Molecular Epidemiology of First Lumpy Skin Disease Outbreak in Odisha, India

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Abstract

Lumpy skin disease virus (LSDV) is the causative agent of lumpy skin disease (LSD) which is a member of Capripoxvirus. It is an economically critical transboundary disease affecting cattle. This study records the first LSD incidence in cattle of Ganjam district and analyses data from LSD outbreak in August 2020 on epidemiological and genetic characterization. Out of 452 animals clinically examined (59 farms), 63 animals were clinically affected with LSD, with a total morbidity rate of 13.93%. The morbidity rates in the villages (ten villages) varied from 5.55 to 21.62%. The multivariable logistic regression showed that grazing of animals (*P*=0.023; OR: 1.90; 95% CI: 1.09-3.32), and lactation and pregnancy status of animals (*P*=0.007; OR: 2.86; 95% CI: 1.32-6.17) were the potential risk factors for the occurrence of lumpy skin disease. Out of 53 clinically suspected animals collected from Ganjam district of Odisha, 18 samples (33.96%) were found positive by PCR for both *P32* and *F* genes of capripox virus. Phylogenetic analysis of *P32* gene of LSD (MW147486) showed 100% similarity with other isolates from India, Bangladesh, Egypt and Saudi Arabia. Additionally, phylogenetic analysis of *F* gene of LSD (MW147485) revealed a similarity of 97.99 %, 97.36%, and 96.60% with, Odisha India (MT074110), Beni Suif Egypt (MN694826) and Marsa Matrouh Egypt (MN699855), respectively.

Introduction

LSD is among one of the most economically significant viral diseases of bovines. LSDV is often referred to as the Neethling virus (Salib and Osman 2011). It causes high economic loss characterized by chronic debility, retarded growth, reduced milk yield, sterility in bulls, infertility, abortion and sometimes death. Generally, it affects the economic value of animals as it affects the production of meat and milk, the quality of hides and reproductive ability (Limon et al. 2020).

It is transmitted mainly by biting insects, biting flies and mosquitoes (Chihota et al. 2001) beside other transmission vectors have been reported. Molecular evidence revealed that Ixodid ticks can transmit LSDV. 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). LSDV is also excreted from infected animals through milk (transmitted to suckling calves), blood, nasal secretion, lachrymal secretion, saliva and semen which act as a significant source of infection for other healthy animals. Nodules which occur on the eye, mouth, nose, udder, rectum, and genital mucous membranes ulcerate and shed viruses that often act as a source of infection (Lefèvre et al. 2010).

The reasons of this disease spread to Odisha are yet to be identified. Odisha is a state which is most susceptible to natural calamities like cyclone and flood. Like every year, Odisha was hit by an extremely severe cyclonic storm “FANI” on 3rd May of 2019. The coastal districts suffered a serious loss of livelihood along with livestock. The scenario was yet to be get worsen. Post FANI, the coastal district animals started getting affected with a nodular skin disease having similar clinical signs to that of lumpy skin disease (LSD). This type of clinical signs was observed for the first time in the bovines in Odisha

post FANI. This may be due to movements of livestock across international borders or may be due to the movements of vectors from neighbouring countries. LSD has been recorded from countries neighbouring to India, such as China and Bangladesh, in recent years. Consequently, identifying the epidemiology of exotic diseases is important for successful disease control to be planned in a timely manner.

India, the world's largest milk producing nation, has a huge population of 192.49 million cattle (DAHD, 2019). In small backyard farms and each contain small number animals (2-10) reared mainly for production of milk. Of the many bovine poxvirus infections, buffalopox outbreaks have been recorded in India in buffaloes and cattle (Gurav et al. 2011), though LSD is considered an exotic disease. The aim of this study was to report the first LSD incidence in cattle and to examine molecular epidemiology and phylogenetic analysis details from LSD outbreak August, 2020 in Ganjam district, India.

Materials And Methods

Area of study

The study was carried in Ganjam district of Odisha (Fig. 1) between 18° 2' and 22° 6' N latitude and 82° 8' and 87° 6' E longitude where the weather is very hot and humid due to its location adjacent to the Bay of Bengal that provides suitable conditions for the development and propagation of vector-borne diseases.

Sample collection

An outbreak was investigated on 59 small farms with five to ten cattle in each farm. The incidence of the outbreak was occurred following cyclone Fani. A total of 452 cattle were included in the study. Animals were examined for the typical clinical signs of LSD, such as fever, skin nodules involving all layers of the skin, brisket edema, enlarged lymph nodes and edema in limbs. We aseptically collected 53 skin lesions from clinically affected animals. The tissue samples were placed in sterile bottles on ice to the department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Bhubaneswar, Odisha within eight hour of collection, and kept at –20°C until processing (OIE 2010).

DNA extraction, polymerase chain reaction and nucleotide sequencing

DNA was extracted from 2-3 gram of scab tissue using DNA mini kit (QIAGEN, USA) available commercially as per the instruction of manufacturer. Extracted DNA samples were preserved at -80°C before further processing. For conventional PCR, LSD specific *P32* and *F gene* primers were used. LSD infected cattle was identified by PCR with a set of primers (Table 1). The total volume of PCR reactions was 25 µl, comprising 12.5 µl of 2X Taq PCR master mix (Himedia, India), 5.0 µl of DNA template, 1.0 µl of each primer (10 pmol), and 5.5 µl of free water nuclease. Amplifications were performed in Bio –Rad Thermal Cycler (Bio-Rad, USA). The conditions started with initial denaturation (one-step) at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 50°C for 30 s, and extension at 72°C for 1 minute, with final extension at 72°C for 5 minutes. Positive control samples were

obtained from Veterinary Clinical Medicine Department, College of Veterinary Science and Animal Husbandry, Bhubaneswar, Odisha.

