SHORT COMMUNICATION

Gross-Morphological, Histopathological and Molecular Investigation of Lumpy Skin Disease in Naturally Affected Cattle in Jaipur, India

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Abstract

Lumpy skin disease (LSD) is a highly contagious transboundary animal disease caused by the Lumpy skin disease virus (LSDV), a member of the *Capripoxvirus* genus within the *Poxviridae* family. This study examined sporadic cases of Lumpy skin disease (LSD) in cattle from India, spanning September 2023 to December 2024 and explored the gross-morphological, histopathological and molecular diagnosis of Lumpy Skin Disease. Affected cattle exhibited multiple cutaneous nodules of varying sizes (2-5 cm) on the head, neck, limbs, udder and other body parts. Post-mortem examination revealed significant thickening of the epidermis, extending into the dermis and involving subcutaneous and muscular layers. Histopathological analysis demonstrated characteristic features, including ballooning degeneration, microvesicle formation and intracytoplasmic inclusion bodies. Molecular diagnostics using PCR assays confirmed the presence of LSDV in tested cases, successfully amplifying the *P32* antigen gene. These findings enhance our understanding of LSD pathology and highlight the importance of molecular techniques for accurate disease confirmation.

Keywords: Cattle, Intra cytoplasmic inclusion bodies, Lumpy skin disease (LSD), *P32*.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.5.31

Introduction

Lumpy skin disease (LSD), also known as pseudo-urticaria, neethling virus disease or knopvelsiekte, is a vector borne, zoonotic viral disease of cattle and buffalo with significant economic implications. Lumpy skin disease virus (LSDV) is a member of the *Capripoxvirus* genus within the *Poxviridae* family has a 151-kilobase double-stranded DNA genome and is structurally an enveloped, ovoid virion. While cattle and buffalo are the primary hosts, the virus has also been detected in wild ruminants, indicating its potential to infect a wide range of species (Tuppurainen *et al.,* 2017; Afzal *et al.,* 2024). LSD is primarily transmitted via blood-feeding arthropods such as flies, mosquitoes and ticks, with secondary transmission occurring through bodily secretions and contaminated environments (Sprygin *et al.,* 2018). The most severe outbreak was reported in Rajasthan in 2022. The disease is classified as a notifiable disease by the World Organization for Animal Health (OIE) due to its rapid transmission, economic impact and threat to livestock industries. This study reports the gross-morphological, histopathological and molecular aspects of lumpy skin disease in naturally affected cattle in Jaipur, India

Materials and Methods

A total of 85 cutaneous nodules were collected from cattle died due to LSD suggestive by the generalized cutaneous nodules all over the body in Jaipur. Samples were obtained immediately after post-mortem and stored in two separate containers. For histopathology, samples were preserved in 10% buffered neutral formalin at room temperature for

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How to cite this article: Longesha, F., Meena, S., Devi, G.., Jyoti, Deepika, Mandawat, S., & Khandelwal, P. (2026). Gross-Morphological, Histopathological and Molecular Investigation of Lumpy Skin Disease in Naturally Affected Cattle in Jaipur, India. Ind J Vet Sci and Biotech, 22(5), 167-170.

Source of support: Nil

Conflict of interest: None

Submitted 14/06/2026 **Accepted** 29/06/2026 **Published** 10/09/2026

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analysis at the Department of Veterinary Pathology, PGIVER, Jaipur. For molecular analysis, the samples were stored in viral transport media and transported to the CDSRZ Laboratory at PGIVER, Jaipur, where they were stored at -80°C.

Histopathological Examination

Formalin-fixed tissues were processed for paraffin embedding using the acetone-benzene technique (Lillie, 1965). Tissue sections (4-6 µm) were stained using the Haematoxylin and Eosin (H&E) method (Suvarna *et al.*, 2018) for microscopic evaluation.

