RESEARCH ARTICLE

Clinico-Haemato-Biochemical Parameters in Sheep (*Ovis aries*) Naturally Infected with Paratuberculosis in Jaipur, India

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Abstract

Paratuberculosis (PTB) is an overlooked disease, recently gaining widespread attention owing to its associated enormous economic impacts. It affects the global livestock industries, including sheep, demanding adequate attention towards *Mycobacterium avium* subspecies *paratuberculosis* (MAP). In the present study, based on the PTB associated clinical signs, 250 blood samples were collected. For the disease diagnosis, single intradermal (SID) test, ELISA and PCR were performed. Further, positive blood samples were compared against the control group for haemato-biochemical analysis. Our findings revealed a marked reduction in haemoglobin, total erythrocyte count and packed cell volume ($p<0.05$-0.01), with increased platelet count ($p<0.01$), indicating the presence of anaemia likely due to chronic infection and impaired nutrient absorption. Total leukocyte count was significantly increased ($p < 0.05$), reflecting an ongoing inflammatory response. Differential leukocyte count showed a significant increase in lymphocytes, monocytes and eosinophils, suggestive of sustained immune stimulation, while neutrophils and basophils remained unchanged. The biochemical profile revealed no significant differences in most enzymatic and renal parameters, suggesting no liver or kidney impairment. Contrastingly, a significant decline in total protein and albumin concentrations was observed ($p = 0.01$), suggestive of chronic intestinal lesions, malabsorption of nutrients and prolonged disease stress caused by paratuberculosis. These findings highlight the chronic inflammatory nature of paratuberculosis in sheep that requires timely surveillance and prophylactic measures.

Keywords: Chronic infection, Disease diagnostics, Hypoalbuminemia, Johne's disease, Nutrient malabsorption.

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Introduction

Paratuberculosis (PTB), also known as Johne's disease (JD), is a chronic infectious disease of zoonotic significance. It majorly affects ruminants leading to granulomatous inflammation of the intestines and caused by bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP) (Borujeni *et al*., 2021). The World Organization for Animal Health (WOAH) has identified PTB as a priority infectious disease due to its substantial economic impact on livestock worldwide (Stabel *et al*., 2020). MAP is a small, rod-shaped, Gram-positive, acid-fast bacillus, obligate intracellular, slow-growing and capable of replicating only within the macrophages of the susceptible host (Shabana and Aljohani, 2020). Clinical signs include poor body condition, weight loss, debility, emaciation and diarrhea, leading to productivity losses such as reduced milk yield and quality, higher somatic cell counts, poor feed efficiency, increased mastitis risk, impaired reproduction, premature culling and lower carcass quality (Singh *et al*., 2016). PTB primarily spreads via the fecal-oral route, where the MAP bacteria target the M cells of the ileum; however, prenatal transmission is also possible. While enteric lesions typically develop within 6-12 months, the disease progression varies, often transitioning from mild (paucibacillary) to severe (multibacillary) forms over several years. Clinical signs generally appear once severe enteritis is established, though some animals may clear the infection during its early stages (Windsor, 2015).

Haematologically, MAP-infected animals manifest signs of anaemia and exhibit significant reductions in overall mean values of haematological parameters (Sharma *et al*., 2023). Among biochemical parameters analysed, only serum albumin levels showed a consistent and significant decrease

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as Johne's disease progressed (McGregor *et al*., 2015). In this study, we report PTB in sheep flock from Jaipur, Rajasthan, India as diagnosed by single intradermal test, serological prevalence and molecular biology techniques. Subsequently haemato-biochemical analysis was performed by comparing against the control group.

Materials and Methods

Animal and Clinical Examination

In this study, 250 sheep without discrimination of age, sex and breeds were selected from different flocks situated in and around Jaipur, Rajasthan. Each of the animals was evaluated using the single intradermal (SID) test, ELISA and PCR. Animals that tested positive in any of these assays were assigned to Group I. For the purpose of comparing haemato-biochemical parameters, 10 clinically healthy sheep, without discrimination of age, sex and breeds were included as Group II (control group). A detailed clinical examination of each animal, including measurements of rectal temperature, respiratory rate, heart rate and assessment of mucous membranes, was also conducted.

Collection of Samples

Blood samples (10 mL) were collected aseptically from the jugular vein by using sterile labelled disposable syringes. The blood sample were collected in two test tubes, one without anticoagulant for Sero-biochemical and another one with anticoagulant for molecular and haematological examination.