Sequencing was performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermofisher Scientific, USA). An automated ABI 3100 Genetic Analyzer was used to perform sequence analysis (Applied Biosystems, Germany). Sequence alignments were conducted with MEGA 6 programme. The sequences of *P32* and *F* partial genes were compared and analysed with that of the genes sequences previously reported in GenBank.

Sequencing results were analyzed by search tool nBLAST present in NCBI database for determining the similarity of LSD *P32* and *F* genes. Using the Clustal X software, the sequences were compared. Using the neighbor-joining process, the phylogenetic tree was established according to the nucleotide alignments (Saitou and Nei, 1987). The Bootstrap analysis was carried out using MEGA 6 program (Kumar et al. 2016). The sequences obtained in this research were registered with the accession number MW147486 and MW147485 into the GenBank database.

Statistical analysis

Statistical analysis was carried using SPSS 26 statistical software. Logistic regression analysis was conducted to detect the association between the prevalence of infection and the potential risk factors. The univariable logistic regression analysis followed by the independent factors with significant *P v*alue ($P<0.1$) were included into the multivariable logistic regression analysis. The results were considered significant at $P<0.05$.

Results

Out of 452 animals clinically examined (59 farms), 63 animals were clinically affected with LSD, with a total morbidity rate of 13.93%. The morbidity rates in the villages (ten villages) ranged from 5.55 to 21.62% (Table 2). During clinical examination, we observed the characteristic clinical signs of LSD like fever, nasal and ocular discharge, salivation, enlarged prescapular and prefemoral lymph nodes and skin nodules (1 cm to 8 cm) involved all layers of the skin on the head, neck, trunk, perineum, udder, teats and throughout body sometimes (Fig. 2, 3, 4 and 5).

Analysis of epidemiological data in the univariable logistic regression (Table 3) between independent variables and clinically affected animals with LSD revealed that the association of age, sex and breed with prevalence was statistically insignificant. In contrast, grazing of animal, lactation and pregnancy status were the potential risk factors for the incidence of LSD. We observed that the incidence of LSD increased with ages. Moreover, the highest prevalence was observed in the age group more than 3 years (22.35%) and the lowest prevalence was observed in animals less than one year (7.69%). Additionally, the highest prevalence (14.31%) was found in crossbred cattle while the lowest prevalence (7.79%) was observed in native breeds. Statistically significant relationship between cows left for grazing outside and the occurrence of LSD infection was found, and the highest prevalence was observed in grazing animals

(20.31%, *P*= 0.015, OR = 1.97, 95% CI: 1.14–3.42). Concerning lactation and pregnancy status and the incidence of LSD, the highest prevalence was recorded in periparturient (17.78%, *P*= 0.005, OR = 2.95, 95% CI: 1.37–6.35), followed by pregnant cows (15.17%, *P*= 0.039, OR = 2.44, 95% CI: 1.04- 5.72), while the lowest prevalence was observed in non pregnant cows (6.8%).

Results of multivariable logistic regression (Table 4) revealed that grazing of animals, lactation and pregnancy status were the potential risk factors for the incidence of LSD. Grazing of animals was significantly associated with highest prevalence of LSD (*P*=0.023; OR: 1.90; 95% CI: 1.09- 3.32). Additionally higher prevalence was noticed in periparturient cows (*P*= 0.007; OR: 2.86; 95% CI: 1.32-6.17).

Out of 53 samples collected from Ganjam district of Odisha, 18 samples (33.96%) were found positive by PCR for both *P32* (Fig. 6) and *F* gene (Fig. 7) of Capripoxvirus. Phylogenetic analysis of *P32* gene of LSD (MW147486) through nBLAST revealed Ganjam LSDV sequences were similar and clustered in LSDV cluster with field strains of LSDV. Nucleotide sequence identity analysis of Ganjam isolate showed 100% similarity with other isolates from India, Bangladesh, Egypt and Saudi Arabia (Fig. 8). No genetic divergence was detected between our isolates and the other isolates from India, Bangladesh, Egypt, Saudi Arabia and China (Fig. 9). Additionally, phylogenetic analysis of *F* gene of LSD (MW147485) through nBLAST revealed a similarity of 97.99 %, 97.36%, and 96.60% with, Odisha India (MT074110), Beni Suif Egypt (MN694826) and Marsa Matrouh Egypt (MN699855), respectively (Fig. 10). A higher genetic divergence was observed between Ganjam isolate and Qalyubia and Dakahlia, Egypt isolates. The lower genetic diversity was recorded between Ganjam isolate and Odisha, India and Beni Suif, Egypt isolates (Fig. 11).

Discussion

Livestock rearing is one of the oldest professions of life sustenance for the peoples of the rural communities. In a state like Odisha nearly 60 percent of the population depends upon livestock production to earn their livelihoods. Diseases that affect population and productivity of the livestock greatly hamper the livelihood of the rural peoples. The study showed the presence LSDV field strains in Ganjam LSD outbreak, according to the laboratory findings of samples obtained from LSD outbreaks and phylogenetic analysis of *P32* and *F* genes partial sequences. This research also offers valuable data on the epidemiological characteristics of LSD outbreaks in cattle reared in small backyard.

LSD affected cattle showed clinical signs including fever, skin nodules on the neck, back, perineum, tail, hind limbs and genital organs, enlarged superficial lymph nodes and, in a some cases, legs and brisket edema along with lameness (Al-Salihi 2014; Abutarbush et al. 2015). Lesions sloughing will produce "sitfast" hole shape, the usual lesion that consequently triggers screwworm fly invasion and invasion of bacteria which can further aggravate to septicaemia (Constable et al. 2017). This was in agreement with our observation during outbreak investigation.

