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Evaluation of Haemato-biochemical Alterations, Antioxidant status and Oxidative stress in *Babesia* Infected Cattle

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ABSTRACT

Babesia is one of the most common causes of anemia in cattle. The present study was designed to determine the changes in haemato-biochemical parameters and biomarkers of oxidative stress in *Babesia* infected cattle. Twelve *Babesia* infected cattle (irrespective of age, sex and breed) were selected for this study. Clinical signs, microscopic findings and PCR findings were recorded and blood samples were collected to investigate the haemato-biochemical parameters and biomarkers of oxidative stress. The most commonly observed symptoms were pyrexia, haemoglobinuria, tick infestation and icteric mucous membrane. Among the haemato-biochemical changes, significant reduction in haemoglobin, PCV, RBC count, albumin and serum glucose along with significant increase in TLC, serum total protein, globulin, ALT, AST, serum total bilirubin and serum creatinine were observed. Assessment of biomarkers of oxidative stress showed significant increase in malondialdehyde (MDA) and total nitric oxide (NO) along with significant reduction in glutathione s-transferase activity (GST activity), catalase and superoxide dismutase (SOD) activities. These findings support the theory that *Babesia* infection causes oxidative stress, which may be linked to the anaemia.

HIGHLIGHTS

- Babesia infection caused oxidative stress in cattle.
- Significant increase in malondialdehyde (MDA) and total nitric oxide (NO).

Keywords: *Babesia*, oxidative stress, anaemia, haemato-biochemical

In India, majority of the rural population own livestock which are kept to generate additional income through the production of milk, meat, egg and wool. India's livestock population is more susceptible to a number of haemoprotozoan diseases such as babesiosis, anaplasmosis and theileriosis which are borne by vectors. Tick-borne haemoprotozoan diseases cause pyrexia, anaemia, loss of body weight and decrease in milk production (Kumar *et al*., 2015). The first intra-erythrocytic piroplasm to be identified by Victor Babes in cattle in 1888 was *Babesia*. Smith and Kilbourne documented in 1893 that the causative agent of Texas fever in cattle was transmitted through ticks. The organism was named *Pyrosum bigemina* (believed to be either *Babesia bigemina* or *Babesia bovis*), making babesiosis the first tick-transmitted disease to be

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documented. Babesiosis is characterized by high fever (41-45.5°C) followed by anorexia, increased respiratory and heart rate, anaemia, jaundice, haemoglobinuria, constipation or diarrhoea, weight loss, abortion, nervousness in calves, coma and death (Hashem *et al*., 2018 and Muniraja *et al.,* 2021).

Haematology of *Babesia* infected cattle reveals a significant decrease in haemoglobin, total erythrocyte count and packed cell volume (Khinchi *et al*., 2016 and Kaur *et al*., 2021). Similarly, biochemical study shows increase in total serum protein and globulin, serum urea, creatinine, AST, ALT, iron and total iron binding capacity in cattle clinically affected with babesiosis (Khinchi *et al*., 2016 and Nasreldin *et al*., 2020). The vast majority of eukaryotic organisms need atmospheric oxygen to survive. Oxygen may turn into free radicals; which is a very hazardous toxic form generated during metabolic reactions in all the cells of aerobic organisms. Free radicals are short-lived reactive atoms, ions or molecules with one or more unpaired electrons in their outer orbitals. The main sources of cellular ROS (reactive oxygen species) production are mitochondria, peroxisomes, cytochrome P450 enzymes and antimicrobial oxidative burst of phagocytic cells. Of these, mitochondria are a major source of free radicals and therefore have the potential to cause oxidative damage. Most of the free radicals are oxygen ROS derivatives and nitrogen (reactive nitrogen species) or RNS species (Terzi, 2020). Haemolytic anaemia is the main feature of the disease which is caused by mechanical damage. Membrane damage, methaemoglobin production, osmotic fragility and cell death are the symptoms of erythrocyte oxidation (Chaudhri *et al*., 2008). Lipids, particularly polyunsaturated fatty acids (PUFA) are susceptible to oxidation, resulting in lipid peroxidation or thiobarbituric acid reactive substances (TBARS), the most abundant of which is malondialdehyde (Crnogaj *et al*., 2010 and Radwan *et al*., 2013). MDA measurement provides for indirect identification of the degree of lipid peroxidation and the level of free oxygen radicals (Deger *et al*., 2009).

MATERIALS AND METHODS

Animals

In the present study, twelve clinically-ill cattle presented with the symptoms of haemoglobinuria, pyrexia, anorexia, depression, weakness, pale mucous membrane, emaciation and weight loss at Veterinary Clinical Complex, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur were included. Confirmation of positive cases was done by the use of microscopy and PCR. Six apparently healthy cattle (free from ticks) from Gir cattle farm at College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur were kept as control.