Haematology and Biochemical Analysis

Blood samples were analysed to determine haematological parameters including haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC). All the above haematological parameters were estimated by using automated Haematology Analyzer (Nihon Kohden). The estimations of differential leukocyte count (DLC) were carried out following standard haematological procedures described in Schalm's Veterinary Haematology (Jain, 1986). Biochemical analysis of serum was carried out to estimate serum total protein, albumin, globulin, serum total bilirubin, serum creatinine, blood urea nitrogen and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase. All the above biochemical parameters were estimated by using automated Biochemistry Analyzer (Merilyzer, AutoQuant 200i^{TM} VET), and assay kits as per the manufacturer's / operator's manuals.

Statistical Analysis

The data of serological, molecular and haemato-biochemical studies were analysed as per standard method described by Snedecor and Cocheran (2004).

Results and Discussion

Clinical Findings

Clinical examination of PTB-positive animals revealed diarrhoea (52.41%), reduced feed intake (48.38%), progressive weight loss or emaciation (37.09%) and poor wool quality or a rough body coat (33.06%). Concurrent occurrence of all clinical signs was observed in 24.19% of the animals (Table 1)

The clinical features noted in this study were comparable to the descriptions given by Idris *et al*. (2021) and Tharwat *et al*. (2025). Earlier researchers have reported that a majority of affected sheep exhibited clinical signs including poor appetite, lethargy, marked loss of body condition, a dull or rough hair coat and soft or semi-formed feces causing matting of tail hairs. The affected sheep typically show progressive weakness, exercise intolerance and a tendency for soft feces that may lose their normal pelleted appearance (Windsor, 2015). According to Balikci and Gurdogan (2015), Johne's disease represents a classic form of protein-losing enteropathy, in which chronic inflammation of the intestinal mucosa leads to degeneration of absorptive epithelium. This impairment results in reduced nutrient absorption and leakage of protein-rich fluid into the intestinal lumen, causing malabsorptive and secretory diarrhea. The disease therefore manifests as severe projectile diarrhea in cattle and soft or loose stools in small ruminants.

As a consequence of these intestinal changes, infected animals suffer from reduced weight gain, poor body condition, decreased milk or fiber yield and progressive emaciation (Borujeni *et al*., 2021; Idris *et al*., 2021). Other studies have confirmed that both small and large ruminant species are susceptible to PTB, a chronic wasting disorder characterized by long-term weight loss, diarrhea, reduced productivity and eventual debilitation (Olsen *et al*., 2002; Idris *et al*., 2021).

Table 1: Clinical signs observed in paratuberculosis-infected sheep, along with the number affected and percentage occurrence

Clinical findings	No. affected (n=124)	Percentage
Diarrhoea	65	52.41
Reduced feed intake	60	48.38
Progressive weight loss/ emaciation	46	37.09
Poor wool quality / rough body coat	41	33.06
Concurrent occurrence of all clinical signs	30	24.19

Comparison of Clinical Parameters

The mean ± SE values of rectal temperature (°F), heart rate (beats/min) and respiration rate (breaths/min) of PTB-positive animals (Group I) and apparently healthy control animals (Group II) are shown in Table 2. The mean rectal temperature of PTB-positive animals was found to be significantly ($p < 0.05$) higher compared to apparently healthy controls. The elevated body temperature in infected animals may be attributed to the infectious and chronic granulomatous

inflammatory nature of paratuberculosis, which can cause intermittent fluctuations in temperature due to prolonged immune response

Table 2: Comparative details of clinical parameters between affected animals and the control group

Parameters	Affected group (n=124)	Control group (n=10)	't' test
Rectal temperature (°F)	102.2±0.1	101.4± 0.1	S*
Heart rate (per min)	79.2±1.6	78.0± 1.6	NS
Respiratory rate (per min)	23.2±0.7	22.8±0.8	NS

*p<0.01 between groups

Haematological Parameters

Sheep affected with paratuberculosis showed significant haematological alterations compared to healthy controls. A marked reduction in haemoglobin, total erythrocyte count and packed cell volume ($p < 0.05$-0.01) indicated the presence of anaemia, likely due to chronic infection and impaired nutrient absorption. Total leukocyte count was significantly increased ($p < 0.05$), reflecting an ongoing inflammatory response. Red cell indices (MCH, MCV and MCHC) did not differ significantly, suggesting a normocytic normochromic type of anaemia. Platelet count was significantly higher in diseased sheep ($p < 0.01$), possibly due to chronic inflammation. Differential leukocyte count showed a significant increase in lymphocytes, monocytes and eosinophils, indicating sustained immune stimulation, while neutrophils and basophils remained unchanged. These findings highlight the chronic inflammatory nature of paratuberculosis in sheep (Hemalatha *et al*., 2013; Sharma *et al*., 2023; Nabi *et al*., 2024).

