



Effect of *Eimeria* Infection on Hematology and Biochemistry of Sheep at Sohag Governorate, Egypt

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ABSTRACT

Eimeria infection is one of the most common protozoal disease affecting sheep worldwide. This study was carried out to investigate the effect of *Eimeria* species (spp.) infection in native sheep breed at Sohag governorate. Total number of investigated sheep was 101. All sheep were examined clinically; blood and fecal samples were collected from each animal. Coprological examination was performed using the standard flotation technique. *Eimeria* oocysts were counted microscopically using the high power. Oocysts numbers were used to classify the degree of infection into mild and severe. Complete blood picture was performed using automatic blood cell counter. Levels of total protein, albumin, and globulin were determined in sera of infected sheep. In addition to the activities of liver enzymes (ALT, AST, ALP) and serum calcium level were measured using the spectrophotometer. Results of hematology and blood biochemistry were analyzed statistically to compare between mild and heavy infections. The total number of sheep infected with *Eimeria* spp. was 67/101 (66.4%). Infected sheep manifested variable clinical signs such as bloody and/or mucoid diarrhea, dehydration, lethargy, emaciation, fever and loss of wool. In this study, total erythrocytic count and hemoglobin concentration were significantly decreased in heavy *Eimeria* spp. infection. Total leucocytes count and granulocytes percentage were significantly elevated in mildly infected sheep. While, lymphocyte percentage was increased in case of heavy infection. Total proteins were significantly decreased in case of heavy infection. Albumin/ globulin ratio was considerably altered. The liver enzymes, ALT and ALP were elevated in heavily infected sheep. AST was significantly higher when compared with mild cases. This study highlighted the risk of heavy Eimeriosis that can result in severe anemia and altered liver functions. Therefore, Eimeriosis is a clinical disease of economic importance in productive animals such as sheep.

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INTRODUCTION

Eimeriosis is an economically important disease of small ruminants. It is one of the major causes of losses in producing countries due to the reduction in animal's weight gains or stunted growth (Daugshies and Najdrowski, 2005; Andrews, 2013; Shaheed and Al-Azizz, 2020). Coccidiosis was described as (*Eimeriosis sensu stricto*) it is a host-specific protozoan infection caused by several species of the genus *Eimeria*, which is an enteric disease affecting small and large intestinal mucosa (Chartier and Paraud, 2012). It affects most domestic animal

species particularly young animals (Constable *et al.*, 2017).

The characteristic clinical signs of *Eimeria* species (spp.) infection include diarrhea, fever, inappetence, weight loss, emaciation, and death in extreme cases. Eimeriosis mainly is subclinical in adult animals and they act as reservoirs of infection. In sheep, *Eimeria* spp. can infect all ages but lambs are more susceptible (Taylor and Catchpole, 1994 ; Taylor, 1995).

In sheep, developmental stages of *Eimeria* 's life cycle, both the sexual and asexual phases occurs in epithelial cells of the intestine (Reeg et al., 2005). Acute Eimeriosis occurs if animals subjected to stressors such as dietary changes, long travel, and sudden changes of temperature or environment and or presence of concurrent infection. Nutritional deficiencies of minerals and or vitamins can also influence animal's resistance and increase liability to such infections (Taylor, 1995). Collectively, the severity of Eimeriosis is depending on the infectious dose, immune status and age of the host (Taylor and Catchpole, 1994 ; Kaya, 2004).

Previous reports emphasized that the exposure of intestinal lining to the destructive effect of *Eimeria* spp. infection results in loss of blood protein and vital components and eventually leads to loss of conditions and illthriftiness in infected animals (Pandey et al., 2010). In native sheep breed of Egypt, *Eimeria* spp. infection has a medical and economic importance because of its distressing effects on animal health (Ghanem and Abd El-Raof, 2005). In addition, El-Manyawe et al., (2010) pointed to the occurrence of significant alterations in liver enzymes upon protozoal infection such as *Eimeria* spp.

Therefore, the objectives of this study were to record the infection rate of *Eimeria* spp. in local sheep breed reared in Sohag governorate. In addition, clinical examination and hemato-biochemical analyses were performed to evaluate the effects of *Eimeria* spp. infection in sheep.

