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Haemato-biochemical alterationas and therapeutic study on babesiosis in cattle of lower Brahmaputra valley region

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Abstract
The study was conducted with an aim to investigate the status of some haematological and biochemical parameters and comparative efficacy of three different treatment regime in the cattle affected with Babesiosis. Blood samples were collected from 239 cattle clinically suspected for Babesiosis. Twenty one cattle found positive for Babesiosis on microscopic examination were utilized for further study. Whole blood and serum samples were analysed for estimation of Haemoglobin (Hb), Total Erythrocyte Count (TEC), Packed Cell Volume (PCV), Total Leucocyte Count (TLC), Thrombocyte Count, Total Serum Protein (TSP), Albumin, Bilirubin, Alanine Transaminase (ALT), Aspartate Transaminase (AST), Blood Urea Nitrogen (BUN) and Creatinine. The study revealed significant decline in the level of Hb, TEC, PCV, Thrombocyte count, total serum protein and albumin whereas, a significant increase in the level of TLC, ALT, AST, bilirubin (Total), BUN and creatinine was observed in the affected cattle. Diminazene diaceturate and oxytetracycline combination was found the most effective among the three treatment regimes.

Keywords: haemato-biochemical alterationas, therapeutic, babesiosis, cattle

Introduction
Among various tick borne diseases, bovine Babesiosis is one of the important haemoparasitic diseases that causes significant morbidity and mortality in cattle (Sharma *et al.*, 2016) [11]. The livestock industry faced about 57.2 million US dollars of annual economic loss due to Babesiosis in India (McLeod and Kristjanson, 1999). It is an emerging disease and included in the OIE's list of 'B' category diseases (OIE Terrestrial Manual, 2014) [7]. Babesiosis is characterized by fever, anorexia, coffee colored urine, anemia, drop in milk production (Barman *et al*., 2018) [2].

The present study area is a hub of dairy industry for entire North Eastern region of India. The area being hot and humid, is conducive for multiplication of tick vector and thus there is higher incidence of Babesiosis and tick borne diseases as per the report of Veterinary practitioners. This pose a threat to the profitability of the dairy farmers in terms of mortality, treatment cost and decreased production.

Pressed by the need and keeping in view of the above facts, this study was conducted to investigate the occurrence, haeamto-biochemical changes and therapeutic management of cattle affected with Babesiosis.

Materials and Method
Study area was in foothills of Himalayan region covering boarder area of two states Assam and Meghalaya of North Eastern India. Clinical cases of cows with symptoms of fever, anemia, haemoglobinuria, inappetence were attended as per call of the farmers as it was not possible for them to bring the animal because of geographical difficulties of farm location. Peripheral blood smears were prepared through ear tip puncture from 239 clinically suspected cows followed by microscopic examination of the Giemsa stained blood smears (Soulsby, E.J.L. 2012) [12]. Twenty one animals, found positive for *Babesia bigemina* on microscopic examination were considered for further study. Three mililitres of blood samples were collected in each of an EDTA and a clot activator vacutainer for haematological estimation in an automatic blood cell counter and biochemical investigation in a semiautomatic biochemistry analyser respectively.

Serum was separated following standard procedure and utilized for estimation of Total Serum Protein (TSP), Albumin, Bilirubin, Aspartate Transaminase (AST), Alanine Transaminase (ALT), creatinine, Blood Urea Nitrogen (BUN) using commercial kits.

The cattle found positive for Babesiosis were equally divided into 3 groups with 7 in each group (A, B and C). Another group (D) comprising of 7 unaffected animals were kept as healthy control. The different therapeutic regime against the affected cases were executed as presented in Table 1. Apart from pre-treatment, Blood samples were also collected on 10th and 30th day post treatment for evaluation of the response to the treatment.