Regarding to the age, in the present study, prevalence rate of LSD indicated that adult cattle were more susceptible to infection than young calves. This finding is in agreement with (Albayrak et al. 2018) who

reported that adult cattle displayed a higher prevalence, than calves under one year of age, 46.34%. On the other hand, this finding is not in agreement with the previous studies that reported higher prevalence in young calves than adult cows (Ahmed and Kawther 2008; Sevik and Dogan 2017).

Despite the higher prevalence of LSD infection in females than in males, there were no significant relationship between the sex and LSD prevalence in our study. Similar results have been reported by some researchers (Tuppurainen and Oura 2012; Ayelet et al. 2014). However, it did not agree with the results of others (Gari et al. 2010; Abera et al. 2015) who suggested that males were more vulnerable to disease than females due to the exposure to stress factors such as fatigue from hard work. Additionally, no significant differences were observed among breed in relation to the prevalence of lumpy skin disease and this agrees with the results of others (Ambilo and Melaku 2013).

In this study, the highest prevalence rate of LSD was observed in grazing animals. This finding is in agreement with previous study (Hailu et al. 2014) who reported that grazing of animals was a potential risk factor for occurrence of LSD. Moreover, there was a significant relationship between the lactation status and pregnancy status of animal with occurrence of the LSD. Higher prevalence of LSD was observed in periparturient cows. This may be due to stress, which considers the main reason in the occurrence of the disease; therefore, recently calved and pregnant cows are at a high-risk for disease occurrence (Ahmed and Kawther 2008).

LSDV transmission occurs mainly by mechanical vector transmission, which is the primary route of disease propagation. The incidences of disease dramatically increased with the advent of rainy and summer seasons in most endemic countries such as sub-Saharan Africa, Egypt and Ethopia, because of the proliferation of vectors (Mulatu and Feyisa 2018). This is in agreement with our results that the outbreak occurred during Mansoon (rainy season in India) which is characterized by very hot and humid conditions making it suitable for vector multiplications and proliferation. Mosquitoes, flies, biting midges and ticks are the main insect vectors present during the monsoon season in Odisha coastal region (Rao et al. 2015; Dehuri et al. 2017). Additionally, LSD outbreak occurred in India after Cyclone Fani and this may be the cause of spread of LSD in India. Although the restrictions of animals movement from Egypt, infection has been observed to spread to Palestine 100 to 200 km away via the movement of air and insects biting (AU-IBAR 2013).

Positive PCR results, indicated by the amplification of a *P32* gene 192-bp and *F* gene 472-bp genome fragment, and were detected in 18 sampled materials out of 53. The results of this research are similar to earlier study in Odisha showed a prevalence of 29.87 % (Sudhakar et al. 2020). This variation in prevalence is undoubtedly associated with the environmental changes and management measures conducted in these regions, including of vectors, and loads.

Phylogenetic analysis of *P32* gene of LSD (MW147486) through nucleotide BLAST revealed Ganjam LSDV sequences were similar and in the same cluster with LSDV field strains. Nucleotide sequence identity analysis of Ganjam isolate showed 100% similarity with other isolates from India, Bangladesh, Egypt and Saudi Arabia. No genetic divergence was detected between our isolates and the other isolates

from India, Bangladesh, Egypt, Saudi Arabia and China. This agreed with previous studies (Doğan et al. 2016; Mafirakureva et al. 2017; Sudhakar et al. 2020). Additionally, phylogenetic analysis of *F* gene of LSD (MW147485) through nucleotide BLAST revealed a similarity of 97.99%, 97.36%, and 96.60% with Odisha India (MT074110), Beni Suif Egypt (MN694826) and Marsa Matrouh Egypt (MN699855), respectively. A higher genetic divergence was observed between Ganjam isolate and Qalyubia and Dakahlia, Egypt isolates. The lower genetic diversity was between Ganjam isolate and Odisha, India and Beni Suif, Egypt isolates. This is in agreement with previous studies (Sudhakar et al. 2020; Allam et al. 2020).

Conclusion

This is the first study on molecular epidemiolgy and phylogenetic analysis of LSDV in Ganjam, India using the *P32* gene and *F* gene. The multivariable logistic regression showed that grazing of animals and lactation and pregnancy status of animals were the potential risk factors for the incidence of lumpy skin disease. The current study showed that the two viral genes were detected from clinical cases in the Ganjam district and verified LSDV circulation in this outbreak. These genes were similar after sequencing to those of the LSDV that had been detected and registered in GenBank.

Declarations

Funding:

Not applicable

Conflict of interest

The authors of this study stated that they have no conflicts of interest.

Code availability

Not applicable

Ethics approval:

Oral permission was taken from owners of the farms before collection of samples. Additionally samples collection was performed by professional field veterinarians.

Consent to participate:

Oral permission was taken from owners of the farms before collection of samples. There is no specific law in India which requires permission from the ethics committee for collection of samples for clinical diagnosis. Additionally samples collection was performed by professional field veterinarians.

Consent for publication:

We know of no conflicts of interest associated with this publication. As Corresponding Author, I confirm that the manuscript has been read and approved for submission by all the named authors.

Availability of data and material:

Data is available within the article to support the findings of this study

Author contributions:

Santosh Senapati and Manoranjan das conceived and planned the study; Ahmed Selim and Rajesh Sethi collaborated in the writing and revision of the manuscript; Rajesh Sethi and Aditya Prasad Acharya conducted laboratory testing; Ahmed Selim and Chinmoy Mishra collaborated in sequencing of genes and phylogenetic analysis. Santosh Senapati and Shuvranshu Biswal revised the manuscript. All authors' read and endorsed the final manuscript.

