Indian J Anim Health (2025), 64(1) - Special Issue: 239-243
DOI: https://doi.org/10.36062/ijah.2025.07624

Short Communication

Outbreak of Lumpy skin disease (LSD) in Mizoram, India

T. K. Dutta[1*], P. Roychoudhury[1], C. Lalsangzuala[1] and Zohmingthangi[2]

[1]Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl- 796 015, Mizoram, India; [2]Deputy Director, Disease Investigation Laboratory, Directorate of Veterinary and Animal Husbandry, Khatla, Aizawl- 796 001, Mizoram, India

Abstract

Lumpy skin disease (LSD) is a newly emerged disease of bovines in India. In India, the first outbreak of LSD was reported from Odisha in 2019. Since then, it has spread over various states of the country. A total of 54 samples from 18 affected animals from Aizawl (n=16) and Champhai (n=2) districts were collected. All the samples were processed for detection of LSD virus by PCR and further analyzed by DNA sequencing. The DNA sequences were analyzed to generate the phylogenetic relationship with other circulating LSDV in this region as well as countries. All the skin scabs from affected animals were recorded as positive for LSDV, and the circulating viruses were found to be related to other LSDV viruses of Assam, Odisha, West Bengal, Andhra Pradesh, as well as Chinese strains. To the best of our knowledge, this is the first report of LSD outbreak in Mizoram.

Keywords: India, Lumpy skin disease, Mizoram

Highlights

- First outbreak of LSD in Mizoram is reported.
- Eight villages in three districts of Mizoram were affected.
- Circulating virus might be transmitted from nearby states or through porous border of Myanmar.

Lumpy skin disease (LSD) is an economically important emerging, infectious, vector-borne, non-zoonotic disease of large ruminants (cattle and buffalo) with transboundary potential. The disease is characterized by moderate to high fever, enlarged lymph nodes, increased nasal and pharyngeal secretions, anorexia followed by emaciation, generalized depression, loss of milk yields in milching animals, nodular skin lesions, and infertility in male animals. Incubation period is varied between 4 to 12 days. The morbidity of the disease remains high ranging from 50 to 100% but mortality is very low (1-5%) (Kumar *et al*., 2021). Although LSDV was first time recorded in Zambia in the year 1929, the first outbreak in India was reported in Odisha in the year 2019 (Sudhakar *et al*., 2019).

Mizoram (23.1645° N, 92.9376° E) is a small, landlock hilly state in the North eastern region of India, sharing large international border with Myanmar and Bangladesh. The economy of the state is largely dependent on agriculture, horticulture and animal husbandry practices by the rural population. Although pigs remain the major livestock, poultry and cattle rearing are quite popular as a source of income. Being closely attached to a porous international border, Mizoram has witnessed many transboundary animal disease outbreaks in the recent past, *viz*., porcine respiratory and reproductive syndrome (PRRS) (Rajkhowa *et al*., 2015), African swine fever (ASF) (Rajkhowa *et al*., 2022). In addition, the state is highly dependent on other parts of the country to supply quality chicks, piglets, eggs, fish, and concentrated animal feed, which increases the risks of transmission of animal diseases in this region from other parts of the country as well as transmission from other countries due to porous international borders.

In the present study, we report the first outbreak of LSD in cattle of Mizoram, India with special emphasis on laboratory diagnosis and phylogenetic analysis of the LSDV associated with the outbreak.

Suspected LSD like symptoms were reported from Aizawl (23.7307° N, 92.7173° E), Champhai (23.4775° N, 93.3237° E) and Mamit (23.9284° N, 92.4907° E) districts of Mizoram during July 2023 to November 2023. A total of 56 dairy cattle were recorded with suspected symptoms like high fever, anorexia, generalized depression, dysgalactia, typical skin nodules, etc., on the body surface. Among the 56 infected cattle, 40, 11 and 5 were recorded from Aizawl (5 villages), Champhai (2 villages) and Mamit (1 village) districts,

*Corresponding Author, E-mail: tapandutta@rediffmail.com

respectively. Overall morbidity and mortality were 50.91% (56/110) and 3.64% (4/110), respectively. All the animals were maintained under a semi-intensive system, where 6-15 cattle were maintained under one shed. The age of the animals ranged between 6 months and 8 years.