Table 1: Grouping of animals and therapeutic trial conducted

Group	Drug	Dose rate (mg/kg)	Route	Duration
A	Diminazene diaceturate	5	Intra-muscular	Single dose
B	Diminazene diaceturate + Oxytetracycline	5 10	Intra-muscular Intravenous	Single dose Twice daily for 5 days
C	Diminazene diaceturate + Doxycycline	5 5	Intra-muscular Oral	Single dose Twice daily for 15 days
D	-	-	-	-

Results and Discussion

Microscopic examination of 239 stained blood smears from clinically suspected cattle revealed intra-erythrocytic pear shaped Babesia organism in 21 cattle.

Haematological alterations

The MEAN ± S.E. values of Haemoglobin (Hb) and total Erythrocyte Count (TEC) were significantly lower in all the affected groups (A, B, C) than the healthy control group. (Table 2, $p<0.01$) It was due to intravascular haemolysis of the red blood cells caused by emerging parasites, increased phagocytic activity of non-infected RBC by Reticulo Endothelial system and suppression of erythropoiesis (Rani *et al*., 2010, Jyotishree *et al*., 2013, Maharana *et al*., 2016) [9, 5, 6].

The TLC increased significantly in all the affected groups in comparison with the healthy control (Table 2, $p<0.01$) which might be attributed to the toxic metabolite of the circulating parasite, phagocytosis of the destructed erythrocytes. Another possible reason behind this elevation might be the associated with stress. Rani *et al.* (2010) [9] and Hussein *et al.* (2007) [4] found a decrease in the level of TLC which could be due to the suppression of leucogenesis resulting from metabolites of infected and lysed RBC. A significant fall in platelet count (Table 2, $p<0.01$) was observed in all the affected animals. Maharana *et al*. (2016) [6] observed a similar finding, which might be due to the immune mediated destruction of platelets occurring as a result of strong antigenicity of the invading organism.

Biochemical alterations

In the present study, the value of TSP and albumin showed a significant reduction (Table 3 and 4, $p<0.01$) in all the affected group than the healthy control. This could be due to decreased protein synthesis by the affected liver cells as well as decreased feed intake. The underlying reason behind low albumin might be attributed to decreased synthesis of the same as well as the release of albumin from destructed RBC and its loss in the urine (Barbara *et al.*, 2008) [1].

There was significant elevation of AST and ALT level in the affected groups (Table 5 and 6, $p<0.01$) which might be associated with hypoxic injury to hepatocyte, skeletal tissue due to haemolytic anemia. Moreover, due to destruction of RBC, which is a store house for AST, leads to its release and an increased level in the blood (Hussein *et al*., 2007 and Ola *et al*., 2010) [4]. The bilirubin value increased significantly (Table 7, $p<0.01$) in all the affected animals, which might be attributed to the massive haemolysis of the red blood cells leading to over production of bilirubin (Rani *et al*., 2010 and Saud *et al*., 2005) [9, 10].

The mean level of BUN and creatinine increased significantly (Table 8 and 9; $p<0.01$) in the affected groups than that of the healthy control. The increase of BUN could be due to massive haemolysis and increased catabolism of protein (Ola *et al*., 2000). This might also be associated with the decreased renal perfusion due to dehydration. The increase of Creatinine might be associated with the sequestration of Babesia infected RBC in renal capillaries and subsequent decrease in glomerular filtration rate. Esmaeilnejad *et al*. (2012) [3] and Sharma *et al*. (2016) [11] also reported an elevation in the level of creatinine in cattle affected with Babesiosis.

Table 2: Mean±SE values of various hematological parameters in different groups at pre-and post-treatment days

