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Haemato-biochemical, electrocardiographic and cardiac biomarker studies in cattle affected with lumpy skin disease

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Abstract
The study was undertaken on 14 cattle affected with Lumpy skin disease (LSD), showing clinical signs like nodular lesions on the body, enlargement of superficial lymph nodes and their tracts, oedema of limbs and brisket. Age of the animals affected was 7-8 years, significant increase in rectal temperature and respiratory rate was recorded. Hematologically significant decrease in TLC, RBC and haemoglobin was seen. Biochemically significant increase in ALT, AST, AST, TP, creatinine and cTn-I, significant decrease in albumin and glucose was evident. Electrocardiography revealed normal sinus rhythm in 57.14%, low amplitude QRS complexes in 21.42%, VPC's in 7.14%, sinus tachycardia in 7.14% and sinus bradycardia in 7.14% of the affected animals. Echocardiography did not reveal any structural abnormalities in the morphology of heart.

Keywords: LSD, lymph nodes, cTn-I, electrocardiography and echocardiography

Introduction
Lumpy skin disease (LSD, Pseudo-urticaria, Neethling virus disease, exanthema nodularis bovis and knopvelsiekte) is a viral disease of cattle caused by lumpy skin disease virus (LSDV). Belonging to Capri pox virus genus in the Poxviridae family (Sudhakar *et al* 2020) [22]. LSDV is a double-stranded DNA genome, which encodes 30 homologues of pox viral proteins known to be structural or non-structural, and it is antigenically and genetically closely related to sheep poxvirus (SPPV) and goat pox virus (GTPV) with nucleotide sequence identities of 96% between species (Tulman *et al* 2002) [23]. LSDV is transmitted by insects like biting flies (*Stomoxys calcitrans* and *Biomyia fasciata*), mosquitoes (genera *aedes* and *culex*) and ticks (Ixodid ticks: *Amblyomma* spp and *Rhipicephalus* spp) (Chihota *et al* 2003: Tuppurainen *et al* 2011: Lubinga 2014) [5, 24, 10]. Clinical signs are characterized by recurrent fever (40-41.5 °C), lethargy, anorexia, tachycardia (120/ minute), cardiac arrhythmia, enlargement of prescapular, precrural lymph nodes, serous ocular and nasal discharges. Characteristic skin nodules of the disease were observed and located particularly on the head and neck, these nodules included the dermis and epidermis of the skin and oedema of dewlap and limbs (Helal *et al* 2019) [8]. LSDV affects both sexes and all ages (young animals may be more susceptible to the severe form of the disease) (Shen *et al* 2011) [20] and it is an important disease causing severe economic losses due to emaciation, decreased or cessation of milk production, low weight gain, abortion, myiasis and permanent damage of hides which causes lowering of their commercial value (Abera *et al* 2015) [1] and the complications of LSD include corneal opacity (Keratitis), recumbency, mastitis, cellulitis and phlegmon, myiasis, abortion, dysentery, lameness, pneumonia (Salib and Osman, 2011) [18].

Materials and Methods
The study was conducted on 14 animals presented to department of veterinary clinical complex veterinary college Bidar- KVAFSU, age ranging from 7 to 8 years and 6 apparently healthy cattle were selected for determining the reference values, animals showing clinical signs like fever, lachrymation, skin nodules (2–5 cm in diameter), enlargement of subscapular and pre-crural lymph nodes, ulcerative lesions on the mucous membranes of eye or oral or nasal cavity and dependent oedema of limbs, brisket region were selected for detailed clinical examination and parameters like temperature, heart rate, respiratory rate and capillary refill time were recorded.

2 ml of blood was collected in EDTA vial and complete blood count was carried out using fully automated haematology analyser (ERMA PCE 210® by AGD biomedicals private limited, Chennai-India) and 4ml of blood was collected in clot activated vial and serum parameters like ALT, AST, ALP, TP, albumin, calcium, glucose and creatinine was estimated using commercially available kits (ERBA Manheim®) in semi-automated biochemical analyser (MICROLAB-300®, Eli Tech Group).

