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Epidemiological, Clinico-haemato-biochemical and Molecular Characterization of Lumpy Skin Disease in Cattle

Garima Rathore[1], S.K. Sharma[2], Monika Joshi[3]

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ABSTRACT

Background: Lumpy skin disease (LSD) is responsible for huge economic losses due to reduced milk production, degradation of hide, abortion, temporary or permanent sterility of bulls and cows and death. It is highly contagious in nature. The clinical entity and morbidity pattern of disease vary considerably therefore present study was undertaken.

Methods: A total of 308 cattle were screened for LSD for a period of one year (October 2021 to September 2022) in Rajasthan state in India and the animals were subjected to thorough physical and clinical examination. Skin nodular scab samples were collected and processed through polymerase chain reaction (PCR) for molecular characterization. Blood samples were also collected for investigations on haemato-biochemical parameters.

Result: Overall prevalence of LSD in cattle was 5.84 per cent. Highest prevalence was observed in cattle above 3 years of age. Higher prevalence of LSD was observed in crossbred cattle and during winter season. Molecular characterization revealed a band size of 192bp in all LSDV positive samples. There was highly significant (P<0.01) difference in PCV, Hb, TEC, TLC, total protein, albumen, globulin, ALT, AST, ALKP, BUN and total bilirubin in LSD affected cattle as compared to healthy control whereas reduction in platelet count was significant (P<0.05).

Key words: Blood, Cattle, Lumpy skin disease, Molecular, PCR, Prevalence.

INTRODUCTION

Lumpy skin disease (LSD) is responsible for huge economic losses due to reduced milk production, degradation of hide, abortion, temporary or permanent sterility of bulls and cows and death. Indirect losses comprised of costs for laboratory diagnosis, treatment, vaccination, disinfection of premises and restrictions on foreign trades. LSD has recently spread beyond its territory into Europe and Asian countries (Tuppurainen *et al*., 2015 and Ince *et al*., 2016). In Asia, India, China and Bangladesh have reported the first LSD outbreaks during 2019 (Sudhakar *et al*., 2020). Huge mortality has been seen in cattle due to LSD in the year 2022 in India.

Lumpy skin disease is highly contagious in nature. LSD virus (LSDV) is highly host specific. LSD is transmitted predominantly by arthropod vectors like mosquito, biting flies and ticks mechanically (Lubinga *et al*., 2015). However, transmission through common grazing, drinking troughs and contaminated fomites is also observed.

The clinical entity, morbidity and mortality of the disease vary considerably, depending on the breed, immune status of the cattle population, virus strain and prevalence of the insect vectors (Tuppurainen *et al*., 2017) therefore it is very difficult to diagnose the disease in early stages. Hence present investigation was undertaken for epidemiological, clinical and molecular characterization of LSD in cattle.

MATERIALS AND METHODS

Study population

Total 308 cattle were screened for skin diseases from various organized and unorganized farms for a period of 12 months (October 2021 to September 2022) in Rajasthan state in India. Cattle of different breed, age and either sex were examined. Ten apparently healthy cattle (n=10) were selected to have base line data on basic clinical and haemato-biochemical parameters for the comparison and analysis.

Clinical examination

Each animal was subjected to thorough physical and clinical examination as per the methods described by Radostitis *et al.* (2007). Entire skin coat of all the animals was examined thoroughly in the day light for lesions. Visible mucous membranes, eyes *etc*. were also examined and relevant clinical data was recorded.

[1]Division of Veterinary Medicine, Indian Veterinary Research institute, Izatnagar, Bareilly-243 122, Uttar Pradesh, India.
[2]Department of Veterinary Medicine, College of Veterinary and Animal Science, Vallabhnagar, Udaipur 313601, Rajasthan, India.
[3]Department of Animal Nutrition, College of Veterinary and Animal Science, Vallabhnagar, Udaipur 313601, Rajasthan, India.

