Study on haematological findings in cattle with clinical Babesiosis

P. Dhanamjayam[1], B. Sudhakara Reddy[1], B. Shobhamani[1] and S. Sivajothi[2*]

[1]Department of Veterinary Medicine; [2]Department of Veterinary Parasitology, College of Veterinary Science, Sri Venkateswara Veterinary University, Proddatur – 516360 Andhra Pradesh INDIA
*Corresponding author: sivajothi579@gmail.com

Journal of Livestock Science (ISSN online 2277-6214) 15: 227-231
Received on 8/4/24; Accepted on 26/5/24; Published on 2/6/24
doi. 10.33259/JLivestSci.2024.227-231

Abstract

The report details hematological observations from a study involving 28 adult cattle afflicted with clinical babesiosis. The study, conducted in the YSR district of Andhra Pradesh from January to December 2023. Diagnosis of babesiosis through symptoms such as fever, hemoglobinuria, tick infestation, and positive results from microscopic examination of stained peripheral blood smears. Whole blood samples were analyzed using an automatic blood analyzer to assess hematological parameters. The hematological findings revealed significant alterations in several parameters among the infected cattle compared to healthy counterparts. Notable reductions were observed in hemoglobin (P=0.001), packed cell volume (P=0.001), total erythrocyte count (P=0.003), mean corpuscular hemoglobin concentration (P=0.046), and absolute lymphocyte count (P=0.042). Conversely, there were no significant changes in mean corpuscular hemoglobin (P=0.063) and absolute monocyte count (P=0.082). Moreover, the infected cattle exhibited significant increases in mean corpuscular volume (P=0.035), total leukocyte count (P=0.021), absolute eosinophil count (P=0.034), and absolute neutrophil count (P=0.007) compared to their healthy counterparts. Assessing the hematological profile in infected cattle provides valuable insights into animal health during infection. The present study concludes the macrocytic anemia with leukocytosis, leucopenia, neutrophilia and eosinophilia in cattle with babesiosis. Complete blood count is essential to assess the extent of the infection and the overall health status of the animal for formulation of treatment and management strategies.

Key words: Cattle; Babesiosis; Haematology; MCV; Leucocyt count; Anemia

Introduction

Hemoprotozoan diseases cause serious economic loss to dairy industry (Farhang, 2017). Babesiosis, caused by *Babesia* parasites, primarily spreads through blood-sucking ticks but can also occur through blood inoculation, insect transmission, surgical procedures, and intrauterine infection. In India, *Rhipicephalus* ticks are the main vectors due to favorable climatic conditions (Akel and Mobarakai, 2017). This disease poses significant economic losses particularly affecting crossbred cattle more than zebu and buffaloes, which serve as carriers. Studies were carried out on the haematological changes during the clinical babesiosis in dogs (Reddy et al. 2014) and in sheep with babesiosis (Sivajothi et al. 2022). Understanding these changes in infected animals is crucial for diagnosis, prognosis, therapy, and assessing infection severity (Abdel et al. 2020). Limited studies are available in the present geographical region in cattle with clinical babesiosis and hence, the study investigated these parameter variations to record the pathological changes during clinical disease in cattle.

Materials and Methods

The research took place in the YSR district of Andhra Pradesh, spanning from January to December 2023. Samples were collected from the five veterinary dispensaries (Proddatur, Chapadu, Khajipeta, Muddanur, Veerapu Nayuni Palli) in YSR district of Andhra Pradesh during this period (Fig.1). All the cattle under the study were belongs to Holstein Friesian and age from 3 years to 11 years. Cattle with signs of fever, lymphadenopathy, pale mucus membranes, icteric mucus membranes and passing of red colour urine were selected for collection of blood for the study. Three thin blood smears were prepared from each sample, fixed in methanol for 5 minutes, and stained with Geimsa stain (dilution ratio: 1:10) for 30 minutes. The slides were examined under an oil immersion objective lens to confirm the presence of piroplasmic stages (Sivajothi et al. 2023). Confirmation of babesiosis was achieved through microscopic identification of the piroplasmic stage of *Babesia* spp. A total of 240 samples were gathered, from which 28 were identified as positive for acute infection based on clinical signs and tick infestation. To ensure comparability, ten samples were randomly selected from healthy cattle for reference. All samples utilized in this study comprised whole venous blood. Aseptic collection of whole blood in EDTA coated vials approximately 6 ml from the jugular vein of suspected animals was performed (Reddy et al., 2022). Complete blood count was carried out by the automatic blood analyzer in the College of Veterinary Science, Proddatur. The obtained results underwent statistical analysis using SPSS version 23.00. Results are expressed as mean ± standard error (SE). Student's t-test was employed to compare the means of infected and non-infected groups.

