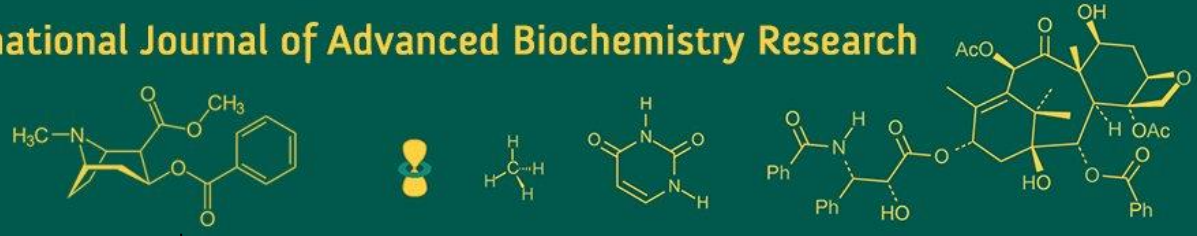
International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(1): 90-95
www.biochemjournal.com
Received: 01-10-2023
Accepted: 07-11-2023

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Genetic insight into lumpy Skin Disease Virus Outbreaks in India: A comparative analysis of 2019 and 2022 strains through L126 (EEV) gene molecular characterization

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DOI: https://doi.org/10.33545/26174693.2024.v8.i1b.311

Abstract
Lumpy skin disease (LSD) is an infectious WOAH notifiable disease of the cattle and water buffalos having capability of rapid transboundary spread and, characterized by eruptive skin lesions, greatly reduced milk production, poor growth, reduced fertility, sometimes abortion and rarely death. It is caused by the lumpy skin disease virus (LSDV) of genus *Capripoxvirus* in the *Poxviridae* family. Lumpy skin disease viruses can be distinguished by their distinctive brick-shaped appearance using electron microscopy. It is an enveloped DNA virus measuring approximately 294 nm × 262 nm in size, with a 151-kbp genome. This study focuses on the molecular characterization of Lumpy Skin Disease Virus (LSDV) during outbreaks in India in 2019 and 2022. The LSD can be transmitted by various arthropods, including blood-feeding insects and ticks, and can also spread through respiratory secretions and saliva. The recent surge in LSD cases in India, particularly in 2022, prompted this study. The outbreak affected a significant number of cattle, leading to an increased morbidity and mortality rate. The study aims to molecularly characterize LSDV strains recovered from outbreaks in different states of India, focusing on the L126 (EEV) gene. This gene-based analysis is crucial for understanding the genetic variations and virulence factors associated with LSDV in India, especially considering recent reports of increased virulence and breed-wise susceptibility differences among cattle. The results of this study contribute valuable insights into the molecular aspects of LSDV circulating in India, providing essential information for effective management and control of the disease.

Keywords: Lumpy skin disease virus, EEV glycoprotein, phylogenetic analysis, *Capripoxvirus*

1. Introduction
Lumpy skin disease (LSD) is an infectious illness of cattle and water buffalo, characterized by eruptive skin lesions, greatly reduced milk production, poor growth, reduced fertility, sometimes abortion and rarely death. It is caused by the lumpy skin disease virus (LSDV), belongs to the genus *Capripoxvirus* in the *Poxviridae* family and shows close antigenic relationship with sheep and goat pox virus (Sprygin *et al*., 2018) [14]. The LSDV is an enveloped DNA virus with a 151-kbp genome that is made up of 156 putative genes and a core coding region that is flanked by identical 2.4-kbp inverted terminal repeats (Tulman *et al*., 2002) [18]. It is carried by a variety of blood-feeding and biting arthropods, including *Stomoxys calcitrans* and *Biomyia fasciata*. Additionally, *Ixodid* hard ticks like *Rhipicephalus appendiculatus* are capable of mechanically transmitting LSDV to other animals (Tuppurainen *et al*., 2013) [17]. Sometimes it can also spread through skin lesions, respiratory secretions, and saliva (Abutarbush *et al*., 2015) [1]. Poor growth, chronic debility in sick animals, infertility, fetal death, diminished milk yield, and rarely death are the disease's hallmarks (Lu *et al*., 2021) [10]. The disease is notifiable to the World Organization for Animal Health (Formerly OIE), as it can spread quickly across borders and result in significant losses in the production of cattle (Klement, 2018) [8]. Zambia became the first nation to disclose a lumpy skin disease pandemic in 1929. LSD has recently been identified as a severe concern to cattle in Southeast Asia. The first country on the Asian continent to report an LSD incidence was Bangladesh.

