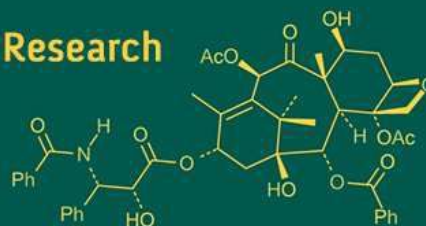


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(4): 341-344
www.biochemjournal.com
Received: 12-01-2026
Accepted: 15-02-2026

Dr. Amit Kumar Meena
Department of Veterinary
Medicine, PGIVER Jaipur,
Rajasthan, India

Dr. Dharm Singh Meena
Professor, Head & Dean
Department of Veterinary
Medicine, Post Graduate Institute
of Veterinary Education and
Research, Jaipur, Rajasthan, India

Dr. Nazeer Mohammed
Assistant Professor, Department of
Veterinary Medicine, Post
Graduate Institute of Veterinary
Education and Research, Jaipur,
Rajasthan, India

Dr. Mukesh Chandra Parashar
Assistant Professor, Department of
Veterinary Surgery and Radiology,
Post Graduate Institute of
Veterinary Education and
Research, Jaipur, Rajasthan, India

Dr. Bhavana Rathore
Assistant Professor, Department of
Veterinary Parasitology, Post
Graduate Institute of Veterinary
Education and Research, Jaipur,
Rajasthan, India

Dr. Rohitash Dadhich
Professor and Head, Department of
Veterinary Pathology, Post
Graduate Institute of Veterinary
and Education Research, Jaipur,
Rajasthan, India

Dr. Jyoti
Teaching Associate, CDSRZ,
Department of Veterinary
Medicine, Post Graduate Institute
of Veterinary and Education
Research, Jaipur, Rajasthan, India

Dr. Neha Meena
Veterinary Officer, Department of
Animal Husbandry Govt. of
Rajasthan, Jaipur, Rajasthan,
India

Corresponding Author:
Dr. Amit Kumar Meena
Department of Veterinary
Medicine, PGIVER Jaipur,
Rajasthan, India

Hemato-biochemical studies on paratuberculosis in caprine

Amit Kumar Meena, Dharm Singh Meena, Nazeer Mohammed, Mukesh Chandra Parashar, Bhavana Rathore, Rohitash Dadhich, Jyoti and Neha Meena

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i4e.8119>

Abstract

Paratuberculosis (Johne's disease) is a chronic granulomatous enteritis of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), resulting in severe production losses and public health concern due to its possible link with Crohn's disease in humans. The present study was conducted to investigate haemato-biochemical alterations in naturally infected goats in Jaipur, Rajasthan. A total of 400 goats aged above one year were screened using single intradermal tuberculin test (SIDT) and ELISA. Out of these, 6.75% were positive by SIDT, 40.75% by ELISA, and 6.25% by both tests. Haematological analysis revealed a significant ($p < 0.01$) decrease in haemoglobin, packed cell volume and total erythrocyte count in infected goats, indicating anemia. Total leukocyte count showed no significant variation, whereas neutrophils were significantly increased ($p < 0.05$) and lymphocytes significantly decreased ($p < 0.05$), suggesting immune modulation and exhaustion due to chronic infection. Biochemical evaluation showed significantly lower ($p < 0.05$) serum total protein, albumin, and globulin in MAP-positive goats, reflecting hypoproteinemia and protein-losing enteropathy. Conversely, liver function indicators including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin were significantly elevated ($p < 0.05$), indicating hepatic dysfunction and systemic involvement. Blood urea nitrogen and creatinine showed no significant differences.

Keywords: Paratuberculosis, biochemistry, haematology, Crohn's, human SID, ELISA

Introduction

Paratuberculosis, also known as Johne's disease is a chronic, fatal intestinal mycobacterial infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease was first described by Johne and Frothington in 1895 and first reported in sheep in Bosnia in 1908 (Bruère and West, 1993) [4]. Clinically manifested by irreversible wasting, therapy resistant diarrhoea and death from cachexia in ruminants and is widely spread world over in domestic and wild ruminant population (Sweeney, 1996 and Windsor *et al.*, 2014) [21, 25]. Johne's disease has shattering effects on the livestock sector in terms of production and economic losses and also has serious impacts on food safety, associated with Crohn's disease in humans (Ott *et al.*, 1999 and Singh *et al.*, 2008) [11, 17]. The disease is found in both ovine and caprine, having worldwide distribution and has been designated as multispecies disease in OIE listed disease (Okwumabua *et al.*, 2010) [10].

