Indian J Anim Health (2024), 63(2): 298- 302
DOI: https://doi.org/10.36062/ijah.2024.16523

Research Article

Haemato-biochemical and oxidative stress evaluation in Beetal goats naturally infected with *Mycobacterium avium* subsp. *paratuberculosis* in Sub-Himalayan region of India

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Abstract

Johne's disease has shattering effects on the livestock sector in terms of production and economic losses. Due to paucity of the implications of Johne's disease on metabolic values, this study was to investigate the haemato-biochemical and oxidative stress indices derangement in goats affected with paratuberculosis. A number of 40 animals with body condition score (BCS) 1-2, positive for multiple tests viz., Intradermal Johnin testing, fecael microscopy, and ELISA were considered affected with Johne's disease and were studied compared with healthy control groups. According to the results obtained, there was a significant decrease in haemoglobin, packed cell volume, lymphocyte count and a significant increase in neutrophil count in affected ones. Biochemical and antioxidant analysis revealed a significant decrease in serum total protein, albumin, calcium, magnesium and superoxide dismutase, glutathione peroxidase, and catalase as compared to the healthy ones. Conclusively, haemato-biochemical and oxidative stress parameters could be used to check the severity of disease in the endemic herds showing signs of paratuberculosis.

Keywords: Body condition score, Intradermal Johnin, Johne's disease, Oxidative stress, Paratuberculosis

Highlights

- Johne's disease leads to significant production and economic losses in the livestock sector.
- Significant decrease in haemoglobin, packed cell volume, and lymphocyte count in affected goats
- Significant increase in neutrophil count in goats with Johne's disease
- Notable reductions in serum total protein, albumin, calcium, magnesium, and antioxidant enzymes
- Haemato-biochemical and oxidative stress values can be indicators of severity in herds showing signs.

INTRODUCTION

Paratuberculosis, also known as Johne's disease (Pronounced 'Yo-nees'), is a chronic, fatal intestinal mycobacterial infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) clinically manifested by irreversible wasting, therapy-resistant diarrhoea and death from cachexia in ruminants, is widely spread world over in domestic and wild ruminant population (Windsor, 2014). Johne's disease has shattering effects on the livestock sector in terms of production and economic losses (Ott *et al.*, 1999) and also has serious impacts on food safety associated with Crohn's disease in humans (Singh *et al.*, 2008). The disease is found in both sheep and goats and is considered to have a worldwide distribution and is also designated as a multispecies disease in OIE-listed diseases (Okwumabua *et al.*, 2010; OIE, 2021). *M. avium* subsp. *paratuberculosis* (MAP) infection generally results from faecal-oral route exposure, with entry via the intestinal tract and then to the lymphatic system, where it resides in M cells overlying Peyer's patches in the ileum. Paratuberculosis results in the

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derangement of many haematological and biochemical parameters and also leads to the generation of reactive oxygen species, leading to oxidative stress, which is a secondary aggravating factor in most diseases. Oxidative stress may be defined as an alteration in the steady-state balance between oxidant and anti-oxidant agents in the cells; when reactive oxygen species are accumulated in cells, several physiological processes may get disturbed. Publications regarding the effect of paratuberculosis on haematological, biochemical and oxidative stress markers in goats are lacking, and no such previous records have been reported that state such derangements during paratuberculosis infection in caprines. This part of study pertains to determine haemato-biochemical and oxidative profile of goat herds infected with MAP diagnosed with the aid of intradermal testing, faecal microscopy and indirect-ELISA technique using indigenous-ELISA kit.

MATERIALS AND METHODS

Study area: Livestock contributes significantly to the economy of local population. The present study was conducted on goat population (Beetal Breed) reared in different geographical areas of Jammu region of Union Territory of Jammu & Kashmir. The Jammu division lies between 33.2778° N latitude and 75.3412° E longitude in the sub-Himalayan region of India. The total goat population in this division is 12.054 lakh out of 78.908 lakh of total livestock population with agriculture and livestock rearing as one of the main occupations of people. The sampling was conducted in and around R. S. Pura Tehsil of Jammu division from organized and unorganized herds after thorough physical examination keeping body condition score under consideration.

