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Research Article

Evaluation of Total Antioxidant Capacity, Malondialdehyde, Catalase, Proteins, Zinc, Copper and IgE Response in Ovine Verminous Pneumonia

Abdel-Saeed H* and Salem NY

[1]Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt
***Corresponding author:** dr.hysam2013@yahoo.com

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ABSTRACT

Pneumonia considered one of the most important and frequent problem facing sheep industry in veterinary practice as it causes high economic losses through mortalities and weight loss leading to poor body condition. Verminous pneumonia is one of main types of pneumonia in Egypt that severely affect sheep with characteristic clinical picture included paroxysmal cough, abnormal lung sound and pulmonary emphysema. This study aimed to detect alterations in total antioxidant capacity, Malondialdehyde, catalase, Protein profile, selected minerals as well as IgE response in verminous pneumonia in sheep. This study conducted on fourteen sheep with age range of approximately 11.5 year. Studied animals divided into seven diseased and seven control animals. Clinical signs were recorded and parasitic involvement was detected using modified Baermann technique. Blood samples were collected and divided into three portions. First part was taken on EDTA for hematological examination. Second, was taken on heparin for estimation of TCA, Malondialdehyde, and catalase. Third portion separated and serum samples were used for estimation of total protein, albumin, zinc and copper. Regarding results, there was significant ($P \leq 0.001$) elevation in total leucocytic count and significant ($P \leq 0.05$) elevation of eosinophils. Significant ($P \leq 0.01$) decrease in total antioxidant capacity was recorded while there was significant ($P \leq 0.01$) elevation in Malondialdehyde level. Significant ($P \leq 0.001$) decrease was recorded among total proteins, globulins, zinc and copper levels in diseased group. IgE response indicates significant (≤ 0.01) elevation in diseased sheep in comparison with control group. Verminous pneumonia appeared to be associated with oxidant-antioxidant imbalance manifested by elevation in MDA and reduction in TAC. Reduction in albumin seems to be correlated with oxidative stress status. IgE elevation is expected in respiratory disease especially that of parasitic factor.

Key words: Total antioxidant capacity, Malondialdehyde, Protein profile, IgE, Verminous pneumonia, Sheep

INTRODUCTION

Pneumonia is a common disorder affecting lower respiratory tract of sheep, many agents involved in this problem as viruses, bacteria or parasites (Scott, 2018). Dictyocaulosis, also known as "lung worm infestation or verminous pneumonia" can induce lower respiratory disease in ovine and other ruminants, with clinical presentation of cough, nasal discharge, loss of weight and dyspnea (Fox *et al*. 2015). Diagnosis is usually depends on clinical picture and finding of first stage larvae in feces of suspected animal using modified Baermann technique (Adem, 2016).

Oxidative stress is a subsequent to disequilibrium among antioxidant and oxidants, in which, oxidant side increase and surplus antioxidant ability to remove it (Celi, 2011). The most voluminous free radicals in body are reactive oxygen species and its metabolites (Miller *et al*., 1993). *Malondialdehyde (MDA)*, one of pro oxidant (Celi, 2011), is a low molecular weight "end-products" produced during decomposition of polyunsaturated fatty acids induced by radical actions (Janero, 1990).

Numerous antioxidant agents present in body either enzymatic as catalase, SOD and GPx or non-enzymatic (Celi, 2011). Among endogenous non-enzymatic antioxidants are proteins as albumin, which was hailed as superior element of extra-cellular defenses (Ueland *et al*., 1996). Total antioxidant capacity estimation may constitute a solution for dilemma of presence of many antioxidants as it symbolizes accumulative action of all antioxidant in plasma (Ghiselli *et al*., 2000).

IgE estimation was postulated to determine activation of Th_2 in numerous conditions as parasitic infection and some immunologic disorders (Souza-Atta *et al*., 1999).

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Parasitic infection was argued to elicit an immune regulatory status which harmonizes allergic conditions (Santiago *et al*., 2014) as parasite could boost TH_2 equilibrium with inclination towards IgE production (Valmonte *et al*., 2012). Although, immunologic response especially that of IgE toward intestinal parasites was recorded in previous studies (Bambou *et al*., 2008) and (Abdel-Saeed and Salem, 2019). Yet, the extend of its response in correlation with other systems not fully established. Therefore, this study aimed to detect alterations in total antioxidant capacity, Malondialdehyde, catalase, Protein profile, selected minerals as well as IgE response in verminous pneumonia in sheep.