Corresponding Author: S.K. Sharma, Department of Veterinary Medicine, College of Veterinary and Animal Science, Vallabhnagar, Udaipur 313601, Rajasthan, India.
Email: drshivsharmavet@rediffmail.com

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Collection of skin nodular scab samples and DNA extraction

Skin nodular scab samples were collected aseptically and placed in clean sterile tube containing phosphate buffer saline for molecular detection of viral DNA. DNA extraction from skin scab homogenates was done using HiPurA viral DNA Purification Kit (HigenoMB Himedia) following the manufacturer's instructions. Concentration and purity of DNA were estimated in UV absorbance spectrophotometer (Eppendorf, Hamburg, Germany).

PCR Reaction for molecular characterization

Convention PCR was carried out to find out the presence of LSDV specific nucleic acid through amplification of 192 bp region in the P^{32} gene as described by Ireland and Binepal (1998).

Forward primer: 5′ -TTTCCTGATTTTTCTTACTAT-3′
Reverse primer: 5′ -AAATTATATACGTAAATAAC-3′

The PCR reaction was set up in a final volume of 25 µl containing: 12.5 µl of 2X Master mix (Genetix Biotech Asia Pvt. Ltd., New Delhi, India) 0.75 µl of each 50 pmol/µlprimer concentration 8.5 µl of nuclease free water (Genetix Biotech Asia Pvt. Ltd., New Delhi, India) 2.5 µl of extracted DNA (Template DNA).

The PCR reaction was carried out in a Bio-Rad T100, Thermo Cycler system (Bio-Rad Pvt. Ltd., California,U S.A.). The PCR conditions had an initial denaturation step of 94°C for 5 minutes, followed by 34 cycles of denaturation at 94°C for one minute, annealing at 50°C for 30seconds, extension at 72°C for 1minute and a final extension step of 72°C for 5 minutes.

The PCR product was subjected to electrophoresis in agarose gel containing ethidium bromide for assessing their integrity. 5 µl of PCR product was mixed with 2 µl of 6X gel loading dye (Thermo Scientific) and was loaded in the wells within agarose gel. To determine the size of the amplified PCR products, 5 µl of Gene Ruler 100 bp DNA ladder (Thermo Scientific) was loaded in first well. Gel electrophoresis was allowed to run for 1 hour at a constant voltage of 70V till the dye migrated 3/4th distance in the agarose gel. The amplified PCR products were visualized under gel documentation system (Biogen Scientific, Cambridge, U.S.A.).

Collection and analysis of blood samples

Blood samples were collected from LSD affected cattle as well as control animals to determine haemato-biochemical parameters. Haematological parameters *viz*. Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), platelet count and differential leukocytes count (DLC) were estimated as per the methods described by Feldman *et al*. (2000). Biochemical parameters *viz*. serum glucose, serum total protein (TP), serum albumin, serum globulin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALKP), creatinine, blood urea nitrogen (BUN) and total bilirubin were estimated by using automated serum biochemistry analyzer (IDEXX Vet test chemistry analyzer).

Statistical analysis

The data obtained in the research work were statistically analysed and compared using standard formula given for mean and standard error as per statistical methods described by Snedecor and Cochran (1996).

RESULTS AND DISCUSSION

Overall prevalence

Out of total 308 cattle screened under present study; 18 animals were found affected with LSD. The overall prevalence of LSD in cattle was 5.84 per cent. The cases were further confirmed by molecular detection of P32 gene of LSDV. Present finding is in agreement with that of Sudhakar *et al*. (2020) and Nayakwadi *et al*. (2021).

The variation in prevalence of lumpy skin disease might be attributed to difference in geographical location, climate and managemental practices (Ramesh, 2014 and Jubara *et al.,* 2019).

Age group-wise prevalence

Age group-wise prevalence of LSD in cattle was 3.27 per cent, 4.9 per cent and 7.8 per cent in cattle below 6 months of age, 6 months to 3 years of age group and cattle above 3 years of age, respectively. Age group-wise percent distribution of LSD was 11.11 per cent in cattle below 6 months of age, 27.78 per cent in age group 6 months to 3 years of age and 61.11 per cent in cattle above 3 years of age. Highest prevalence was observed in cattle above 3 years of age whereas lowest prevalence was observed in calves *i.e*., below 6 months of age. It was revealed that prevalence of LSD increased with advancement of age in cattle.