A total of 18 skin scabs, 18 whole blood and 18 nasal swabs from 18 affected animals from Aizawl (n=16) and Champhai (n=2) districts were collected following aseptic conditions and were transported to the laboratory on ice.

DNA was extracted from all the samples using commercial DNA extraction kits (QIAGEN) following the instructions of the manufacturer. Extracted DNA were further checked for its purity and quantity by using a Bio-spectrophotometer (Eppendorf, Germany) and stored at -20°C until further use. All the samples were subjected to amplification of p32 gene of LSDV virus by polymerase chain reaction (PCR) using specific oligonucleotide primers (LSDF: 5'-ACTAGTGGATCCATGGACAGAGCTTTATCA-3' and LSDR: 5'-GCTGCAGGAATTCTCATA GTGTTGTACTTCG-3') (Sudhakar *et al*., 2019). PCR assays were performed in 0.2 mL thin-walled tube (Eppendorf, Germany) with total volume of 25 µL containing 12.5 µL PCR mastermix (TaKaRa), 1 µL (20 pmol) of each primer, 5 µL of extracted DNA and adjusted the volume with 5.5 µL of nuclease free water. The PCR assays were performed in a thermal cycler (Eppendorf, Germany) with the following cycling regime: initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 45 second, annealing at 50°C for 30 second and extension at 72°C for 45 second followed by final extension at 72°C for 5 min. Genomic DNA extracted from commercially available goatpox virus vaccine was used as positive control. All the PCR products were analyzed by agarose gel electrophoresis on 1.0% agarose gel in 1X TAE buffer at 50V for 2 hours. The gel was stained with ethidium bromide (2 µg/mL) and visualized the amplicon under UV transilluminator, and documented by a gel documentation system (Eppendorf, Germany). One 100 bp DNA ladder was used as positive marker to compare the size of the amplicons. Four representative samples were subjected to sequencing of partial sequence of p32 gene at Delhi University, South Campus using Sanger sequencing method. The nucleotide sequence data were verified with BLAST n software in GenBank to know the sequence identity with GenBank sequence data. The phylogenetic analysis of the sequences was done using MEGA X software (https://www.megasoftware.net) by adopting the maximum likelihood method using the Kimura 2-parameter model.

The affected animals exhibited typical symptoms like high fever, anorexia, generalized depression, dysgalactia, typical skin nodules, etc., on the body surface (Fig. 1). Amongst the three affected districts, Aizawl district was mostly affected (40/56), followed by Champhai (11/56) and Mamit (5/56). On analysis of the clinical specimens by PCR, all the scab samples were found to be positive for 192 bp amplicon, whereas none of the whole blood samples were recorded as positive by PCR (Fig. 2). The p32 gene sequence data

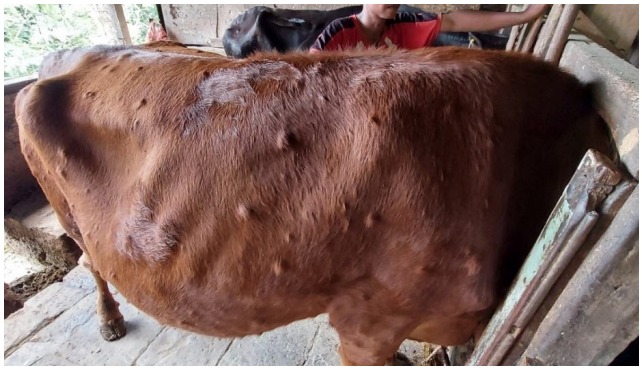

Fig. 1. Clinical signs of lumpy skin disease in a cow suffering from severe erosions and nodules

