



Research Article

Clinical, Hematologic, Sero-Biochemical and IgE Response in Lambs with Diarrhea Caused by *Eimeria*

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ABSTRACT

Diarrhea is a frequently encountered problem especially in lambs. *Eimeria* is one of major causes of diarrhea in young animals. It has a negative impact on health status and growth. This study aimed to investigate changes in hematologic, selected sero-biochemical and IgE response in lambs diarrhea caused by *Eimeria* infection. This study enrolled 20 lambs, eleven diseased and 9 apparently healthy lambs. Detection of *Eimeria* oocyte was done via salt floatation technique. Blood samples were withdrawn from each animal for clinical hematology and serum biochemistry (total protein, albumin, zinc, copper, T₄ and IgE). Significant elevation in hemoglobin, WBCs and neutrophils along with significant reduction in lymphocytes were recorded. Eosinophils and PCV showed numerical elevation, however, this elevation was considered to be non-statistically significant. Significant reduction in copper level along with insignificant decrease in total protein, albumin, and zinc were recorded. T₄ showed no significant alteration in diseased group when compared with control data. IgE response showed significant elevation in diseased lambs compared with control lamb. *Eimeria spp* has great impact on hematology and serum biochemistry of infected lambs as well as it appears to have an effect on immune response presented by an elevation in IgE level. Further studies needed to be performed to investigate the effect of *Eimeria spp* infection on immune response in lambs.

Key words: Lambs; Diarrhea; *Eimeria*; IgE; Hematology, Sero-biochemistry.

INTRODUCTION

Diarrhea is commonly encountered problem in ovine medicine practice especially in lambs. Diarrhea has a significant impact on health status and consequently affect animal's growth performance (Sargison, 2004).

Diarrhea of parasitological nature is frequently identified in young lambs. One of those parasitological etiologies is *Eimeria*; numerous *Eimeria* species were linked to clinical illness in ovine especially *E. crandallii* and *E. ovinoidalis*, which are believed to be most pathogenic particularly in lambs (Constable, 2018). Coccidiosis is not only cause diarrhea, growth reduction and high morbidity, but it can cause mortality also (Hashemnia *et al.*, 2014).

Coccidiosis main sign is diarrhea either watery or mucoid, often tinged with blood (Ghanem, and Abd El-Raof, 2005). Other signs are inappetance, dullness, abdominal discomfort and dehydration (Constable, 2018).

Although *Eimeria spp.* usually found in normal sheep; however, of scanty number, when host exposed to high quantities of infective stage, clinical coccidiosis is ensued

(Al-dujaily *et al.*, 2017). Diagnosis should relies on history, clinical signs as well as oocyte count of more than 20,000/g (Tylor, 1995; Constable, 2018). Several hematologic and serum biochemical alterations were recorded depend on severity of infection and infective dose (Hashemnia *et al.*, 2014).

Parasite infestation is known to be a major stimulus for immunoglobulin E (IgE) (Shaw *et al.*, 1999). Though, immune response against intestinal nematodes is established in sheep (Miller and Horohov, 2006; Bambou *et al.*, 2008; Lee *et al.*, 2011). It was proposed that parasite specific IgE, IgG and IgA are produced in response to nematode infection in sheep (Pfeffer *et al.*, 1996). IgE was also found to be elevated in correlation with low egg count in sheep (Huntley *et al.*, 2001) and plays a significant role in nematode resistance (Lee *et al.*, 2011).

Unfortunately, there is low data available about response of IgE in other parasites invading sheep intestine; therefore, this study was designed to investigate alterations in hemotologic, sero-biochemical along with evaluation of IgE response in lambs naturally infected with *Eimeria spp.*

MATERIALS AND METHODS

This study was conducted on twenty Baladi lambs with age range of 3-6 months. Eleven diarrheic lambs and nine apparently healthy animals serve as control were enrolled in this study. Clinical signs were recorded at time of admission, physical and clinical examinations performed.

Fecal samples

Fresh fecal samples were taken from each animal and examined microscopically (X100 and 400X) using salt flotation methods for detection of Oocysts (Constable, 2018).

Blood samples

Blood samples were withdrawn from jugular vein on a sterile EDTA containing tube for hematologic estimation and a different tube for serum separation. Serum was separated and divided into two portions, first portion was used for detection of Total protein, albumin, zinc, copper (spectrum diagnostic, Egypt), and T₄ (BIOS, Chemux BioScience, INC. San Francisco, CA, USA). Second portion was stored at -20°C and it was used for estimation of IgE.

