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ORIGINAL ARTICLE

Hematological Findings in Nellore Brown Sheep Infected with Babesiosis

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ABSTRACT

Background: Babesia infection in small ruminants especially in sheep can cause a wide range of clinical and laboratory presentations. *Babesia ovis* plays an important role in causing anemia and other hemotological changes in affected sheep.

Aim: Therefore, the present study was conducted to analyze and compare hematological parameters between blood profiles of Nellore brown sheep naturally infected and uninfected with *Babesia ovis*, the main causative agent of ovine babesiosis.

Material and Methods: The present study was carried out to record the haematological changes in Nellore brown sheep affected with babesiosis. Total 22 adult sheep were identified from a sheep flock (n=187), suffering with the clinical babesiosis. Microscopic examination of the stained peripheral blood smears confirmed the presence of Babesia organisms. Affected sheep showed fever, anorexia, suspended rumination, weakness, lethargy, haemoglobinuria and progressive emaciation.

Results: Significantly reduced hemoglobin concentration, packed cell volume (PCV), red blood cell count, whereas significantly elevated total leukocyte count and eosinophil count were noticed. All the affected sheep were successfully treated with diminazene aceturate, oxytetracycline dihydrate, hematinic along with the electrolyte therapy.

Conclusion: The Present study reveal that a significant reduction in erythrocyte count, hemoglobin concentration and PCV, a significant increase in total leukocyte count and eosinophil count in sheep affected with babesiosis.

KEYWORDS

• Babesiosis • Haematology • Sheep

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INTRODUCTION

More number of animals including small ruminants are suffering from haemoprotozoan diseases may be due to presence of the vectors such as ticks. Vector-borne diseases are caused by different groups of pathogens, including bacteria, parasites and viruses transmitted by the bite of arthropods when they feed on the blood of the host animals. The prevalence of vector-borne diseases has been increasing during the last few years. Globalization, animal migrations, change in the climate and modifications in the location and distribution of the vectors favors the prevalence of this vector-borne diseases (Villanueva-Saz *et al.,* 2022). Babesiosis is a tick-born disease (Moreau *et al.,* 2009). *Babesia ovis* is one of the important parasitic diseases in small ruminants including sheep which causes severe economic losses to the formers. *Babesia ovis* being the most prevalent species with a worlwide distribution (Mira *et al.,* 2019). However, there could be differences in prevalence caused by the geographical distribution of each *Babesia* species and vector species. Differences in pathogenicity have also been observed in the same *Babesia* species (Villanueva-Saz *et al.,* 2022). *Babesia* parasite is intra-erythrocytic in nature and diameter is approximately 1 to 1.5 µm (Sevinc *et al.,* 2007). The significant effect of *Babesia* infections have been published in dogs, cattle as well as in small ruminants (Sivajothi et al., 2022). It causes acute, sub acute or chronic form of infection.

Small ruminants affected with clinical babesiosis shows the variable clinical signs including fever, anemia and hemoglobinuria (Sevinc *et al.,* 2007). Duration of babesiosis varies with the individual animal with acute shock, a long convalescent period, progressive renal failure and death. *Babesia ovis* causes haemolytic anemia and kidney dysfunction in affected animals. Several causes have been associated with haemolytic anaemia, with haemoparasites being one of the most important causes included. The Babesia parasites attach to the red blood cell membrane and enter the red blood cells after infected ticks have fed on sheep blood. Following that, haemoparasites multiplication occurs with formation of merozoite and red blood cell lysis, these are the signs of Babesia parasite growth. This condition is accompanied by blood cell membrane lesions because of intravascular and extravascular hemolysis as well as cell membrane fragility. Following the destruction of red blood cells with tremendous multiplication of Babesia parasites in the cell, new hemoparasites invade fresh erythrocytes in the bloodstream, hence extending the parasite's widespread growth (Villanueva-Saz *et al.,* 2022). There are few extensive studies about hematological and biochemical findings of small ruminants' babesiosis. Analysis of the haematological components provides the information of the health status of individual animal, helps in diagnosis, differential diagnosis and formulation of therapeutic regimen. In the present study, describes the clinical and haematological findings induced by naturally occurring Babesia infection in sheep. In the previous literature reported the haemolytic anaemia, haemoglobinuria and polychromasia, which coincide with observations in other animals with babesiosis.

