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Research Article

IMPACT OF LUMPY SKIN DISEASE ON SERUM BIOCHEMICAL PARAMETERS IN CATTLE

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ABSTRACT

Lumpy skin disease (LSD) has emerged as a worldwide infectious disease in cattle, leading to significant devastation in the livestock industry. It is characterized by high fever and widespread nodules on the mucous membranes or skin, severely impacting the growth of the cattle industry and international trade. A total of 214 cattle affected by LSD were studied during year 2023-2024, with clinical progression, age and breed of the affected animals recorded. Out of 214 samples, 92 serum samples were found positive for LSD virus (74 in ELISA test and 8 in PCR). Among the total cattle affected by LSD, 31% were Holstein Friesian (HF), 28% were nondescript breeds, 27% were Rathi, 7% were Gir, 4% were Jersey and 3% were Tharparkar. Of the total cattle affected by LSD, 49% were over 5 years old, 39% were between 2 and 5 years, and 12% were under 2 years of age. Serum biochemical analysis showed a significant rise in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, along with hypoalbuminemia in cattle affected by LSD. This study contributes to a better understanding of the disease's pathogenesis, aiding in the early management and treatment of affected animals.

Keywords: Biochemical, Cattle, Jaipur, Lumpy skin disease

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Lumpy Skin Disease is characterised by fever, enlargement of lymph nodes, anorexia, depression, dysgalactia, emaciation and development of skin nodules, eventually resulting in abortion in pregnant cattle, and sterility in bulls (Tuppurainen *et al.*, 2017). LSD is a infectious viral disease of cattle caused by Lumpy skin disease virus (Murphy *et al.*, 2008). Lumpy skin disease virus is a double-stranded DNA containing virus of around 150 kilobase pairs (kbp) with relatively large sizes (230-260 nm), enclosed in a lipid envelope, belongs to Genus *Capripox*, which is genetically related to the sheep pox (SPPV) and goat pox (GTPV) viruses (Buller *et al.*, 2005; Givens, 2018). Lumpy skin disease virus (LSDV) is classified under Genus *Capripox*, Family *Poxviridae* and subfamily *Chordopoxvirinae* (Tuppurainen and Oura, 2012).

Depending on the virulence of the strains and the sensitivity of the cow breed, the severity of the clinical indications of LSD ranges from asymptomatic to lethal. Several clinical symptoms, including skin nodules, swollen lymph nodes, fever, nasal discharge, lacrymation, edema of mucous membrane and internal organ might be seen in infected animals (Abutarbush *et al.*, 2015). Viremia and fever are the first symptoms of the generalized type, later localization in the skin and production of inflammatory nodules can be noticed (Constable *et al.*, 2017). The severity of the early clinical manifestations of LSD varies, depending on the herd management, irrespective of the sex or age of the animal. The animal develops several solid, bounded nodules in epidermis (Beard *et al.*, 2016). Nodules may emerge within 1-2 days, which may remain as localized to a few lesions or may be extensive, can be seen at different locations including the head, neck, perineum, genitalia, udders and limbs (Al-Salihi *et al.*, 2014).

Increased levels of aspartate aminotransferase, alkaline phosphatase, and creatinine concentrations in serum biochemical analysis of infected animals, suggest the liver and kidney failures occur during LSDV infection (Murat *et al.*, 2016).

MATERIAL AND METHODS

Animal source: For this study, a total of 214 blood samples from LSD suspected cases of cattle were collected during year 2023-2024, from various parts of Rajasthan, for detection of LSD Virus. Out of 214 samples, 92 samples were found positive for LSD virus (74 in ELISA test and 18 in PCR, kept under Group-I. Twenty visibly healthy cattle, kept under optimal management conditions, were selected as the healthy control group (Group-II) to assess normal biochemical parameters. Cattle suspected of having Lumpy Skin Disease (LSD), based on symptoms such as skin nodules, fever, palpable superficial lymph nodes, brisket edema, lameness, excessive salivation and reduced feed and water intake, underwent a thorough physical examination. Clinical evaluation included measuring rectal temperature, assessing the conjunctival mucous membrane, palpating superficial lymph nodes, and recording other LSD-related symptoms, including limb swelling, brisket edema, nasal discharge, excessive tearing, and lumps on different body parts. Serum samples

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from each group were obtained for biochemical assessment.

Blood collection: Whole blood of 5 ml was collected aseptically, from the jugular vein of the animal with a 10 ml syringe, in clot activator tube taking all precautions to avoid haemolysis.

