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Genetic and Pathogenic Characterization of a Lumpy Skin Virus Disease Strain from a Diseased Cattle in Northern Vietnam

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ABSTRACT

Lumpy skin disease (LSD) is a highly contagious transboundary viral disease that causes substantial economic losses to the global cattle industry, including Vietnam. This study was conducted to diagnose, analyze the genome sequence, and evaluate the pathogenicity of the Lumpy skin disease virus (LSDV) strain responsible for lumpy skin disease (LSD) outbreaks in Vietnam in 2021. Real-time PCR and next-generation sequencing (NGS) techniques were employed to detect LSDV in clinical samples and to sequence the viral genome. LSDV DNA was successfully detected in clinical specimens using real-time PCR. The complete genome sequence of the LSDV/Vietnam/21L10 strain, comprising 146,083bp and 149 open reading frames (ORFs), was successfully obtained. Comparative genomic analysis revealed a high level of nucleotide similarity between the LSDV/Vietnam/21L10 strain and other virus strains circulating in Vietnam (99.99%) and China (99.99%). Phylogenetic analysis further demonstrated that the LSDV strain identified in this study belongs to the same genetic clade as strains circulating in Vietnam and China, and is distinct from vaccine strains. Experimental infection of cattle (n=3) with LSDV/Vietnam/21L10 resulted in typical clinical manifestations, including fever, depression, anorexia, lacrimation, the development of cutaneous nodules that ruptured and left scars, and regional lymphadenitis, whereas mock-infected cattle (n=3) were clinically healthy. These findings confirm that the LSDV/Vietnam/21L10 strain is a virulent field isolate.

Keywords: Complete genome; Genomic characterization; LSDV; Pathogenicity; Vietnam.

INTRODUCTION

Lumpy skin disease (LSD) is known as a highly infectious, transboundary viral disease impacting cattle, water buffaloes, giraffes, impalas, and camels. The disease is causing significant economic repercussions to the global livestock industry (Abutarbush et al. 2015; Al-Salihi & Hassan 2015; Khan et al. 2022; Akther et al. 2023; Bianchini et al. 2023). LSD virus (LSDV) belongs to the Poxviridae family, under the Chordopoxvirinae subfamily and the Capripoxvirus genus, which also contains Sheeppox virus (SPPV) and Goatpox virus (GTPV). LSDV primarily affects cattle, with occasional infections reported in goats and sheep (Tuppurainen et al. 2017; McInnes et al. 2023).

LSDV is a double-stranded DNA poxvirus. The genome of LSDV contains about 156 open reading frames (ORFs) and is bordered by two identical inverted terminal repeats (ITRs), each measuring roughly 2.4kbp. The LSDV genome is categorized into two primary clusters (1.1 and 1.2) based on the whole genome sequences (Biswas et al. 2020; Van Schalkwyk et al. 2022). Multiple genomic regions, including P32, G-protein-couple receptor (GPCR), EEV glycoprotein, RNA polymerase 30kDa subunit (RPO30) have been used as a tool to differentiate LSDV isolates belonging to the main clusters, and to distinguish Neethling-derived vaccines strains from field isolates (Le Goff et al. 2009; Lamien et al. 2011; Koirala et al. 2022). However, the recent outbreak involving recombinant LSDV strains highlights the likelihood of genetic variation and evolutionary changes within the LSDV genome (Krotova et al. 2022). Regarding to the GPCR gene, it is considered crucial due to its high sensitivity to polymorphism (Seerintra et al. 2022). It has also been applied to identify the LSDV strains carrying a 12 nucleotide insertions, a characteristic feature shared with the vaccine strains (Haegeman et al. 2021).

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As reported previously, the LSDV-infected cattle exhibit a high fever (39-41ºC), nodular lesions on the skin, mucus membrane, and swollen lymph nodes. In some cases, affected animals may developed firm skin nodules that merge and become necrotic masses and the disease can occasionally result in death (Mathewos et al. 2022). Furthermore, the diseased cattle also reduce milk yield and body weight. The morbidity rate of LSD was reported to average approximate 10%, ranging from 3% to 85%; while the mortality rate is typically between 1 and 3% (Bianchini et al. 2023). LSD has become a disease of global concern due to its continuous expansion of geographical borders, with reports of outbreak in the Middle East, Asia, and Europe (Al-Salihi & Hassan 2015; Tasioudi et al. 2016; Zeynalova et al. 2016; Sameea Yousefi et al. 2017; Tuppurainen et al. 2017; Mercier et al. 2018; Sprygin et al. 2018; Manic et al. 2019; Ren et al. 2023; Suwankitwat et al. 2023; Sudhakar et al. 2025; Yao et al. 2025). According to the World Organization for Animal Health, LSD is classified as a transmissible disease due to its highly infectious nature. LSDV has spread in different ways, such as indirect contact between animals via vectors, uterine environment, semen, natural nursing, biting insects, and iatrogenic transmission (Pitkin et al. 2009; Annandale et al. 2010; Magori-Cohen et al. 2012; Rouby & Aboulsoud 2016; Molla et al. 2017).

In Vietnam, LSD was first reported in 2020 in Lang Son and Cao Bang provinces (Tran et al. 2021a). Then, the disease has been occurred in cattle in some provinces (Tran et al. 2021b; Bich et al. 2024; Tran et al. 2024a; Tran et al. 2024b; Tran et al. 2024c) and a giraffe (Dao et al. 2022). The genetics analysis have been demonstrated mainly based on GPCR and RPO30 gene sequences of the Vietnamses LSDV strains. However, additional information on the molecular characterization and pathogenicity of LSDV strains is needed to fulfill. This study was conducted to investigate the molecular characterization based on the complete genome and pathogenicity of the LSDV strain circulating in Vietnam. The data obtained in this study may provide important insights to support the diagnosis, prevention, and control strategies of LSD in Vietnam.

MATERIALS AND METHODS

Samples

In this study, circumscribed nodules were collected from a LSD-suspected cattle aged 18-month-old in Lang Son province in 2021. The sample was stored in viral transferred buffer at 2ºC to 6ºC and send to the laboratory. After reaching the laboratory, the sample were homogenized and stored at -80°C until use.

DNA extraction, real-time PCR

For DNA extraction, TopPURE® Viral DNA/RNA Extraction Kits (ABT, Hanoi, Vietnam) was used to demonstrate DNA from the homogenized samples according to the manufacturer's instruction.

For LSDV detection, real-time PCR method was performed using specific primers, a probe (Table 1) and Luna® Universal Probe qPCR Master Mix (NEB, United Kingdom). The 20µL reaction mixture included 10µL of Luna® Universal Probe qPCR Master Mix, 0.8µL of each forward and reverse primer (10µM), 0.4µL of a probe, 5µL of DNA, and 3µL of distilled water. The real-time PCR process for LSDV detection was performed at 95°C for 1min, followed by 40 cycles at 95°C for 10s, 60°C for 30s. A Ct-value was less than 35 regarded as positive.

