Genomic characterization of Lumpy Skin Disease virus (LSDV) from 2019 outbreak in India reveals circulation of Kenyan-like LSDV strains with unique kelch-like proteins

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Abstract

Lumpy skin disease (LSD) is an economically important poxviral disease endemic to Asia, Europe, and Africa. Recently, LSD has spread to naïve countries, including India, China, Bangladesh, Pakistan, Myanmar, Vietnam, and Thailand. Here, we describe the complete genomic characterization of LSDV from India, LSDV-WB/IND/19 isolated from a calf in Vero cells determined by Illumina next-generation sequencing (NGS). The LSDV-WB/IND/19 has a genome size of 150969 bp encoding 156 putative ORFs. Phylogenetic analysis based on complete genome sequence suggested that LSDV-WB/IND/19 is closely related to Kenyan LSDV strains with 10-12 variants with non-synonymous changes confined to LSD_019, LSD_049, LSD_089, LSD_094, LSD_096, LSD_140, and LSD_144 genes. In contrast, to complete kelch-like proteins in Kenyan LSDV strains, LSDV-WB/IND/19 LSD_019 and LSD_144 genes were found to encode truncated versions (019a, 019b, and 144a, 144b). LSD_019a and LSD_019b proteins of LSDV-WB/IND/19 resemble that of wild-type LSDV strains based on SNPs and the C-terminal part of LSD_019b except for deletion at K229, whereas the LSD_144a and LSD_144b proteins resemble that of Kenyan LSDV strains based on SNPs, however, C-terminal part of LSD_144a resembles that of vaccine-associated LSDV strains due to premature truncation. The NGS findings were confirmed by Sanger sequencing of these genes in Vero cell isolate as well as in the original skin scab along with similar findings in another Indian LSDV from scab specimens. LSD_019 and LSD_144 genes are thought to modulate virulence and host range in capripoxviruses. This study demonstrates the circulation of unique LSDV strains in India and highlights the importance of constant monitoring of the molecular evolution of LSDV and associated factors in the region in light of the emergence of recombinant LSDV strains.

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ABSTRACT

Lumpy skin disease (LSD) is an economically important poxviral disease endemic to Asia, Europe, and Africa. Recently, LSD has spread to naïve countries, including India, China, Bangladesh, Pakistan, Myanmar, Vietnam, and Thailand. Here, we describe the complete genomic characterization of LSDV from India, LSDV-WB/IND/19 isolated from a calf in Vero cells determined by Illumina next-generation sequencing (NGS). The LSDV-WB/IND/19 has a genome size of 150969 bp encoding 156 putative ORFs. Phylogenetic analysis based on complete genome sequence suggested that LSDV-WB/IND/19 is closely related to Kenyan LSDV strains with 10-12 variants with non-synonymous changes confined to LSD_019, LSD_049, LSD_089, LSD_094, LSD_096, LSD_140, and LSD_144 genes. In contrast, to complete kelch-like proteins in Kenyan LSDV strains, LSDV-WB/IND/19 LSD_019 and LSD_144 genes were found to encode truncated versions (019a, 019b, and 144a, 144b). LSD_019a and LSD_019b proteins of LSDV-WB/IND/19 resemble that of wild-type LSDV strains based on SNPs and the C-terminal part of LSD_019b except for deletion at K229, whereas the LSD_144a and LSD_144b proteins resemble that of Kenyan LSDV strains based on SNPs, however, C-terminal part of LSD_144a resembles that of vaccine-associated LSDV strains due to premature truncation. The NGS findings were confirmed by Sanger sequencing of these genes in Vero cell isolate as well as in the original skin scab along with similar findings in another Indian LSDV from scab specimens. LSD_019 and LSD_144 genes are thought to modulate virulence and host range in capripoxviruses. This study demonstrates the circulation of unique LSDV strains in India and highlights the importance of constant monitoring of the molecular evolution of LSDV and associated factors in the region in light of the emergence of recombinant LSDV strains.

Keywords: Lumpy skin disease virus, kelch-like proteins, Phylogenetic analysis, whole genome

