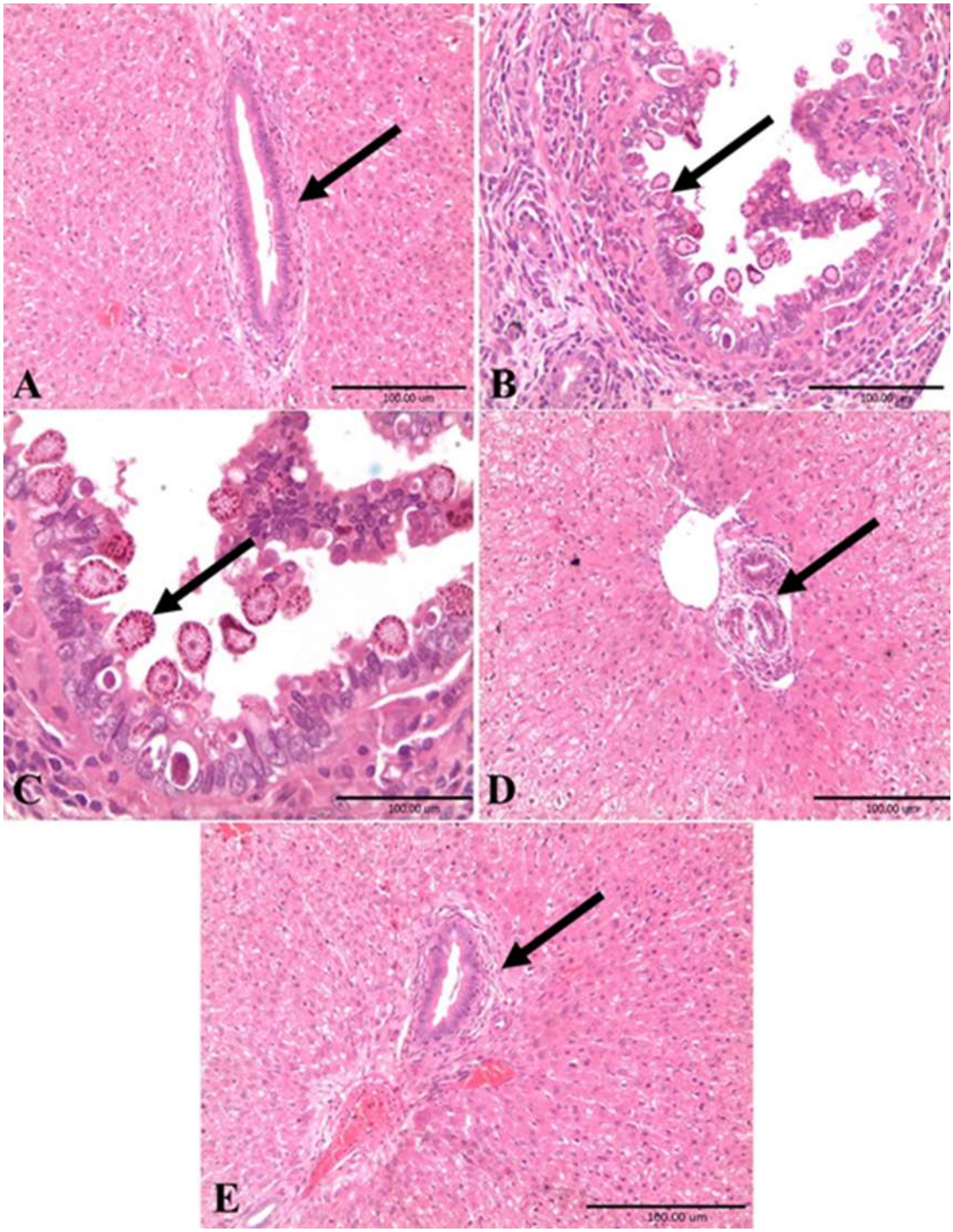

Figure (2): Light micrograph of the livers of the rabbits at day 18 PI. A. Control negative group showing normal tissue architecture with the normal structure of bile duct in the portal area (arrow) (H&E x10). B. Group experimentally infected with *E. stiedae* showing proliferation of bile duct epithelium forming finger-like projection in the lumen with the presence of different developmental stages of coccidia in the bile duct epithelium and the lumen of the bile duct (arrow), there are mononuclear cells infiltration and proliferation of connective tissues around the bile duct in the portal area (H&E x 20). C. Higher magnification of B. (H&E x 40). D. Group protected by diclazuril then experimentally infected with *E. stiedae* showing normal tissue architecture with the normal structure of bile duct in the portal area (arrow) (H&E x10). E. The liver of a rabbit from the diclazuril-treated group showed normal tissue architecture with the normal structure of the bile duct in the portal area (arrow) (H&E x10).

