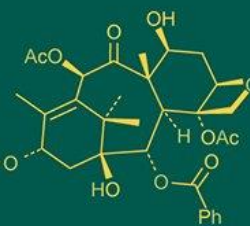
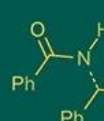


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(8): 781-785
www.biochemjournal.com
Received: 25-05-2026
Accepted: 29-06-2026

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Comparative analysis of hematological changes in bovine species infected with Haemoprotozoans in Bikaner region of Rajasthan, India

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i8j.9776>

Abstract

Haemoprotozoans are arthropod-transmitted parasites that proliferate within the blood cells of vertebrate hosts, causing hematological alterations. The present study aimed to investigate hematological changes associated with haemoprotozoan infections in dairy animals from the Bikaner region of Rajasthan, India, over a nine-month period (January-September 2021). A total of 200 blood samples (117 cattle and 83 buffaloes) were analysed using Geimsa stained blood smears. The overall cases of *Theileria* species (25) was reported to be highest followed by *Anaplasma* species (6), *Babesia* species (3) and mixed infections (3). However the distribution of infection is did not vary significantly between the two animal species (chi-square = 2.841, $p > 0.05$). Infected animals although non-significant, exhibited lower haemoglobin (8.39 ± 2.10 g/dL) and packed cell volume ($26.64 \pm 6.19\%$) when compared to healthy animals in which values were (10.42 ± 2.10 g/dL and $31.26 \pm 5.96\%$, respectively). Among individual infections Bovine babesiosis was reported to have lowest mean haemoglobin concentration (7.00 ± 2.60 grams per decilitre) and packed cell volume ($23.67 \pm 10.30\%$). The total leukocyte counts was found to be significantly higher ($p \leq 0.01$) in infected animals ($13,376.08 \pm 5,179.95$ cells per cubic millimetre) as compared to non-infected animals $11,238.80 \pm 3,633.83$ cells per cubic millimetre. Animals having mixed infection of multiple haemoprotozoa species exhibited highest mean neutrophil proportion and lowest Lymphocyte proportion. Above findings conclude that presence of haemoparasites infection can cause marked alterations in erythrocyte and leukocyte profile in bovines and have specific association with anaemia related changes.

Keywords: Haemoprotozoan infection, cattle, buffaloes, haematological alterations, anaemia, leukocyte profile

Introduction

Haemoprotozoan diseases, the majority of which are transmitted by tick vectors, represent a major challenge in the dairy sector. Among these bovine babesiosis, tropical theileriosis, and bovine anaplasmosis are of particular importance in India. Overall the clinicopathology associated with these infections lead not only to high mortality rates but also affect physiological functions, feed consumption and productive and reproductive performance. Genetically improved cattle breeds are particularly vulnerable to these infections and often have lower survival rates (Radostitis *et al.*, 1994) [26], resulting in increased costs associated with control measures (Makala *et al.*, 2003) [16]. Although advanced diagnostic techniques are now available, conventional diagnostic methods still primarily rely on the microscopic identification of infective parasite stages in blood or tissue fluids (Terkawi *et al.*, 2011) [31]. The widespread epidemiology of these diseases is of particular importance in Indian subcontinent mainly due to high prevalence of vector population owing to subtropical climatic conditions. (Vahora *et al.*, 2012; Velusamy *et al.*, 2014; Bhatnagar *et al.*, 2015; Maharana *et al.*, 2016) [32, 33, 5, 15].

In their mammalian hosts all the above mentioned haemoparasites have tropism towards Red blood cells so they proliferate inside them and due to their repeated invasion cause their phagocytosis or extravascular lysis by immune system of host that lead to haemolytic anaemia and decrease in blood indices, further complex pathogenic mechanisms also involve various erythropoietic and lymphoid organs. These parasites often induce clinical signs such as pyrexia, lymphadenopathy, nasal and ocular discharges, anorexia, weakness,

haemoglobinuria and severity of disease vary with host susceptibility and stage of infection (El-Deeb and Younis, 2009) [9]. With the exception of trypanosomiasis, which typically causes leukopenia, most haemoprotozoan infections are characterized by decreased hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), and elevated total leucocyte count (TLC) as a consequence of the aforementioned pathological processes (Maxie *et al.*, 1976; Aziz *et al.*, 2020) [17, 3].