Introduction

Lumpy skin disease is a contagious viral disease caused by lumpy skin disease virus (LSDV) belonging to the *Capripoxvirus* genus of the*Poxviridae* family and affects cattle and domestic water buffaloes. It incurs huge economic losses to the livestock industry due to a decrease in milk production, infertility, abortions, hide damage as well as trade restrictions (Tuppurainen et al., 2017). The disease was originally described in Zambia in 1929, later spreading to South Africa and Kenya. Initially, the disease was contained to Africa and the Middle East (Davies, 1982), later spreading to Europe followed by outbreaks in several Asian countries including India (Sudhakar et al., 2019), Nepal (Acharya and Subedi, 2020), Bangladesh (Hasib et al., 2021), Vietnam (Tran et al., 2021), Thailand (Arjkumpa et al., 2021), Myanmar (Maw et al., 2022), China (Lu et al., 2021), and Hongkong (Flannery et al., 2021) has raised serious concerns. The clinical manifestations of lumpy skin disease include pyrexia, characteristic nodules on different body parts, lymphadenopathy, drop in milk production, weight loss, infertility, abortion, and sometimes death (Tuppurainen et al., 2017). The morbidity rate can be as high as 100% whereas the mortality rate is usually less than 10%. LSDV is mainly transmitted by arthropod vectors. LSDV has a genome size of ˜151 kbp encoding putative 156 proteins (Tulman et al., 2001; Biswas et al., 2020). LSDV is antigenically related to other members of Genus *Capripoxvirus* , sheeppox virus (SPPV), and goatpox virus (GTPV). Full genome sequences of LSDV strains circulating across the world are available (Table 1). The information on the genomic sequence of LSDV strains circulating in India has not been widely available yet, although previous sequence analyses were based on partial gene sequence data (Kumar et al., 2021; Sudhakar et al., 2021, Sethi et al., 2022). In this study, we have therefore determined the complete genome sequence of LSDV from cattle in India to offer additional insights into molecular epidemiology and the factors affecting the evolution of capripoxviruses.

2. Materials and Methods

2.1. Clinical samples

Samples (including whole blood, skin scrapings, and nasal plaques) were received at Pox Virus Laboratory, ICAR-IVRI Mukteswar from Bankura, West Bengal, India collected from a one-month-old calf suffering from a high rise in temperature, numerous nodules all over the body, enlarged lymph nodes along with mucopurulent nasal discharge and respiratory distress. The scab samples were processed to make 10% suspension in PBS along with antibiotics followed by freeze-thawing, filtration, and storage at -80°C for further use.

2.2. Preliminary confirmation and virus isolation

DNA was extracted from scab suspensions using QIAGEN Blood and tissue DNA isolation kit. Isolated DNA was subjected to CaPV-specific PCR as described earlier (Ireland and Binepal, 1998). After preliminary confirmation, Vero cells grown in EMEM supplemented with antibiotics and 10% fetal calf serum were infected with filtered scab suspension using the adsorption method. The cells were observed daily for the appearance of cytopathic effect (CPE). After preliminary isolation of LSDV into Vero cells, further propagation was continued up to seventy passages (P70).

2.3. Whole genome sequencing

2.3.1. Library preparation, sequencing, and genome assembly

The Vero cell-adapted virus (passage-5) was harvested followed by freeze-thawing three times and partially purified as described elsewhere with slight modifications (Babiuk et al., 2009). The DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen, Germany) as per the manufacturer's instructions. The quality of DNA was checked on 0.8% agarose gel for the single intact band and DNA concentration was determined using Qubit(r) 2.0 Fluorometer. The paired-end library was prepared from 200ng DNA using Truseq Nano DNA Library prep kit and sequenced on Illumina (HiSeq) next generation sequencing (NGS) platform using paired-end sequencing reads with 150-nucleotide (nt) length. The filtered reads obtained were mapped to LSDV-NI2490 (GenBank accession number AF325528.1) with 99.99% coverage using the CLC genomics workbench (v. 20.0). After reference mapping, the consensus was called from the resultant BAM file. In addition, the denovo assembly method was also used in which three major contigs were obtained which were aligned with bowite2 on the complete genome of LSDV-NI2490. The consensus sequence of all contigs was then extracted to obtain the draft of the assembled genome of LSDV-WB/IND/19. All variants were manually validated.

2.3.2. Genome Annotation

Annotation was done using a combination of annotation transfer using LSDV-NI2490 when similarity was >70% and ORF finding using homology searches on the NCBI 'Basic Local Alignments Search Tool' (BLAST) server with ORF >120 nucleotides and ATG as a start codon. ORFs of less than forty amino acids in length were omitted unless previously characterized in CaPVs. A total of 156 ORFs were annotated as putative genes and numbered from left to right as described previously (Tulman et al., 2001; Biswas et al., 2020). Protein identity analysis was performed using BLAST at the NCBI server. The annotated sequence of LSDV-WB/IND/19 was submitted to GenBank (Accession No. OP297402). Confirmation of nt changes in LSD_019 and LSD_144 genes was confirmed by Sanger sequencing in the same sample (Vero, P5 isolate), as well as in the original scab as well as in another scab sample, LSDV-UK/IND/21 from India.

2.4. Genome analysis, alignment, and phylogeny

The complete genome sequence of LSDV-WB/IND/19 was aligned with available genome sequences of other LSDVs from different countries as well as GTPV and SPPV sequences retrieved from GenBank included in Table 1 using MAFFT (Katoh et al., 2019). A phylogenetic tree was made with RaxML using the Maximum Likelihood construction method based on the GTR G+I with 100 bootstrap iterations (Stamatakis, 2014) and visualized by iTOL (Letunic and Bork, 2021). The nucleotide identities between CaPV genomes were calculated using the MegAlign program of DNASTAR as per the Tamura-Nei method (Tamura and Nei, 1993). The pairwise comparison between LSDV genomes was calculated using the Diffseq program

(https://www.bioinformatics.nl/cgi-bin/emboss/diffseq) whereas Base-by-Base was used to visualize and depict the indels/SNPs (Tu et al., 2018). Prediction of protein domains was done by InterPro (Blum et al., 2020).

