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Paper

Molecular characterization of lumpy skin disease virus in North Central Vietnam during 2021 and early 2022

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Abstract

In October 2020, the first outbreaks of lumpy skin disease (LSD) in Lang Son Province, Vietnam were reported by our laboratory. The disease had rapidly spread to the South, and it was reported in 55 of 63 provinces and cities of Vietnam by the end of 2021. The most economic loss caused by this disease occurred in the north-central region in 2021 where approximately 46,788 LSD virus (LSDV) infected cattle and buffaloes have been reported and 8,976 animals have been culled. However, the information on this pathogen circulating in this region is missing. Here, we describe the molecular characterization of LSDV circulating in north-central Vietnam in 2021 and early 2022. In total, 155 LSDV samples were collected during this period and three of these samples from each province were further characterized by Sanger sequencing analysis based on three key maker genes (GPCR, RPO30, and p32). Sequence comparison and phylogenetic analysis based on GPCR, RPO30, and p32 genes indicated that LSDV strains circulating in north-central Vietnam are closely related to previously reported strains in Vietnam regions which bordered China and all LSDV strains were 100% identical. These results show the importance of continuous monitoring and characterization of circulating LSDV strains and are important for vaccine development for the control and eradication of LSD in Vietnam.

Keywords

Lumpy skin disease, Vietnam, Diagnosis, GPCR, Vaccine

Introduction

Lumpy skin disease (LSD) is one of the most important transboundary viral diseases of cattle and buffaloes and it is listed as a notifiable disease by the World Organization for Animal Health . Previous studies have indicated that LSD virus (LSDV) had rapidly spread throughout neighboring provinces and the country through hematophagous vectors (mosquitoes and flies), which facilitate the rapid spread of the virus in optimal climatic conditions (Acharya and Subedi 2020; Beard 2016; Sanz-Bernardo et al. 2022). Recently LSD outbreaks have been reported in Africa, Europe, and Asia affecting thousands of cattle and buffaloes with a significant economic loss. This loss, including a reduction of total animals, decreased productivity, cost of disease control and prevention campaigns, and the loss of export markets (Casal et al. 2018), depends on the strains of LSDV, the age and immunological status, as well as the breed of cattle. The clinical signs of LSD range from mild (nodules and lesions in the skin) to severe symptoms that are sometimes fatal. Research studies indicate a mortality rate of <10% and a morbidity of up to 90% (OIE 2017; Sprygin et al. 2018a; Sprygin et al. 2018b; WOAH 2021). LSD was first reported in Zambia in 1929 and then spread into Botswana by 1943 and then was seen in southern and eastern Africa . From 1970 to 1986, LSD was epidemic and

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spread into most African countries such as Sudan (1970), Nigeria (1974), Mauritania, Mali, Ghana, and Liberia (1977). Another epizootic event of LSD occurred between 1981 and 1986, affecting Tanzania, Kenya, Zimbabwe, Somalia, and Cameroon, with reported mortality rates in affected cattle of 20% . The LSD outbreak outside sub-Saharan Africa was first reported in Egypt and Israel between 1988 and 1989 and has spread into southeast Europe, the Middle East, and South Asia . The outbreaks of LSD in Asia were first reported in China in 2019 and then spread into a large portion of Asian countries including India , Nepal , Bangladesh (Hasib et al. 2021), Thailand (Singhla et al. 2022), Myanmar (Maw et al. 2022), Mongolia (Zan et al. 2022), and Hongkong (Flannery et al. 2022) has raised serious concerns.

In Vietnam, the first LSD outbreak occurred in October 2020 and was reported by our laboratory with additional reports focusing on LSD outbreaks in the north of Vietnam . The most economic loss caused by LSD occurred in north-central Vietnam where approximately 46,788 LSDV-infected cattle and buffaloes had been reported and 8,976 animals had been culled in 2021. With the situation, the vaccination program with the live-attenuated LSDV vaccine based on the Neethling strain of the virus has been a key to the control of the disease in Vietnam since the middle of 2021. However, information about this pathogen is lacking in north-central Vietnam. It is important to understand the molecular characteristics and epidemiology of circulating LSDV for the control and prevention of LSDV in Vietnam. This study aims to determine the molecular characterization of LSDV circulating in north-central Vietnam (NCV) in 2021 and early 2022.

