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A clinical study on Hemato-biochemical changes in cows affected with Babesiosis

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Abstract
Babesia parasites, the causative agent of Babesiosis, are transmitted by ticks and are able to invade the red blood cells of the vertebrate hosts. They cause a febrile disease of domestic and wild animals characterized by an extensive erythrocytic lysis leading to anaemia, icterus and hemoglobinuria and can be fatal. Babesia parasites are among the most widely distributed blood pathogens with a considerable economic, veterinary and medical impact worldwide. In the present study, 23 cows (irrespective of age and breed) found positive of babesiosis were taken for study. Two cows died before they were evaluated. The remaining 21 cows were evaluated for Hemato-biochemical estimations. Hemato-biochemical studies revealed decrease in Hb, PCV, TEC, Lymphocytes, Neutrophils, TP, Albumin, Globulin and Glucose and increase in TLC, ALKP, Bilirubin, AST, ALT and BUN. Moreover, marked changes in haematological and serum biochemical profile observed in babesiosis infected cows may be useful in understanding disease pathogenesis and undertaking necessary corrective measures.

Keywords: Anaemia, Babesia, Hemato-biochemical, Hemoglobinuria, Icterus, Ticks

Introduction

Babesiosis is a disease caused by intra-erythrocytic apicomplexan parasites of the genus Babesia, and transmitted by blood-sucking ticks of the Ixodidae family (hard ticks). It is a one-host tick; all stages are spent on one animal. The transmission occur transovarially through several generations. The parasites can be readily transmitted experimentally by blood inoculation, mechanical transmission by insects or during surgical procedures. Intrauterine infection has also been reported but is rare. Piroplasmosis is a disease with an international distribution affecting many species of mammals with a prime impact on cattle and human. It has increasingly more been diagnosed throughout the sector as public health problems (Zanet *et al.*, 2014) [33]. Babesia is the second most common parasite found in the blood of mammals after trypanosomes (Yabsley and Shock, 2013) [30]. India is situated in tropical region (Agrawal *et al*., 2003) [1], so the climatic conditions are conducive to the multiplication and growth of ticks which are most important ectoparasites of livestock both in tropical and sub-tropical countries (Ibrahim *et al.,* 2012) [13]. Ticks not only cause direct losses by sucking blood of the host animal, but are also responsible for various blood-borne diseases such as anaplasmosis, babesiosis and theileriosis (Durrani *et al.,* 2008) [7]. Hematology and serum biochemistry of infected animals are very touchy indicators for the degree of hepatic damage and the parasitic infection severity, wherein liver damage upsets the vital metabolic methods for regular health and most fulfilling productiveness of the animal.

Material and Methods

In the present investigation 23 Holstein Friesian cows (irrespective of age and breed) found positive for babesiosis were taken for study. Two cows died before being evaluated. The remaining 21 cows were evaluated for Hemato-biochemical estimations. For comparison of hemato-biochemical analysis, a total of ten apparently healthy cows were also included in this study to serve as control. For hematological studies blood was collected from jugular vein with all aseptic precautions in sterilized test tubes. Blood was collected in sterile tubes having disodium salt of ethylene diamine tetra acetic acid (EDTA) as an anticoagulant added at the rate of 1 mg/ml of blood. The blood samples were subjected for estimation of hemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, differential leukocyte count. These parameters were analyzed as per the methods described by Jain (1986). For biochemical studies, blood was collected in other sterile tubes having no anticoagulant. The blood slants were made and incubated for 1 hour at 37 ^{0}C. Blood clots were broken and tubes were

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centrifuged at 2,500 rpm for 30 min. The serum was pipette out in small Pyrex tubes. Biochemical estimations for serum total protein, serum albumin, serum globulin, serum alkaline phosphatase, serum bilirubin, serum aspartate aminotransferase, serum alanine aminotransferase, blood urea nitrogen and glucose were analyzed by chemistry analyzer (IDEXX vet test TM kit, manufactured by IDEXX laboratories, USA) as per standard diagnostic protocol.

Table 1: Mean±SE value of hematological parameters in apparently healthy and babesiosis infected cattle.

S. No.	Parameters	Healthy cows (n=10)	Babesiosis affected cows (n=21)
1	Hb (g/dl)**	11.07±0.3538b	6.77±0.2390a
2	PCV (per cent)**	33.36±1.0590b	20.80±0.7183a
3	TEC (million/cmm)**	5.56±0.1942b	3.43±0.1166a
4	TLC (10^3/μL)*	7.65±0.2511a	9.53±0.2968b

*($P<0.05$), **($P<0.01$) Means with different superscripted letters in the same row differ significantly.

Table 2: Mean±SE value of Differential leucocyte count in apparently healthy and babesiosis infected cows

S. No.	Parameters	Healthy cows (n=10)	Babesiosis affected cows (n=21)
1	Lymphocytes (*)	64.50±0.7637b	61.33±2.0602a
2	Monocytes	2.50±0.2687a	2.29±0.2197a
3	Neutrophils (*)	29.80±0.9637b	33.77±2.2274a
4	Eosinophils	3.00±0.3651	2.43±0.2347
5	Basophils	0.2±0.1333	0.34±0.0878

*($P<0.05$), **($P<0.01$) Means with different superscripted letters in the same row differ significantly.