3. Results and discussion

3.1. Preliminary confirmation and virus isolation

DNA isolated from suspected samples yielded 192 bp PCR product in CaPV-specific PCR (Data not shown). After infection into Vero cells, cells were observed for 6 days for the appearance of CPE. After the 4^{th} passage, CPE characterized by cell clustering was observed at 3 days post-infection followed by rounding and degeneration at the 6^{th} day. After 6 days, cells were freeze-thawed three times and used for further infection. Passage-5 (P5) was used for DNA extraction.

3.2. Genome size, organization, and ORFs

The libraries were prepared from the isolated DNA sample by Truseq Nano DNA Library preparation kit. The number of filtered reads obtained was 24,657,026 out of which 1,241,516 reads were mapped to LSDV-NI2490 strain with 99.9% coverage using the CLC genomics workbench. The average size of libraries was 442 bp, whereas the average coverage depth was ~2500 X. After reference mapping and denovo assembly and calling the consensus from the resultant BAM file, the full-length genome size of LSDV-WB/IND/19 was 150,969 bp with a 145,938 bp central coding region flanked by two inverted terminal repeats (ITRs) of 2613 bp and 2418 bp. The genome has a high A+T content of 74.1% and a compact gene arrangement with no large regions of non-coding sequence as noted previously (Tulman et al., 2001; Biswas et al., 2020). The putative concatemer resolution sequence (CRS) element (5'- ATTTATAGGCTAAAAAAAAAGTA-3') was present at nt positions 86-108 which is a highly conserved sequence necessary for concatemer resolution during genome replication. A total of 156 ORFs were annotated as putative genes and numbered from left to right as previously described (Tulman et al., 2001; Biswas et al., 2020). Four genes each, viz. LSD_001 and LSD_156, LSD_002 and LSD_155, LSD_003 and LSD_154, and LSD_004 and LSD_153 are identical and appear twice due to their location within the ITRs. Like other LSDVs, LSDV-WB/IND/19 encodes nine genes (LSD_002, LSD_004, LSD_009, LSD_013, LSD_026, LSD_126, LSD_132, LSD_153, LSD_155) that have been disrupted by accumulated mutations in GTPV and SPPV. Although, the total sequence length remains largely unchanged among capripoxviruses. Two short open reading frames located between genes 28/29 and120/121, encoding 49-a.a. and 41-a.a. proteins, respectively were numbered LSD_028.5 and LSD_120.5 as described previously (Biswas et al., 2020).

3.3. Comparison with other LSDV, GTPV, and SPPV genomes

In comparison with other LSDV genomes, the intraspecies nucleotide identity of LSDV genomes was 98.13%-100%. The LSDV-WB/IND/19 strain was found to be most closely related to the Kenyan LSDV-NI2490 strain with an nt identity of 99.86% whereas, 99.79% and 99.5% nt identities were seen with LSDV-KSGO 240 and LSDV-Kenya/58 strains, respectively. Earlier reports based on EEV glycoprotein (LSD_126), RPO30, and GPCR (LSD_011) genes showed high similarity to Kenyan LSDV strains (Sudhakar et al., 2022). Identical to Indian strains, LSDV strains from Bangladesh (Badhy et al., 2021), Nepal (Koirala et al., 2022), and Myanmar (Maw et al., 2022), showed a high resemblance to Kenyan LSDV strains based on EEV glycoprotein, RPO30, and GPCR genes indicating the common source of infection through illegal trade of biologicals, animal products or animals. LSDV strains from Vietnam, Thailand, China, and Russia are not related to Kenyan strains but instead are recombinant strains.

With the wild-type, vaccine, and recombinant LSDV strains, LSDV-WB/IND/19 shared 99.44%-99.86%, 98.13%-98.25%, and 98.59%-99.08% nt identities, respectively. Wild-type LSDV strains had 99.44%-99.86% nt identity among each other, whereas 98.18%-98.5% and 98.64%-99.17% nt identities were observed with vaccine and recombinant LSDV strains, respectively. LSDV vaccine strains showed 99.72%-100% nt identity among each other as compared to 98.97%-99.53% nt identity with recombinant LSDV strains. Recombinant LSDV strains shared 98.96%-100% nt identity among each other. With GTPV and SPPV strains, 95.72%-

96.74% identities were observed. Due to high nucleotide identity and serological cross-reactivity within the*Capripoxvirus* genus, heterologous GTPV vaccines have been used in cattle with equal protection against LSDV (Gari et al., 2015; Zhugunissov et al., 2020; Tuppurainen et al., 2022).