MATERIALS AND METHODS

1. Study area and sample collection:

Study areas included several villages at Sohag governorate which located at southern Egypt. Total number of samples were 101. Numbers of sheep sampled are described as following, number of villages/ town, numbers of samples (8/ Sohag city, 25; 3/ Akhmim, 11; 1/ El Maragha, 3; 4/ El Mansha, 22 and 5/ El Salaa, 40 samples). Fecal and blood samples were collected from local sheep breed maintained in small holders and owned by farmers. Sample's collection had been performed in period between Feb. 2018 and Oct. 2020. Blood samples were taken by jugular vein puncture using a disposable plastic syringe and needle into EDTA anticoagulant containing tubes for hematological analysis (Coles, 1986).

2. Hematological and biochemical analyses:

The EDTA- anti coagulated blood was used to perform complete blood count (CBC) using automated blood cell counter (Exigo, produced by boule medical

AB, Domnarvsgatan, Sweden). The machine measures the following hematological parameters, erythrocyte count, leukocyte count, hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and differential leukocyte count.

For biochemical analysis, amount of 5 mL blood was collected onto tubes without anticoagulant, left to coagulate and centrifuged at 3000 rpm for 5 minutes to obtain the serum. Sera were preserved at -20°C until performing analysis. Blood biochemical analyses involved the estimation of liver enzymes activities including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphate (ALP). The activity of liver enzymes was kinetically determined by using Digital Ultraviolet Spectrophotometer (CE 790 KD NO 19 v4.74A. Shenzhen Kindle Medical Devices Co., Ltd, China) and according to the manufacturer kit instructions of each enzyme (Wiesbaden, Germany). Additionally, serum total protein, albumin and calcium level were calorimetrically evaluated by using the same machine and the specific kit of each parameter (Hannover, Germany).

3. Fecal analysis:

The fecal samples were collected from the rectum of sheep using sterile disposable glove, placed in a plastic cup, and transferred directly to the laboratory. The samples were stored at 4°C until examination time. The characters of each fecal sample were inspected grossly, presence of blood or mucous was recorded. After that, samples were screened by direct smear and then subjected to standard floatation test for detection of *Eimeria* spp. using saturated sucrose solution (Blagburn and Butler, 2006, Kaplan and Nielsen, 2010). A quantitative analysis of *Eimeria* oocysts per high power field was conducted on all *Eimeria* spp. positive fecal floatation to determine the severity of infection.

4. Statistical analysis:

Data were statistically analyzed by a student *t*-test to compare means between groups using GraphPad prism software (La Jolla, CA, USA). The results were presented as means and standard deviation (SD). Data were significant if $P < 0.05$.

RESULTS

Clinical presentation of investigated sheep:

This study focused on clinical cases of sheep live in small-scale farms distributed in several villages and small towns that belong to Sohag governorate. Number of sampled sheep was 101 and total number of

sheep infected with *Eimeria* spp. was 67/101 (66.4%). The signalment of all infected sheep including the age, sex and physiological status is illustrated in table 1. Sheep infected with *Eimeria* spp. suffered from variable clinical manifestations such as diarrhea, dehydration, lethargy, emaciation, fever and easily detached wool. In addition, some cases were recumbent or unable to walk (Figs.1, 2 and 3). Infected sheep with *Eimeria* spp. were categorized into two groups, lambs less than one year (18/67) and adults aged between 1 and 5 years (49/67). The most encountered clinical manifestations were organized in table 2. Numbers and percentages of cases affected by each sign were tabulated.

Table 1: Signalment of infected sheep with *Eimeria* spp.

