RESEARCH ARTICLE

Molecular Diagnosis of Lumpy Skin Disease Outbreak in Cattle of Bidar, Karnataka

Shilpa*[1], Sandeep Halmandge[2], Vivek Kasaralikar[3], Ravindra B G[4], Mallinath K C[5], Rajendrakumar T[6]

Abstract

Lumpy skin disease (LSD) is an infectious, arthropod born disease caused by lumpy skin disease virus (LSDV) belongs to genera *Capripox virus* family poxviridae. In the recent past, Karnataka experienced several outbreaks of LSD with severe economic losses. Based on clinical signs the disease was tentatively diagnosed as LSD and confirmed by PCR and phylogenetic analysis. Out of the total 226 suspected cases of LSD with varying degree of clinical signs and nodular lesions,blood and tissue samples were collected from 26 animals. Conventional capripox generic PCR detected 17 (65.38 %) of P32 gene and F gene in skin biopsy samples. Phylogenetic analysis of P32 gene showed that the isolates from this study showed the similarity with other Indian isolates.

Key words: Capripox virus, Lumpy skin disease, Phylogenetic analysis.

Ind J Vet Sci and Biotech (2024): 10.48165/ijvsbt.20.3.20

Introduction

Lumpy skin disease (LSD) is an infectious transboundary disease of cattle of all age groups and breeds. The disease is listed by OIE (2018) in "List A" due to its rapid spread and severe economic losses such as hide damage, decrease in milk production, and weight gain, mastitis, infertility in males and females, decreased semen quality and death. In India, Lumpy skin disease had been first reported from Mayurbhanj and Bhadrak districts in Odisha in August 2019 (Sudhakar et al.,2020) and has spread rapidly across the country which may indirectly correlate the productivity due to stress by associated viral infection (Ahmed *et al.,* 2020).

Clinical signs in LSD has two febrile phases (Al-Salihi, 2014) and varying incubation period of 4-12 days. Infected animals showed temperature about 40-41.5°C (which persists up to 6-72 h or more, rarely up to 10 days), general depression, anorexia, lacrimation, increased nasal and pharyngeal secretions, dysgalactia, disinclination to move. Eruption of multiple skin nodules which are firm, raised on the skin may be widespread or restricted to just few lesions measuring about 5-50 mm in diameter (Al-Salihi, 2014). The main predilection sites are head, neck, perineum, genitalia, udder and limbs (Salib and Osman, 2011). Regional lymph nodes are enlarged 3-5 times the normal size which are easily palpable (Tuppurainen *et al.,* 2017). Muzzle shows typical ring like necrotic lesions separated from surrounding healthy epithelium. After 2-3 weeks skin lesions become necrotic and harder, later harder skin lesions become sit fast typical to LSD. In chronic cases skin of oedematous limbs may peel off leaving large holes which is susceptible to infection and myasis (Edelsten,2014). Keratitis is a common complication. Common sequel of LSD is pneumonia, mastitis, anoestrous in cows, infertility in bulls, abortion in pregnant cows. Recovered animals may show weakness and debility up to 6 months. 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).

In India several outbreaks were reported from 2019 (Sudhakar *et al.,*2020) in different states causing severe economic losses due to uncontrolled movement of cattle and lack of vaccination. The current investigation was devised to validate the occurrence of a Lumpy Skin Disease (LSD) outbreak in cattle in Bidar, Karnataka, utilizing both skin lesion-based clinical observations and molecular diagnostic techniques. Additionally, the study aimed to conduct molecular characterization of the Lumpy Skin Disease virus.

Materials and Methods

The present study was undertaken at the Department of Veterinary Clinical Complex (VCC), Veterinary College, Bidar from

[1,2,3]Department of Veterinary Medicine, Veterinary College, Nandinagar, KVAFSU, Bidar-585226, Karnataka, India

[4]Department of Veterinary Medicine, Veterinary College, Vinobanagar, Shivamogga, Karnataka, India

[5]Department of Veterinary Microbiology, Veterinary College, Nandi Nagar, KVAFSU, Bidar-585226, Karnataka, India

[6]Department of Veterinary Pathology, Veterinary College, Nandinagar, KVAFSU, Bidar-585226, Karnataka, India

Corresponding Author: Shilpa, Department of Veterinary Medicine, Veterinary College, Nandinagar, KVAFSU, Bidar, Karnataka, India. E-mail: shilpatonpe45@gmail.com

How to cite this article: Shilpa, Sandeep Halmandge, Vivek Kasaralikar, Ravindra B G, Mallinath K C, & Rajendrakumar T. (2024). Molecular Diagnosis of Lumpy Skin Disease Outbreak in Cattle of Bidar, Karnataka. Ind J Vet Sci Biotech, 20(3), 102-105.