Johne's disease causes major problems for the livestock sector, leading to reduced production and large economic losses (Ott *et al.*, 1999) [11]. It also raises public health concerns because of its possible link with Crohn's disease in humans (Singh *et al.*, 2008) [17]. The disease affects both sheep and goats, is widespread globally, and is listed by the OIE as a multispecies disease (Okwumabua *et al.*, 2010; OIE, 2021) [10, 9]. Animals usually become infected with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) by eating or drinking contaminated material. After entering the intestine, the bacteria move to the lymphatic system and settle in M cells located above Peyer's patches in the ileum. Paratuberculosis results in the derangement of many haematological and biochemical parameters.

Materials and Methods

Study area

The Jaipur is located in the semi-arid region of Rajasthan, India, at 26°55' N latitude and 75°49' E longitude. The area is characterized by hot summers, low annual rainfall, and relatively mild winters.

Animals

In present study, a total of 400 goat animals aged above 1 year, without discrimination the sex and breed were selected from different flocks located in and around Jaipur, Rajasthan. All the 400 animals were screened by using single intradermal (SID) and ELISA tests and the animals that showed positive reaction either in tests were included in Group-I. For comparison of haemato-biochemical parameters, 20 apparently healthy animals aged above 1 year without discrimination the sex and breed, were taken as Group-II or control animals.

Single intradermal skin test

The purified protein derivatives (PPD) were procured from Biological Product Division (B.P. Division), Indian Veterinary Research Institute (IVRI), Izatnagar. The PPD was stored at 4 °C before and after use. The test was performed according to Ritchie (1953) [12]. After complete restraint of animal, an area of 1 square inch will be prepared for SIDT exactly 4 inches below crest of neck was shaved using shaving blade under aseptic conditions. The thickness of skin fold was measured in millimeters using digital vernier calipers. Tuberculin syringe of 1 ml (40 Units) capacity was used for the administration of 0.1 ml (4 Units) Johnin PPD intradermally. The correction of the injection was confirmed by palpation of a small pea-like swelling at the site. After 72 hours, the thickness at the injection sites was measured again and interpreted. The result was interpreted as 3 mm- negative, swelling between 3 to 4 mm-doubtful and swelling of 4 mm and above- positive.

Enzyme linked immuno-sorbent assay

A total of 400 serum samples were tested for antibodies against *Mycobacterium avium* subsp. *Paratuberculosis* (MAP). ELISA was carried out by using *Mycobacterium paratuberculosis* (MAP) Test Kit AsurDxTM (Biostone Animal Health, USA). The test was performed as per manufacturer's protocol.

Hematological examinations

The blood samples were subjected for estimation of the haematological parameters viz. haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC). These parameters were analysed as per standard haematological methods cited by Schalm's veterinary haematology (Jain, 1986) [6].

Biochemical estimations

Biochemical analysis of serum was carried out to estimate serum total protein, albumin, globulin, serum total bilirubin, serum creatinine, blood urea nitrogen (BUN) and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). All the above biochemical parameters were estimated by using the —Turbo Chem. 100I (Automatic blood Biochemistry Analyzer), in the Department of Veterinary

Physiology and Biochemistry of Post Graduated Institute of Veterinary Education and Research (PGIVER), Jaipur. The principles, reagents required, procedure, calculation and precautions used for each of them were followed as per operator's manual.

Comparative statistical analysis

The compared using standard formulas given for mean, standard error, t- test as per the procedures explained by Snedecor and Cochran (2004) [18].

Results and Discussion

Out of 400 goats a total of 27 (6.75%) were found positive tuberculin reactors and 163 (40.75%) showed positive reaction by ELISA test and 25 (6.25%) animals showed positive reaction in both tests. These 163 goats that show positive reaction in either test, were subjected to further process for haematological and biochemical investigation.

Haematological parameters

The Mean $\pm$ SE values of hematological parameters of caprine paratuberculosis positive animals (Group-I) and apparently healthy animals (Group-II) are presented in Table 1.