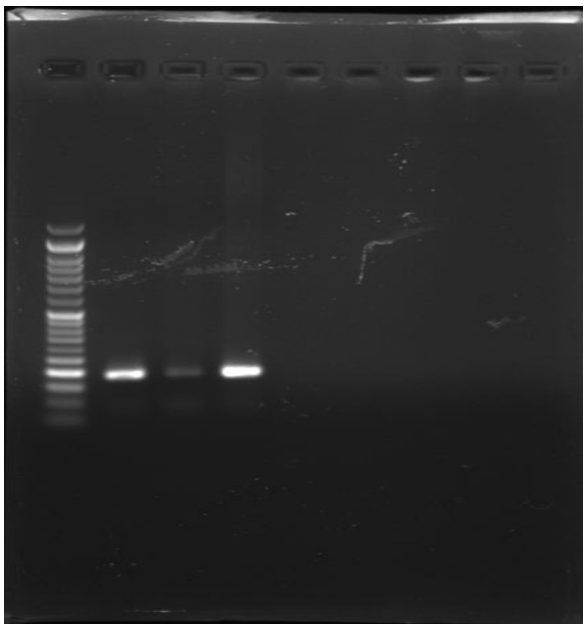

Fig. 2. PCR amplification of 192 bp fragment of partial P32 gene of LSDV

[Lane-M :50bp DNA ladder; Lane-1, 2 and 3 (positive amplicons); lane-4 (NTC)]

were analyzed for phylogenetic relationship with other LSDV as well as the closely related sheeppox and goatpox viruses circulating in this region. The representative 4 sequence data were submitted to Gene bank and assigned the accession number PP464711, PP464712, PP464713 and PP464714. The four representative sequences were used for generation of phylogenetic tree and found that all the four samples were under same cluster of LSDV along with the Neethling vaccine virus, isolates from West Bengal, Karnataka and also from Egypt (Fig. 3).

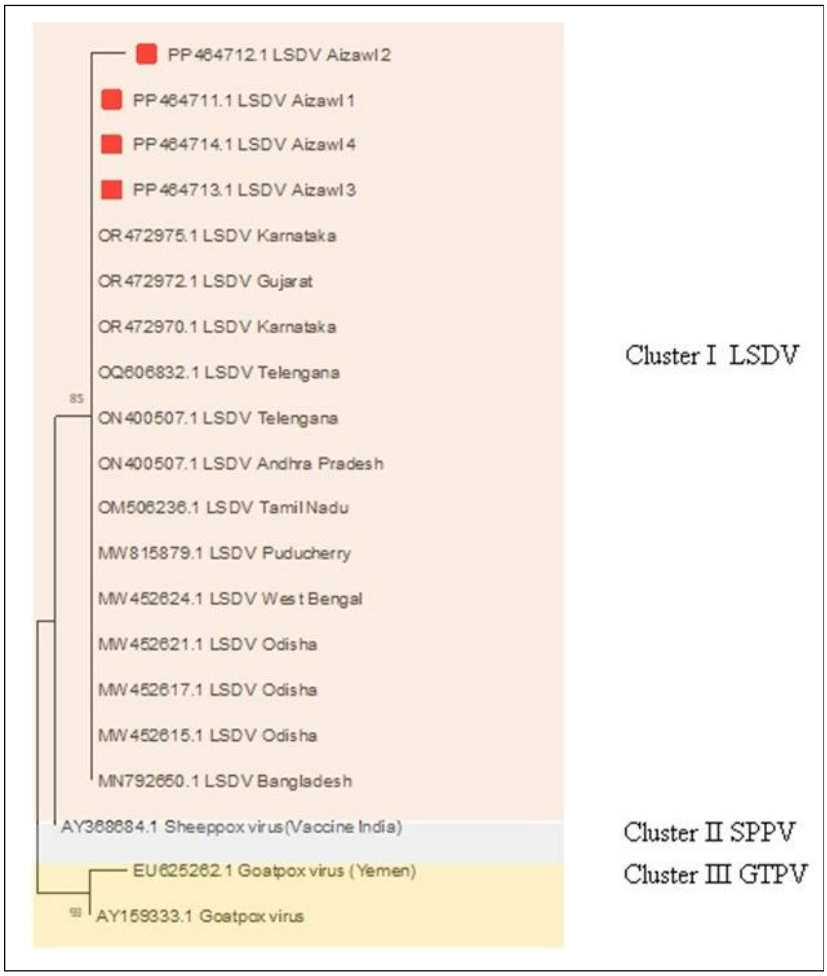

Fig. 3. Phylogenetic tree of LSDV strains circulating in Mizoram based on genetic sequence of ORF 117 gene of LSDV