IgE Estimation

Serum IgE level was estimated using Immunoglobulin E ELISA kit (RSHAKRIE011R, Bio Vendor-Laboratorni Medicina A.S., Japan) on a ELISA reader (BIO TEK ELX 808, USA).

Statistical analysis

All the data was added to Microsoft Excel sheet and results were shown as mean \pm SE. Diseased animal's data were compared with control animal's data using student T-Test through SPSS® program version 16, USA. P value \leq 0.05 were considered significant.

RESULTS

Diarrhea along with depression, inappetance dehydration, elevation of respiratory and pulse rate were most recorded signs shown in Table 1. Confirmation of diagnosis was done via visualization of *Eimeria spp.* under a light microscope (Figure 1).

Hematologic alterations results are given in Table 2. Significant elevation in hemoglobin, WBCs and neutrophils

along with significant reduction in lymphocytes were recorded. Eosinophils, PCV showed elevation, however, this elevation was considered to be non-statistically significant.

Serum biochemical alterations, thyroid functions and IgE response are shown in Table 3. There was significant reduction in copper level along with insignificant decrease in total protein, albumin, and zinc. T₄ showed no significant alteration in diseased group when compared with control data. IgE response showed significant elevation in diseased lambs compared with control lamb.

Table 1: Physical and clinical examinations results of the lambs.

Parameters	Animals lambs	Control lambs	Diseased lambs
Respiration (Time/min)		20 \pm 0.30	25 \pm 0.66***
Pulse (Pulse/min)		83 \pm 0.53	87 \pm 1.4*
Temperature (°C)		39.3 \pm 0.11	39.4 \pm 0.09

***P \leq 0.001; *P \leq 0.05.

Table 2: Hematological results of the lambs.

Parameters	Animals lambs	Control lambs	Diseased lambs
PCV (%)		28.1 \pm 0.91	33 \pm 2.73
hemoglobin (g/dl)		10.9 \pm 0.06	12.6 \pm 0.53**
RBCs ($\times 10^6/\mu$ l)		6.90 \pm 0.52	7.79 \pm 0.38
MCV (fl)		42.6 \pm 3.82	41.8 \pm 1.53
MCH (Pg)		16.5 \pm 1.24	16.2 \pm 0.58
MCHC (%)		39.2 \pm 1.24	39.5 \pm 2.4
WBCs ($\times 10^3/\mu$ l)		9.92 \pm 0.68	15.4 \pm 1.44**
D.L.C			
Neutrophils (%)		54.6 \pm 0.93	72.8 \pm 1.83***
Lymphocytes (%)		39.6 \pm 0.83	21.4 \pm 1.49***
Monocytes (%)		2.8 \pm 0.17	2.1 \pm 0.38
Eosinophils (%)		2.7 \pm 0.14	3.5 \pm 0.62
Basophils (%)		0 \pm 0.00	0 \pm 0.00

***P \leq 0.001; **P \leq 0.01.

Table 3: Sero-biochemical results of the lambs

Parameters	Animals lambs	Control lambs	Diseased lambs
Total proteins (g/dl)		5.5 \pm 0.34	5.35 \pm 0.30
Albumin (g/dl)		2.6 \pm 0.22	2.63 \pm 0.21
Globulin (g/dl)		2.9 \pm 0.24	2.72 \pm 0.25
A/G ratio		0.93 \pm 0.12	1.06 \pm 0.18
Zinc (μ mol/l)		17.9 \pm 1.41	16.9 \pm 2.95
Copper (μ mol/l)		32.3 \pm 5.4	18.7 \pm 3.36*
T ₄ (nmol/l)		60.9 \pm 7.6	68.7 \pm 5.17
IgE (μ g/ml)		1.89 \pm 0.75	8.85 \pm 2.36*

*P \leq 0.05.

