



ISSN (E): 2277-7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2022; SP-11(10): 2176-2180

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Received: 08-08-2022

Accepted: 14-09-2022

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## Study on clinical, haemato-biochemical changes in lumpy skin disease affected cattle in Bidar

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### Abstract

Lumpy skin disease is an infectious, emerging viral disease of cattle caused by Lumpy skin disease virus (LSDV) belonging to genus capripoxvirus of poxviridae family. Lumpy skin disease was first seen in Zambia in 1929. In India, Lumpy skin disease had been first reported from Mayurbhanj and Bhadrak districts in Odisha in August 2019. Totally 226 affected cattle were taken into study, based upon the course of clinical signs, early stage and late stage was recorded in 49.55 percent and 50.44 percent respectively in Lumpy skin disease affected cattle. Haematology revealed leukopenia, lymphopenia in early stage of Lumpy skin disease affected cattle. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values in early stage were elevated whereas reduced in late stage indicating macrocytic anaemia in early stage and hypochromic microcytic anaemia in late stage. Serum biochemical analysis revealed significant increase in aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels and hypoalbuminemia in LSD affected cattle. So, this study helps to understand more about pathogenesis so that early management and treatment of affected cattle.

**Keywords:** Cattle, lumpy skin disease, clinical, haematology, biochemical, Bidar

### Introduction

Lumpy skin disease (LSD, Pseudo-urticaria, Neethling virus disease, exanthema nodularis bovis and knopvelsiekte) is an infectious transboundary disease of cattle and buffaloes of all age groups and breeds. The disease listed by OIE in "List A" due to its rapid spread and economic significance across the world characterized by distinctive nodular lesions causing temporary to permanent damage to the skin leading to detrimental effect on the commercial value of hides (Davies, 1991; Irons *et al.*, 2005) [12, 16].

The disease is primarily vector borne, biting insects are thought to be the primary route for transmitting the disease and ticks have also been implicated (Tuppurainen *et al.*, 2011; Lubinga *et al.*, 2014) [29, 21]. Although not commonly seen, the disease can be transmitted by direct contact (cutaneous lesions, saliva, respiratory secretions, milk and semen) and using of contaminated needles (Davies, 1991; Hunter and Wallace, 2001) [12, 15]. Rare transmission occurs through direct contact and contaminated feed and water (Ali *et al.*, 2012) [5].

The clinical disease is characterized by pyrexia, enlarged superficial lymph nodes, nasal discharge, watery eyes and characteristic generalized, firm, flat topped papules and nodules of 0.5–5.0 cm size all over the body including head, neck, udder, scrotum, perineum and buccal mucosa; oedema of limbs and brisket causing lameness, severe emaciation, conjunctivitis, keratitis, corneal opacity (Babiuk *et al.*, 2008; Tageldin *et al.*, 2014) [8, 27]. Skin lesions or nodules are known to be the most significant source of infection for healthy animals, as the virus can live for long periods in the lesions or scabs and has heavy tropism towards dermal tissues (Babiuk *et al.*, 2008) [8].

LSD causes high morbidity and low-to-moderate mortality, and the severity of the disease may vary from subclinical infection to death which is based on the virus strain, vector prevalence, age and immune status of the host (OIE, 2018; Tuppurainen *et al.*, 2017) [23, 31]. Hence this study performed is to describe the haematological and biochemical changes in naturally infected lumpy skin disease in cattle in Bidar

### Material and methods: Study of healthy cattle

Six apparently healthy cattle which were maintained in good managerial condition were

taken as healthy control group (group I) to study the normal haematological and biochemical parameters.

Suspected cases of Lumpy skin disease presented to Veterinary Clinical Complex, Veterinary College, Bidar with history of nodules over the body, reduced feed and water intake, salivation, brisket oedema and lameness were subjected to physical examination. In clinical evaluation rectal temperature, conjunctival mucus membrane, palpation of superficial lymph nodes and other symptoms suggestive of LSD i.e., swelling of limbs, brisket oedema, nasal discharge, lacrimation, lumps on various sites of body were recorded. Based on history and clinical signs suspected cases were divided into early (acute) that is group II and late (chronic) stage group III. Blood samples from each group were collected for complete blood count, serum biochemical analysis. Further samples were confirmed by PCR.