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Tables

Table 1 List of the primers used in this study

Primer Name	Sequence (5'→3')	Amplicon Size	Assay	Reference
P32 –F	TCCGAGCTCTTTCCTGATTTTTCTTACTAT	192 bp	LSD Specific	(Ireland & Binepal, 1998)
P32-R	TATGGTACCTAAATTATATACGTAAATAAC			
F gene-F	ACTAGTGGATCCATGGACAGAGCTTTATCA	472 bp		
F gene –R	GCTGCAGGAATTCTCATAGTGTTGTACTTCG			

Table 2 Epidemiological characteristics of LSD outbreak in cattle in Ganjam district , India in August 2020

Village	No of Farms	No. of examined animals	No of clinically affected animals	Morbidity rate (%)	Mortality rate
Badakhandi	7	56	8	14.28	0.00
Badiambagon	5	42	5	11.90	0.00
Sasanambagon	8	50	5	10	0.00
Sompur	5	36	2	5.55	0.00
Samarajhol	5	45	7	15.55	0.00
Sikiri	8	60	9	15	0.00
Makarajhola	7	56	7	12.5	0.00
Sardhapur	5	46	8	17.39	0.00
Konchuru	4	24	4	15.38	0.00
Saru	5	37	8	21.62	0.00
Total	59	452	63	13.93	0.00

Table 3 Univariable logistic regression model for the potential risk factors associated with LSD in cattle

P value	C.I	OR	S.E	B	Number of clinically affected animals	Number examined of animals	Variable
					63 (13.93)	452	Age
0.10	0.78-15.28	3.45	0.75	1.24	38 (22.35)	170	>3 year
0.82		1.18	0.76	0.16	23(8.98)	256	1–3 years old
	0.26-5.33	-	-	-	2 (7.69)	26	< 1 year old
							Sex
0.55	0.45-4.27	1.39	0.57	0.33	4(18.18)	22	Male
					59(13.72)	430	Female
							Breed
0.35	0.46-8.70	2	0.74	0.69	61(14.31)	426	Crossbreeds
					2(7.69)	26	Native breeds
							Grazing
0.015*	1.14-3.42	1.97	0.28	0.68	26(20.31)	128	Yes
-	-	-	-	-	37(11.41)	324	No
							Pregnancy and lactation status
							Periparturient
0.005*	1.37-6.35	2.95	0.39	1.08	37(17.78)	208	Pregnant
0.039*	1.04-5.72	2.44	0.43	0.89	17(15.17)	112	Non pregnant
-	-	-	-	-	9(6.8)	132	

β; regression coefficient; SE; standard error CI; confidence interval at 95%; OR; odds ratio; P; *P* value is <0.05

Table 4 Multivariable logistic regression form for the potential risk factors associated with LSD in cattle.

P value	CI	OR	S.E	B	Variable
					Grazing
0.023*	1.09- 3.32	1.90	0.28	0.64	Yes
-	-	-	-	-	No
					Pregnancy and lactation status
					Periparturient
0.007*	1.32- 6.17	2.86	0.39	1.05	Pregnant
0.047*	1.01-5.58	2.37	0.43	0.86	Non pregnant
-	-	-	-	-	

P value is <0.05

Figures

Figure 1

Collection sites of Odisha indicated by red triangles Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

