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Research Article

Molecular Characterization of Emerging Lumpy Skin Disease Virus in Western Uttar Pradesh, India

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Abstract

Context: Lumpy skin disease, a transboundary disease recently spread across the India during 2022-2023 and caused unexpected mortality in the range of 2-10% in different states. It imposed a severe economic stress on the livestock based rural economy and adversely affected the export of animal based products. Government of India tried to prevent it by vaccinating more than 87 million animals with existing Goat pox vaccine. However, there are reports of infection in spite of vaccination. These might be because of variation of strain prevalent in particular state.

Objective: It is imperative to study the virus involved in the infection in particular state due to climatic and geographical variation. With this objective, the present study involved the molecular characterization and phylogeny analysis of LSD virus prevalent in Uttar Pradesh to ascertain any variation in virus.

Results: Samples collected from the clinical cases revealed the presence of LSD virus with WAHO recommended PCR based amplification. The further validation of the virus with amplification of ORF011 encoding G-protein-coupled receptors (GPCRs) led to phylogeny study of the virus.

Conclusions: The virus of UP aligned with other reported viruses of country and also showed highest resemblance with the reported viruses of the neighbouring countries like Myanmar, Nepal, Bangladesh and china.

Keywords: Lumpy Skin Disease Virus; Phylogeny; Cattle; Uttar Pradesh

Introduction

Emerging Lumpy skin disease (LSD) posed a serious threat not only to cattle but also to the nation as a whole since they have a negative impact on the economy. Over a brief period, LSD spread quickly. The most appropriate illustration of an infectious disease that started in Africa and spread to China and Southeast Asia is LSD. The LSD virus (LSDV) is a member of the Poxviridae family, which includes the genus capripoxvirus with a genome size of ~151 kbp long [1]. LSD affects cattle of all ages and breeds and has a significant morbidity but a low fatality rate. Reduced milk production, loss of meat and draught animals, abortion, infertility, loss of condition, and damage to the hide all result in substantial economic concerns [2]. Blood-feeding insects such stable flies, mosquitoes, and ticks can spread LSDV [3]. Nodular skin lesions, enlarged lymph nodes, and fever are the symptoms of LSD.

In 1929, LSD was found in Zambia, from where it soon spread to the majority of Africa. Consequently, the Mediterranean basin, Europe, the Middle East, and Asia all witnessed a slow spread of the disease [4,5]. Bangladesh, China, and India recorded LSDV cases in 2019. The disease later found in Nepal, Sri Lanka, Vietnam, Myanmar, Thailand, Malaysia, Laos, and Cambodia. LSD, which was initially identified in the country in 2019 [6], has since spread to approximately all of the states as well as the Andaman and Nico-

bar Islands. In the recent outbreak of 2022, LSD has killed over 90,000 cattle in 23 states across the nation [7]. According to earlier findings, phylogenetic analysis and sequencing of the G-protein-coupled chemokine receptor (GPCR) gene revealed a close genetic relationship between the Russian LSDV strain and the LSDVs circulating in Asian countries [8]. Recent study in Nepal revealed that the Nepali isolates resemble those from Kenya in the past as well as those from India and Bangladesh [9].

The livestock industry is significant to the Indian economy, contributing 28% and 5%, respectively, to the country's agricultural output and overall gross domestic product. India has the largest number of cattle in the world, with 192.49 million total cattle, including 142.11 million native cattle and 50.42 million crossbred/exotic cattle (20th Livestock census, Govt of India). From July to September 2022, the Lumpy Skin Disease claimed the lives of more than 70,000 cattle and expanded to 23 states, with a death rate of more than 15% [7]. In India, Uttar Pradesh is the state with the largest population of cattle; in the month of September, there have been more than 25,000 cases reported in 2,600 villages across 25 districts, and more than 1.5 million animals are in the infection zone. As a result, the objective of this work was to perform the molecular characterization and phylogenetic analysis of LSDV using the GPCR gene isolated from cattle in Uttar Pradesh exhibiting clinical indications identical to those of LSD.