Materials and Methods

Samples collection

During January 2021 to April 2022, outbreak of LSD in north-central Vietnam, a total of 155 samples (whole blood, scabs, nasal, and oral swabs) were collected by the Region Animal Health Office 3 (RAHO3). These were collected from 6 provinces as shown in Table 1 and Figure 1, respectively. Samples were sent to the Vietnam National Institute of Veterinary Research for further pathogen studies, including molecular characterization of the lumpy skin disease virus.

DNA extraction and agent identification

Genomic DNA from the 155 field samples was extracted by using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). LSDV in samples was identified by conventional PCR according to standard protocol recommended by WOAH and real-time PCR for viral p32 gene detection (Alexander et al. 2019; Tran et al. 2021).

Outbreak date	Location	Number of samples collected	Type of sample				Number of PCR/Realtime PCR positive samples	Number of amplicons sequenced		
			Whole blood	Scabs	Nasal	Swabs		RPO30 gene	GPCR gene	p32 gene
April 2021	Hue	3	1		1	1	3	3	3	3
April-May, 2021	Nghe An	88	10	62	8	3	88	3	3	3
April 2021	Quang Binh	12	10		2		12	3	3	3
April-May, 2021	Thanh Hoa	41	9	7	3	22	41	3	3	3
April 2021	Ha Tinh	4	2		1	1	4	3	3	3
April 2021	Quang tri	4	2		2		4	3	3	3
Jan-Apr, 2022	Nghe An	3		3			3	3	3	3

Table I Samples were analyzed for PCR and sequencing. Samples were collected from six provinces of the North Centre region of Vietnam affected by lumpy skin disease from 2021 to early 2022

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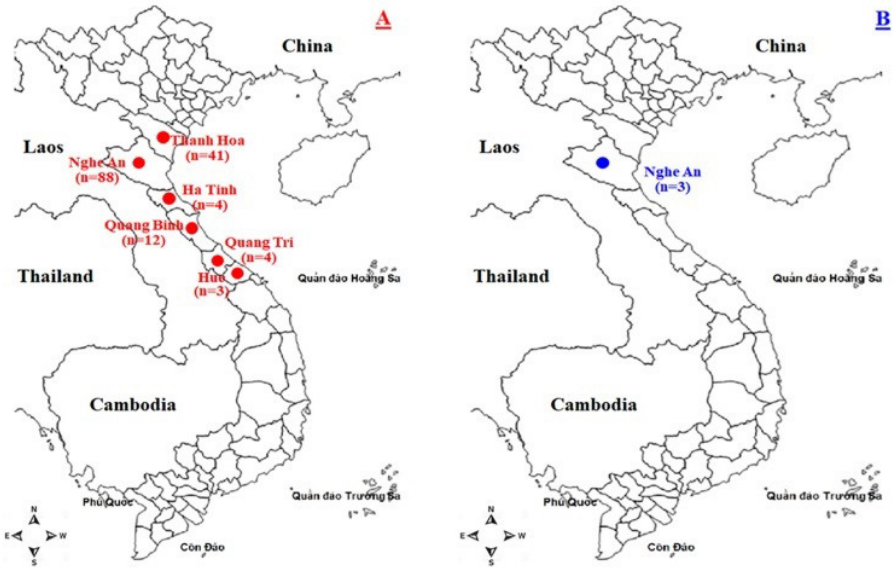

Figure 1 Map of Vietnam showing the samples collected in the North Central Vietnam that reported LSD outbreaks from 2021 to early 2022. (A) 2021; (B) Early 2022