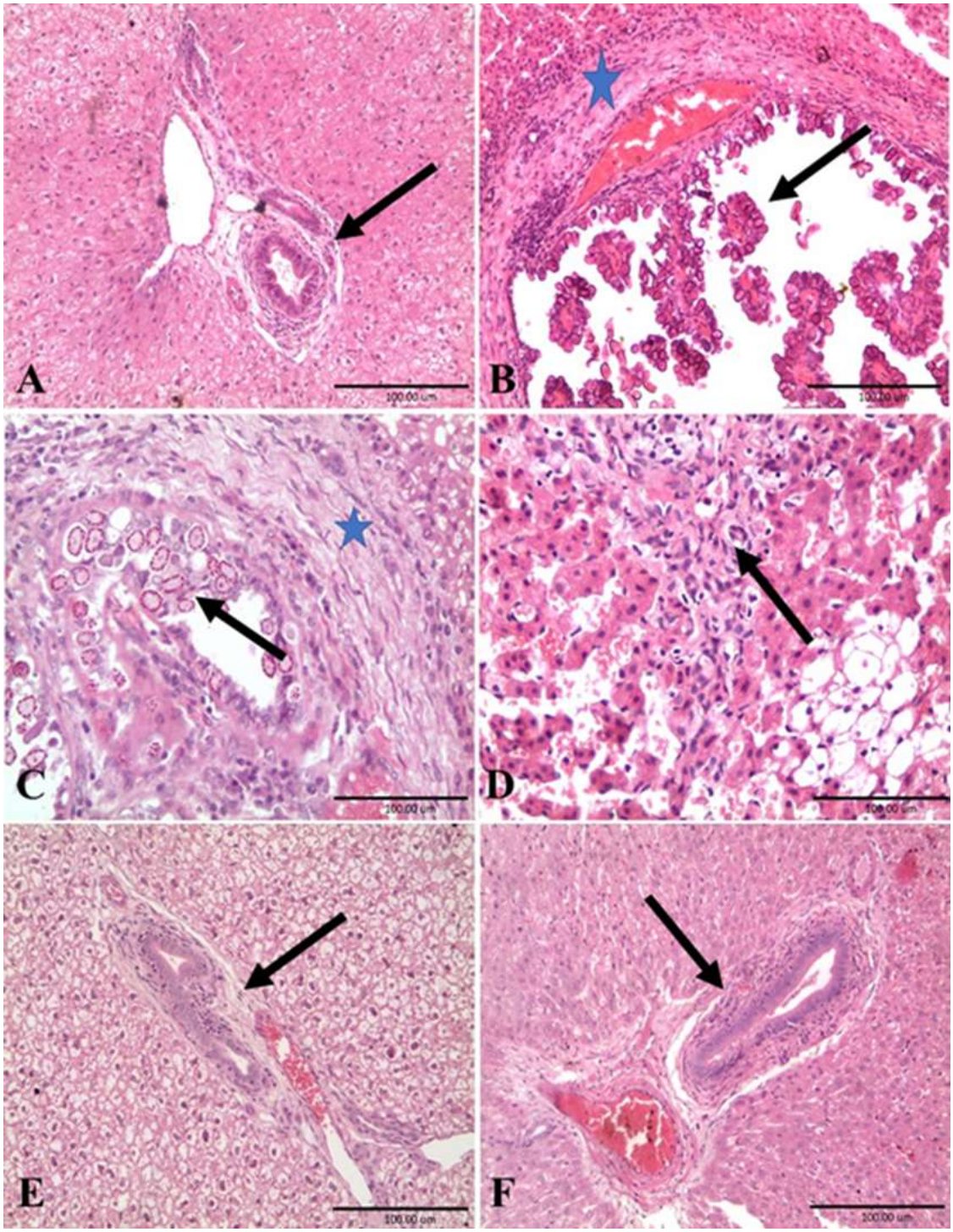

Figure (3): Light micrograph of the livers of the rabbits at day 39 PI. A. Control negative group showing normal tissue architecture with the normal structure of bile duct in the portal area (H&E x10). B. Group experimentally infected with *E. stiedae* showing proliferation of bile duct epithelium forming finger-like projection in the lumen with the presence of different developmental stages of *Eimeria* in the bile duct epithelium and the lumen of the bile duct, there are mononuclear cells infiltration and proliferation of connective tissues around the bile duct in the portal area with severely congested blood vessels. (H&E x 10). C. Higher magnification of B. (H&E x 20). D. liver of rabbit from infected group show focal area of necrosis and mononuclear cell infiltration in hepatic parenchyma also there is fatty change. (H&E x20). E. Liver of the group protected by diclazuril showing normal tissue architecture with the normal structure of the bile duct in the portal area (H&E x10). F. liver of group experimentally infected with *E. stiedae* and treated with diclazuril showing normal tissue architecture with the normal structure of bile duct in the portal area (H&E x10).

.

Table 3: Effect of diclazuril on leukogram in rabbits experimentally infected with *E. stiedae*

Parameters	Groups (n=5)	Days Post-Infection (DPI)			
		18	25	32	39
TLC ($\times 10^3$ /µl)	G1	6.52 ± 0.94 $^{b\ A}$	6.82 ± 0.38 $^{bc\ A}$	8.21 ± 0.46 $^{a\ A}$	8.21 ± 0.46 $^{a\ A}$
	G2	10.18 ± 0.60 $^{a\ AB}$	10.96 ± 1.21 $^{a\ A}$	10.41 ± 0.61 $^{a\ AB}$	7.58 ± 1.18 $^{a\ B}$
	G3	8.90 ± 1.03 $^{ab\ A}$	8.50 ± 0.74 $^{ab\ A}$	9.91 ± 2.13 $^{a\ A}$	6.84 ± 0.41 $^{a\ B}$
	G4	7.92 ± 0.55 $^{ab\ AB}$	5.12 ± 0.75 $^{c\ B}$	8.70 ± 1.38 $^{a\ A}$	8.20 ± 1.19 $^{a\ AB}$
N (%)	G1	32.20 ± 0.66 $^{a\ AB}$	30.60 ± 0.68 $^{b\ AB}$	33.00 ± 1.22 $^{b\ A}$	28.20 ± 2.13 $^{b\ B}$
	G2	32.80 ± 0.80 $^{a\ B}$	41.40 ± 1.03 $^{a\ A}$	42.20 ± 1.16 $^{a\ A}$	31.20 ± 0.80 $^{ab\ B}$
	G3	29.00 ± 2.28 $^{a\ A}$	31.20 ± 0.86 $^{b\ A}$	32.00 ± 2.55 $^{b\ A}$	32.40 ± 0.93 $^{a\ A}$
	G4	31.80 ± 0.73 $^{a\ A}$	30.80 ± 0.73 $^{b\ A}$	32.40 ± 0.93 $^{b\ A}$	31.60 ± 1.12 $^{ab\ A}$
L (%)	G1	64.40 ± 0.87 $^{a\ A}$	66.00 ± 1.30 $^{a\ A}$	62.60 ± 1.60 $^{a\ A}$	66.00 ± 1.30 $^{a\ A}$
	G2	63.00 ± 1.00 $^{a\ A}$	52.20 ± 0.86 $^{b\ B}$	50.80 ± 0.86 $^{b\ B}$	65.00 ± 1.30 $^{a\ A}$
	G3	64.60 ± 0.87 $^{a\ A}$	65.40 ± 0.98 $^{a\ A}$	62.40 ± 1.08 $^{a\ A}$	64.00 ± 1.41 $^{a\ A}$
	G4	64.80 ± 0.97 $^{a\ A}$	65.60 ± 1.66 $^{a\ A}$	62.80 ± 1.07 $^{a\ A}$	65.00 ± 1.48 $^{a\ A}$
M (%)	G1	2.40 ± 0.51 $^{a\ A}$	2.40 ± 0.81 $^{a\ A}$	3.00 ± 0.32 $^{a\ A}$	2.80 ± 0.58 $^{a\ A}$
	G2	2.60 ± 0.51 $^{a\ AB}$	1.40 ± 0.51 $^{a\ B}$	3.00 ± 0.32 $^{a\ A}$	2.20 ± 0.20 $^{a\ AB}$
	G3	3.00 ± 0.32 $^{a\ A}$	2.40 ± 0.24 $^{a\ A}$	2.40 ± 0.51 $^{a\ A}$	2.60 ± 0.24 $^{a\ A}$
	G4	2.40 ± 0.51 $^{a\ A}$	2.40 ± 0.75 $^{a\ A}$	3.20 ± 0.37 $^{a\ A}$	2.60 ± 0.24 $^{a\ A}$
E (%)	G1	1.00 ± 0.45 $^{a\ A}$	1.20 ± 0.80 $^{b\ A}$	1.40 ± 0.24 $^{b\ A}$	1.00 ± 0.32 $^{a\ A}$
	G2	1.60 ± 0.51 $^{a\ B}$	5.00 ± 0.45 $^{a\ A}$	4.20 ± 0.73 $^{a\ A}$	1.60 ± 0.40 $^{a\ B}$
	G3	1.40 ± 0.51 $^{a\ A}$	1.00 ± 0.32 $^{b\ A}$	1.20 ± 0.49 $^{b\ A}$	1.00 ± 0.32 $^{a\ A}$
	G4	1.00 ± 0.32 $^{a\ A}$	1.20 ± 0.58 $^{b\ A}$	1.60 ± 0.40 $^{b\ A}$	0.80 ± 0.20 $^{a\ A}$

