Journal of Animal Research: v.15 n.01, p. 37-40. February 2025

**DOI:** 10.30954/2277-940X.01.2025.7

# Inactivation of Lumpy Skin Disease Virus Isolate Obtained from the Field Outbreaks using Binary Ethylenimine

**Bommena Sushma[1*], M. Srinivas[2], L. Gopala[1], Kalyani Putty[3] and G.B. Manjunatha Reddy[4]**

[1]*Department of Veterinary Microbiology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, INDIA*
[2]*Department of Veterinary Microbiology, College of Veterinary Science, Korutla, Telangana, INDIA*
[3]*Department of Animal Biotechnology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, INDIA*
[4]*Senior Scientist, ICAR-NIVEDI, Bengaluru, Karnataka, INDIA*

**Corresponding author: B Sushma; E-mail: bommenasushma2017@gmail.com*

**Received:** 19 Jan, 2025 **Revised:** 25 Feb., 2025 **Accepted:** 28 Feb, 2025

**ABSTRACT**

Present study was aimed to isolate and inactivate the lumpy skin disease virus from the field outbreaks, clinically suspected samples like skin lesions, skin scabs, skin nodules and blood were collected from the affected bovine species. The samples were homogenised and processed, DNA was isolated and they were initially amplified for *Capripoxvirus* specific partial P32 gene, followed by LSDV-specific EEV glycoprotein gene-LSDV126 using respective forward and reverse primers. The PCR positive samples are subjected for isolation and propagation MDBK cells. Once the CPE was noticed in infected flasks, the cells were lysed by three cycles of freeze and thaw, the culture fluid was collected and stored at -20°C. The infected cell culture fluid was subjected for molecular detection by conventional PCR by primers targeting LSDV126 gene which is specific for LSDV. After molecular confirmation the viral isolates were assayed for titres and the infectivity titre was calculated by Reed and Muench (1938) and subjected for inactivation using BEI.

**HIGHLIGHTS**

- Detection of LSD virus in skin lesions, nodules, scabs and blood using Real time PCR.
- Isolation of LSD virus using MDBK cell lines.
- Inactivation of LSD virus using Binary Ethylenimine (BEI).

**Keywords:** LSD virus, P32 gene, EEV glycoprotein, MDBK cells, inactivation, Binary ethylenimine

India's dairy sector is a pivotal component of the nation's agricultural economy, serving as a dependable source of nutrition and income for millions of farmers and pastoralists. As the world's largest milk producer, India reported a total milk production of 230.58 million tons in the fiscal year 2022-2023 (BAHS, 2022-2023). Despite this, the susceptibility of livestock, especially dairy animals, to various bacterial and viral diseases poses a significant challenge. A notable contemporary issue is the outbreak of Lumpy Skin Disease (LSD), caused by the Lumpy Skin Disease Virus (LSDV), belonging to the *Capripoxvirus* genus.

LSD manifests through severe clinical symptoms such as high fever, painful cutaneous nodules, and a marked decline in productivity. This disease incurs considerable economic losses by impacting cattle health and reducing milk yield, thereby threatening rural livelihoods and nutritional security which are heavily dependent on agriculture and livestock farming. In India, the first confirmed case of Lumpy Skin Disease (LSD) was recorded in November 2019 in Odisha. Initially, sporadic outbreaks were observed, but a significant surge in cases occurred by 2022, starting in states such as Gujarat and

**How to cite this article:** Sushma, B., Srinivas, M., Gopala, L., Putty, K. and Manjunatha Reddy, G.B. (2025). Inactivation of Lumpy Skin Disease Virus Isolate Obtained from the Field Outbreaks using Binary Ethylenimine. *J. Anim. Res.,* **15**(01): 37-40.

**Source of Support:** None; **Conflict of Interest:** None

Rajasthan, eventually impacting cattle across 15 Indian states. Between July and September 2022, LSD resulted in the mortality of over 97,000 cattle. This major outbreak led to substantial economic losses, including reduced milk production and restrictions on cattle movement, placing a strain on the dairy industry (Gupta *et al*., 2020; Pankaj *et al*., 2023).