### Blood collection

After proper restraining of the animal, whole blood was aseptically collected from the jugular vein. Two ml of blood was collected in EDTA (ethylene diamine tetra acetic acid) vials (QUANTUM BIOMEDICALS INDIA, CB-PLUS) for complete blood count.

**Haematological examination:** Whole blood sample of about 2 ml collected in EDTA coated vials was used for estimation of following parameters:

- Total leucocyte count (TLC) ( $10^3/\mu\text{L}$ )
- Total erythrocyte count (TEC) ( $10^6/\mu\text{L}$ )
- Haemoglobin concentration (Hb) (g/dL)
- Packed cell volume (PCV) (%)
- Platelet count (PLT) ( $10^3/\mu\text{L}$ )
- Mean corpuscular volume (MCV) (fL)
- Mean corpuscular haemoglobin (MCH) (pg)
- Mean corpuscular haemoglobin concentration (MCHC) (g/dL)

Fully automatic haematology analyser (ERMA PCE 210® fully automatic blood cell counter, manufactured by ERMA Inc., Tokyo, Japan) was used for analysis of above blood parameters.

Differential leucocyte count (DLC) was done using Giemsa stain as per standard protocol (Coles, 1986) [10].

### Biochemical parameters

Four ml of venous blood was collected in clot activator tube taking all precautions for avoiding haemolysis. The blood was allowed for clotting then centrifugation @ 4500 rpm for 5 min, serum was separated and used for the estimation of serum AST (serum aspartate transaminase), ALP (serum alkaline phosphatase), creatinine, total protein and albumin using Microlab 300® (semi-automated clinical chemistry analyser, manufactured by ELI Tech Group Biomedical systems, France).

### Results and Discussion

A total of 1,199 cattle were presented to Veterinary Clinical Complex from July to December 2020, among them, 226 cattle were observed with clinical signs of Lumpy skin disease suggesting a point prevalence of LSD to be 18.85 percent. Out of 226 LSD suspected cattle, early stage was recorded in 49.56 percent (112/226) and late stage was noted in 50.44 percent (114/226).

The apparent clinical signs in LSD affected cattle were fever ( $>102^{\circ}\text{F}$ ), skin nodules (localised and generalised), lymph node enlargement, anorexia, edema, respiratory distress, corneal opacity, lameness, an and recumbency were observed in cattle with LSD (table 1). Table 1a indicating distribution of localised skin nodules. Fig. 1, 2, 3, 4 showing pictures of LSD affected cattle with skin nodules, brisket edema, calf with generalised skin nodules and corneal opacity in bullock. Haematological analysis of LSD affected cattle showed decline in values of TEC, Hb, PCV in early stage. MCV decline observed in late stage. MCH and MCHC were elevated in early stage whereas decline in the values observed in the late stage. Leukopenia and lymphopenia were observed in early stage of the disease. Eosinophil and monocyte count showed no alteration in the values. Platelet count showed slight increase in number in late stage of the disease. Serum biochemical analysis revealed increased levels of serum AST, ALP in early and late stage of the disease. Decrease in albumin level observed in both early and late stage affected cattle. Whereas TP, globulin, creatinine levels were in normal limits no such alteration was observed in LSD affected cattle. Table 2 & 3 indicating mean $\pm$ SE of haematological and serum biochemical values in the control group and Lumpy skin disease affected groups.



