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SHORT COMMUNICATION

Serum Biochemical Alterations in Sheep Affected by Ovine Pneumonic Pasteurellosis

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ABSTRACT

Ovine Pneumonic Pasteurellosis is one of the most economically important infectious disease in sheep. The infection is caused by group of bacteria belonging to family *Pasteurellaceae*. In present study, blood samples were collected from ten (n=10) pasteurellosis affected sheep after molecular confirmation through PCR assay using 16S rRNA universal primer. Additionally, blood samples were also collected from ten (n=10) apparently healthy sheep to serve as a control group. The overall mean value of biochemical parameters *viz.* AST, ALT, calcium, phosphorus, magnesium and CPK were 96.11 ± 0.96 IU/L, 62.61 ± 2.77 IU/L, 10.99 ± 0.25 mg/dL, 7.60 ± 0.20 mg/dL, 1.90 ± 0.86 mg/dL and 9.31 ± 0.23 IU/L, respectively, which were obtained from Pasteurellosis affected sheep. Affected sheep exhibited significantly ($p<0.01$) elevated serum AST and ALT levels as compared to healthy sheep. Serum calcium and magnesium levels were significantly lower ($p<0.05$ and $p<0.01$, respectively), while phosphorus levels were significantly higher ($p<0.05$) in affected sheep. Serum CPK levels were also found higher in affected sheep although this change was non-significant. This study highlights significant alterations in serum biochemical parameters in sheep affected by Pneumonic Pasteurellosis. Elevated AST, ALT and phosphorus, along with reduced calcium and magnesium in affected sheep indicate the systemic impact of the infection. These findings contribute to understanding the patho-physiological effects of the disease and may assist in diagnostic and therapeutic approaches in pasteurellosis affected sheep.

Keywords: ALT, AST, CPK, ovine pneumonic pasteurellosis

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Sheep are highly valued in the Indian agrarian economy. They are particularly well-suited to arid and semi-arid tropical regions with marginal and sub-marginal lands. Their adaptability makes them ideal small ruminants for efficiently utilizing sparse vegetation in dryland areas through methods like rangeland management and reseeded pastures[1]. According to the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT, 2019), the leading countries in terms of sheep population are Mainland China, with 163.48 million heads, followed by India with 74.26 million heads and Australia with 65.75 million heads. India ranks second globally in sheep population, contributing over 4.03% to the global total with a recorded population of 74.26 million sheep[2]. As per the 20th Livestock Census, Rajasthan accounted for a sheep population of 7.9 million, ranking fourth in the country with a share of 10.64%.

The lungs, being in direct contact with the external environment are continuously exposed to airborne particles such as feed dust, pollutants, microorganisms and harmful chemicals, all of which can contribute to respiratory distress and disease in animals. In sheep, pneumonia represents a multifactorial syndrome, often triggered by a combination of physiological stressors and various infectious agents, making it a complex and significant respiratory condition[3]. Ovine pneumonic pasteurellosis is highly fatal infectious disease caused by group of bacteria belonging to family *Pasteurellaceae*. Bacteria belonging to this family includes *Pasteurella multocida*, *Pasteurella haemolytica* (now considered as *Mannheimia haemolytica*) and *Biberstenia trehalosi*. These are gram negative, cocco-bacilli shaped non-spore forming bacteria. High morbidity and mortality caused by the organism result in significant economic losses among sheep farmers in arid and semi-arid region of Rajasthan.

During the septicemic stage of ovine pneumonic pasteurellosis, disruptions in serum biochemical parameters may impose physiological stress on affected animals, ultimately contributing to decreased productivity. Given the sparseness of literature addressing the impact of pasteurellosis on the serum biochemical profile of sheep, the current investigation was undertaken to evaluate these alterations in naturally infected sheep with *Pasteurella* spp.

Table 1. Detail of 16s rRNA primer gene for identification of *Pasteurella* spp.