Electrocardiography was carried out using base apex lead system in lead I were the positive electrode of lead I (left arm) was attached to the skin of the fifth intercostal space just caudal to olecranon and the negative electrode (right arm) on the jugular furrow about 1/3rd of the left side of the neck (Rezakhani *et al* 2004) [16] and lead II were the positive electrode (Left Leg) was placed on the skin over the left fifth intercostal space at the level of the elbow; the negative electrode (right arm was placed on the skin over the right jugular furrow roughly 30 cm from the thoracic inlet and the ground electrode (left arm) was attached to the neck or withers (Peak and McGuirk, 2008) [13] using BPL™ (CARDIART 6108T® machine).

Echocardiography was performed as described by Braun *et al* (2001) [4] using portable ultrasound machine (CHISON®, China). In this study the probe used was 2.5-5 MHz convex abdominal probe. Animals affected with LSD were prepared by shaving 25 x 25 cm area on left and right side from third to seventh intercostal space. Ultrasound gel was applied to make good contact with the surface, 2-D image was obtained.

Cardiac troponin-I (cTn-I) was estimated by ELISA using commercially available kits (Bovine Cardiac Troponin I®, cTn-I Genlisa Elisa™, Krishgen Biosystems).

All the data obtained were statistically analysed as described by Snedecor and Cochran (1994) [21]. The data were analysed by student t-test using SPSS software 20.0 (SPSS Inc. Chicago, IL, USA). Difference at $p \leq 0.05$ was considered statistically significant.

Results

Clinical signs in affected cattle were presence fever, lachrymation, skin nodules (2–5 cm in diameter), enlargement of subscapular and pre-crural lymph nodes, ulcerative lesions on the mucous membranes of eye or oral or nasal cavity and dependent oedema of limbs, brisket region (Figure 1, 2, 3 and 4).

There was significant increase in rectal temperature and respiratory rate in cattle affected with LSD compared to healthy control. No change in heart rate and capillary refill time in affected cattle (Table 1).

Hematologically there was leukopenia, erythrocytopenia and decreased haemoglobin, non-significant changes in PCV and platelet count were recorded. DLC revealed no-significant changes (Table 2).

Biochemically significant increase in ALT, AST, ALP, TP and creatinine was recorded, significant reduction in concentrations of albumin and glucose were noticed in affected cattle compared to healthy cattle, there was non-significant changes in concentration of calcium in LSD affected cattle compared to healthy control (Table 3).

Electrocardiographic study revealed normal sinus rhythm in 8 (57.14%), ventricular premature complexes (VPC's) in 1 (7.14%), sinus tachycardia in 1 (7.14%), sinus bradycardia in 1 (7.14%) and low voltage QRS complexes in 3 (21.42%) in LSD affected cattle (Figure 6, 7, 8, 9 and 10).

Echocardiography in the affected animals did not reveal any structural changes (Figure 11).

There was significant increase in the concentration of cardiac troponin-I in cattle affected with LSD compared to healthy control (Table 3).

Table 1: Mean±SE values of vital parameters in healthy and LSD affected cattle

Parameter	Healthy cattle	LSD affected cattle
Temperature (^{0}F)	100.28±0.14^a	101.87±0.34^b
Heart rate (bpm)	75.33±2.81^a	68.57±3.84^a
Respiratory rate (breaths/min)	15.0±0.85^a	21.00±1.18^b
Capillary refill time (sec's)	2.33±0.42^a	3.64±0.37^a

Table 2: Mean±SE values of haematological parameters in healthy and LSD affected cattle

Parameters	Healthy control	LSD affected
TLC (10^3/µL)	9.06±0.84^a	6.48±0.51^b
RBC (million/µL)	7.31±0.87^a	6.10±0.20^b
Hb (g/dL)	9.03±0.18^a	7.02±0.16^b
PCV (%)	36.70±4.65^a	30.35±1.02^a
PLT (x10^3/µL)	136.50±30.45^a	162.14±19.16^a
Neutrophil's (%)	39.33±1.80^a	44.71±3.55^a
Lymphocyte's (%)	60.00±1.73^a	54.42±3.72^a
Monocyte's (%)	0.66±0.33^a	0.78±0.26^a
Eosinophil's (%)	0.00±0.00^a	0.07±0. 07^a

Table 3: Mean±SE values of biochemical parameters in healthy and LSD affected cattle