Values are means ± SE Means in the same column without a common small letter differ significantly at (P<0.05). Means in the row without a common capital letter differ significantly at (P<0.05). **TLC=** total leukocytic counts **N=**neutrophils **L=**lymphocytes **M=**monocytes **E=**eosinophils **G1**: Control negative non-infected non-treated group **G2:** Infected non-treated group **G3:** Infected treated with diclazuril group **G4:** Protected by diclazuril then infected group

Table 4: Effect of diclazuril on liver biochemical markers in rabbits experimentally infected with *E. stiedae*

Parameters	Groups (n=5)	Days post infection			
		18	25	32	39
Total protein (g/dl)	G1	6.74 ± 0.43 $^{b\ B}$	7.51 ± 0.15 $^{a\ AB}$	8.13 ± 0.33 $^{a\ A}$	7.61 ± 0.21 $^{a\ AB}$
	G2	8.30 ± 0.28 $^{a\ A}$	6.28 ± 0.36 $^{b\ B}$	6.61 ± 0.16 $^{b\ B}$	6.18 ± 0.28 $^{b\ B}$
	G3	7.24 ± 0.23 $^{ab\ A}$	7.46 ± 0.43 $^{a\ A}$	7.77 ± 0.29 $^{a\ A}$	7.57 ± 0.58 $^{a\ A}$
	G4	7.25 ± 0.50 $^{ab\ A}$	7.49 ± 0.27 $^{a\ A}$	7.61 ± 0.46 $^{a\ A}$	7.25 ± 0.16 $^{a\ A}$
Albumin (g/dl)	G1	3.80 ± 0.16 $^{a\ A}$	3.86 ± 0.17 $^{a\ A}$	3.75 ± 0.15 $^{a\ A}$	3.74 ± 0.11 $^{a\ A}$
	G2	3.86 ± 0.11 $^{a\ A}$	3.42 ± 0.06 $^{b\ B}$	3.20 ± 0.10 $^{b\ B}$	3.35 ± 0.05 $^{b\ B}$
	G3	3.75 ± 0.19 $^{a\ A}$	3.51 ± 0.05 $^{ab\ A}$	3.61 ± 0.05 $^{ab\ A}$	3.70 ± 0.10 $^{a\ A}$
	G4	3.55 ± 0.27 $^{a\ A}$	3.45 ± 0.12 $^{ab\ A}$	3.53 ± 0.17 $^{ab\ A}$	3.52 ± 0.12 $^{ab\ A}$
Globulin (g/dl)	G1	2.94 ± 0.43 $^{b\ B}$	3.65 ± 0.12 $^{ab\ AB}$	4.38 ± 0.45 $^{a\ A}$	3.87 ± 0.32 $^{a\ AB}$
	G2	4.45 ± 0.34 $^{a\ A}$	2.86 ± 0.41 $^{b\ B}$	3.42 ± 0.25 $^{a\ AB}$	2.82 ± 0.26 $^{a\ B}$
	G3	3.50 ± 0.29 $^{b\ A}$	3.95 ± 0.46 $^{a\ A}$	4.15 ± 0.32 $^{a\ A}$	3.87 ± 0.48 $^{a\ A}$
	G4	3.71 ± 0.73 $^{b\ A}$	4.04 ± 0.33 $^{a\ A}$	4.08 ± 0.31 $^{a\ A}$	3.73 ± 0.27 $^{a\ A}$
Total bilirubin (mg/dl)	G1	1.16 ± 0.04 $^{a\ A}$	1.17 ± 0.03 $^{a\ A}$	1.18 ± 0.03 $^{b\ A}$	1.16 ± 0.02 $^{b\ A}$
	G2	1.32 ± 0.24 $^{a\ A}$	1.20 ± 0.04 $^{a\ A}$	1.90 ± 0.04 $^{a\ A}$	1.88 ± 0.07 $^{a\ A}$
	G3	1.26 ± 0.07 $^{a\ A}$	1.21 ± 0.02 $^{a\ A}$	1.19 ± 0.20 $^{b\ A}$	1.20 ± 0.02 $^{b\ A}$
	G4	1.18 ± 0.02 $^{a\ A}$	1.15 ± 0.02 $^{a\ A}$	1.16 ± 0.01 $^{b\ A}$	1.20 ± 0.01 $^{b\ A}$
Direct bilirubin (mg/dl)	G1	0.27 ± 0.02 $^{a\ A}$	0.22 ± 0.01 $^{b\ A}$	0.24 ± 0.02 $^{b\ A}$	0.24 ± 0.02 $^{b\ A}$
	G2	0.24 ± 0.01 $^{a\ C}$	0.30 ± 0.03 $^{a\ BC}$	0.49 ± 0.04 $^{a\ AB}$	0.63 ± 0.09 $^{a\ A}$
	G3	0.24 ± 0.01 $^{a\ A}$	0.22 ± 0.01 $^{b\ A}$	0.23 ± 0.01 $^{b\ A}$	0.26 ± 0.00 $^{b\ A}$
	G4	0.25 ± 0.02 $^{a\ A}$	0.21 ± 0.04 $^{b\ A}$	0.24 ± 0.01 $^{b\ A}$	0.27 ± 0.01 $^{b\ A}$
Indirect bilirubin (mg/dl)	G1	0.89 ± 0.04 $^{a\ A}$	0.95 ± 0.03 $^{a\ A}$	0.94 ± 0.04 $^{b\ A}$	0.92 ± 0.01 $^{b\ A}$
	G2	0.75 ± 0.10 $^{a\ C}$	0.90 ± 0.04 $^{a\ C}$	1.41 ± 0.15 $^{a\ B}$	1.25 ± 0.03 $^{a\ A}$
	G3	0.91 ± 0.08 $^{a\ A}$	0.98 ± 0.03 $^{a\ A}$	0.94 ± 0.01 $^{b\ A}$	0.94 ± 0.02 $^{b\ A}$
	G4	0.92 ± 0.03 $^{a\ A}$	0.94 ± 0.05 $^{a\ A}$	0.98 ± 0.01 $^{b\ A}$	0.93 ± 0.02 $^{b\ A}$
Cholesterol (mg/dl)	G1	228.83 ± 7.00 $^{a\ A}$	224.55 ± 4.52 $^{a\ A}$	221.17 ± 4.72 $^{a\ A}$	220.95 ± 13.04 $^{a\ A}$
	G2	202.25 ± 12.99 $^{a\ A}$	206.98 ± 8.00 $^{a\ A}$	178.38 ± 7.83 $^{b\ A}$	172.30 ± 10.28 $^{b\ A}$
	G3	200.68 ± 7.97 $^{a\ A}$	208.56 ± 5.08 $^{a\ A}$	209.32 ± 7.04 $^{a\ A}$	210.08 ± 6.43 $^{a\ A}$
	G4	220.50 ± 16.34 $^{a\ A}$	209.69 ± 21.41 $^{a\ A}$	224.77 ± 11.15 $^{a\ A}$	217.57 ± 10.92 $^{a\ A}$

