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Haematological and biochemical profile of cattle naturally infected with lumpy skin disease (LSD) virus

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Abstract

Lumpy skin disease is an important viral disease of cattle and buffaloes caused by lumpy skin disease virus that belonging to genus *Capri pox* virus. The present study was conducted in Karnataka during the outbreak of LSD to determine the changes in haematological and serum biochemical values of cattle naturally infected with lumpy skin disease virus (LSDV). Blood in EDTA and serum samples were collected from cattle suffering from LSD (n=152) and those which are healthy (n=10). The results of blood parameters in LSD affected animals showed a significant decrease in the total erythrocyte count, haemoglobin and haematocrit values. Serum biochemical analysis showed significant increase in aspartate aminotransferase ($p<0.05$). In addition, Serum gamma glutamyl transferase, albumin and creatinine values were significantly decreased in serum of infected animals compared to healthy animals. However, there were no significant differences in the other blood and biochemical parameters observed. The results of the current study suggest severe inflammatory process and disease complications such as anorexia and reduced muscle mass associated with natural LSD infection. These findings may help in developing treatment strategies, identification of biomarkers for early detection and for assessing the prognosis LSD.

Keywords: Lumpy skin disease, lumpy skin disease virus, *Capri pox*, haematology, serum biochemistry

Introduction

Lumpy skin disease a viral disease of cattle and buffalo caused by lumpy skin disease virus belongs to genus *Capri pox* virus, sub family *Chordopoxvirinae* of the family Poxviridae. LSDV is host specific and affects cattle, buffalo and wild ruminants. Apart from lumpy skin disease virus (LSDV) genus *Capri pox* virus includes goat pox virus (GTPV) and sheep pox virus (SPPV) which causes diseases in goat and sheep, respectively.

LSD is a subacute to acute OIE "List A" bovine illness, characterized by extensive cutaneous lesions and signs typical to generalized poxvirus diseases (Coetzer *et al.*, 1994)^[6]. Extensive cutaneous lesions and signs typical to generalized poxvirus diseases. LSDV mainly affects cattle, but LSD is also observed in domestic Asian water buffaloes (Ali *et al.*, 1990; Tuppurianen *et al.*, 2017)^[3, 26]. In wildlife, clinical disease has been suspected in springbok (*Antidorcas marsupialis*) in Namibia, oryx (*Oryx gazelle*) in South Africa and Arabian oryx (*Oryx leucoryx*) in Saudi Arabia (Greth *et al.*, 1992; OIE, 2013)^[10, 17].

First outbreak of LSD was noticed in Zambia in the year 1929. Later the disease spread to other African countries, European countries and Asian countries such as Bangladesh, China and Pakistan. In India first outbreak of LSD in cattle was reported in Odisha State in August 2019.

The transmission of LSDV from infected to susceptible cattle is assumed to be predominantly via biting and blood-feeding arthropod vectors. Intravenously feeding arthropods are most responsible for the transmission of LSDV. The mosquito *Aedes aegypti* has been shown to transmit LSDV mechanically from infected to susceptible animals, under experimental conditions (Chihota *et al.*, 2001)^[5]. Ixodid ticks have also shown to be capable of transmitting LSDV (Tuppurainen *et al.*, 2011)^[25].

LSD is a one the economically important disease because it affects the hide quality and decreases milk production along with high fever, enlarged pre-scapular and pre-femoral lymph nodes, swollen limbs, brisket oedema, presence of skin nodules on surface of the body, mainly seen on head, thorax, back and sides, tail region, mammary gland, scrotum, gums and tongue of the affected animals.

The aim of this study was to describe the haematological and serum biochemical changes associated with natural clinical infection of lumpy skin disease in cattle. The evaluation of the haematological and biochemical profile may aid in the understanding of the disease's pathophysiology, prognosis and for making informed decision on treatment.

Materials and Methods

Study site and selection of the animals and sample size

The present study was undertaken in Karnataka state during October 2020 to September 2022 when the LSD outbreak was reported. The LSDV affected clinically suffering cattle presenting with the skin nodular lesions on the different parts of the body and found positive for LSDV by PCR were included in the study (n=152). Those animals present in the LSD outbreak area showed clinical signs such as nodular skin lesions, pyrexia, loss of appetite, lachrymation, nasal discharge, open mouth breathing, excessive salivation, enlargement of superficial lymph nodes, conjunctivitis and oedema mainly involving limbs and brisket region were selected for the study. In addition, another 10 cattle which were apparently healthy and not showing clinical symptoms of LSD were selected as healthy controls for the study.

Collection of samples

From each animal 2 ml volume of blood in EDTA and 5 ml serum samples were collected from left jugular vein using sterile vacutainer needles and tubes. The blood in serum tubes was allowed to stand at ambient temperature for 1 h followed by centrifugation at 2000 rpm for 10 min for separation of serum. The blood in EDTA was stored at 4 °C and serum samples at -20 °C till further processing.