With intraspecies nucleotide similarity of 98.13%-100% amongst LSDV strains over the length of their genomes, several insertions, deletions, and substitutions were observed in the comparison of InDel/SNP patterns (Fig. 1) which indicates a high genetic variation among different LSDV strains globally. The wild-type LSDV cluster comprises subclusters of Kenyan LSDV strains, and wild-type LSDV strains from Africa, the Middle East, Europe, and Asia. Vaccine LSDV strains form another cluster. Recombinant strains could be divided into 4 groups, R1 to R4 as described previously with slight differences (Van Schalkwyk et al., 2022; Krotova et al., 2022b; Vandenbussche et al., 2022). Group R1 consists of LSDV-Russia/Saratov/17 and LSDV-Russia/Saratov/19. LSDV-Russia/Tyumen/2019 and LSDV-Udmurtiya/19 form groups R2 and R3, respectively. LSDV strains from China, Vietnam, Hongkong, and Thailand, as well as LSDV-Russia/Tomsk/2020 and LSDV-Russia/Khabarovsk/2020 strains, form group R4. Most LSDV strains from Russia and a larger part of Asia appear to be vaccine-like recombinant strains with signatures from Neethling and KSGP-based vaccines (Vandenbussche et al., 2022).

3.4. Comparison of ORFs including LSD_ 019 and LSD_ 144

As compared to LSDV-NI2490, 6 single nucleotide polymorphisms (SNPs), 5 small indels (2 single nt deletions, 1 single nt insertion, and 1 double nt deletion) were observed in LSDV-WB/IND/19, whereas as compared to LSDV-KSGO 240, 6 single nucleotide polymorphisms (SNPs), 6 small indels (3 single nt deletions, 1 single nt insertion and 1 double nt deletion) were observed (Table 2). Out of 156 ORFs, LSDV-WB/IND/19 showed 100% amino acid identity in 148 ORFs with Kenyan LSDV strains except for eight ORFs viz. LSD_019 (all Kenyan strains), LSD_049 (LSDV-KSGO 240, LSDV-KSGO 240/cattle), LSD_-089 (LSDV-KSGO 240/cattle), LSD_094 (LSDV-NI2490, LSDV-KSGO 240, LSDV-Kenya/58), LSD_096 (LSDV-NI2490, LSDV-Kenya/58), LSD_134 (LSDV-KSGO 240), LSD_140 (all Kenyan strains) and LSD_-144 (all Kenyan strains). Out of eight ORFs, five ORFs viz. LSD_049 (Q393P), LSD_089 (I24V), LSD_094 (P85H, H536N, I537N), LSD_096 (R23E), LSD_140 (N203K) showed 99.4%-99.9% aa identities owing to non-synonymous changes (Table 2). LSD_134 is 100% identical except LSDV-KSGO 240 in which it has a truncated version. LSD_019 and LSD_144 showed 51% aa identity to that of Kenyan LSDV strains due to truncation of both kelch-like proteins in LSDV-WB/IND/19 (Fig. 2, 3).

12 bp nt insertion present in GPCR gene (LSD_011) of LSDV vaccine strains, recombinant LSDV strains as well as Kenyan strains was also observed in LSDV-WB/IND/19. LSD_126 having 27 bp nt insertion in wild-type LSDV strains including Kenyan strains was also present in LSDV-WB/IND/19 whereas deletion is present in vaccine and recombinant LSDV strains (Erster et al., 2017). Similar findings were observed in the GPCR and LSD_126 genes of LSDV strains from Bangladesh (Badhy et al., 2021). LSDV-KSGO 240 genome isolated from the Kenyavac vaccine vial (JOVAC) shared 99.9% homology with LSDV-NI 2490 strain differing by single aa substitution in LSD_ 049 and single nt deletion causing frameshifting and further truncation of LSD_134 gene (Vandenbussche et al., 2016). LSDV-KSGO 240 isolated from cattle has 99.99% identity to the parent KSGO 240 strain isolated from the parent vaccine vial with seven variants out of which 3 genes viz. LSD_026, LSD_089, and LSD_094 are affected (Bamouh et al., 2021). Also, LSDV-KSGO 240 isolated from cattle demonstrated intact LSD_134 protein. LSDV-WB/IND/19 also has intact LSD_134 protein similar to LSDV-KSGO 240 from cattle, LSDV-NI2490, and LSDV-Kenya/58. Genomes of three vaccine strains isolated from Lumpyvax, Herbivac LS, and LSDV-OBP share 99.9% homology (Mathijs et al., 2016b). LSD_134 is also disrupted in LSDV-LW1959 and OBP vaccines by a+1 frameshifting whereas Herbivac LS vaccine shows restoration/merging of LSD_134a and LSD_134b into single LSD_134 (Mathijs et al., 2016b). Also, Herbivac LS 008 vaccine batch showed disruption of 134 into 134a, 134b along with AT deletion in LSD_131 (Douglas et al., 2019). Among SPPV strains, frameshifting has been demonstrated in LSD_ 134 in case of SPPV-SA and SPPV-AG vaccine strains, whereas GTPV-G20-LKV vaccine strain also reveals a + 1 frameshift leading to disruption of LSD_134 (Biswas et al., 2020). LSD_134 belongs to the poxviral B22R family of proteins. Monkeypox B22R homolog MPXV197 is associated with inhibition of

T-cell responses and its deletion leads to attenuation (Alzhanova et al., 2014).