Table 1: Mean $\pm$ SE values of haematological parameters of positive animals (Group-I) and apparently healthy animals (Group-II)

S. No.	Parameters	Group-I (N-163)	Group-II (N-20)	Statistical analysis (T test)
1	Hb (g/dl)	9.47 $\pm$ 0.08	10.05 $\pm$ 0.17	S**
2	PCV (%)	29.94 $\pm$ 0.33	31.35 $\pm$ 0.54	S*
3	TEC ($\times 10^6/\mu$ l)	7.51 $\pm$ 0.12	8.01 $\pm$ 0.15	S*
4	TLC ($\times 10^3/\mu$ l)	6.18 $\pm$ 0.14	5.62 $\pm$ 0.23	NS
5	DLC			
5.1	N (%)	41.50 $\pm$ 0.54	37.50 $\pm$ 1.20	S*
5.2	L (%)	54.60 $\pm$ 0.52	58.10 $\pm$ 1.20	S*
5.3	M (%)	2.46 $\pm$ 0.06	2.75 $\pm$ 0.21	NS
5.4	E (%)	1.31 $\pm$ 0.04	1.45 $\pm$ 0.11	NS
5.5	B (%)	0.12 $\pm$ 0.02	0.20 $\pm$ 0.11	NS

*= indicate significant ($p < 0.05$) difference | **= indicate highly significant ($p < 0.01$) difference | S indicate Non-significant ($p > 0.05$) difference

The mean values of Hb, PCV and TEC were significantly ($p < 0.01$) decreased in positive animals as compare to healthy animals. These findings were in agreement with the study conducted by Weiss and Goodnough, (2005) [24], Senturk *et al.* (2009) [15]; Tharwat *et al.* (2012) [23]; Suman *et al.* (2016) [20] and Nabi *et al.* (2024) [8]. Sravani and Ganesan (2025) [19] reported significant anaemia and lower levels of Hb, probably as a result of in-appetence leading to poor body condition of animals. On contrary, Senturk *et al.* (2009) [15], Bhardwaj *et al.* (2011) [3], El-Deeb *et al.* (2014) [5] and Nabi *et al.* (2024) [8] who observed decrease erythrocyte count and PCV in affected animals with PTB. The mean values of total leucocyte count did not change significantly in the affected group as compare to healthy animals group. On statistical analysis there was no significant change in total leucocyte count of affected goats as compared to healthy control animals. The findings of present investigation are agreement with Nabi *et al.* (2024) [8] who also reported non-significant changes in total leucocyte count.

The mean values of neutrophil were significantly ($p < 0.05$) increased in positive animals as compare to healthy animals. The increased neutrophil count in the affected group could be attributed to the nature of neutrophils which are one of the most abundant and important mediators of innate immunity and are professional phagocytes which mount the acute inflammatory response and act as the first line of defense against invading pathogens Segal, (2005) [14]; El-Deeb *et al.* (2014) [5] and Nabi *et al.* (2024) [8].

The mean values of lymphocyte were significantly ($p < 0.05$) decreased in positive animals as compare to healthy animals. According to Sharma *et al.* (2020) [16] and Nabi *et al.* (2024) [8] a decreased levels of lymphocytes in MAP-positive animals and mentioned chronic infections are known to induce 'immune exhaustion'. The mean values of monocyte, eosinophil and basophil revealed non-significant changes in positive animals as compare to healthy animals.

Biochemical Parameters

The Mean $\pm$ SE values of biochemical parameters of positive animals (Group-I) and apparently healthy animals (Group-II) are presented in Table 2.

Table 2: Mean $\pm$ SE values of biochemical parameters of positive and apparently healthy animals

S. No.	Parameters	Group-I (n=163)	Group-II (n=20)	Statistical analysis (T test)
1	Total Protein (g/dl)	7.04 $\pm$ 0.11	7.86 $\pm$ 0.20	S*
2	Albumin (g/dl)	3.68 $\pm$ 0.05	4.04 $\pm$ 0.12	S*
3	Globulin (g/dl)	3.35 $\pm$ 0.09	3.82 $\pm$ 0.14	S*
4	ALT (IU/L)	31.92 $\pm$ 0.59	27.48 $\pm$ 1.72	S*
5	AST (IU/L)	71.85 $\pm$ 1.53	65.60 $\pm$ 2.13	S*
6	ALP (IU/L)	103.24 $\pm$ 0.61	91.11 $\pm$ 5.64	S*
7	BUN (mg/dl)	11.61 $\pm$ 0.57	9.55 $\pm$ 1.01	NS
8	Creatinine (mg/dl)	0.97 $\pm$ 0.02	0.93 $\pm$ 0.04	NS
9	Total bilirubin (mg/dl)	0.27 $\pm$ 0.01	0.22 $\pm$ 0.01	S*

