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Investigation and Molecular Detection of Lumpy Skin Disease during an Outbreak in Cattle in the State of Tripura, India

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ABSTRACT

The study was conducted during August, 2023 at the Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Agartala, Tripura, India to establish the cause of a suspected lumpy skin disease outbreak in the cattle population at different villages of the Khowai district of Tripura. Lumpy skin disease is a contagious viral disease of cattle caused by lumpy skin disease virus, of the genus *Capripoxvirus* and family *Poxviridae*. The disease spreads mainly via biting insects and is characterized by fever, anorexia, enlarged lymph nodes, and development of firm, round skin nodules. It causes significant economic losses due to reduced milk yield, weight loss, production of inferior quality hides, and mortality in cattle. Tripura is a state located in the northeastern part of India with a 0.735 million cattle population. Animal husbandry is a major mode of income for a substantial part of the rural population, mainly marginal farmers. A large number of animals were affected. The disease was characterized by fever, anorexia, and the development of skin nodules that begin as firm, raised lumps ranging from a few millimeters to several centimeters in diameter, mostly in the neck, ears, limbs, and perineal regions. Over time, some of these nodules underwent necrosis and ulcerated, leading to secondary bacterial infections. In calves, the disease was severe and often fatal. The outbreak of the disease caused significant economic losses to the farmers. As per the clinico-pathological analysis and molecular detection method using samples collected from affected cattle, the disease was confirmed to be LSD.

KEYWORDS: Lumpy skin disease, diagnosis, polymerase chain reaction

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1. INTRODUCTION

Lumpy skin disease (LSD) is an infectious, vector-borne, transboundary viral disease of cattle (Di Giuseppe et al., 2024). It is a notifiable disease as per the World Organization for Animal Health's international epizootic office (Akther et al., 2023). Lumpy skin disease virus (LSDV) belongs to the genus *Capripoxvirus*, subfamily *Chordopoxvirinae*, and family *Poxviridae* (Hidayatik et al., 2025). The virus is enveloped, with its nucleic acid being double-stranded DNA (Namazi and Tafti, 2021). It is also genetically related to sheeppox and goatpox viruses (Givens, 2018; Norian et al., 2019; Pandey et al., 2022). LSDV is very stable and can persist for long periods of time in necrotic tissues, crusts, and air-dried hides (Namazi and Tafti, 2021). Biting flies, mosquitoes, and ticks act as vectors for the virus (Paslaru et al., 2022; Wang et al., 2022; Lubinga et al., 2015). However, transmission via close contact with infected animals or contaminated feed and water troughs was reported (Moudgil et al., 2024). Hot and humid weather is favorable for vector multiplication and activity; therefore, the occurrence of LSD is higher during the summer, monsoon, and autumn (Mulatu and Feyisa, 2018). LSDV is host-restricted and mainly affects. cattle, buffalo, and water buffalo naturally (Ahmed et al., 2021). Animals of all age groups are susceptible (Moudgil et al., 2024). However, younger animals suffer from the severe clinical form of the disease (Ratyotha et al., 2022; Elhaig et al., 2017). The incubation period in natural cases of LSD in cattle ranges from 2 to 5 weeks (Hidayatik et al., 2025; Wolff et al., 2020). LSD is characterized by high fever, swelling of lymph nodes, development of firm, circumscribed skin nodules, and chronic debility. Nodular skin lesions are mostly observed in the muzzle, nares, back, legs, scrotum, perineum, eyelids, lower ear, nasal and oral mucosa, and tail (Beard, 2016; Roy et al., 2025; Sevik and Dogan, 2017). Secondary bacterial infections often complicate the condition, causing suppurating ulcers and abscesses (Kumar et al., 2025; Whittle et al., 2023). LSD is characterized by high morbidity of up to 90%; the mortality rate is generally less than 10% in affected cattle (Liang et al., 2022; Khan et al., 2021). The disease causes significant economic losses as it adversely affects the growth, milk and meat production, quality of hides, and reproductive efficiency of the animal (Naidu et al., 2025; Whittle et al., 2023). Trade restrictions imposed by different countries and outbreak control measures further amplify the economic losses (Tuppurainen et al., 2021). The outbreak of LSD in India during 2022 affected 2.4 million cattle, caused more than 110,000 deaths, and resulted in a financial loss of approximately 60 million USD (Sharma and Kumar, 2025). Since there is no specific treatment for the disease, measures such as surveillance, vector control, hygiene, biosecurity, quarantine, movement restriction, vaccination, and stamping out is important for effective control of LSD (Eom et al., 2023). In India, the disease was first reported from the state of Odisha in August 2019 (Sudhakar et al., 2020). A substantial part of the rural population of the state of Tripura is dependent on animal husbandry, especially the rearing of cattle, for their livelihood. During the year 2023, the state experienced a severe suspected outbreak of LSD at different localities. Incidents of LSD have adversely affected the marginal farmers of the state financially in terms of loss of production, cost of treatment, and mortality. In this study, we have visited a few of the affected villages of the Khowai district of Tripura. Clinico-pathological analysis and molecular detection methods using samples from affected animals confirmed the disease to be LSD.