Values are means ± SE Means in the same column without a common small letter differ significantly at (P<0.05).
Means in the row without a common capital letter differ significantly at (P<0.05). **G1**: Control negative non-infected non-treated group
G2: Infected non-treated group **G3:** Infected treated with diclazuril group **G4:** Protected by diclazuril then infected group

Table 5: Effect of diclazuril on liver enzymes and renal biochemical markers in rabbits experimentally infected with *E. stiedae*

Parameters	Groups (n=5)	Days post infection			
		18	25	32	39
ALT (U/L)	G1	17.67 ± 0.67 $^{a\,B}$	21.67 ± 1.45 $^{b\,AB}$	18.67 ± 1.76 ^{bAB}	22.33 ± 0.33 $^{a\,A}$
	G2	19.00 ± 3.06 $^{a\,C}$	27.33 ± 0.88 $^{a\,B}$	35.33 ± 2.73 $^{a\,A}$	23.00 ± 1.53 $^{a\,BC}$
	G3	17.67 ± 0.67 $^{a\,A}$	20.00 ± 2.65 $^{b\,A}$	19.33 ± 3.28 $^{b\,A}$	20.67 ± 0.88 $^{a\,A}$
	G4	14.00 ± 2.00 $^{a\,A}$	20.67 ± 1.20 $^{b\,A}$	16.33 ± 3.28 $^{b\,A}$	21.00 ± 0.58 $^{a\,A}$
AST (U/L)	G1	12.00 ± 1.00 $^{a\,A}$	7.00 ± 2.31 $^{b\,B}$	4.00 ± 1.15 $^{b\,B}$	5.33 ± 0.88 $^{a\,B}$
	G2	10.00 ± 2.65 $^{a\,B}$	19.33 ± 2.33 $^{a\,A}$	25.00 ± 2.52 $^{a\,A}$	7.67 ± 1.45 $^{a\,B}$
	G3	14.33 ± 2.19 $^{a\,A}$	10.67 ± 1.76 $^{b\,AB}$	4.33 ± 1.33 $^{b\,C}$	6.00 ± 0.00 $^{a\,BC}$
	G4	11.00 ± 0.58 $^{a\,A}$	4.67 ± 0.33 $^{b\,C}$	3.67 ± 0.88 $^{b\,C}$	7.67 ± 1.33 $^{a\,B}$
ALP (U/L)	G1	221.37 ± 1.56 $^{a\,A}$	173.93 ± 7.60 $^{b\,C}$	203.07 ± 6.48 $^{b\,B}$	194.93 ± 2.40 $^{a\,B}$
	G2	214.70 ± 1.92 $^{a\,B}$	325.37 ± 2.80 $^{a\,A}$	344.13 ± 2.55 $^{a\,A}$	192.07 ± 5.21 $^{a\,B}$
	G3	229.23 ± 4.28 $^{a\,A}$	168.13 ± 8.21 $^{b\,B}$	208.37 ± 4.99 $^{b\,A}$	200.20 ± 1.74 $^{a\,A}$
	G4	215.80 ± 5.75 $^{a\,A}$	179.47 ± 5.52 $^{b\,B}$	216.33 ± 4.48 $^{b\,A}$	189.90 ± 4.62 $^{a\,B}$
GGT (U/L)	G1	25.87 ± 5.72 $^{a\,A}$	27.93 ± 2.16 $^{b\,A}$	24.39 ± 2.19 $^{b\,A}$	29.58 ± 1.92 $^{b\,A}$
	G2	28.16 ± 0.79 $^{a\,B}$	36.65 ± 1.09 $^{a\,AB}$	44.01 ± 6.07 $^{a\,A}$	44.03 ± 0.69 $^{a\,A}$
	G3	28.56 ± 3.15 $^{a\,A}$	26.53 ± 3.30 $^{b\,A}$	32.84 ± 5.02 $^{b\,A}$	27.13 ± 1.67 $^{b\,A}$
	G4	27.29 ± 5.17 $^{a\,A}$	26.02 ± 2.52 $^{b\,A}$	30.94 ± 2.38 $^{b\,A}$	30.13 ± 1.45 $^{b\,A}$
Urea (mg/dl)	G1	43.55 ± 0.83 $^{a\,AB}$	40.21 ± 1.28 $^{a\,B}$	44.58 ± 1.69 $^{a\,A}$	43.22 ± 0.35 $^{b\,AB}$
	G2	42.74 ± 1.81 $^{a\,A}$	43.11 ± 4.22 $^{a\,A}$	45.58 ± 0.18 $^{a\,A}$	47.57 ± 0.69 $^{a\,A}$
	G3	45.31 ± 1.77 $^{a\,A}$	41.09 ± 0.62 $^{a\,B}$	41.64 ± 1.26 $^{a\,AB}$	40.77 ± 0.65 $^{b\,B}$
	G4	41.72 ± 1.90 $^{a\,A}$	39.20 ± 0.73 $^{a\,A}$	42.87 ± 0.40 $^{a\,A}$	41.65 ± 1.52 $^{b\,A}$
Creatinine (mg/dl)	G1	1.30 ± 0.23 $^{a\,A}$	1.56 ± 0.14 $^{a\,A}$	1.46 ± 0.04 $^{a\,A}$	1.28 ± 0.07 $^{a\,A}$
	G2	1.02 ± 0.21 $^{a\,A}$	0.97 ± 0.33 $^{a\,A}$	1.21 ± 0.13 $^{a\,A}$	1.35 ± 0.01 $^{a\,A}$
	G3	1.23 ± 0.03 $^{a\,A}$	1.49 ± 0.12 $^{a\,A}$	1.37 ± 0 .11 $^{a\,A}$	1.21 ± 0.08 $^{a\,A}$
	G4	1.27 ± 0.05 $^{a\,A}$	1.21 ± 0.13 $^{a\,A}$	1.47 ± 0.11 $^{a\,A}$	1.15 ± 0.09 $^{a\,A}$