Polymerase Chain Reaction (PCR)

DNA was extracted from the nodular samples using the HiPurA Multi-Sample DNA Kit (HiMedia) as per the manufacturer's instructions. PCR amplification targeted the *P32* gene with 10 µL reaction mixture consisting of 1 µL 10X buffer, 1 µL dNTPs, 0.5 µL each of forward (F: 5'-TCCGAGCTCTTTCCTGATTTTTCTTACTAT-3') and reverse (R: 5'-TATGGTACC TAAATTATATACGTAAATAAC-3') primers (Ireland and Binepal, 1998), 0.4 µL Taq polymerase, 4 µL DNA template, and 2.6 µL nuclease-free water.

PCR conditions included an initial denaturation at 94°C for 7 min, followed by 42 cycles each of denaturation at 94°C for 60s, annealing at 52°C for 30s and extension at 72°C for 60s. A final extension was performed at 72°C for 10 min. PCR products were analyzed using 1.5% agarose gel electrophoresis and the presence of a 192 bp product confirmed *LSDV* infection.

Results and Discussion

Gross Pathology and Histopathology

Multiple cutaneous nodules of varying sizes (2-5 cm) were observed on the head, neck, limbs, udder and other body parts (Fig. 1). Post-mortem examination revealed significant thickening of the cutaneous nodule, epidermis extending into the dermis and involving subcutaneous and muscular layers (Fig. 2).



Fig. 1: Multiple nodules observed on the various part of body including neck, thorax, abdomen and rest part of body.



Fig. 2: Gross photograph of cutaneous nodule demonstrating the significant thickening of the epidermis extending into the dermis and involving subcutaneous and muscular layers.

Microscopic examination disclosed characteristic histopathological changes in the epidermis, including ballooning degeneration, hyperkeratinization and the presence of eosinophilic intracytoplasmic inclusion bodies (Figs. 3, 4, 5). In the dermis, significant alterations were observed, such as extensive connective tissue proliferation, vascular congestion, Zenker's necrosis and sparse infiltration of inflammatory cells (Figs. 6, 7). These observations were consistent with findings reported in multiple studies, including Amin *et al.* (2015), Vaskovic *et al.* (2019) and Trinh *et al.* (2022), which collectively document comparable histopathological features. Specifically, these studies describe ballooning degeneration of epithelial cells, predominantly within the stratum spinosum and basal cells in affected epidermal cells. Furthermore, eosinophilic intracytoplasmic inclusion bodies were frequently noted within epidermal epithelial cells and keratinocytes of the keratin layer, a feature widely recognized as pathognomonic for poxvirus infections, thereby substantiating the viral origin of these lesions.

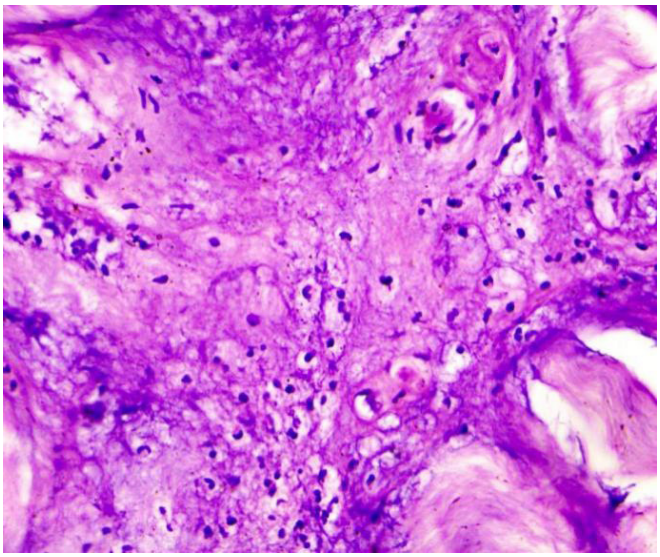


Fig. 3: Photomicrograph of skin showing vacuolar degeneration, accompanied by the infiltration of a few inflammatory cells. (H & E, 100X).