Results

The presence of babesiosis was confirmed by the presence of intra-erythrocytic piroplasms of *Babesia* in the form of pyriform or pear-shaped in the present study (Fig.2). Haematological parameters were haemoglobin (g/dL) ranging from 5.35 to 6.92, packed cell volume (%)ranging from 18.65 to 22.45, total erythrocyte count ($\times 10^6$ /µL) ranging from 2.98 to 3.35, mean corpuscular volume (fL) ranging from 62.6 to 69.8, mean corpuscular haemoglobin (pg) ranging from 16.6 to 21.8, mean corpuscular haemoglobin concentration (g/dL) ranging from 26.67 to 33.08, total leucocyte count ($\times 10^3$ /µL) ranging from 13.02 to 16.01, absolute neutrophil count ($\times 10^3$ /µL) ranging from 6.86 to 7.42, absolute lymphocyte count ($\times 10^3$ /µL) ranging from 5.41 to 6.35, absolute eosinophil count ($\times 10^3$ /µL) ranging from 0.72 to 0.85 and absolute monocyte count ($\times 10^3$ /µL) ranging from 0.28 to 0.37. A significant reduction in in haemoglobin (P=0.001), packed cell volume (P=0.001), total erythrocyte count (P=0.003), mean corpuscular haemoglobin concentration (P=0.046) and absolute lymphocyte count (P=0.042) was noticed. There is no change in mean corpuscular haemoglobin (P=0.063) and absolute monocyte count (P=0.082). Significant increase in mean corpuscular volume (P=0.035), total leucocyte count (P=0.021), absolute eosinophile count (P=0.034), absolute neutrophile count (P=0.007) observed when compared to the apparently healthy cattle. Cattle with babesiosis showed the significant reduction (P<0.01) in haemoglobin concentration, packed cell volume percentage, total erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin, total leucocyte count and absolute neutrophil count; a significant increase (P<0.05) in absolute eosinophil count and absolute monocyte count; no significant changes in mean corpuscular haemoglobin concentration when compared to the apparently healthy cattle.

Therapy was initiated by administered with injection diminazene aceturate @ 5 mg/kg body weight intramuscularly along with fluid therapy, injection flunixine meglumine @ 0.2 mg/kg body weight intra muscularly, injection pheniramine maleate @ 0.2 mg/kg body weight intra muscularly, injection containing Vitamin A (300000 IU), D3 (100000 IU) and Vitamin E (50 mg) intra muscularly, injection B complex, each ml contains methylcobalamin 500 mcg, pyridoxine 50 mg and nicotinamide 50 mg were given intramuscularly; oral administration of syrup containing liver tonic fortified with yeast, vitamin (Brotone Vet) as per requirement; syrup 3D RED (containing the calcium gluconate 140 mg, ferric ammonium citrate containing elemental iron eq to 54 mg, copper sulphate 40 mg, cobalt chloride 20 mg, niacinamide 18 mg, biotin 30 mcg folic acid 3mg,

vitamin B 12 125 mcg and pyridoxine 4 mg) are nutritional feed supplement as per the manufacture recommendations (Aulakh et al. 2005; Reddy et al. 2014 ; Sivajothi et al. 2022).