MATERIALS AND METHODS

Animals

This study conducted on 14 sheep with age range of approximately 1- 1.5 year. Studied animals divided into 7 diseased and seven control animals. Clinical signs were recorded and parasitic involvement was established using modified Baermann technique (Adem, 2016).

Samples

Blood samples were collected from jugular vein on three tubes from each animal. First tube contains EDTA for hematologic estimation. Second heparin-containing tube was used to determine total antioxidant capacity (TAC), Malondialdehyde (MDA) and catalase (Bio-diagnostic, Egypt) from plasma. Third plain tube was used for serum separation to estimate total protein, albumin, zinc and copper (spectrum-diagnostic, Egypt).

Serum IgE was determined via "Immunoglobulin-E" ELISA kit (RSHAKRIE011R, Bio Vendor-Laboratorni Medicina A.S., Japan) using ELISA reader (BIO TEK ELX 808, USA).

Statistical analysis

The data was added to Excel sheet and results were recorded as mean ± SE. Diseased data were compared with control data by aid of student T- Test, SPSS®program ver., 16, USA. P value ≤0.05 were considered significant.

RESULTS

The recorded signs in diseased group were slight elevation in respiratory rate, cough which was of moderate intensity, weight loss and inappetance. Upon physical examination, abnormal lung sounds were detected in affected sheep.

Alterations in hematologic parameters are shown in Table 1. Significant elevation in WBCs and Eosinophils were recorded in affected animal compared control animals. No statistical changes were recorded in other hematologic parameters.

Abnormalities in oxidative status, protein profile and IgE are shown in Table 2. Significant elevation in MDA and IgE values were recorded in diseased sheep compared with control data. Significant reduction in TAC, total protein, albumin, zinc and copper were recorded in diseased group compared with control group. Catalase showed elevated value, though; this elevation was not considered to be of statistical significance.

DISCUSSION

Small Ruminants as sheep has a great economic impact, though, their productivity could be influenced by many factors among them is the parasitic infection (Adem, 2016). One of these parasites is known as lung worm, which is a nematode infection caused mainly by *Dictyocaulus* and characterized by respiratory involvement.

Table 1: hematologic alterations in diseased group compared with control group

Parameter	Group	Control group	Diseased sheep	Significance
Hematological parameters	PCV (%)	26.33 ± 0.33	23.83 ± 1.62	no
	Hb (g/dl)	10.96 ± 0.20	9.2 ± 0.51	no
	RBCs count ($\times10^6$/µl)	6.98 ± 0.59	6.51 ± 0.50	no
	MCV (Fl)	38.36 ± 3.70	36.83 ± 1.70	no
	MCH (Pg/l)	15.86 ± 1.04	14.23 ± 0.61	no
	MCHC (%)	41.63 ± 1.25	38.78 ± 1.11	no
	WBCs count ($\times10^3$/ µl)	9.11 ± 1.99	17.91 ± 0.71	0.001***
	Neutrophils (%)	56 ± 2.30	60 ± 2.88	no
	Lymphocytes (%)	38.33 ± 2.02	26.16 ± 3.70	no
	Monocytes (%)	3 ± 0.28	3.66 ± 0.49	no
	Eosinophils (%)	2.66 ± 0.16	10 ± 1.57	0.05*
	Basophils (%)	0 ± 0.00	0 ± 0.00	no

*P≤0.05; **P≤0.01; ***P≤0.001.

Table 2: oxidative stress, protein profile and IgE in diseased and control group

Parameter	Group	Control group	Diseased sheep	Significance
Biochemical parameters	TAC (Mm/L)	0.626 ± 0.10	0.305 ± 0.03	0.01**
	MDA (nmol/L)	1.61 ± 0.23	11.34 ± 1.67	0.01**
	Catalase (U/L)	251.18 ± 44.59	386.45 ± 42.16	no
	Total proteins (g/dl)	6.7 ± 0.208	4.53 ± 0.18	0.001***
	Albumin (g/dl)	3.53 ± 0.41	2.91 ± 0.07	no
	Globulins	3.56 ± 0.29	1.60 ± 0.17	0.001***
	Zinc (ug/dl)	79.33 ± 2.72	25.93 ± 3.09	0.001***
	Copper (ug/dl)	122 ± 8.18	29.86 ± 2.43	0.001***
	IgE (µg/ml)	3.20 ± 1.79	15.95 ± 2.11	0.01**

*P≤0.05; **P≤0.01; ***P≤0.001.