Nucleotide sequencing

The LSDV positive sample was sequenced for four genes (GPCR, RPO30, P32, and 126) with the specific primer sets (Table 1). The PCR reactions were performed as described previously using Gotaq® Green Master Mix (Promega, WI, USA). A total of 25µL of reactions contained 12.5µL of Gotaq® Green Master Mix, 1µL of each forward and reverse primer (10µM), 2µL of DNA template, and 8.5µL of distilled water. The amplified successfully PCR products were purified using QIAquick PCR Purification Kits (MP Biomedicals, Santa Ana, CA, USA) and send for Sanger sequencing at 1st BASE (Selango, Malaysia). Nucleotide sequence was deposited into GenBank with the accession number PX127682.

Table 1: Primers used for real-time PCR and PCR in this study

Purpose/Gene	Name of primers	Nucleotide sequence (5'-3')	PCR product (bp)	References
Detection	LSDr-F	CAAAAACAATCGTAACTAATCCA		(Alexander et al. 2019)
	LSDr-R	TGGAGTTTTTATGTCATCGTC		
	LSDr-P	FAM- TCGTCGTCGTTTAAAACTGA-BHQ1		
Sequencing/126	LSDV126-seqF1	ACAAGGCATGTGTAGAGGCA	1302	(Nandi et al. 2023)
	LSDV126-seqR1	TGCTTTAGTGCAAAAACAAACTCA		
	LSDV126-seqF2	ATGGGAATAGTATCTGTTGTATACGTCGTA	519	
	LSDV126-seqR2	TTAACAACAATTATAATAGTTTGACTCGG		
Sequencing/P32	LSD074-F3	GTTGGTCGCGAAATTTCAGATGTA	780	(Tursunov et al. 2022)
	LSD074-R3	GTAAGAAAAATCAGGAAATCTATG		
	GPCR-seqF1	TGAAAAATTAATCCATTCTTCTAAACA	617	(Gelaye et al. 2015)
Sequencing/GPCR	GPCR-seqR1	TCATGTATTTTATAACGATAATGCAAA		
	GPCR-seqF2	TTAGCGGTATAATCATTCCAAATA	603	
	GPCR-seqR2	GCGATGATTATGATGATTATGAAGTG		
	GPCR-seqF3	CACAATTATATTTCCAAATAATCCAA	684	
	GPCR-seqR3	TGTACATGTGTAATTTTAATGTTCGTA		
Sequencing/RPO30	LSD011-F2	CAGCTGTTTGTTTACATTTGATTTTT	554	(Gelaye et al. 2015)
	LSD011-R2	TCGTATAGAAACAAGCCTTTAATAGA		
	LSD011-F2	TTTGAACACATTTTATTCCAAAAAG	520	
	LSD011-R2	AACCTACATGCATAAACAGAAGC		

For Next generation sequencing (NGS): LSDV isolated in MDBK cells. Viral DNA was extracted using GeneJET™ Genomic DNA Purification Kit (ThermoFisher Scientific). Purified DNA products were sent and sequenced the complete genome of LSDV isolate by NGS. NGS method was performed by 1st BASE (Selango, Malaysia).

Nucleotide sequences obtained were analysis and aligned by the Clustal W multiple alignment tool in BioEdit v.7.7.1.0 (Thompson et al. 1994; Hall 1999). The GENETYX v.10 software (GENETYX Corp., Tokyo, Japan) was used to evaluate the homology in nucleotide of the obtained LSDV strain and other strains downloaded from GenBank (Table 2). Comparison of the LSDV strains was determined by the BLAST program. The confidence values on phylogenetic trees were assessed based on bootstrapping with 1,000 replicates using the MEGA X software (www.megasoftware.com/).

Recombination analysis: analysis of putative recombination events among the Vietnamese LSDV strains was performed using RDP (Beta version 4.97) and SimPlot (version 3.5.1) (Lole et al. 1999; Martin et al. 2015).

Virus isolation and titration

Viral isolation was performed using a Madin-Darby bovine kidney (MDBK) cell line cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cytopathic effects (CPE) were observed under a microscope every days after virus inoculation.

Viral titration was performed in MDBK cells. In brief, the process follows at: (i) prepation of MDBK cells with a density of 2×10^5cells/mL in DMEM medium supplemented with 10% FBS; (ii) virus dilution: serial dilutions (10^{-1} to 10^{-9}) in DMEM medium without FBS; (iii) transfer 100µL/well of the diluted virus sample according to the successive dilutions into the corresponding wells of the 96-well plate. Repeat each concentration for 5 wells. For the cell control wells , add 100µL DMEM/well; (iv) Add 100µL/well of the prepared cell suspension into the wells. The cells are then cultured at at 37°C with 5% CO_2. The infected cells ware monitor daily, read result after 72 infected hours. $TCID_{50}$ was caluculated accroding to Spearman Kärber method (Kärber 1931).

Table 2: Description of goat, sheep poxvirus and lumpy skin virus used in this study

No.	GenBank accession no.	Strain	Location	Host	Year	Type
1	MK302073	Arsi/b1	Ethiopia	Cattle	2011	LSDV
2	KX757824	Evros	Greece	Cattle	2015	LSDV
3	KY829023	Evros	Greece	Cattle	2015	LSDV
4	MK302072	B624	Bungoma	Cattle	2010	LSDV
5	KJ818281	SGP O-240	Kenya			LSDV
6	KY595106	RNOA-15	Russia	Cattle	2015	LSDV
7	MK302082	North	Sudan	Cattle	2008	LSDV
8	MK765544	Kazakhstan/2016	Kazakhstan	Cattle	2016	LSDV
9	AF409138	Neethling vaccine/LW	South Africa	Cattle	1958	LSDV
10	KX764643	SIS-Lumpyvax vaccine	South Africa	Cattle	1999	LSDV
11	MK441838	Herbivac LS	Africa	Cattle	2011	LSDV
12	MN636839	LSD-103-GP-RSA	South Africa	Cattle	1991	LSDV
13	OM793604	33-KZN-RSA	South Africa	Cattle	1977	LSDV
14	MW656252	Haden/RSA	South Africa	Cattle	1954	LSDV
15	OM793606	LSDV-Potter-RSA	South Africa	Cattle	1958	LSDV
16	KX683219	KSGP 0240	Kenya	Sheep	1974	LSDV
17	OM793607	Fourie-FS_RSA	South Africa	Cattle	1959	LSDV
18	MT643825	210LSD-249	Bulgaria	Cattle	2016	LSDV
19	MW631933	LSD	Morocco		2017	LSDV
20	KF495253	Maharashtra	India	Sheep	2010	GTPV
21	KJ818280	Kedong	China	Goat		GTPV
22	AY077836	G20 - LKV	Kazakhstan	Goat	2020	GTPV
23	FJ869347	01_18P2	Tunisia	Sheep	2001	SPPV
24	MG731222	Isparta-Yalvac	Turkey	Lamb	2017	SPPV
25	MW699032	Dagestan	Russia	Cattle	2015	LSDV
26	OP688128	V392.1	Bangladesh	Cattle	2021	LSDV
27	OK318001	V281	Nigeria	Cattle	2018	LSDV
28	OP297402	WB	India	Cattle	2019	LSDV
29	OM530217	Saratov	Russia	Cattle	2019	LSDV
30	MH646674	Saratov	Russia	Cattle	2017	LSDV
31	MT992618	KZ-Kostanay	Kazakhstan	Cattle	2018	LSDV
32	MT134042	Udmurtiya	Russia	Cattle	2019	LSDV
33	OL542833	Tyumen	Russia	Cattle	2019	LSDV
34	MW355944	GD01	China	Cattle	2020	LSDV
35	MW732649		HongKong	Cattle	2020	LSDV
36	MZ577076	20L81 Bang-Thanh	Vietnam	Cattle	2020	LSDV
37	MZ577073	20L42 Quyet-Thang	Vietnam	Cattle	2000	LSDV
38	OM033705	YST	Thailand	Cattle	2021	LSDV
39	OM984485	XJ201901	China	Cattle	2019	LSDV
40	OP508345	Xinjiang	China	Cattle	2019	LSDV
41	OR232413	S1	Indonesia	Cattle	2022	LSDV
42	OM793602	Tomsk	Russia	Cattle	2020	LSDV