Separation of serum: Serum was separated, by keeping the vial in slanting position for 1 hour at room temperature and centrifugation at 3000 rpm for 20 minutes.

ELISA assay: LSD suspected cases of cattle, during year 2023-2024 were subjected to rapid detection by ELISA assay, using Bovine Lumpy Skin disease (LSDV) ELISA Kit (SunLong Biotech Co., LTD).

Genomic DNA isolation: The viral DNA extraction was done by using HiPura Multi- Sample DNA purification kit (Himedia).

Molecular detection of LSD virus: Conventional polymerase chain reaction (PCR) was performed for the amplification of the VP32 gene of lumpy skin disease virus (LSDV). The forward primer sequence used was 5′-TTTCCTGATTTTTCTTACTAT-3′, while the reverse primer sequence was 5′-AAATTATATACGTAAATAAC-3′, yielding an expected amplicon size of 192 bp.

PCR amplification was carried out in a 10 µL reaction volume comprising 1 µL of 10X PCR buffer, 1 µL of 2 mM dNTP mix, 0.5 µL each of forward and reverse primers, 0.4 µL of Taq DNA polymerase (5 U/µL), 2 µL of template DNA, and 4.6 µL of nuclease-free water to make up the final volume. The PCR cycling conditions consisted of an initial denaturation at 94° C for 7 minutes, followed by 42 cycles of denaturation at 94° C for 1 minute, annealing at 52° C for 30 seconds, and extension at 72° C for 1 minute, with a final extension at 72° C for 10 minutes. The amplified products were subsequently held at 4° C until further processing.

The PCR products were resolved by agarose gel electrophoresis and visualized using a gel documentation system. Known LSDV-positive DNA procured from the National Centre for Veterinary Type Culture (NCVTC), ICAR-NRCE, Hisar, was used as the positive control, while nuclease-free water served as the negative control.

Biochemical estimations: Biochemical parameters included the estimation of serum total protein, albumin, globulin, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP), estimated by using TURBOCHEM 100 Biochemistry Analyzer (CPC DIAGNOSTICS PVT. LTD).

RESULTS AND DISCUSSION

A total of 214 blood samples from LSD suspected cases of cattle were collected during year 2023-2024. Out of 214 samples, 92 samples were found positive for LSD virus (74 in ELISA test and18 in PCR). Fig. 2 displays that among the total cattle affected by LSD, 31% were Holstein Friesian (HF), 28% were nondescript breeds, 27% were Rathi, 7% were Gir, 4% were Jersey and 3% were Tharparkar. Fig. 3 displays that out of the total cattle affected by LSD, 49% were over 5 years old, 39% were between 2 and 5 years, and 12% were under 2 years of age. In Fig. 1 Graph is showing level of Serum biochemical parameters in the control group and Lumpy skin disease affected cases of various places. The serum biochemical analysis revealed an increase in serum AST and ALT levels, accompanied by a decrease in albumin levels across all age groups of affected cattle. Among them, older animals were the most susceptible to LSD, followed by younger ones, with very young animals being the least affected. However, no significant changes were observed in the levels of albumin, globulin, creatinine, and alkaline phosphatase (ALP), which remained within the normal range in LSD-affected cattle. Table 1 displays the Mean ± SE values of serum biochemical parameters for both the control group and LSD-affected cattle from different locations. Among the LSD-affected animals, region-wise variation, with exceptionally high AST levels in Udaipur followed by, Shriganganagar, Jaipur and Bikaner. ALT activity was elevated predominantly in animals from Jaipur, followed by Shriganganagar, Bikaner and Udaipur regions.

Levels of AST and ALT were significantly ($p<0.05$) elevated in LSD affected cases compared to healthy control group (Group-II) highlighted the influence of disease severity, management practices and environmental stressors on biochemical responses. The lowest mean albumin levels were recorded in Udaipur, followed by Shriganganagar, Jaipur and Bikaner suggested impaired hepatic synthetic function, increased vascular permeability and enhanced protein loss due to systemic inflammation. Alkaline phosphatase activity showed moderate elevation without significant regional differences, indicating possible involvement of hepatobiliary tissues and bone metabolism, though its diagnostic sensitivity appeared limited compared to transaminases. Mild to moderate elevation in serum creatinine, especially in older and Holstein Friesian cattle, pointed towards renal involvement or dehydration secondary to prolonged illness. Overall, the findings of the present study confirm that Lumpy Skin Disease induces characteristic biochemical alterations involving hepatic, muscular, renal and immune systems.

