Lumpy skin disease (LSD): Pathomorphological features and molecular detection in dairy cattle of west coastal India

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Abstract

Lumpy skin disease (LSD) is an emerging pox viral disease affecting cattle population worldwide. In India, the first outbreak of LSD is reported during August 2019 in Odisha state, which then followed by outbreaks in crossbred and indigenous cattle population of other states. Present investigation designed to study the prevalence, pathomorphological changes and molecular detection of LSD virus in naturally infected cattle. The overall morbidity of LSD was 4.48% among 30 dairy farms. Skin nodular biopsy, whole blood and serum samples (n= 66) were collected for the diagnosis of LSD by histopathology, PCR and sequencing. The envelope protein gene (P32), Fusion protein (F) and DNA dependent RNA polymerase 30 kDa subunit (RPO30) genes were targeted for PCR testing. Out of 66, 46 cattle showed generalized skin nodules and papules of various sizes (0.5 - 6.5cm) on the skin particularly at neck, face, nose, tail, perineum and udder. Microscopic examination of the skin nodule biopsy tissue revealed presence of diffuse granulomatous inflammation, hyperkeratosis, focal to diffuse vasculitis and lymphangitis, vacuolar degeneration, spongiosis and acanthosis. The inflammatory cells typically comprised of macrophages, lymphocytes, neutrophils and eosinophils along with diffuse necrosis in dermis in chronic cases. The eosinophilic intracytoplasmic viral inclusions in keratinocytes and epithelial cells were detected in few cases. Gel-PCR assay detected P32 gene in 83%, F gene in 72% and RPO30 gene in 77% of skin biopsy samples. Three blood samples were also found positive for P32 gene by PCR. Whereas TaqMan probe Real Time PCR targeting EEV glycoprotein gene (LSDV126) detected LSDV in 94% of biopsy samples and three blood samples which indicated its higher sensitive for the diagnosis of LSDV. Phylogenetic analysis of RPO30 gene sequence showed that the isolates from this study were grouped in same cluster with LSDV isolates of Bangladesh, Kenya and other Indian isolates detected during 2019-20.

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Abstract:

Lumpy skin disease (LSD) is an emerging pox viral disease affecting cattle population worldwide. In India, the first outbreak of LSD is reported during August 2019 in Odisha state, which then followed by outbreaks in crossbred and indigenous cattle population of other states. Present investigation designed to study the prevalence, pathomorphological changes and molecular detection of LSD virus in naturally infected cattle. The overall morbidity of LSD was 4.48% among 30 dairy farms. Skin nodular biopsy, whole blood and serum samples (n= 66) were collected for the diagnosis of LSD by histopathology, PCR and sequencing. The envelope protein gene (P32), Fusion protein (F) and DNA dependent RNA polymerase 30 kDa subunit (RPO30) genes were targeted for PCR testing. Out of 66, 46 cattle showed generalized skin nodules and papules of various sizes (0.5 - 6.5cm) on the skin particularly at neck, face, nose, tail, perineum and udder. Microscopic examination of the skin nodule biopsy tissue revealed presence of diffuse granulomatous inflammation, hyperkeratosis, focal to diffuse vasculitis and lymphangitis, vacuolar degeneration, spongiosis and acanthosis. The inflammatory cells typically comprised of macrophages, lymphocytes, neutrophils and eosinophils along with diffuse necrosis in dermis in chronic cases. The eosinophilic intracytoplasmic viral inclusions in keratinocytes and epithelial cells were detected in few cases. Gel-PCR assay detected P32 gene in 83%, F gene in 72% and RPO30 gene in 77% of skin biopsy samples. Three blood samples were also found positive for P32 gene by PCR. Whereas TaqMan probe Real Time PCR targeting EEV glycoprotein gene (LSDV126) detected LSDV in 94% of biopsy samples and three blood samples which indicated its higher sensitive for the diagnosis of LSDV. Phylogenetic analysis of RPO30 gene sequence showed that the isolates from this study were grouped in same cluster with LSDV isolates of Bangladesh, Kenya and other Indian isolates detected during 2019-20.

Keywords: Lumpy Skin Disease Virus (LSDV), pathomorphology, transboundary, poxvirus, TaqMan probe Real-Time PCR, eosinophilic intracytoplasmic viral inclusions

Introduction:

Lumpy skin disease (LSD) is an emerging viral disease affecting bovine population world including India. It is spreading rapidly in various parts of world and causing severe economic losses in dairy farming due to reduced milk yield, abortion and hide damage. In recent years, it is believed to be spreading from African region and is an emerging threat to cattle populations in Europe and Asia (Sanz-Bernardo et al., 2020). The incubation period of LSD under field conditions is two to four weeks while, in the experimental infection, it is between 4 to 14 days (Prozesky and Barnard, 1982; Carn and Kitching, 1995). LSD is an arthropod-borne disease and is indirectly transmitted mechanically by biting insects (*Aedes aegypti*) and Culicoides, and hard Ixodid ticks (Chihota et al., 2001; Lubinga et al., 2013; Hussein, et al., 2017). Direct transmission also occurs via contact with infected animals. LSD infection in bovines is clinically characterized by high fever (104°F), lacrimation, nasal discharge, and hypersalivation, anorexia, leg edema, enlarged superficial lymph nodes, diffuse papules and raised subcutaneous nodules of 0.5-7 cm all over the body specifically on the neck, head, scrotum, perineum, udder and oral mucosa (Tageldin et al., 2014; OIE, 2018; Sudhakar et al., 2019; Sanz-Bernardo et al., 2020). Microscopic lesions of the LSD include granulomatous inflammation in the dermis and hypodermis with necrotizing and fibrinoid vasculitis, lymphangitis and infarction. In acute to subacute LSD infection presence of intracytoplasmic eosinophilic inclusions in skin histiocytes, keratinocytes, sebaceous gland cells and dermal epithelial cells can be seen (Prozesky, & Barnard, 1982; Al-Salihi and Hassan, 2015; Abdallah, et al., 2018; Sanz-Bernardo et al., 2020).