Similar findings were obtained by Elhaig *et al.* (2017) and Sahoo (2020). Higher prevalence of LSD seen in adult animals might be due to production stress. It may also be due to less exposure of young calves to disease transmitting vectors like mosquito, biting flies *etc*.

Breed-wise prevalence

Breed-wise prevalence of LSD in cattle was 4.3 percent and 8.2 per cent in indigenous and crossbred cattle, respectively. Breed-wise percent distribution of LSD was 44.44 per cent in indigenous breeds (Gir and non-descript) and 55.56 per cent in crossbred cattle. Higher prevalence was observed in crossbred cattle as compared to indigenous breeds *i.e*., Gir and non-descript. Similar findings were reported by Tageldin *et al.* (2014) and Sahoo (2020).

Sex-wise prevalence

Sex-wise prevalence of LSD in cattle was 6.66 and 4.42 per cent in female and male cattle, respectively. Sex-wise percent distribution of LSD was 72.22 and 27.78 per cent in female and male animals, respectively. The prevalence was higher in female cattle as compared to male. Findings of

present investigation are in agreement with that of Elhaig *et al.* (2017) and Sahoo (2020).

Higher prevalence rate in female animals might be due to production stress in females.

Season-wise prevalence

Season-wise prevalence of LSD in cattle was 8.73 and 6.48 per cent in winter and rainy season, respectively. Season-wise per cent distribution of LSD was 61.11 and 38.89 per cent in winter and rainy season, respectively. In present study, no any case of LSD was recorded during summer season. The prevalence was highest in winter season followed by rainy season.

The higher prevalence of LSD was recorded during winter season which might be attributed to close confinement of animals during winters leading to easier transmission of disease. Vector population also enhances the prevalence of disease in winter season (Hameed *et al.,* 2017).

Clinical signs

General signs

There was highly significant increase (P<0.01) in the mean values of body temperature and respiration in LSD affected animals as compared to control whereas significant increase (P<0.05) was observed in the mean values of heart rate in LSD affected cattle (Table 2). Similar findings were reported by Sahoo (2020) and Rouby *et al*. (2021).

Fever in LSD affected cattle might be due to viraemia which leads to release of endogenous pyrogens and febrile reaction (Vorster and Mapham, 2008). The increase in heart rate in LSD might be associated with febrile and anaemic condition whereas increased respiration rate could be due to secondary bacterial infection resulting in pneumonia. Respiration rate may also be increased because of fever and brisket oedema.

Other clinical signs

All the affected cattle (100 per cent) showed presence of nodules over the skin. Enlargement of superficial lymph nodes and elevation of rectal temperature each was recorded in 88.88 per cent animals. Oedema of limbs was observed in 72.22 per cent LSD affected cattle while brisket oedema was found in 38.89 per cent animals. Anorexia was observed in 66.67 per cent cases. Nasal and lacrimal secretions were seen in 61.11 per cent animals. Among all affected cattle, 27.78 per cent exhibited lameness. Pneumonia was recorded in 11.11 per cent cases. Out of total lactating cows affected with LSD, 91.67 per cent cows showed reduced milk production. Kerato-conjunctivitis and corneal opacity each was recorded separately in only 5.55 per cent cows. Abortion was recorded in 14.28 per cent pregnant cattle out of total pregnant cows screened under present investigation (Table 1).

Presence of nodules over the skin was most consistent clinical finding of LSD in cattle. It was followed by fever and enlarged lymph nodes, oedema of limbs and anorexia. Reduction in milk yield was also commonly observed in lactating cows. However, pneumonia, kerato-conjunctivitis and corneal opacity were recorded with lower occurrence. Occasionally, abortion was also observed. The nodules were distributed all over the body *viz*. on face, neck, abdomen, legs, udder, perineum and scrotum. In few severe cases, nodules were observed on muzzle, oral and nasal mucosa which later on appeared as ulcerative lesion. The diameter of these nodules varied largely ranging from 0.5 cm to 5.5 cm.

