



Full Length Research Paper

Biochemical changes investigated by psoroptic mange infestation in buffaloes

^{1*}Mervat E.I. Radwan, ²Reham Samir E., ³Mohamed Abd-ElAziz M.

¹Infectious Diseases Department with Veterinary Hospital, Faculty of Veterinary Medicine

²Parasitology Department, Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh, 13736, Egypt

³Physiology Department, Faculty of Veterinary Medicine, Assuit University, New valley, Egypt

*Corresponding Author's E-mail: Dr_Mervat19@yahoo.com

Abstract

The present study was executed on fifty buffaloes (*Bubalus bubalus*), aged 1-3 years, suffering from signs of pruritus and alopecia throughout the period from January to December 2015. Upon parasitological enquiry, they were proved to be infested by *Psoroptes* species. The changes in the sero-biochemical parameters were compared in two groups, infested group and non infested healthy group, each of 30 buffaloes. The following parameters were assessed: serum total protein (TP), serum albumin (ALB), blood urea nitrogen (BUN), creatinine levels, serum enzymatic activities levels as glutamic-pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), Oxidative and anti oxidative biomarkers as malondialdehyde (MDA), Glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT), inflammatory parameters as C-reactive protein (CRP), pro inflammatory cytokines (IL6) and tumor necrosis factor (TNF). The results concluded a significant difference in the serum constituents ($P < 0.05$) between the infested buffaloes and the non-infested ones.

Keywords: Psoroptic mange, Oxidant and antioxidant bio markers, CRP and enzymatic changes.

INTRODUCTION

Psoroptic mites are imperative etiological causes of mange that have a significant economic impact among wild and domesticated ungulates (Syed, 2009). They are non-burrowing mites that inhabit the superficial layer of the skin and by means of their mouthparts, they actively abrade and scrape the epidermis rather than piercing it (Bates 1999; Van Den Broek and Huntley, 2003). Mainly, Psoroptic mange seems to be one of the highly important skin affections of buffaloes in tropical countries, where the disease is highly widespread and contagious, transmits among animals by direct and indirect contact resulting in outbreaks (Radostits et al., 2007; Jones et al., 2008). It results in increasing the fattening time in the herd, decreasing daily weight gain, destructing the hides and causing sporadic mortalities (Lonneux et al. 1998; Rehbein et al., 2002).

Among dairy cattle, the milking process is hampered and the milk yield is decreased due to the restlessness

effect of the parasite (Schoett et al., 2002). The disease produced by animals acariasis is not restricted topically to the skin but can lead to a generalized illness that affects the different tissues of the animal and causes severe cell damage due to the biochemical changes induced (Shang, et al; 2014).

In Egypt, *Psoroptes* sp. is highly prevailing among buffaloes in Nile Delta (El-Khodery et al., 2009); however, the risky consequences of the psoroptic infestation, the changes in the biochemical parameters, oxidant/antioxidant and inflammatory markers are still unobvious. The identification of these risk factors assist the practitioner to establish the most proper control programmes (El-Khodery et al., 2010).

Therefore, the aim of this study is to recognize of biochemical changes associated with psoroptic infestation by serum analysis of different biochemical constituent as TP, ALB, BUN, creatinine, GPT, GOT,

ALP, and LDH, MDA, GST, SOD, CAT, CRP, IL6 and TNF.

MATERIAL AND METHODS

Animals

Fifty buffaloes, aged 1-3 years suffering from pruritus and alopecia were admitted to Benha education hospital in faculty of veterinary medicine, Benha University throughout the period from January to December 2015. They were proved to be infested by *Psoroptes* sp. upon screening of skin scraps.

Skin scraping

Psoroptic infestations were diagnosed by microscopic inspection of superficial scrapings of the skin which were obtained from different areas of the body. On the edge of active cutaneous lesion, glycerin was applied and the scrapings were done using a scalpel. The collected scraps were located in closed tubes. Ten percent of potassium hydroxide (KOH) was added to the scrapings, the sediment was spread on a glass slide, microscopically examined under $\times 10$ magnification and identified according to Soulsby (1986).

Animal grouping for sero-biochemical assessment

A total of 60 buffaloes were grouped into Positive and negative group, each of 30 animals. The infested group was chosen from the psoroptic infested buffaloes. Whereas, the healthy group were selected apparently healthy and were proved to be free from any infection by cautious clinical and parasitological examination.

Blood sampling and serum preparation

Ten ml of blood was collected from the jugular vein of each buffalo using dry syring and poured on the wall of non heparinized tubes. The blood samples were left to stand in a slant position for 30- 60 minutes at room temperature for clotting. The clotted blood was centrifuged at 3,000 rpm for 10 min for complete serum separation; all the sera were stored at -20°C till further analysis (Tuck et al., 2009).