The LSD virus (LSDV) is highly host-specific, belongs to genus Capripox of the family *Capripoxviridae* and is 96% antigenically related to goat pox (GTPV) and sheep pox (SPPV) viruses (Tulman et al., 2002; OIE,

2019). The genome of LSDV is a double-stranded DNA with 151 kbp size containing 156 putative genes. The genes regulating host range, virulence and immune evasions are situated at the terminal parts of the genome (Tulman et al., 2001). The serological assays are not useful to distinguish different strains of Capri pox viruses (CaPVs), therefore, molecular studies are useful to distinguish LSDV, SPPV and GTPV (Tulman et al., 2002). Sequencing the host-specific G-protein-coupled chemokine receptor (GPCR), or RNA polymerase (RPO30) genes, species-specific molecular assays have been developed and validated for differentiation of CaPVs and also for phylogenetic grouping of field strains of LSDV (Lamien et al., 2011; Kumar et al., 2021).

Natural infection of LSDV in bovines is diagnosed based on typical clinical symptoms of lumpy skin nodules followed by laboratory detection of virus by PCR, virus isolation, electron microscopy and serological assays. In Asian continent, China and Bangladesh have reported the first LSD outbreaks during 2019 (OIE, 2019) due to uncontrolled movement of cattle and complete lack of vaccination program against LSD. In India, the first outbreak of LSD was reported in Odisha state on the East coast of India during August 2019 affecting 182 of 2,539 cattle with morbidity rate of 7.1% and no mortality (Sudhakar et al., 2020). Presently, in India, there are limited epidemiological and diagnostic studies of LSD. LSD in India is affecting mainly dairy cattle (both crossbreed and indigenous) thereby causing significant economic losses to small and medium farmers. There is no vaccination and any serological assays available in India. Many states in India have reported LSDV occurrence which included Maharashtra, Gujarat, MP, Goa, Karnataka, Kerala, Andhra Pradesh, Odisha, West Bengal and North-Eastern states. Based on phylogenetic analysis, the strains circulating in India were reported to be genetically close to South African NI2490/KSGP-like strains (Sudhakar et al. 2020) and Kenyan LSDV strains (Kumar et al., 2021). Various workers demonstrated gel-based PCR and real-time PCR assays for the detection of LSDV in bovines and differentiation between wild-type LSDV and vaccine virus strains (Lamien, et al., 2011; Vidanovic et al., 2016; Pestova et al., 2018). Sudhakar et al (2020) used Taqman probe-based real-time PCR for rapid diagnosis of LSD outbreak in bovines by targeting the EEV glycoprotein gene (LSDV126) which is specific for field strains of LSDV developed by earlier workers (Pestova et al., 2018). More studies are required to understand the pathology, pathogenesis and to develop suitable in-house diagnostics and vaccine against this disease. In this paper, we have evaluated morbidity, significant pathological changes and the detection of viral genome in bio-samples by employing gel-PCR, teal-time probe PCR assay and sequencing.

Materials and methods

2.1 Ethical clearance: All samples were collected as per standard procedures given by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi, India without causing any stress to animals. Skin biopsy specimens were collected under local anesthesia (2% Lignocaine at the site of skin nodule) and blood samples were drawn from jugular vein of the animal. The due consent was obtained from farm owner before collection of the specimens. The laboratory work was carried out at ICAR-Central Coastal Agricultural Research Institute, Goa, India.

2.2 Sample collection and processing:

Total 30 affected dairy farms (backyard -13 and organized -17) rearing both crossbred and indigenous cows were investigated. The size of backyard farms was 2-10 animals with limited floor space and close contact between animals. The organized dairy farms consisted of 10 to 600 animals. Sampling was done from 66 cows showing varying degree of clinical signs of LSDV and 15 healthy/asymptomatic animals which were in close contact with affected animals. Skin biopsy specimens were collected under local anesthesia (2% lignocaine administration at the nodule site). Blood in EDTA and without anticoagulant was collected from all the animals. The samples were transported to laboratory on ice.

2.3 Histopathology:

Skin biopsy specimens of size 5-10mm were cut and fixed in 10% buffered formalin for 48 hours. The tissues were sequentially dehydrated in ethanol (65-100%) and cleared in three changes in xylene. The paraffin blocks were prepared and 5μm sections were prepared for staining with hematoxylin and eosin staining method. The histological lesions were documented under 10x and 40x objective in compound microscope

(DM3000, Leica, Germany)

2.4 Extraction of DNA from Skin biopsies:

DNA extraction from skin biopsies and blood samples was done by using Wizard® Genomic DNA purification kit (Promega #A1120, USA) by following manufacturer's instructions. DNA samples were stored at -20°C for further testing.

2.5 PCR amplification of LSDV P32 and F gene:

For amplification of LSDV genes, OIE recommended primers were employed (Table -1) (OIE, 2018; Sudhakar et al., 2020). For PCR, 12.5µl of 2X GoTaq Green master mix (GoTaq® DNA polymerase in 2X Green GoTaq® reaction buffer of pH 8.5, 400µM of each dNTPs and 3mM $MgCl_2$) (Promega, USA), 1µl of each forward and reverse primer (10pmol), 150ng of DNA with final volume of 25µl was prepared using nuclease-free water. The reaction was carried out in gradient thermocycler (Eppendorf, Germany) with the conditions mentioned in Table-1. The PCR products were electrophoresed in 1.5% agarose gel and visualized under UV transilluminator. DNA from goat pox vaccine used as positive control in PCR reaction.