Parameters	Healthy control	LSD affected
ALT (U/L)	30.58±1.52^a	43.95±2.70^b
AST (U/L)	46.35±4.46^a	117.90±21.70^b
ALP (U/L)	47.83±12.70^a	264.42±52.38^b
TP (g/dL)	9.36±0.81^a	11.15±0.39^b
Albumin (g/dL)	3.85±0.40^a	2.69±0.21^b
Calcium (mg/dL)	10.10±0.67^a	8.68±0.34^a
Glucose (mg/dL)	64.90±1.61^a	53.63±2.33^b
Creatinine (mg/dL)	0.93±0.05^a	1.91±0.14^b
cTn-I (ng/dL)	0.29±0.08^a	1.13±0.12^b

Note: Mean±SE values bearing different superscript differ significantly at ($p \leq 0.05$)

Fig 1: Lumps on the body in a cow affected with LSD

Fig 2: Brisket oedema in a bullock affected with LSD

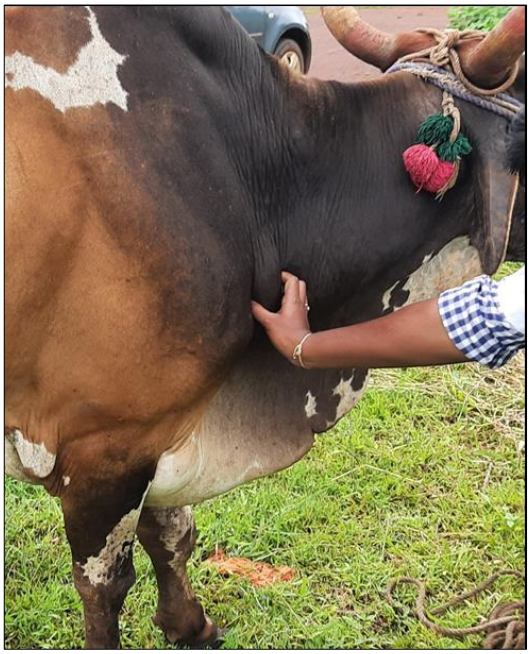

Fig 3: Lymph node enlargement in a bullock affected with LSD

Fig 4: Nasal secretions in a calf having lung involvement in LSD

Fig 5: ECG of healthy cow (Lead II)

Fig 6: ECG showing low amplitude QRS complexes in a cow affected with LSD

Fig 7: ECG showing ventricular premature complex in a bullock affected with LSD

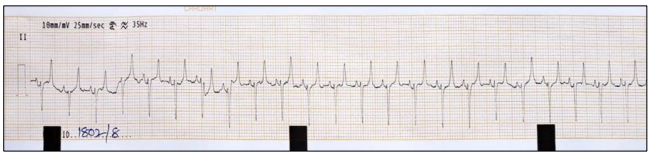

Fig 8: ECG showing sinus tachycardia in a bullock affected with LSD

Fig 9: ECG showing sinus bradycardia in a cow affected with LSD

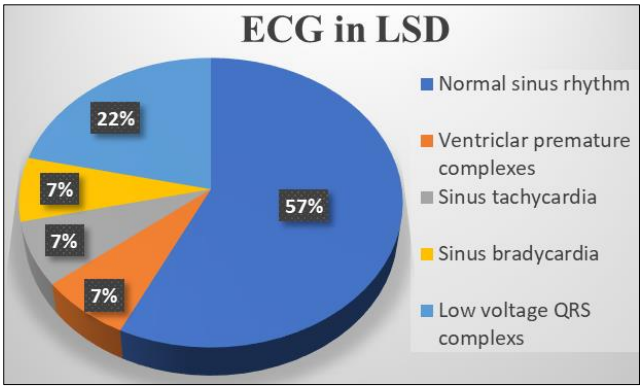

Fig 10: Electrocardiographic interpretation in LSD affected cattle

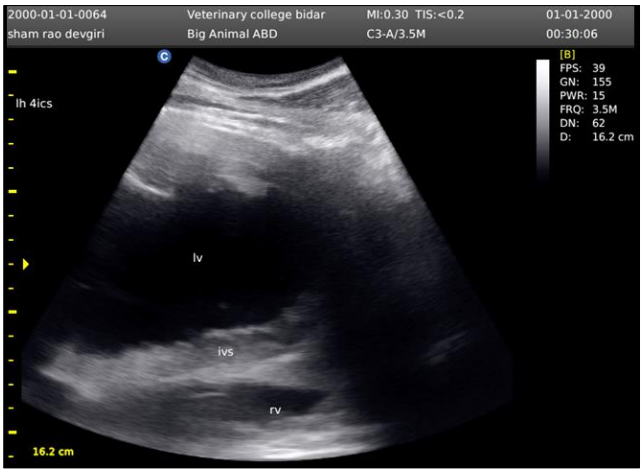

Fig 11: Echocardiogram of a bullock affected with LSD showing absence of structural changes in the heart (LV-Left ventricle, IVS-Interventricular septum and RV-Right ventricle)