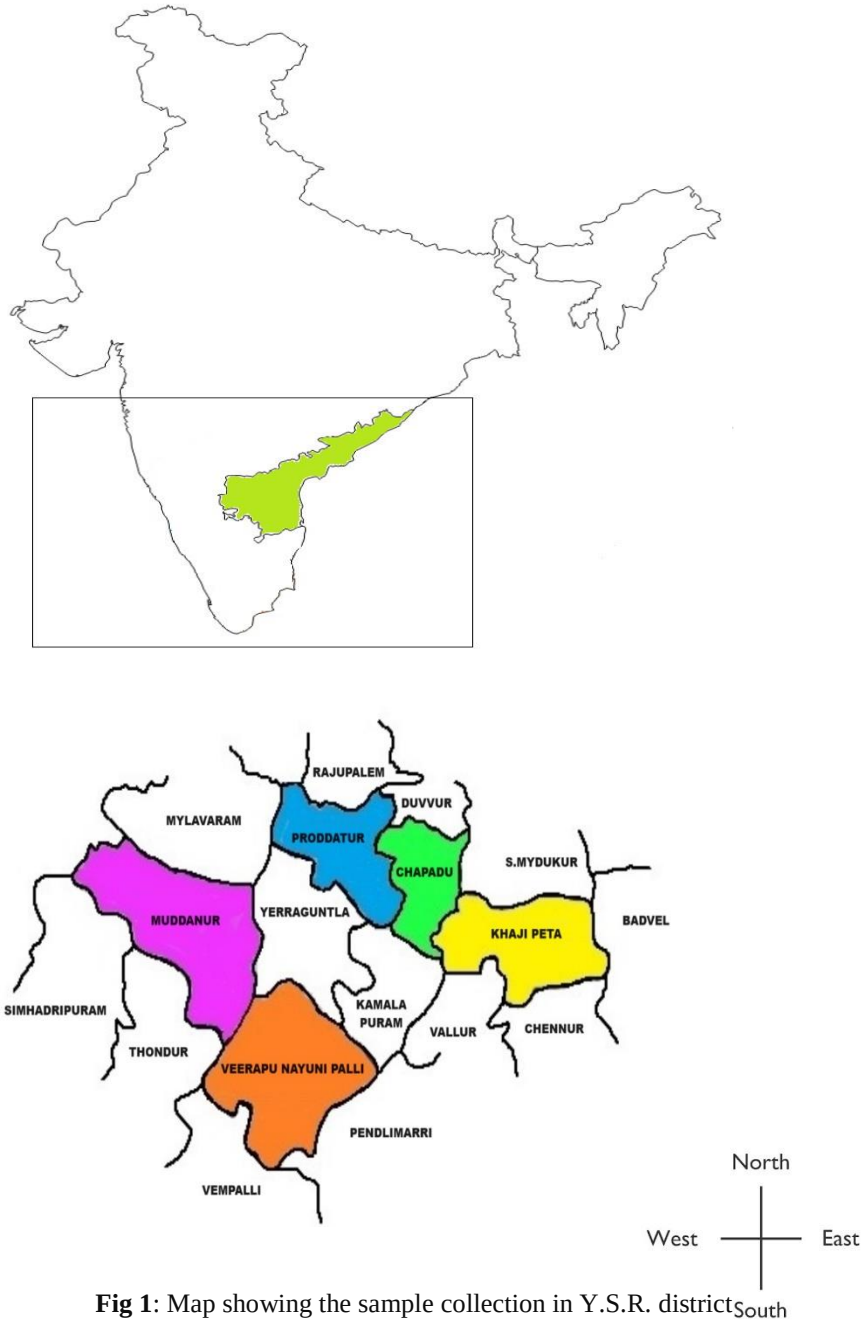

Fig 1: Map showing the sample collection in Y.S.R. district

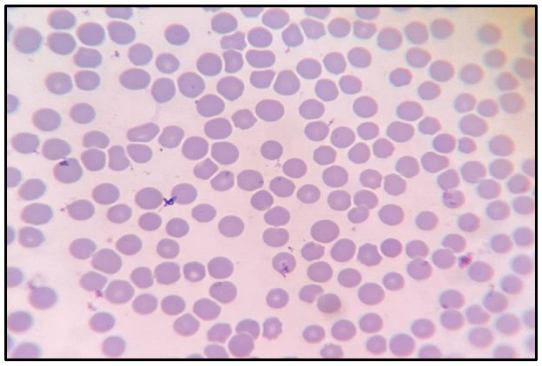

Fig 2: Presence of Babesia piroplasms within the erythrocytes (1000x)