Species (Target gene)	Primer Sequence (5′-3′)	Annealing Temperature	Amplicon Size (bp)	Reference
Pasteurella spp. Universal primer (*16s rRNA* gene)	Fp AGAGTTTGATCMTGGCTCAG Rp CGGTTACCTTGTTACGACTT	52^{O}C	1466	Lane (1991)[18]

A cross-sectional study was carried out under field conditions, wherein all animals were maintained within the same geographical region and subjected to uniform climatic and environmental factors. Study population consist of sheep suffering from pneumonia irrespective of their age, sex and breed. Also, there was no history of vaccination among the herd. The study was conducted from July 2024 to December 2024. The research on live sheep was conducted in accordance with the guidelines and with the prior approval of the Institutional Animal Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India vide letter no. CVAS/IAEC/2024-25/41.

Blood samples and nasal swab samples were collected aseptically from ten (n=10) affected sheep. Additionally, blood samples were also collected from ten (n=10) apparently healthy sheep to serve as control. Blood samples were collected by jugular venipuncture and collected in non-EDTA vials for serum separation. Serum samples were separated by making blood slant and incubated for 1 hour at 37^{O}C. Blood clots were broken and tubes were centrifuged at 2500 rpm for 30 minutes. Serum samples were pipetted out in small Pyrex tubes and were stored at -20^{O}C in deep freeze till biochemical parameters assessment.

All the nasal swab samples were individually processed. Isolation and purification of DNA was performed using HiPurA® Multi-Sample DNA Purification Kit, HiMedia (Maharashtra, India) according to the manufacturer's instructions by spin column protocol.

16S rRNA universal primer was selected for identification of *Pasteurella* spp. Primer was synthesized by Bioserve Biotechnologies (India) Private Limited, Hyderabad, India. Detail of primer is mentioned in Table 1.

Molecular identification of the target genes was conducted through PCR amplification using the Sure Cycler 8800 (Agilent Technologies, California, USA) and universal primer. PCR conditions, including the annealing temperature for gene was optimized during the initial standardization process. The PCR amplification reaction consisted of 2 µL of DNA template combined with 18 µL of reaction mixture, which included 7 µL of nuclease free water, 0.005 µM of each forward and reverse primer and 10 µL of 2X PCR Master Mix (Hi-Chrom PCR REDy Master Mix, Himedia, Maharashtra, India).

For serum biochemical examination, serum samples were analyzed for different biochemical parameters *viz.* Alanine amino transferase (ALT), Aspartate amino transferase (AST), Creatinine Phosphokinase (CPK), Calcium, Phosphorus and Magnesium by using commercial kit of 'Meril Diagnostics Pvt. Ltd.' in Spectra Lab Genie Semi-Automatic Biochemistry Analyzer.

The data obtained from both apparently healthy and Pasteurellosis affected sheep were analyzed using Software Package for Social Sciences (SPSS) Version 27.0 (IBM Corp., 2020) with significance level taken at $p \leq 0.05$ and $p \leq 0.01$.

Mean values of biochemical parameters *i.e.* serum AST, ALT, CPK, calcium, phosphorus and magnesium of Pasteurellosis affected and clinically healthy sheep are presented in Table 2.

In sheep that were found affected by pasteurellosis, overall mean serum levels of AST and ALT were significantly ($p<0.01$) elevated compared to those in clinically healthy sheep. This increase in serum AST and ALT levels may be attributed to hepatic inflammatory, degenerative and necrotic changes induced by the bacterial infection and its associated circulating toxins[4-9]. The increased serum AST activity observed in

Table 2. Values of serum biochemical parameters of clinically healthy and Pasteurellosis affected sheep (Mean±SE).

S.No.	Parameters (Units)	Clinically Healthy Sheep	Pasteurellosis Affected Sheep
1.	Serum AST/SGOT (IU/L)	78.17 ± 1.35	96.11 ± 0.96**
2.	Serum ALT/SGPT (IU/L)	24.48 ± 1.28	62.61 ± 2.77**
3.	Serum Calcium (mg/dL)	12.06 ± 0.27	10.99 ± 0.25*
4.	Serum Phosphorus (mg/dL)	6.39 ± 0.29	7.60 ± 0.20*
5.	Serum Magnesium (mg/dL)	2.54 ± 0.11	1.90 ± 0.86**
6.	Serum Creatinine Phosphokinase (IU/L)	9.12 ± 0.31	9.31 ± 0.23^{NS}