Table 3: Mean±SE value of biochemical parameters in apparently healthy and babesiosis infected cows

S. No	Parameters	Healthy Cows (n=10)	Babesiosis affected cows (n=21)
1	T.P. g/dl (**)	6.70±0.1771b	5.16±0.1915a
2	Alb. g/dl (**)	3.55±0.0815b	2.64±0.1376a
3	Glb. g/dl	3.15±0.2009	2.53±0.2367
4	ALKP IU/L (**)	84.78±8.9346a	210.61±1.2982b
5	Bil. IU/L (**)	0.62±0.0452a	2.44±0.0740b
6	AST mg/dl (**)	77.02±2.0863a	124.09±2.4368b
7	ALT mg/dl (**)	31.96±1.9382a	63.75±1.3884b
8	BUN mg/dl (**)	18.45±0.9384a	30.49±0.8174b
9	Glucose mg/dl (**)	73.15±0.6205b	34.65±0.7213a

*($P<0.05$), **($P<0.01$) Means with different superscripted letters in the same row differ significantly.

Results and Discussion

The mean values of haemato-biochemical parameters in affected and healthy cows are presented in the tables. Present investigation showed significant decrease in values of Hemoglobin, Packed cell volume and Total erythrocyte count in the babesia affected cows as compared to healthy cows. The results of the hematological investigation in cows suffering from babesiosis revealed significant reduction in erythrocytic count, hemoglobin content and packed cell volume, all this might be due to destructive effect of the parasite on erythrocytes in infected cows compared to that of apparently healthy one and this result was in agreement with Radostits et al. (2007) [22]; Mahmoud et al. (2015) [19].

The total leucocyte count was significantly increased in

babesiosis infected cows as compared to healthy cows which may be due to response of the host mounted during the infection (Aulakh et al., 2005) [4]. Leukocytosis may be due to stress associated with acute babesiosis (Bhikane et al., 2001) [5]. The neutrophils in babesiosis infected cows were significantly higher as compared to healthy cows. Similar findings were recorded by Saud et al. (2005) [24], Aulakh et al. (2005), Wadhwa et al. (2008) [28], Mahmoud et al. (2015) [19], Tufani et al. (2015) [7], Gungi et al. (2016) [11], Ganguly et al. (2017) [10]. The Lymphocytes in babesiosis infected cows were significantly lower as compared to healthy cows. Similar findings were recorded by Saud et al. (2005) [24], Aulakh et al. (2005) [4], Wadhwa et al. (2008) [28], Tufani et al. (2015) [7], Ganguly et al. (2017) [10]. Non-significant changes were observed in eosinophil, monocyte and basophil counts in babesiosis infected cows as compared to non-infected cows. Similar findings were recorded by Bhikane et al. (2001) [5], Aulakh et al. (2005) [4], Saud et al. (2005) [24], Wadhwa et al. (2008) [28], Tufani et al. (2015) [7], Gungi et al. (2016) [11], Ganguly et al. (2017) [10]. In the present study a significantly higher number of neutrophils was observed indicating neutrophilia and a significant decrease in the number of lymphocyte was observed indicating lymphopenia. This may be due to stress associated with babesia infection. These findings are consistent to that Bhikane et al. (2001) [5] and Aulakh et al. (2005) [4]. No significant difference was observed in other leucocyte count.

The serum total protein was significantly lower in babesiosis affected cows as compared to healthy cattle. Similar findings were recorded by several workers including Hussein et al. (2007) [12], Alam and Nasr (2011) [3], Gungi et al. (2016) [11], Mahmoud et al. (2016) [18], Salem et al. (2016) [23], Ganguly et al. (2017) [10], Jayalakshmi et al. (2017) [14]. The decrease in serum total protein in cattle infected with Babesia species might be due to decrease in albumin and globulin as a result of liver insufficiency (Laiblin et al., 1978) [16].

The serum albumin was significantly lower in babesiosis affected cattle as compared to healthy cattle. Similar findings were recorded by several workers including Bhikane et al. (2001) [5], Aulakh et al. (2005) [4], Hussein et al. (2007) [12], Talkhan et al. (2010) [26], Alam and Nasr (2011) [3], Mahmoud et al. (2016) [28], Salem et al. (2016) [23], Ganguly et al. (2017) [10], Jayalakshmi et al. (2017) [14]. The 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 affected liver or prolonged insufficient caloric intake (Talkhan et al., 2010) [26]. Low albumin levels may be due to pronounced hemolytic crises, proteinuria associated with renal failure and anorexia in relation to high rise of body temperature (Esmaeilnejad et al., 2012) [8]. Babesia can cause disruption in liver function that leads to decreased albumin synthesis (Werner et al., 2004) [29].

The serum globulin in babesiosis infected cows was non-significantly lower as compared to healthy cows. Similar findings were recorded by Ganguly et al. (2017) [10].