Accessment of pathogenecity of the LSDV isolate

Animals

A total of six healthy cattle aged 6-month-old were purchased from a farm where has seen LSD outbreak historically in Hanoi, Vietnam. Cattle were housed in rooms with a strictly biosecurity control in Central Veterinary Medicine JSC No. 5, Hanoi, Vietnam. Blood and swab samples were collected to examine presence of LSDV-antibody and viral DNA by using ELISA and realtime PCR methods. Six cattle were confirmed to be negative for LSDV DNA detection and LSDV-antibody.

Experimental design

Six healthy and LSDV-negative cattle were randomly divided into two groups, with three animals in each group: LSDV-infected group: cattle were intramuscularly inoculated with 1mL of viral suspension containing $10^{6.5}\times TCID_{50}$ of LSDV per animal. Following infection, the animals were monitored daily for clinical signs, including body temperature, behavioral changes, gait, respiratory patterns, and other relevant indicators. Blood samples and oropharyngeal (throat) swabs were collected on days 3, 7, 10, 14, 21, and 28 post-infection for the detection of the LSDV genome using real-time PCR. On day 28 post-infection, all infected animals were euthanized and necropsied to assess gross pathological changes.

Mock-infected group: cattle were intramuscularly injected with 1mL of Dulbecco's Modified Eagle Medium (DMEM) into the neck muscle. The animals were managed and monitored in the same manner as the LSDV-infected group throughout the study period.

Statistical analysis

Student's t-tests were employed to compare the mean body temperature between LSDV-infected and mock-infected cattle by using the Statistical Package for the Social Sciences software version 25.0 (IBM Corp., Armonk, NY, USA). Differences were considered statistically significant at $P<0.05$.

RESULTS

Detection of the LSDV DNA, isolation and genetic characterization of the LSDV/Vietnam/21L10 strain

The sample obtained was positive for the LSDV DNA by using PCR. LSDV/Vietnam/21L10 strain was successfully recovered in MDBK cell lines.

The complete genome sequence of the LSDV/Vietnam/21L10 (21L10) strains obtained in this study was sequenced. Length of genome was 146,083bp with the rate G and C content of 20%. Neither deletions nor insertions were found in the 21L10 strain. 149 open reading frames were found in the genome of the 21L10 strain.

Comparison of the complete genome of the 21L10 strain with that of other strains in Vietnam and abroad was conducted. Among Vietnamese LSDV strains, the highest nucleotide identity was 99.83% (the 21L10 vs. 20L70_Dinh-To and 20L81 Bang-Thanh) (Table 3). Comparison the four gene sequences, GPCR, RPO30, P32 and LSDV126, was also established. The highest nucleotide identity was 100% when comparing GPCR, P32 and LSDV126 gene sequences of the 21L10 strain with those of 20L70_Dinh-To and 20L81 Bang-Thanh strains, reported in Vietnam in 2020. Whereas, the highest nucleotide identity was 99.83% (Table 3). The highest nucleotide identity of the complete genome sequences was 99.99% (the 21L10 strain vs. China/LSDV/XJ01/2020 (OM105589)). Among selected four gene sequences, the highest nucleotide identity was 100% (the 21L10 strain vs. China/LSDV/XJ01/2020 (OM105589)) for the GPCR, P32 and LSDV126 sequences, when this rate was 99.83% for the RPO30 gene sequence (Table 4).

Phylogenetic analysis was constructed based on the complete genome of the 21L10 and other sequences from GenBank. Results indicated that the 21L10 belonged to subcluster 2.5 within LSDV. The Vietnamese LSDV strain obtained in this study was clustered with that of other Vietnamese LSDV strain (20L81-Bang-Thanh/2020 (MZ577076), 20L70_Dinh-To/2020 (MZ577075), LangSon0LS16 (PQ859561)). These strains were

Table 3: Comparisons of nucleotide identity of the full-length GPCR, RPO30, P32, and LSDV126 gene sequences of the LSDV/Vietnam/21L10 strain and other Vietnamese viral strains

Complete genome/ Gene name	Virus with the highest nucleotide identity				
	GenBank accession number	Strain name	Source	Year	% identity
Complete genome	MZ577076	20L70_Dinh-To	Cattle	2020	99.99
	MZ577076	20L81 Bang-Thanh			99.99
GPCR	MZ577076	20L70_Dinh-To	Cattle	2020	100
	MZ577076	20L81 Bang-Thanh			
RPO30	MZ577075	20L70_Dinh-To			99.83
	MZ577076	20L81 Bang-Thanh			
P32	MZ577075	20L70_Dinh-To			100
	MZ577076	20L81 Bang-Thanh			
LSDV126	MZ577075	20L70_Dinh-To			100
	MZ577076	20L81 Bang-Thanh			

Table 4: Comparisons of nucleotide identity of the full-length GPCR, RPO30, P32, and LSDV126 gene sequences of the LSDV/Vietnam/21L10 strain with sequences from GenBank database

Complete genome/ Gene name	Virus with the highest nucleotide identity					
	GenBank accession number	Country	Strain name	Source	Year	% Identity
Complete genome	OM105589	China	XJ01	Cattle	2019	99.99
GPCR	OM105589	China	XJ01	Cattle	2019	100
RPO30						99.83
P32						100
LSDV126						100

genetically closed to XJ01 Chinese viral strains (OM105589), reported in China in 2019 and differed from vaccine strains (Fig. 2). Phylogenetic trees, constructed based on GPCR, RPO30, P32, and LSDV126 gene sequences, supported that the 21L10 and other Vietnamese LSDV strains formed in a cluster that differed from vaccine strains (Fig. 2).