Genomic characterization of LSDV strains

Three samples from each province were further characterized by Sanger sequencing analysis based on three key maker genes (GPCR, RPO30, and p32). Briefly, these marker genes were amplified and electrophoresed on a 1.5% agarose gel against a 100 bp DNA leader marker (Thermo Scientific) and visualized by UV irradiation with ethidium bromide staining (Sigma-Aldrich, St. Louis, MS, USA) . The PCR products were purified using the QIAQuick gel extraction kit (Qiagen, Germany) according to the manufacturer's specifications for sequencing (1st BASE, Selangor, Malaysia). The BioEdit and DNAstar program (DNASTAR Inc. Madison, WI, USA) were employed to analyze the chromatograms of nucleotide sequences. The Blast tool at the National Center for Biotechnology Information (NCBI) was used to identify the LSD virus strains in Vietnam in comparison with the information of published sequences. The multiple sequence alignment was performed using the Lasergene software (DNASTAR Inc.). A phylogenetic tree of LSDV p32, GPCR, and RPO30 genes was constructed using the neighbor-joining method with a bootstrap value of 1,000 in the MEGA7 program (Kumar et al. 2016).

Results and discussion

In 2021, a total of 34,711 family farms in 5,296 villages of 1,142 communes of 84 districts in 6 provinces in north-central Vietnam officially reported the LSDV outbreak. A total of 46,788 cattle and buffaloes were infected, and 8,976 animals have been culled, indicating the substantial economic impact on the cattle industry in NCV. In this study, we aimed to determine the molecular characterization of the lumpy skin disease virus (LSDV). From January 2021 to April 2022, a total of 155 clinical samples from cattle and buffaloes from 6 provinces of the NCV, including Thanh Hoa, Nghe An, Ha Tinh, Quang Binh, Quang Tri and Hue, were collected and a conventional PCR recommended by WOAH was used to identify of LSDV viral DNA in these samples. Furthermore, a real-time PCR was employed to re-confirm the LSDV genome in the clinical samples as previously described (Alexander et al. 2019; Tran et al. 2021). The results indicated that all of the samples were positive by both diagnostic methods.

To understand the molecular genetics of LSDV strains circulating in NCV, three samples from each province of north central region were used. The main marker genes, including partial p32 (390 bp), full-length RPO30 coding sequence (1272 bp), and full-length GPCR coding sequence (1146 bp) genes of the LSDVs were sequenced by the Sanger sequencing method for further molecular analysis. A total of 63 sequences of three maker genes were determined and submitted to the GenBank database under the accession numbers OQ948267 - OQ948329. For each of the targeted genes, the sequences of LSDV in NCV showed 100% identity among each other. On the phylogenetic trees for the p32 (Figure 2), RPO30 (Figure 3), and GPCR (Figure 4) of LSDV circulating in NCV clustered together.