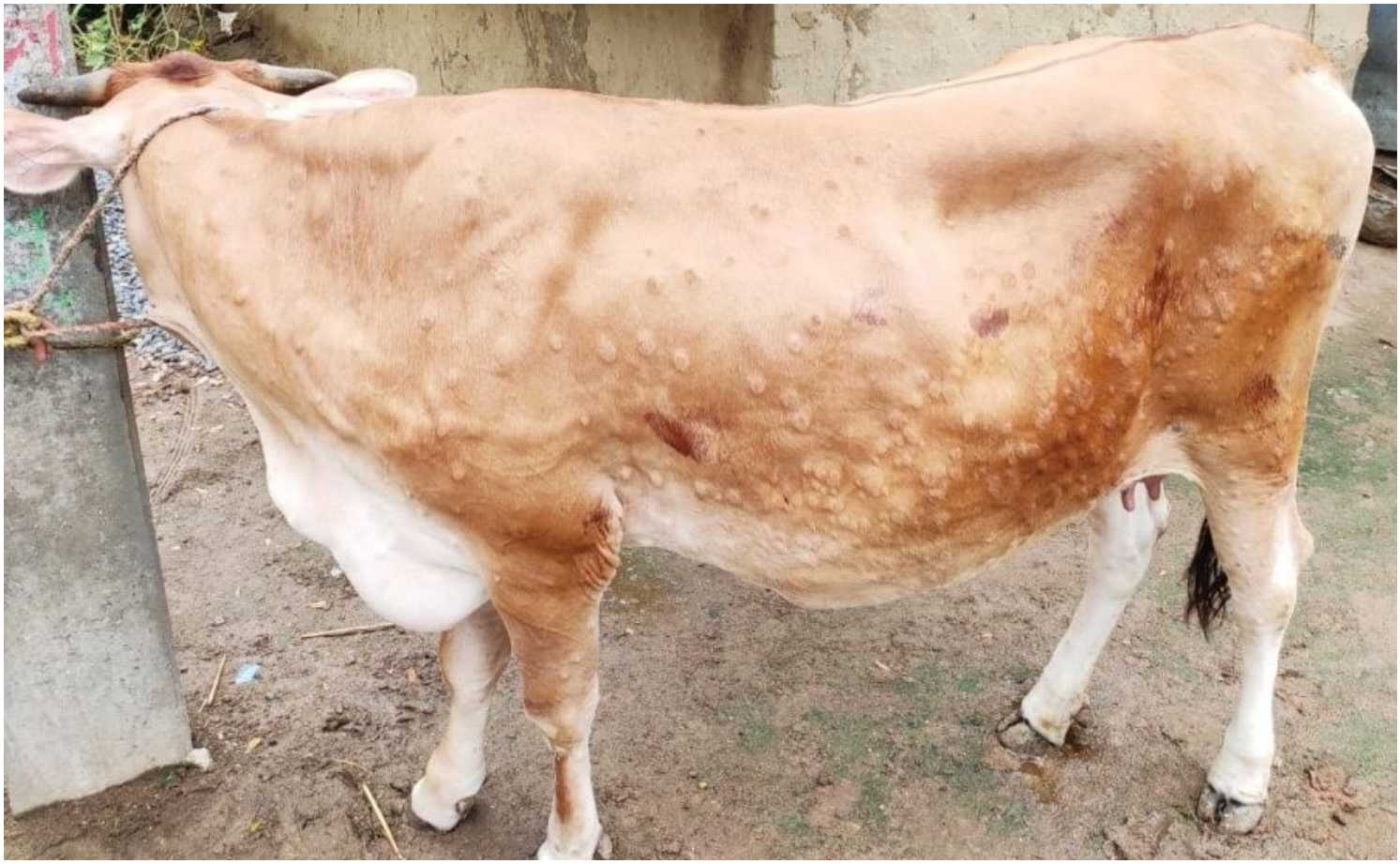

Figure 2

Cow showed skin nodules in all over the body

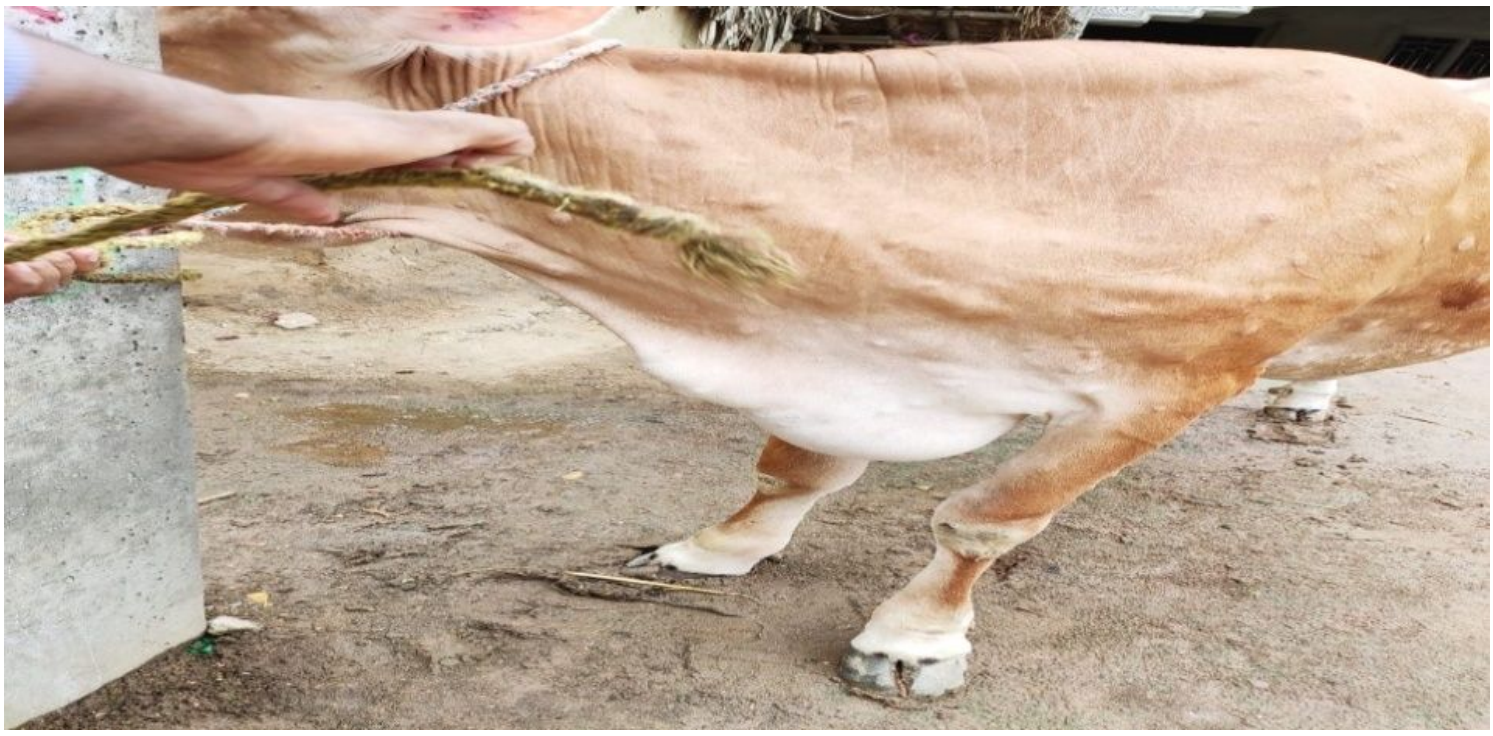

Figure 3

Cow showed skin nodules and brisket edema

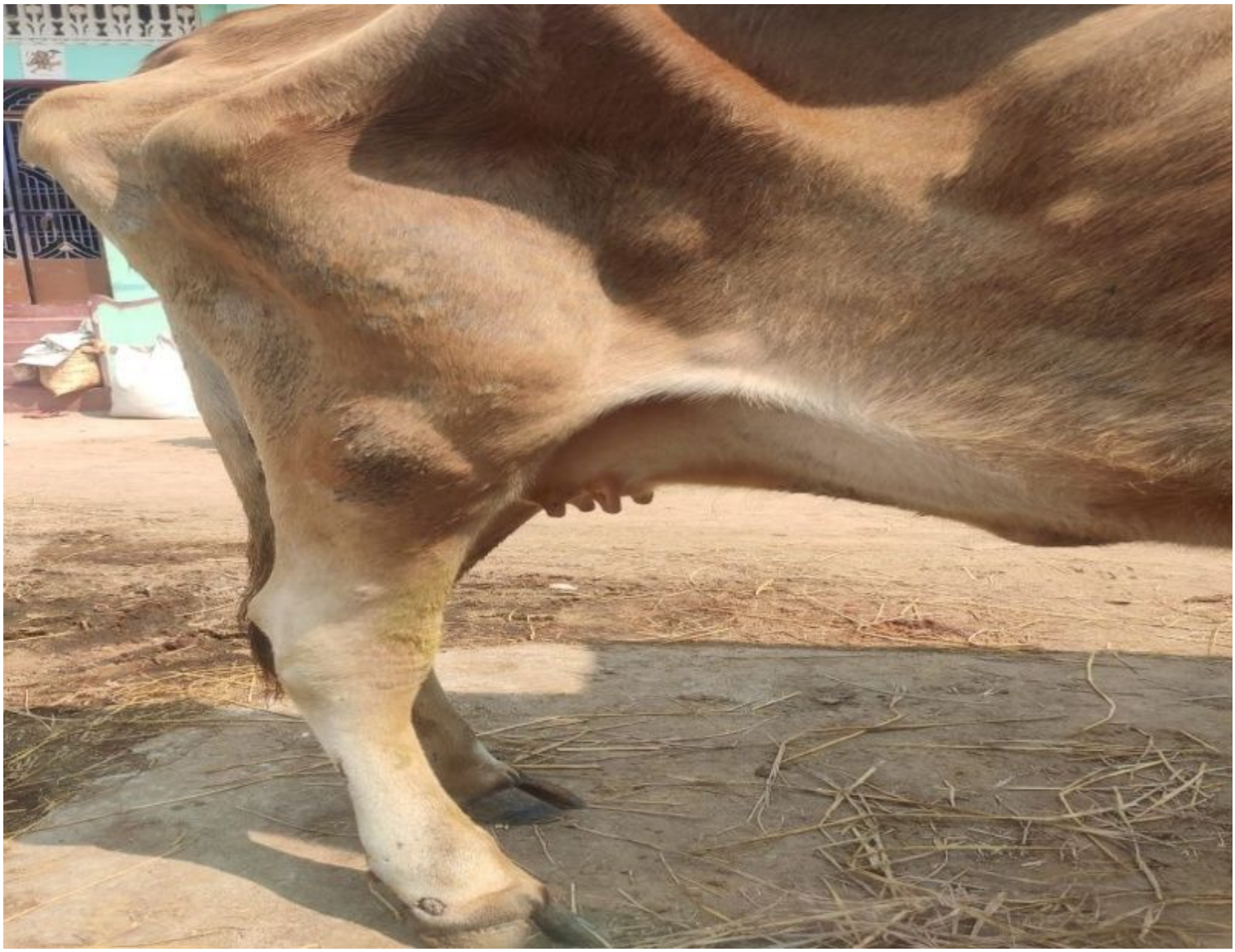

Figure 4