Pathogenic characterization of the LSDV/Vietnam/21L10 strain

In this study, body temperature was observed during 28 days post-infection (dpi). Results indicated that body temperature increased in the LSDV-infected group (39.9ºC±0.1), significant higher (P<0.05) than that of mock-infected group (38.77ºC±0.38) at 5dpi. During period of 6 to 14dpi, cattle were recorded with a high body temperature in LSDV-infected group, while a lower (P<0.05) body temperuture was in the mock-infected group (Fig. 3). Body temperuture was insignificant at other time points when comparing LSDV-infected and mock-infected groups (Table 3).

Clinical signs were observed in both LSDV-infected and mock-infected groups. Cattle began to exhibit clinical signs such as reduced appetite, weakened physical condition, lacrimation, and inflammation of the lymph nodes at 7dpi. Subsequently, skin nodules appeared between days 17 and 19dpi and then these nodules later ruptured and formed sunken scabs. On the other hand, no clinial signs were observed in the mock-infected group.

All cattle in the LSDV-infected group were nescopsied at 30dpi. Cattle infected with the LSDV/Vietnam/21L10 strain developed caseous necrosis in the cutaneous nodules (Fig. 1B). The peripheral lymph nodes, including the cervical, inguinal, and mesenteric lymph nodes, were enlarged and showed signs of necrosis (Fig. 5C-5G).

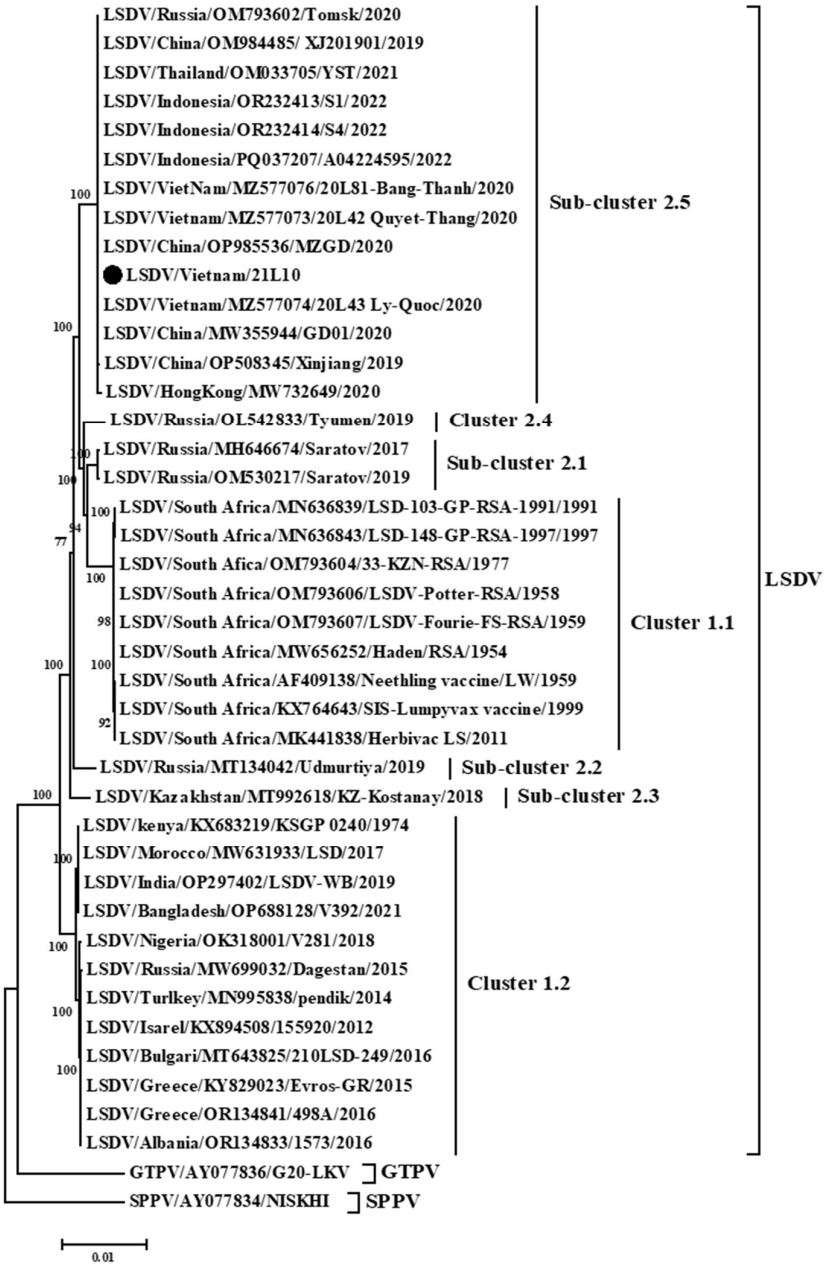

Fig. 1: Phylogenetic tree of the complete genome sequence of the 21L10 strains and other sequences downloaded from GenBank database. Numbers at each branch indicate bootstrap values of ≥50% by the bootstrap interior branch test. Vietnamese strains from this study are indicated by solid black circle.