Values are means ± SE Means in the same column without a common small letter differ significantly at (P<0.05).
Means in the row without a common capital letter differ significantly at (P<0.05). **G1**: Control negative non-infected non-treated group
G2: Infected non-treated group **G3:** Infected treated with diclazuril group **G4:** Protected by diclazuril then infected group

Table 6: Effect of diclazuril on minerals and electrolytes in rabbits experimentally infected with *E. stiedae*

Parameters	Groups (n=5)	Days Post Infection			
		18	25	32	39
Ca (mg/dl)	G1	10.50 ± 0.45 $^{a\,A}$	10.78 ± 0.32 $^{a\,A}$	10.94 ± 0.31 $^{a\,A}$	10.66 ± 0.14 $^{a\,A}$
	G2	10.27 ± 0.31 $^{a\,A}$	9.26 ± 0.39 $^{b\,A}$	9.50 ± 0.26 $^{b\,A}$	9.66 ± 0.51 $^{b\,A}$
	G3	10.07 ± 0.75 $^{a\,A}$	11.25 ± 0.57 $^{a\,A}$	10.64 ± 0.12 $^{a\,A}$	10.55 ± 0.22 $^{a\,A}$
	G4	9.43 ± 0.65 $^{a\,A}$	10.45 ± 0.26 $^{a\,A}$	10.35 ± 0.34 $^{a\,A}$	10.60 ± 0.17 $^{a\,A}$
iP (mg/dl)	G1	5.65 ± 0.09 $^{a\,A}$	5.37 ± 0.35 $^{a\,A}$	5.48 ± 0.40 $^{a\,A}$	5.64 ± 0.11 $^{a\,A}$
	G2	5.36 ± 0.27 $^{a\,A}$	5.37 ± 0.04 $^{a\,A}$	5.11 ± 0.10 $^{a\,A}$	5.31 ± 0.18 $^{a\,A}$
	G3	5.23 ± 0.11 $^{a\,A}$	4.82 ± 0.29 $^{a\,A}$	5.17 ± 0.11 $^{a\,A}$	5.36 ± 0.27 $^{a\,A}$
	G4	5.33 ± 0.24 $^{a\,A}$	5.48 ± 0.25 $^{a\,A}$	4.87 ± 0.04 $^{a\,A}$	5.15 ± 0.19 $^{a\,A}$
Sodium (MEq/L)	G1	100.82 ± 6.80 $^{a\,A}$	105.88 ± 4.90 $^{a\,A}$	112.91 ± 10.29 $^{a\,A}$	105.07 ± 8.31 $^{a\,A}$
	G2	101.31 ± 8.60 $^{a\,A}$	111.11 ± 11.35 $^{a\,A}$	120.73 ± 5.72 $^{a\,A}$	108.22 ± 4.75 $^{a\,A}$
	G3	113.69 ± 15.14 $^{a\,A}$	108.17 ± 6.05 $^{a\,A}$	113.47 ± 11.19 $^{a\,A}$	124.02 ± 10.21 $^{a\,A}$
	G4	111.93 ± 6.23 $^{a\,A}$	112.09 ± 5.80 $^{a\,A}$	116.60 ± 13.09 $^{a\,A}$	119.11 ± 10.50 $^{a\,A}$
Potassium (MEq/L)	G1	4.68 ± 0.25 $^{a\,A}$	4.52 ± 0.05 $^{a\,A}$	4.33 ± 0.50 $^{a\,A}$	4.05 ± 0.10 $^{a\,A}$
	G2	4.23 ± 0.24 $^{a\,A}$	4.22 ± 0.17 $^{a\,A}$	4.14 ± 0.10 $^{a\,A}$	4.07 ± 0.12 $^{a\,A}$
	G3	4.18 ± 0.21 $^{a\,A}$	4.38 ± 0.21 $^{a\,A}$	3.99 ± 0.20 $^{a\,A}$	3.16 ± 0.66 $^{a\,A}$
	G4	4.48 ± 0.23 $^{a\,A}$	4.15 ± 0.13 $^{a\,A}$	3.89 ± 0.07 $^{a\,A}$	4.09 ± 0.21 $^{a\,A}$
Chloride (mg/dl)	G1	66.98 ± 4.54 $^{a\,A}$	56.23 ± 5.30 $^{a\,A}$	55.92 ± 7.82 $^{a\,A}$	52.90 ± 0.61 $^{a\,A}$
	G2	57.31 ± 2.13 $^{a\,A}$	51.82 ± 2.03 $^{a\,B}$	61.95 ± 1.40 $^{a\,A}$	60.44 ± 0.07 $^{a\,A}$
	G3	57.77 ± 2.56 $^{a\,A}$	51.12 ± 1.25 $^{a\,A}$	50.81 ± 5.49 $^{a\,A}$	59.24 ± 2.05 $^{a\,A}$
	G4	59.16 ± 1.80 $^{a\,A}$	58.81 ± 5.82 $^{a\,A}$	58.39 ± 2.75 $^{a\,A}$	60.25 ± 6.66 $^{a\,A}$

Values are means ± SE Means in the same column without a common small letter differ significantly at (P<0.05).
Means in the row without a common capital letter differ significantly at (P<0.05). **G1**: Control negative non-infected non-treated group
G2: Infected non-treated group **G3:** Infected treated with diclazuril group **G4:** Protected by diclazuril then infected group