2.6 PCR and Sequencing of RPO30 gene for phylogenetic analysis:

PCR amplification of RPO30 gene was carried out by using the specific primers (**Table 1**) and sequenced using Sanger's sequencing method. The sequence was submitted to NCBI GenBank and obtained accession number MW590715. The reference sequences of LSDV from China, Bangladesh, Kenya, Indian states of Odisha and Haryana, GTPV and SPPV virus strains retrieved from GenBank and the phylogenetic analysis of RPO30 gene generated in present investigation. The sequences were aligned and compared using BioEdit and the phylogenetic analysis was carried out using MEGA-X.

TaqMan^{TM} Probe Real-Time PCR Assay:

For this assay, 25µl reaction mixture containing 12.5µl of 2X TaqMan^{TM} Fast Universal PCR Master Mix, 1µl of each forward (f2) and reverse (r33) primers, 1µl of TaqMan^{TM} probe was prepared. 150ng of template DNA was added to the reaction mixture and nuclease-free water was used to make up the total volume of 25 µl. The polymerase chain reaction was carried out in Real-time PCR system (Applied Biosystems). The conditions for real-time PCR were as follows; 50°C for 2 min for Uracyl -D- glycosylase (UDG) activation, 1 cycle at 95°C, for 10 min for DNA polymerase activation, 40 cycles of 95°C for 15s of denaturation and 60°C for 1 min of annealing and extension. One positive control DNA from goat pox vaccine and one negative control (NTC) were also included in the reaction panel.

Results

3.1 Clinical infection and morbidity:

The major clinical findings observed in affected animals includes fever (upto 105°F), inappetence and drastic reduction in milk yield with lacrimation, nasal discharge, superficial lymph node enlargement, localized/generalized subcutaneous nodules, leg edema and pustular lesions in feet with lameness. The overall morbidity of LSDV infection in both backyard and organized dairy farms (n=30) was 4.48% (66/1471) with no mortality (Table 2). The cattle in backyard farms showed higher morbidity (91.6%) and the risk factors were attributed to limited floor space and close contact with infected animals. In organized farms, though the number of susceptible animals was more, however, early detection of clinical signs and timely separation of affected animals by farmers resulted in lower morbidity (3.17%).

3.2 Pathological observations:

Out of 66 clinical cases, 46 cattle (69.7%) showed generalized skin nodules and papules of various sizes ranged from 0.5 cm to 6.5 cm located all over the body particularly, neck, head, face, nose, tail, perineum and udder (**Fig. 1A).** Twenty showed few small patchy nodules at the neck region, lumbar and face region. The number of nodules varied from few to more than a hundred based on the severity of the disease. These subcutaneous nodules were round, circumscribed, firm and raised above the skin and diffusely present on

the body (**Fig. 1B)** . In severe cases, these nodules were ruptured, and whitish-grey serous fluid was oozing out of lesions. In few cases, nodules at the vulva, perineum and hindquarters were ruptured leading to ulcers and suppurative wounds (**Fig. 1C)** . The prescapular lymph nodes were enlarged in nineteen cases (19/66, 38%). Edema of the ventral body parts of the body (brisket edema) and legs was consistent in some cases. Nasal and ocular discharge, conjunctivitis and salivation were noticed in all the animals. Lameness was observed due to severe edema and pustular lesions on the feet of affected animals (**Fig. 1D)** .

Microscopic lesions observed in the skin nodules includes hyperkeratosis (46, 69.7%), diffuse granulomatous reaction in dermis and epidermis (39, 59%) (**Fig. 1E)** , focal to diffuse vasculitis and lymphangitis in dermis and epidermis (39, 59%) (**Fig. 1F)** , spongiosis (34, 51.5%), vacuolar degeneration of epithelial cells (15, 22.7%) and acanthosis (12, 18%) (**Fig. 1G)** . The inflammatory cell types were mainly comprised of macrophages, neutrophils, lymphocytes and eosinophils along with diffuse coagulative necrosis and infarction in blood vessels in the dermis (15.1%) (**Fig. 1H)** . The characteristic eosinophilic intracytoplasmic viral inclusions in keratinocytes and epithelial cells were observed in 2 (3.03%) cases which were a pathognomonic feature of LSDV infection **(Table 3)** .

3.3 Detection of LSDV nucleic acid:

PCR assay targeting capripoxvirus-specific P32 gene, detected viral genome in 83% and LSDV- specific F gene in 72% skin biopsy specimens**(Fig. 2A)** . Whole blood, nasal swabs and flies were found negative for both the genes. Blood samples collected from close contact animals were negative for both the genes.

3.4 Amplification and phylogenetic analysis of RPO30 gene:

PCR amplification of RPO30 gene detected genome in 77% skin biopsy samples **(Table 4)** . Nucleotide sequencing of RPO30 gene revealed that LSDV isolate LSDV/CCARI-Goa1/India/2021 (Acce.No.MW590715) showed highest similarity (˜100%) with Neethling (NI-2490) strain (Acce.No.AF325528) and Kenyan strain (Acce.No.MN072619), however it was distinctly different from GTPV and SPPV isolates of India. The phylogenetic tree generated five clusters. Cluster I had the LSDV isolates from India, Kenya and Bangladesh. Cluster II contained LSDV from China, cluster III with GTPV isolates and cluster IV with SPV isolates **(Fig. 2B)** . The recovered isolate in this study was similar to LSDV isolates reported from field outbreaks in Odisha and other parts of India during 2019**.**

3.5 Detection of LSDV by TaqMan^{TM} probe:

The TaqMan probe real time PCR assay detected LSDV in 62 skin biopsy samples (94%), 2/66 blood samples and 3/5 ocular swab samples, which proved that this assay could be very sensitive and rapid in the detection of LSDV from field outbreaks **(Table 4) (Fig. 2C)** . Average threshold cycle (Ct) values were 15.2±11.2 in tissue biopsy samples, 29.4±11.6 in blood and 25.6±10.9 in ocular swabs.

