

Hemato-biochemical alterations in Sheep affected with Brucellosis in Anantapur district, Andhra Pradesh, India

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ABSTRACT

Brucellosis, a zoonotic disease caused by *Brucella* spp., significantly impacts the health and productivity of sheep, leading to economic losses and public health concerns. This study evaluated the hemato-biochemical alterations in sheep affected with brucellosis in Anantapur district, Andhra Pradesh, India. A total of 100 serum samples from 100 animals (50 male and 50 female) were collected and screened for brucellosis using the Rose Bengal Plate Test (RBPT) and Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA), revealed seroprevalence rates of 16% and 30%, respectively. Hematological and biochemical analyses were conducted on 20 seropositive sheep, with results compared to seronegative controls. Significant hematological changes ($P < 0.01$) included reduced hemoglobin, packed cell volume and total erythrocyte count, accompanied by increased total leukocyte count, neutrophils and eosinophils. Lymphocytes, monocytes and basophils were reduced. Biochemical profiles showed elevated aspartate aminotransferase and alanine aminotransferase with decreased total protein and albumin ($P < 0.05$).

Keywords: ALT, AST, *Brucella*, hemoglobin, I-ELISA, neutrophils, PCV, RBPT

INTRODUCTION

Sheep and goats played a crucial role in the livestock sector, serving as vital sources of nutrition and supporting the rural economies of developing nations through their ability to convert forages and residues into valuable products like meat, wool and milk¹. In India, the sheep population was estimated at 65.07 million and goats at 135.2 million, constituting 12.71% and 26.4% of the total livestock population, respectively². However, small ruminants faced numerous diseases, including brucellosis, a major zoonotic bacterial infection caused by *Brucella* spp., which resulted in substantial economic losses and posed risks to human health^{3,4}. Brucellosis transmission occurred through direct contact with infected animals, consumption of contaminated products or exposure to aborted materials, affecting both animals (e.g., abortions, infertility) and humans, particularly farmers and Veterinarians^{5,6}. Although bacterial isolation remained the diagnostic gold standard, its limitations-such as the need for BSL-3 facilities-necessitated alternative methods like serological tests (RBPT, ELISA) and PCR, which offered sensitivity and practicality⁷⁻⁹.

MATERIALS AND METHODS

The present study was conducted from April to December 2024 in various villages of Anantapur District, Andhra Pradesh, India with sample analyses performed at the Department of Veterinary Pathology, Sri Venkateswara Veterinary University, Tirupati and ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bangalore with prior approval of IAEC under proposal no. 281/go/ReBi/S/2000/CPCSEA/CVSc/TPTY/049/Veterinary Pathology.

A total of 100 blood samples (male & female each 50) were collected aseptically from the jugular vein of sheep across eight villages. Samples were drawn into serum clot activator tubes, allowed to clot for 2-3 hours and

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centrifuged at 3000 rpm for 2-4 minutes. Serum was stored at -20°C until analysis.

From seropositive samples, 20 blood samples were selected for hematological analysis using EDTA-collected blood and serum was separated from blood for biochemical analysis using plane vial. Parameters included Hb (Sahli's method), PCV (microhematocrit), TEC and TLC (dilution technique) and DLC (Field's stain, Battlement technique)^{10,11}. Biochemical analysis assessed ALT, AST, total protein and albumin in serum centrifuged at 4000 g for 20 minutes, using an Auto Span analyzer with ERBA and Biosystem kits. Statistical

Table 1. Hematological values of sheep affected with brucellosis.

Parameter	Control Sheep (N = 6)	Sheep affected with Brucellosis (N = 20)
Hb - Haemoglobin (g/dl)	10.60 ± 0.20*	7.90 ± 0.20*
PCV - Packed Cell Volume (%)	36.50 ± 0.70*	30.40 ± 0.50*
TEC - Total erythrocyte count (10 ⁶ /μl)	10.90 ± 0.45*	4.15 ± 0.18*
TLC - Total leukocyte count (10 ³ /μl)	5.97 ± 0.45*	22.53 ± 2.52*
MCV - Mean Corpuscular Volume	30.0 ± 0.49*	37.20 ± 0.09*
MCHC - Mean Corpuscular Haemoglobin Concentration	32.40 ± 0.40*	52.70 ± 1.70*
MCH - Mean Corpuscular Haemoglobin	10.60 ± 0.30*	19.60 ± 0.60*
DLC - Differential leukocyte count		
Neutrophils (%)	39.50 ± 0.22*	62.15 ± 2.82*
Lymphocytes (%)	58.17 ± 0.17*	39.33 ± 0.36*
Eosinophils (%)	1.25 ± 0.18*	2.45 ± 0.18*
Monocytes (%)	1.33 ± 0.21*	1.90 ± 0.20*
Basophils (%)	0.58 ± 0.26*	0.83 ± 0.11*
Band cells (%)	0.00 ± 0.00	0.00 ± 0.00