Haematology

The haematological analysis was performed using Mindray BC-2800VET Auto Haematology Analyser as per manufacturer instruction.

The parameters such as total leukocyte count (TLC), total erythrocyte count (TEC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total platelet count (Plt) were analysed.

Serum biochemistry

Serum biochemical parameters such as AST, ALT, ALP, BUN, total protein, creatinine, GGT, albumin and cholesterol were estimated using automatic biochemical analyser (Erba).

Data analysis

Haematological and serum biochemical parameters values were entered into a Microsoft Excel 2007 spreadsheet. For descriptive statistical analysis, SPSS software version 17 was used. In addition, the same software was used to compare the mean values of cattle affected with LSD and healthy animals using the t- test. A p value less than or equal to 0.05 was considered significant and p value less than or equal to 0.001 was considered as highly significant.

Results and Discussion

Haematological profile

It was observed that there was a significant decrease in the mean erythrocyte count ($p < 0.001$), haemoglobin ($p < 0.001$) and haematocrit ($p < 0.05$) among LSD affected cattle in comparison to the respective haematological parameters of normal, healthy cattle. In addition, there was significant increase in mean corpuscular volume ($p < 0.05$). The mean value of total leukocyte count, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration and platelet counts did not differ between LSD affected and healthy cattle. However, an appreciable numerical decrease in the mean value of platelet count was observed in affected animals compared to healthy animals but without any significant difference.

Our study was in agreement with those of Abutarbush (2015)^[1]; Katsoulos *et al.*, (2016)^[11] and Keshta *et al.*, (2020)^[13] who also observed similar findings. Decreased red blood cell count, haemoglobin and haematocrit encountered in affected animals was indicative of anaemia. In Some diseases, stress and inflammatory processes, cause an increase in free radical (reactive oxygen species (ROS) and nitric oxide (NO)) generation, which in turn causes lipid peroxidation leading to cellular damage (Gaschler and Stockwell, 2017)^[9]. Platelet count was also significantly reduced in the affected animals. However, MCV values significantly increased in affected animals which is in contrary to the findings of Abutarbush (2015)^[1]. This change could be attributed to macrocytic anaemia.

The reduction in RBC count probably attributed to a slight haemolysis due to the vasculitis, which is a consistent finding at LSD lesions (Tageldin *et al.*, 2014 and Katsoulos *et al.*, 2016)^[24, 11].

The mean of total leukocyte count, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration between affected and control group did not show significant changes. However, variation in TLC, MCH and MCHC was reported by Abutarbush (2015)^[1]. This is most likely connected to the stage and intensity of infection or could be due to wide variation in stage of sample collection during disease progression and due to variation in factors with respect to age, gender and physiological status of the infected animals.

Rouby *et al* (2021)^[18] found leucocytosis accompanied with neutrophilia, lymphocytosis and monocities as compared to control group. This result in agreement with Abutarbush (2015)^[1]. On the contrary, El-Shoukary *et al.* (2019)^[7] reported leukopenia in LSD infected bull. This is probably related to the stage and severity of infection (Ahmed, 2015)^[2]. In cattle, leukopenia is usually seen in the developmental stage of the acute infection, after which the production of neutrophils is intensified, leading to leucocytosis (Morris, 2002). Leucocytosis could be due to secondary acute bacterial infections, especially pyogenic bacterial infections as reported by Ahmed (2015)^[2].

Student t-test was carried out using SPSS software version 17 to calculate P value in test with Equal variances not assumed. In affected animals, the mean \pm SE values. (Table-1).

Table 1: In affected animals, the mean \pm SE values.

Haematology parameters	Control	LSD affected	p-value
WBC ($10^3/\mu\text{l}$)	11.311 \pm 1.059	11.721 \pm 0.327	0.765
RBC ($10^6/\mu\text{l}$)	8.262 \pm 0.221	5.492 \pm 0.093***	0
HGB (g/dl)	10.102 \pm 0.293	7.605 \pm 0.132***	0
HCT (%)	34.565 \pm 1.631	27.596 \pm 0.676*	0.006
MCV (fl)	41.224 \pm 0.845	47.374 \pm 0.588*	0.012
MCH (pg)	15.688 \pm 0.552	16.902 \pm 0.551	0.595
MCHC (g/dl)	34.922 \pm 0.253	35.973 \pm 1.22	0.836
PLT ($10^4/\mu\text{l}$)	40.411 \pm 13.502	31.8335 \pm 15.78	0.17

* - Significant at $p < 0.05$, *** - Highly significant at $p < 0.001$

LSD = Lumpy skin disease, WBC = white blood cells, RBCs = red blood cells, HGB = hemoglobin, HCT = haematocrit, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, PLT = Platelet count

Serum Biochemical profile

In the current study, biochemically the mean \pm SE values of ALT, AST, ALP, BUN, GGT, total protein, albumin, creatinine and cholesterol in lumpy skin disease confirmed cases were estimated. The mean \pm SE values of LSD affected animals and healthy animals (Table 2).