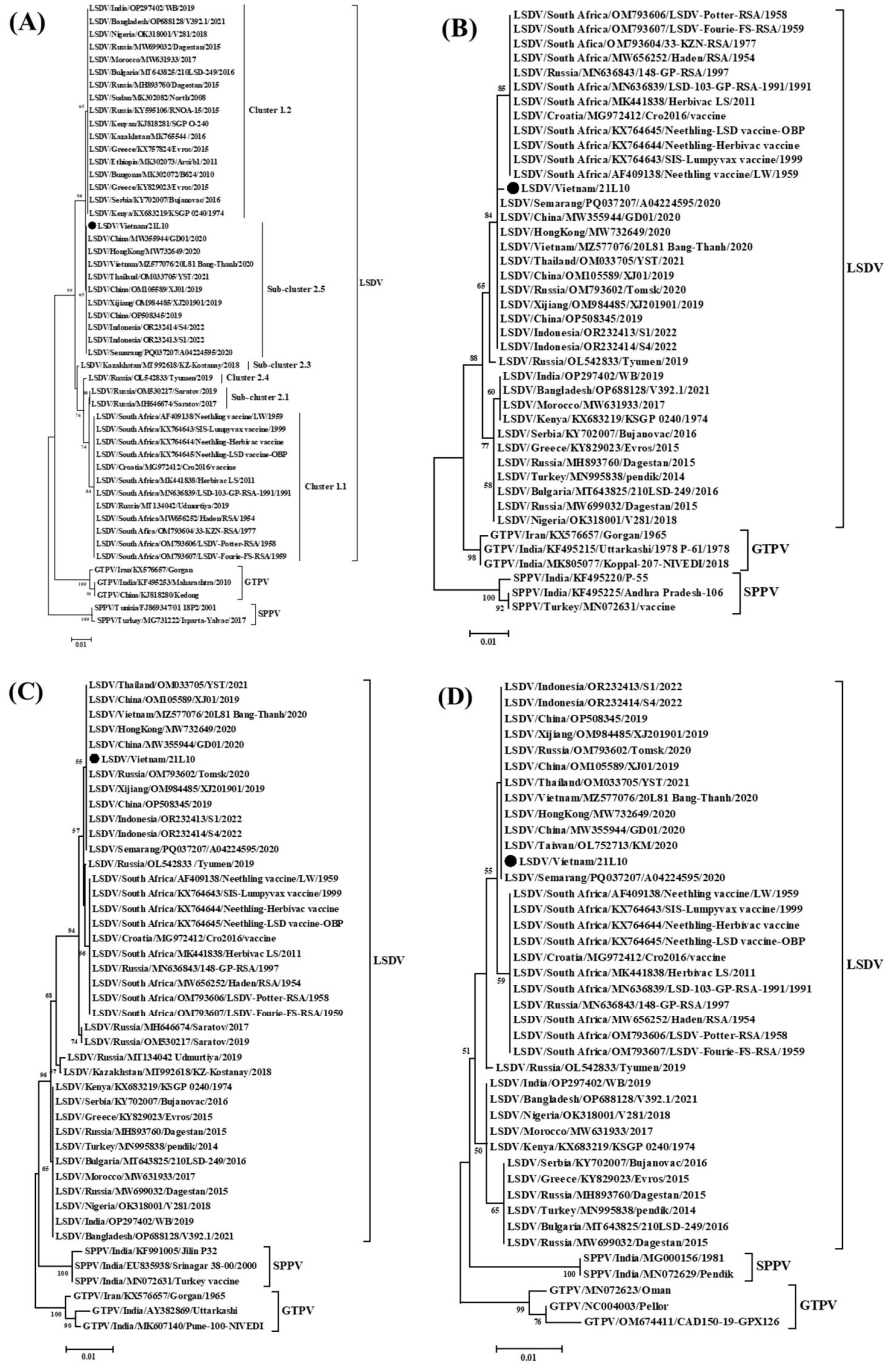

Fig. 2: Phylogenetic trees based on the full-length (A) GPCR, (B) RPO30, (C) P32, and (D) LSDV126 gene sequences of the LSDV/Vietnam/21L10 strain and other viral strains downloaded from GenBank database. Vietnamese strains from this study are indicated by solid black circle.

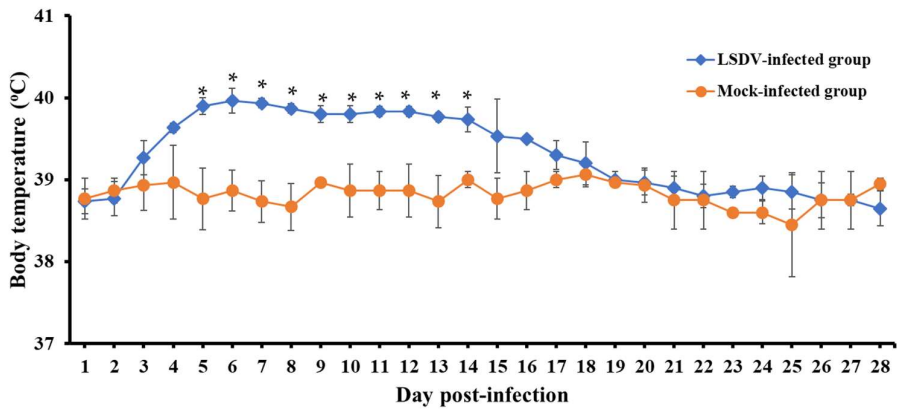

Fig. 3: Body temperature measurement of cattle infected with LSDV compared with those of mock-infected group. Asterisks (*) indicate that data is significantly different.

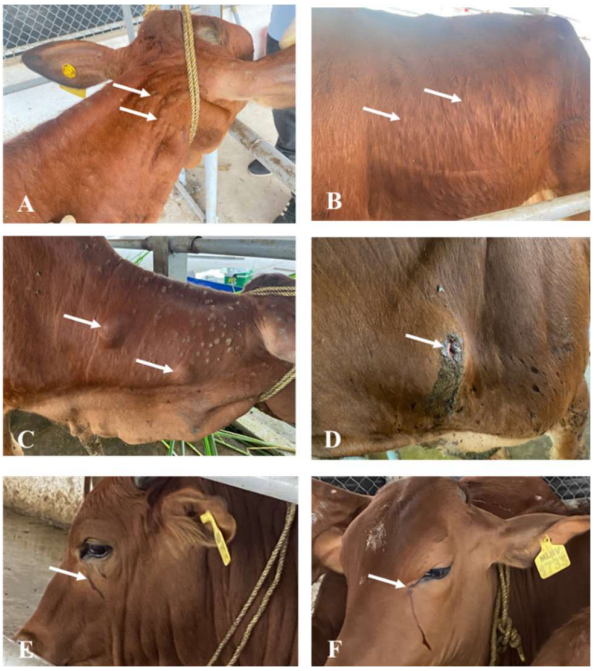

Fig. 4: Observation of clinical signs, (A-D) cutaneous nodules, (E-F): lacrimation, of cattle infected with the LSDV/Vietnam/21L10 strain.

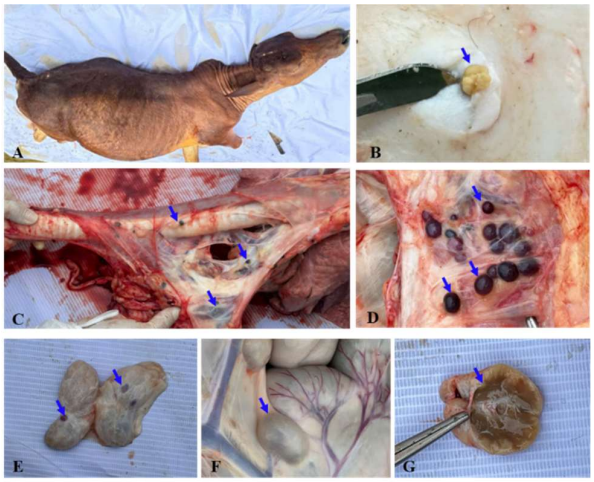

Fig. 5: Gross lesions, (A) dead cattle, (B) caseous necrosis, (C-G) enlargement and necosis of regional lymph nodes, of cattble infected with the the LSDV/Vietnam/21L10 strain.

Regarding viral shedding, saliva samples from infected cattle were collected at several time points. Results indicated that viral DNA was detected in saliva samples after 3dpi (3/3 cattle). The viral DNA still appeared in saliva until 7dpi. After that virus was not determined at 10, 13 and 28dpi (Table 5).

Table 5: Detection of viral shedding in cattle infected with the LSDV/Vietnam/21L10 strain

Cattle number	Days post-infection					
	0	3	7	10	13	28
TN01	-	+	+	-	-	-
TN02	-	+	+	-	-	-
TN03	-	+	+	-	-	-

"+": Positive for the LSDV genome; "-": Not detected.