Source of support: Nil

Conflict of interest: None

Submitted 31/01/2024 **Accepted** 28/02/2024 **Published** 10/05/2024

July to December 2020, with prior approval of the Animal Ethical Committee (IAEC) of Veterinary College, KVAFSU, Bidar under IAEC supervision (20/2020-21/VCB/VMD dated 29.06.2021).

In the present study total 226 suspected cases of LSD with varying degree of clinical signs and nodular lesions were included. Out of 226 animals, sampling was done from 26 animals. Blood was collected in EDTA coated vials and after due consent obtained from the farmers, skin biopsy specimens were collected under local anaesthesia (2% lignocaine) with disposable punch biopsy instrument. Samples were transported immediately to the laboratory on ice for further processing.

DNA from blood sample was extracted using the HiPurA blood genomic DNA miniprep purification kit (HIMEDIA MOLBIO, MB504) by following manufacturer instructions and extracted DNA samples were stored at -20˚C for further process. Skin scab / nodule samples were collected and preserved in phosphate buffer saline glycerol (PBSG) and stored at -20 °C for further process. Tissue DNA extraction was done by using Boiling method (Chadranaik *et al.,*2014).

PCR: Conventional Capri Pox generic PCR was carried out for the presence of LSD virus in 26 blood and tissues samples separately using the primers (Table 1) for the fusion (F) protein gene and P32 gene as recommended by OIE regulations (OIE,2018).

Table 1: Primer sequences of P32 and F gene

Gene	Primer sequence	Band size
P32 (LSDV074)	F: 5′-TCCGAGCTCTTTCCTGATTTTTCTTACTAT-3′ R: 5′-TATGGTACCTAAATTATATACGTAAATAAC-3′	192bp
F (LSDV117)	F: 5′-ACTAGTGGATCCATGGACAGAGCTTTATCA-3′ R: 5′-GCTGCAGGAATTCTCATAGTGTTGTACTTCG-3′	472bp

25µL PCR mixture composition for amplification of P32 and F gene separately for nodular tissue DNA sample containing 12.5 µL of Taq PCR Master Mix (HiMedia Laboratories Pvt. Ltd.), 1 µL of each forward and reverse primers, 5µL template DNA and rest of the volume (5.5 µL) was made up with nuclease free water.

Polymerase chain reaction was carried out in thermal cycler with thermal conditions *viz.,* 95˚C for 2 min for initial denaturation, 34 cycles of 95˚C for 45 sec denaturation, 50˚C for 50 sec annealing, 72˚C for 1minute extension, and final extension of 72˚C for 2 min. The PCR product was analyzed and examined by agarose gel (1.5%) electrophoresis and visualized under ultraviolet illumination after stained with ethidium bromide.

Sequencing and Phylogenetic Analysis: The representative PCR positive samples for P32 gene were sequenced by Sanger sequencing method in a commercial gene sequencing lab. To determine the sequence identity with the available GenBank sequences, the BLASTn software in GenBank was used and the per cent identity / homology was determined. The phylogenetic analysis of the sequence was done by the Neighbor joining method in MegaX software (http://www.megasoftware.net). The branch reliability was assessed by the bootstrap method with 1000 replications. The evolutionary distances were computed using the p-distance method and all the gaps were completely removed and a phylogenetic tree was constructed.

Results and Discussion

LSD is an exotic, exhausted viral disease affecting cattle of all ages resulting economically great losses due to the high rates of morbidity, chronic debilitation in diseased cattle, abortion, reduction in milk production, damage to hides, and temporary or permanent sterility. In this study, provisional disease diagnosis relied on clinical manifestations, subsequently validated through Polymerase Chain Reaction (PCR) and detection of Lumpy Skin Disease Virus (LSDV) infection. Further analysis encompassed DNA sequencing and phylogenetic investigations.