2. MATERIALS AND METHODS

The study was conducted during the month of August, 2023 at Department of Veterinary Microbiology, College of Veterinary Sciences & A.H., Agartala, Tripura, India, to establish the cause of a suspected lumpy skin disease (LSD) outbreak in cattle population at various villages of the Khowai district of Tripura. Clinico-pathological analysis and polymerase chain reaction were carried out for confirmatory diagnosis of the disease.

2.1. Disease outbreak

A suspected lumpy skin disease (LSD) outbreak was reported in the state of Tripura during August, 2023, affecting a large number of cattle throughout the state. The disease was characterized by high fever, anorexia, and swelling of superficial lymph nodes, followed by the development of circumscribed skin nodules of 0.5–5 cm in diameter. The number of skin nodules ranged from a few to several hundred and was mostly observed in the nares, back, perineum, ear, nasal and oral mucosa, and tail (Figure 1). The skin nodules often ulcerated and formed abscesses as a result of secondary bacterial infection (Figure 2). Severe clinical form accompanied by secondary bacterial infection was observed in calves, resulting in higher mortality than in older cattle (Figure 3). For confirmation of the disease, scabs from skin lesions (n=20) in 50% glycerol saline were collected, chilled on ice, and transported to the laboratory under refrigeration. The samples were stored at -20°C until further processing.

2.2. Processing of samples and nucleic acid extraction

Samples were processed in the laboratory as previously described by Rouby (2018). Scab samples were triturated and homogenized with 50% phosphate buffer saline (PBS) and made into 10% suspension. Then, DNA extraction was performed using a commercial DNA extraction kit following the manufacturer's protocol (NucleoSpin® Tissue, Macherey-Nagel, Germany). After extraction of the DNA,

Figure 1: Calf showing circumscribed skin nodules in neck and ear

Figure 2: Adult cattle showing ulcerated skin lesions

Figure 3: Calf with skin lesions accompanied with severe secondary bacterial infection

purity and concentration were evaluated using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, USA). The extracted DNA was stored at -20°C until further processing.

2.3. Polymerase chain reaction (PCR)

PCR was performed as described by Lamien et al. (2011), using primers (Table 1) targeting 172 bp of the 30 kDa RNA polymerase subunit (RPO30) gene. The PCR was carried out in a 200 µl PCR tube containing 25 µl of PCR Master Mix (EmeraldAmp GT PCR Master Mix, Takara-Bio Inc., USA), forward and reverse primer (0.4 µM each), 5 µl of extracted DNA, and nuclease-free water (NFW) to a 50 µl reaction volume. The resultant reaction mixture was subjected to a precise thermal profile: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 45 sec, and final extension at 72°C for 10 min. The amplification reaction was performed in a T100™ Thermal Cycler (Bio-Rad Laboratories, Inc., USA). For analysis of resultant PCR amplicons, 5 µl of PCR product was electrophoresed on 1.5% agarose gel in Tris-EDTA buffer stained with ethidium bromide (0.5 µg ml^{-1}), in comparison with a 100 bp DNA ladder (HiMedia, Cat. No. MBT049), and visualized and photographed under UV illumination in a GelDoc Go Gel Imaging System (Bio-Rad Laboratories, Inc., USA).

3. RESULTS AND DISCUSSION

Lumpy skin disease (LSD) is an important, vector-borne, contagious disease of cattle. Though the disease was first reported during the year 1929 in Zambia (MacDonald, 1931), it was considered to be mostly confined to the African countries until 1986 (Al-Salihi and Hassan, 2015). After 1986, the disease was reported from various other parts of the world, including Israel, the Middle East, Eastern Europe, and Russia (Davies, 1991). During the year 2019, LSD was reported from various countries in South and East Asia, including China, Bangladesh, and India (Das et al., 2021; Roche et al., 2020). In India, the disease was first reported in the state of Odisha during August 2019 (Sudhakar et al., 2020). Later on, during mid-2022, the disease spread to other parts of the country, including Gujarat and Maharashtra (Bhatt et al., 2023; Kumar and Tripathi, 2022).