DISCUSSION

Since the first report in Vietnam in 2020 (Tran et al. 2021a), LSD has had a significant impact on cattle farming across the country. This study provided additional data on the genome sequence of the LSDV strain responsible for the 2021 outbreak in Lang Son province of northern Vietnam. The viral genome was approximately 146,083bp in length, contains 149 ORFs, and does not exhibit any deletions or insertions in the protein-coding regions. The genome of strain 21L10 shared a high identity with LSDV strains associated with outbreaks in Vietnam and was genetically related to strains originating from China. This is the first report to describe certain pathogenicity of a field-isolated LSDV strain. Experimental infection of cattle with the LSDV/Vietnam/21L10 strain obtained in this study demonstrated its ability to induce disease, producing typical clinical signs such as fever, depression, anorexia, lacrimation, the formation of skin papules that rupture and leave scars, and lymphadenitis. The findings from this study lay the groundwork for the development and assessment of future vaccine candidates.

Based on genome sequences in GenBank, LSDV strains can be divided into subgroups, including 1.1 and 1.2. The LSD outbreak was first reported in several northern provinces of Vietnam in 2020 (Tran et al. 2021a) and subsequently spread to many provinces in both the northern and southern regions across the country (Trinh et al. 2022; Bich et al. 2024; Tran et al. 2024b; Tran et al. 2024c). Previous studies primarily focused on sequencing specific genes such as GPCR, RPO30, and p32 (Trinh et al. 2022; Bich et al. 2024; Tran et al. 2024b; Tran et al. 2024c). However, only a few studies have sequenced and analyzed the complete genome sequences of LSDV strains circulating in Vietnam. In this study, the complete genome sequence of strain LSDV/Vietnam/21L10 was successfully obtained. The results indicated that the viral genome, along with strains from China and some other Vietnamese LSDV strains, belonged to a similar subgroup. This suggested that LSDV strains may share a common origin. Given that Vietnam and China share a long border and engage in daily trade, cross-border transmission of infectious diseases is plausible. Another perspective was that the LSDV strains circulating in both Vietnam and China belong to the same group because this particular virus strain was widely distributed across Asian countries. Further researches into the evidence of cross-border transmission are essential to provide more detailed insights for controlling the spread of this disease in the future.

Investigation of the pathogenicity of field LSDV strains in experimental animals is of critical practical significance, particularly for informing disease control strategies, guiding vaccine development, and assessing vaccine efficacy in the context of Vietnam's current epidemiological landscape. Previous studies have reported that certain LSDV strains exhibit low or no pathogenicity in animals, with infected individuals displaying only subclinical signs of infection (Tuppurainen et al. 2005; Sanz-Bernardo et al. 2021). Carn and Kitching (1995) noted that the proportion of animals developing cutaneous nodules varied depending on the route of viral inoculation, highlighting the importance of the infection route in determining disease manifestation (Carn & Kitching 1995).

Recently, Ren et al. (2023) conducted experimental infections via jugular vein inoculation with strain LSDV/FJ/CHA/2021 (Ren et al. 2023). Their findings demonstrated that infected cattle developed high fever, lethargy, anorexia, lacrimation, and cutaneous nodules. Additionally, gross pathological examination revealed pulmonary lesions and enlargement of superficial lymph nodes. Consistent with these observations, cattle experimentally infected with 21L10 strain in the present study exhibited comparable clinical signs and pathological features. These results confirm the virulence of the 21L10 isolate. This constitutes a significant advancement in the characterization of circulating LSDV strains and provides a crucial foundation for the development and evaluation of effective vaccines in Vietnam.

Recombination, a form of genetic evolution, plays an important role in the evolution of viruses, including LSDV. Recombination events have been reported between LSDV strains in several countries, such as Russia and China, particularly within protein-coding regions of the viral genome, leading to the emergence of novel strains (Krotova et al. 2022; Tursunov et al. 2022; Ren et al. 2023). In the present study, no evidence of recombination even was detected among the LSDV strains circulating in Vietnam. To gain a more comprehensive understanding of LSDV evolution in the country, it is necessary to expand both the geographic scope and the number of samples analyzed in future investigations.

Conclusion

In this study, LSDV was detected in clinical samples collected during a suspected outbreak in Lang Son Province in 2021. The complete genome sequence of LSDV/Vietnam/21L10 strain was successfully sequenced using next-generation sequencing technology, revealing a genome size of 146,083 base pairs. Comparative analysis demonstrated that the genome of strain LSDV 21L10 shared a high rate of nucleotide identity with virus strains currently circulating in both Vietnam and China. Experimental infection of cattle with the LSDV/Vietnam/21L10 strain induced clinical signs including fever, depression, anorexia, lacrimation, and the development of cutaneous papules that ruptured and formed scars, accompanied by inflammation and swelling of the lymph nodes. These findings indicate that the LSDV 21L10 strain isolated in Vietnam in 2021 was a virulent field isolate.

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Author's Contribution: Dai Quang Trinh, Hieu Van Dong, Dao Anh Tran Bui designed the study. Dai Quang Trinh collected the sample. Dai Quang Trinh, Nga Thi Pham, Tham Hong Thi Nguyen, Yen Thi Tran, Thai The Le, My Hoa Thi Tran, Thanh Thi Vu analyzed data. Dai Quang Trinh, Hieu Van Dong wrote the manuscript. All authors read and approved the final manuscript.

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REFERENCES

Abutarbush SM, Ababneh MM, Al Zoubi IG, Al Sheyab OM, Al Zoubi MG, Alekish MO and Al Gharabat RJ, 2015. Lumpy skin disease in Jordan: disease emergence, clinical signs, complications and preliminary-associated economic losses. Transboundary and Emerging Diseases 62: 549-554. https://doi.org/10.1111/tbed.12177

Akther M, Akter SH, Sarker S, Aleri JW, Annandale H, Abraham S and Uddin JM, 2023. Global burden of Lumpy skin disease, outbreaks, and future challenges. Viruses 15: 1861. https://doi.org/10.3390/v15091861

Al-Salihi KA and Hassan IQ, 2015. Lumpy skin disease in Iraq: study of the disease emergence. Transboundary and Emerging Diseases 62: 457-462. https://doi.org/10.1111/tbed.12386

Alexander S, Olga B, Svetlana K, Valeriy Z, Yana P, Pavel P and Aleksandr K, 2019. A real-time PCR screening assay for the universal detection of lumpy skin disease virus DNA. BMC Research Notes 12: 371. https://doi.org/10.1186/s13104-019-4412-z

Annandale CH, Irons PC, Bagla VP, Osuagwuh UI and Venter EH, 2010. Sites of persistence of lumpy skin disease virus in the genital tract of experimentally infected bulls. Reproduction in Domestic Animals 45: 250-255.