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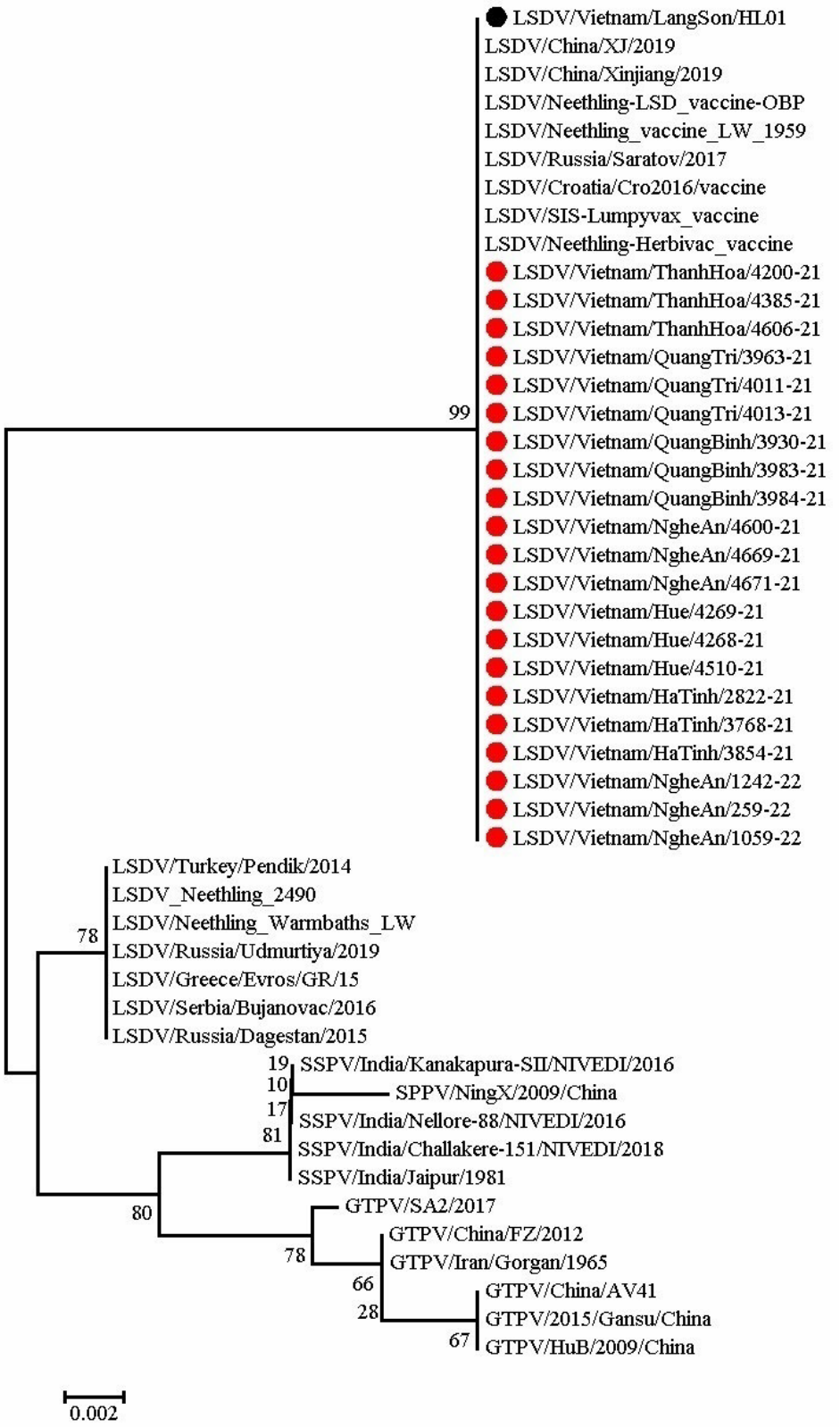

Figure 2 Phylogenetic trees with Neighbor-Joining (NJ) show the relationship between LSDV p32 genomic sequences of LSDV isolated in the North Central Vietnam, marked with red round, with other Capripoxvirus gene sequences from GenBank.