Test: T - Test Significance: (P < 0.05); *Values were significantly different at (P < 0.05)

significance was determined by T-tests for hematological/ biochemical data.

RESULTS

Hematological analysis (Table 1, Fig. 1) showed significant reductions (P < 0.05) in hemoglobin (Hb), packed cell volume (PCV) and total erythrocytes count (TEC) in infected sheep compared to controls, indicating anemia and decreased red blood cell counts. Total leukocytes count (TLC) was significantly elevated (P<0.05) in infected sheep, with differential counts showing significant increases (P<0.05) in neutrophils, eosinophils and monocytes compared to control sheep, while lymphocytes decreased significantly (P<0.05) in positive sheep compared to *Brucella* negative sheep.

Basophils elevated slightly in seropositive sheep compared to seronegative sheep (P<0.05). Red blood cell indices also shifted: MCV, MCHC and MCH increased in sheep confirmed with brucellosis compared to control sheep (P<0.05).

Biochemical analysis identified significant changes (P<0.05) in infected sheep with elevated alanine aminotransferase (ALT) in seropositive sheep and increased aspartate aminotransferase (AST) in sheep infected with brucellosis compared to controls, indicating hepatic stress. Significant decrease in total protein and serum albumin was observed in seropositive sheep compared to seronegative sheep (P<0.05), suggesting metabolic disruption.

Hematological changes in Brucella affected animals

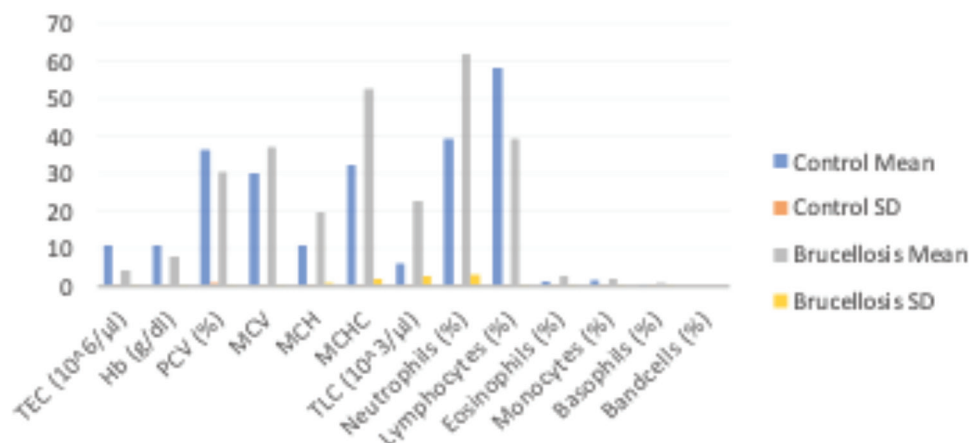


Fig. 1. Hematological values of sheep affected with brucellosis.