Bianchini J, Simons X, Humblet MF and Saegerman C, 2023. Lumpy skin disease: a systematic review of mode of transmission, risk of emergence and risk entry pathway. Viruses 15: 1-8. https://doi.org/10.3390/v15081622

Bich TN, Trung LQ, Hieu TV, Huyen VTK, Hieu TQ, Giang HT and Chien NTP, 2024. Characterization and molecular identification of the lumpy skin disease virus in cattle in the Mekong Delta of Vietnam. Open Veterinary Journal 14: 1877-1895. https://doi.org/10.5455/OVJ.2024.v14.i8.16

Biswas S, Noyce RS, Babiuk LA, Lung O, Bulach DM, Bowden TR, Boyle DB, Babiuk S and Evans DH, 2020. Extended sequencing of vaccine and wild-type capripoxvirus isolates provides insights into genes modulating virulence and host range. Transboundary and Emerging Diseases 67: 80-97. https://doi.org/10.1111/tbed.13322

Carn VM and Kitching RP, 1995. An investigation of possible

routes of transmission of lumpy skin disease virus (Neethling). Epidemiology and Infection 114: 219-226. https://doi.org/10.1017/s0950268800052067

Dao TD, Tran LH, Nguyen HD, Hoang TT, Nguyen GH, Tran KVD, Nguyen HX, Van Dong H, Bui AN and Bui VN, 2022. Characterization of Lumpy skin disease virus isolated from a giraffe in Vietnam. Transboundary and Emerging Diseases 69: e3268-e3272. https://doi.org/10.1111/tbed.14583

Gelaye E, Belay A, Ayelet G, Jenberie S, Yami M, Loitsch A, Tuppurainen E, Grabherr R, Diallo A and Lamien CE, 2015. Capripox disease in Ethiopia: genetic differences between field isolates and vaccine strain, and implications for vaccination failure. Antiviral Research 119: 28-35. https://doi.org/10.1016/j.antiviral.2015.04.008

Haegeman A, De Leeuw I, Mostin L, Campe WV, Aerts L, Venter E, Tuppurainen E, Saegerman C and De Clercq K, 2021. Comparative evaluation of Lumpy skin disease virus-based live attenuated vaccines. Vaccines (Basel) 9: 473. https://doi.org/10.3390/vaccines9050473

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Kärber G, 1931. Beitrag zur kollektiven behandlung pharmakologiseher reihenversuche. Naunyn-Schmiedeberg's Archives of Pharmacology 162: 480–484. https://doi.org/10.1007/BF01863914

Khan A, Du XX, Hussain R and Kwon OD, 2022. Lumpy skin disease: A threat to the livestock industry - A review. Agrobiological Records 9: 22-36. https://doi.org/10.47278/journal.abr/2022.015

Koirala P, Meki IK, Maharjan M, Settypalli BK, Manandhar S, Yadav SK, Cattoli G and Lamien CE, 2022. Molecular characterization of the 2020 outbreak of Lumpy skin disease in Nepal. Microorganisms 10: 539. https://doi.org/10.3390/microorganisms10030539

Krotova A, Byadovskaya O, Shumilova I, van Schalkwyk A and Sprygin A, 2022. An in-depth bioinformatic analysis of the novel recombinant lumpy skin disease virus strains: from unique patterns to established lineage. BMC Genomics 23: 396. https://doi.org/10.1186/s12864-022-08639-w

Lamien CE, Le Goff C, Silber R, Wallace DB, Gulyaz V, Tuppurainen E, Madani H, Caufour P, Adam T, El Harrak M, Luckins AG, Albina E and Diallo A, 2011. Use of the Capripoxvirus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: development of a classical PCR method to differentiate Goat poxvirus from Sheep poxvirus. Veterinary Microbiology 149: 30-39. https://doi.org/10.1016/j.vetmic.2010.09.038

Le Goff C, Lamien CE, Fakhfakh E, Chadeyras A, Aba-Adulugba E, Libeau G, Tuppurainen E, Wallace DB, Adam T, Silber R, Gulyaz V, Madani H, Caufour P, Hammami S, Diallo A and Albina E, 2009. Capripoxvirus G-protein-coupled chemokine receptor: a host-range gene suitable for virus animal origin discrimination. Journal of General Virology 90: 1967-1977. https://doi.org/10.1099/vir.0.010686-0

Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, Ingersoll R, Sheppard HW and Ray SC, 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. Journal of Virology 73: 152-160. https://doi.org/10.1128/JVI.73.1.152-160.1999

Magori-Cohen R, Louzoun Y, Herziger Y, Oron E, Arazi A, Tuppurainen E, Shpigel NY and Klement E, 2012. Mathematical modelling and evaluation of the different routes of transmission of lumpy skin disease virus. Veterinary Research 43: 1. https://doi.org/10.1186/1297-9716-43-1

Manic M, Stojiljkovic M, Petrovic M, Nisavic J, Bacic D, Petrovic T, Vidanovic D and Obrenovic S, 2019. Epizootic features and control measures for lumpy skin disease in south-east Serbia in 2016. Transboundary and Emerging Diseases 66: 2087-2099. https://doi.org/10.1111/tbed.13261

Martin DP, Murrell B, Golden M, Khoosal A and Muhire B, 2015. RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evolution 1: vev003. https://doi.org/10.1093/ve/vev003

Mathewos M, Dulo F, Tanga Z and Sombo M, 2022. Clinicopathological and molecular studies on cattle naturally infected with lumpy skin diseases in selected districts of Wolaita Zone, Southern Ethiopia. BMC Veterinary Research 18: 297. https://doi.org/10.1186/s12917-022-03403-4

McInnes CJ, Damon IK, Smith GL, McFadden G, Isaacs SN, Roper RL, Evans DH, Damaso CR, Carulei O, Wise LM and Lefkowitz EJ, 2023. ICTV virus taxonomy profile: Poxviridae 2023. Journal of General Virology 104: 001849. https://doi.org/10.1099/jgv.0.001849

Mercier A, Arsevska E, Bournez L, Bronner A, Calavas D, Cauchard J, Falala S, Caufour P, Tisseuil C, Lefrancois T and Lancelot R, 2018. Spread rate of lumpy skin disease in the Balkans, 2015-2016. Transboundary and Emerging Diseases 65: 240-243. https://doi.org/10.1111/tbed.12624

Molla W, Frankena K and MCM DEJ, 2017. Transmission dynamics of lumpy skin disease in Ethiopia. Epidemiology and Infection 145: 2856-2863. https://doi.org/10.1017/S0950268817001637

Nandi S, Mahajan S, Mishra R, Nautiyal S, Agrawal RK, Singh M, Biswas SK, Chander V, Singh KP and Sharma GK, 2023. LSDV126 gene based molecular assays for specific detection and characterization of emerging Lumpy Skin Disease virus. Journal of Virological Methods 312: 114665. https://doi.org/10.1016/j.jviromet.2022.114665

Pitkin A, Deen J and Dee S, 2009. Further assessment of fomites and personnel as vehicles for the mechanical transport and transmission of porcine reproductive and respiratory syndrome virus. Canadian Journal of Veterinary Research 73: 298-302.