Day	A				B				C				D			
	Hb (gm/dl)	TEC (million /cmm)	TLC (Thousand / cmm)	THR (Thousand / cmm)	Hb (gm / dl)	TEC (million / cmm)	TLC (Thousand / cmm)	THR (Thousand / cmm)	Hb (gm/ dl)	TEC (million / cmm)	TLC (Thousand / cmm)	THR (Thousand / cmm)	Hb (gm/ dl)	TEC (million / cmm)	TLC (Thousand / cmm)	THR (Thousand / cmm)
0	5.01±0.32^{a} ^{A}	4.09 ± 0.10 ^{aA}	15.21 ± 0.59 ^{aA}	90.28 ± 2.56 ^{aA}	5.03 ± 0.32 ^{aA}	4.07 ± 0.13 ^{aA}	15.28 ± 0.50 ^{aA}	89.43 ± 2.41 ^{aA}	5.04 ± 0.37^{a} ^{A}	4.02± 0.19 ^{aA}	15.27 ± 0.60 ^{aA}	89.71 ± 2.26 ^{aA}	9.90 ± 0.29^{a} ^{B}	9.80 ± 0.32 ^{aB}	8.73 ± 0.32 ^{aB}	152.14 ± 2.31 ^{aB}
10	6.31±0.37 ^{bA}	5.34 ± 0.10 ^{bA}	14.22 ± 0.60 ^{bA}	108.85 ± 2.81 ^{bA}	6.2 ± 0.32 ^{bA}	5.43 ± 0.11 ^{bA}	14.33 ± 0.49 ^{aA}	108.72 ± 2.45 ^{bA}	6.3 ± 0.40 ^{bA}	5.31 ± 0.10 ^{bA}	14.37 ± 0.63 ^{bA}	107.14 ± 2.66 ^{bA}	9.88 ± 0.30 ^{aB}	9.84 ± 0.32 ^{aB}	8.80 ± 0.30 ^{aB}	153.71 ± 2.20 ^{aB}
30	9.65±0.42 ^{dA}	8.87 ± 0.18 ^{dA}	9.67 ± 0.62 ^{cA}	152.57 ± 2.41 ^{dA}	9.94 ± 0.28 ^{dA}	9.54 ± 0.08 ^{dB}	8.61 ± 0.43 ^{cA}	157.71 ± 2.35 ^{dA}	9.71 ± 0.32 ^{dA}	8.84 ± 0.20 ^{dA}	9.54 ± 0.62 ^{cA}	148.14 ± 3.05 ^{dB}	9.85 ± 0.27 ^{aA}	9.88 ± 0.32 ^{aB}	8.75 ± 0.21 ^{aA}	154.14 ± 2.17 ^{aA}

Table 3: Mean±SE values of TSP (gm/dl) in different groups at pre-and post-treatment days

Day	Group			
	A	B	C	D
0th	5.86 ± 0.13 aA	5.82 ± 0.13 aA	5.87 ± 0.13 aA	7.08 ± 0.12 aB
10th	6.34 ± 0.14 bA	6.34 ± 0.12 bA	6.23 ± 0.13 aA	7.05 ± 0.08 aB
30th	7.08 ± 0.12 cA	7.24 ± 0.09 dA	6.94 ± 0.14 cA	7.07 ± 0.05 aA

Table 4: Mean±se values of albumin (gm/dl) in different groups at pre-and post-treatment days

Day	Group			
	A	B	C	D
0th	1.88 ± 0.05 aA	1.84 ± 0.06 aA	1.87 ± 0.04 aA	3.00 ± 0.12 aB
10th	2.17 ± 0.07 bA	2.26 ± 0.07 bA	2.21 ± 0.04 bA	2.97 ± 0.10 aB
30th	2.77 ± 0.06 dA	3.03 ± 0.08 dB	2.78 ± 0.06 dA	3.01 ± 0.08 aB

Table 5: Mean±se values of bilirubin (mg/dl) in different groups at pre-and post-treatment days

Day	Group			
	A	B	C	D
0th	1.58 ± 0.10 aA	1.55 ± 0.15 aA	1.54 ± 0.14 aA	0.26 ± 0.04 aB
10th	1.43 ± 0.07 bA	1.10 ± 0.08 bA	1.23 ± 0.10 aA	0.25 ± 0.03 aB
30th	0.50 ± 0.05dA	0.39 ± 0.04 dB	0.60 ± 0.07 aB	0.24 ± 0.02 aC