Biochemical assessment

Estimation of biochemical changes was done by analyzing the serum samples of the animals in each group for serum total protein (TP), serum albumin (ALB),

blood urea nitrogen (BUN) levels, serum enzymatic activities levels as glutamic-pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), Alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) using high performance liquid chromatography method (HPLC, Sykam 1125 pump system, Germany) (Miller and Yang, 1985; Zaspel and Csallany, 1983; Reynolds and Judd, 1984).

Moreover, spectrophotometric method using commercial kits (bio-diagnostic, Egypt) was used for measuring oxidative and anti oxidative biomarkers (serum malondialdehyde (MDA), glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and the inflammatory biomarkers (CRP, IL6 and TNF).

Statistical analysis

The statistics was applied by means of SPSS soft ware (SPSS ver. 16, Inc., Chicago, IL). T-test was used for each group at a significant value at $p < 0.05$ (Steel, 1997).

RESULTS

Buffaloes infested with psoroptic mite manifested apparent loss of body weight, emaciation, severe itching, the buffaloes attempted to rub the infested areas of the body against walls and fences, alopecia, crust formation and exudation (Figure. 1. A-C). Psoroptic mites had a tendency to attack the longhaired areas of the body, wither, the back, head of the tail or the entire parts of the body in severe infestation. The positively infested buffaloes revealed the presence of psoroptic mites upon microscopic examination of skin scraps (Figure. 1. D- F).

In table 1, the biochemical assessment revealed that TP and ALB were efficiently ($P < 0.001$) decreased (6.18 ± 0.25 and 3.46 ± 0.22 respectively) among infested buffaloes as compared with the healthy ones. An elevation in the level of enzymatic activity among infested buffaloes which was expressed by high significant levels of GPT (77.80 ± 5.95) and GOT (142.6 ± 11.17), ALP (129.0 ± 6.01) and LDH (137.2 ± 10.66). A noticed increase of BUN and creatinin (56.20 ± 3.81 and 1.99 ± 0.23) levels was also recorded due to psoroptic infestation.

The oxidative stress biomarkers, CAT, GST and SOD activities were declined from 50.00 ± 4.10 , 241.0 ± 26.24 and 41.00 ± 3.51 in healthy animals to 24.80 ± 3.48 , 107.6 ± 26.71 and 16.00 ± 1.30 in infested animals. Conversely, the level of MDA was significantly activated to 160.4 ± 7.51 . The inflammatory biomarkers as CRP, IL6 and TNF were significantly raised to 10.70 ± 0.82 , 84.6 ± 3.86 , and 123.4 ± 5.30 correspondingly in the infested group.

Table 1. Serum biochemical changes induced by psoroptic mange

Bio chemical parameters	Positive group Mean \pm SE	Negative group
TP	6.18 \pm 0.25 ***	8.463 \pm 0.22
ALB	3.46 \pm 0.22 ***	4.545 \pm 0.17
GPT	77.80 \pm 5.95 ***	18.75 \pm 2.29
GOT	142.6 \pm 11.17***	56.50 \pm 5.33
ALP	129.0 \pm 6.01***	20.25 \pm 3.57
LDH	137.2 \pm 10.66***	43.50 \pm 4.27
BUN	56.20 \pm 3.81 ***	24.50 \pm 2.66
CREATININE	1.99 \pm 0.23***	0.52 \pm 0.04
CAT	24.80 \pm 3.48**	50.00 \pm 4.10
GST	107.6 \pm 26.71 ***	241.0 \pm 26.24
SOD	16.00 \pm 1.30***	41.00 \pm 3.51
MDA	160.4 \pm 7.51***	64.50 \pm 4.25
CRP	10.70 \pm 0.82**	5.743 \pm 0.34
IL6	84.6 \pm 3.86***	81.50 \pm 5.36
TNF	123.4 \pm 5.30**	33.25 \pm 2.06