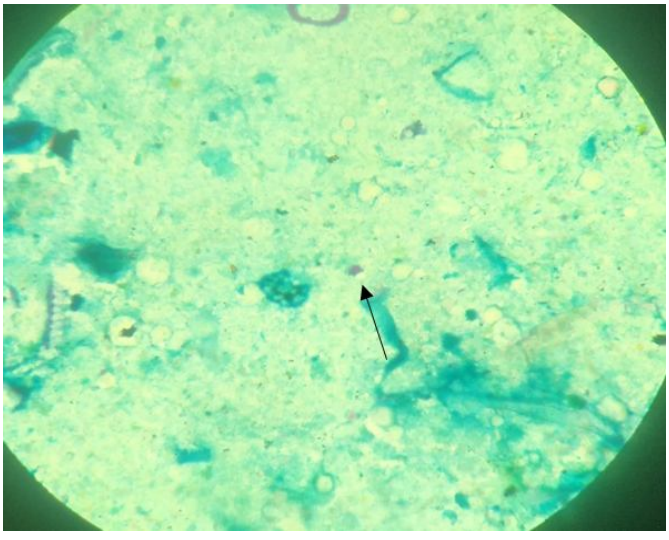

Fig. 1. Acid fast bacilli after Ziehl-Neelsen staining procedure (arrow pointed)

Ethical clearance: The sampling procedures were approved by the Faculty of Veterinary and Animal Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India (Institutional Animal Ethics Committee; approval. No: 07/IAEC-20/2021).

Sampling technique: Random and spatial sampling was done among the goat population of age group >1 years old.

Single intra-dermal test (SIT): Single intra-dermal test with Johnin (procured from Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India) was carried out as per manufacturers' instructions and interpretation was done as described by Ritchie (1953) after a period of 72 hours, the measurement and character of the swelling at the place of inoculation was recorded using digital Vernier calliper as; Negative (swelling under 3 mm), Doubtful (Swelling between 3 mm and 4 mm), Positive (Swelling of 4 mm and over).

Faecal microscopy: The faecal samples collected for diagnosis of MAP were first examined by direct microscopy. Approximately 2 g of faecal material was finely grounded in a sterilized pestle and mortar with sterilized distilled water (10-12 mL) and centrifuged at 4000 rpm for 40 min at room temperature (RT; 37°C). Smear was prepared from portions of sediment, stained by Ziehl-Neelsen (ZN) staining (Singh *et al*., 2011) and examined for the presence of acid-fast bacilli (AFB) indistinguishable to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) as shown in Fig. 1.

Serum Enzyme Linked Immunosorbent Assay (ELISA): Serum samples collected from suspected animals from common herd were screened using i_ELISA kit (Developed by Department of Biotechnology, Institute of Applied Sciences & Humanities, GLA University, Mathura, UP, India) for detecting antibodies against MAP having sensitivity and specificity as 53.7 and 86.0%, respectively (Singh *et al.*, 2009). Samples were evaluated on the basis of S/P ratio as negative (0.00-0.09), suspected (0.1-0.24), low positive (0.25-0.39), positive (0.4-0.99) and strong positive (1-10.00) for MAP infection (Singh *et al.*, 2009). Only samples of

positive and strong positive were selected for this study.

Haemato-biochemical and oxidative stress evaluation: Goats (n=40) with BCS 1 or 2 positive on intradermal testing, faecal microscopy, and ELISA were considered JD affected, and for statistical equality same number of animals with BCS >3 (n=40) negative on all tests mentioned were considered clinically healthy for comparison of haemato-biochemical and oxidative stress parameter. Haematological parameters studied included haemoglobin (Hb) using Drabkin's Method, packed cell volume (PCV) by Microhematocrit method, differential leucocyte count (DLC) by the method described by Jain (1986), total leucocyte count (TLC) by the method described by Jain (1986), biochemical parameters and oxidative parameters studied were total protein (g/dL), albumin (g/dL), calcium (mg/dL), phosphorus (mg/dL), magnesium (mg/dL) using semi-automated ERBA biochemistry analyzer and superoxide dismutase (Aebi, 1983); glutathione peroxidase, GSH-Px (Hafeman *et al*., 1974); catalase (Marklund and Marklund, 1974), respectively.