According to the OIE situation report and current academic works, eight countries in this designated region, including Bangladesh, India, Nepal, China, Bhutan, Hong Kong, Vietnam, and Myanmar, have reported the disease outbreak (Das *et al*., 2021) [5]. According to the WOAH, India has seen three significant LSD epidemics in 2019. The first one was found in five coastal districts of the state of Odisha in August 2019. From August to December 2019, LSD outbreaks followed in various parts of Odisha and the neighboring state West Bengal (Sudhakar *et al*., 2020) [15]. Over the following two years, sporadic cases continued to emerge, with instances reported in Maharashtra and Gujarat. However, in the preceding year (2022), the disease exhibited a notable increase in its geographical spread, reaching both western and northern states, including the Andaman and Nicobar Islands. The first case of Lumpy Skin Disease (LSD) during 2022 was identified in Gujarat and has since propagated to encompass eight states and union territories. Multiple LSD outbreaks have ensued in India, extending to Rajasthan, Gujarat, Maharashtra, Punjab, Haryana, Himachal Pradesh, Uttarakhand, Uttar Pradesh, Madhya Pradesh, Jammu and Kashmir, and Delhi, with Rajasthan and Gujarat reporting the highest incidences. The recent surge, beginning in May-June 2022, was remarkable not only for the heightened morbidity rates and the rapid transmission among animals but also for the increased mortality rates. The 2022 outbreak affected approximately 29.45 lakh cattle, resulting in nearly 1.55 lakh deaths. The disease has now spread to 251 districts across 15 states in India (Bhadauria *et al*., 2023) [4]. In India the LSD is emerging transboundary disease and very less is known about Indian LSDV isolates. According to previous reports virulence of LSD is moderate or low but, recently thousands of deaths have been reported from Rajasthan (India) in animals, suffering from LSD and, the virulence determining factor is still unknown. In India the breed -wise variation in susceptibility to LSDV infection among Indian cattle were seen. The crossbred cattle are more susceptible to LSDV infection while the Zebu cattle is less, Among zebu cattle some breeds are more vulnerable to LSDV infection. The mechanism involved in this breed wise susceptibility difference is not understood, as all of these need molecular characterization of infecting/circulating LSDV strains in India. In the present study we performed L126 (EEV) gene based molecular characterization of LSDV recovered from 2019 and 2022 outbreaks from different states of India.

2. Materials and Methods

2.1 Clinical Samples

In between 2019-2022, Lumpy skin disease (LSD) suspected samples (Skin scab) were collected from various outbreaks from different Indian states namely Uttar-Pradesh, Madhya-Pradesh, Punjab, Rajasthan, Uttarakhand, Jammu & Kashmir and West-Bengal. The animals were showing typical clinical signs of LSD *viz*, high fever (40-41.5 °C), lacrimation, inappetence, lethargy, presence of large, firm, and elevated skin nodules covering almost the entire part of body. The samples were collected in sterile viral transport media (50% Phosphate buffered glycerol) and transported to the virus laboratory CADRAD. All the samples were processed after reaching the laboratory using standard protocol recommended by WOAH. The samples were stored at -20 °C till further processing.