PCR for the detection of P32 and F gene: Conventional capripox generic PCR for the detection of LSDV showed the amplification products of expected size of 192 bp and 472 bp for fragment P32 and F gene as shown in Figure 1 &2 respectively. Among 26 skin samples tested for PCR, 17(65.38%) samples yielded expected amplicon. No blood samples were positive for PCR product among 26 samples tested. Similar findings were recorded by previous workers (Sudhakar *et al.,*2020, El-Khabaz,2014, Ochwo *et al.,*2020, Sethi *et al.,*2022). The reason for negative result from a blood sample taken from a clinically sick animal could be that the virus is known to be present in blood for a short time 4-11 days after infection before the appearance of generalized skin nodules hence may have been missed (Tuppurainen *et al.,*2005). Zeynalova *et al.,* (2016) reported that skin nodules are better samples for PCR detection of LSDV than blood samples. LSD virus has high tropism to skin cells with low detection rate in blood due to its presence in blood is confined to fever only. Biopsies of the scabs or skin lesions are more suitable, distinguishable and represented sample for diagnosis of LSD as they are substantial, progress to sit-fasts and contain abundant DNA of LSDV (Amin *et al.,*2015). Negative samples could be due to either of the complete absence of LSDV in a specimen or deficient level of virus that is present in tissue sample below the sensitivity of the assay (El-kholy *et al.,*2008, Awadin *et al.,*2011).

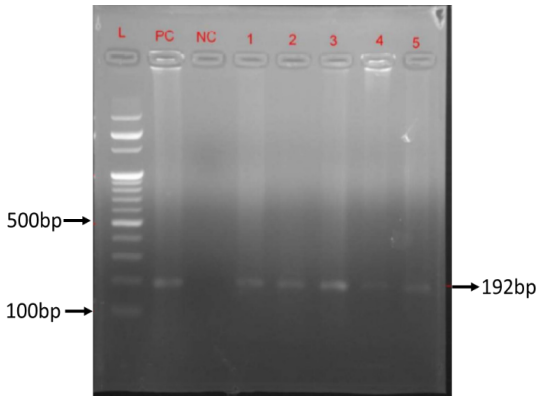

Fig.1: PCR showing bands of 192bp encoding P32 gene of LSDV

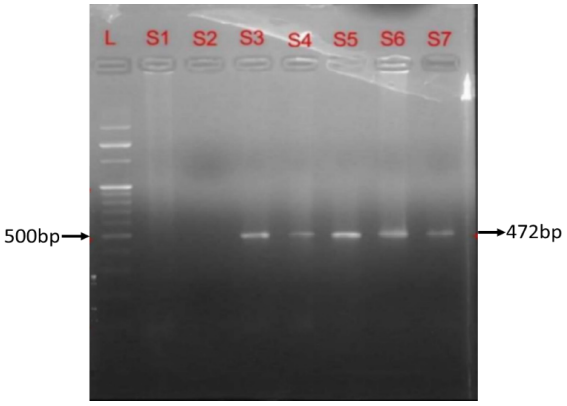

Fig. 2: PCR showing bands of 472bp encoding F gene of LSDV

DNA sequencing and Phylogenetic analysis: To determine the genetic relationship between LSDV strains from India and those circulating in other countries results revealed of nucleotides of P32 gene of LSDV isolates Cattle/LSDV/P32gene/TB/BIDAR-KVAFSU/KARNATAKA/INDIA/2020 and Cattle/LSDV/P32gene/6/BIDAR-KVAFSU/KARNATAKA/INDIA/2020 were aligned with other sequences registered with Genebank and results revealed close sequence identity (100%) with MW147486, MT074103, MT074106 (India) and MT050465(Iran), KX033498 and KX033495 (Zimbabwe). The P32 gene neighbor joining tree showed that all Indian LSDV sequences were identical and clustered in LSD cluster with field strains of LSDV. Nucleotide identity with other field strains of LSDV was 100 % as depicted in Fig.3.