** Value of $p < 0.01$ and *** value of $p < 0.001$.

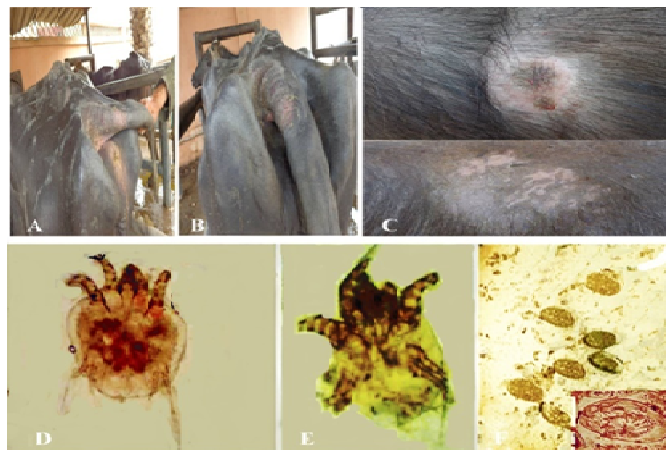


Figure 1. A-C Alopecia and skin crusts due to psoroptic mange in different parts of the body , D,E Psoroptes mites obtained by skin scraping, F. Eggs of mites (magnified X40 at lower right corner)

DISCUSSION

The Infested buffaloes in the contemporary study

suffered from loss of body weight, emaciation, itching where the buffaloes attempted to rub the infested areas of the body against the walls and fences, alopecia, crusts

formation and exudation. These findings came in agreement with Vishe et al., (2012) and Syed (2009). The significant decrease in TP and ALB levels and the increase in CRP levels among infested buffaloes were considered good markers of inflammation induced by mites. The elevation of cytokines levels in response to such inflammation stimulated the liver to retort by producing a great number of positive acute-phase proteins and reducing the production negative acute-phase ones (Abbass et al; 2012) as Albumin and some other protein. The physiological interpretation for decreasing of negative proteins synthesis is mostly to save and protect amino acids for forming positive acute-phase proteins more competently (Chua et al., 1999). Whereas, CRP is considered positive acute phase protein which serves different physiological functions for the immune system as a part of innate immunity (Herpers et al; 2009).

Moreover, the free radicals produced during psoroptes infestation caused protein oxidation and DNA impairment (Allaam et al., 2014; Kanbur 2008 ; Dimri 2008). In this study, buffaloes with psoroptic mange were found in an oxidative stress condition which was indicated by decreased SOD, CAT and GST activities and high MDA level. The inflammation induced by psoroptic mange among infested buffaloes activated the inflammatory cells, which recruit macrophages and neutrophils with reactive oxygen substance, as hypochlorite, hydrogen peroxide (H_2O_2) and oxygen radicals. These substances show forceful cytotoxic effects on the parasites (Gurgoze et al., 2003). Both catalase and SOD had a combating effect on the produced oxidative stress. The over consumption of SOD was attributed to its activity to counter the free radicals created during the infestation (Beigh et al., 2014).

Moreover, SOD plays a role in catalyzing the super oxidizedismutation to hydrogen peroxide (H_2O_2) and oxygen. The over utilization of SOD leads to an increase in H_2O_2 level in the cells. Subsequently, CAT activity is consumed in neutralizing H_2O_2 produce (Clemen and Waller, 1987).

Similarly, the severe exhaustion of antioxidant system could explain the decreased GST activity encountered in this study (Kocyigit et al., 2005). The early phase of libeartion of pro inflammatory cytokine due to psoroptic infestation led to elevation of lipid peroxides and hence the level of MDA increased. The significant change in BUN, GPT, GOT, ALP, and LDH among infested buffaloes were previously recorded by Allaam et. al.,(2014) who attributed this change to the increase of poisonous product of oxidative stress in circulation which have a systematic effect on other tissues than the skin. The elevation of cytokines (IL-6, TNF) levels as compared with healthy group maybe accredited to the severe allergic inflammatory condition induced by Psoroptes mange which stimulated the release of already secreted and newly synthesized cytokines and

contributed to the parasite pathology (Majewska et al., 2016). Mainly, TNF plays an important role in linking adaptive and innate immunity in chronic inflammatory disease (Pasparakis et al; 1996) and extreme secretion of TNF is related to the vulnerability to allergies (Stanley and Lacy, 2010). Such release of cytokines or inflammatory factors can exacerbates the condition by extra activation of the immune system (Shang et al., 2013).

CONCLUSION

Though psoroptic mites commence as a topical disease, it has the ability to persuade changes in the tissue activities, inflammatory reaction and the oxidant/antioxidant stability of animals with the progression of the illness which may lead to systemic disease. This was expressed well in our study, where there were significant changes between psoroptic infested and healthy groups in the levels of protein, albumin, different enzymes (GPT, GOT, ALP, and LDH) oxidative stress factors (MDA, GST, SOD, CAT) and inflammatory biomarkers (CRP, IL6 and TNF). Therefore, these are highly endorsed to be used as bio-indicator of the tissue reaction, inflammatory status and oxidative stress in psoroptic infestation.

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