In this study moderate cough and respiratory distress along with abnormal lung sounds were recorded in affected animals. Verminous pneumonia was described to have a wide array of symptoms ranged from elevated respiratory rate, cough to persistent cough with severe respiratory distress and open mouth, weight loss and abnormal lung sounds were also recorded (Abebe *et al*., 2016) and (Ballweber, 2018). (Malone, 2007) reported that verminous pneumonia signs severity relies upon infection level. Diagnosis is usually depends on clinical picture and finding of first stage larvae in feces of suspected animal using modified Baermann technique (Adem, 2016).

Hematologic alterations showed significant elevation in WBCs and Eosinophils, these findings came in agreement with other reports (Yacob *et al*., 2004) and (Dutta *et al*., 2017). It was recorded that larvae could induce damage with subsequent obstruction of bronchioles with mucus and eosinophils (Nashiruddullah *et al*., 2007). Elevation of both WBCs and Eosinophils could be attributed to inflammation and parasite itself (Dutta *et al*., 2017). In human model, "pulmonary larvae migrans" caused also by nematode (*T.canis)* was recorded to be associated with eosinophilia and elevated IgE level (Magnaval *et al*., 2001) and (Cheepsattayakorn and Cheepsattayakorn, 2014).

Oxidative stress biomarkers showed elevation in MDA along with reduction in TAC, Zn and Cu. Lipids are most prone component to damage of free radicals, with MDA as lipid peroxidation end-product (Aktas *et al*., 2017), it was elevated in numerous diseases as coccidiosis (Yilmaz *et al*., 2014), Thileriosis (Baghshani *et al*., 2011) and more recently in *Psoroptes Ovis* (Aktas *et al*., 2017). This study showed elevation in MDA level in diseased animals compared to control group, this elevation may be associated with free radical release as a consequent to parasite.

Reduction in TAC was observed in this study, the test defined as determination of antioxidant constituents in a given sample in general, as estimation of each constituent may prove to be expensive and time-consuming (Rubio *et al*., 2016). TAC was found to be reduced in association with *Psoroptes Ovis* and was attributed to utilization of antioxidant components in clearing free radicals released in response to infection (Aktas *et al*., 2017).

Zinc and Copper levels showed significant reduction in this study, though their levels may be affected by physiologic status of animal (Salem, 2017), zinc and copper levels could be affected by presence of oxidative process. It is postulated that zinc act as an antioxidant, as various transcription factors could be up regulated by Zn and the antioxidant, detoxifying molecules (for example, glutathione, SOD) could be generated (Lee, 2018). Cu and Zn are thought to play a key role in antagonize damage effect elicited by oxidant in response to infection (Rakhshandehroo *et al*., 2014).

Reduction in total protein and albumin were recorded in this study, Albumin is one of antioxidant pool as it plays a role in scavenging free radicals (Halliwell, 1988). SH group of proteins are important component of defense mechanism (extracellular antioxidant) against possible oxidants (Celi, 2011).

Elevation in IgE was observed in this study, in nematode infection, Parasite particles adhesive by "Cell-surface IgE" could induce mast cell degradation (Lee *et al*., 2011). Moreover, in one study dealing with wither the parasitic infection could be linked to elevated IgE, elevation in IgE was proposed to be increased in consequence to "polyclonal IgE" elicitation by helminthes or Th2 auxiliary consequence of helminthes (Santiago *et al*., 2014). In intestinal nematode model, host response are generally characterized by TH_2 response with increased levels of parasite (Ag-specific/non-specific) IgE along with eosinophilia (Mukai *et al*., 2016), though, response may differ depending on parasite involved, IgE is expected to rise in many parasitic disease, although, IgE is frequently not specific for parasitic antigen per Se (Pritchard, 1993). Furthermore, one of leading stimulus for IgE is parasitic infection (Shaw et al. 1999) and it also played a key role in parasite resistance (Mukai *et al*., 2016).

Conclusions

Verminous pneumonia appeared to be associated with oxidant-antioxidant imbalance manifested by elevation in MDA and reduction in TAC. Reduction in albumin seems to be correlated with oxidative stress status. IgE elevation is expected in respiratory disease caused by parasitic factor.

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