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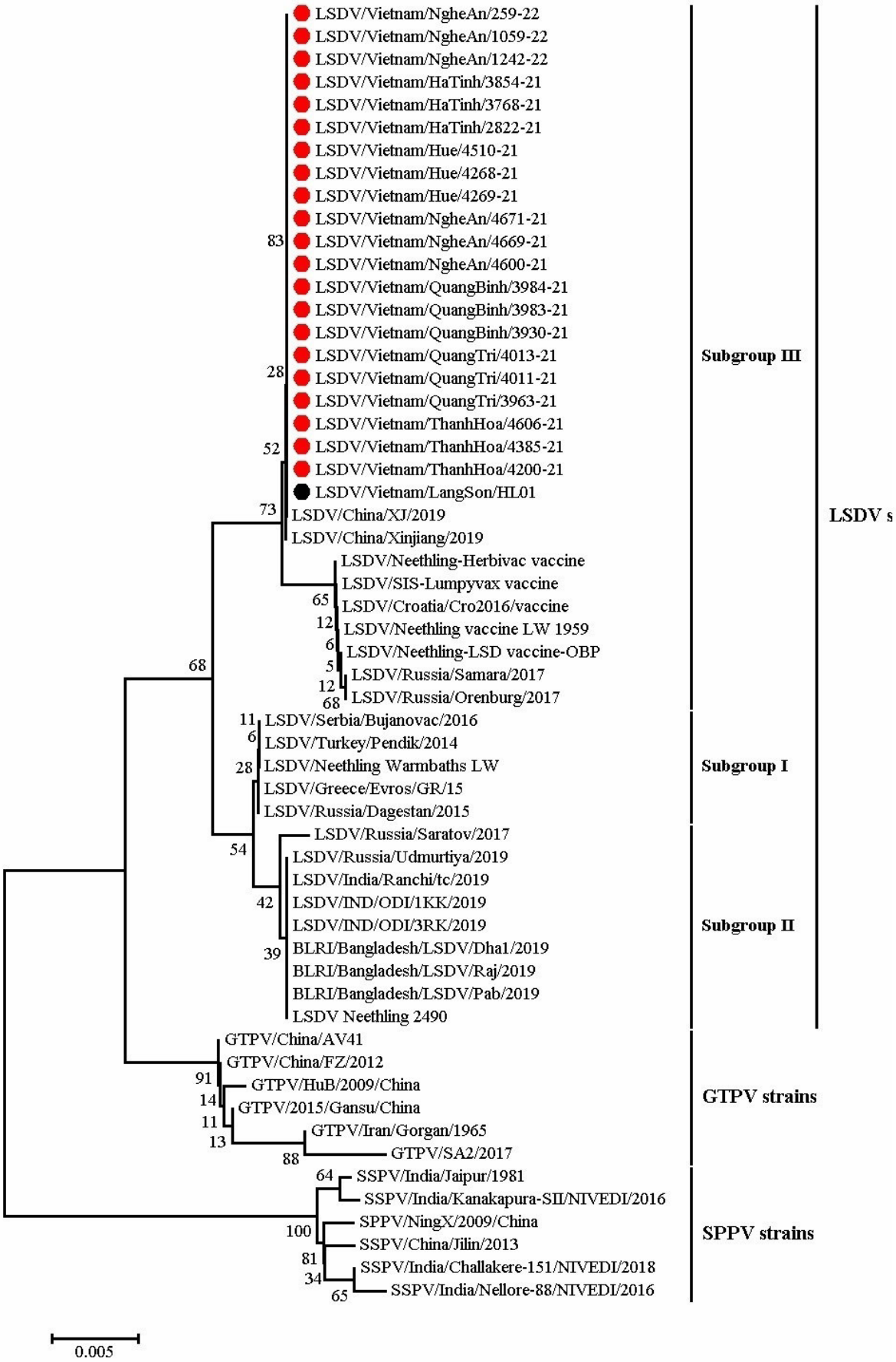

Figure 3 Phylogenetic trees with Neighbor-Joining (NJ) show the relationship between LSDV RPO30 genomic sequences of LSDV isolated in the North Central Vietnam, marked with red round, with other Capripoxvirus gene sequences from GenBank.