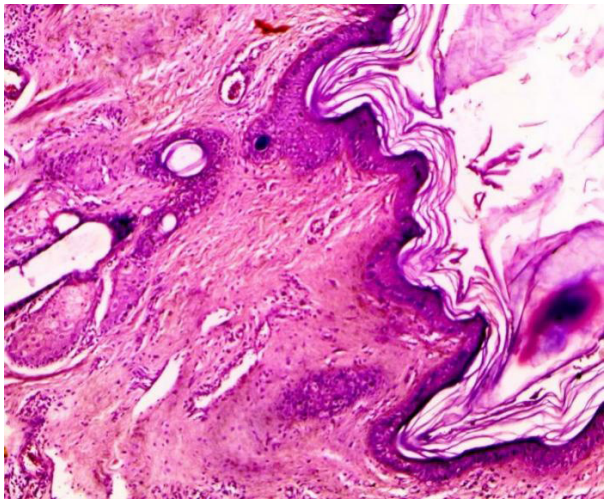


Fig. 4: Photomicrograph of skin demonstrating prominent hyperkeratinization. (H & E, 100X).

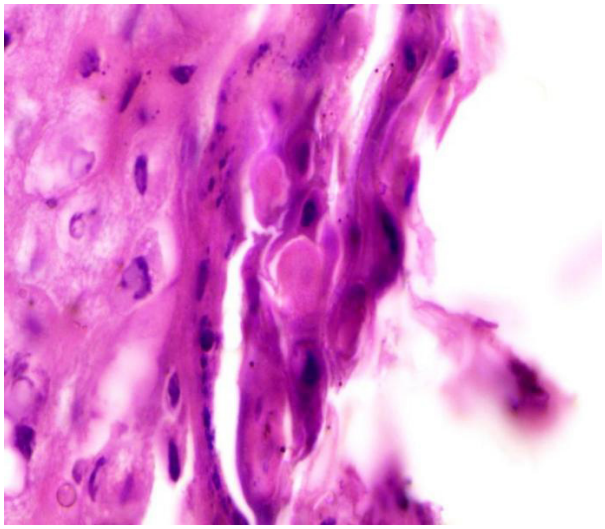


Fig. 5: Photomicrograph of skin tissue revealing the presence of the intracytoplasmic inclusion bodies in keratinocyte of keratin layer. ((H & E, 1000X).

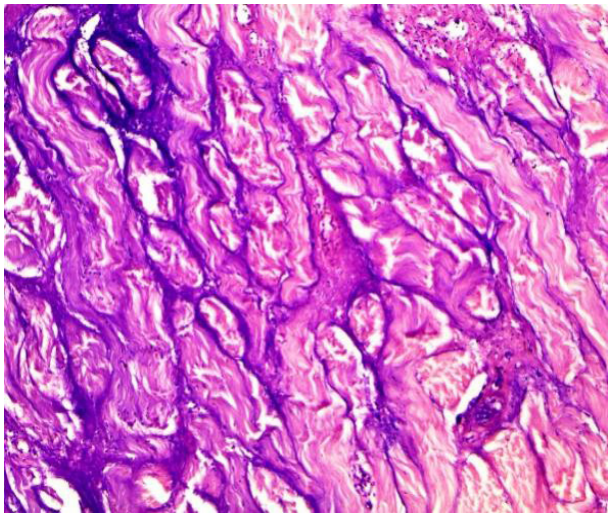


Fig. 6: Photomicrograph of a skin section showing Zenker's degeneration of connective tissue with a few inflammatory cells. (H & E, 100X).

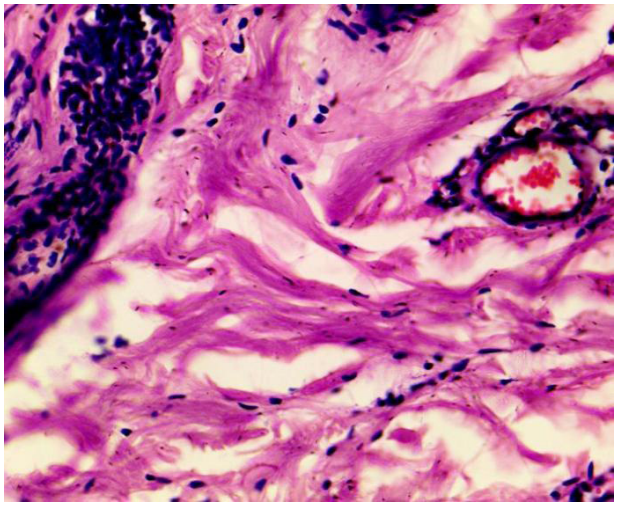


Fig. 7: Photomicrograph of skin showing congestion of blood vessels, hyaline degeneration of connective tissue and infiltration of a few inflammatory cells. (H & E, 400X).