MATERIAL AND METHODS

The study was carried out at Anantapur district (Andhra Pradesh) where an outbreak occurred in 2024. Twenty two sheep in a flock of 187 sheep showed the passing of red colored urine, anorexia and dull and death of the two sheep with similar signs. Clinical signs recorded are elevated rectal temperature, pale mucus membranes, increased capillary refill time, suspended rumination and haemoglobinuria. Clinical samples including faecal samples, whole blood, peripheral blood smears were collected for laboratory analysis. Blood collected in ethylene diamine tetraacetic acid (EDTA) tubes was used to estimate haematological parameters including red blood cell count, haemoglobin, packed cell volume, white blood cell count, differential count were estimated as per standard procedures. RBC indices i.e., mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and MCH concentration (MCHC) were evaluated (Schalm *et al.,* 1986). Peripheral blood smears was stained with Giemsa and screened for the haemoprotozonas. Faecal samples were analyzed by the standard procedures to screen for gastro enteric parasitic ova. The results were analyzed by one-way analysis of variance (ANOVA) followed by pair-wise comparisons using the Duncan tests. Difference were considered significant when $P < 0.05$. The computer software, SPSS version

22.0 for windows was used for analysis. When studying vector-borne diseases, it is crucial to integrate data and findings from various clinical methods, such as thorough case history and an in-depth physical examination of the affected animals. The tentative diagnosis relies on epidemiological information, the identification of ticks, the clinicopathological results observed, and a positive outcome from a validating diagnostic method. In these diseases, prompt diagnosis and effective treatment are vital for effective control. (Ranjbar-Bahadori *et al.,* 2011).

After confirmation of the disease, affected sheep were treated with injection diminazene aceturate (@ 3.5 mg/ kg, deep IM, OD for two consecutive days), injection oxytetracycline dehydrate (@ 10 mg/ kg, IM, OD for 3 days), hematinic (Inj. Feritas, Intas Pharmaceuticals) @ 1 ml/50 kg, IM, twice weekly for 2 weeks along with oral electrolyte solution for 2 weeks (Sevinc *et al.,* 2007).

RESULTS AND DISCUSSION

The recorded clinical findings were including fever, pale mucus membrane (Fig.1), tachycardia, increased capillary refill time and haemoglobinuria. These findings were in association with the previous studies (Sivajothi *et al.,* 2022). *Babesia* organisms were detected in the stained peripheral blood smears. Sheep with acute babesiosis typically present clinical signs such as fever, jaundice, haemoglobinuria and pale mucous membranes due to anaemia. Other non-specific clinical signs such as lethargy, anorexia, weakness and a poor general condition could be detected during the physical examination due to wasting condition (Stuen, 2020). Out of twenty two sheep, twenty showed the presence of the organisms in the stained blood smears (Fig.2). The degree of *Babesia* infection was assessed based on the percentage of RBCs infected with the organisms. These observations were in accordance with the findings by (Sevinc et al., 2007, Aktas *et al.,* 2007). The haematological findings were presented in Table 1. Significantly ($P < 0.05$) reduced hemoglobin concentration, packed cell volume (PCV), red blood cell count, whereas significantly elevated total leukocyte count and eosinophil count were noticed.

Fig. 1: Pale mucus membrane in Sheep affected with Babesia

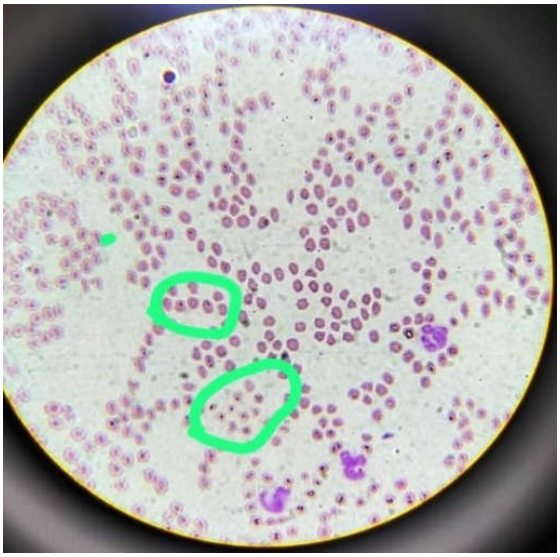

Fig. 2: *Babesia* parasites in sheep blood

Microcytic hypochromic anaemia was recorded in the present study may be due to the destruction of red blood cells haemolysis resulting from the replication of *Babesia* in intra cytoplasm of the RBCs of affected sheep. In some animals, the massive intracellular parasite multiplication in the red blood cells, together with the presence of haemolytic anaemia, could cause more severe clinical signs, even causing other alterations such as haemostasis, thrombocytopenia and haemoglobinuria (Rahbari *et al.,* 2008). Polychromatophilic erythrocytes in blood smears indicated a hemolytic anemia. Haemolysis results in profound anaemia, jaundice, and haemoglobinuria. A significant