Recent estimates indicate that the outbreak caused economic losses amounting to INR 18,337.76 crores (Bhadauria *et al*., 2023). The morbidity rate of LSD is reported to vary between 50-100%, while the mortality rate is generally low (1-5%). Nonetheless, in certain instances, higher mortality rates have been documented (Kumar *et al*., 2021). Lumpy Skin Disease is classified as a notifiable disease by the World Organisation for Animal Health (WOAH) due to its rapid transmissibility and significant economic impact (WOAH, 2023). Clinically, LSD manifests through symptoms such as fever, cutaneous nodules, lymphadenopathy, edema, reduced milk yield, infertility, abortions, and damage to hides, all of which contribute to substantial economic losses (Tuppurainen and Oura, 2012). Affected animals may also exhibit hypersalivation, nasal discharges, and cachexia (Matsiela *et al*., 2022).

Transmission of LSD can occur through various routes, including indirect contact, shared water sources, and arthropods. The virus is capable of molecular evolution, producing novel phylogenetically distinct variants due to selective pressures. Recombination between field and homologous vaccine strains in cell culture can generate virulent recombinants, posing diagnostic challenges (Mazloum *et al*., 2023).

Vaccination is the most effective method for preventing and controlling Lumpy Skin Disease (LSD) in the absence of specific treatments for viral infections. In India, only live attenuated vaccines are available, which pose risks such as viral shedding, transmission, and potential recombination with field strains, leading to new viral variants. These vaccines are not used in LSD-free countries, highlighting the need for safer alternatives. Efforts have been made to isolate and propagate the Lumpy Skin Disease Virus (LSDV) from field outbreaks, followed by chemical inactivation using Binary Ethylenimine (BEI).

## MATERIALS AND METHODS

Study was carried out in the Department of Veterinary Microbiology and Biotechnology, College of Veterinary Science, Rajendranagar PVNRTVU in collaboration with ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Benguluru. A total of 10 LSDV affected clinically suspected samples like blood, nodules from skin lesions, skin scabs were collected aseptically and are immediately transported to laboratory in the sterile 1X PBS.

### Processing of samples

The tissue samples were homogenised with PBS using mortar and pestle while the blood samples were directly processed for genomic isolation using Quiagen DNeasy Blood and Tissue kit (Cat No:69504 and 69506).

### Molecular detection

After DNA extraction, they were initially amplified for *Capripoxvirus* specific partial P32 gene, followed by LSDV- specific EEV glycoprotein gene-LSDV126 using respective forward and reverse primers where the target amplicon lengths were 237bp for partial P32 gene (Reference: Reddy *et al.* (2015)) and 287bp for LSDV126 (Reference: Inhouse by ICAR-NIVEDI, Bengaluru). On agarose gel electrophoresis, out of 10 samples 6 samples were showing amplification for P32 gene and LSDV126 gene. Ireland and Binepal (1998) and Rashid *et al*. (2017) have targeted the same P32 gene for identification of *Capripoxvirus* and LSD virus and they confirmed that this gene can be successfully targeted for diagnosis of LSDV. Erster *et al*., (2019), Badhy *et al*. (2021) and Menasherow *et al*. (2014) worked on the importance of partial EEV glycoprotein (encoded by ORF LSDV126) which plays an important role in the ability of *Capripoxvirus* to infect cattle also helps to differentiate field strain from the vaccine strain.

### Infection and propagation of LSDV samples

The PCR positive samples were syringe filtered and inoculated into MDBK cells and the infected cell culture flasks were observed daily for five days for CPE

formation and were given three blind passages. After three blind passages the CPE was noticed as rounding, granular formation and aggregation of cells in only three positive samples. The positive CPE flasks were once again confirmed for LSDV by PCR. Similarly, Fay *et al*. (2020) isolated a wild type strain of LSD virus in MDBK cells and LSDV formed foci on MDBK cells, which subsequently developed irregular shapes with long, thin projections

### Assay and inactivation of virus

The virus titre was assessed by infecting the monolayers of MDBK cells in flat bottomed wells and the titres was below $10^{4.5}$ $TCID_{50}$/ml and inactivated using 3 mM BEI at 37C for 24 hrs.

### Test for residual infectivity

An aliquot was collected to check the residual infectivity in MDBK cell lines. No CPE was observed after three consecutive blind passages. Later an aliquot of inactivated virus was kept for sterility check by PDA, BHI and TSA. No contamination was noticed.

## CONCLUSION

It may be concluded that the titres of LSDV which were isolated from the field outbreaks were low (less than $10^{4.5}$ $TCID_{50}$/ml) and the virus was completely inactivated with 3mM BEI. Some authors have also reported low titres. Saber *et al*. (1996) have also reported a low titre of $10^{4.43}TCID_{50}$/ml.

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