Discussion

This study mainly described the overall morbidity, pathomorphological features, molecular detection and phylogenetic analysis of LSD in cattle of organized and backyard dairy cattle in the west coastal region of India. The overall morbidity of LSDV infection in cattle was 4.48% with no mortality. It was observed that the backyard farms were more affected due to lack of adequate floor space to keep the affected animals isolated, lack of timely diagnosis and lack of awareness about the disease, presence of stray cattle around and grazing practice. Our findings were similar to the outbreak report from Europe, where higher morbidity has been reported in cattle in small farms with fewer animals than in medium and large farms (EFSA, 2017; Sevik & Dogan, 2017).

The pathology investigation in present study revealed that the animals exhibited characteristic lesions of LSD such as fever, cutaneous nodules, lymphadenopathy, pustules on various body parts, lameness, etc., which were described earlier also (Prozesky, & Barnard1982; Abdallah, et al., 2018; Sanz-Bernardo et al., 2020; Sudhakar et al., 2020; Kumar et al., 2021). This study observed morbidity rate of 4.48%, which is in the range of the LSD morbidity rate reported earlier (Sudhakar et al., 2020; Kumar et al., 2021). Higher

morbidity rate of 40%–75% is also reported by some workers (Babiuk et al., 2008; Tuppurainen&Oura, 2012). The characteristic microscopic lesions of LSD were vasculitis, perivascular infiltration of inflammatory cells around the dermal blood vessels, spongiosis and necrosis (Tageldin et al., 2014; Abdallah et al., 2018; Vaskovic, et al., 2019; Prozesky and Barnard,1982). The characteristic lesions of pox viral infection*viz.,* vacuolar degeneration of keratinocytes, spongiosis, and intracytoplasmic eosinophilic inclusion bodies were also observed in our study (Body, et al., 2012; Tageldin, et al., 2014; Sanz-Bernardo et al., 2020).

Three major genes namely, capri pox specific -P32 and LSD specific fusion protein gene (F) and RPO30 were targeted for PCR amplification to detect the LSDV nucleic acid. The P32 gene encodes envelope protein which is homologous to vaccinia virus H3L gene located on the membrane surface of a mature virion (Tulman et al*.,* 2001). Several studies demonstrated that the P32 gene is highly conserved among capripox viruses and therefore it has been used as a diagnostic tool for the detection of LSDV (Tuppurainen et al. 2005; El-Kholy et al. 2008; Shooshtari et al., 2009), SPPV and GTPV (Shooshtari et al., 2009). Hosamani et al. (2006) carried out the differentiation of SPPV, GTPV and LSDV by sequence analysis of the P32 gene. Sudhakar et al (2020) reported PCR based diagnosis of LSD targeting P32, F and RPO30 genes of LSDV in DNA isolated from blood and skin biopsies of cattle.

LSD is an emerging transboundary viral disease for India as neighboring countries such as China and Bangladesh have also evidenced its impact during the same time and there is always a risk of spread of diseases across the borders due to uncontrolled movement of animals, animal products and presence of porous boundaries (EFSA 2020; Sudhakar et al., 2020). After the report of the first outbreak of LSD in Odisha state in the west coast region of India in August 2019, many states situated in the southern region of India witnessed large outbreaks. Although LSDV, SPPV and GTPV have high antigenic similarity and genetic identity, genome sequence analyses have shown that, they are phylogenetically distinct (Le Goff et al., 2009; Lamien, et al., 2011). In this study, LSDV isolate LSDV/CCARI-Goa1/India/2021 (Acce.No.MW590715) was distinctly different from SPPV and GTPV isolates from India and showed highest similarity with Kenyan, Bangladesh and other Indian LSD isolates. Phylogenetic analysis of RNA polymerase subunit (RPO30) gene showed clustering of LSDV, GPTV & SPPV into three different clusters and our isolate exhibited close relation with LSDV isolates of India, Kenya, China and Bangladesh. In comparison with other Indian isolates, our isolate showed similarity with the isolates of Odisha and Haryana states. RPO30 gene was also been used to differentiate lumpy skin disease virus and sheep poxvirus field and vaccinal strains (Rouby, 2018).

In addition to the diagnostic application of real time PCR in LSDV infection, it has been highly recommended for the differentiation of wild-type LSDV and vaccine virus strains (Vidanovic et al., 2016; Pestova et al., 2018). Real time PCR is significantly recognized as a sensitive technique than traditional gel-based PCR in contrast with detection and quantification of the LSD viral gene (Zeynalova et al., 2016; Pestova et al., 2018). In this study, gel-based PCR could not detect LSDV genome in blood samples, however, few blood samples were positive in TaqMan^{TM} Probe-based real time PCR assay. As per earlier studies, samples collected during active viraemic phase of infection were positive for viral genome either by gel PCR or Real time PCR (Babiuk et al., 2008; Sudhakar et al., 2020).

Conclusion

This study was primarily focused on the prevalence of LSDV infection in small and organized farms, its pathological features and molecular detection using gel-based and real-time PCR assays. LSDV causes significant economic losses to dairy cattle and there is an urgent need to develop diagnostic assays and homologous vaccine for the control of the disease in the Indian subcontinent.

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Conflict of Interests: None to declare.