Table 7: Effect of diclazuril on serum malondialdehyde and total antioxidant capacity in rabbits experimentally infected with *E. stiedae*

Parameters	Groups (n=5)	Days Post-Infection			
		18	25	32	39
MDA (nmol/ ml)	G1	2.60 ± 0.11 $^{a\,A}$	1.74 ± 0.21 $^{b\,B}$	1.87 ± 0.34 $^{b\,B}$	1.47 ± 0.14 $^{b\,B}$
	G2	3.51 ± 1.44 $^{a\,B}$	2.88 ± 0.21 $^{a\,B}$	5.52 ± 1.77 $^{a\,A}$	2.92 ± 0.81 $^{a\,B}$
	G3	2.07 ± 0.68 $^{a\,A}$	1.62 ± 0.15 $^{b\,A}$	1.42 ± 0.10 $^{b\,A}$	0.96 ± 0.11 $^{b\,A}$
	G4	1.40 ± 0.29 $^{a\,A}$	1.38 ± 0.08 $^{b\,A}$	1.68 ± 0.53 $^{b\,A}$	1.48 ± 0.46 $^{b\,A}$
Total antioxidant capacity (mM/L)	G1	1.41 ± 0.02 $^{a\,A}$	1.27 ± 0.05 $^{a\,A}$	1.27 ± 0.12 $^{a\,A}$	1.36 ± 0.00 $^{a\,A}$
	G2	1.30 ± 0.06 $^{a\,A}$	1.33 ± 0.08 $^{a\,A}$	1.37 ± 0.08 $^{a\,A}$	1.37 ± 0.06 $^{a\,A}$
	G3	1.35 ± 0.10 $^{a\,A}$	1.26 ± 0.04 ^{aA}	1.39 ± 0.02 $^{a\,A}$	1.31 ± 0.10 $^{a\,A}$
	G4	1.46 ± 0.07 $^{a\,A}$	1.20 ± 0.16 $^{a\,A}$	1.39 ± 0.11 $^{a\,A}$	1.22 ± 0.08 $^{a\,A}$

Values are means ± SE. Means in the same column without a common small letter differ significantly at (P<0.05).
Means in the row without a common capital letter differ significantly at (P<0.05).**G1**: Control negative non-infected non-treated group
G2: Infected non-treated group G3: Infected treated with diclazuril group **G4:** Protected by diclazuril then infected group

bilirubin, indirect bilirubin, and cholesterol in diclazuril treated and the protected groups contrasted with the negative control. While animals in group 2 had significant increases in the rates of total bilirubin, direct bilirubin and indirect bilirubin, and significant decrease in value of cholesterol at 32- and 39-days post-infection (Table 4).

There were no significant changes in ALT, AST, ALP, and GGT values between diclazuril treated and the protected groups and the negative control. While there is a significant rise in these values in group 2 at 25- and 32-days post-infection, besides a significant rise in the values of GGT was observed at day 39 post-infection (Table 5). For urea and creatinine values, only a significant rise in the values of urea was detected in group 2 at day 39 post-infection when compared with other groups (Table 5). The mean values of serum inorganic phosphorus, sodium, potassium, and chloride showed insignificant changes in all groups at any time during the experiment (Table 6) while group 2 had a significant decrease in serum calcium levels at 25, 32-, and 39-days post-infection (Table 6). By comparing all groups with each, animals of group 2 showed significant elevation of MDA at 25-, 32-, and 39-days post-infection. Instead, insignificant variations were detected in serum total antioxidant capacity in all groups along the entire time of the experiment (Table 6).

Histopathological findings

Gross lesions

No abnormal macroscopic findings were observed in the liver of rabbits in diclazuril treated (Fig. 1 C) or protected (Fig. 1 D) in contrast to negative control (Fig. 1 A) groups. While typical gross lesions of hepatic coccidiosis were observed in rabbits of group 2. The lesions included great enlargement of the liver. There were also various yellowish to white nodules of different sizes upon the liver surface. Cross-section of these nodules revealed yellowish to creamy color fluid (Fig. 1, B).

Microscopical lesions

The structure of liver parenchyma and portal areas in diclazuril treated (G3) (Fig 2. D and Fig.3 E) and protected (G4) (Fig 2. E and Fig.6 F) groups had normal liver structure contrasted with the negative control (Fig 2. A and Fig.3 A) while the liver of rabbits in group 2 showed bile duct epithelium hyperplasia forming finger-like projections in the lumen. It contained different developmental stages of *Eimeria* (microgametes, macrogametes, and oocyst); furthermore, cholangitis and pericholangitis with mononuclear cells infiltration and engorged blood vessels were evidenced (Fig. 3 B and Fig. 3 C) in contrast to the negative control group (Fig.1 A and Fig. 2 A). Besides, the liver parenchyma exhibited focal areas of necrosis and mononuclear cell infiltrations. Other microscopical features included the fatty change of hepatocytes, hemorrhages in hepatic parenchyma, and dilatation of hepatic sinusoids were also observed (Fig. 1, D).

4. Discussion

The clinical signs in infected non-treated rabbits were similar to those previously reported (Cam et al., 2008; Singla et al., 2000) while diclazuril treated and protected groups develop very mild clinical signs indicating its efficacy to control the infection.

Diclazuril treated and protected groups had similar red blood cell count, hemoglobin and packed cell volume compared with negative control while there is a significant decrease for their levels in the group 2 infected with *E. stiedae* indicating the presence of anemia (Weiss and Goodnough, 2005) which might be attributed to the severity of infection, malnutrition, and anorexia. The significant decrease of MCV and MCHC on 39-day post-infection in group 2 indicated that the anemia is of inflammatory disease described microcytic hypochromic as a part of the immune response that occurs with infectious and inflammatory diseases, immune cells release cytokines which interfere with the body's capability to engage and utilize iron. Moreover, cytokines may affect the output and regular activity of EPO (Agarwal and Prchal, 2009).