In past studies low haemoglobin, packed cell volume has been reported from naturally infected bovines (Nazifi *et al.*, 2012) [20]. The magnitude of haematological changes vary with species of parasite, its tissue tropism and host factors such as systemic immune response, nutritional status, age, breed. Also concurrent infection with multiple parasites can further complicate disease pathology and clinical signs. Haematogram analysis of both infected and non infected animals help in comparative analysis of physiological impacts of haemoparasites in host's body. Hematological and coagulation parameters serve as valuable tools for diagnosis, prognosis, and therapeutic monitoring, providing critical information regarding the severity and progression of infections. Consequently, the present study focused on evaluating changes in these parameters to better understand the pathogenesis of haemoprotozoan infections in dairy animals.

Earlier studies conducted on bovine haemoprotozoans in arid region of Rajasthan have reported the prevalence rate, various epidemiological factor associated with it were mainly focused on a particular haemoprotozoan species. However the limited data is available on the comparative analysis of haematogram and leukogram of infected and non infected animals. We have documented data on prevalence of individual protozoans provided information about the prevalence rate of different haemoprotozoan in Bovines however haematological changes associated with the infections were not discussed in detail (Bharti *et al.*, 2022) [4]. Thus present study was undertaken to evaluate the erythrocytic and leucocytic profiles of naturally infected animals (Kachhawa *et al.*, 2016; Goyal *et al.*, 2018; Bharti *et al.*, 2022) [14, 11, 4].

Materials and Methods

Study Area and Sample Collection

The study was conducted in Bikaner, located in arid western plain zone of Rajasthan. A total of 200 samples were collected from bovines (cattle and buffaloes) at selected Gaushalas, polyclinics, and the TVCC of the College of Veterinary and Animal Science (CVAS), Rajasthan University of Veterinary and Animal Sciences (RAJUVAS), Bikaner. Both infected as well as apparently healthy animals were selected based on their availability during study period from field and as well as clinically presented animals. Aseptic blood collection was done using disposable syringes and immediately transferred into EDTA-containing vials and gently mixed to prevent clot formation. The samples were properly labelled and transported to the laboratory for further examination. If required, fresh blood smears were prepared from peripheral circulation for microscopic examination.

Haematological Analysis

Haemoglobin (Hb) was estimated by Sahli's acid haematin method (Cohen and Smith, 1919) [7] and expressed as g/dL.

Packed cell volume (PCV) was determined by Wintrobe's method (Wintrobe, 1929) [34] and expressed as percentage. Total leucocyte count (TLC) was estimated using a haemocytometer as described by Coles (1974) and expressed as cells/mm³. Differential leucocyte count (DLC) was determined by counting 100 leucocytes in stained blood smears following Parida *et al.* (2012) [21] and expressed as percentage.

Statistical Analysis

Data were classified according to month, area, age, sex, and breed. Statistical analysis was performed using IBM SPSS Statistics version 20.0 following Snedecor and Cochran (1994) [30].

Results

Alterations in Haemoglobin (Hb)

In animals infected with haemoprotozoa, lower average haemoglobin values were observed, with the lowest (7 g/dl) recorded in *Babesia* spp. infection. Healthy animals had an average Hb of 10.42 g/dl, while infected animals had 8.39 g/dl, although the difference was statistically non-significant ($p > 0.05$).

Alterations in Packed Cell Volume (PCV)

PCV values were lower in infected animals (26.6%) compared to healthy animals (31.2%), with the lowest PCV (23.6%) recorded in babesiosis cases. The difference was statistically non-significant ($p > 0.05$).

Alterations in Total Leucocyte Count (TLC):

A highly significant increase ($p < 0.01$) in TLC was observed in infected animals compared to healthy ones. Although values remained within the normal range, the highest leucocytosis was found in animals infected with *Anaplasma* spp. (Table 1, Figure 1).

Alterations in Differential Leucocyte Count (DLC):

In infected animals, a highly significant decrease ($p < 0.01$) in neutrophil count was observed. A significant increase in lymphocyte count and a significant decrease in monocyte count ($p < 0.05$) were recorded, while eosinophil count showed a non-significant ($p > 0.05$) increase. Notably, neutrophil and monocyte counts were higher in co-infected animals, whereas the highest lymphocyte and eosinophil counts were observed in *Theileria* spp. and *Anaplasma* spp. infections, respectively (Table 2, Figure 2).