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Tables & Legends:

Table 1: Primers and probes used in the study

No	Gene	primers details (5' to 3')	Size (bp)	Cyclic conditions	Cyclic conditions	Cyclic conditions	Reference
				Denaturation	**Annealing**	**Extension**	

No	Gene	primers details (5' to 3')	Size (bp)	Cyclic conditions	Cyclic conditions	Cyclic conditions	Reference
1	RPO30-F	CTC TGT TCC AAA CTA AAT CAT	1385	95°C for 1 min	49°C for 1 min	72°C for 1 min	**OIE, 2018; Sudhakar et al., 2020**
2	RPO30-R	TTT TTG TAT TAC CAA TTT CTG					
3	P32-F	TCC GAG CTC TTT CCT GAT TTT TCT TAC TAT	192	95°C for 1 min	49°C for 1 min	72°C for 1 min	**OIE, 2018; Ireland &Binepal, 1998**
4	P32-R	TAT GGT ACC TAA ATT ATA TAC GTA AAT AAC					
5	F gene (LSDV117)-F	ACT AGT GGA TCC ATG GAC AGA GCT TTA TCA	470	95°C for 1 min	49°C for 1 min	72°C for 1 min	
6	F gene (LSDV117) R	GCT GCA GGA ATT CTC ATA GTG TTG TAC TTC G					
	TaqMan Probe and primers	**TaqMan Probe and primers**	**UDG Incubation**	**Polymerase activation**	**Denaturation**	**Annealing/Extension**	**OIE, 2018; Pestova et al., 2018**
7	LSD f2	TAG GAA AAT GGA TGT ACC ACA AAT ACA G	50°C 2 min	95°C for 10 min	95°C for 15 sec	60°C for 1 min	

No	Gene	primers details (5' to 3')	Size (bp)	Cyclic conditions	Cyclic conditions	Cyclic conditions	Reference
8	LSD r33	TTG TTA CAA CTC AAA TCG TTA GGT G					
9	TaqMan probe	FAM - ACC ACC TAA TGA TAG TGT TTA TGA TTT AC-31 BHQ1					

Table 2: Morbidity of natural LSDV in backyard and organized dairy farms

Farms	LSD affected farms	Susceptible animals	Affected	Age group	Morbidity (%)
Backyard farms	13	24	22	3-6 years	91.6
Organized farms	17	1447	46	4- 13 years	3.17
Overall	30	1471	66		4.48

Backyard farms <10 animals (2 to 10); Organized farms -10 to 600 animals; ND- Non-descript; HF; Holstein Friesian

Table 3: Clinical, gross and histological changes in clinical cases of LSDV

No	Clinical and Gross lesions	Clinical signs (%) (n=66)	Clinical si
1	Lacrimation and nasal discharge	34 (51.5)	34 (51.5)
2	Fever, inappetence and reduced milk yield	41 (62.1)	41 (62.1)
3	Superficial lymph node enlargement	19 (28.7)	19 (28.7)
4	Leg edema and pustular lesions in feet with lameness	10 ((15.1)	10 ((15.1)
5	Localized skin/subcutaneous nodules	20 (30.3)	20 (30.3)
6	Diffuse/generalized skin/subcutaneous nodules and papules all over the body	46 (69.7)	46 (69.7)
7			
8			

Table 4: Gel-based PCR and TaqMan™ probe Real-Time PCR assay for the detection of LSDV in cattle

Bio-samples	LSDV PCR	LSDV PCR	LSDV PCR	TaqMan™ probe Real-Time PCR	Average Ct value
	P32 gene	**F gene**	**RPO30**		
Skin biopsy (n=66)	55 (83%)	48 (72%)	51 (77%)	62 (94%)	15.2±1.2
Blood (n=66)	-	-	-	02 (3%)	29.4±1.6
Flies (n=10)	-	-	-	-	-
Nasal swabs (n=20)	-	-	-	-	-

Bio-samples	LSDV PCR	LSDV PCR	LSDV PCR	TaqMan^{TM} probe Real-Time PCR	Average Ct value
Ocular swabs (n=5)	-	-	-	03 (60%)	25.6±0.9

Figures:

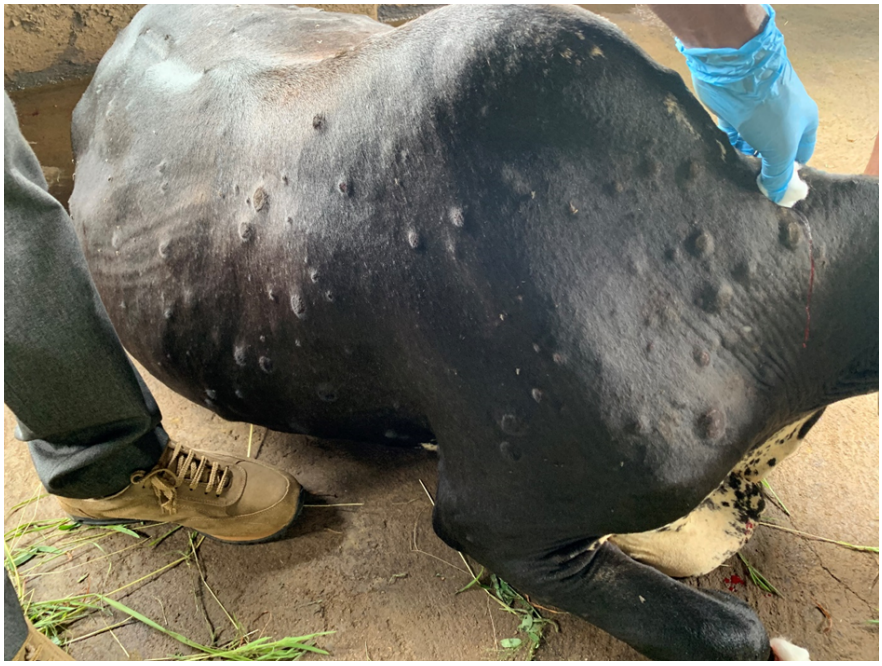

Figure 1: (A) Generalized skin nodules and papules of various sizes (1-6cm) present all over the body