Table 1. Haematological findings in cattle with babesiosis

S.N.	Parameter	Apparently healthy Cattle (10)	Cattle with babesiosis (28)	P value
1.	Haemoglobin (g/dL)	10.94^{b}±0.33	6.02^{a}±0.86	0.001**
2.	Packed Cell Volume (%)	33.14^{b}±1.08	20.58^{a}±1.21	0.001**
3.	Total Erythrocyte Count ($\times10^{6}$ µl)	6.45^{b}±0.24	3.11^{a}±0.21	0.003**
4.	Mean Corpuscular Volume (fL)	51.08^{a}±1.72	66.23^{b}±0.94	0.035*
5.	Mean Corpuscular Haemoglobin (pg)	17.86±0.45	18.45±0.71	0.063^{NS}
6.	Mean Corpuscular Haemoglobin Concentration (g/dL)	33.09^{b}±1.85	29.32^{a}±1.97	0.046*
7.	Total Leucocyte Count ($\times10^{3}$ µl)	10.62^{a}±0.65	14.15^{b}±1.08	0.021*
8.	Absolute neutrophil count ($\times10^{3}$ µl)	3.21^{a}±0.24	7.14^{b}±0.18	0.007**
9.	Absolute lymphocyte count ($\times10^{3}$ µl)	6.73^{b}±0.07	5.85^{a}±0.09	0.042*
10.	Absolute eosinophil count ($\times10^{3}$ µl)	0.48^{a}±0.08	0.81^{b}±0.02	0.034*
11.	Absolute monocyte count ($\times10^{3}$ µl)	0.25±0.03	0.34±0.01	0.082^{NS}

*P<0.05; **P<0.01; ^{NS}P>0.05; ^{ab}Columns bearing different

In this study, babesiosis confirmation was conducted through microscopic examination of stained blood smears, revealing the presence of pear-shaped piroplasms inside red blood cells. This finding serves as a confirmatory diagnosis, particularly in the acute stages of the disease (Sivajothi et al. 2022, Sivajothi et al. 2023).

The results of the hematological investigation in cattle suffering from babesiosis revealed significant reduction in erythrocytic count, hemoglobin concentration and packed cell volume percentage. The results were in association with the previous studies carried out by Mahmoud et al. (2015), Sharma et al. (2000), Aulakh et al. (2005). Anemia development is a characteristic feature of babesiosis in the present study, where the parasite causes the destruction of red blood cells. Parasitemia during the pathogenesis significantly alters the osmotic fragility of red blood cells, making even unaffected RBCs prone to destruction, leading to hemolysis. In some cases, auto-antibodies against circulating red cells have been proposed. These antibody-coated red cells are targeted by macrophages and complement proteins, leading to hemolysis. *Babesia* has the ability to impair the red cell membrane rich in polyunsaturated fatty acids, making it susceptible to oxidative damage, further compromising the integrity of red cells (Omar et al. 2015; Sedar et al. 2009. Significant declines in RBC count, hematocrit percentage, hemoglobin concentration, and mean corpuscular hemoglobin concentration along with an increase in mean corpuscular volume were observed, indicative of macrocytic hypochromic anemia. These findings may be attributed to mechanical damage caused by trophozoite cleavage within RBCs, production of anti-erythrocyte antibodies targeting structural erythrocyte membranes, and increased erythrophagocytosis by activated macrophages (Goes et al. 2007; Salem et al. 2016). Sometimes the newly produced red blood cells may not mature fully before entering circulation, leading to variations in cell size (anisocytosis), including larger cells (macrocytes), and reduced hemoglobin content (hypochromia) (Sivajothi et al. 2015).