2.2 DNA extraction

After the processing of tissue samples all the samples were subjected for DNA extraction and total DNA was extracted. In the present study initially the DNA extraction was carried out by QIAamp® DNA Mini Kit as per manufacturer protocol. The extracted DNA was preserved at -20 °C for further use.

2.3 Screening of samples

After the extraction of DNA all the samples were screened for the presence of capripoxvirus genome using PCR amplification. The WOAH recommended published primers (Ireland & Binepal, 1998) [6] targeting the viral attachment protein gene. The reactions were performed using 2X DreamTaq PCR Master Mix (Thermo Fisher Scientific, USA). The reaction was carried out at a final reaction volume of 25µL using thin-walled PCR tubes (0.2mL) comprising of 12.5µL of 2X master mix, 1µL of both forward (10pM) and reverse (10pM) primers (GCC Biotech India Pvt. Ltd.), 2µL of template DNA (50ng/ µL) and 8.5µL of nuclease free water. The cycling conditions were as follows: Initial denaturation at 95 °C for 3 minutes, followed by 35 amplification cycles of denaturation at 95 °C for 10 seconds, Annealing at 56 °C for 30 seconds, extension at 72 °C for 30 seconds. The amplification was completed by an ultimate elongation of 10 minutes at 72 °C. Products were visualized on 1.5% agarose gel with ethidium bromide.

2.4 Amplification of L126 (EEV) gene

For the amplification of L126 gene of LSDV gene-specific published primers targeting the L126 (EEV) was used (Nandi *et al*., 2023) [11]. The Phusion™ High-Fidelity DNA polymerase (Thermo Fisher Scientific, USA) enzyme was used for PCR amplification. PCR reaction was then performed at a final reaction volume of 50 µL using 0.2 mL thin-walled PCR tubes comprising of 10 µL of 5X Phusion HF buffer, 1µL of dNTPs (10mM), 2µL of both forward (10pM) and reverse (10pM) primers (GCC Biotech India Pvt. Ltd.), 4µL of template DNA (50ng/ µL) and 30.5µL of nuclease free water. The cycling conditions were as follows: Initial denaturation at 98 °C for 30 seconds, followed by 35 amplification cycles of denaturation at 98 °C for 10 seconds, Annealing at 56 °C for 30 seconds, extension at 72 °C for 1.5 minutes. The amplification was completed by an ultimate elongation of 10 minutes at 72 °C. Products were visualized on 1% agarose gel with ethidium bromide.

2.5 Sequencing of amplified products

The amplicons were gel purified using a commercially available gel extraction kit (MinElute® Gel Extraction Kit, Qiagen, Germany) as per the manufacturer's instructions. The gel purified products having concentration between 30-50 ng/µL were selected and commercially sequenced using the chain termination or Sanger Sequencing method at Barcode Biosciences, Bangalore, India. The sequence data generated was received as colored electropherogram and text files.

2.5 Annotation and Submission to NCBI

The sequences having good quality reads were processed using Sequencher 5.0 software, the primer ends were trimmed off and the consensus sequences were generated merging both forward and reverse reads. The sequence

similarities were analyzed using BLAST analysis. The final sequence editing was done by using the EditSeq software of DNA STAR and sequences were submitted to NCBI Genbank through the BankIt submission portal.

2.6 Phylogenetic Analysis
A thorough phylogenetic analysis was performed to determine the evolutionary dynamics in terms of temporal and spatial distribution of LSDV targeting the L126 (EEV) gene. The reference sequences targeting L126 gene were retrieved from National Center for Biotechnology Information (NCBI) and were used for comparative phylogenetic analysis. In order to ensure the accuracy of the dataset thorough processing of these sequences, encompassing alignment and exclusion of any artifacts, was performed using ClustalW Software. Then the Fasta files containing the aligned sequences were created, and aligned sequences were used for the creation of a phylogenetic tree using IQTREE-1.6.12-Windows software (Nguyen *et al*., 2015) [12], a flexible program for studying molecular evolutionary genetics. Afterwards, phylogenetic trees were created to illustrate the evolutionary connections between the targeted genes.