Figure 1: (B) Diffuse skin nodules on the face, eyes, near muzzle, neck and ears

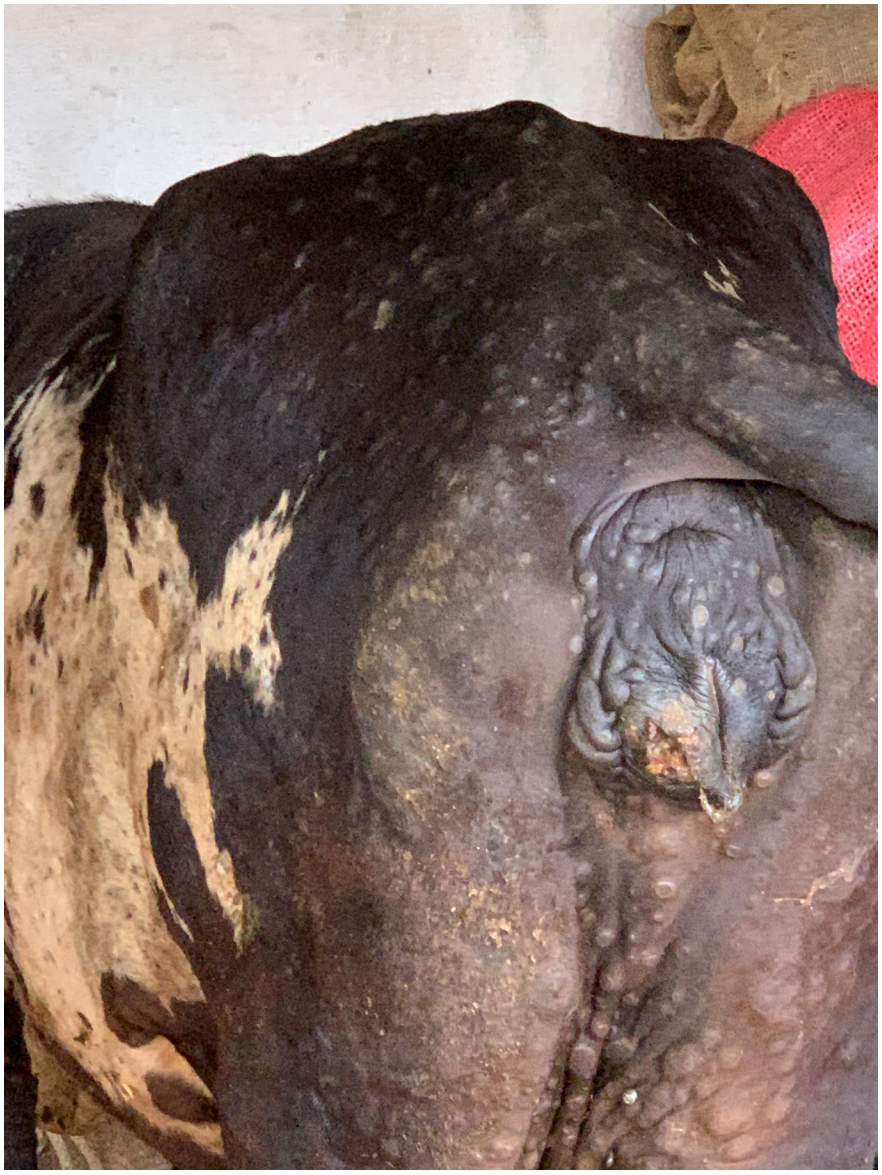

Figure 1: (C) Diffuse necrotic nodular lesions at perineum with a suppurative wounds at vulva

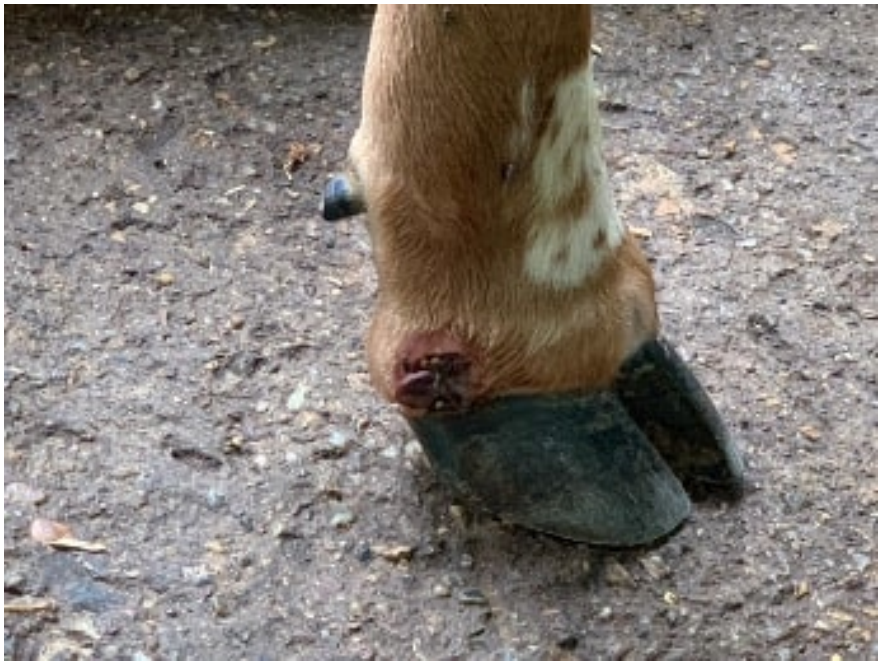

Figure 1: (D) Oedema and pustule formation at later stages in foot lead to lameness