*= indicate significant ($p < 0.05$) difference | **= indicate highly significant ($p < 0.01$) difference | NS indicate Non-significant ($p > 0.05$) difference

The mean values of serum total protein, albumin and globulin were significant ($p > 0.05$) decrease in positive animals as compare to healthy animals. The findings are in agreement with Bhardwaj *et al.* (2011) [3]; El-Deeb *et al.* (2014) [5]; (Balikci and Gurdogan 2015) [2] and Nabi *et al.* (2024) [8] who observed lower value of total protein in affected group as compared to healthy group. The hypoproteinemia might be attributed to decrease of albumin in serum. Mishra *et al.* (2024) [7] studied decrease in serum total protein in PTB affected goats that could be attributed to dehydration caused by recurrent diarrhea. There was a significant decrease in serum albumin levels, which might be attributed to albumin leakage via injured tissues, resulting in a damaging granulomatous inflammatory response that finally leads to intestinal malabsorption and protein losing enteropathy (Sweeney *et al.*, 2012; Balikci and Gurdogan, 2015) [22, 2]. Hypoglobulinemia could all be attributed to the malabsorption diarrhea. Other factors such as reduced appetite and emaciation as the disease progressed may have contributed to changes in these serum parameters. The decrease level of globulin in PTB affected goats could be attributed to dehydration caused by recurrent diarrhoea (Schroeder *et al.*, 2001) [13].

The mean values of ALT, AST and ALP were significant ($p > 0.05$) increase in positive animals as compare to healthy animals. The finding of study is in collaboration with finding of El-Deeb *et al.* (2014) [5] and Mishra *et al.* (2024). Significant increase in ALT concentrations were indicators of certain systemic affections, indicating liver damage caused by chronic infections of acid-fast bacilli (Balikci and Gurdogan, 2015) [2]. A significant increase in aspartate aminotransferase (AST) concentrations serves as an indicator of systemic involvement, reflecting hepatic damage associated with chronic infections caused by MAP Balikci and Gurdogan (2015) [2]. Increased activities of ALP can be related to muscle cell damage as a result of weight loss and anorexia in *Mycobacterium* infection Askar *et al.* (2018) [1]. The mean values of BUN and creatinine were revealed non-significant ($p > 0.05$) in positive animals as compare to healthy animals. These findings are similar with Balikci and Gurdogan (2015) [2].

The mean values of total bilirubin were significant ($p > 0.05$) increase in positive animals as compare to healthy animals. These findings in accordance with Balikci and Gurdogan (2015) [2]. El-Deeb *et al.* (2014) [5] reported hepatic involvement in animals infected with *Mycobacterium avium* subspecies *paratuberculosis* (MAP), characterized by a significant increase in serum bilirubin levels.

Conclusion

The study revealed that caprine paratuberculosis-positive goats exhibited significant haematological and biochemical alterations compared to healthy animals. Affected goats showed marked reductions in hemoglobin, PCV, TEC, total protein, albumin, and globulin, alongside increased neutrophils, liver enzymes (ALT, AST, ALP), and total bilirubin, while TLC, monocytes, eosinophils, basophils, BUN, and creatinine remained non-significant. These findings indicate anemia, hypoproteinemia, hepatic dysfunction, and immune alterations in MAP-infected goats, consistent with earlier studies, suggesting systemic involvement and metabolic disruption associated with chronic infection.

Acknowledgements

Authors are thankful to Dean, PGIVER, Jaipur and Principal investigator "Centre for Diagnosis, Surveillance & Response of Zoonotic Diseases (CDSRZ)" for financial support and provided facilities.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

1. Askar S, Askar TK, Guzel M. Effect of *Mycobacterium bovis* infection in cattle on changes in appetite-related hormones. *Austral J Vet Sci.* 2018;50(3):119-123.
2. Balikci E, Gurdogan F. Some biochemical parameters and oxidative stress biomarkers in sheep with paratuberculosis. *Med Weter.* 2015;71(11):679-682.
3. Bhardwaj RK, Randhawa CS, Randhawa SS, Singla VK. Haematobiochemical profiles of cows shedding acid-fast bacilli vis-à-vis normal cows. *Indian J Anim Sci.* 2011;81(1):19-22.
4. Bruère AN, West DM. The sheep: health, disease & production. 1st ed.