Collection of blood samples

Approximately, 5 ml of blood was collected from jugular vein as per standard protocols for haematological study in a dry vial containing 10 per cent EDTA. For the preparation of the blood smear, blood was collected from ear vein. For estimation of biochemical parameters blood was collected in sterile non-EDTA vial for the separation of serum from apparently healthy cattle and *Babesia* infected cattle.

Estimation of haematological parameters

The blood samples collected before and after treatment were analysed for the determination of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocytes count (DLC) in healthy and *Babesia* affected cattle as per standard techniques (Feldman *et al.,* 2000).

Estimation of biochemical parameters

Biochemical parameters such as serum total protein, serum albumin, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total bilirubin, serum creatinine, and serum glucose were estimated colorimetrically by using kits supplied by Biolab diagnostics, Tarapur, Boisar, MS, India.

Estimation of biomarkers of oxidative stress

The plasma MDA and NO activity were assayed by using commercially available kits (EZAssay TM TBARS Estimation Kit, Product Code: CCK023, Himedia, Mumbai, India and EZAssay Nitric oxide Estimation Kit, Product Code: CCK061-20, Himedia, Mumbai, India) and the value were expressed in µM. SOD and catalase were estimated as per the method described by Marklund & Marklund (1974) and Slaughter and O'Brien (2000).

STATISTICAL ANALYSIS

The data obtained in the research work were statistically analyzed and compared using standard formulae for mean, standard error and analysis of variance as per the methods described by Snedecor and Cochran (2004).

RESULTS AND DISCUSSION

All the blood samples (n=12) which were positive for *Babesia* organism by Giemsa staining were also found to be positive by PCR. The main clinical signs exhibited were haemoglobinuria, pyrexia, decreased milk production, pale mucous membrane, tick infestation and anorexia. Haematological variations in *Babesia* infected cattle are mentioned in Table 1.

Table 1: Mean±SE values of haematological parameters in healthy cattle and *Babesia* infected cattle

Sl. No.	Parameters	Healthy cattle (n=6)	Babesia infected cattle (n=12)
1	Haemoglobin (gm/dl)	11.79±0.407	5.4±0.133**
2	Packed cell volume (%)	36.1±0.607	27.14±1.347**
3	Total erythrocyte count (million/mm^3)	6.82±.0.406	4.73±0.220**
4	Total leucocyte count ($10^3/mm^3$)	7.59±0.457	8.5±0.165*
	Differential leukocyte count		
5	Neutrophil (%)	30.67±0.803	36.5±0.557**
6	Lymphocyte (%)	62.84±0.601	58.42±0.645**
7	Monocyte (%)	3.5±0.428	2.42±0.358
8	Eosinophil (%)	3±0.365	2.83±0.271

*: Means differ significantly (P<0.05) with healthy cattle group; **: Means differ highly significant (P<0.01) with healthy cattle group.

The mean values of haemoglobin, packed cell volume and total erythrocyte counts were significantly (P<0.01) decreased in cattle infected with *Babesia* as compared to healthy cattle. These findings were in accordance with Khinchi *et al.* (2016), Aziz *et al.* (2020), Saravanan *et al*. (2020) and Kaur *et al*. (2021). This could be due to the parasite's damaging effect on erythrocytes in infected cattle (Radostits *et al*., 2010, Mahmud *et al.,* 2015 and Aziz *et al*., 2020). In acute infections, there is large scale destruction of erythrocytes with intravascular hemolysis, indiscriminate phagocytosis of infected/non-infected erythrocytes by activated macrophage system and suppression of erythropoietic activity of bone marrow, which leads to anaemia (Rani *et al.,* 2010).

The total leucocyte count was significantly increased in *Babesia* infected cattle as compared to healthy cattle which may be due to immune response of the host mounted during the infection. This result was in agreement with Aulakh *et al.* (2005) and Khinchi *et al.* (2016). The leukocytosis may be attributed to the stress associated with acute babesiosis (Bhikane *et al*., 2001)

The differential leucocyte count (%) in *Babesia* infected cattle showed significant (P<0.01) neutrophilia and lymphopenia as compared to healthy cattle. Similar findings were reported by Tufani *et al*. (2015), Ganguly *et al.* (2017) and Aziz *et al.* (2020). The leukocytosis associated with neutrophilia and lymphopenia may be due to the stress in *Babesia* infected cattle (Bhikane *et al*., 2001). A non-significant (P>0.05) difference in the mean values of monocyte and eosinophils was recorded in *Babesia* infected cattle as compared to healthy cattle. Similar findings were also recorded by Gungi *et al*. (2016), Khinchi *et al.* (2016) and Ganguly *et al.* (2017).