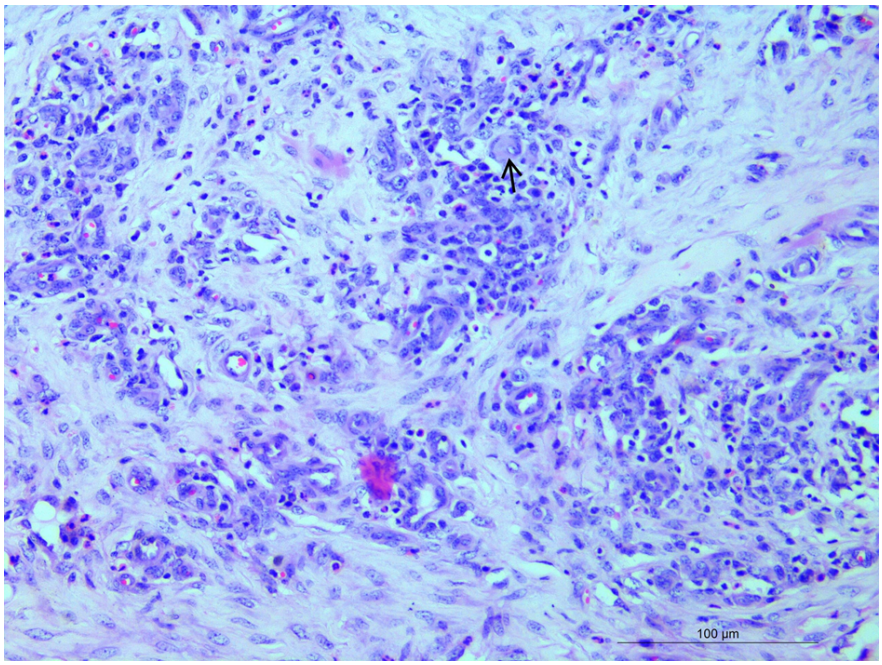

Figure 1: (E) Diffuse granulomatous reaction in the dermis with necrotic inflammatory cells and giant cells (Arrow) H&E, 10X

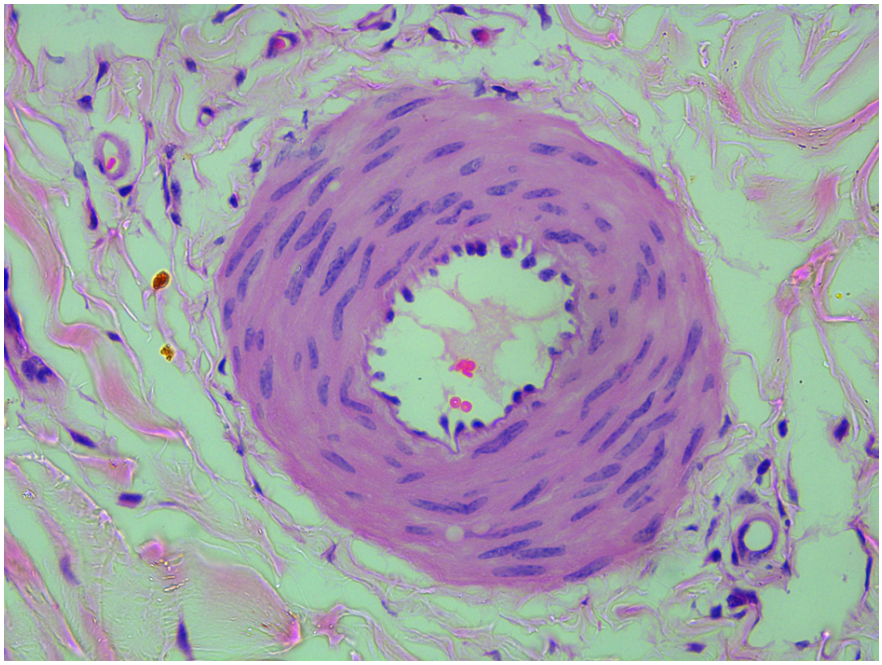

Figure 1: (F) Vasculitis with endothelial cell thickening, H&E, 40X

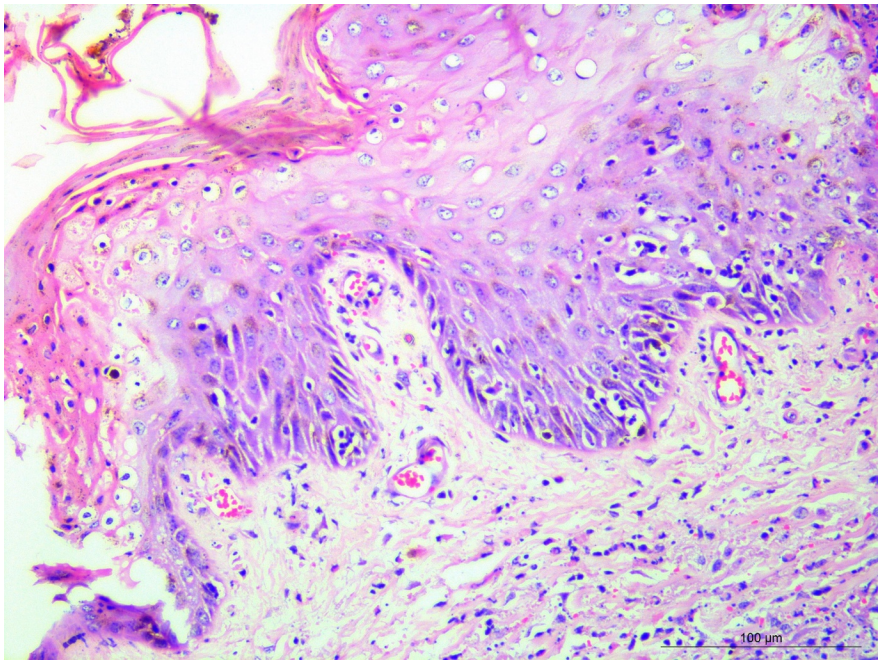

Figure 1: (G) Proliferation of epidermal cells with hydrophic/vacuolar degeneration of cells H&E, 20X;