The significant rise in total leukocytic count in group 2 was identified by neutrophilia and eosinophilia with a substantial decrease in lymphocyte count. These outcomes are steady with the earlier reports which ascribed these effects to an inflammatory response to infection (Cam et al., 2008). Neutrophilia is pathologically induced by infections but also might be a possible marker of the stress response because of the endogenous release of corticosteroids which have a crucial function in regulating the circulating concentration of leukocytes (Merlot, 2004; Zahorec, 2001). Lymphopenia could be attributed to the control of adrenocortical hormones upon lymphoid tissues and lymphocytes, resulting in the increased dissolution of the cells during the disease (Anwar et al., 1999). Eosinophilia in rabbits rarely occurs but may be associated with parasitism. Eosinophilia occurs as a pathophysiologic response to infection with parasites through participation in the immune response by discharging their cytotoxic granular contents onto the parasites, which kills them (Capron, 1991; Rothenberg and Epthtein, 1998).

The significant rise in serum total protein values in the second group at 18th-day post-infection may be due to the release of proteins from harmed hepatocytes (Barriga and Arnoni, 1979). This primary increase in serum total protein was followed by significant decreases at 25, 32-, and 39-days post-infection. A similar reduction in serum albumin values was noticed at the same experimental periods. Serum albumin may be diminished due to reduced albumin production resulting from hepatic degeneration related to the infection as the liver is the main site for protein synthesis (Cam et al., 2008). Also, Albumin is deliberated as a negative acute-phase protein and its concentration is likely to decrease mostly during inflammation (Hashemnia et al., 2014). Hyperglobulinemia was noticed at 18th day post-infection suggesting a specific result of liver injury in liver disease and globulin levels may rise in compensation to declined albumin levels due to liver injury. Furthermore, the significant rise in serum total bilirubin, direct bilirubin, and indirect bilirubin in addition to liver enzymes activities ALT, AST, ALP, and GGT in infected non-treated rabbits suggesting that the liver might be adversely affected by coccidiosis. Elevation of bilirubin might be caused by the sexual multiplication of *E. stiedae* in the ductal epithelium of the liver (Barriga and Arnoni, 1979) or might be attributed to ductal obstruction (Martine and Yvoré, 1974). The increase in serum enzymatic activity may be related to cell destruction due to the parasitic infection, which caused the escape of enzymes into the bloodstream (Hanada et al., 2003). High levels of ALT and AST might be a sign of the broken epithelial covering of the bile channel due to increased numbers of parasite oocysts (Sanyal and Sharma, 1990). Elevated levels of ALP and GGT might be the result of the inflammatory reaction that causes degenerative and necrotic changes in the epithelial lining of the bile duct. These findings were further supported by the histopathological picture of the liver, which showed severe hydropic degeneration and necrotic changes of hepatocytes (Abu-Akkada et al., 2010). Besides, the reduction in serum cholesterol level could be attributed to liver failure and decreased cholesterol synthesis (Matsuoka et al., 2009). While in diclazuril groups the levels of these values were not changed indicating that diclazuril protected the liver from damage induced by *E. stiedae* infection.

The increase in blood urea in group 2 only at the ending of the study was accredited to a disorder in the urea cycle in the liver (Yaplito-Lee et al., 2013). Serum calcium levels were meaningfully reduced in group 2 throughout the study this may be due to hypoalbuminemia which results in decreasing protein-bound calcium and may contribute to hypocalcemia (Sharp et al., 2009).

The rise in serum concentration of malondialdehyde in the group 2 might be attributed to the destruction of the liver parenchyma and bile duct (Cam et al., 2008) and was likewise seen in *E. tenella* infected chicks

(Ersalan et al., 2004). While its levels were not increased in diclazuril treated or protected groups suggesting its protective effect from oxidative stress in hepatic coccidiosis.

The histopathological changes in both post-mortem lesions of hepatic coccidiosis and microscopical changes in group 2 were consistent with the previous research (Abu-Akkada et al., 2010; Cam et al., 2008; Singla et al., 2000). In contrast, diclazuril treated or protected groups had a normal post-mortem and microscopical architecture of the liver indicating its protective effect against *E. stiedae* infection.

Conclusions

In conclusion, *E. stiedae* caused various significant changes in hematological, blood biochemical, and oxidative stress biomarkers, in addition to altered histopathological changes included macroscopical and microscopical pictures. Either the treated and protected groups showed no significant changes in most of these parameters implicated that diclazuril appeared to be a potent anticoccidial drug against *E. stiedae* infection in rabbit either for treatment or as prophylaxis, however further studies may be required to evaluate the pharmacokinetics of the drug to explore its tissue residues for safety for consumption. Interestingly, the treatment was recorded not to be successful unless a sanitation program was instituted simultaneously because infected animals spread millions of oocysts and seriously contaminate their environment.

Competing Interests

The authors have no conflict of interest.

References

Abu-Akkada, S.S., Oda, S.S., Ashmawy, K.I. 2010. Garlic and hepatic coccidiosis: prophylaxis or treatment? Trop. Anim. Health Prod. 42, 1337-1343.

Agarwal, N., Prchal, J.T. 2009. Anemia of chronic disease (anemia of inflammation). Acta Haematol. 122(2–3), 103–108.

Al-Mathal, E.M. 2008. Hepatic coccidiosis of the domestic rabbit *Oryctolagus cuniculus domesticus* L. in Saudi Arabia. World J. Zool. 3, 30-35.

Al-Rukibat, R.K., Irizarry, A.R.; Lacey, K., Lazacos, K.R., Storandt, S.T., Denicola, D.B. 2001. Impression smears of liver tissue from a rabbit. Vet. Clin. Pathol. 30, 57–61.

Anwar, A. H.; Kazmi, S. I. H., Khan, M. N. 1999. Effects of experimentally induced coccidiosis on some blood parameters of buffalo calves. Pakistan J. Biol. Sci. 2, 1024-1026.

Bancroft, J.; Stevens, A., Turner, D. 1996. Theory and practice of histopathological techniques.4th ed. New York, USA.

Barriga, O.O., Arnoni, J.V. 1979. *Eimeria stiedae*: Weight, oocyst output, and hepatic functions of rabbits with graded infection. Exp. Parasitol. 48, 407-414.

Bhat, T.K., Jithendran, K.P., Kurade, N.P. 1996. Rabbit coccidiosis and its control: a review. World Rabbit Sci. 4, 37–41.

Çam, Y., Alasever, A., Eraslan, G., Kibar, M., Atalay, O., Beyaz, L., Inci, A., Liman, B.C. 2008. *Eimeria stiedae*: experimental infection in rabbits and the effect of treatment with toltrazuril and ivermectin. Exp. Parasitol. 119, 164-171.

Capron, M.1991. Eosinophils and parasites. Ann. Parasitol. Hum. Comp. 66(1), 41-5.