Table 2: The mean \pm SE values of LSD affected animals and healthy animals

Biochemical Parameters	Control (n=10)	LSD (n=152)	p-Value
ALT (U/I)	27.862 \pm 2.548	30.618 \pm 0.765	0.271
AST (U/I)	66.312 \pm 7.446	96.207 \pm 3.474*	0.007
ALP (U/I)	70.889 \pm 3.856	97.671 \pm 4.486	0.055
BUN (mg/dl)	14.156 \pm 2.024	14.601 \pm 0.542	0.804
GGT (U/I)	23.688 \pm 0.747	13.938 \pm 0.572***	0
Total protein (g/dl)	7.077 \pm 0.157	7.427 \pm 0.097	0.25
Albumin (g/dl)	3.549 \pm 0.081	2.139 \pm 0.036***	0
Creatinine (mg/dl)	1.3571 \pm 0.047	1.075 \pm 0.019***	0
Cholesterol (mg/dl)	126.75 \pm 1.174	123.488 \pm 3.834	0.783

* - Significant at $p < 0.05$, *** - Highly significant at $p < 0.001$

LSD = Lumpy skin disease group. Alanine transaminase (ALT). Aspartate transaminase (AST) and Alkaline phosphatase (ALP), Blood urea nitrogen (BUN), Gamma glutamyl transferase (GGT)

There was a significant increase ($p < 0.005$) in mean value of AST and a significant ($p < 0.001$) decrease in GGT, albumin and creatinine values in LSD affected animals compared to control animals. No significant change was observed in the mean values of total protein, BUN, ALT and cholesterol between affected and healthy animals. However, a numerical increase in the mean values of ALP was observed in affected compared to healthy cattle without significant variation.

Hypoalbuminemia in LSD cases similar to our study was also observed by Neamat-Allah (2015) [16] who opined that it could be due to loss of appetite, decreased protein synthesis and higher catabolic rate with progressive emaciation in the affected animals. However, on the contrary, increase in albumin concentration and total protein was reported by Abutarbush (2015) [1] and Roubly *et al.* (2021) [18] who stated that hyperproteinaemia with increased albumin value was due to dehydration that occurred in the affected animals. However, in the present study no significant alteration in the total protein value was observed in affected animals in spite of decrease in albumin level which could be due to increased globulin level especially γ globulins consequent to infection in the affected cattle which was also the observation of Neamat-Allah (2015) [16], El-Mandrawy and Alam (2018) [22]

and Roubly *et al.*, (2021) [18] who recorded elevation in the globulin level in LSD cases.

Similar to the current study increased mean AST values was also observed by Neamat-Allah (2015) [16] and Roubly *et al.*, (2021) [18] Kauppinen (1984) Meyer and Harvey (1998) [14] who stated that elevation AST could be due to the LSD induced injuries to hepatocytes, skeletal muscles and muscular cardiac cells which are the normal sites of AST. LSDV induced vasculitis causing extracutaneous injuries in muscle, liver, rumen, lung kidney, small intestine and cardiac muscle was reported by Sanz-Bernardo *et al.* (2020), Roubly and Aboulsoud (2016) [19], Vasatova *et al.*, 2013 [27], Sevik *et al.* (2016) [21] which was also the observation in the current study which explains the reason for elevation of AST.

Elevated GGT concentration was observed in LSD cases by Stockham and Scott 2008 [23], Sheefa *et al.*, 2018 [22]; Sevik *et al.* 2016) [21] who attributed it to damaged hepatocytes surrounding the bile duct, intrahepatic cholestasis and biliary disease. However, in the present study a significant decrease in the GGT mean value was observed in spite of cholestasis observed microscopically in some of the cases which needs to explain on further exploration.

An increased creatinine level was reported by Neamat-Allah (2015) [16], El-Mandrawy and Alam (2018) [22] and Roubly *et al.*, (2021) [18]. However, in the present study a significant decrease in creatinine value was observed which was also the observation of Abutarbush (2015) [1]. The absolute muscle mass and level of physical activity influence the rate of creatinine production and thus the serum concentration. In the present study the chronically affected cattle with LSD were emaciated and debilitated with decreased muscle mass and were weak with no physical activity which could be the reasons for decreased creatinine observed in the present study (Carlson 2002) [4].

Conclusion

Anaemia, thrombocytopenia, decreased HCT, creatinine concentration, GGT, albumin, increased in AST were the main findings observed in cattle naturally infected with LSDV. These are likely due to the associated severe disease complications, such as anorexia and decreased muscle mass. Understanding the haematological and serum profile picture of cattle affected by LSD gives further insight into the pathogenesis of the disease and can help in the treatment of LSD affected cattle.

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