Statistical analysis: Statistical analysis of data was done by using paired t-test (Ross and Willson, 2017).

RESULTS

Haematological parameters: The mean±S.E ($p<0.05$) of haematological values of the infected and healthy group are shown in Table 1. Clinically affected goats showed a significant decrease in values of haemoglobin (Hb), packed cell volume (PCV), and lymphocyte count and a significant increase in neutrophil count values as compared to the control group (healthy ones). Meanwhile, total leucocyte count, monocyte, eosinophil, and basophil revealed non-significant changes as compared to control ones.

Biochemical parameters: The mean±S.E of biochemical values ($p<0.05$) of the infected and healthy group are shown in Table 2. Clinically affected goats showed a significant decrease in values of serum total protein, albumin, calcium and magnesium values and a non-significant decrease in phosphorus values as compared to the control group (healthy ones).

Table 1. Haematological values of *Mycobacterium avium* subsp. *paratuberculosis* infected and healthy animals

Parameter	Haematological values	
	Non-infected Group (n=40)	Infected Group (n=40)
Haemoglobin (g/dL)	10.22±0.20	9.01±0.19*
Packed Cell Volume	30.70±0.61	27.15±0.57*
TLC × /µL	9860.00±250.95	9262.50±234.12
Neutrophil (%)	31.83±0.57	36.00±0.55*
Lymphocyte (%)	59.45±0.59	55.40±0.57*
Monocyte (%)	4.13±0.23	4.50±0.20
Eosinophil (%)	4.15±0.17	3.70±0.22
Basophil (%)	0.45±0.11	0.40±0.11

*Differ significant ($p<0.05$)

Table 2. Biochemical values of *Mycobacterium avium* subsp. *paratuberculosis* infected and healthy animals

Parameter	Biochemical values	
	Non-infected Group (n=40)	Infected Group (n=40)
Total protein (g/dL)	7.00±0.17	6.11±0.15*
Albumin (g/dL)	3.33±0.08	2.90±0.07*
Calcium (mg/dL)	10.50±0.23	9.18±0.17*
Phosphorus (mg/dL)	6.32±0.19	5.81±0.19
Magnesium (mg/dL)	3.26±0.09	2.65±0.08*

*Differ significant ($p<0.05$)

Antioxidant status/oxidative stress parameters: The mean±S.E ($p<0.05$) of oxidative stress values of the infected and healthy group are shown in Table 3. Clinically affected goats showed a significant ($p<0.5$) decrease in values of blood superoxide dismutase, glutathione peroxidase, and catalase values as compared to the control group (healthy ones).

Table 3. Oxidative stress values of *Mycobacterium avium* subsp. *paratuberculosis* infected and healthy animals

Parameter	Oxidative stress values	
	Non-infected Group (n=40)	Infected Group (n=40)
Glutathione peroxidase (unit/mg of Hb)	4.68±0.15	2.57±0.17*
Catalase (µmole H_2O_2 decomposed/min/mg Hb)	54.43±0.95	47.04±1.43*
Superoxide dismutase (unit/mg of Hb)	825.43±18.56	733.46±17.04*

*Differ significant ($p<0.05$)

DISCUSSION

Mycobacterium avium subsp. *paratuberculosis* poses a significant risk to animal health. It results in enormous economic losses for livestock animals all around the world. Limited research was conducted in India and outside India to study the effect of MAP infection on haemato-biochemical and oxidative stress parameters in caprine, which drew our attention to this disease and its negative impact on metabolic values. Several haemato-biochemical and antioxidant changes in cattle and sheep have been documented as a result of Johne's disease, but such studies are very limited to goats.