LSD_019 and LSD_144 encode kelch-like proteins with BTB (broad-complex, tramtrack and bric a brac)/POZ domain, BTB/Kelch-associated (BACK) domain, and kelch-type beta-propeller with C-terminal kelch repeats. Kenyan LSDV strains as well as vaccine-associated LSDV strains from South Africa have a single LSD_019 protein (569 aa), whereas some LSDV vaccine strains (LW1959, Herbivac, OBP, and Cro2016) show inactivation of LSD_019 into 019a (440 aa) and 019b (150 aa) due to +1 frameshift mutations (Kara et al., 2003; Mathijs et al., 2016b) (Fig. 2). GTPV G20-LKV vaccine strain has also demonstrated similar findings (Biswas et al., 2020). Some LSDV wild-type strains encode other mutant forms of LSD_019 viz. 019a (290 aa) and 019b (270 aa). The amino acid pattern at different positions is the same in Kenyan LSDV strains, vaccine-associated LSDV strains, wild-type LSDV strains as well as in LSDV-WB/IND/19. LSDV vaccine strains show amino acid changes at these positions (Fig. 2). However, unlike Kenyan and vaccine-associated LSDV strains, LSDV-WB/IND/19 strain possesses a pattern of LSD_019 similar to wild-type strains viz. 019a (290 aa) and 019b (269 aa) based on the C-terminal part of LSD_019b except for the deletion of lysine (K) at position 229. SPPV_019 gene is required for virulence after intranasal and intradermal inoculation (Balinsky et al., 2007).

The amino acid pattern at different positions is the same in Kenyan LSDV strains, wild-type LSDV strains, and recombinant LSDV strains, as well as in LSDV-WB/IND/19. LSDV vaccine and vaccine-associated strains show amino acid changes at these positions (Fig. 3). Kenyan LSDV strains have a single LSD_144 protein with 547 aa length, whereas wild-type, as well as recombinant LSDV strains, also possess a single LSD_144 protein with 550 aa length due to additional 3 amino acids (VKT) at extreme C-terminus of the protein. All of the related LSDV vaccines (LW1959, Herbivac, OBP, and Cro2016), as well as vaccine-associated LSDV strains, encode LSD_144a and LSD_144b proteins of 269/270 aa and 281 aa, respectively. LSDV vaccine strains have a deletion of phenylalanine (F) at position 252 as compared to vaccine-associated LSDV strains. LSDV-WB/IND/19 encodes two ORFs 144a and 144b of 270 aa and 281 aa, respectively. LSD_144a and LSD_144b proteins resemble that of Kenyan LSDV strains based on amino acid patterns, however, the C-terminal part of LSD_144a resembles that of vaccine-associated LSDV strains due to premature truncation. LSDV/Evros/GR/15, the first LSDV reported in Europe, showed 99.8% homology with LSDV field isolate NI-Warmbaths-LW with frameshift in LSD_144. (Agianniotaki et al., 2017). LSDV-Russia/Saratov/17 showed 8 aa changes in LSD_144 leading to the merging of LSD_144a and LSD_144b to single LSD_144 like wild-type LSDVs (Sprygin et al., 2018). GTPV Gorgon vaccine isolate has a 1.6 kbp deletion spanning the 3' end of LSD_144 and LSD_145 and appears to inactivate both genes. China/GD01/2020 shows LSD_019 gene identical to Neethling vaccine LW 1959 and LSD_144 gene identical to Neethling 2490 strain. The unique variants in LSD_019 and LSD_144 have been confirmed by Sanger sequencing of the same sample as well as from original scab material to rule out nucleotide change arising due to passaging of virus for five passages. Interestingly, the same variants in both the genes were also present in another field sample, LSDV-UK/IND/21 in our study as well as other isolates from India, LSDV/Cattle/India/2019/Ranchi/P10, P30, and P50 (Acc. No. OK422492, OK422493, OK422494) (Fig. 4, 5). These findings suggest that LSDV strains circulating in India from 2019-2021 carry the same variants.