Phylogenetic tree analysis revealed that the current study isolate was having 100 % sequence homology with LSDV isolated from Odisha state. Genetic characterization of circulating LSDV strains provides useful information on molecular epidemiology, tracing of origin of outbreaks, vaccine design and selection of appropriate vaccine for control of LSD. The results agreed with the previous studies of Sudhakar *et al.,* (2020) and Sethi *et al.,* (2022).

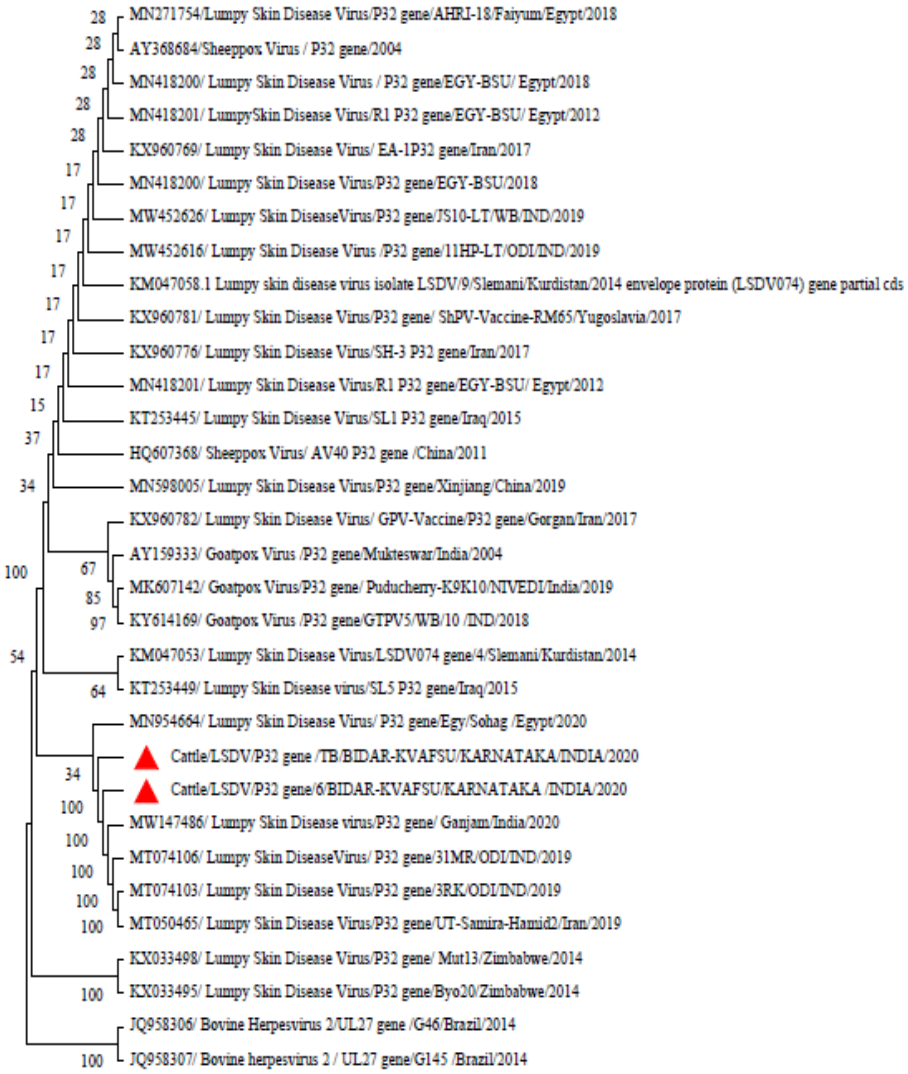

Fig. 3: Phylogenetic analysis of P32 gene of LSDV. The sequence obtained from this study are labelled in filled triangles

Conclusion

In the Bidar region, no previous study on phylogenetic analysis of LSD sample was reported so, the sequences were compared with other known sequences in phylogenetic tree. Existing review suggestive of first report of phylogenetic confirmation of LSDV from the Bidar region, Karnataka. The study confirms circulation of LSDV identical to the virus of Odisha outbreak among the cattle population of this region.

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