In our study, there were decreased Hb, PCV and lymphocyte count values; similar results have been reported previously in paratuberculosis-affected animals, which indicated the presence of anaemia (Weiss and Goodnough, 2005; Senturk *et al.*, 2009; Tharwat *et al.*, 2012). These changes are perhaps associated with decreased serum iron and iron binding capacity (Senturk *et al.*, 2009). Sharma *et al.* (2020) reported a decreased levels of lymphocytes in MAP-positive animals and mentioned chronic infections are known to induce 'immune exhaustion'. 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).

In this study, significantly low total protein, albumin, Ca and Mg levels were reported to be in connection with cases of clinical diseases. Hypoproteinaemia might be attributed to decline in albumin levels (El-Deeb *et al.*, 2014). Also, hypoproteinaemia may be associated to protein-losing enteropathy, which may be caused by compromised mucosal integrity of the gastrointestinal tract or by an increase in gut motility with decreased absorption, resulting in an increase in intestinal protein loss (Sweeney, 2011; Tharwat *et al.*, 2012). Contrary to our findings, Shalini *et al*. (2017) reported decreased levels of total protein values in paratuberculosis-affected goats, mentioning the variation in the protein level is related to the stage of the disease.

In the present study, 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). Moreover, decreased levels of calcium in the infected group as compared to normal ones can be attributed to hypoalbuminemia and a decreased proportion of calcium bound with albumin (Delaney *et al.*, 2003). Bhardwaj *et al.* (2011) also reported significantly lower levels of total protein and albumin in paratuberculosis-affected animals than in the normal control group. Also, McGregor *et al.* (2015) reported a significant impact on albumin levels of animals affected with Johne's disease. The albumin levels had a significant association with the severity of intestinal lesions due to MAP. There are a number of physiological mechanisms that may affect serum albumin concentration. These may be due to the reduced nutrient intake, mal-absorption of nutrients across the small intestine, an increased rate of loss of albumin, change in rate of synthesis of albumin in the liver, an increased rate of turnover of albumin.

The ROS may be formed as a result of bacterial infection (paratuberculosis), and if they are not eliminated by the animal's endogenous enzymatic (SOD, CAT, GPx) and non-enzymatic (RGSH) antioxidant defences, oxidative stress will result, as demonstrated by the current findings (Sies, 1991). Balikci and Gurdogan (2015) also reported significant changes in the oxidative stress parameters, thereby cognizance of our findings. Furthermore, a reduction in SOD and GSH-Px in the study can be attributed to significant damage to the erythrocyte membrane and other cellular components caused by an inability to effectively detoxify oxygen free radicals. The decrease in SOD, GSH-Px, and GSH activity indicates that the anti-oxidant defence mechanism remains insufficient as a result of increasing oxidative stress. These alterations demonstrate the presence of oxidative stress during paratuberculosis since SOD, GSH-Px, and GSH are involved in the conversion of radicals into less effective metabolites (Balikci *et al.*, 2013).

Conclusively, haemato-biochemical and oxidative stress parameters could be used to check the severity of disease in the endemic herds showing signs of paratuberculosis. Also, the current study's findings are more cognitive than practical. However, they may be included in the differential diagnosis.

Conflict of interest: It is hereby mentioned there is no conflict of interest over any part of the study and preparation of the manuscript.

Author's contributions: BN, SRU, SVS: Conceptualized the work and conducted research; SR, AAN: Helped in sampling and writing of the manuscript; RS, JY, RY, IML: Helped in the editing of the manuscript.

Data availability statement: The data mentioned in the article will be available with the corresponding author.

Ethical statement: The work has been approved by the

IAEC having IAEC approval No. 07/IAEC-20/2021.

ACKNOWLEDGEMENT

Authors are thankful to the Department of Biotechnology, Institute of Applied Sciences and Humanities, GLA University, Mathura, UP for providing necessary help and support.

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Received- 10.12.2023, Accepted- 05.08.2024, Published- 20.08.2024 (Online), 01.12.2024 (Print)

Section Editor: Dr. A. K. Patra, Associate Editor