The mean values of serum total protein and globulin were significantly (P<0.01) increased and mean values of albumin were significantly (P<0.01) decreased in *Babesia* infected cattle as compared to healthy cattle. Similar findings were reported by several workers including Talkhan *et al.* (2010) and Nasreldin *et al*. (2020). Serum biochemical changes are mentioned in Table 2.

The major place of synthesis of the serum protein is liver. The increased value of globulin is due to the stimulation of immune system by the antigens of invaded parasite and decreased value of the albumin is associated with the acute phase of the disease and albumin may be decreased due to decreased protein synthesis capacity of infected liver or prolonged insufficient caloric intake (Talkhan *et al*., 2010).

The mean values of serum ALT and AST were significantly(P<0.01) higher in cattle infected with *Babesia* as compared to healthy cattle.The findings recorded in present study are in agreement with previous reports of Khinchi *et al*. (2016), Aziz *et al*. (2020) and Debbarma *et al.* (2020). The values of serum AST and ALT are the indicators of hepatic function and the increase in serum

ALT and AST may be caused due to alteration of liver function as a result of bovine babesiosis (Zulfiqar *et al*., 2012). These enzymes are present in high concentrations in the liver and muscles. Elevation of these enzymes in the blood is an indicator of organ necrosis or damage (Murray *et al*., 1990). *Babesia* infection involves an increase in enzyme activity which causes severe anaemia that lead to hypoxic and toxic liver damages (Talkhan *et al*., 2010). There was significant (P<0.01) increase in mean value of bilirubin in *Babesia* infected cattle as compared to healthy cattle. These observations are in agreement with Aziz *et al*. (2020), Abdel-Hamied *et al.* (2020) and Debbarma *et al*. (2020). In the present study, the significantly increased values of bilirubin might be due to intravascular hemolysis resulting in hyperbilirubinemia and icterus (Rani *et al*., 2010 and Hashem *et al.,* 2018).

Table 2: Mean±S.E values of biochemical parameters in healthy cattle and *Babesia* infected cattle

Sl. No.	Parameter	Healthy cattle (n=6)	Babesia infected cattle (n=12)
1	Serum total protein (g/dl)	6.36±0.131	7.606±0.233**
2	Albumin (g/dl)	3.58±0.084	2.62±0.202**
3	Globulin (g/dl)	2.78±0.091	4.98±0.209**
4	AST (IU/L)	76.34±4.582	85.34±1.66*
5	ALT (IU/L)	26.25±4.552	45.39±1.076**
6	*T. bilirubin* (mg/dl)	0.64±0.071	2.33±0.27**
7	Serum glucose (mg/dl)	58.11±4.799	41.19±0.971**
8	Serum creatinine (mg/dl)	0.96±0.068	1.48±0.154*

*: Means differ significantly (P<0.05) with healthy cattle group; **: Means differ highly significant (P<0.01) with healthy cattle group.

The mean values of serum creatinine were significantly (P<0.05) increased in *Babesia* infected cattle as compared to healthy cattle. These observations are in agreement with the findings reported by Abdel-Hamied *et al.* (2020) and Debbarma *et al*. (2020). Significant increase of serum creatinine in *Babesia* infected cattle may be attributed to indirect renal tissue damage and the existence of globin catabolites released from haemoglobin breakdown by the reticuloendothelial system through erythrophagocytosis (Ismael *et al.,* 2016) accompanied by hypoxia leading to glomerular dysfunction as well as nephropathy and immune-mediated glomerulonephritis (Mathe *et al*., 2006 and Ganguly *et al.,* 2017).

The mean values of serum glucose were significantly (P<0.01) lower in cattle infected with *Babesia* as compared to healthy cattle. The observations recorded in the present study were in agreement with the findings of Debbarma *et al.* (2020) and Nasreldin *et al.* (2020). A significant decrease in blood glucose level in infected animals was recorded in our study. A reduction in blood glucose level might be caused due to the utilization of glucose by parasite as well as the liver damage in large ruminants infected by *Babesia* (Zulfiqar *et al.,* 2012).

The mean value of MDA and NO in *Babesia* infected cattle were significantly (P<0.01) increased as compared to healthy cattle (Table 3). Similar findings were reported by Osman and Gaadee (2012), Radwan *et al*. (2013), Kumar *et al*. (2019) and Nasreldin *et al*. (2020). There was significant decrease (P<0.01) in the mean values of glutathione s-transferase activity (GST activity) in *Babesia* infected cattle as compared to healthy cattle. Similar findings were reported by El-Far *et al*. (2014). The present study revealed that *Babesia* infected cattle showed decrease in antioxidants (SOD and Catalase) as compared with healthy cattle and which are in agreement with the findings reported by Xiao *et al.* (2001), Siemieniuk *et al.* (2008), Osman and Gaadee (2012), Omnia *et al.* (2014) and Kumar *et al.* (2019).