Discussion

Lumpy skin disease is considered as transboundary animal disease due to its significant impacts on trade and food security as well as its capacity to spread to other countries (Rossiter and Al Hammadi 2009) [17]. The clinical signs in affected cattle were presence nodular skin lesions on the body and mucus membranes, fever, anorexia, lethargy, enlargement prescapular, prefemoral lymph nodes and their tracts, presence of oedema of limbs extending up to the brisket region similar clinical findings were recorded by (Sevik *et al* 2016: El-Mandrawy and Alam 2018) [19, 6]. There was significant leucopenia in affected cattle the results were in accordance with (Abutarbush *et al* 2015 and El-Mandrawy and Alam 2018) [2, 6], this could be due to leukopenia is usually seen in the developmental stage of the acute viral infection. There was significant reduction in erythrocyte count and haemoglobin concentration indicating anaemia the results were in agreement with Neamat-Allah and Mahmoud (2019) [12], the anaemia might be due to oxidative stress or reduction in the phosphorus concentration, which is the integral part phospholipid of erythrocyte membrane leading to haemolysis.

Significant increase in the levels of ALT was recorded in the present study, these results were in agreement with Neamat-Allah (2015) [11], Sevik *et al* (2016) [19], Helmy *et al* (2017) [9] and Helal *et al* (2019) [8]. Increase in the ALT concentrations might be due to impaired hepatic function as a consequence of hepatic damage. The levels of AST were higher in affected cattle similar findings were recorded by El-Mandrawy and Alam (2018) [6] and Helal *et al* (2019) [8], this could be due to severe inflammation resulting in muscular or cardiac muscle damage. Significant increase in levels of ALP was recorded the findings were in agreement with Helmy *et al* (2017) [9] and El-Mandrawy and Alam (2018) [6], this might be attributed to hepatic damage. Increase in the concentration of total protein was seen similar results were recorded by Sevik *et al* (2016) [19] and El-Mandrawy and Alam (2018) [6], this might be due to inflammatory changes in the body and activation of immune response of host following infection. hypoalbuminaemia was recorded the results were in accordance with Neamat-Allah (2015) [11] and Helmy *et al* (2017) [9]. Might be due to hepatic dysfunction leading to reduced albumin synthesis. Hypoglycaemia was seen in LSD affected animals this is in agreement with El-Mandrawy and Alam (2018) [6], hypoglycaemia could be attributed to anorexia and subsequent hepatic damage. Increase in the concentration of creatinine was recorded this is in accordance with Helal *et al* (2019) [8] this could be due to reduced renal blood flow, reduction in the glomerular filtration and increased catabolic rate of protein causing renal impairment. Nonsignificant changes in serum calcium levels were recorded similar findings were recorded by Neamat-Allah and Mahmoud (2019) [12].

Electrocardiographic study revealed low voltage QRS complexes, this might be due to peripheral oedema leading to hypovolaemia Radostits *et al* (2007) [15] and Vershney (2020) [25]. Ventricular premature complexes were seen this could be due to myocarditis as a result of focal areas of inflammation or fibrosis or hypertrophy consequent to viral myocarditis may slow down the action potential leading to formation of re-entry circuits and ventricular arrhythmia Baksi *et al* (2015) [3] and Priyanka *et al* (2019) [14]. Sinus tachycardia in the affected group might be due to anaemia subsequently leading to hypoxia and even can occur due to electrolyte imbalances Hasanpour *et al* (2008) [7] and Varshney (2020) [25] and sinus bradycardia could be due to hypoglycaemia (Varshney 2020) [25].

Echocardiography in the affected animals did not reveal any structural changes in the cardiac tissue.

Significant increase in the concentration of cTn-I in affected group similar results were recorded by Helal *et al* (2019) [8], this might be due to myocardial damage.

Conclusion

Based on above findings *i.e*., variation in the ECG (electrical disturbances) and increased concentration of cTn-I it can be concluded that LSDV is having direct effect on injury to the cardiac muscle.

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