3. Results
3.1 Screening of samples
In the present study a total of 55 samples collected from different time periods and locations of outbreak were screened to detect the presence of *Capripoxvirus* (LSDV) genome. A total of 31 representative samples were selected in screening, as confirmed by visualization of 195 bp PCR products on agarose gel electrophoresis (Fig. 1).The positive samples were further used for the amplification of targeted gene.

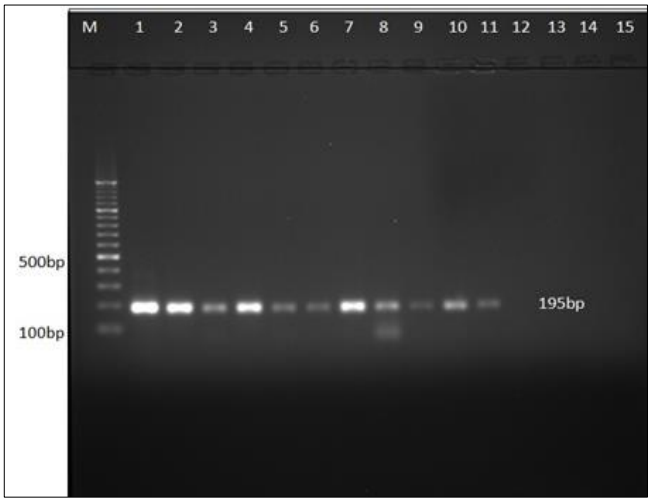

Fig 1: Agarose gel showing amplification of LSDV genome (Product size- 195bp), Lane M: 100bp ladder, Lane 1: Positive control, Lane 2-11: Samples

3.2 Amplification and Sequencing of L126 (EEV) gene
In the present study a total of 31 samples underwent conventional PCR to generate 1300 bp products. The visualization of these products was achieved through gel electrophoresis, as illustrated in Fig. 2. Subsequently, the gel-purified products were forwarded for sequencing. A total of 17 representative samples were sent for sequencing. These successfully processed sequences were then employed for a comprehensive sequence analysis, aiming to achieve meaningful insights from the obtained genetic information.

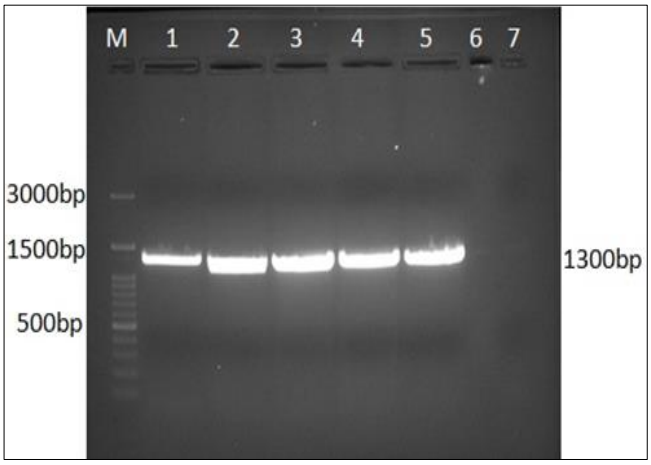

Fig 2: Agarose gel showing amplification of L126 (EEV) gene of LSDV genome (Product Size-1300bp), Lane M: 100bp plus ladder, Lane 1-4: Samples, Lane 5: Positive control, Lane 6: Negative control

3.3 Annotation and submission to NCBI
The sequences were annotated using BioEdit software and 17 consensus sequences were submitted to National Center for Biotechnology Information (NCBI) GenBank database. The submitted sequences were reviewed by NCBI and the GenBank accession numbers (OR983215-OR98323) were assigned to the submitted sequences.