3.5. Phylogenetic Analysis

Phylogenetic analysis based on complete genomes clustered the CaPVs into 3 distinct species – LSDV, GTPV, and SPPV (Fig. 6). Among LSDV, two monophyletic clusters viz. cluster 1.1 and 1.2 and several novel recombinant LSDV strains have been shown as described previously (Biswas et al., 2020; Van Schalkwyk et al., 2020; 2022) Cluster 1.1 contains LSDV vaccine strains as well as vaccine-associated field strains from South Africa. All LSDV vaccines share a common ancestry with the Neethling 1959 vaccine (AF409138). Second cluster 1.2 consists of wild-type strains. The wild-type LSDV cluster comprises of subclusters of Kenyan LSDV strains and wild-type LSDV strains from other parts of Africa, the Middle East, Europe, and Asia. LSDV-WB/IND/19 clustered with Kenyan LSDV strains providing further support for the incursion of LSDV into India through illegal trade of animals, animal products, or biologicals. Kenyan strains have caused outbreaks in Kenya, but however never been reported in subsequent epidemics in Africa, the Middle

East, and Europe (Tulman et al., 2001). Since 2017, recombinant LSDV strains have been detected in infected cattle in Russia (Sprygin et al., 2018; Sprygin et al., 2020; Shumilova et al., 2022); Krotova et al., 2022a), China (Ma et al., 2021), Hongkong (Flannery et al., 2021), Taiwan (Huang et al., 2022) and Vietnam (Mathijs et al., 2021; Tran et al., 2021). In phylogenetic analysis, recombinant strains formed four monophyletic groups R1, R2, R3, and R4 as described in section 3.3. These recombinant LSDV strains have demonstrated an increase in virulence as compared to other field LSDV strains and can be transmitted in vector-independent mode (Aleksandr et al., 2020; Kononov et al., 2020). Even, LSDV-Saratov/19 has been demonstrated to persist in winter in absence of vectors through direct contact or fomites (Shumilova et al., 2022). LSDV has also been reported in a giraffe in Vietnam which is closely related to recombinant Vietnamese LSDV strains (Dao et al., 2022). Evidence of genetic recombination between capripoxviruses has been established in-vitro based on RFLP (Gershon et al., 1989). The use of homologous LSDV vaccines has not been authorized in Russia but the Neethling-based Lumpyvax vaccine (KEVE-VAPI) was used in Kazakhstan near border areas of Russia where recombinant LSDV strains have been detected (Sprygin et al., 2018; Sprygin et al., 2020). It was suggested that these vaccines might be possible for the emergence of recombinant strains in adjoining regions (Sprygin et al., 2018). In addition, a commercial Lumpyvax vaccine has been demonstrated to contain Neethling-like LSDV, KSGP-like LSDV, and GTPV-like genomes have been described (Haegman et al., 2021; Vandenbussche et al., 2022). Combining closely related viruses may increase the risk of recombination either in naturally infected animals or during seed virus production (Vandenbussche et al., 2022).

4. CONCLUSION

In view of the recent outbreaks of LSD in Asia, it is imperative to characterize the circulating LSDV strains in the region. We have sequenced and characterized the complete genome of LSDV, LSDV-WB/IND/19 isolated from an outbreak in 2019 in the West Bengal region of India. Phylogenetic analysis demonstrated clustering of LSDV-WB/IND/19 with Kenyan LSDV strains, however with unique kelch-like proteins (LSD_-019 and LSD_144). Unique variants in kelch-like proteins have been confirmed by Sanger sequencing of these genes in LSDV-WB/IND/19 as well as other Indian LSDV strains. LSD_019 and LSD_144 genes are thought to modulate virulence and host range in capripoxviruses. It is important to sequence other LSDV strains circulating in the region associated with recent outbreaks in 2022 to get a better picture of molecular epidemiology and the factors driving the evolution. This will help in a better understanding of factors affecting the host range of these viruses to develop precise diagnostics and vaccines.

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AUTHORS' CONTRIBUTIONS

AK and GV conceptualized and designed the study. AK and GV performed data analysis and drafted MS. AK, AK, PG performed the laboratory work. TS collected the samples. MAR, PD, GSK and RKS provided the necessary support to carry out the work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The calf samples were collected following animal welfare guidelines of CPCSEA.

DATA AVAILABILITY STATEMENT

The data underlying the results presented in the study are available from GenBank under Accession No. OP297402.

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Figures

Fig. 1. Distribution of polymorphic sites in LSDV genomes. The sequence of LSDV-NI-2490 strain was used as the reference. Green, blue, and red bars depict nucleotide insertions, substitutions, and deletions, respectively. LSDV-WB/IND/19 shows very high similarity with Kenyan LSDV strains with 10-12 variants.