Findings of present investigation are in agreement with that of Sahoo (2020) and Gayal *et al*. (2022).

Haematological changes

There was highly significant (P<0.01) reduction in the mean value of PCV, Hb, TEC and TLC in LSD affected cattle as compared to control whereas reduction in mean value of platelet count was significant (P<0.05). Further, non-significant variation was observed in the mean values of DLC (Table 2).

Allam *et al*. (2021) and Keshta *et al*. (2020) also found reduced PCV and TEC in LSD affected cattle whereas marked decline in mean Hb and TLC has also been reported

Table 1: Per cent distribution of clinical signs of LSD in cattle.

Clinical finding	Number of affected cattle (n=18)	Percentage (%)
Anorexia	12	66.66
Pyrexia	16	88.88
Cutaneous nodules	18	100
Nasal and lacrimal secretions	11	61.11
Enlargement of lymph nodes	16	88.88
Limb oedema	13	72.22
Brisket oedema	7	38.88
Pneumonia	2	11.11
Lameness	5	27.78
Kerato-conjunctivitis	1	5.55
Corneal opacity	1	5.55
Reduction in milk yield (Lactating cattle n=12)	11	91.66
Abortion (Pregnant cattle n=7)	1	14.28

by Jalali *et al*. (2017). El-Mandrawy and Alam (2018) and Xavier *et al*. (2020) also showed reduction in TLC and platelet count in cattle affected with LSD.

Decreased PCV, Hb and TEC amy be the result of anorexia and destruction of RBCs. Increased production of reactive oxygen species has adverse effect on membrane and macromolecules of erythrocytes (Nashwa *et al*., 2017). Thrombocytopenia may be a result of lower production of platelets in bone marrow or sequestration of platelets. Leukopenia in acute viral infection could be attributed to increased tissue demand and neutrophilic margination.

Biochemical changes

There was highly significant increase (P<0.01) in the mean value of serum TP, serum globulin, ALT, AST, ALKP, BUN and total bilirubin in LSD affected cattle as compared to control group whereas mean value of serum albumin was significantly decreased (P<0.01). Non-significant variation was observed in the mean values of serum creatinine and serum glucose (Table 2).

Similar findings were reported by Jalali *et al*. (2017), El-Mandrawy and Alam (2018), Keshta *et al*. (2020) Rouby *et al*. (2021) and Allam *et al*. (2021).

Lower serum albumin in LSD affected cattle might be due to poor nutritional status as a consequence of anorexia whereas serum globulin level is associated with immune response of infected cattle. Increase in hepatic serum enzymes is attributed to virus induced degenerative changes. The increase in serum ALKP might be due to injuries in cardiac and skeletal muscles as a result of degenerative changes in heart, muscles, lymph nodes and skin (Jalali *et al*., 2017). The increased level of total bilirubin is attributed to hepatocyte and bile duct damage due to vasculitis and vascular thrombosis (Tageldin *et al*., 2014). Increased level of BUN could be attributed to the effect of LSDV on renal tissues resulted in renal damage or reduction of glomerular filtration rate and protein catabolism (Neamat-Allah, 2015).