Discussion

Haematological analysis revealed variation in blood parameters of infected animals as compared to healthy. The value of haemoglobin and packed cell volume were declined in parasitic animals as compared to non parasitic animals, though the observation was non significant. This could be due to multiplication of blood protozoans in red blood cells which were phagocytised by reticuloendothelial cells and cause haemolytic anaemia (Jayalakshmi *et al.*, 2019) [13]. A highly significant increase was observed in total leucocyte count of infected animals as compared to healthy ones. In differential leucocyte count a significant decrease was seen in values of Neutrophil ($p \leq 0.01$) and monocytes ($p \leq 0.05$) while the values of lymphocytes ($p \leq 0.05$) were increased. These results may be due to immune response of body of animals to ongoing parasitic infection. Across the world

similar results were obtained by Muraleedharan *et al.* (2005) ^[19] from Karnataka, Aulakh and Singla (2006) ^[2] from Punjab, Ananda *et al.* (2009) ^[1] from Karnataka, Shahnawaz *et al.* (2011) ^[27] from Pakistan, Chaudhri *et al.* (2013) ^[6] from Eastern Haryana, Sitotaw *et al.* (2014) ^[29] from Central Eithopia, Paul *et al.* (2016) ^[25] from Nigeria, Ganguly *et al.* (2017) ^[10] from Haryana, Patel *et al.* (2017)

^[23] from Gujarat, Jaiswal *et al.* (2019) ^[12] from Rajasthan, Jayalakshmi *et al.* (2019) ^[13] from Tamil Nadu, Parmar and Chandra (2019) ^[22] from Uttar Pradesh, Patil and Satbige (2019) ^[24] from Karnatka, Aziz *et al.* (2020) ^[3] from Rajasthan, Sharma *et al.* (2020) ^[28] from Rajasthan and Muniraja *et al.* (2021) ^[18] from Andhra Pradesh.

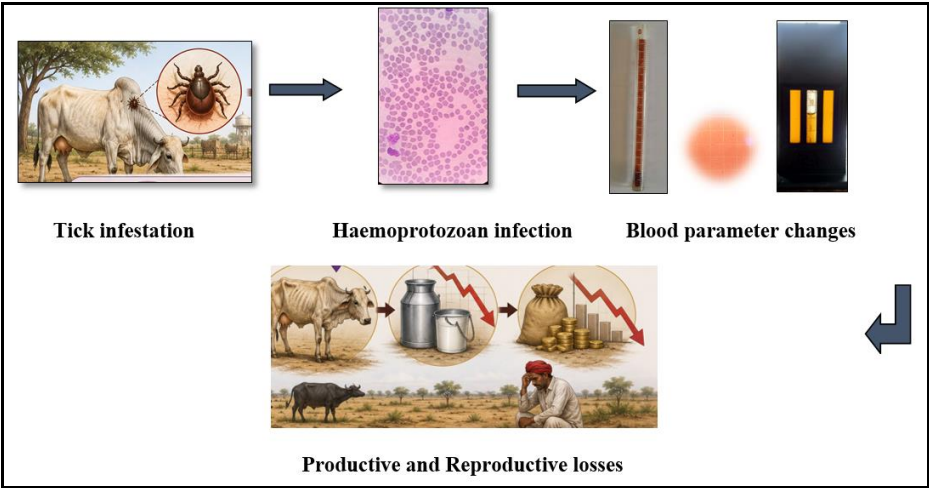


Table 1: Values of total leucocyte counts (TLC) in infected and non infected animals

Sr. No.	Type of Infection	Total leucocyte count(TLC)/(cumm)
1.	<i>Theileria</i> spp.	13158 ± 5915.30
2.	<i>Babesia</i> spp.	13483.33 ± 3695.38
3.	<i>Anaplasma</i> spp.	14907.50 ± 3378.19
4.	Mixed Infection	12023.33 ± 3241.39
5.	Overall	11634.20 ± 4036.80
6.	Infected samples	13376.08 ± 5179.95
7.	Non-infected samples	11238.80 ± 3633.83
	F value	0.49**

**-Highly significant ($p \leq 0.01$)

Table 2: Values of differential leucocyte counts (DLC) in infected and non infected animals