3.4 Phylogenetic Analysis
In the present research investigation, a comprehensive examination of L126 (EEV) gene sequences, comprising a dataset of 60 nucleotide sequences including 17 sequences from present study, sourced from diverse global origins was undertaken. The sequences derived from goat pox virus (GTPV) and sheep pox virus (SPPV) were also included in reference. To discern the genetic affiliations and elucidate the evolutionary relationships among them, our set of 17 nucleotide sequences was systematically compared with reference sequences derived from various geographical regions worldwide. For the inference of evolutionary history, the best fit model was predicted and Maximum Likelihood tree was constructed using the TPM3+F+I model with 10,000 bootstrap replicates in IQTREE-1.6.12-Windows software (Nguyen *et al*., 2015) [12]. The sequence

derived from samples collected during the initial outbreak of Lumpy Skin Disease (LSD) in India in 2019 clustered in previously reported clade 1 (Indian clade 1), displayed a distinct genetic profile when compared to other Indian sequences in the current study. Notably, this particular sequence exhibited a close genetic relationship with Kenyan LSDV strain. Additionally, it shared genetic affinities with isolates originating from the first LSD outbreaks in Bangladesh, Nepal, Myanmar, and India. The resulting phylogenetic tree, illustrated in Fig. 3, encapsulates the intricate relationships among these sequences. The sequences derived from the samples collected during the 2022-2023 form a separate new clade (Indian clade 2) and supported by strong bootstrap values along with other currently circulating LSDV isolates of India. This finding highlights the distinct genetic composition of the LSDV variant linked to the first outbreak in India as compared with recent Indian LSD outbreaks (2022) and implies related evolutionary connections with viral strains from various regions during the beginning stages of LSD emergence in these nations.

Fig 3: Phylogenetic tree based on L126 (EEV) gene of LSDV showing different clades of LSDV globally

The Maximum Likelihood tree was constructed using TPM3+F+I model. The nodes represent the bootstrap value (10,000 replicates).The generated sequences used in present study are highlighted as red triangle. The SPPV and GTPV were also included in analysis

5. Discussion and Conclusion

The LSD is endemic in nature in Africa as well as in the Middle East, but recently has started to spread in Asian and other countries, mainly affecting Asian breeds of cattle (*Bos indicus*) and Asiatic water buffaloes (*Bubalus bubalis*). China and Bangladesh, which share borders with India, have reported some LSD outbreaks since 2019. Morbidity rate varies between 10 to 20%, while mortality rates of 1 to 5% are considered usual. The first breakout of Asia was reported from Bangladesh, in July 2019, where 66 cattle were affected (Badhy *et al*., 2021) [3]. Other than these countries various outbreaks have been reported from Nepal, Bhutan, Sri Lanka, Thailand, Malaysia, Laos and Cambodia (Azeem *et al*., 2022) [2]. India too observed the first case of LSD in the same year (2019) in five districts of Odisha (Sudhakar *et al*., 2020) [15]. In total, 182 out of 2,539 cattle were affected, indicating an apparent morbidity rate of 7.1%, with no recorded mortality. Subsequently, over the following two years, sporadic cases emerged, including instances in Maharashtra and Gujarat. However, in the preceding year (2022), the disease was reported in western and northern states, as well as the Andaman and Nicobar Islands. The first case of Lumpy Skin Disease (LSD) during 2022 outbreaks was documented in Gujarat and has since spread to eight states and union territories. India witnessed several LSD outbreaks, extending to Rajasthan, Gujarat, Maharashtra, Punjab, Haryana, Himachal Pradesh, Uttarakhand, Uttar Pradesh, Madhya Pradesh, Jammu and Kashmir, and Delhi, with Rajasthan and Gujarat reporting the maximum cases. The recent surge, since May-June 2022, was unusual not only for the increased morbidity rates and the rapid transmission of the disease but also for