El-Raffa, A. M., Youssef, Y. K., Iraqi, M. M., Khalil, M. H., García, M. L., Baselga, M. 2005. Developing rabbit lines for meat production in Egypt and Saudi Arabia: overview, synthesizing plan, descriptive performance, and future prospects. In Proc.: The 4th International Conference on Rabbit Production in hot climates. Genetics Section N4. Sharm El-Sheik, Egypt, 24-27 February. ERSA.

Eraslan, G., Çam, Y., Eren, M., Liman, B.C. 2004. Changes in malondialdehyde level and catalase activity and effect of toltrazuril on these parameters in chicks infected with *Eimeria tenella*. Bull. Vet. Inst. Pulawy 48, 251–255.

Gomez-Bautista, M., Garcia, M.V., Rojo-Vazquez, F.A. 1986. The levels of total protein fractions in the serum of rabbits infected with *Eimeria stiedae*. Ann.Parasitol. Hum. Comp. 61, 393–400.

Hanada, S., Omata, Y., Umemoto, Y., Kobayashi, Y., Furuoka, H., Matsui, T., Maeda, R., Saito, A. 2003. Relationship between lier disorders and protection against *Eimeria stiedai* infection in rabbits immunized with soluble antigens from the bile of infected rabbits. Vet. Parasitol. 111, 261-266.

Hashemnia, M.; Khodakaram-Tafti, A.; Razavi, S.M., Nazifi, S. 2012. Hematological and serum biochemical analyses in experimental caprine coccidiosis. J. Parasit. Dis. 38(1), 116–123.

Hauptman, K., Tichy, F., Knotek, Z. 2001. Clinical diagnostics of hepatopathies in small mammals: Evaluation of importance of individual method. Acta Vet. Brno 70, 297- 311.

Jarvinen, J.A. 1999. Prevalence of *Eimeria macusaniensis* (Apicomplexa: Eimeriidae) in midwestern Lama Spp. J.Parasitol.85, 373–376.

Karaer, Z. 2001. *Evcil Tavsanlarda* (*Oryctolagus cuniculus*) Coccidiosis. In: Dincer, S (Ed.), Coccidiosis Türkiye Parasitoloji Derneği Yayinlari, no. 17, Basim, M., Izmir,B. pp. 269-278.

Lebas, F., Coudert, P., De Rochambeau, H., Thebault, RG. 1986. The rabbit: husbandry, health and production. FAO Animal Production and Health Series, 21. FAO Rome.

Lebdah, M.A., Shahn, A.M. 2011. Closteridia as an etiological agent of mucoid enteropathy in rabbits. Nat. Sci., 9:63–72.

Li, M.H., Ooi, H. K. 2009. Fecal occult blood manifestation of intestinal *Eimeria* spp. infection in rabbit. Vet. Parasitol. 161, 327-329.

Long, P.L.; Millard, B.J.; Joyner, L.P., Norton, C.C. 1976. A guide to laboratory techniques used in the study and diagnosis of avian coccidiosis. Folia Vet. Lat. 6: 201–217.

Martine, G., Yvore, P. 1974. Aspects pathogeniques de la coccidiose hepatique du lapin domestique. Cahiers Med. Veferin. 43, 147-158.

Matsuoka, T., Mizuguchi, H., Mizoguchi, Y., Endho, T., Kamata, R., Fukuda, K. et al. 2009. Effects of restricted feeding on blood parameters in pregnant rabbits. J. Toxicol. Sci. 34, 129–137.

Merlot, E. 2004. Conséquences du stress sur la fonction immunitaire chez les animaux d'élevage. INRA Prod. Anim. 17, 255-264.

Pakandl, M. 2009. Coccidia of rabbit: a review. Folia Parasitol. 56,153-166.

Rothenberg, M. E. 1998. Eosinophilia. New England J.Med. 338(22), 1592-1600.

Sanyal, P.K., Sharma, S.C. 1990. Clinicopathology of hepatic coccidiosis in rabbits. Indian J. Anim. Sci. 60, 924-928.

Sharp, C.R., Kerl, M.E., Mann, F. 2009. A comparison of total calcium, corrected calcium, and ionized calcium concentrations as indicators of calcium homeostasis among hypoalbuminemic dogs requiring intensive care. J. Vet. Emerg. Crit. Care 19, 571-578.

Singla, L.D., Juyal, P.D., Sandhu, B.S. 2000. Pathology and therapy in naturally *Eimeria stiedae*-infected Rabbits. J. Protozool. Res.10, 185–191.

Sivajothi, S.; Rayulu, V. C., Reddy, B. S. 2013a. Haematological and biochemical changes in experimental *Trypanosoma evansi* infection in rabbits. J. Parasitic Dis. 39(2), 216-220.

Sivajothi, S.; Reddy B.S., Rayulu, V.C. 2013b. Intestinal coccidiosis infection in domestic rabbits. Inter. J. Biol. Res., 2(2):48–50.

Snedecor, G. W., Cochran, W. G. 1980. Statistical Methods.7th ed., Iowa State University Press. USA.

Van Praag, E. 2011. Protozoal enteritis: Coccidiosis. http://www.medirabbit.com/EN/GI_diseases/Protoz

Vanparijs, O., Marsboom, R., Desplenter, L. 1989b. Diclazuril, a new broad spectrum anticoccidial drug in chickens. I. Dose titration studies and pilot floor pen trials. Poultry sci. 68(4), 489-495.

Vanparijs, O., Hermans, L., Van Der Flaes, L., Marsboom, R. 1989a. Efficacy of diclazuril in the prevention and cure of intestinal and hepatic coccidiosis in rabbits. Vet. Parasitol. 32, 109-117.

Vennen, K.M., Mitchell, M.A. 2009. Rabbits. In: Mitchell, M.A., Tully Jr., T.N. (Eds.), Manual of Exotic Pet Practice. B. Saunders, St. Louis. pp. 375–405.

Weiss, G., Goodnough, LT. 2005. Anemia of chronic disease. N. Engl. J. Med., 352, 1011-23.

Wiid, I.; Seaman, T.; Hoal, E.; Benade, A. J. S., Van Helden, P. D. 2004. Total antioxidant levels are low during active TB and rise with anti-tuberculosis therapy. IUMB Life 56,101-106.

Yaplito-Lee, J., Chow, C.W., Boneh, A. 2013. Histopathological findings in livers of patients with urea cycle disorders. Mol. Genet. Metabol. 108, 161–165.

Zahorec, R. 2001. Ratio of neutrophil to lymphocyte counts—rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl. Lek. Listy., 102: 5-14.