STUDDIES ON CLINICAL MARKERS, HEMATO-BIOCHEMICAL AND OXIDATIVE STRESS PARAMETER ALTERATIONS IN THEILERIOSIS AFFECTED BUFFALOES FROM SEMI ARID ZONE OF NORTHERN PLAINS OF INDIA

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ABSTRACT

The study was carried out on 36 naturally infected buffaloes with *Theileria annulata*, to determine the clinical markers, hemato-biochemical and oxidative stress parameters alterations. The clinical markers recorded in infected buffaloes were fever, enlargement of pre-scapular lymph nodes, pale mucosae, anorexia, thin mucoid nasal discharge along with melanic faeces and corneal opacity as clinical signs which might be considered as clinical markers of theleriosis in buffaloes in the semi arid zone of the region. Hemato-bichemical and oxidative stress parameters alterations revealed significant decrease in red blood cells (RBC) and haemoglobin (Hb) concentration in the infected animals, compared to the control ones. Increased lipid peroxidation (LPO) and decrease in the levels superoxide dismutase (SOD) and catalase (CAT) in infected animals, compared to the control ones. It can be concluded that theleriosis in buffaloes may lead to anemia which might be attributed due to increased oxidative stress.

Keywords: *Bubalus bubalis*, buffaloes, theileriosis, clinical markers, hemato-biochemical, oxidative stress

INTRODUCTION

Tropical theleriosis is a major threat for water buffaloes, caused by *Theileria annulata*, a protozoan parasite transmitted by ticks of the *Hyalomma* genus, causing significant economic losses as well as reduced production (Razavi *et al*., 2015). Stained blood Smear and lymph node smears are accepted as a method of laboratory diagnosis in cattle and buffaloes (Ramazan and Ugur, 2006), however microscopic examination requires skilled technicians and has poor sensitivity and lower diagnostic efficacy. Therefore, to diagnose the disease veterinarians in the field requires some clinical markers which can help them to diagnose without applying any diagnostic procedure although diagnosis of *Theileria annulata* infection in buffaloes based on clinical signs, is difficult, because of the wide variety of the disease clinical picture that may be mistaken with other diseases. Therefore, keeping in the view of the above facts present study was planned to determine the clinical markers of theileriosis and hemato- biochemical

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and oxidative stress parameters alterations in naturally infected buffaloes reported to the TVCC of university from different areas of semi arid zone of northern plains of India.

MATERIALS AND METHODS

The study was performed at teaching veterinary clinical complex (TVCC), DUVASU, Mathura, on 36 positive buffaloes and 6 parasites free healthy buffaloes used as control. Diagnosis of theileriosis was done based on giemsa stained thin blood smear examination prepared from the ear tip veins of all animals. Three thin blood films from every sample were prepared, fixed with absolute methanol (5 minutes), stained with 10% giemsa solution (45 minutes) and examined under oil immersion (×1000), to observe abnormal red blood cells (RBCs) and intra-erythrocyte forms of *Theileria* sp.

Detailed clinical examinations were performed on all the positive animals and the individual clinical signs were observed and recorded. Clinical markers were determined based on frequency distribution of different clinical signs and their combinations (clinical markers) in positive animals and evaluated as % frequency.

To evaluate the haemato-biochemical alterations blood samples were collected from all the infected buffaloes and the parasite-free control, through jugular vein puncture, in EDTA tubes for routine blood tests and in heparinized glass-stoppered tubes for other analysis from both infected and control group of buffaloes. The RBCs and WBCs counts were determined using a hemocytometer, whereas packed cell volume (PCV) and Hb concentration were determined by microhematocrit and cyanomethemoglobin methods, respectively. Differential leukocyte counts were estimated manually.

For biochemical estimations plasma was separated from blood by centrifugation at 3000 rpm for 15 minutes and was used for estimation of total protein (Biuret method), albumin (BCG Dye method), globulin (Total protein-Albumin), creatinine (Jaff's method), blood urea nitrogen (NED-dye method) and ALT by Modified UV (IFCC) with the help of BS-120 Chemistry Analyzer (Shenzhen Mindray Biochemical Electronics Co. Ltd.) using Span diagnostic kits (Span Diagnostics Ltd, Sachin, Surat, India).

For evaluating the oxidative stress parameters, plasma was harvested by centrifugation of blood samples at 2000 rpm for 10 minutes and buffy coat were removed and erythrocyte pellet washed thrice with 0.15 M NaCl and further RBCs were suspended in PBS used for the estimation of lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activity using standard methodology (Pandey *et al*., 2015). The data obtained were analysed using Student's t-test with a P value of <0.05 considered as significant.

RESULTS AND DISCUSSIONS

To determine the clinical markers of theleriosis in buffaloes in present investigation the frequencies of clinical signs in diseased were estimated (Table 1). It had been found that predominant clinical signs observed (clinical markers) in theleriosis were high fever (100%), enlarged pre-scapular lymph nodes (100%), pale mucous membranes (100%), anorexia/inappetance (100%), Thin mucoid nasal discharge and respiratory distress (100%) in all positive cases and Corneal opacity (94.44%), Melanic feces

(88.88) along with earlier clinical signs indicative of confirmatory diagnosis in the semi arid plains of India. Lacrimation (69.44%), reduced milk yield (77.78), exophthalmia (66.66%) should also be taken into consideration in the diagnosis. Constipation (33.33%), Diarrhea (11.11%) and skin nodules (11.11%) can also be observed in some cases along with other predominant manifestation and sometimes they should also be taken into consideration while making a clinical diagnosis of theleriosis in buffaloes. These clinical manifestations (clinical markers) observed in theleriosis in buffaloes are in corroboration with the clinical manifestations earlier observed (Osman and AL-Gaabary, 2007). Therefore, these signs can be considered in concert can be relied upon to diagnose theleriosis in field conditions as a clinical markers where laboratory diagnosis facility are lacking.