Oxidative stress is a general mechanism whereby free radicals induce oxidative damage and reduce the antioxidant defense of biological system (Tskahara, 2007). Oxidative stress resulting from increased production of free radicals and decreased antioxidant defense leads to the disruption of normal metabolism and physiology and contribute to health disorders in animals (Ranjan *et al*., 2005).

Oxidative stress has been reported in babesiosis affected animals (Kumar *et al*., 2019). In Bovine babesiosis, activation of inflammatory cells caused by Babesia infection is an important part of the host defense against the parasite (Bock *et al*., 2004; Saleh, 2009) resulting in excessive proinflammatory cytokines production from mononuclear cells/macrophages (Shoda *et al*., 2000; Goff *et al*., 2002). The overproduced cytokines activate oxidant-generating enzymes in inflammatory cells causing production of high levels of reactive oxygen and

nitrogen species that primarily attack and kill the parasite (Beckman and Koppenol, 1996; Goff *et al*., 2002). The reactive species induce damage to membrane, nucleic acid and protein of these parasites causing their death (Stich *et al*., 1998; Kumar *et al*., 2006; Saleh, 2009).

Table 3: Mean±S.E values of biomarkers of oxidative stress in healthy cattle and *Babesia* infected cattle

Sl. No.	Parameter	Healthy cattle (n=6)	*Babesia* infected cattle (n=12)
1	MDA (µM)	2.18±0.130	7.27±0.376**
2	NO (µM)	1.72±0.165	2.77±0.238**
3	GST activity (µM ml-^{1} Min-^{1})	1.92±0.104	0.46±0.012**
4	Catalase (Unit/ mg Hb)	7.79±0.481	3.8±0.33**
5	SOD (unit/mgHb)	33.46±1.651	22.62±0.561**

*: Means differ significantly (P<0.05) with healthy cattle group; **: Means differ highly significant (P<0.01) with healthy cattle group.

Estimation of MDA remains to be a trustworthy method to assess the degree of oxidative damage to cell membrane, as it is the principal aldehyde formed as a by-product during this process (Gurer *et al*., 1998). Erythrocytes are highly susceptible to peroxidative damage due to abundance of fatty acids and presence of powerful transition metal catalyst (Ranjan *et al*., 2005). Nitric oxide (NO) is produced by a number of different cell types in response to cytokine stimulation and is reported to play an important role in immunological mediated protection against protozoan parasites (Rivero, 2006). SOD and catalase are principal antioxidant enzymes present in mammalian cells. SOD augments the formation of O_2 from reactive oxygen species. A co-product of SOD activity is H_2O_2, which is converted to H_2O by catalase (Fang *et al*., 2002). The severity of parasitemia leads to decrease in GST activity in infested group as babesiosis deletes the antioxidant capacity of erythrocytes (Esmaeilnejad *et al*., 2012 and Esmaeilnejad *et al*., 2020).

The results revealed that significantly decreased antioxidants in affected cattle might be attributed to depletion of antioxidants during neutralization of excessive reactive species produced during course of the disease (Salem *et al*., 2016; Kumar *et al*., 2019).

CONCLUSION

The mean values of haemoglobin, packed cell volume, total erythrocyte counts were significantly decreased and the mean values of total leucocyte counts were significantly increased in cattle infected with *Babesia*. Among biochemical parameters, the mean values of serum ALT, AST, total bilirubin and serum creatinine were significantly higher and the mean value of serum glucose was significantly lower in infected animals. There was significant increase in serum total protein and globulin and significant decrease albumin in infected animals. Among biomarkers of oxidative stress, there was a significant increase in MDA and NO, whereas GST activity, catalase and SOD were found significantly decreased in *Babesia* infected cattle as compared to healthy cattle. The *Babesia* organism can interfere with the protective antioxidant mechanism of RBC leading to enhanced erythrocyte fragility causing haemolytic anaemia. Thus, it can be concluded that the *Babesia* infection is remarkably associated with the induction of anaemia, oxidative damage and inflammation in cattle.

The significant rise in lipid peroxidation with reduction of the antioxidants in diseased animals in this study proved that babesiosis affected cattle experienced severe oxidative stress during course of the disease. Oxidative stress and lipid peroxidation appear to be involved in the progression of bovine babesiosis. Therefore, the use of antioxidants as supportive therapy beside Babesicidal drugs is recommended for better and fast recovery.

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