Sex	Male		Female				
Animals infected with <i>Eimeria</i> Spp. (67)	23		44				
	< 1 year	1-5 years	1-5 years				
			34				
	< 1 year	1-5 years	< 1 year	Pregnant	Non pregnant		
				1	33		
	< 1 year	1-5 years	< 1 year	Lactating	Non lactating		
				-	1	7	26
8	15	10	-	1	7	26	

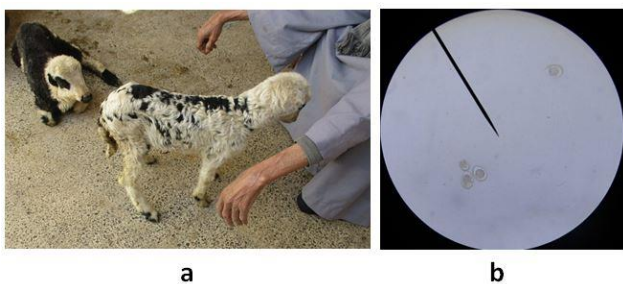


Fig. 1: Infected lambs with *Eimeria* spp. show signs of dullness (a). Microscopic examination of fecal flotates using the high power (x 40) revealed the unsporulated oocysts of *Eimeria* spp. and mild degree of Eimeriosis (b).

Fecal analysis of infected sheep:

Fecal samples of all infected sheep were examined microscopically for the presence of oocysts

of *Eimeria* spp. after performing the flotation technique using the high power (X 40). In this study, we counted *Eimeria* spp. oocysts per high power field to monitor oocyst shedding in each case as an indication for the severity of infection. The data generated from oocysts counting was used to classify the infected sheep into two groups, mild and heavy degree of infection. The mild infection was considered if oocysts number ranged between 1-10 and severe infection if oocysts were 11 or more. Mildly infected sheep had demonstrated signs of diarrhea, dullness and dehydration as shown in Figure (1a & b). While heavily, infected ones were suffered from marked clinical illness that included recumbency and inability to stand plus the deeply stained hind quarters and tail with fecal matter due to profuse diarrhea (Fig. 2 a, b, c and d). Adult sheep infected with *Eimeria* spp. mainly did not suffer from diarrhea but showed marked dullness, roughness of coat, easily detached wool, variable degree of emaciation and poor body conditions (Fig. 3 a and b).

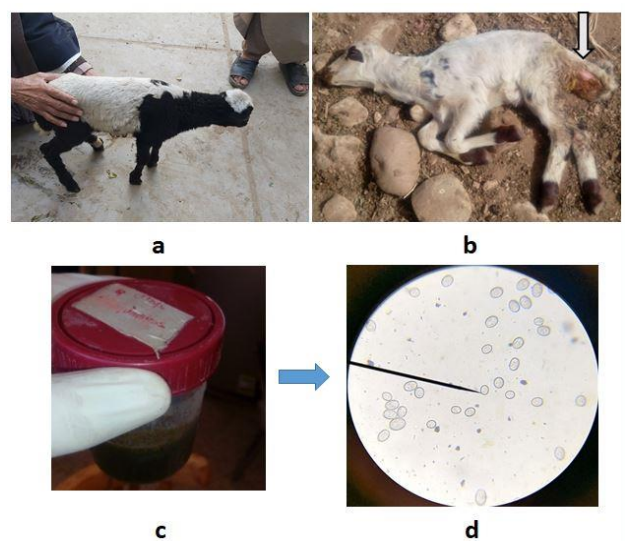


Fig. 2: Severe diarrhea, dullness, depression and inability to stand (a). Lateral recumbency (b) and bloody diarrhea (c) observed in lamb heavily infected with Eimeriosis. Microscopic examination of fecal sample revealed abundant *Eimeria* spp. oocysts using the high power (x 40) (d).



Fig. 3: Dullness, rough coat (a), emaciation and poor body condition (b) in adult sheep infected with *Eimeria* spp.

Table 2: Clinical signs observed in infected sheep with *Eimeria* spp.