Materials and Methods

Study area and sample collection

The study was carried out in the Uttar Pradesh province's Muzaffarnagar, Bulandshar and Meerut districts. Using 6mm biopsy punches (Accumen Schimatle, Gujarat), multiple skin nodules were collected in sterile vials having 10% GPBS as per described by OIE (OIE terrestrial manual 2017) after which the region where the biopsies were collected was cleaned. The samples were transported to the laboratory over ice and stored at – 80°C for further molecular processes.

Sample processing and DNA extraction

The samples kept at -80°C underwent three washings with PBS (pH 7.4). The skin nodules were cut into small pieces and pulverized in Hank's Balanced Salt Solution (HBSS) with a mortar and pestle. After centrifugation of the homogenate at 7000 rpm for 5 minutes, the supernatant was collected into a fresh micro-centrifuge tube. 200 µl of the supernatant was added to an equal volume of lysate, along with 100 µg/ml of proteinase K, vortexed, and incubated at 56°C for 45 minutes. Post incubation the solution was allowed to cool to room temperature then an equal volume of Phenol chloroform and isoamyl alcohol (P:C: I) was added and mixed by inverting. Centrifugation for 10 minutes at a speed of 7500 rpm was used to separate the two phases. Ethanol and sodium acetate were used to precipitate the DNA from the aqueous phase. Isolated DNA was stored at -20°C for further analysis.

LSD Virus confirmation

The PCR was used to validate the presence of Capripoxvirus by amplifying a 192 bp region in the viral attachment protein gene using the primers (Table 1) as per the previously prescribed method [10] with slight modifications. 5.0 µl of extracted DNA, 12.5 µl of EmeraldAmp GT PCR Master Mix (Takara Bio Inc., Japan), 5.5 µl of nuclease free water, and 1.0 µl of each 10 µM primer concentration were added to a total mixture volume of 25 µl to prepare the PCR reactions. The PCR cycle was programmed on the PCR Thermal Cycler Dice Touch (Takara Bio Inc, Japan) under the following conditions: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 45 secs, annealing at 50 °C for 50 s, extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. The amplicons were then separated in 1% Agarose gel and visualized using a UV transilluminator to determine the presence of the band of 192bp to confirm the sample.

GPCR gene amplification

All of the positive samples were subjected to the amplification of ORF011 encoding G-protein-coupled receptors (GPCRs) using previously prescribed primers (Table 1) in order to further validate the virus' identification [11]. A 25- µl PCR reaction including 20pmol of forward and reverse primers, 200ng of DNA (template), and 12.5 µl of EmeraldAmp GT PCR Master Mix (Takara Bio Inc., Japan) was set up to amplify the gene. The following conditions were used in the thermocycler: a denaturation step lasting 5 min at 98°C, 35 cycles of amplification lasting 30 s at 98°C, 30 s at 52°C, and 80°C, and a final extension step lasting 10 min at 72°C. The amplicons were separated on a 1% agarose gel, and an amplified product of 1134 bp was observed.

Nucleotide sequencingand analysis

In order to confirm the identity of the virus GPCR amplicon bands were trimmed and purified using geneJET Gel Extraction Kit (Thermo Fisher MA, USA). 50ng of the purified amplicons were subjected to direct Sanger sequencing using both forward and reverse primers at Biokart India Pvt. Ltd, Bangalore. After that, the quality of the obtained sequences was checked, and the ends of the sequences were trimmed using Molecular Evolutionary Genetics Analysis (MEGA) version X software (Pennsylvania, USA). The nucleotide sequences of the GPCR were subjected to multiple sequence alignment using CLUSTAL W, together with other representative nucleotide sequences of the LSDV and sheeppox virus available on NCBI. Phylogenetic analysis was performed using MEGA X. The sequences of one of the amplicon were submitted to GenBank database.

Gene	Sequence	Reference
PCRA	Forward: 5'TCCGAGCTCTTTCCT-GATTTTTCTTACTAT3'	[10]
	Reverse: 5'TATGGTACCTAAATTATATACGTA-AATAAC-3'	
GPCR	Forward: 5'-ATGAATTATACTCTTAGYACAGT-TAG-3'	[11]
	Reverse: 5'-TTATCCAATGCTAATACTAC-CAG-3'	

Table 1: Primers used to amplify the LSDV specific gene segment.

Results

The multiple clinical samples were collected from the three districts (Meerut, Muzaffernagar, Bulandshar) from the cattle showing the clinical signs of LSD (Figure 1).