Table 6: Mean±se values of alt in different groups at pre-and post-treatment days

Day	Group			
	A	B	C	D
0th	129.61 ± 1.81aA	128.94 ± 0.97 aA	129.13 ± 1.06 aA	58.53 ± 1.50 aB
10th	110.97 ± 1.25cA	107.93 ± 1.16 cA	112.81 ± 1.24 cB	58.58 ± 1.45 aC
30th	58.83 ± 0.74 eA	58.56 ± 0.88 eA	59.18 ± 1.09 eA	58.80 ± 1.39 aA

Table 7: Mean±se values of ast in different groups at pre-and post-treatment days

Day	Group			
	A	B	C	D
0th	28.94 ± 1.02 aA	28.80 ± 0.96 aA	28.43 ± 0.92 aA	7.66 ± 0.30 aB
10th	24.14 ± 0.75 bA	22.78 ± 0.78 bA	22.66 ± 0.76 bA	7.64 ± 0.32 aB
30th	8.27 ± 0.47 dA	7.67 ± 0.31 dA	8.05 ± 0.37 dA	7.77 ± 0.32 aA

Table 8: Mean±se values of bun (gm/dl) in different groups at pre-and post-treatment days

Day	Group			
	A	B	C	D
0th	29.46 ± 0.91 aA	29.16 ± 1.29 aA	29.18 ± 0.78 aA	15.18 ± 0.53 aB
10th	25.38 ± 0.76 bA	23.81 ± 1.13 bA	24.98 ± 0.57 bA	15.15 ± 0.51 aB
30th	15.26 ± 0.79 dA	14.23 ± 0.51 dA	15.06 ± 0.37 dA	15.17 ± 0.53 aA

Table 9: Mean±se values of creatinine (mg/dl) in different groups at pre-and post-treatment days

Day	Group			
	A	B	C	D
0th	1.70 ± 0.07 aA	1.74 ± 0.08 aA	1.68 ± 0.08 aA	0.70 ± 0.05 aB
10th	1.50 ± 0.07 aA	1.50 ± 0.08 bA	1.45 ± 0.45 bA	0.68 ± 0.05 aB
30th	1.01 ± 0.061 cA	0.94 ± 0.05 dA	1.08 ± 0.07 dA	0.68 ± 0.05 aA

In all tables (2 to 9), Means bearing same superscript (A, B) do not differ significantly within a row. Means bearing same superscript (a, b, c, d) do not differ significantly within a column.

Therapeutic efficacy of different drugs

The therapeutic evaluation was carried out based on clinical recovery and improvement in the hematological and biochemical parameters as well as negative result on parasitological examination on post-treatment days (10th and 30th). The percent efficacy of various drugs at different post-treatment days has been presented in Table 13. Highest and earlier recovery was noticed in Group B. Moreover, in Group B the rate of improvement in hematological and biochemical parameters were more than that of Group A and Group C. It might be due to the synergistic effect between the two drugs (oxytetracycline and diminazene diaceturate) used in Group B. Another possible reason might be the presence of other organism sensitive to oxytetracycline that were not detected. Urquhart *et al.* (1996) [13] also recorded the efficacy of oxytetracycline against babesiosis in cattle to reduce parasitemia. However, the lower efficacy in treatment group C (diminazene + doxycycline) might be associated with the limited oral bioavailability of doxycycline due to degradation by the ruminal microflora.

It may be concluded that besides the drug of choice, incorporation of some therapeutic measures based on the haematological and biochemical alterations seen in this study,

the therapeutic efficacy will be enhanced in terms of early recovery thus preventing loss of productivity. Moreover, a detailed molecular study on the detection of haemoparasitic infection will give a clear idea on the prevalence and further interventions regarding control measures of the disease in this area.

Table 10: Percent therapeutic efficacy of different treatment regime

Group	No. of cattle treated	No. of cattle recovered (on 30th day post treatment)
A	7	5 (71.43)
B	7	7 (100)
C	7	4 (57.14)
D	7	–

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