The present study elucidates significant serum biochemical alterations in cattle naturally infected with lumpy skin disease virus (LSDV), reflecting the multisystemic nature of the disease. Marked elevation of hepatic and muscle-associated enzymes, along with

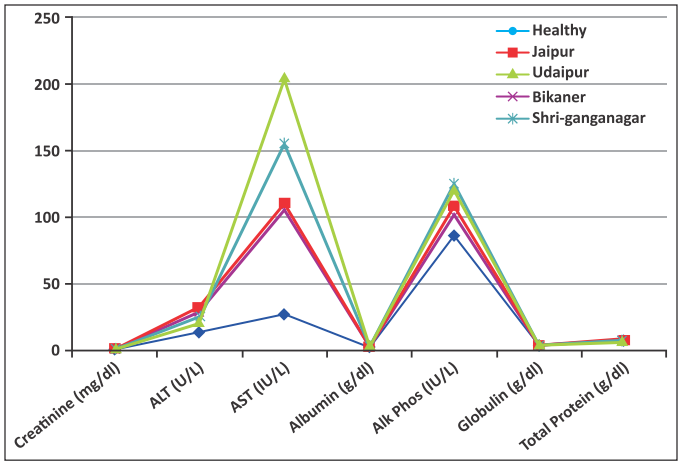

Fig. 1. Graph showing level of Serum biochemical parameters in the control group and Lumpy skin disease affected cases of various places.

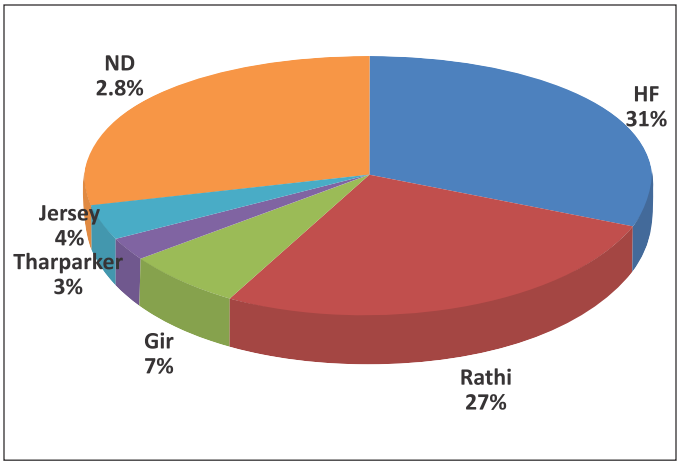

Fig. 2. Breed-wise distribution of infection of LSDV in Cattle during Year 2023-24.

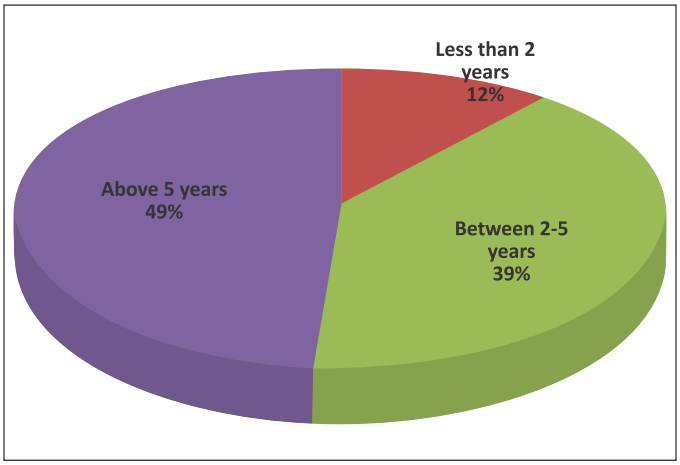

Fig. 3. Age-wise distribution of infection of LSDV in Cattle during Year 2023-24.

alterations in protein fractions and mild renal stress, highlights the extensive metabolic and inflammatory impact of LSDV infection. Significant elevation of alanine aminotransferase (ALT) in LSD-affected cattle, particularly in animals from Jaipur and in Holstein Friesian cattle, indicates hepatocellular injury associated with viral replication, hypoxia, and inflammatory mediator release. The progressive increase in ALT activity with advancing age further suggests diminished hepatic regenerative capacity and increased susceptibility to liver injury in older animals. Similar ALT elevations in LSD have been reported earlier and are attributed to hepatocellular degeneration and leakage of intracellular enzymes into circulation during active infection (Kaneko *et al.*, 2008; OIE, 2019; Elhaig *et al.*, 2019).