The clinical signs recorded in this study including marked painful skin lesions all over the body in all affected animals, but the young animals exhibited more severe lesions. In adult cattle, skin lesions were distributed mainly on the head, neck, jowl, back, udder, and teats (Fig. 1). The lesions were initiated as small nodules, which eventually ruptured and showed marked bleeding areas. The other clinical signs observed are enlargement of lymph nodes and pneumonia. These clinical signs were in agreement with Salib and Osman (2011). In the present study, the clinical signs were observed without any distinction between the age and/or breeds of the affected animals, but the clinical symptoms were more severe in calves compared to the adults. Nearly similar observations were also cited by Al-Salihi (2014) and Kavitha *et al.* (2021). During the present study, we could not trace the exact mode of transmission of the virus to this region. In another study, Kavitha *et al.* (2021) recorded the first outbreak of LSD in Andhra Pradesh and concluded that there was no tick population responsible for the transmission of the LSD virus. As a result, the possible role of other vectors, *viz.*, biting lies, and other modes of horizontal transmission cannot be ruled out (Ali *et al.*, 2012).

Although the clinical symptoms were very prominent to assume the tentative diagnosis of the disease, but being the first outbreak, it was further confirmed by PCR based technique followed by sequencing of the amplicons. PCR was performed for amplification of p32 gene, which is specific for LSDV (Sudhakar *et al.*, 2019) using specific oligonucleotide primers. As mentioned earlier, all the skin swabs were found to be positive for 192 bp amplicon, which is specific for LSDV (Sudhakar *et al.*, 2019). None of the blood or nasal swabs were recorded as positive by PCR. A similar observation was also recorded by other workers in India (Kavitha *et al.*, 2021) and abroad (Zeynalova *et al.*, 2016), which might be due to the non-viremic stage of the animals during sample collection and predilection of the virus in the skin. In addition, during the collection of samples, none of the animals showed high body temperature, which is also in corroboration of the observation made by Zeynalova *et al.* (2016) and Kavitha *et al.* (2021).

PCR based detection of the LSDV is found to be useful in early diagnosis and tracing the origin of the outbreak. In the present study, the isolates were found to be closely related to other LSDV circulating in other states of India as well neighboring countries (Fig. 3).

To the best of our knowledge, this is the first report of the existence and molecular characterization of LSDV circulating in Mizoram state of India. The p32 gene was studied from four different districts of Mizoram, which revealed that all four samples were under the same cluster of LSDV along with the Neethling vaccine virus, isolates from West Bengal, Karnataka and also from Egypt (Fig. 3).

The present study probably offers a very good insight about the spread of LSDV in Mizoram through some unknown sources and rapid spread of the disease in different districts within a short period of time. Earlier, Kavitha *et al*. (2021) also highlighted the similar concern about the rapid spread of the disease in Andhra Pradesh from its neighboring state Odisha coastal district, which is well supported by the evidence of phylogenetic analysis of the circulating LSDV strains. The present outbreaks in Mizoram might be due to the spread of the vector from neighboring states like Assam through the transport of live animals or animal containers, which need to be ascertained for proper disease monitoring and designing of effective control measures. But Mizoram, being the closest bordering state with a porous international border with Myanmar, remains vulnerable to many transboundary animal diseases. Therefore, transmission of the LSDV through illegal movement of animals from Myanmar cannot be ruled out. In the past also, a similar incidence happened when PRRS was first time reported in Mizoram and found to be transmitted from Myanmar due to the illegal movement of pigs through the porous international border (Rajkhowa *et al*., 2015). Early diagnosis and reporting system may also help in containment of the diseases within small geographical region. In addition, in Mizoram, the goatpox virus vaccine was used in the peripheral region just after the report of the outbreak to control further transmission. However, many scientific reports recommended the use of a homologous vaccine because of its host specificity. The cross-protection of SPPV/GTPV-based vaccines in cattle used to develop lower efficacy than live attenuated LSDV vaccines (Tulman *et al*., 2002; Lamien *et al*., 2011; Tuppurainen *et al*., 2014: Sprygin *et al*., 2020).

Therefore, the present investigation revealed the first outbreak of LSDV infection among the bovine population of Mizoram, India. The disease was probably transmitted from Assam or Myanmar through some vectors of fomites. The phylogeny of the p32 gene sequences revealed that viruses were in close association with circulating viruses from Assam, Odisha, Andhra Pradesh, and Bangladesh.

Conflict of interest: The authors certify that there is no conflict of interest in the publication of the article.

Author's contribution: TKD: Conceptualization, manuscript preparation; PRC: Laboratory work for detection of organisms, data analysis; CL: Collection and processing of samples; Z: Collection of metadata and collection of samples.