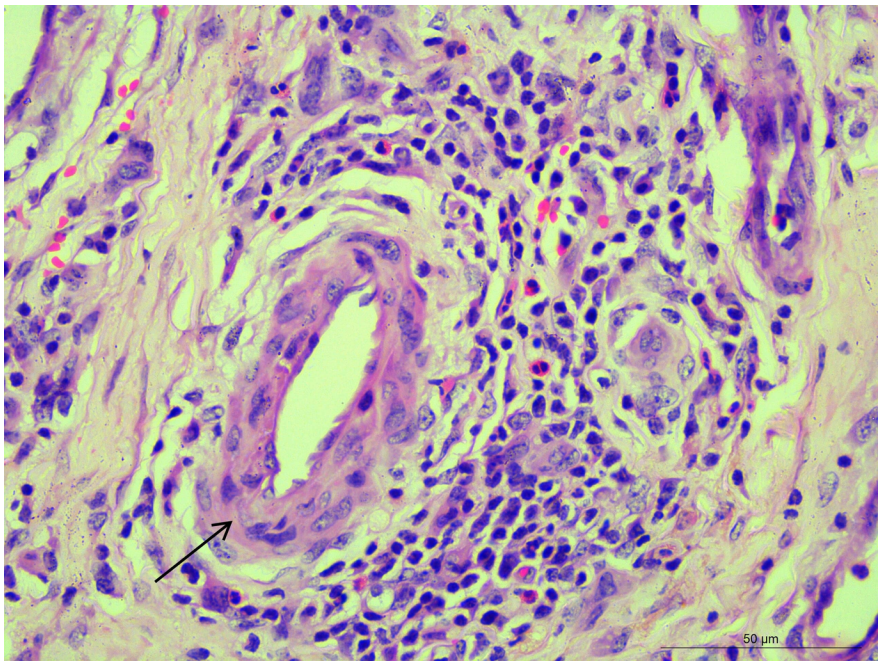

Figure 1: (H) Perivascular lymphocytes and macrophage cell reaction, H&E, 40X; E.

Figure 2: (A) Gel based PCR detected capripoxvirus-specific P32 gene and LSDV- specific F gene in skin biopsy samples

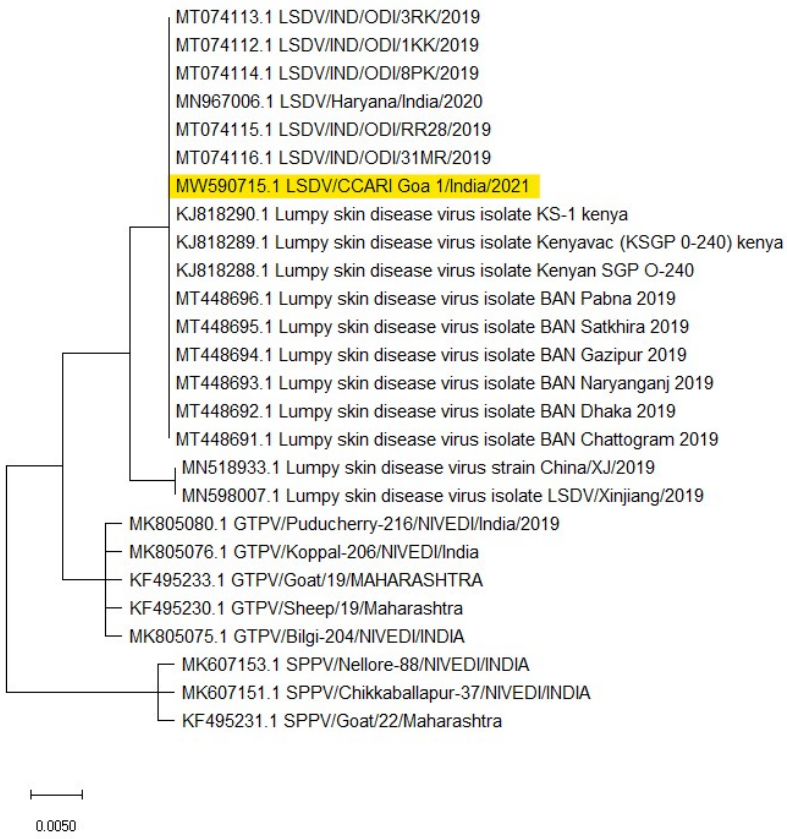

Figure 2: (B) Phylogenetic analysis based on nucleotide sequencing of RPO30 gene of LSDV showed close similarity with other Indian isolates

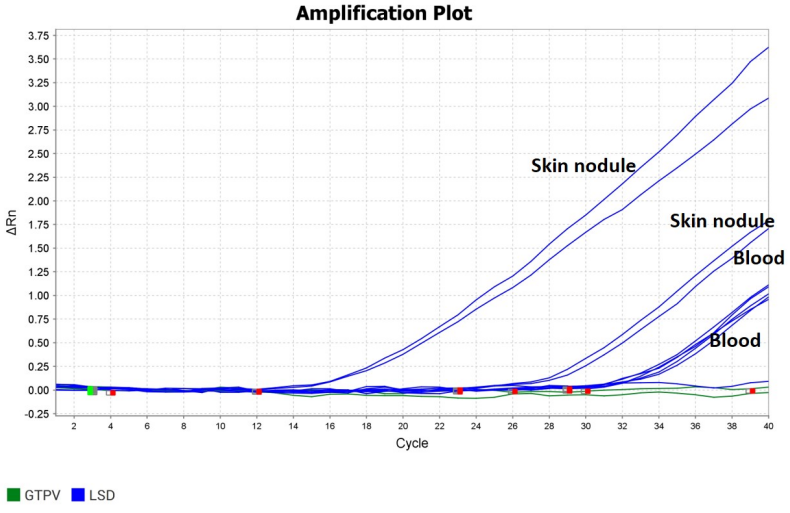

Figure 2: (C) TaqManprobe Real Time PCR targeting EEV glycoprotein gene (LSDV126) detected LSDV in skin biopsy and blood samples