Clinical picture		Less than 1 year (18 lambs)		More than 1 year (49 adults)	
		No.	%	No.	%
Diarrhea	Yes	7	38.89	9	18.38
	No	11	61.11	40	81.63
Dehydration	Yes	2	11.11	1	2.04
	No	16	88.89	48	97.96
Lethargy	Yes	3	16.67	5	10.20
	No	15	83.33	44	89.80
Emaciation	Yes	7	38.89	11	22.45
	No	11	61.11	38	77.55
Fever	Yes	1	5.56	8	16.33
	No	17	94.44	41	83.67
Loss of wool	Yes	11	61.11	34	69.39
	No	7	38.89	15	30.61

Laboratory evaluation of blood parameters of infected sheep with *Eimeria* spp.:

Blood picture of infected sheep was analyzed automatically as mentioned above in methods. According to the oocyst number of *Eimeria* spp. and severity of infection, blood parameters of the mild and heavy infected cases were statistically compared (Fig. 4). Both RBCs count ($10^{12}/l$) and hemoglobin content (g/dl) were significantly higher in mildly infected sheep than heavily infected ones at ($P= 0.013$ and 0.019 respectively). PCV percentages were around 30 in mild infection and less than 30 in heavy infection however, this difference was not significant statistically ($P= 0.076$). WBCs count ($10^9/l$) and granulocytes percentage were relatively elevated in mild infected sheep ($P= 0.149$ and 0.081) while, lymphocyte percentage was higher in case of heavily infected ones with *Eimeria* spp.

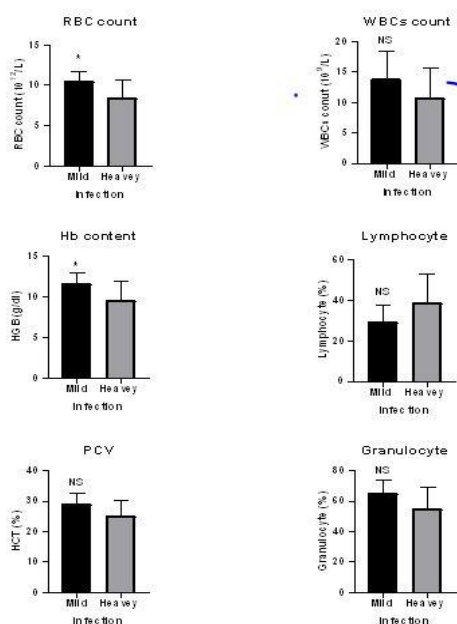


Fig. 4: Hematological parameters of mild and heavy *Eimeria* spp. infections in sheep. Data is shown as mean \pm SD.

Laboratory evaluation of serum biochemistry in infected sheep with *Eimeria* spp.:

In this study, the levels of blood proteins (total protein, albumin, and globulin) were compared in case of mild and heavy infections. In addition to the activities of liver enzymes as ALT, AST, ALP, and total calcium level (Fig. 5). In both mild and heavy infections, albumin (g/dl) levels were nearly similar ($P=0.53$). While, globulin values (g/dl) showed significant increase in mild-infected sheep ($P= 0.0055$). Albumin/ globulin ratio was significantly altered ($P= 0.016$). Thus levels of total protein (g/dl) were also higher in mild-infected sheep ($P= 0.0017$). Evaluation of liver enzymes showed a significantly higher value of AST in heavily infected sheep ($P=0.042$). Although ALT and ALP enzymes were also increased in case of heavy infection but they were statistically not significant ($P= 0.091$ and 0.173). In addition, serum calcium levels in both mild and heavy infected sheep were similar ($P= 0.274$).