Poxviruses are known to replicate within the cytoplasm of host epithelial cells (Murphy *et al.*, 1999), leading to the formation of eosinophilic intracytoplasmic inclusions through the accumulation of viral particles. These inclusions represent a hallmark histopathological feature essential for the diagnosis of Lumpy Skin Disease Virus (LSDV) infections. Additionally, hyperkeratinization, characterized by a marked increase in the thickness of the keratin layer, emerged as a recurrent finding in LSD lesions, a pattern corroborated by Vaskovic *et al.* (2019) and Trinh *et al.* (2022). These studies underscore the progressive nature of LSDV-induced cutaneous pathology.

In addition, our investigation identified Zenker's degeneration within the dermal connective tissue, defined by coagulative necrosis of collagen fibers. This finding suggests severe tissue injury, likely attributable to the cytopathic effects of the virus and the ensuing inflammatory response. This observation aligned with previous reports by Amin *et al.* (2015) and Vaskovic *et al.* (2019), reinforcing the association between these histopathological changes and LSDV infection. Collectively, these findings enhance the understanding of the tissue-level manifestations of LSDV and their diagnostic implications.

Molecular Analysis

In this study, tissue samples were obtained during postmortem examinations of cattle that succumbed due to LSD and were subsequently analyzed using conventional capripox-specific polymerase chain reaction (PCR) techniques. The PCR assay successfully amplified an approximately 200-base-pair fragment of the *P32* gene, which serves as a reliable diagnostic marker for LSD. A total of 85 tissue samples underwent molecular analysis, with 36 (42.35%) testing positive for the *P32* gene. The PCR assays confirmed the presence of LSDV in the tested samples, yielding specific bands at 192 base pairs (Fig. 8).

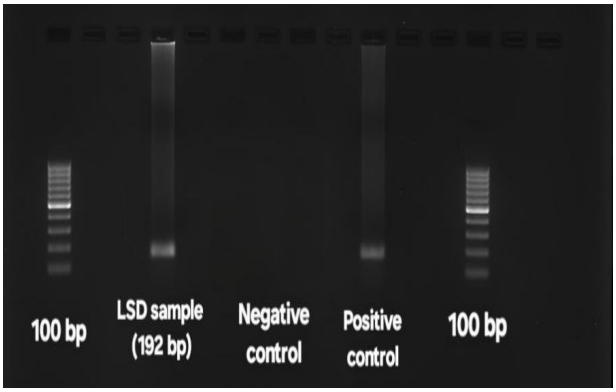


Fig. 8: PCR band 192 bp after Gel electrophoresis indicating presence of lumpy skin disease virus tested in tissue sample.

Scabs and skin lesions were identified as the most suitable and representative samples for LSD diagnosis due to their distinct appearance, progression into sit-fasts and high concentration of LSDV DNA (Amin *et al.*, 2015). Negative results may be attributed to either the complete absence of LSDV in the sample or viral loads below the assay's detection threshold (El-Kholy *et al.,* 2008; Awadin *et al.,* 2011). The *P32* enveloped protein gene encodes a highly antigenic structural component present in all capripoxvirus strains. This gene is integral to LSDV pathogenicity and diagnosis, making it a widely adopted target for both serological and molecular detection methods (Tian *et al.,* 2010). These findings underscore the efficacy of targeting the *P32* gene in the molecular confirmation of LSDV infection and highlight the importance of sample selection in achieving accurate diagnostic outcomes.