Cow showed skin nodules, enlarged prefemoral lymph node and hind limb edema

Figure 5

Calf skin showing lumpy skin disease infection scars in the late stage

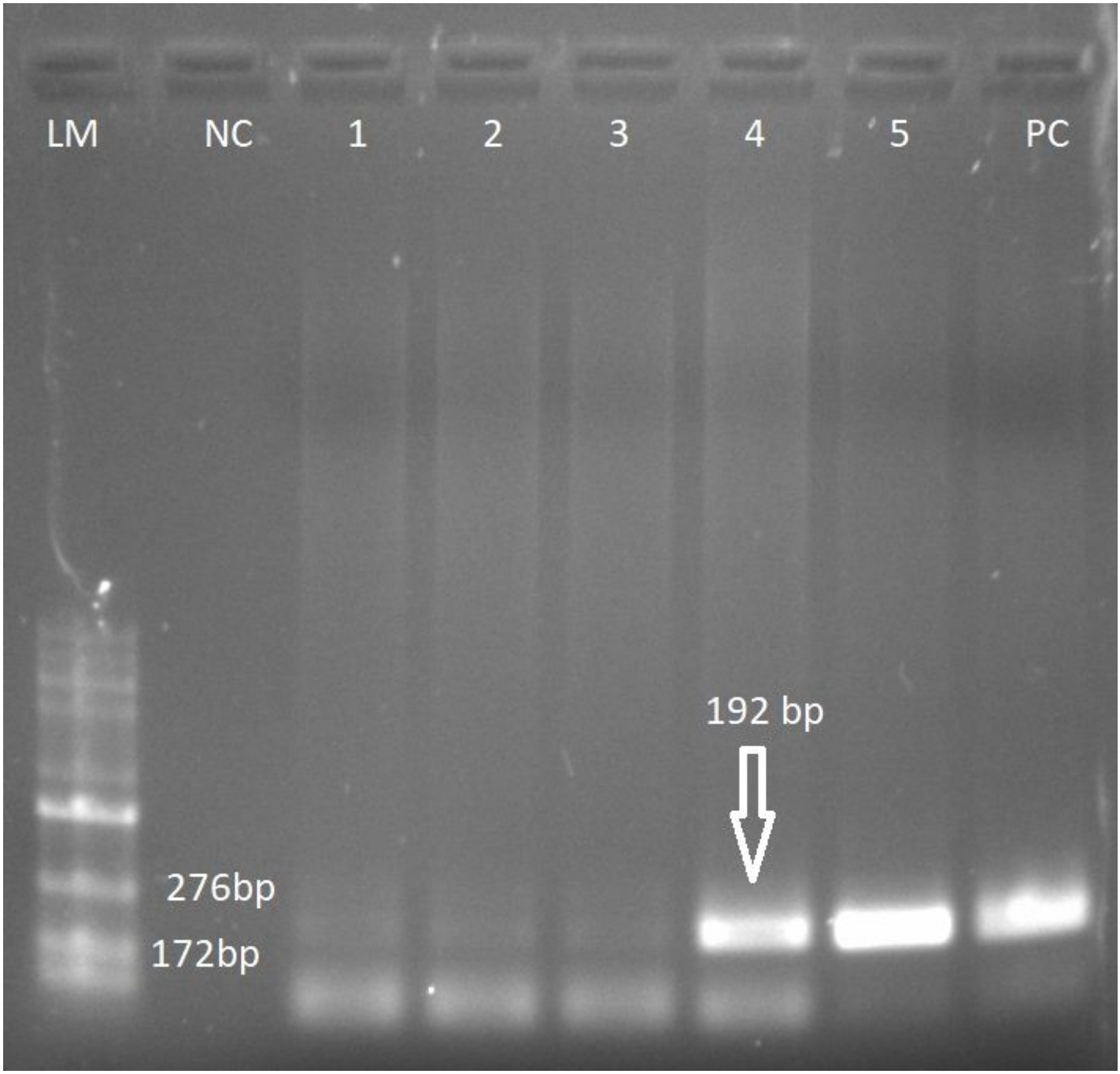

Figure 6

PCR amplification of P32 gene of LSD showing an amplified target of 192 bp. Lane with sample number 4 and 5 