Aspartate aminotransferase (AST) exhibited the most pronounced elevation among all biochemical parameters, with significantly higher values observed in severely affected animals, particularly from Udaipur. Elevated AST likely reflects extensive muscle involvement due to nodular lesions, necrotizing dermatitis and myositis, in addition to hepatic damage. Higher AST levels in older cattle and Holstein Friesian breed further support the role of disease severity, muscle damage, and systemic inflammation in enzyme elevation. These findings are consistent with earlier reports describing AST as a sensitive indicator of muscular and hepatic involvement in LSD (Tuppurainen *et al.*, 2017; Zeedan *et al.*, 2019).

Alkaline phosphatase (ALP) activity showed mild to moderate elevation without consistent statistical significance, suggesting limited involvement of biliary tissues in LSD pathogenesis. The relatively stable ALP levels observed across age groups and breeds align with previous studies indicating that ALP alterations in LSD are usually mild and non-specific, reflecting secondary hepatic congestion or steroid-induced metabolic changes rather than primary cholestasis (Ali *et al.*, 2022; El-Kenawy *et al.*, 2018).

Hypoalbuminemia was a consistent biochemical abnormality observed across regions, breeds and age groups, particularly in older cattle. Reduced albumin levels likely result from impaired hepatic synthesis, negative acute-phase response, decreased feed intake, and increased vascular permeability during systemic inflammation. These findings corroborate earlier observations from LSD outbreaks in India and Africa, where hypoalbuminemia was associated with disease severity and prolonged clinical course (Abutarbush, 2015; Gamal *et al.*, 2020).

Conversely, globulin concentrations were markedly elevated in many LSD-affected cattle, particularly in adult animals, indicating strong humoral immune activation and prolonged antigenic stimulation. The concurrent increase in total protein levels and reversal of the albumin-to-globulin ratio further supports the presence of chronic inflammatory processes. Similar hyperglobulinemia and elevated total protein levels have been reported in LSD-positive cattle and are attributed to increased immuno-globulin synthesis

Table 1. Mean±SE of Serum biochemical values in the control group and Lumpy skin disease affected cattle of various places.

	HEALTHY (N= 20)	Jaipur region (N= 18)	Udaipur region (N= 22)	Bikaner region (N= 25)	Shriganganagar region (N=27)	Significance
Creatinine (mg/dl)	1.236 ± 0.082	1.653 ± 0.163	1.377 ± 0.116	1.623 ± 0.114	1.544 ± 0.131	NS
ALT (IU/L)	13.877^{a} ± 2.244	32.519^{c} ± 3.397	20.686^{ab} ± 3.032	29.348^{bc} ± 2.461	25.478^{bc} ± 3.043	**
AST (IU/L)	26.832^{a} ± 3.895	110.863^{b} ± 25.01	204.632^{c} ± 44.468	105.985^{ab} ± 15.107	155.467^{bc} ± 25.329	**
Albumin (g/dl)	2.909^{bc} ± 0.15	3.074^{c} ± 0.129	2.425^{a} ± 0.092	3.096^{c} ± 0.087	2.683^{ab} ± 0.112	**
Alk Phos (IU/L)	87.041 ± 10.578	108.419 ± 15.645	120.941 ± 12.399	102.056 ± 11.151	124.922 ± 12.004	NS
Globulin(g/dl)	3.672 ± 0.632	4.668 ± 0.82	4.402 ± 0.37	5.456 ± 0.639	4.728 ± 0.398	NS
Total Protein(g/dl)	6.64 ± 0.639	7.741 ± 0.846	6.826 ± 0.37	8.552 ± 0.655	7.411 ± 0.396	NS

Note: Means bearing different superscripts in same row differ significantly ($p \leq 0.05$). N : Number of samples.

during sustained viral infection (El-Nahas *et al.*, 2017). Serum creatinine concentrations showed mild, non-significant elevation in affected animals, particularly in Holstein Friesian and older cattle, suggesting transient renal stress rather than overt renal dysfunction. These changes are likely secondary to dehydration, fever, and systemic inflammatory response rather than direct viral nephropathy. Comparable observations have been reported in previous studies, indicating that renal function is generally preserved in LSD, except in severe or complicated cases (Sevik & Dogan, 2017; Sudhakar *et al.*, 2020).

CONCLUSION

The present study highlights the biochemical alterations in cattle affected by Lumpy Skin Disease (LSD), revealing significant increases in serum AST and ALT levels and a notable reduction in albumin levels. These changes indicate hepatic involvement and possible muscle damage in LSD-affected animals. Among the affected cattle, older animals were the most susceptible. Additionally, crossbred cattle exhibited a higher susceptibility to LSD. This knowledge can contribute to improved disease management, early diagnosis and the development of effective control strategies to mitigate the economic losses associated with LSD.

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