Molecular characterization of lumpy skin disease

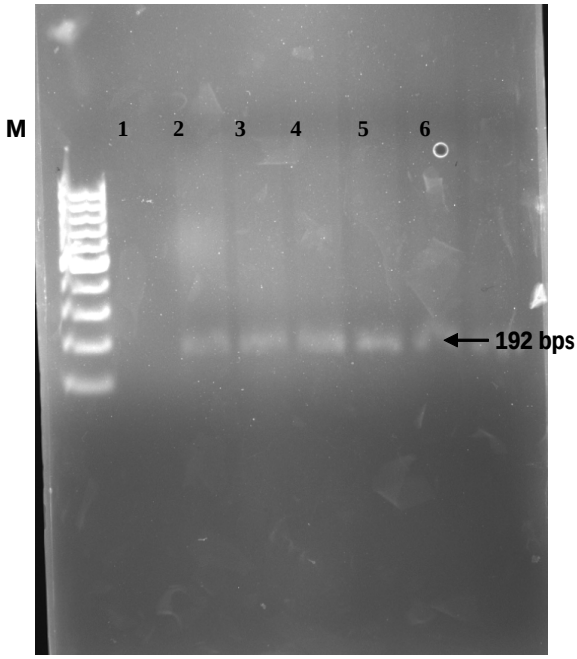
In present study, 18 skin scab samples were aseptically collected from the cattle showing characteristic clinical signs of lumpy skin disease infection. DNA was extracted from the collected samples. All the 18 collected samples were processed through conventional PCR, targeting the viral attachment protein encoding P32 gene of lumpy skin disease virus. The expected amplicon size (192 bp) was found in all examined samples (Plate 1). All LSDV positive samples had a band size of 192bp. All the skin scab samples (100 per cent) were positive for the PCR assay (Plate 1).

The findings of present investigation are in agreement with that of Awad *et al.* (2010), Ün *et al*. (2019) and Allam *et al.* (2021). They reported 100 per cent positive results in molecular detection of lumpy skin disease virus using PCR assay for skin biopsy samples from clinically infected cows.

Table 2: Mean±SE values of various clinical and haemato-biochemical parameters in LSD affected cattle and control animals.

Parameter	Group 1 (Control) (n=10)	Group 2 (LSD) (n=18)
Body temperature**	100.33±0.21	104.72±0.26
Heart rate*	54.44±1.94	66.56±2.47
Respiration rate**	18.11±0.97	32.89±2.55
Packed cell volume (PCV)**	35.96±1.21	29.36±1.25
Haemoglobin (Hb)**	11.12±0.40	9.79±0.26
Total erythrocyte count (TEC)**	7.45±0.20	6.23±0.15
Platelet count*	348±19.39	274.11±23.95
Total leucocyte count (TLC)**	9.73±0.48	7.52±0.26
Lymphocytes	59.40±1.70	54.89±1.22
Neutrophils	33.30±1.36	37.28±1.12
Eosinophils	4.10±0.52	3.33±0.22
Monocytes	3.20±0.32	3.89±0.29
Basophils	0.10±0.10	0.17±0.09
Serum total protein**	7.15± 0.23	8.37±0.24
Serum albumin**	3.01±0.09	2.78±0.07
Serum globulin**	4.14±0.28	5.57±0.21
Alanine transaminase (ALT)**	27.60±1.68	45.28±2.97
Aspartate transaminase (AST)**	67.90±2.73	121.89±11.89
Alkaline phosphatase (ALKP)**	41.50±2.01	65.33±4.45
Serum creatinine	0.95±0.08	1.13±0.07
Blood urea nitrogen (BUN)**	17.42±1.14	22.56±1.34
Total bilirubin**	0.30±0.05	0.52±0.04
Serum glucose	64±2.91	73.50±2.79

*Significant at 5% level (P<0.05); **Significant at 1% level (P<0.01).



Lane M: 100 bp DNA ladder, Lane 1-6 positive samples (amplicon size 192 bp).

Plate 1: Electrophorectic pattern of lumpy skin disease virus (LSDV) PCR assay.

CONCLUSION

LSD was first time reported in Rajasthan, India. There were variations in the prevalence of LSD with age, breed, sex and season. Molecular characterization revealed a band size of 192bp in all LSDV positive samples. Marked alterations were observed in haemato-biochemical parameters in LSD affected cattle. Estimation of clinico-haemato-biochemical parameters will pave way in better understanding the pathogenesis and deciding the suitable therapy.

Conflict of interest

There is no conflict of interest.

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