The haematological examination (Table 2) revealed significant decreases in the Hb content, PCV, TEC and TLC counts in infected buffaloes, compared to the healthy control. These findings suggest that theleriosis in buffaloes leads to anaemia (Carlson, 1990). Differential leukocyte count revealed significant leukocytosis with lymphopenia and neutrophilia in infected buffaloes compared to healthy control animals in present study. The stimulation of lymphoid tissues and stem cells in the bone marrow by the parasite and their toxins may have resulted into leukocytosis (Youssef *et al*., 2015). The release of endogenous corticosteroid due to acute disease or stress and or inflammation accounted for significant neutrophilia observed in infected animal, while lymphopenia recorded in infected animals may be due to lysis of lymphocytes caused by release of protozoal merozoites into blood stream (Feldman *et al*., 2000).

Biochemical alterations revealed (Table 2) significant increase in aspartate transaminase (AST) in infected buffaloes in comparison to healthy ones. However slight rise in total plasma protein, albumin, and slight decline in blood urea nitrogen and creatinine were observed in infected animals than healthy ones, but the variations were non-significant (Pandey *et al*., 2017).

Oxidative stress parameters alterations revealed (Table 2) significant increases ($P \leq 0.05$) in the levels of lipid peroxidation (LPO) in *Theileria annulata* infected buffaloes, compared with healthy buffaloes. However, there was a significant reduction ($P \leq 0.05$) in the levels of super oxide dismutase (SOD) and catalase (CAT) activity in infected buffaloes, compared with healthy buffaloes. These findings of present investigations may be an indicator of high oxidative stress in theileriosis in buffaloes (El-Deeb and Younis, 2009). Free oxygen radicals cause lipid peroxidation and give MDA as the finished product. The determination of MDA is the mirror for the degree of lipid peroxidation and the level of free oxygen radicals (Owen, 1996). The excessive free radical productions observed in the present investigation might leads to severe anemia in infected buffaloes as the oxidative damage to the RBCs might play an important role in the pathogenesis of anaemia in bovine theileriosis (Asri-Rezaei and Dalir-Naghadeh, 2006). The reduced levels of SOD and catalase in present investigation may be attributed to the reduction in antioxidant enzymes as they are consumed by excessive free radicals in the infected animals (El-Deeb and Younis, 2009).

It could be concluded from the present investigation that clinical markers such observed during present investigation can be used as sure shot diagnostic clinical signs and these signs could be utilised in the field conditions particularly in

Table.1. Clinical markers of theileriosis in buffaloes from semi arid zone of northern plains of India.

Clinical signs	Number of affected animals/total sick ones	% Frequency
Fever	36/36	100
Enlarged pre-scapular lymph nodes	36/36	100
Pale mucous membranes	36/36	100
Anorexia/Inappetance	36/36	100
Thin mucoid nasal discharge and respiratory distress	36/36	100
Corneal opacity	34/36	94.44
Melanic faeces	32/36	88.88
Lacrimation	25/36	69.44
Reduced milk yield	28/36	77.78
Exophthalmia	24/36	66.66
Constipation	12/36	33.33
Diarrhea	4/36	11.11
Skin lesions (nodules)	4/36	11.11

Table 2. Hemato-biochemical and oxidative stress parameters alterations in theileriosis in buffaloes.

Parameters	Healthy control (n=6)	Infected buffaloes (n=36)
Hb gm/dl	12.60±0.37	7.48±0.92*
PCV%	37.92±0.42	29.21±0.98*
TEC (x10^6/ µl)	6.67±0.66	5.02±0.48*
TLC(×10^3/µl)	7.5±0.34	5.46±0.39*
Neutrophils %	36.78±0.85	53.00±0.92*
Lymphocytes %	57.82±0.93	43.38±0.78*
Total protein (g/dl)	7.82±0.38	6.74±0.42
Albumin (g/dl)	3.24±0.14	2.40±0.15
BUN (mg/dl)	13.17±0.48	12.80±0.64
Creatinine (mg/dl)	1.20±15	1.05±0.12
ALT (IU/L)	29.67±1.54	28.06±1.36
AST (IU/L)	15.16±1.10	62.80±6.52*
Lipid peroxidation (nM MDA/ml packed RBCs)	2.38±0.18	5.74±0.38*
Superoxide dismutase (U/mg protein)	8.38±0.76	4.60±0.36*
Catalase (mM H2O2 utilized/min/mg protein)	5.65±0.62	2.64±0.42*

*Means bearing superscript differ significantly (P≤0.05).

semi arid plane zone of the region as markers of theleriosis in buffaloes where laboratory diagnostic facilities are lacking. Anaemia is the most important pathological alterations of theleriosis in buffaloes which might be attributed due to excessive oxidative stress.

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