The babesiosis-infected cattle, a significant increase in total leukocyte count was observed compared to healthy cattle, likely reflecting the host's response to infection (Aulakh et al. 2005). When cattle are infected with *Babesia* parasites, their immune system mounts a response to combat the infection. This immune response often involves an increase in the production and release of white blood cells.

Neutrophil levels were notably elevated in infected cattle, indicating neutrophilia. Similar findings were reported by Wadhwa et al. (2008), Mahmoud et al. (2015), Tufani et al. (2015) and Ganguly et al. (2017). In babesiosis, neutrophilia can commonly occur as part of the host response to the *Babesia* parasites. The immune system recognizes the presence of the parasites as foreign invaders and initiates a series of immune responses to combat the infection. This includes the release of various cytokines and chemokines that attract neutrophils and other immune cells to the site of infection. Conversely, lymphocyte counts were significantly lower in infected cattle, indicative of lymphopenia. This aligns with findings reported by Saud et al. (2005), Aulakh et al. (2005), Wadhwa et al. (2008), Tufani et al. (2015), and Ganguly et al. (2017). During the acute phase of babesiosis, white blood cells may migrate to the site of infection, such as the spleen or other organs, leading to a temporary decrease in circulating leukocytes and resulting in leucopenia. In the present study, eosinophilia was noticed and it may be due to secondary response to tissue damage or inflammation caused by the *Babesia* infection. *Babesia* infection triggers an allergic or hypersensitivity reaction in the host, eosinophils may become elevated as part of that response. Non-significant changes were observed in absolute monocytic count in infected cattle compared to non-infected cattle. This corresponds with findings reported by Aulakh et al. (2005), Saud et al. (2005), Wadhwa et al. (2008), Tufani et al. (2015) and Ganguly et al. (2017).

Some of the authors reported leucopenia in babesiosis and it might be due to binding of platelets to endothelial cells and causing neutrophil endothelial interactions which contribute to leucopenia (Barman et al. 2021). Few researchers, documented neutropenia in acute babesiosis and it is due to direct damage to hematopoietic precursor cells, splenic sequestration, increased neutrophil adherence, or a combination of factors (Aziz et al. 2020). Lymphopenia observed in *Babesia* infected cattle may be attributed to stress (Mathe et al. 2006). The present study concludes the macrocytic anemia with leukocytosis, leucopenia, neutrophilia and eosinophilia in cattle with babesiosis. Complete blood count is essential to assess the extent of the infection and the overall health status of the animal for formulation of treatment and management strategies.

Author contribution: All authors read and approved the manuscript.

Ethical approval: All the procedures were carried out in full compliance with the recommendation in the guidelines of Institute animal ethics committee (IAEC) of Sri Venkateswara Veterinary University.