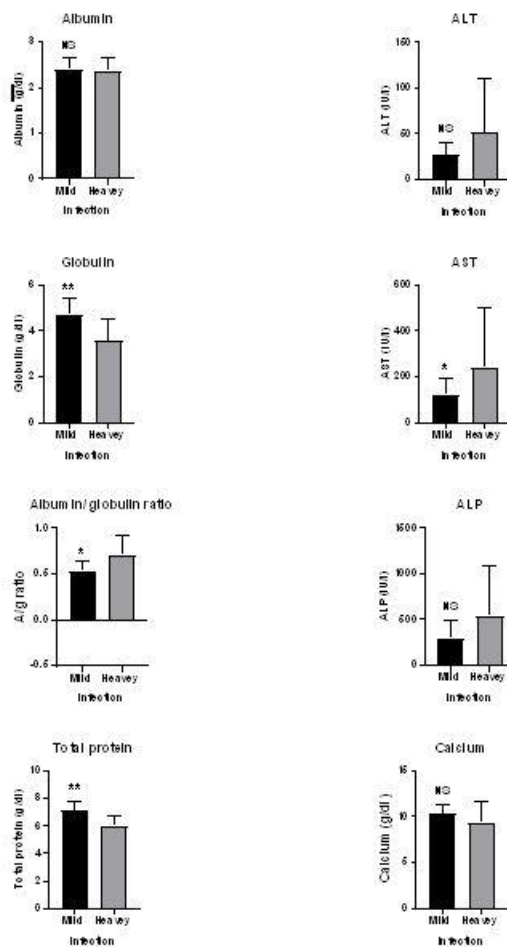


Fig. 5: Biochemical results of mild and heavy *Eimeria* spp. infections in sheep. Data is shown as mean \pm SD.

DISCUSSION

The *Eimeria* spp. infection is one of the most encountered protozoal infection in sheep worldwide. In Egypt, assessment of this parasite prevalences and the associated risk factors were important research topics in many studies and involved several regions all over Egypt (Mohamaden *et al.*, 2018; Abdelaziz *et al.*, 2021). Sultan *et al.* (2016) reported a high incidence of *Eimeria* spp. infection in Kafrelsheikh governorate and in Dakahlia governorate, *Eimeria* spp. infection was 68.4% (El-Alfy *et al.*, 2020). In this study, coprological examination of sheep fecal samples confirmed the infection rate of *Eimeria* spp. in Sohag governorate was 66.4%. Although there are differences between the northern and southern Egyptian governorates in weather, the infection rates are comparable because farming conditions in Egyptian villages are similar.

In this study, among 67 sheep infected with *Eimeria* spp. there were 18 lambs (26.8%) and 49 (73.1%) adults. *Eimeria* spp. infection was recorded in adults more than in young sheep. Whereas, Taylor (1995) stated that all ages of sheep are susceptible to *Eimeria* spp. infection and younger lambs are more susceptible. Previous researches also mentioned that young small ruminants, less than one year age have higher susceptibility to Eimeriosis than adult ones (Sharma *et al.*, 2017; El-Alfy *et al.*, 2020 and Abdelaziz *et al.*, 2021).

Based on the clinical observations in the current study, diarrhea, dehydration, lethargy and emaciation were the most predominant manifestations in lambs. On the other hand, fever and loss of wool were mostly encountered in adult sheep (Table. 2). In agreement, previous reports also mentioned that diarrhea with or without mucus or blood, abdominal pain, fever, weight loss, anemia and fleece damage were among the most common signs associated with *Eimeria* spp. infection (Taylor, 1995; Aitken, 2007 and Constable *et al.*, 2017).

To compare between mild and heavy infections of *Eimeria* spp. among sheep, numbers of oocysts were used as an indication for the severity of infection. Previously, fecal oocysts counting was used to calculate the oocyst shedding in different seasons (Sharma *et al.*, 2017). Quantitative analysis of *Eimeria* spp. oocysts in fecal samples were used to determine the oocysts per gram (OPG) by McMaster's technique (Akyüz *et al.*, 2019). Based on both clinical examination of infected sheep cases, gross and microscopic examination of fecal samples and demonstration of *Eimeria* spp. oocysts, the differentiation between mild and severely infected cases was made.

Regarding the pathogenic effect of *Eimeria* spp. infection on blood parameters, RBCs count and hemoglobin concentration, both were considered slightly lower in infected sheep than the apparently healthy non-infected local Egyptian breed of sheep (RBCs, $10.2 \times 10^6/\text{mm}^3$, hemoglobin, 11.1 g/dl) (Ebrahim, 2015). Significant decreases in RBCs count and hemoglobin were obvious in heavy infections with *Eimeria* spp. that might reflect a state of anemia accompanying heavy Eimeriosis. Our findings were comparable to the results of other research group at Kalubia Governorate, Egypt. Authors studied the *Eimeria* spp. infection in lambs that was also manifested by bloody diarrhea and substantial changes in blood parameters as reduction in RBCs count ($8.16 \pm 0.19 \times 10^6/\text{mm}^3$) and Hb concentration (8.4 ± 0.47 g/dl). (Ghanem and Abd El-Raof, 2005).