5. El-Deeb WM, Fouda TA, El-Bahr SM. Clinico-biochemical investigation of paratuberculosis of dromedary camels in Saudi Arabia: proinflammatory cytokines, acute phase proteins and oxidative stress biomarkers. *Pak Vet J.* 2014;34(4).
6. Jain NC. Schalm's veterinary hematology. 4th ed. Philadelphia: Lea and Febiger; 1986. p.1.
7. Mishra PS, Rath PK, Panda SK, Mishra BP, Biswal S, Mishra J, et al. Hemato-biochemical and pathomorphological alterations due to *Mycobacterium avium* subsp. *paratuberculosis* infection in goats. *Indian J Small Rumin.* 2024;30(2):268-274.
8. Nabi B, Upadhyay SR, Singh SV, Rasool S, Najar AA, Singh R, et al. Haemato-biochemical and oxidative stress evaluation in Beetal goats naturally infected with *Mycobacterium avium* subsp. *paratuberculosis* in sub-Himalayan region of India. *Indian J Anim Health.* 2024;63(2):298-302.
9. OIE (Office International des Epizooties). Manual of diagnostic tests and vaccines for terrestrial animals. 5th ed. Paris: OIE; 2021.
10. Okwumabua O, Shull E, O'Connor M, Moua TV, Danz T, Strelow K. Comparison of three methods for extraction of *Mycobacterium avium* subspecies *paratuberculosis* DNA for polymerase chain reaction from broth-based culture systems. *J Vet Diagn Invest.* 2010;22(1):67-69.
11. Ott SL, Wells SJ, Wagner BA. Herd-level economic losses associated with Johne's disease on US dairy operations. *Prev Vet Med.* 1999;40(3-4):179-192.
12. Ritchie JN. Control of bovine tuberculosis in cattle. *FAO Agric Stud.* 1953;25:25-34.
13. Schroeder C, Seeliger F, Gaede W, Westermeier G, Ganter M. Diagnosis, epidemiology, signs, and pathology of paratuberculosis in a goat herd in Germany. *Tierarztl Prax.* 2001;29:27-34.
14. Segal AW. How neutrophils kill microbes. *Annu Rev Immunol.* 2005;23:197-223.
15. Senturk S, Metcitoglu Z, Ulgen M, Borum E, Temizel E, Kasap S. Evaluation of serum iron and iron binding capacity in cows with paratuberculosis. *Tierarztl Prax G.* 2009;37(6):375-378.
16. Sharma S, Gautam A, Singh SV, Chaubey KK, Mehta R. Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* infection in suspected diarrhoeic buffaloes and cattle reporting at veterinary university in India. *Comp Immunol Microbiol Infect Dis.* 2020;73:101533.
17. Singh SV, Singh AV, Singh R, Sharma S, Shukla N, Misra S, et al. Sero-prevalence of bovine Johne's disease in buffaloes and cattle population of North India using indigenous ELISA kit based on native *Mycobacterium avium* subspecies *paratuberculosis* 'Bison type' genotype of goat origin. *Comp Immunol Microbiol Infect Dis.* 2008;31(5):419-433.
18. Snedecor GW, Cochran WG. Statistical methods. 8th ed. Ames: Iowa State University Press; 2004. p.591.
19. Sravani G, Ganesan PI. Studies on the prevalence of paratuberculosis in Holstein-Friesian cows in an organized dairy farm and its hematological variations. *Int J Vet Sci Anim Husb.* 2025;10(1):230-232.
20. Suman V, Jain VK, Sindhu N, Kumar T, Jhambh R, Kumar M. Clinical and hematological studies in buffaloes suffering from diarrhea. *J Anim Res.* 2016;6:1025-1029.
21. Sweeney RW. Transmission of paratuberculosis. *Vet Clin North Am Food Anim Pract.* 1996;12:305-312.
22. Sweeney RW, Collins MT, Koets AP, McGuirk SM, Roussel AJ. Paratuberculosis (Johne's disease) in cattle and other susceptible species. *J Vet Intern Med.* 2012;26(6):1239-1250.
23. Tharwat M, Al-Sobayil F, Ali A, Hashad M, Buczinski S. Clinical, ultrasonographic, and pathologic findings in 70 camels (*Camelus dromedarius*) with Johne's disease. *Can Vet J.* 2012;53(5):543.
24. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med.* 2005;352(10):1011-1023.
25. Windsor PA, Eppleston J, Dhand NK, Whittington RJ. Effectiveness of Gudair™ vaccine for the control of ovine Johne's disease in flocks vaccinating for at least 5 years. *Aust Vet J.* 2014;92(7):263-268.