Fig. 2. Pattern of LSD_019 proteins in different LSDV strains along with predicted domains. Amino acid

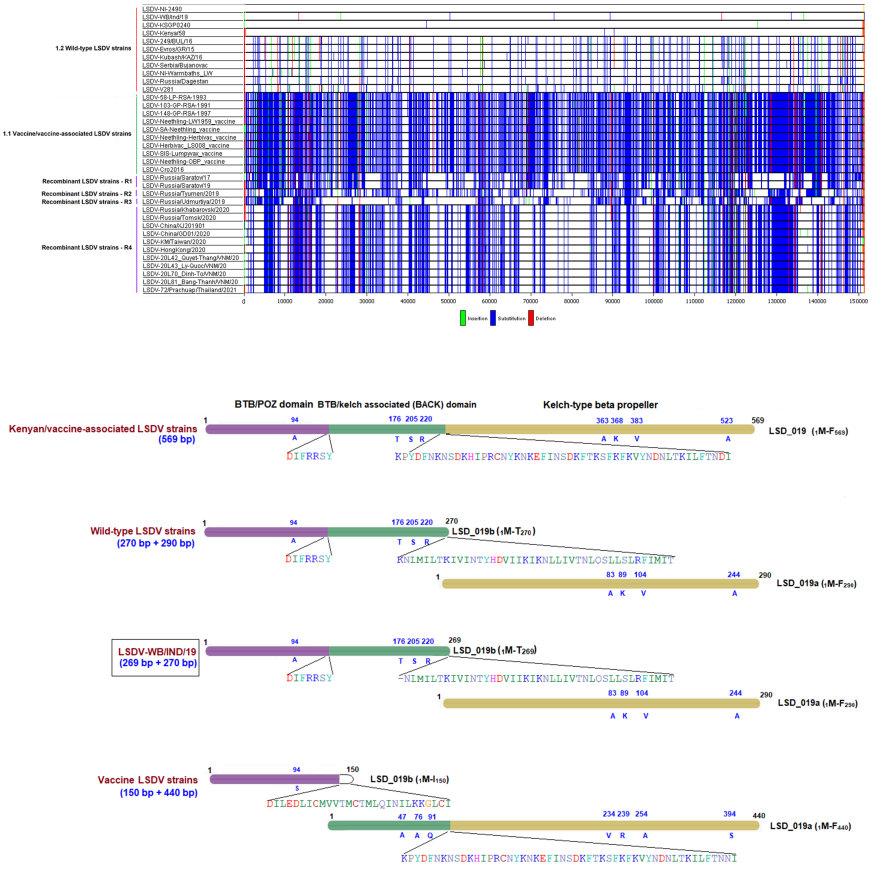
(aa) differences are depicted below the protein along with the respective position at the top.

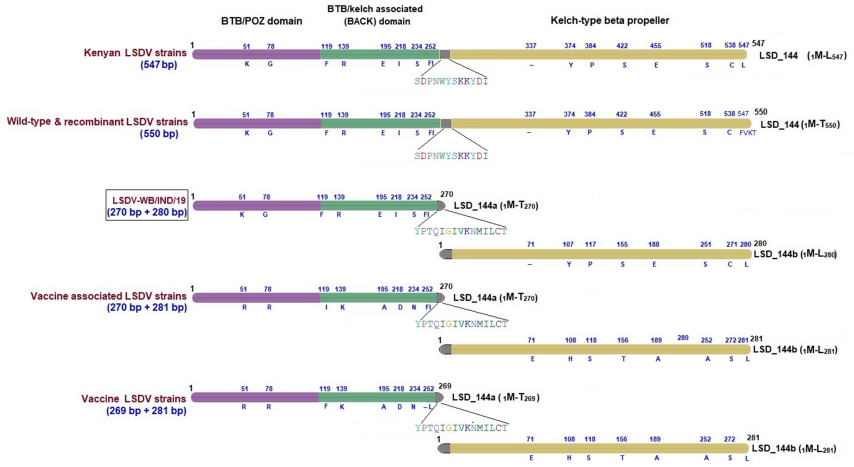
Fig. 3. Pattern of LSD_144 proteins in different LSDV strains along with predicted domains. Amino acid (aa) differences are depicted below the protein along with the respective position at the top.

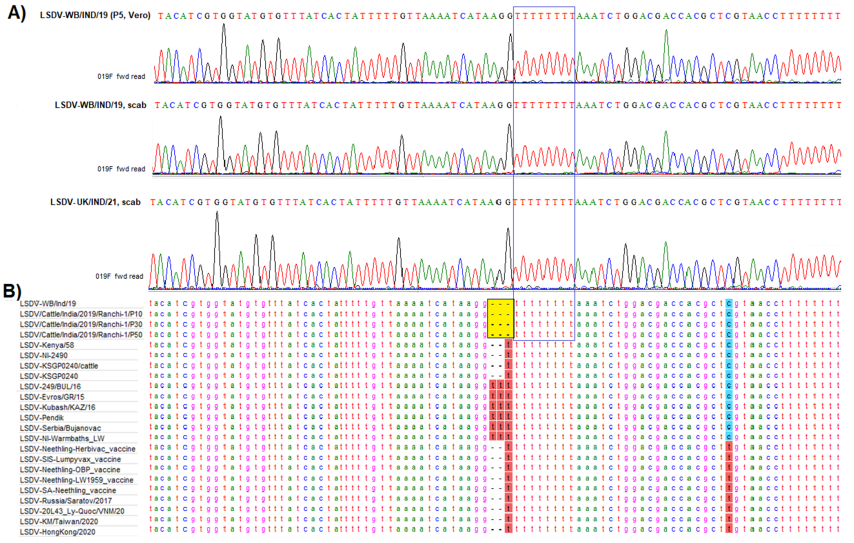
Fig. 4. (A) Sanger sequencing across the mutation (yellow highlighted box) detected in LSD_019 gene of LSDV-WB/IND/19 (P5, Vero as well as original scab) and scab of LSDV-UK/IND/21 (B) alignment of the corresponding DNA sequences with that of other LSDV strains.