**Fig 1:** Picture showing localised skin lesions on the body of a cow



**Fig 2:** Picture showing brisket edema in LSD affected bullock



**Fig 3:** Calf showing generalised skin nodules with prefemoral lymph node enlargement



**Fig 4:** LSD affected bullock showed corneal opacity in late stage of disease

**Table 1:** Frequency of clinical signs in Lumpy skin disease affected cattle (n=226)

SI. No.	Clinical signs	Number of animals affected (n=226)	Percent
1	Skin nodules	226	100
	Localised 153 (67.70%)		
	Generalised 73 (32.30%)		
2	Lymph node enlargement	203	89.82
3	Fever (>102oF)	141	62.39
4	Edema	106	46.90
	Brisket: 26 (24.53%)		
	Brisket and limb: 25(23.58%)		
	Limb: 49 (46.22)		
	Umbilicus and ventral abdomen: 4(3.77%)		
Facial: 2(1.89%)			
5	Inappetence and anorexia	91	40.27
6	Lameness	32	14.16
7	Respiratory Distress	6	2.65
8	Corneal Opacity	4	1.77
9	Recumbency	2	0.88

**Table 1a:** Distribution of localised skin nodules in LSD affected cattle

Muzzle	Head and neck	Body (Thorax and abdomen)	Limbs	Total
12 (7.84%)	45 (29.41%)	78 (50.98%)	18 (11.76%)	153

**Table 2:** Mean±SE of haematological values in the control group and Lumpy skin disease affected groups

SI. No	Parameter	Healthy control	LSD affected cattle	
			Early stage	Late stage
1	WBC (x103/μL)	10.85±1.34a	5.46±1.02b	8.23±1.53ab
2	RBC (x106/μL)	7.07±0.39	5.49±0.27	7.62±1.32
3	Hb (g/dL)	9.41±0.41	7.87±0.49	8.31±0.71
4	PCV (%)	34.36±1.47	27.46±1.56	32.03±3.80
5	MCV (fL)	48.78±1.39	49.78±0.92	44.52±2.75
6	MCH (pg)	13.33±0.36ab	14.25±0.30b	11.88±0.94a
7	MCHC (g/dL)	27.36±0.25ab	28.57±0.36b	26.52±0.93a
8	PLT (x103/μL)	143.66±13.70a	167.87±21.00ab	250.00±42.07b
9	Lymphocyte count (%)	76.33±1.14a	46.80±7.90b	59.00±7.54ab
10	Neutrophil count (%)	22.00±1.06a	48.37±7.60b	39.37±7.50ab
11	Eosinophil count (%)	1.17±0.30	4.00±2.12	1.00±0.50
12	Monocyte count (%)	0.50±0.20	0.75±0.31	0.60±0.32

**Note:** Means bearing different superscripts in same row differ significantly ( $p < 0.05$ )

**Table 3:** Mean±SE of Serum biochemical values in the control group and Lumpy skin disease affected groups

SI. No	Parameter	Healthy control	LSD affected cattle	
			Early stage	Late stage
1	AST (IU/L)	53.50±3.27a	100.00±9.60b	128.50±16.24b
2	ALP (IU/L)	77.50±11.22a	200.36±25.54b	257.75±31.55b
3	TP (g/dL)	6.70±0.19	6.47±0.44	6.47±0.27
4	Albumin (g/dL)	2.85±0.13a	2.13±0.08b	2.10±0.30b
5	Globulin (g/dL)	3.85±0.12	4.35±0.40	4.37±0.33
6	Creatinine (mg/dL)	1.76±0.15	1.53±0.06	1.475±0.08