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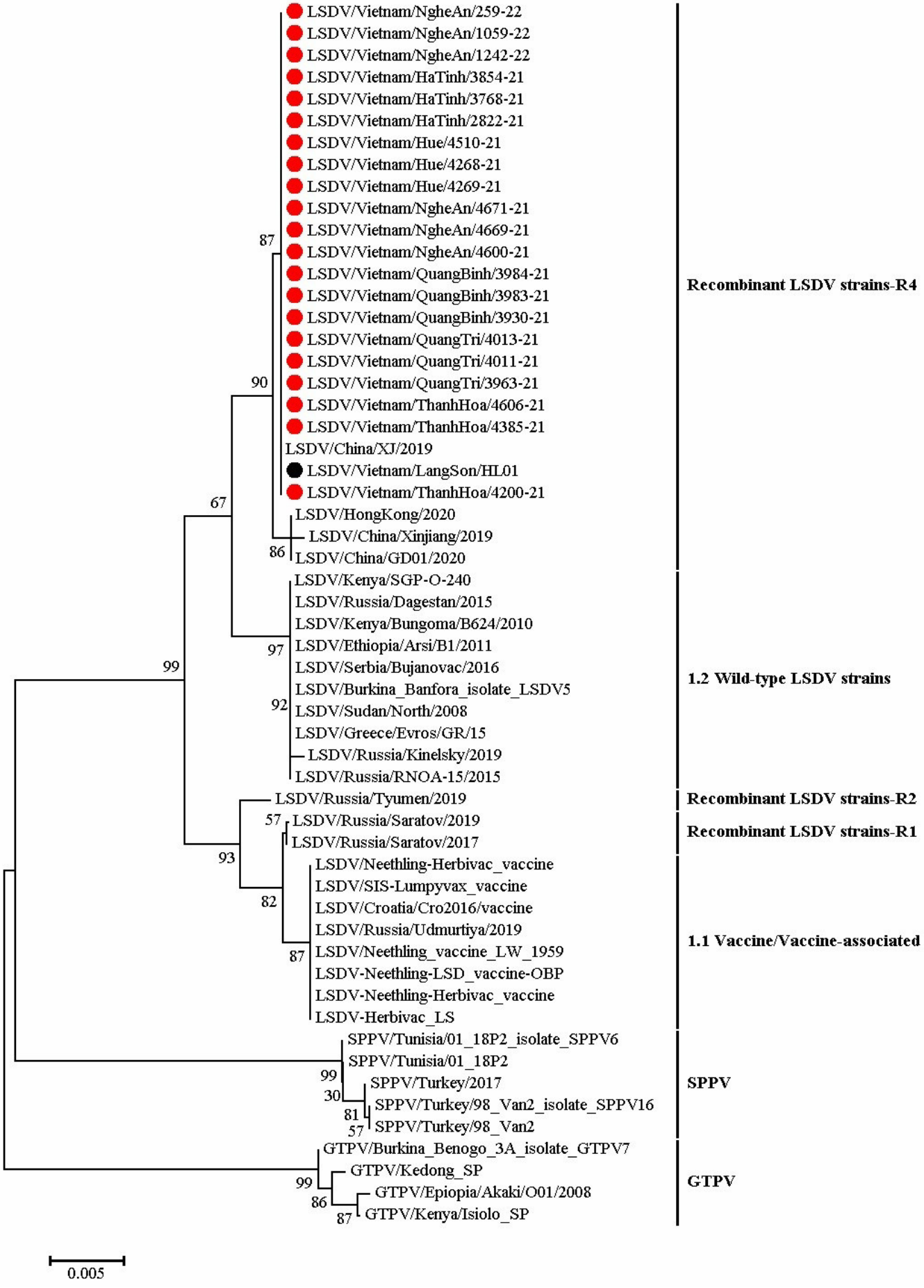

Figure 4 Phylogenetic trees with Neighbor-Joining (NJ) show the relationship between LSDV GPCR genomic sequences of LSDV isolated in the North Central Vietnam, marked with red round, with other Capripoxvirus gene sequences from GenBank.