(P < 0.05) reduction in the levels of erythrocytes, Hemoglobin and PCV was recorded and these findings were in association with the previous reports (Sivajothi *et al.,* 2022, Hadadazadeh e*t al.,* 2002). An increase (P < 0.05) in the levels of total leukocytes and esinophils was recorded and these findings were in association with the previous reports (Sivajothi *et al.,* 2022). In addition, macrophage activation is known to occur during babesiosis and a protective role has been documented for macrophages during infection with several *Babesia* species (Rubino *et al.,* 2006).

Table 1: Hematological findings in sheep affected with babesiosis (Mean±S.E)

Parameters	Healthy sheep (n=12)	Babesia infected sheep (n=18)
TEC x106/mm3	6.38 ± 0.52a	4.27 ± 0.81b
TLC x103/ mm3	8.27 ± 0.46a	12.5 ± 0.24b
Hb (g/dl)	12.1 ± 0.94a	7.87 ± 1.10b
PCV (%)	35.4 ± 2.18a	22.56 ± 2.01b
Neutrophils (%)	30.12 ± 1.31a	24.26 ± 0.91a
Lymphocytes (%)	64.8 ± 2.12a	65.6 ± 1.92a
Eosinophils (%)	2.8 ± 0.41a	7.8 ± 0.82b
Monocytes (%)	2.2 ± 0.3a	2.3 ± 0.2a
MCV (fl)	55.5±0.41a	52.8±0.12a
MCH (pg)	18.9±0.18a	18.4±0.2a
MCHC (g/dl)	34.1±0.46a	34.8±0.23a

Different superscripts of the same row indicate a significant difference at P < 0.05

Recorded anemia might be due to immunomediated phenomena by the autoantibodies directed against component of membrane of infected and uninfected erythrocytes (Rubino *et al.,* 2006), production of toxic hemolytic factors of the parasite, mechanical damage by trophozoite intra-erythrocytic binary fission, erythrophagocytosis and through of release vasoactive molecules such as kallikrein (Sajid *et al.,* 2023). Red blood cell abnormalities were also recorded in our study (polychromatophilic erythrocytes and spherocytosis / anisocytosis). The most common laboratory alteration is hypochromic microcytic anaemia in sick animals, while in sheep with chronic course disease, it is possible to detect macrocytic and hyperchromic anaemia. There is also a decrease in haemoglobin values and detection of thrombocytopenia in the case of disseminated intravascular coagulation (Sevinc *et al.,* 2013). These abnormalities have the role in development of the abnormal RBC indices (Esmaeilnejad *et al.,* 2012 and Shiono *et al.,* 2003). The results of RBC indices i.e., mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and MCH concentration (MCHC) in our study were non-significant (p > 0.05). Whereas, Sajid *et al.,* 2023 reported a minor non-significant (p > 0.05) increase in MCV of infected sheep while a minor, non-significant (p > 0.05) decrease in MCH and MCHC values in infected sheep. Reduction in MCV level may be due to decrease in PCV level may be attributed to the dilution of blood and subsequently MCV could decrease.

A significant increase in total leukocyte count was in accordance with the previous studies (Sivajothi *et al.,* 2022). This increase might be due to extended tissue damage by the babesia parasites, maturation of the neutrophil and lymphocyte. In the present study, eosinophilia was due to the presence of babesia parasites and sensitivity to the foreign protein of a parasite which may be a part of an immune phenomenon (Okon *et al.,* 2011). Clinical recovery is possible in some animals after clinical disease, but they can act as asymptomatic carriers without evident clinical signs, perpetuating the parasite cycle in nature. These carriers act as infection reservoirs and may infect ticks. However, under certain conditions, the carrier animal can suffer an immunosuppression status leading to the appearance of the clinical signs after parasite multiplication (Horta *et al.,* 2014). Death in Babesiosis may be due to hypoxia caused by severe anemia and hemoglobinuria.

CONCLUSION

The Present study reveal that a significant reduction in erythrocyte count, hemoglobin concentration and PCV, a significant increase in total leukocyte count and eosinophil count in sheep affected with Babesiosis. All the affected sheep were successfully treated with diminazene aceturate, oxytetracycline dihydrate, hematinic along with the electrolyte therapy.

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