positive samples. PC; positive control; NC; negative control

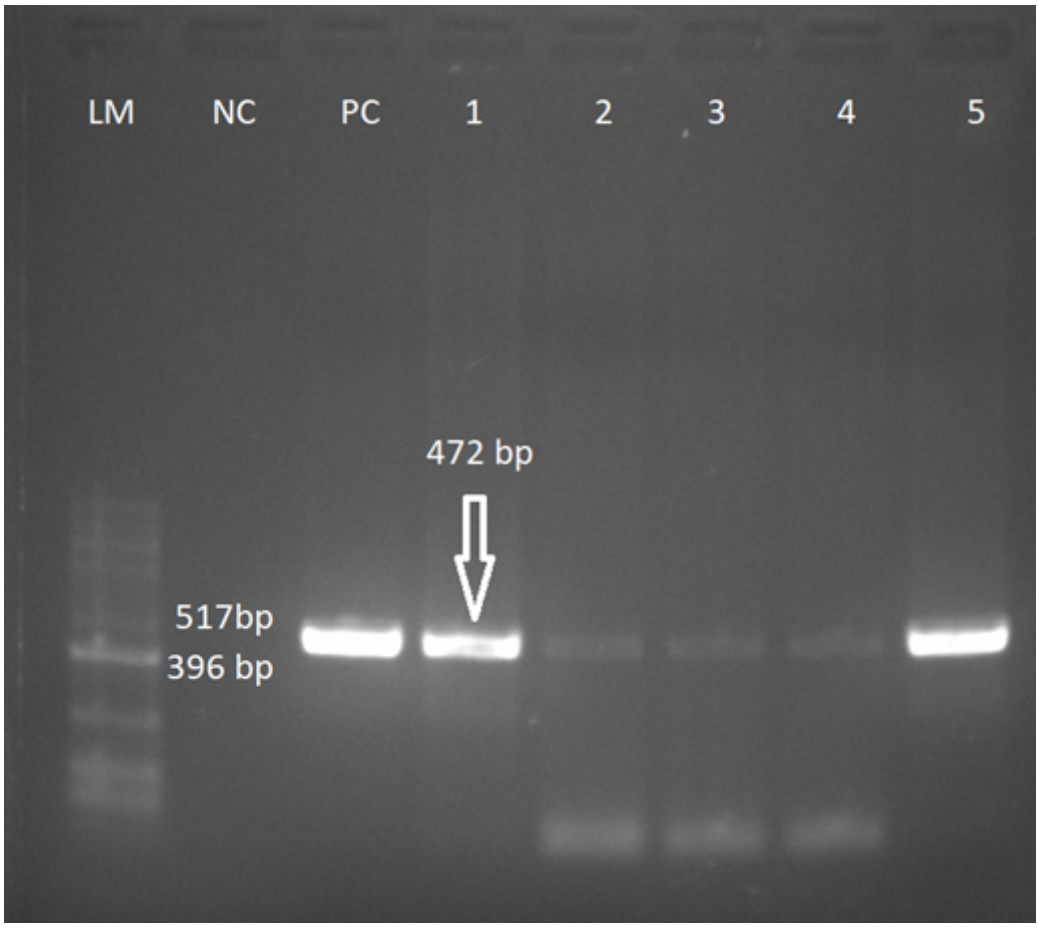

Figure 7

PCR amplification of F gene of LSD showing an amplified target of 472 bp. Lane with sample number 1 and 5 positive samples. PC; positive control; NC; negative control