Conflict of interest: The authors declare that they have no conflict of interest

References

1) Abdel-Hamied E, Arafa W, Mahmoud M, 2020. Oxidative stress, hemogram, hepatorenal function evaluation and molecular diagnosis of babesiosis in crossbred cows naturally infected with *B bigemina.* Advances in Animal and Veterinary Science 8(12): 1402-1409.
2) Akel T, Mobarakai N, 2017. Hematologic manifestations of Babesiosis. Annals of Clinical Microbiology and Antimicrobials 16: 6. 2–7.
3) Aulakh GS, Singla LD, Kaur P, Alka, 2005. Bovine babesiosis due to Babesia bigemina: Haematobiochemical and therapeutic studies. Indian Journal of Animal Science. 75(6):617-622.
4) Aziz PR, Marodia S, Ganesan PI, Sharma CS, 2020. A clinical study on haemato-biochemical changes in cows affected with babesiosis. The Pharma Innovation Journal 9(2): 242-245.
5) Barman U, Arup Das, Abhijit Deka, Hazorika M, Arif S A, Deepa Lahkar and Borpujari D, 2021. Haemato-biochemical alterations and therapeutic study on babesiosis in cattle of lower Brahmaputra valley region. The Pharma Innovation Journal 10(12): 1091-1094.
6) Farhang, H.H. 2017. Development of IFA test to detect Theileria annulata and seroprevalence of the parasite in Tabriz area of Iran. Journal of Livestock Science 8: 169-171
7) Ganguly A, Bisla RS, Ganguly I, Singh H, Bhanot V, Chaudhri SS, 2017. Direct blood PCR detection of *Babesia bigemina* and its effect on haematological and biochemical profile in crossbred cattle of eastern Haryana, Indian Journal of Animal Research 51 (1): 141-145.
8) Goes TS, Goes VS, Ribeiro MF and Gontijo CM, 2007. Bovine babesiosis: anti-erythrocyte antibodies purification from the sera of naturally infected cattle. Veterinary Immunoloy and Immunopathology 116: 215–18.
9) Mahmoud MS, Kandil OM, Nasr SM, Hendawy SH, Habeeb SM, Mabrouk DM, 2015. Serological and molecular diagnostic surveys combined with examining hematological profiles suggests increased levels of infection and hematological response of cattle to babesiosis infections compared to native buffaloes in Egypt. Parasites & Vectors 8(1):319.
10) Mathe A, Voros K, Papp L, Reiczigel J, 2006. Clinical manifestations of canine babesiosis in Hungary (63 cases). Acta Veterinaria Hungarica 54: 367–85.
11) Omar AOJ, Sadat M, Emam FA, 2015. Determination of oxidative stress markers associated with Babesia Bigemina. African Journal of Biotechnology Research 3: 150–5.
12) Reddy BS, Sivajothi S, Reddy LSSV, Raju KGS, 2014. Clinical and laboratory findings of *Babesia* infection in dogs. Journal of Parasitic Diseases 40(2):268- 272.
13) Reddy BS, Sivajothi S, Swetha K 2022. Electrocardiographic findings and alterations of cardiac troponin in dogs with *Babesia gibsoni.* The Pharma Innovation Journal. SP-11(11): 2376-2378.
14) Salem NY, Yehia SG, Farag HS, Elkhiat MA, 2016. Clinical, hemato-biochemical alterations and oxidant–antioxidant biomarkers in *Babesia* infected calves. International Journal of Veterinary Science and Medicine 4(1):17-22.
15) Saud N, Ahmed FA, Sheikh IU, Bhattacharya M, 2005. Prevalence of bovine babesiosis in Dirang valley of Arunachal Pradesh. Indian Veterinary Journal 82:1011-1012.
16) Sedar D, Yeter BK, Ozdaland NAG, 2009. Status of lipid peroxidation, antioxidants and oxidation products of nitric oxide in equine babesiosis: status of antioxidant and oxidant in equine babesiosis. Journal of Equine Veterinary Science 29:743–74.
17) Sharma AK, Katoch RC, Nagal KB, Kishtwaria RS, Sharma SK, 2000. Bovine Babesiosis in Palam valley of Himachal Pradesh. India Veterinary Journal 77: 731–732
18) Sivajothi S, Reddy BS, Reddy LSSVP, Naik BR, 2022. Electrocardiographic and haemato-biochemical findings in sheep with babesiosis, The Pharma Innovation Journal SP 11(9): 1189-1191.
19) Sivajothi S, Reddy BS, Swetha K, 2023. Cerebrospinal fluid analysis and haemato-biochemical variations in young buffalo calves with cerebral babesiosis. Journal of Parasitic Diseases https://doi.org/10.1007/s12639-023-01628-0.
20) Sivajothi S, Rayulu VC, Reddy BS. Haematological and biochemical changes in experimental *Trypanosoma evansi* infection in rabbits. Journal of Parasitic Diseases 39(2):216–220 DOI 10.1007/s12639-013-0321.
21) Tufani NA, Fazili MR, Malik HU, Beigh SA, Dar KH, 2015. Clinico haematological Profile and therapeutic management of acute babesiosis in a Holstein-friesian crossbred cow. Veterinary Clinical Science 3(3):11-14.
22) Wadhwa DR, Pal B, Mandial RK, 2008. Epidemiological and clinico-therapeutic study of babesiosis in cattle. Indian Journal of Veterinary Research 17(2):22-24.