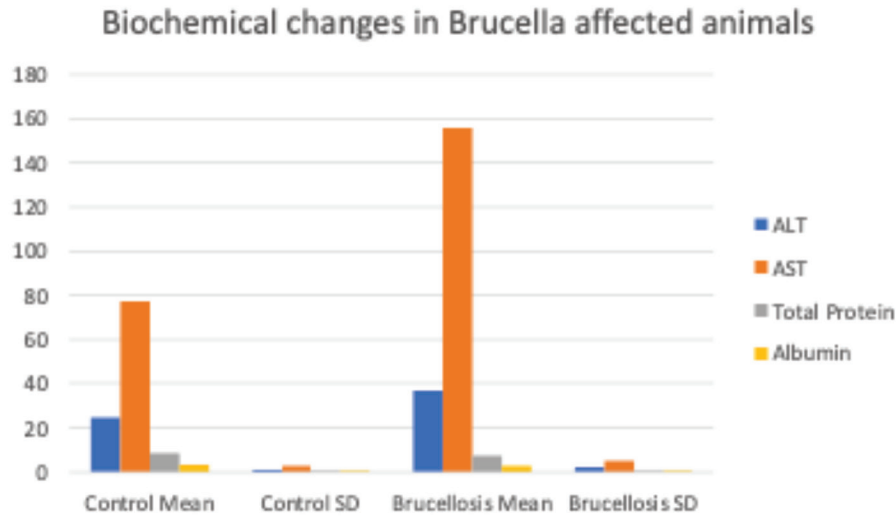


Fig. 2. Serum Biochemical results of Brucella seropositive animals.

DISCUSSION

The hematobiochemical alterations observed in this study revealed the systemic impact of brucellosis on sheep physiology, revealing patterns of anemia, inflammation and hepatic dysfunction. The significant reduction in Hb, PCV and

TEC in infected sheep resembled findings by previous reports¹², who documented similar declines in *Brucella*-infected cattle, attributing them to anemia from impaired erythropoiesis or oxidative stress induced by inflammatory cytokines¹³. Previous findings^{14,15} also reported anemia in 43.5% and 74% of human brucellosis cases, respectively, supported that *Brucella* disrupted red blood cell production across species. However,¹⁶ observed stable Hb and PCV in infected cattle, suggesting variability possibly linked to infection stage or host-specific responses.

The elevated MCV, MCHC and MCH in infected sheep indicated larger, hemoglobin-rich erythrocytes, likely a compensatory mechanism for reduced TEC, as suggested by¹⁷ in cows. This contrasted with¹⁶ who noted stable MCHC in cattle, highlighting potential species differences or dehydration effects in sheep¹⁵. The marked increase in TLC and neutrophilia reflected an acute inflammatory response aligning with¹⁸, who reported neutrophilia in cattle (26.6%), though at lower levels. The lymphocytopenia in this study agreed with¹⁰ in cattle and human studies^{14,15}, suggesting immune suppression or bone marrow exhaustion, unlike the lymphocytosis observed in cattle¹⁸ and horses¹⁹. Elevated eosinophils and monocytes further supported an active immune response, differing from¹⁶, who noted only mild changes in cattle. The slight rise in basophils was less

Table 2. Serum Biochemical results of Brucella seropositive animals.

S.No.	Parameters	Control Sheep (N = 6)	Sheep affected with brucellosis (N = 20)
1.	ALT	24.80 ± 0.77*	36.87 ± 1.86*
2.	AST	77.13 ± 2.70*	155.63 ± 5.16*
3.	Total Protein	8.62 ± 0.18*	7.36 ± 0.11*
4.	Albumin	3.04 ± 0.02*	2.65 ± 0.18*

Test: T - Test Significance: (P < 0.05); *Significant level of significance (P < 0.05)

pronounced but consistent with systemic inflammation. These discrepancies likely stemmed from species-specific immune dynamics or the intracellular nature of *Brucella*, which might suppress certain leukocyte populations while stimulating others^{20,16}.

Biochemically, the increase in AST and ALT in infected sheep indicated hepatic injury, supporting^{21,13,22} who correlated liver enzymes to inflammation and hepatocyte necrosis driven by *Brucella*-induced oxidative stress. The reduced total protein and albumin aligned with^{23,21}, reflecting impaired hepatic synthesis or renal loss as hypothesized by²⁴. In contrary, ²⁵reported increased total protein in cattle, highlighted species-specific metabolic responses.

CONCLUSION

This study described the hematobiochemical alterations in sheep affected by brucellosis in Anantapur District, revealing a 30% seroprevalence and significant physiological impacts. The marked reductions in Hb, PCV and TEC, coupled with elevated TLC and shifts in leukocyte populations, confirmed anemia and inflammation as hallmarks of the disease in sheep. Elevated AST and ALT was associated with decreased total protein and albumin, highlighting hepatic dysfunction and metabolic disruption, consistent with *Brucella*'s

systemic pathogenicity. These findings aligned with prior research in ruminants and humans, though variations demonstrated species-specific responses and infection stages. The higher sensitivity of I-ELISA over RBPT reinforced its value in epidemiological surveillance. Collectively, these results emphasized brucellosis as a multifaceted disease necessitating integrated diagnostic and management strategies to mitigate its economic and zoonotic toll in India's livestock sector.

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