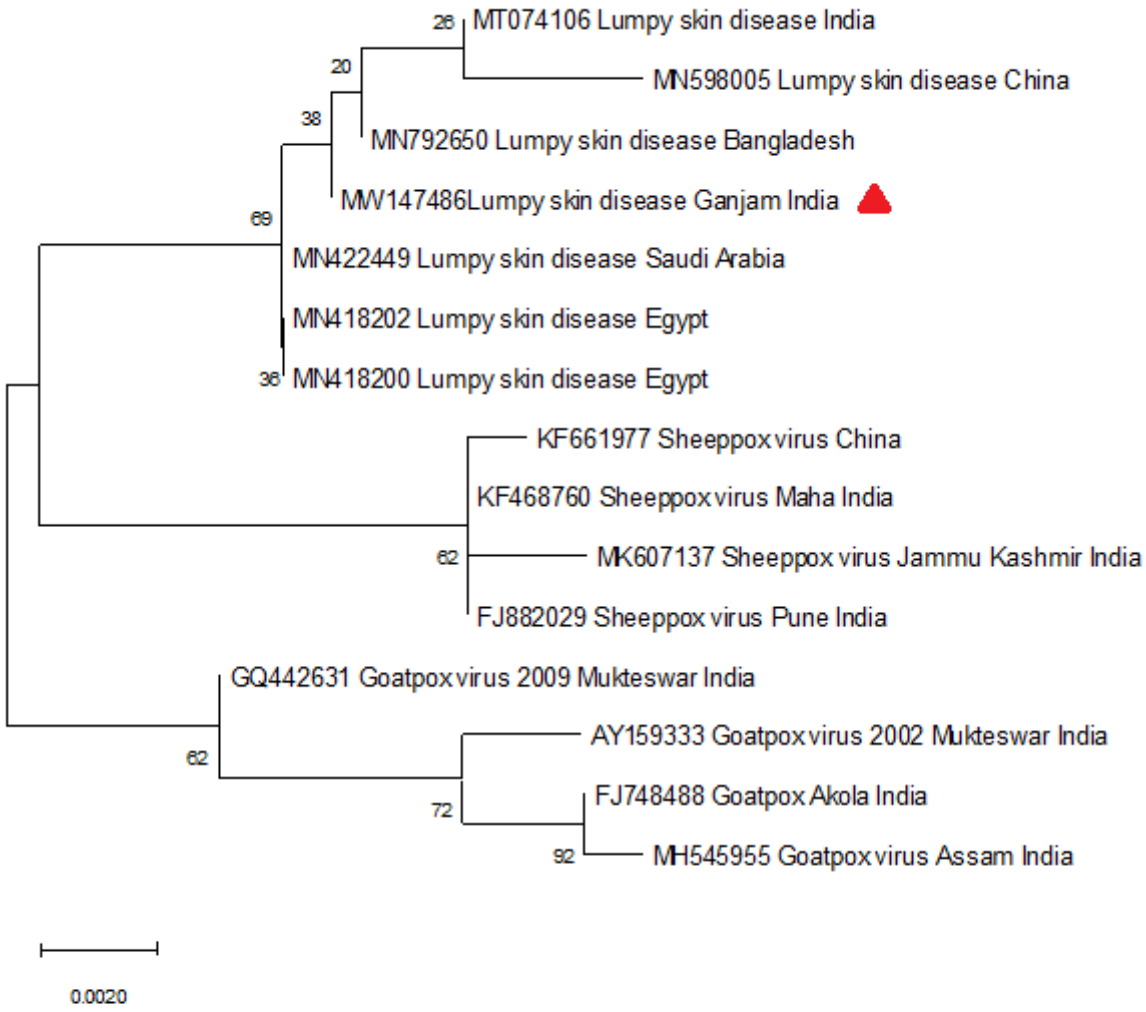

Figure 8

Phylogenetic analysis based on the partial nucleotide sequences (174 bp) of the P32 gene with other isolates from Capripox virus available in GenBank. Sequence isolated in Ganjam is indicated with the red triangle.

	1	2	3	4	5	6	7
1. MW147486Lumpy skin disease Ganjam India		0.00	0.00	0.00	0.00	0.00	0.00
2. MN792650 Lumpy skin disease Bangladesh	0.00		0.00	0.00	0.00	0.00	0.00
3. MT074106 Lumpy skin disease India	0.00	0.00		0.00	0.00	0.00	0.00
4. MN418202 Lumpy skin disease Egypt	0.00	0.00	0.00		0.00	0.00	0.00
5. MN418200 Lumpy skin disease Egypt	0.00	0.00	0.00	0.00		0.00	0.00
6. MN422449 Lumpy skin disease Saudi Arabia	0.00	0.00	0.00	0.00	0.00		0.00
7. MN598005 Lumpy skin disease China	0.00	0.00	0.00	0.01	0.01	0.01	

Figure 9

Genetic Divergence analysis of P32 gene of LSD from Ganjam, India.

Figure 10

Phylogenetic analysis based on the partial nucleotide sequences (447 bp) of the F gene with other isolates from Capripox virus available in GenBank. Sequence isolated in Ganjam India is indicated with the red triangle.

	1	2	3	4	5	6	7	8
1. MW147485 lumpy skin disease virus Ganjam India		0.007	0.007	0.009	0.011	0.284	0.348	0.486
2. MT074110 Lumpy skin disease virus India	0.020		0.000	0.004	0.005	0.282	0.351	0.481
3. MT074111 Lumpy skin disease virus India	0.020	0.000		0.004	0.005	0.282	0.351	0.481
4. MN694826 Lumpy skin disease virus BeniSuif Egypt	0.029	0.007	0.007		0.000	0.223	0.301	0.448
5. MN699855 Lumpy skin disease virus Marsa Matrouh Egypt	0.035	0.006	0.006	0.000		0.210	0.188	0.239
6. MK182356 Lumpy skin disease virus Nigeria	1.336	1.336	1.336	1.277	1.218		0.004	0.006
7. MH051299 Lumpy skin disease virus Dakahlia Egypt	1.373	1.373	1.373	1.330	1.194	0.005		0.006
8. LC486408 Lumpy skin disease virus Qalyubia Egypt	1.469	1.469	1.469	1.433	1.243	0.013	0.013	

Figure 11

Genetic Divergence analysis of F gene of LSD from Ganjam, India.