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No	Genbank ID	Abbreviation name	Year	Origin	Wildtype/ vaccine	Identity to the NCV strain		
						GPCR	RPO30	p32
1	MH893760.2	LSDV/Russia/Dagestan/2015	2015	Russia	Wildtype	99.3%	98.78%	98.01%
2	MK302072.1	LSDV/Kenya/Bungoma/B624/2010	2010	Kenya	Wildtype	99.29%	99.17%	NI
3	MK302073.1	LSDV/Ethiopia/Arsi/B1/2011	2011	Ethiopia	Wildtype	99.29%	99.34%	NI
4	KY702007.1	LSDV/Serbia/Bujanovac/2016	2016	Serbia	Wildtype	99.29%	98.78%	98.10%
5	FJ869352.1	LSDV/Burkina-Banfora-isolate-LSDV5	2009	Burkina Faso	Wildtype	99.29%	99.34%	NI
6	KJ818281.1	LSDV/Kenya/SGP-O-240	2014	Kenya	Wildtype	99.30%	99.17%	NI
7	MK302082.1	LSDV/Sudan/North/2008	2008	Sudan	Wildtype	99.29%	99.34%	NI
8	KY829023.3	LSDV/Greece/Evros/GR/15	2015	Greece	Wildtype	99.29%	98.70%	98.10%
9	MK452255.1	LSDV/Russia/Kinelsky/2019	2019	Russia	Wildtype	99.20%	99.34%	NI
10	MK765544.1	LSDV/Kazakhstan/2016	2016	Kazakhstan	Wildtype	99.74%	99.39%	NI
11	KY595106.1	LSDV/Russia/RNOA-15/2015	2015	Russia	Wildtype	99.29%	NI	NI
12	OM530217.1	LSDV/Russia/Saratov/2019	2019	Russia	Recombinant R1	98.73%	99.01%	100%
13	MH646674.1	LSDV/Russia/Saratov/2017	2017	Russia	Recombinant R1	98.86%	98.97%	100%
14	OL542833.1	LSDV/Russia/Tyumen/2019	2019	Russia	Recombinant R2	98.86%	99.01%	100%
15	MT134042.1	LSDV/Russia/Udmurtiya/2019	2019	Russia	Recombinant R3	98.69%	99.17%	98%
16	MN508357.1	LSDV/China/XJ/2019	2019	China	Recombinant R4	100%	100%	100%
17	MN598005.1	LSDV/China/Xinjiang/2019	2019	China	Recombinant R4	99.73%	100%	100%
18	MW355944.1	LSDV/China/GD01/2020	2020	China	Recombinant R4	99.82%	100%	100%
19		LSDV/Vietnam/LangSon/HL01	2020	Vietnam	Recombinant R4	100%	100%	100%
20	MW732649.1	LSDV/HongKong/2020	2020	HongKong	Recombinant R4	99.82%	100%	100%
21	KX764644.1	LSDV/Neethling-Herbivac Vaccine	U	South Africa	Vaccine	98.69%	99.39%	100%
22	KX764643.1	LSDV/SIS-Lumpyvax-Vaccine	U	South Africa	Vaccine	98.69%	99.39%	100%
23	MG972412.1	LSDV/Croatia/Cro2016/vaccine	U	Croatia	Vaccine	98.69%	99.39%	100%
24	KX764645.1	LSDV-Neethling-LSD-Vaccine-OBP	U	South Africa	Vaccine	98.69%	99.39%	100%
25	KX764644.1	LSDV-Neethling-Herbivac-Vaccine	U	South Africa	Vaccine	98.69%	99.39%	100%
26	MK441838.1	LSDV-Herbivac-LS	U	South Africa	Vaccine	98.50%	99.31%	100%
27	AF409138.1	LSDV/Neethling-Vaccine-LW-1959	U	South Africa	Vaccine	98.69%	99.31%	100%

Table II The nucleotide homology between LSDV isolated from the North central region of Vietnam and other LSDV reference sequences. NI: No information, U: Unknown

Previous studies have described that the RPO30 gene was homologous with the vaccinia virus E4L gene, encoding for a DNA-dependent RNA polymerase subunit and this gene plays an important role in virus replication (Lamien et al. 2011; Santhamani et al. 2014). Based on the phylogenetic analysis of RPO30, it is seen that the LSDV strains were divided into 3 subgroups. Subgroup I consisted of the LSDV isolates from Russia in 2015 and Turkey in 2014. Subgroup II is the commonly circulating field isolates in Africa, the Middle East, Europe, Bangladesh, or India such as recombinant LSDV field isolates from Russia, LSDV-Russia/Udmurtiya/2019 (MT134042) and LSDV-Russia/Saratov/2017 (MH646674). Subgroup III was recognized by some LSDV Neethling-derived vaccine strains and the LSDV field isolates from China (Lamien et al. 2011; Ma et al. 2022; Santhamani et al. 2014; Suwankitwat et al. 2022; Zan et al. 2022). The findings in the current study indicated that the LSDV strain circulating in NCV was 100% identical to the LSDV previously reported strains in northern Vietnam, China strains Xinjiang/2019 (GenBank accession no. MN598007) and China/XJ/2019 (MN518933) based on RP030 genes. Additionally, it had a close genetic relationship with the Russian LSDV strain (LSDV/Russia/Saratov/2017), and the identity using the BLAST tool from the National Center for Biotechnology Information was 98.97% for the RP030 gene (Figure 3 and Table 2). Additionally, analysis of the RPO30 gene sequence of LSDV strains in NCV showed that it was 99.66% identical to the LSDV vaccine strains presented in South Africa. Our results demonstrated that based on the RPO30 gene, the LSDV isolated from NCV were clustered within subgroup III and close relationship with the Vietnam reference strain, LSDV/Vietnam/LangSon/HL01, and the LSDV strains circulating in China.