Figure 1: Nodular skin lesions all over the body and limbs.

The result of PCR for detection of LSDV was found positive for all the samples with the amplification of a product size of 192bp (Figure 2). In-order to establish the phylogenetic relationships of the viruses, ORF011 region was amplified with a product size of 1134bp (Figure 3).

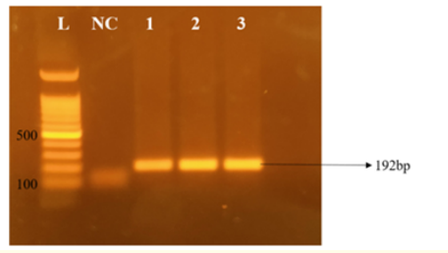

Figure 2: Representative samples showing detection of LSDV with amplicons of 192bp.

The comparison of the sequences of all the sequences obtained by the amplifications of ORF011region revealed 100% homology. One of these sequences were uploaded in GenBank database as Meerut LSDVs' GPCR sequences and are accessible under the accession number OQ029679. The phylogenetic tree of the present LSDV isolate Meerut (OQ029679) revealed a well-resolved clustered

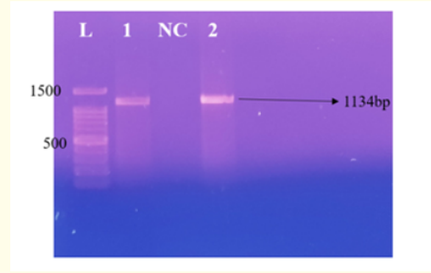

Figure 3: Representative samples with amplification of ORF011 region of GPCR genes.

with same clade with close relationship to the previously identified LSDV isolates (OP056772, OP056769, OM674471, OL689601, MT448700, MN508357, OM250060, OK258136, OL752713, MW732649, OM793603, MT992618, ON005067 and MN072619) (Figure 4 and 5). NJ-based phylogenetic analysis of the LSDV showed consistent clustering of isolates. They all suggest a close relationship between the Indian LSDV isolate Meerut and other LSDV isolates, supported by high posterior probability values.

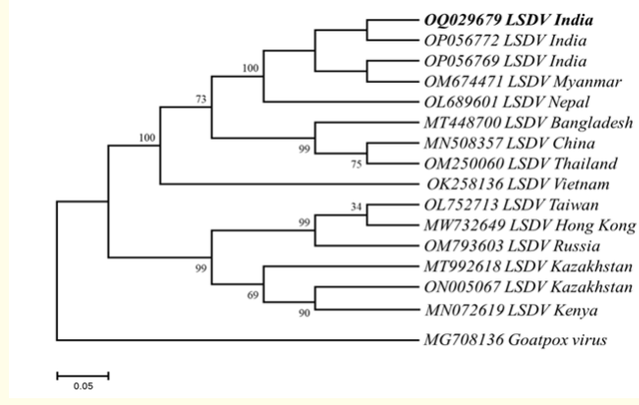

Figure 4: Phylogeny tree of the Meerut isolate of LSD virus.

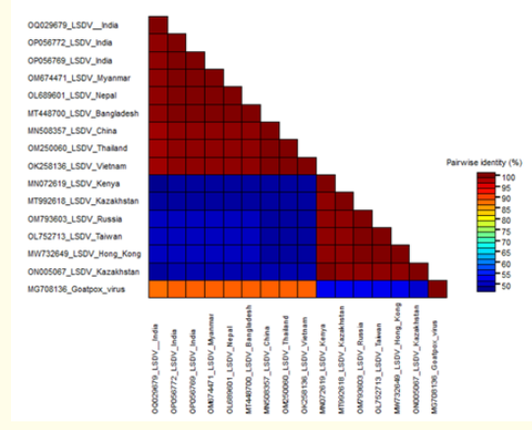

Figure 5: Identity matrix of Meerut LSD virus with previously reported LSD sequences.