The Mean value of Serum Alkaline Phosphatase in babesiosis infected cattle was significantly higher as compared to that in healthy cattle. The observations recorded in the present study are in agreement with findings of Alam and Nasr (2011) [3], Lotfollahzadeh et al. (2012) [17] and Jayalakshmi et al. (2017) [14]. Bork et al., (2004) [6], stated that the elevation in liver enzymes in babesiosis may be due to the hepatic damage and lesions induced by the parasite during its multiplication in the blood followed by disturbed liver function.

The Mean values of serum bilirubin in babesiosis infected cows was significantly higher as compared to that in healthy cows. The observations recorded in the present study are in agreement with Bhikane *et al.* (2001) [5], Suilaman *et al.* (2010) [25], Alam and Nasr (2011) [3], Khan *et al.* (2011) [15], Gungi *et al.* (2016) [11], Mahmoud *et al.* (2016) [18], Ganguly *et al.* (2017) [10]. In the present study bilirubin level was increased significantly in babesiosis infected cows as compared to healthy cows. A significant increase in total bilirubin in *Babesiosis* is due to hepatic damage and the, presumably hemolytic anaemia was confirmed by (Yeruham *et al.,* 2003) [31].

The Mean values of Serum Aspartate aminotransferase in babesiosis infected cows was significantly higher as compared to that in healthy cows. The observations recorded in the present study are in agreement with findings of Hussein *et al.* (2007) [12], Talkhan *et al.* (2010) [26], Lotfollahzadeh *et al.* (2012) [17], Zulfiqar *et al.* (2012) [34], Gungi *et al.* (2016) [11], Ganguly *et al.* (2017) [10], Jayalakshmi *et al.* (2017) [14]. Serum AST and ALT concentrations are the indicators of hepatic function and the rise in serum ALT and AST may be due to alteration of liver function as a result of bovine babesiosis. (Zulfiqar *et al.*, 2012) [34].

These enzymes are present in high concentrations in the muscles and liver. Elevation of these enzymes in the blood is indicator of organ necrosis or damage (Murray *et al.,* 1990) [21]. *Babesia bigemina* infection causes increase in enzyme activity which may attribute to severe anaemia that leads to hypoxic and toxic liver damages. Also massive haemolysis may occur which in conjuction with hypoxia may lead to hepatic cell degeneration leading to increase in AST and ALT (Talkhan *et al.*, 2010) [26].

The Mean values of Serum Alanine aminotransferase in babesiosis infected cows were significantly higher as compared to that in healthy cows. The observations recorded in the present study are in agreement with findings of Hussein *et al.* (2007) [12], Talkhan *et al.* (2010) [26] and Sulaiman *et al.* (2010) [25]. Alam and Nasr (2011) [3], Khan *et al.* (2011) [15], Zulfiqar *et al.* (2012) [34], Ganguly *et al.* (2017) [10], Jayalakshmi *et al.* (2017) [14]. The rise in serum ALT concentration may be due to alteration of liver function as a result of bovine babesiosis (Yuksek *et al.*, 2007).

The Mean values of Blood urea nitrogen in babesiosis infected cows was significantly higher as compared to that in healthy cows. The observations recorded in the present study are in agreement with Bhikane *et al.* (2001) [5], Aulakh *et al.* (2005) [4], Talkhan *et al.* (2010) [26] and Khan *et al.* (2011) [15], Gungi *et al.* (2016) [11], Salem *et al.* (2016) [23], Ganguly *et al.* (2017) [10]. In the present study, blood urea nitrogen level was increased significantly in babesiosis infected cows which may be attributed to rapid destruction of RBCs by phagocytosis in reticulo-endothelial system (Ajayi *et al.*, 1978) [2] and so the massive haemolysis occured during the period of infection with *Babesia bigemina* and hypoxia leads to hepatic cell degeneration and glomerular dysfunction resulting in increased level of blood urea nitrogen (Talkhan *et al.*, 2010) [26]. Babesia can cause degeneration and necrosis in kidney convoluted tubules, consequently a rise in BUN is expected (Mosqueda *et al.,* 2012) [20].

The Mean values of glucose in babesiosis infected cows were significantly lower as compared to that in healthy cows. The observations recorded in the present study are in agreement with Hussein *et al.* (2007) [12], Ganguly *et al.* (2017) [10], Jayalakshmi *et al.* (2017) [14]. The recorded hypoglycemia in *babesiosis* can be attributed to severe depletion of stored glycogen and persistent feverish condition associated with *babesiosis,* resulting in anorexia and consequently hypoglycemia (Fujinaga, 1981) [9] in *Babesia* infected cattle.

Conclusion
In the present study, babesia infection affects the haemato-biochemical parameters which is manifested as anaemia, low hematocrit value, leukocytosis, lymphopenia, neutrophilia, hypoalbuminemia, hypoglycemia, hypoproteinemia, hyperbilirubinemia. Besides this high level of AST, ALT, ALKP and BUN were also evaluated. These findings suggest that Babesiosis affects the liver and kidney of the affected animals which is shown in the present study. These valuable data obtained in the present study may be helpful in the diagnosis and treatment (symptomatic) of the disease.

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