Sr. No.	Type of Infection	Differential leucocyte count (DLC) (%)			
		Neutrophill	Lymphocyte	Eosinophill	Monocyte
1.	<i>Theileria</i> spp.	27.44 ± 4.47	61.80 ± 4.97	6.56 ± 1.78	4.20 ± 2.86
2.	<i>Babesia</i> spp.	30 ± 10.58	60 ± 9.85	7 ± 2	3 ± 1
3.	<i>Anaplasma</i> spp.	29.17 ± 6.11	60.50 ± 7.89	8 ± 1.55	2.33 ± 2.07
4.	Mixed Infection	36.33 ± 11.85	55.33 ± 10.69	3.67 ± 1.53	4.67 ± 1.53
5.	Overall	30.26 ± 8.98	60.01 ± 8.39	5.52 ± 2.32	4.22 ± 2.03
6.	Infected samples	28.65 ± 6.22	60.92 ± 6.33	6.59 ± 1.96	3.84 ± 2.61
7.	Non-infected samples	30.62 ± 9.47	59.80 ± 8.80	5.27 ± 2.33	4.31 ± 1.87
	F value	2.32**	1.98*	1.43	0.54*

**-Highly significant ($p \leq 0.01$) *-Significant ($p \leq 0.05$)

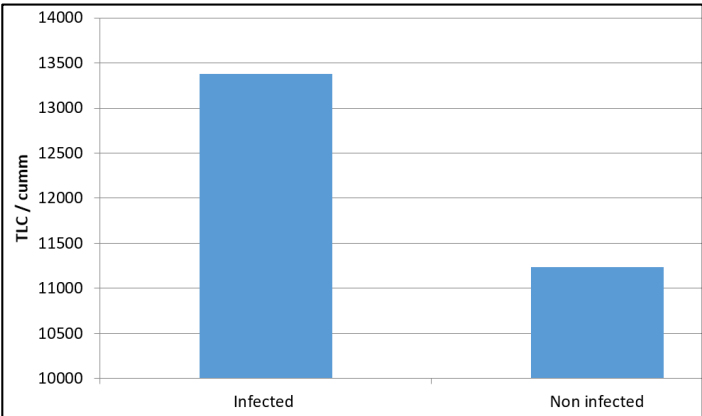


Fig 1: Alterations in Total leucocyte count (TLC) value of animal infected with haemoprotozoan parasites

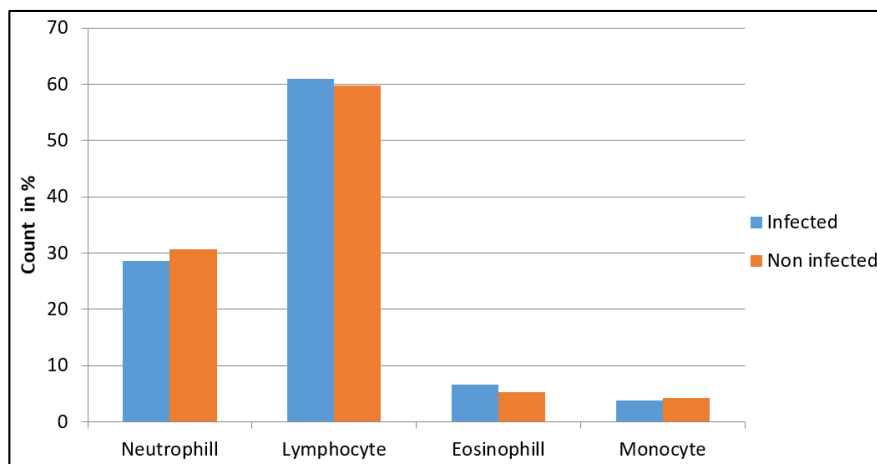


Fig 2: Alterations in Differential leucocyte count (DLC) value of animal infected with haemoprotozoan parasites

Conclusion

The present study highlighted the impact of haemoprotozoan infection on the physiology of animals. The parameters included for the study provided the valuable information on how the presence of infection can cause changes in animal body and how they can act as supportive tools for assessing health status of animals under field conditions. This regular screening and early detection can help timely management and reduction of adverse impact on bovine health and productivity.

Conflict of Interest

The authors declare that there is no conflict of interest.

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