Discussion and Conclusion

The LSD is causing a severe economic loss to the rural economy and industry based on livestock and livestock products with unexpected mortality rate. The recent casualty number has crossed 97,453 and that number doubled only in 3 weeks time. The data available at the website of Ministry of Fisheries, Animal Husbandry and Dairying shows the spread of disease across 251 districts in 15 states and affected over 20 lakhs animals until September 23, 2022. However, as per the information submitted to Parliament on February 7, 2023, 184,447 cattle died due to LSD during 2022-23 across the country. It happened in spite of 87 million-goat pox vaccination in susceptible population in 2022-23. It is considered that Goat pox virus has more than 95% homology with current LSD virus and its cross protection is expected to produce desirable immune response in the vaccinated animals [12]. There are reports of protection, however sporadic cases of failure of vaccination are also reported from the different geographical regions. There are many published studies suggesting Goat pox vaccination provide sufficient protection against active LSD [12]. These capripox viruses are so antigenically closed to each other that these can only be differentiated by using advanced molecular characterization [13,14]. The Sheep and Goat pox vaccines have been successfully used to obtain protection against LSD virus in cattle in various LSD endemic countries [12,15]. Some of the scientists recommended heterologous vaccine instead of homologous strain as use of homologues vaccine might have induced recombinant strain that can challenge the existing heterologous vaccine. Such genomic changes due to recombination have been recorded in recent outbreaks [16].

In recent past, LSD has spread across the globe particularly central to eastern and southern Asia and there is no explanation of this rapid spread. However, the explanation of use of heterologous vaccine to avoid development of recombinant LSD virus might have some clue on it. The first report of such recombination came from Russia where a recombinant LSD virus Saratov/2017 spread very rapidly in the large animal population and it was found the recombinant of a live attenuated virus used for vaccination and the Southern African LSD virus. The recombinant virus was also reported with more aggressive multiplication abilities leading to more rapid spread in susceptible population [16].

In India, virus spread very rapidly across the country with unprecedented high mortality rate. It imposed the pressure of the system to adopt for the available vaccine and based on the available reports and literature Department of Dairying and Animal Husbandry released the guidelines for the use of Goat pox vaccine in susceptible population. However, due to long incubation period of LSD virus, there were always chances of vaccination of animals without clinical disease but in incubation. Such cases provide undesirable interaction of vaccine strain with wild virus strain and may lead to development of recombinant virus. It is therefore, it is imperative to continuous molecular surveillance on the virus prevalent in the various regions of the country. Considering these, the samples were collected from three districts of Uttar Pradesh and subject to confirmation of LSD virus (Figure 2) and for genotyping subject to the amplification of ORF011 region for G-Protein coupled chemokine receptor (GPCR) gene (Figure 3). These genes are frequently used to study nucleotide polymorphism in the genome of LSD virus and recommended for phylogenetic studies between the viruses of genus Capripox [17]. These are also recommended to detect the presence of vaccine and wild strains of viruses [18,19]. All the sequences of the amplicons of samples collected from three different districts of Western Uttar Pradesh revealed 100% homology suggesting the involvement of a common virus in the western Uttar Pradesh.

These also revealed close identity with isolates other part of country as well as the viruses prevalent in the neighbouring countries like Myanmar, Nepal, Bangladesh and china 9 (Figure 4, 5). These align far distinct to the viruses of Kazakhstan and Russia. Similarly, similarity matrix revealed the differences from the viruses of East Asian countries like Taiwan and Hongkong. The findings are suggestive of different lineage for virus of Africa, south East Asia, East Asia and Russia or the development of mutants due to recombination. There are also reports of involvement of recombinant vaccine-like strain of LSD causing low-level infection in cattle due to virus inoculation [20]. Thus, the use of homologous strain still has the difference of opinion in scientific community. The heterologous vaccine reduces the chances of recombination and also the mild disease as induced by the vaccine strain.

As per the available literature, there are possibilities of genomic rearrangements between wild and vaccine strains [14,21]. Further, there are reports of use of contaminated vaccine batch leading to widespread disease on subsequent vaccination [22,23]. Under such circumstances it is difficult to understand a single cause involved in the rapid spread of the disease in India. Thus, the continuous monitoring on the virus involved in the spread of the disease is essential along with the vaccination of variable heterologous vaccine like Goat Pox vaccine to minimize the chances of recombination as well as vaccine induced clinical conditions.

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Conflict of Interest

Authors declare no conflict of interest.

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