elevated mortality. The 2022 outbreak affected approximately 29.45 lakh cattle, resulting in nearly 1.55 lakh deaths, and it has now spread to 251 districts in 15 states across India (Bhadauria *et al*., 2023) [4]. The molecular characterization of LSDV from cattle in Ranchi, India and revealed that the isolated virus (LSDV/Bos taurus-tc/India/2019/Ranchi) was closely related to Kenyan LSDV strains (Kumar *et al*., 2021) [9]. Molecular characterization of LSDV from LSD outbreaks happened in Andhra-Pradesh state of India and revealed that the isolates were showing similarity with the isolates of India (Odisha), Bangladesh, Russia, Egypt, and Kenya (K *et al*., 2021) [7]. Molecular characterization of LSDV isolated from the earliest and subsequent Indian LSD outbreaks during 2019 using GPCR, RPO30, P32 and EEV gene sequences and observed their close relation with Kenyan NI-2490/Kenya/KSGP-like field LSDV strains (Sudhakar *et al*., 2022) [15]. Molecular epidemiology of lumpy skin disease outbreak in Ganjam district of Odisha, India during August 2020 using P32 and F gene was carried out and phylogenetic analysis of the P32 gene of LSDV showed 100% similarity with other isolates from India, Bangladesh, Egypt and Saudi Arabia and phylogenetic analysis of the F gene of LSDV revealed a similarity of 97.99%, with Odisha India isolate and located in the same cluster with other Indian isolates (Sethi *et al*., 2022) [13]. Previous analyses primarily indicated the presence of a single clade, mainly related to the Kenyan strain. However, recent observations of changes in morbidity and mortality patterns in various new Indian LSD outbreaks prompted further investigation. To discern any alterations in the virus genome, we focused on the most targeted gene based on available literature and conducted a phylogenetic analysis covering samples from both old and new outbreaks. The analysis uncovered an entirely different lineage circulating in the 2022 outbreaks, closely related to each other and a few other published sequences from contemporaneous outbreaks in India. It's important to note that this phylogenetic analysis was solely based on a single gene, and to validate the presence of a distinct lineage in the Indian subcontinent, whole-genome sequencing is essential.

6. Acknowledgement

The work was conducted under the ICAR funded project entitled “Genetic characterization of lumpy skin disease virus and host immune response in natural infections”. All the authors are thankful to the Director, ICAR-Indian Veterinary Research Institute, Izatnagar, India, for facilitating the carrying out of this work.