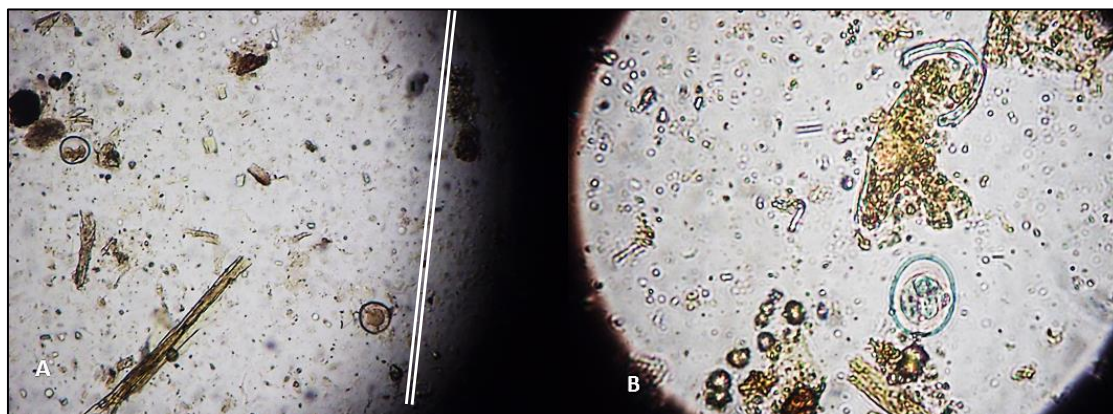


Fig. 1: *Eimeria* species oocyst in fecal samples examined by concentration floatation technique [A: X100 and B: X400].

DISCUSSION

Coccidiosis is a worldwide intestinal affection of both caprine and ovine members, with substantial effect on young animals, economic impact is considerable (Khodakaram-Tafti and Hashemnia, 2017).

In this study, diarrhea, dehydration, inappetance and alterations in physical examination findings were recorded. These clinical manifestations were established to be correlated with *Eimeria* infection (Al-dujaily *et al.*, 2017). The ability of apicomplex to conquer and demolish intestinal cells subsequently leads to electrolyte loss and poor absorption and manifested by diarrhea, weakness and reduction in growth (Wang *et al.*, 2010). The infection tends to be more profound in young lambs, the fact that epithelial turnover ability is to some extent lessened in this age, making lambs more prone to infection (Jolley and Bardsley, 2006). Diagnosis should rely on history, signs and presence of oocysts, however, when severe diarrhea (hemorrhagic or even with no blood) happens in young lamb, coccidiosis should be in top of the suspicion list (Taylor, 1995).

Elevation in hemoglobin and PCV were recorded in this study, these findings were consistent with those described by Dai *et al.*, (2006) and Hashemnia *et al.*, (2014), however, it contradicts other reports (Anumol *et al.*, 2012). Moreover, 'Ghanem and Abd El-Raof (2005) recorded a reduction in hemoglobin along with elevation in PCV and attributed these changes to hemorrhagic enteritis and dehydration. On other hand the elevation of both hemoglobin and PCV were found to be correlated with the reduction of circulating blood in response to diarrhea (Dai *et al.*, 2006).

Leucogram of diseased lambs showed significant elevation in TLC. Enteritis and intestinal inflammation might be incriminated for TLC elevation ('Ghanem, and Abd El-Raof, 2005). Reduction in lymphocytes is the result of depletion of lymphocytes and peyer's patches ileal follicle shrivel (Aleksandersen *et al.*, 2002).

Insignificant decrease in total protein and albumin were recorded, these findings agreed with other reports (Anumol *et al.*, 2012; Hashemnia *et al.*, 2014). Significant reduction in copper level along with insignificant reduction in zinc were recorded. Though, their reduction were recorded in response to physiologic conditions (Salem, 2017), their reduction was also expected in association with numerous diseases. Micronutrients as copper and zinc contribute in extensive assortment of animals' physiological procedures (Richards *et al.*, 2010), they are often described as "anti-oxidant trace minerals", and those minerals play an integral role in supporting antioxidant reactions to counteract the triggered oxidant damage by the effect of *Eimeria* (Rakhshandehroo *et al.*, 2014).

Significant elevation in circulating serum IgE levels found in lambs affected with coccidiosis compared to control animals. IgE response has been extensively researched in nematodes affection in sheep (Lee *et al.*, 2011) and cattle (Miller *et al.*, 1996). In the nematode infection model, tying of parasite molecules by "cell-surface IgE" is the chief elicit for degradation of mast cell, and hence IgE is associated accordingly in nematode resistance (Lee *et al.*, 2011). A link between high IgE level and reduction in egg count has been established in lambs

(Huntley *et al.*, 2001). Furthermore, numerous reports recorded an elevation in total and parasite Ag-specific IgE in association with gastrointestinal nematodes (Shaw *et al.*, 1998; Bendixsen *et al.*, 2004; Pernthaner *et al.*, 2005). In the experiment designed by Mackinnon *et al.*, (2010), lower fecal egg count was associated with high PCV and IgE levels at day 27th post inoculation.

Conclusions

Eimeria spp appears to have an effect on immune response presented by an elevation in IgE level as well as hemato-biochemical parameters.

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