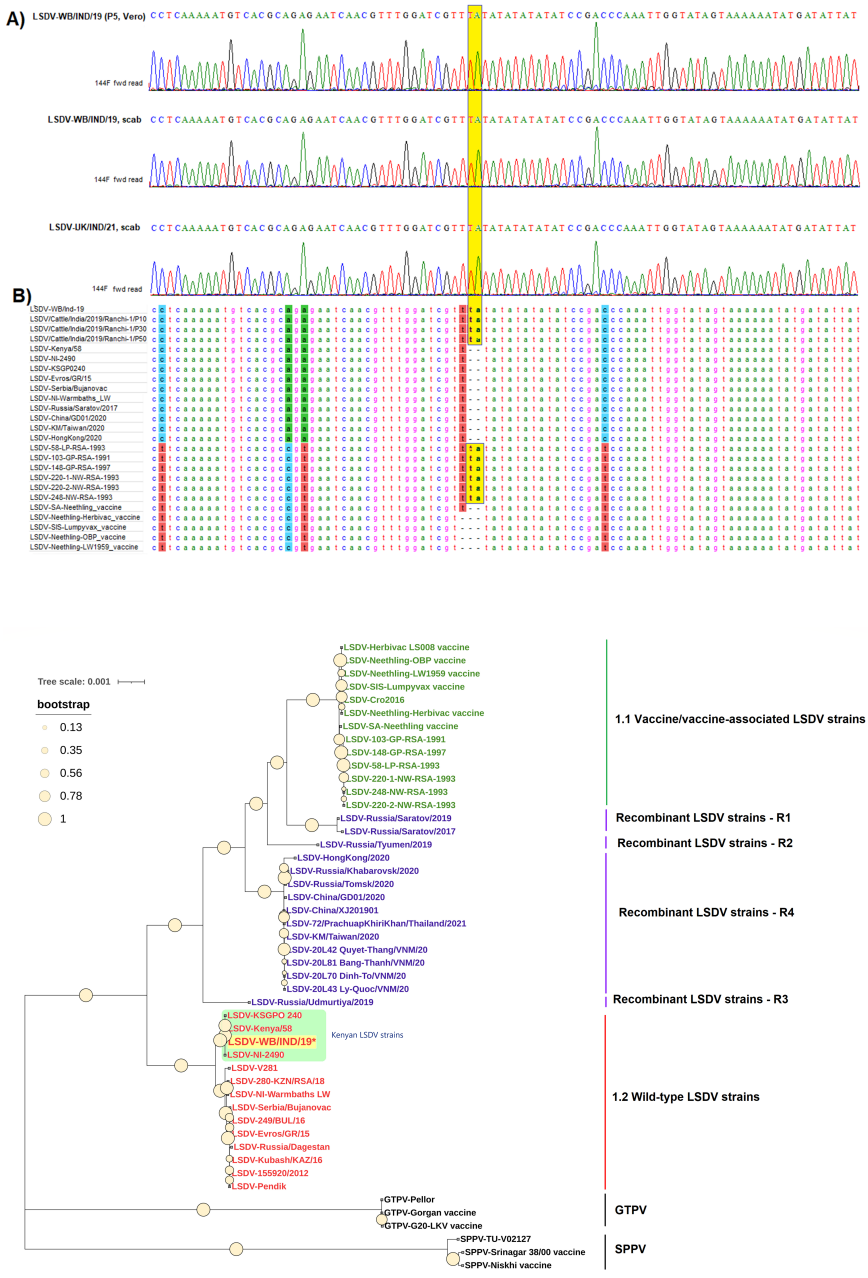
Fig. 5. (A) Sanger sequencing across the mutation (yellow highlighted box) detected in LSD_144 gene of LSDV-WB/IND/19 (P5, Vero as well as original scab) and scab of LSDV-UK/IND/21 (B) alignment of the corresponding DNA sequences with that of other LSDV strains.

Fig. 6. Phylogenetic analysis of LSDVs based on complete genomes constructed using the Maximum Likelihood method using RaXML with a bootstrap setting of 100. LSDV-WB/IND/19 strain is depicted in a yellow box with an asterisk (*) clustering with Kenyan LSDV strains (green box). Among LSDV, two monophyletic clusters viz. cluster 1.1 having vaccine/vaccine-associated LSDV strains and 1.2 having wild-type LSDV strains are observed along with several novel recombinant LSDV strains (R1, R2, R3, and R4).









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