7. References

1. Abutarbush SM, Ababneh MM, Al Zoubi IG, Al Sheyab OM, Al Zoubi MG, Alekish MO, *et al*. Lumpy Skin Disease in Jordan: Disease Emergence, Clinical Signs, Complications and Preliminary-associated Economic Losses. Transboundary and Emerging Diseases. 2015;62(5):549–554. doi:10.1111/tbed.12177
2. Azeem S, Sharma B, Shabir S, Akbar H, Venter E. Lumpy skin disease is expanding its geographic range: A challenge for Asian livestock management and food security. The Veterinary Journal. 2022;279:105785. doi:10.1016/j.tvjl.2021.105785
3. Badhy SC, Chowdhury MGA, Settypalli TBK, Cattoli G, Lamien CE, Fakir MAU, *et al*. Molecular characterization of lumpy skin disease virus (LSDV) emerged in Bangladesh reveals unique genetic features compared to contemporary field strains. BMC Veterinary Research. 2021;17(1):61. doi:10.1186/s12917-021-02751-x
4. Bhadauria P, Singh BP, Makhdoomi DM, Singh T, Brijvanita, Singh S, *et al*. Lumpy Skin Disease: Emerging Threat to Livestock Industry. In: Lumpy Skin Disease: Emerging Threat to Livestock Industry. ICAR-Agricultural Technology Application Research Institute; c2023. p. 56.
5. Das M, Chowdhury M, Akter S, Mondal A, Uddin M, Rahman M, *et al*. An updated review on lumpy skin disease: A perspective of Southeast Asian countries. Journal of Advanced Biotechnology and Experimental Therapeutics. 2021;4(3):322. doi:10.5455/jabet.2021.d133
6. Ireland DC, Binepal YS. Improved detection of capripoxvirus in biopsy samples by PCR. Journal of Virological Methods. 1998;74(1):1–7. doi:10.1016/S0166-0934(98)00035-4
7. K LK, B S, K R. Clinico-molecular diagnosis and characterization of bovine lumpy skin disease virus in Andhra Pradesh, India. Tropical Animal Health and Production. 2021;53(4):424. doi:10.1007/s11250-021-02872-3
8. Klement E. Economic Impact of Lumpy Skin Disease. In: Tuppurainen ESM, Babiuk S, Klement E, editors. Lumpy Skin Disease. Springer International Publishing; 2018. p. 7–9. doi:10.1007/978-3-319-92411-3_3
9. Kumar N, Chander Y, Kumar R, Khandelwal N, Riyesh T, Chaudhary K, *et al*. Isolation and characterization of lumpy skin disease virus from cattle in India. PLOS ONE. 2021;16(1):e0241022. doi:10.1371/journal.pone.0241022
10. Lu G, Xie J, Luo J, Shao R, Jia K, Li S. Lumpy skin disease outbreaks in China, since 3 August 2019. Transboundary and Emerging Diseases. 2021;68(2):216–219. doi:10.1111/tbed.13898
11. Nandi S, Mahajan S, Mishra R, Nautiyal S, Agrawal RK, Singh M, *et al*. LSDV126 gene based molecular assays for specific detection and characterization of emerging Lumpy Skin Disease virus. Journal of Virological Methods. 2023;312:114665. doi:10.1016/j.jviromet.2022.114665
12. Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Molecular Biology and Evolution. 2015;32(1):268–274. doi:10.1093/molbev/msu300
13. Sethi RK, Senapati SK, Selim AM, Acharya AP, Mishra C, Das M, *et al*. Molecular epidemiology of lumpy skin disease outbreak in Odisha, India. Veterinary Research Communications. 2022;46(3):711–717. doi:10.1007/s11259-022-09886-8
14. Sprygin A, Artyuchova E, Babin Y, Prutnikov P, Kostrova E, Byadovskaya O, *et al*. Epidemiological characterization of lumpy skin disease outbreaks in Russia in 2016. Transboundary and Emerging Diseases. 2018;65(6):1514–1521. doi:10.1111/tbed.12889
15. Sudhakar SB, Mishra N, Kalaiyarasu S, Jhade SK, Hemadri D, Sood R, *et al*. Lumpy skin disease (LSD) outbreaks in cattle in Odisha state, India in August 2019: Epidemiological features and molecular studies.

Transboundary and Emerging Diseases. 2020;67(6):2408–2422. doi:10.1111/tbed.13579
16. Sudhakar SB, Mishra N, Kalaiyarasu S, Jhade SK, Singh VP. Genetic and phylogenetic analysis of lumpy skin disease viruses (LSDV) isolated from the first and subsequent field outbreaks in India during 2019 reveals close proximity with unique signatures of historical Kenyan NI-2490/Kenya/KSGP-like field strains. Transboundary and Emerging Diseases. 2022;69(4). doi:10.1111/tbed.14322
17. Tuppurainen ES, Lubinga JC, Stoltsz WH, Troskie M, Carpenter ST, Coetzer JAW, *et al*. Mechanical transmission of lumpy skin disease virus by Rhipicephalus appendiculatus male ticks. Epidemiology and Infection. 2013;141(2):425-430. doi:10.1017/S0950268812000805.
18. Tulman ER, Afonso CL, Lu Z, Zsak L, Sur JH, Sandybaev NT, *et al*. The genomes of sheeppox and goatpox viruses. Journal of virology. 2002 Jun 15;76(12):6054-61.