**Note:** Means bearing different superscripts in same row differ significantly ( $p < 0.05$ ).

The present clinical findings recorded in the study agreed with earlier reports of Tuppurainen and Oura (2012) [28, 29], Al-Salihi (2014) [6] and Keshta *et al.* (2020) [20]. Lumpy skin disease is characterised by nodules all over body with various size. Early stage cases showed one or two lumps on the body whereas, late stage cases showed generalised nodules covering whole body in random pattern. The results were in accordance with Weiss (1968) [33]; Agag *et al.* (1992) [2] and Al-Salihi (2014) [6]. The virus causes skin lesions due to its rapid replication in specific cells such as endothelial cells of lymphatic and blood vessel walls with development of inflammatory nodules on the skin (Vorster and Mapham, 2008) [32]. Appearance of the quintessential skin nodules which are very characteristic for the disease which is attributed to the virus tropism to skin tissues and internal organs (Tuppurainen *et al.*, 2005; Gari *et al.*, 2010; Constable *et al.*, 2017) [16, 21, 13, 11]. In the present study, 89.82 percent cases showed enlarged superficial lymph nodes. Similar findings were noticed by Prozesky and Barnard (1982) [24]; Ali *et al.* (1990) [4] and Tuppurainen *et al.* (2017) [31]. LSDV virus reaches the regional lymph node and causes lymphadenitis (Vorster and Mapham, 2008) [32]. In LSD affected cattle fever is first noticeable clinical sign. In the present study 62.39 percent cattle showed high temperature (>102oF). Similar findings were observed by Ayre-Smith (1960); Al-Salihi (2014) [6] and Jalali *et al.* (2017) [18]. LSDV has epitheliotropic property enters the host body through skin or gastrointestinal tract mucosa resulting in viremia accompanied by febrile reactions which persist for two weeks. Prolonged fever is probably associated with secondary bacterial infections (Agag *et al.*, 1992; Coetzer and Tustin, 2004) [2, 9]. The disease is generalised epitheliotropic disease causing vasculitis and lymphadenitis that results in edema and necrosis (Tuppurainen *et al.*, 2017) [31].

In the present study, significant decrease in TLC was observed in animals of LSD affected cattle. Leukopenia observed in the initial phases of acute infectious diseases in ruminants is also associated with increased tissue demand and neutrophil margination (Jalali *et al.*, 2017) [18]. Leukopenia is usually seen in the developmental stage of the acute infection, after which the production of neutrophils is intensified, leading to leucocytosis (Abutarbush, 2015) [1]. In the present study, no significant difference found in TEC, Hb and PCV were observed. MCV showed no statistical significant decrease in values observed in late stage. Whereas MCH and MCHC values were significantly elevated in early stage; reduced values observed in late stage. In the present study, significant increase in the platelet count was observed in late stage whereas no statistical significant difference in platelet count was observed in early stage. Differential leucocytic count in present study showed significant lymphopenia and



neutrophilia in early stage of the disease, whereas no significant change in eosinophil and monocyte count. LSDV-infected cattle produced leukopenia with eosinopenia, lymphopenia and monocytopenia, which may result from viral infections (Coles, 1986) [10]; a release of high quantities of corticosteroid hormones also induce lymphopenia (Ismail and Yousseff, 2006) [17].

In the present study, significant increase in AST in LSD affected cattle compared to healthy control. Whereas no statistical difference was observed in early and late stage of LSD affected cattle. The increase in AST also may be due to the breakdown of the heart muscle and or secondary bacterial infection (Agag *et al.*, 1989) [3]. Significantly higher concentrations of serum AST is a sensitive indicator of hepatocellular damage, even if the damage is of a subclinical nature (Kauppinen, 1984; Meyer and Harvey 1998; Stockham and Scott, 2013) [19, 22, 25]. There was also significant increase in ALP in LSD affected cattle compared to healthy control. Whereas no statistical difference observed in early and late stage of LSD affected cattle. The elevated ALP in LSDV infected cattle may be related to viremia induced hepatic injury (Sevik *et al.*, 2016) [26]. Significant decrease in the albumin level observed in LSD affected group compared to healthy control. Hypoalbuminemia resulted in LSD affected cattle may be due to decreased synthesis and higher catabolic rate of protein as well as damage of liver parenchyma (Hassan *et al.*, 2011) [14]. In the present study, no significant change in total protein, serum globulin and creatinine were observed.

### Conclusion

Present study concludes that the apparent clinical signs observed were skin nodules (100%), lymph node enlargement (89.82%), fever (62.39%) and edema (46.90%). Haematology analysis revealed leukopenia, lymphopenia, macrocytic anaemia in early stage whereas hypochromic microcytic anaemia and thrombocytosis were observed in late stage of the disease. Serum biochemical analysis revealed increase in AST and ALP levels and hypoalbuminemia in LSD affected cattle. Further this helps in diagnosis and treatment of diseased animals.

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