In general, this study offers critical insights into the pathology and molecular diagnosis of Lumpy Skin Disease (LSD) in cattle. Examination of affected cattle in Jaipur, India, revealed characteristic lesions, including cutaneous nodules, ballooning degeneration, eosinophilic intracytoplasmic inclusion bodies, and Zenker's degeneration. The presence of widespread cutaneous lesions strongly indicated LSD infection, with histopathological evidence of eosinophilic intracytoplasmic inclusion bodies providing further confirmation. PCR analysis substantiated the presence of the LSDV by amplifying the *P32* gene, yielding a 192 bp fragment in 42.35% of the tested cases. Samples from scabs and skin lesions proved to be reliable substrates for diagnosis, underscoring the efficacy of PCR as a diagnostic tool.

Acknowledgement

We thank the Dean, PGIVER, Jaipur, for providing facilities, and the CDSRZ staff for their support.

References

Afzal, H., Umar, M., Hussain, W., & Cheng, L.T. (2024). Lumpy skin disease: An encroaching risk in cattle farming. *Taiwan Veterinary Journal, 49*(01), 1-11.

Amin, A., El-Nahas, E., & El-Mashed, A.E. (2015). Pathological and virological studies on an outbreak of lumpy skin disease among cattle in Kalubia Governorate-Egypt. *Journal of Advanced Veterinary Research, 5*(4), 165-175.

Awadin, W., Hussein, H., Elseady, Y., Babiuk, S., & Furuoka, H. (2011). Detection of lumpy skin disease virus antigen and genomic DNA in formalin-fixed paraffin-embedded tissues from an Egyptian outbreak in 2006. *Transboundary & Emerging Diseases, 58*(5), 451-457.

El-Kholy, A.A., Soliman, H.M.T., & Abdelrahman, K.A. (2008). Polymerase chain reaction for rapid diagnosis of a recent lumpy skin disease virus incursion to Egypt. *Arab Journal of Biotechnology, 11*(2), 293-302

Ireland, D.C., & Binepal, Y.S. (1998). Improved detection of Capripoxvirus in biopsy samples by PCR. *Journal of Virology Methods, 74*(1), 1-7.

Lillie, R.D. (1965). *Histopathological Technique and Practical Histochemistry*. New York and London: McGraw Hill Book Co.

Murphy, F.A., Gibbs, E.P.J., Horzinek, M.C., & Studdert, M.J. (1999). *Veterinary Virology*. 3rd edn., Elsevier, pp: 87-285.

Sprygin, A., Artyuchova, E., Babin, Y., Prutnikov, P., Kostrova, E., Byadovskaya, O., & Kononov, A. (2018). Epidemiological characterization of lumpy skin disease outbreaks in Russia in 2016. *Transboundary and Emerging Diseases, 65*(6), 1514-1521..

Suvarna, K.S., Layton, C., & Bancroft, J.D. (2018). *Bancroft's Theory and Practice of Histological Techniques E-Book*. 7th edn., Elsevier health sciences, pp. 4-128.

Tian, H., Chen, Y., Wu, J., Shang, Y., & Liu, X. (2010). Serodiagnosis of sheeppox and goatpox using an indirect ELISA based on synthetic peptide targeting for the major antigen *P32. Virology Journal, 7*(1), 1-4.

Trinh, T.B.N., Nguyen, V.T., Nguyen, T.T.H., Mai, N.T.A., Le, P.N., Lai, T.N.H., Phan, T.H., Tran, D. H., Pham, N.T., Dam, V.P., Nguyen, T.L., Ambagala, A., Babiuk, S., & Le, V.P. (2022). Molecular and histopathological characterization of lumpy skin disease in cattle in northern Vietnam during the 2020–2021 outbreaks. *Archives of Virology, 167*(11), 2143-2149.

Tuppurainen, E.S.M., Venter, E.H., Shisler, J.L., Gari, G., Mekonnen, G.A., Juleff, N., Lyons, N.A., Clercq, K.D., Upton, C., Bowden, T.R., Babiuk, S., & Babiuk, L.A. (2017). Capripoxvirus diseases: current status and opportunities for control. *Transboundary and Emerging Diseases, 64*(3), 729-745.

Vaskovic, N., Debeljak, Z., Vidanovic, D., Sekler, M., Matovic, K., Anicic, M., & Marinkovic, D. (2019). Morphological characteristics of skin lesions in cattle naturally infected with lumpy skin disease virus in Serbia. *Acta Veterinaria, 69*(4), 369-378.