Ren S, Chen H, Yuan L, Yang X, Afera TB, Rehman ZU, Wang H, Wang X, Ma C, Lin Y, Qiu X, Yin X and Sun Y, 2023. Phylogenetic and pathogenic characterization of lumpy skin disease virus circulating in China. Virology 585: 127-138. https://doi.org/10.1016/j.virol.2023.06.008

Rouby S and Aboulsoud E, 2016. Evidence of intrauterine transmission of lumpy skin disease virus. The Veterinary Journal 209: 193-195. https://doi.org/10.1016/j.tvjl.2015.11.010

Sameea Yousefi P, Mardani K, Dalir-Naghadeh B and Jalilzadeh-Amin G, 2017. Epidemiological study of Lumpy skin disease outbreaks in North-western Iran. Transboundary and Emerging Diseases 64: 1782-1789. https://doi.org/10.1111/tbed.12565

Sanz-Bernardo B, Haga IR, Wijesiriwardana N, Basu S, Larner W, Diaz AV, Langlands Z, Denison E, Stoner J, White M, Sanders C, Hawes PC, Wilson AJ, Atkinson J, Batten C, Alphey L, Darpel KE, Gubbins S and Beard PM, 2021. Quantifying and modeling the acquisition and retention of Lumpy skin disease virus by hematophagus insects reveals clinically but not subclinically affected cattle are promoters of viral transmission and key targets for control of disease outbreaks. Journal of Virology 95: e02239-02220. https://doi.org/10.1128/JVI.02239-20

Seerintra T, Saraphol B, Wankaew S and Piratae S, 2022. Molecular identification and characterization of Lumpy skin disease virus emergence from cattle in the northeastern part of Thailand. Journal of Veterinary Medical Science 23: e73. https://doi.org/10.4142/jvs.22111

Sprygin A, Pestova Y, Prutnikov P and Kononov A, 2018. Detection of vaccine-like lumpy skin disease virus in cattle and Musca domestica L. flies in an outbreak of lumpy skin

disease in Russia in 2017. Transboundary and Emerging Diseases 65: 1137-1144. https://doi.org/10.1111/tbed.12897

Sudhakar SB, Mishra N, Kalaiyarasu S, Rai S and Sanyal A, 2025. Genetic characterization of lumpy skin disease virus (LSDV) from 2022 to 2023 outbreaks in yaks (*Bos grunniens*) and cattle in the Himalayan state of Sikkim, India, reveals dominant circulation of the LSDV 1.2.1 variant strain. Comparative Immunology, Microbiology and Infectious Diseases 125: 102432. https://doi.org/10.1016/j.cimid.2025.102432

Suwankitwat N, Deemagarn T, Bhakha K, Songkasupa T, Vitoonpong R, Trakunjaroonkit P, Rodphol S, Nuansrichay B, Chintapitaksakul L, Wongsarattanasin K, Kwon OK, Kang HE and Shin YK, 2023. Complete genomic characterization of Lumpy skin disease virus isolates from beef cattle in Lopburi province, Central Thailand, during 2021-2022. Veterinary Sciences 11: 10. https://doi.org/10.3390/vetsci11010010

Tasioudi KE, Antoniou SE, Iliadou P, Sachpatzidis A, Plevraki E, Agianniotaki EI, Fouki C, Mangana-Vougiouka O, Chondrokouki E and Dile C, 2016. Emergence of lumpy skin disease in Greece, 2015. Transboundary and Emerging Diseases 63: 260-265. https://doi.org/10.1111/tbed.12497

Thompson JD, Higgins DG and Gibson TJ, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673-4680. https://doi.org/10.1093/nar/22.22.4673

Tran HTT, Truong AD, Dang AK, Ly DV, Nguyen CT, Chu NT, Hoang TV, Nguyen HT, Nguyen VT and Dang HV, 2021a. Lumpy skin disease outbreaks in vietnam, 2020. Transboundary and Emerging Diseases 68: 977-980. https://doi.org/10.1111/tbed.14022

Tran THG, Dang HA, Nguyen VG and Huynh TML, 2021b. Molecular characterization of Lumpy skin disease virus emerged in northern Vietnam in 2020 based on RPO30 and GPCR genes. Philippine Journal of Veterinary Medicine 59: 155-163.

Tran AT, Tran HTT, Truong AD, Dinh VT, Dang AK, Chu NT, Phan L, Phan HT, Nguyen HT, To NBT and Dang HV, 2024a. Molecular characterization of lumpy skin disease virus in North Central Vietnam during 2021 and early 2022. Veterinaria Italiana 60(1): . https://doi.org/10.12834/VetIt.3233.22342.2

Tran HTT, Truong AD, Tran AT, Chu NT, Nguyen VT and Dang HV, 2024b. A new variant of lumpy skin disease virus circulating in Vietnam based on sequencing analysis of GPCR gene. Open Veterinary Journal 14: 1701-1707. https://doi.org/10.5455/OVJ.2024.v14.i7.19

Tran NB, Le QT, Truong VH, Vo TKH, Thai QH, Huynh TG and Nguyen TPC, 2024c. Characterization and molecular identification of the lumpy skin disease virus in cattle in the Mekong Delta of Vietnam. Open Veterinary Journal 14: 1877-1895. https://doi.org/10.5455/OVJ.2024.v14.i8.16

Trinh TBN, Nguyen VT, Nguyen TTH, Mai NTA, Le PN, Lai TNH, Phan TH, Tran DH, Pham NT, Dam VP, Nguyen TL, Ambagala A, Babiuk S and Le VP, 2022. Molecular and histopathological characterization of lumpy skin disease in cattle in northern Vietnam during the 2020-2021 outbreaks. Archives of Virology 167: 2143-2149. https://doi.org/10.1007/s00705-022-05533-4

Tuppurainen E, Alexandrov T and Beltrán-Alcrudo D, 2017. Lumpy skin disease field manual – A manual for veterinarians. FAO Animal Production and Health Manual No. 20. Rome. Food and Agriculture Organization of the United Nations (FAO).

Tuppurainen ES, Venter EH and Coetzer JA, 2005. The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. Onderstepoort Journal of Veterinary Research 72: 153-164. https://doi.org/10.4102/ojvr.v72i2.213

Tursunov K, Tokhtarova L, Kanayev D, Mustafina R and Mukantayev K, 2022. Effect of thioredoxin on the immunogenicity of the recombinant P32 protein of lumpy skin disease virus. Veterinary World 15: 2384-2390. https://doi.org/10.14202/vetworld.2022.2384-2390

Van Schalkwyk A, Byadovskaya O, Shumilova I, Wallace DB and Sprygin A, 2022. Estimating evolutionary changes between highly passaged and original parental lumpy skin disease virus strains. Transboundary and Emerging Diseases 69:e486-e496.

Yao J, Li X, Tan L, Gao L, Zhu P, Xie J, Yan R, Yu T, Xia F, Chen Q, Zeng B and Yang J, 2025. Molecular detection and phylogenetic analysis of lumpy skin disease virus in Yunnan Province, China from 2019 to 2023. Veterinary Research Communications 49: 297. https://doi.org/10.1007/s11259-025-10879-6

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