Table 3: Haematological parameters in sheep affected by paratuberculosis compared with the healthy control group (Mean ± SE)

Parameter	Healthy sheep (n=10)	PTB affected sheep (n=124)	p-value
Hb (g/dL)	10.57 ± 0.21	9.56 ± 0.35	0.018*
TLC (x10^3/µL)	10.79 ± 1.22	15.56 ± 1.33	0.013*
TEC (x10^6/µL)	10.61 ± 0.19	8.87 ± 0.79	0.038*
PCV (%)	35.02 ± 0.83	27.53 ± 0.41	0.000**
MCH (pg)	10.36 ± 0.21	12.06 ± 0.87	0.063
MCV (fL)	33.50 ± 0.44	33.84 ± 0.51	0.614
MCHC (g/dL)	30.21 ± 0.17	30.86 ± 0.57	0.277
Platelets (x10^5/µL)	286.92±14.12	396.63±35.03	0.005**
Neutrophils (%)	34.33 ± 1.28	32.56 ± 0.90	0.274
Lymphocytes (%)	53.87 ± 1.22	59.38 ± 1.05	0.002**
Monocytes (M)	2.66 ± 0.22	4.38 ± 0.36	0.000**
Eosinophils (E)	3.10 ± 0.35	4.13 ± 0.27	0.032*
Basophils (B)	0.04 ± 0.01	0.05 ± 0.01	0.721

*p<0.05, **p<0.01 between groups

Biochemical Parameters

The biochemical profile of sheep affected with paratuberculosis showed no significant differences in most enzymatic and renal parameters when compared to healthy control animals. Serum levels of SGOT, SGPT, ALP, blood urea nitrogen, creatinine, urea, total bilirubin, and globulin remained statistically comparable between the two groups ($p > 0.05$), suggesting the absence of pronounced liver or kidney impairment. In contrast, a significant decline in total protein and albumin concentrations was observed in the diseased group ($p = 0.01$). The reduced protein and albumin levels may be associated with chronic intestinal lesions, malabsorption of nutrients and prolonged disease stress caused by paratuberculosis. These biochemical alterations highlight the chronic and progressive nature of the infection and indicate that changes in protein metabolism are key features of the disease in sheep (Balikci and Gurdogan, 2015; McGregor *et al*., 2015; Askar *et al.,* 2018; Rizwan *et al.* 2019; Sharma *et al*., 2023; Nabi *et al.,* 2024).

Table 4: Details of biochemical parameters in sheep affected by paratuberculosis compared with the healthy control group (Mean ± SE)

Parameter	Healthy sheep (n=10)	PTB affected sheep (n=124)	P value
SGOT (U/L)	126.56 ± 10.17	129.10 ± 2.83	0.814
SGPT (U/L)	21.88 ± 1.82	23.29 ± 0.73	0.485
ALP (U/L)	150.46 ± 47.12	164.17 ± 9.05	0.781
BUN (mg/dL)	18.75 ± 1.79	24.02 ± 0.53	0.50
Creatinine (mg/dL)	3.10 ± 2.97	1.66 ± 0.73	0.50
Urea (mg/dL)	38.07 ± 2.24	51.43 ± 1.14	0.50
TP (g/dL)	8.10 ± 0.27	5.80 ± 0.10	0.01**
TBIL (mg/dL)	0.37 ± 0.07	0.52 ± 0.03	0.50
Albumin (g/dL)	3.20 ± 0.23	2.70 ± 0.05	0.01**
Globulin (g/dL)	4.58 ± 0.23	4.98 ± 0.10	0.50

**p<0.01 between groups

In context to the zoonotic potential of MAP and its detection in animal products, often alarms the enormous economic concerns associated with and urges prophylaxis in the paratuberculosis for pathogen elimination. Our study highlights the high prevalence of the paraTB in sheep, revealing chronic wasting diarrhoea and reduced appetite along with significant haematological alterations, suggestive of anaemia and chronic inflammation. The observed hypoproteinaemia and hypoalbuminemia further confirm the role of MAP-induced protein-losing enteropathy. These changes collectively contribute to poor growth, reduced productivity and huge economic burden. Early diagnosis, segregation or removal of infected animals with improved farm hygiene and awareness among farmers are essential components for disease control.

Conclusion

Overall, our findings provide important baseline data for the area that highlights the critical need for integrated diagnostic strategies, routine screening programs and efficient control measures that will lessen the burden of paratuberculosis in sheep populations and thus, hold significant role in safeguarding both animal and human health and related food safety.

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