There was a suspected outbreak of LSD during August 2023 in different localities of all eight districts of the state of Tripura, and affected cattle irrespective of age, breed, and sex. The timing of the outbreak is in accordance with previous reports that state that the disease mostly occurs during the summer, monsoon, and autumn (Mulatu and

Table 1: Primer set for PCR

Primer	Annealing	Target	Reference
F 5´-TCTATGTCTTGATATGTGGTGGTAG-3´	55°C	RPO30 gene	Lamien et al., 2011
R 5´- AGTGATTAGGTGGTGTATTATTTTCC-3´			

Feyisa, 2018). The severe form of the disease was mostly recorded in younger animals, which is also consistent with previous reports (Ratyotha et al., 2022; Elhaig et al., 2017). The disease was characterized by fever, anorexia, a drop in milk production, enlargement of lymph nodes, and development of firm, round skin nodules, which is consistent with earlier findings (Roy et al., 2025; Kumar and Tripathi, 2022; Ratyotha et al., 2022). Morbidity and mortality rates in cattle reached approximately 25–30% and 5–10%, respectively; similar to previous reports by different workers (Jamil et al., 2022; Liang et al., 2022; Khan et al., 2021). Secondary bacterial infection often resulted in ulcer and abscess formation, complicating the case, consistent with earlier reports (Kumar et al., 2025; Whittle et al., 2023).

On agarose gel electrophoresis of the PCR amplicon generated by using primers targeting the 30 kDa RNA polymerase subunit (RPO30) gene of *Capripoxvirus, the* expected band size of 172 bp was visualized and documented under UV transillumination (Figure 4) in the GelDoc Go Gel Imaging System (Bio-Rad Laboratories, Inc., USA). All the samples collected from clinically affected animals were positive for LSDV.

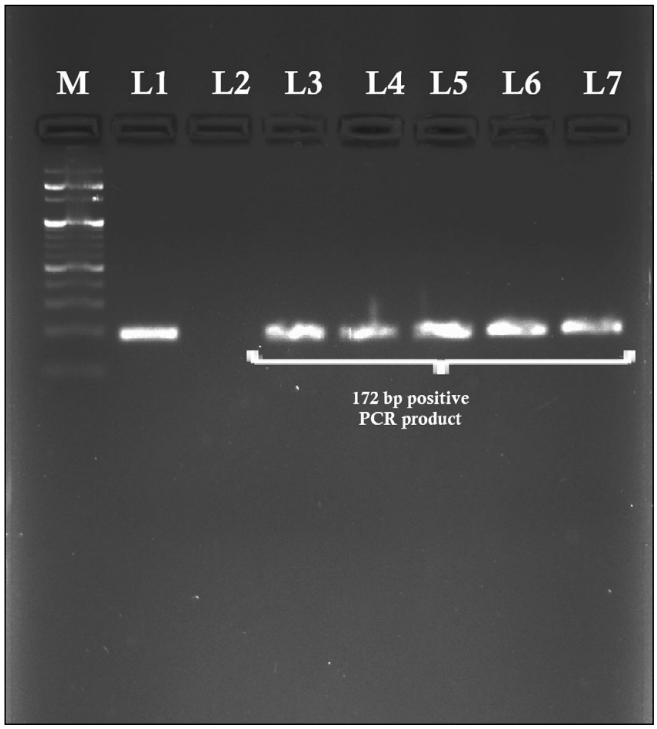

Figure 4: Result of PCR using primers targeting 172 bp of the RPO30 gene of *Capripoxvirus*, confirming presence of LSDV, M: 100 bp DNA Ladder (Himedia, Cat. No. MBT049), Lane 1: Positive control, L2: Negative control, L3-L7: PCR products from samples

Viremia in LSD is considered to be short and has a limited window period for detection, i.e., 4–11 days before development of the skin lesions. Whereas, skin lesions serve as a more suitable sample for LSD as it provides a longer window period of up to 92 days for detection (Sudhakar et al., 2020; Tuppurainen et al., 2005). The 30 kDa RNA polymerase subunit (RPO30) gene of *Capripoxvirus* has been used by several workers before for detection as well as phylogenetic analysis of lumpy skin disease virus (Ma et al., 2022; Makoga et al., 2024; Omoniwa et al., 2023; Rouby, 2018; Sudhakar et al., 2020).

4. CONCLUSION

Suspected lumpy skin disease (LSD) outbreak in cattle at different villages of Khowai district of Tripura was investigated. Scab samples from a suspected animal, known to provide a longer window for detection of the virus was collected and subjected to polymerase chain reaction (PCR) targeting 172 bp of the 30 kDa RNA polymerase subunit (RPO30) gene of *Capripoxvirus*. Clinico-pathological findings and the result of PCR confirmed the disease to be LSD.

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