Data availability statement: All the data supporting the research findings have been presented in this paper. The corresponding author is willing to provide the raw data upon reasonable request.

ACKNOWLEDGEMENTS

The authors are thankful to the DBT project on Establishment of a Consortium for One Health to address Zoonotic and Transboundary Diseases in India, including the Northeast Region (BT/PR39032/ADV/90/285/2020 dated 06/08/2021) for providing all the facilities. They are also highly thankful to the Dean of the college for extending all the help in conducting the study.

REFERENCES

Ali H, Ali AA, Atta MS and Cepica A, 2012. Common, emerging, vector-borne and infrequent abortogenic virus infections of cattle. Transbound Emerg Dis, 59(1): 11-25, doi: 10.1111/j.1865-1682.2011.01240.x

Al-Salihi K, 2014. Lumpy skin disease: Review of the literature. Mirror Res Vet Sci Anim, 3(3): 6-23

Kavitha Kl, Sreedevi B and Rajesh K, 2021. Clinico-molecular diagnosis and molecular characterization of bovine lumpy skin disease virus in Andhra Pradesh, India. Trop Anim Health Prod, 53(4): 424, doi: 10.1007/s11250-021-02872-3

Kumar N, Chander Y, Kumar R, Khandelwal N, Riyesh T *et al*., 2021. Isolation and characterization of lumpy skin disease virus from cattle in India. PLoS One, 16(1): e0241022, doi: 10.1371/journal.pone.0241022

Lamien CE, Lelenta M, Goger W, Silber R, Tuppurainen E *et al*., 2011. Real time PCR method for simultaneous detection, quantitation and differentiation of capripox viruses. J Virol Method, 171: 134-140, doi: 10.1016/j.jviromet.2010.10.014

Rajkhowa TK, Mohanarao GJ, Gogoi A, Hauhnar L and Isaac L, 2015. Porcine reproductive and respiratory syndrome virus (PRRSV) from the first outbreak of India shows close relationship with the highly pathogenic

variant of China. Vet Q, 35(4): 186-193, doi: 10.1080/01652176.2015.1066043

Rajkhowa TK, Kiran J, Hauhnar L, Zodinpui D, Paul A *et al*., 2022. Molecular detection and characterization of African swine fever virus from field outbreaks in domestic pigs, Mizoram, India. Transbound Emerg Dis,69(4): e1028-e1036, doi: 10.1111/tbed.14384

Salib FA and Osman AH, 2011. Incidence of Lumpy skin disease among Egyptian cattle in Giza Governorate, Egypt. Vet World, 4(4): 162-167, doi: 10.5455/VETWORLD 2011.162-167

Sprygin A, Pestova Y, Bjadovskaya O, Prutnikov P, Zinyakov N *et al*., 2020. Evidence of recombination of vaccine strains of lumpy skin disease virus with field strains, causing disease. PLoS One, 15(5): e0232584, doi: 10.1371/journal.pone.0232584

Sudhakar SB, Mishra N, Kalaiyarasu S, Jhade SK, Hemadri D *et al*., 2019. Lumpy skin disease (LSD) outbreaks in cattle in Odisha state, India in August 2019: epidemiological features and molecular studies. Transbound Emerg Dis, 67(6): 2408-2422, doi: 10.1111/tbed.13579

Tulman ER, Afonso CL, Lu Z, Zsak L, Sur JH *et al*., 2002. The genomes of sheeppox and goatpox viruses. J Virol, 76(12): 6054-6061, doi: 10.1128/jvi.76.12.6054-6061.2002

Tuppurainen ESM, Lubinga JC, Stoltsz WH, Troskie M, Carpenter ST *et al*., 2013. Mechanical transmission of lumpy skin disease virus by *Rhipicephalus appendiculatus* male ticks. Epidemiol Infect, 141(2): 425-430, doi: 10.1017/S0950268812000805

Zeynalova S, Asadov K, Guliyev F, Vatani M and Aliyev V, 2016. Epizootology and molecular diagnosis of lumpy skin disease among livestock in Azerbaijan. Front Microbiol, 7: 1022, doi: 10.3389/fmicb.2016.01022

Received- 22.05.2024, Accepted- 25.11.2024, Published- 18.03.2025 (Online), 01.06.2025 (Print)
Section Editor: Prof. S. N. Joardar, Associate Editor