** : Highly Significant at ($p<0.01$); * : Significant at ($p<0.05$); NS : Non-Significant

affected sheep may also be attributed to reduced feed intake and starvation, as well as to its muscle origin, resulting from dystrophic muscle damage associated with prolonged recumbency or increased respiratory muscle exertion in severe cases of disease[10-12]. These alterations are indicative of systemic involvement and underlying metabolic imbalances frequently linked to infections caused by *Pasteurella multocida* and *Mannheimia haemolytica* infections. The observations of the present study regarding serum AST and ALT levels are consistent with previous reports. El-Latif and El-Dessouky[4] documented mean serum AST activities of 38.67 ± 1.04 IU/L in clinically healthy lambs and 43.04 ± 0.90 IU/L in lambs with respiratory disorders, along with corresponding ALT values of 24.73 ± 0.98 IU/L and 29.86 ± 1.09 IU/L, respectively. Comparable observations have been reported in bovines[10], with mean serum AST activities of 1.1 ± 0.4 μkat/L in healthy calves and 2.1 ± 1.4 μkat/L in diseased calves. Another report[6] recorded AST values of 68.1 ± 0.4 IU/L in healthy bovines and 71.16 ± 0.69 IU/L in affected animals.

A highly significant ($p<0.01$) effect was observed in serum magnesium levels and a significant ($p<0.05$) effect were noted in serum calcium and phosphorus levels in sheep affected by pasteurellosis. The overall mean serum calcium and serum magnesium levels were lower, while serum phosphorus levels were higher in affected sheep compared to clinically healthy controls. The decreased calcium and magnesium levels may be attributed to anorexia and impaired intestinal absorption associated with the disease. Altered mineral profiles, particularly hypocalcemia and hypomagnesemia may be linked to anorexia, intestinal malabsorption and systemic stress[13]. Hyperphosphatemia, often seen in inflammatory and renal dysfunction, highlights the reciprocal regulatory relationship between calcium and phosphorus wherein hyperphosphatemia leads to a reduction in serum ionized calcium through mass action interactions between phosphate and calcium ions[14]. This imbalance is further exacerbated by impaired renal synthesis of 1, 25-dihydroxyvitamin D, which plays a crucial role in maintaining calcium homeostasis[15]. The present findings on serum calcium, magnesium and phosphorus levels are in agreement with earlier studies. El-Latif and El-Dessouky[4] reported mean serum calcium concentrations of 12.06 ± 0.27 mg/dL in clinically healthy lambs and 10.99 ± 0.25 mg/dL in affected lambs. Similarly, another finding[15] observed mean serum calcium levels of 8.643 ± 0.601 mg/dL in healthy goats and 7.280 ± 0.234 mg/dL in infected goats. They also recorded mean serum magnesium concentrations of 2.473 ± 0.387 mg/dL in healthy goats compared to 1.347 ± 0.167 mg/dL in infected goats, along with mean serum phosphorus levels of 6.213 ± 0.854 mg/dL in healthy goats and 4.173 ± 0.187 mg/dL in infected goats.

The overall mean serum CPK level was non-significantly higher in affected sheep compared to the mean values observed in clinically healthy sheep suggesting sub-clinical muscular damage or stress-related myopathy, as previously reported in severe cases involving respiratory distress and prolonged recumbency[10-12,16,17].

CONCLUSIONS

A comparative serum biochemical analysis between pasteurellosis affected sheep and clinically healthy controls revealed notable differences, indicative of systemic involvement. Affected sheep showed significantly elevated levels of serum AST, ALT and phosphorus, alongside significantly reduced serum calcium and magnesium concentrations. Additionally, a non-significant increase in serum CPK levels was observed. These biochemical changes suggest multi-organ dysfunction and metabolic disturbances often associated with bacterial infections such as those caused by Pasteurella multocida and Mannheimia haemolytica. These findings highlight the systemic nature of pasteurellosis and targeted therapeutic strategies. The incorporation of supportive mineral supplementation, prompt administration of appropriate antibiotic therapy and the implementation of effective vaccination strategies can collectively aid in reducing the adverse effects of the disease, thereby enhancing animal health and overall productivity in sheep production systems.

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