Paul *et al.*, (2020) studied the effect of gastrointestinal parasite infestation in small ruminants and categorized animals based on the severity of infection; author estimated a low percent of PCV (< 27%) in anemic heavily infected sheep and a high PCV (> 27%) in non-anemic cases. In accordance, in this study, the mildly infected sheep with *Eimeria* spp. had higher PCV percentages (about 30%) than heavily infected ones (25%). In spite of the insignificance in the statistics of PCV in mild and heavy infections, both values are lower than PCV in normal sheep (32.3% estimated by Ebrahim (2015)). WBCs count ($10^9/l$) percentage was significantly elevated in both mild and severely infected sheep with *Eimeria* spp. Furthermore, both parameters are higher in case of mild infection when compared to WBCs count in healthy sheep ($8.97 \times 10^9/l$, Ebrahim, 2015). Whereas, lymphocyte percentage was higher in case of heavily infected sheep with *Eimeria* spp. In the same way, Ghanem and Abd El-Raof (2005) detected leucocytosis, neutrophilia, eosinophilia and lymphocytopenia in lambs suffered from Eimeriosis.

Literally, intestinal parasites lead to variations in serum biochemistry of infected sheep and can indicate the degree of host tissues damage and the severity of parasitic infection. (Pandey *et al.*, 2010). In this study, in both mild and heavy infections, albumin levels were nearly similar (less than 2.5 g/dl) that is considered low comparing to the albumin in normal sheep 3.48 g/dl (Ebrahim, 2015). In addition, serum total protein in normal sheep is around 6.42 g/dl as estimated in previous report (Ebrahim, 2015).

In this study, we measured the total protein as 7 and less than 6 g/dl in mild and heavy infections respectively. Serum total protein and globulin values were significantly higher in mild than severe infections. Mohamaden *et al.*, (2018) also detected a significant

reduction in serum total proteins and altered albumin/globulin ratio of infected sheep with *Eimeria* spp. **Pandey et al. (2010)** detected changes in albumin/globulin ratio in infected animals when compared with healthy ones. It is well-known that intestinal tissues can be damaged by *Eimeria* spp. infection, subsequently these results in hemorrhage and protein leakage into intestinal lumen. Malabsorption through injured gut results in reduction of plasma proteins and other nutrients. (**Pandey et al., 2010**).

Concerning the normal values of the liver enzymes ALT, AST, and ALP that were recorded as 27.1, 71.9 and 118.5 U/l respectively (**Ebrahim, 2015**). Evaluation of the liver enzymes in this study revealed a slight elevation in case of mild and extremely significant increase in level of AST (above 200 U/l) in heavily infected sheep. In addition, mild Eimeriosis was resulted in slight elevation in ALT and ALP levels, heavy infection induced marked elevations of both enzymes. In addition, total calcium levels in both mild and heavy infections were almost similar and considered within the normal ranges of the native sheep (8.64 mg/dl) according to previous studies (**Ghanem and Abd El-Raof, 2005 ; Ebrahim, 2015**). *Eimeria* spp. infection in sheep did not alter the serum calcium level, while a significant reduction was detected in infected goats (**Mohamaden et al., 2018**).

Clinical Eimeriosis is likely to occur if animals are subjected to heavy dose of infection or if their resistance is lowered by the overcrowdness and stress as stated by **Taylor (1995)**. Therefore, the levels of infection should be controlled by reducing the contamination and paying more attention to the hygiene especially in food and water mangers. Adequate nutrition of ewes prior to parturition should be ensured and avoidance of malnutrition will help to reduce the risk of heavy infection.

CONCLUSION

Eimeriosis is an intestinal parasitic disease affecting small ruminants, in particular, sheep. Its significant effect on the general health is caused by the parasite damage to the intestinal epithelium. This study summarized the hematological and biochemical alterations that occur upon *Eimeria* spp. infection in sheep, in both mild and severe forms of infection. Heavily infected sheep with *Eimeria* spp. is suffering from illthriftiness and therefore poor production efficiency.

Declaration of Conflicting Interests:

The authors revealed that there is no potential conflicts of interest.

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