The G-protein-coupled chemokine receptor (GPCR) of *Capripoxvirus* family genomes plays an important gene in capripoxvirus discrimination because the GPCR gene of LSDV field-type strains deleted 12 nucleotides in comparison with the LSDV vaccine strain . Previous studies have demonstrated three major groups of LSDV, including vaccine/vaccine-associated LSDV strains group, wild-type LSDV strains group, and recombinant LSDV strains group (Flannery et al. 2022; Krotova et al. 2022b). Additionally, recombinant LSDV strain groups can also be classified into 4

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groups, identified as R1, R2, R3, and R4 with slight differences as described previously (Krotova et al. 2022a; Krotova et al. 2022b; Van Schalkwyk et al. 2022a; van Schalkwyk et al. 2022b). Group R1 consists of LSDV-Russia/Saratov/17 and LSDV-Russia/Saratov/19; whereas the LSDV-Russia/Tyumen/2019 and LSDV-Udmurtiya/19 were identified as groups R2 and R3, respectively (Flannery et al. 2022). Group R4 included some LSDV strains from China, Hong Kong, and Thailand (Flannery et al. 2022; Paungpin et al. 2022). 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 (Flannery et al. 2022; Shumilova et al. 2022). The data from the current study also showed that all LSDV strains circulating in the NCV have a 12-bp insertion and it is the highest homology with previously reported strains in Vietnam and China/XJ/2019 (MN518933) (100% nucleotide identify). These strains shared 99.73%, 99.82%, and 99.82% nucleotide identity with China strains Xinjiang/2019 (MN598007), Hong Kong/2020 (MW732649.1) and China/GD01/2020 (MW355944.1), respectively as shown in Table 2.

In comparison with other LSDV based on the GPCR gene, the intraspecies nucleotide identity of LSDV from the NCV was shared 99.29% - 99.74%, 98.50% - 98.69%, and 98.69% - 99.29% nucleotide identical to the wild-type, vaccine, and other recombinant LSDV strains, respectively. With GTPV and SPPV reference strains, 94.06% - 96.07% of identities were recognized as shown in Table 2. 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; Tuppurainen et al. 2021). Our findings suggest that the LSDV strain isolated from NCV belongs to the same sub-cluster as LSDV/Vietnam/LangSon/HL01, China/XJ/2019 (MN518933), China/GD01/2020 (MW732649.1) and LSDV/Hong- Kong/2020 (MW732649.1) as presenting the recombinant LSDV group R4 (Figure 4). Recent studies demonstrated that recombinant LSDV strains increased the virulence when compared to the field LSDV strains and it can be transmitted in vector-independent mode (Aleksandr et al. 2020; Kononova et al. 2020). Additionally, the LSDV strains in NCV in the same lineages were grouped into a new cluster, which is slightly different from the LSDV vaccine strains and the current wild-type LSDV strains. This suggests the multiple recombination mechanism of involvement of least one wild-type strain and one vaccine LSDV strain, resulting in these recombinant LSDV strains circulating in China, Russia, and Vietnam (Aleksandr et al. 2020; Kononova et al. 2020).

It is critical to understand and characterize the circulating LSDV strains for control and prevention of this disease in the most economic loss region in Vietnam. We have sequenced and characterized the LSDV isolated in north-central Vietnam from an outbreak in 2021 and early 2022. Phylogenetic analysis demonstrated the same clustering of LSDV in north-central Vietnam with China LSDV strains. Additionally, the data from the current study is important to support the national vaccination program against the LSD virus and the strategies of the effective LSD virus vaccine development in Vietnam.

Ethical Statement

The study was conducted in compliance with the institutional rules for the care and use of laboratory animals and a protocol approved by the Ministry of Agriculture and Rural Development, Vietnam (TCVN 8402:2010).

Acknowledgement

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Conflict of interest statement

There are no potential conflicts of interest.

Data Availability

All sequences generated in this study were submitted to GenBank under Accession Nos. OQ948267 - OQ948287 for p32 genes, OQ948288 - OQ948308 for GPCR genes, and OQ948309 - OQ948329 for RPO30 genes, respectively.

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