Veterinary Integrative Sciences 2025; 23(3): e2025081-1-13. DOI: 10.12982/VIS.2025.081

Veterinary Integrative Sciences
ISSN: 2629-9968 (online)

Research article

Molecular characterization and phylogenetic analysis of lumpy skin disease virus from the field outbreaks in Ben Tre province of Vietnam in 2023

Hieu Van Truong[1], Quyen Thi Kim Nguyen[1], Ut Danh[1], Chien Tran Phuoc Nguyen[2], Trung Quang Le[2] and Bich Ngoc Tran[2,*]

[1] *Department of Husbandry and Veterinary Studies, School of Agriculture and Aquaculture, Tra Vinh University, Tra Vinh 87000, Vietnam*
[2] *Faculty of Veterinary Medicine, College of Agriculture, Can Tho University, Can Tho 94000, Vietnam*

Abstract

Lumpy skin disease (LSD) is an emerging transboundary viral disease and an economically important disease of domestic ruminants worldwide caused by the LSD virus (LSDV). LSD was first confirmed in Vietnam on 13 October 2020 and continuously spread to almost every province. This study highlighted the molecular characterization and phylogenetic analysis of LSDV occurring in Ben Tre province of Vietnam during 2023. Nasal swabs were collected in cattle from 70 outbreaks of LSD in seven districts of Ben Tre province to amplify the GPCR gene via the PCR method. The nucleotide and amino acid identities of LSDV between the field sequences in Ben Tre province and the reference sequences in GenBank were very high (98.4–99.6% and 99.7–100%, respectively), however, the nucleotide and amino acid identities between the field sequences and vaccine sequence were low (78.7–79.0% and 78.0–78.2%, respectively). Phylogenetic analysis according to the GPCR gene revealed that all the field sequences were clustered into the same group with the LSDV collected from cattle in other provinces in Vietnam and those in the neighboring countries including China and Thailand, and distinguished from the GTPV and SPPV recruited from the GenBank database. Moreover, multiple alignments of the field LSDV sequences in Ben Tre province and reference sequences from the previous studies in China, Thailand, and Vietnam showed a tight homology in the GPCR region. The present study expanded basic knowledge of the molecular characteristics of LSDV in Ben Tre province and Vietnam.

Keywords: Ben Tre, Cattle, Epidemiology, Lumpy skin disease, Phylogeny.

*Corresponding author: Bich Ngoc Tran, Faculty of Veterinary Medicine, College of Agriculture, Can Tho University, Can Tho 94000, Vietnam. E-mail: tnbich@ctu.edu.vn.

Article history; *received manuscript: 2 October 2024, revised manuscript: 20 November 2024, accepted manuscript: 8 January 2025, published online: 13 January 2025,*
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Lumpy skin disease (LSD) is an emerging transboundary infectious viral disease and an economically important disease of domestic ruminants including cattle and water buffalo globally. The average morbidity rate of LSD oscillates from 3–85% and the mortality rate is roughly 5%. Cattle infection with LSD shows clinical signs of skin nodules in the head, neck, limbs, and genital areas; fever; loss of appetite; increased nasal secretion; salivation; weakness; etc. (Tuppurainen et al., 2005; Tuppurainen and Oura, 2012; Tuppurainen et al., 2017; Sudhakar et al., 2022). LSD could be transmitted through the arthropods (mosquitoes, flies, midges) and the hematophagus (ticks) (Tuppurainen et al., 2017; Gupta et al., 2020). Since the first report of LSD in Zambia in 1929, the outbreaks have been frequently reported in several geographical regions worldwide despite numerous disease control strategies including vaccination programs being applied to control LSD (Tuppurainen and Oura, 2012; Klement et al., 2020; Selim et al., 2021; Anwar et al., 2022). Especially, the LSD outbreaks were rapidly increased in Asian countries over the current decade and seriously affected the income of small–scale households (Anwar et al., 2022; Chouhan et al., 2022; Maw et al., 2022; Sariya et al., 2022; Wang et al., 2022; Rafe–Ush–Shan et al., 2025). There was an estimation that the average economic loss was approximately 110.40 US per LSD case in Bangladesh (Chouhan et al., 2022). Therefore, studies focusing on the field outbreaks of LSD in different geographical regions should be promoted to clearly illustrate the molecular characteristics of LSD globally.

LSD is caused by the LSD virus (LSDV) which is a double–stranded DNA virus belonging to the *Poxviridae* family, *Capripoxvirus* genus, and shares a very close genetic proximity with sheeppox virus (SPPV) and goatpox virus (GTPV). The genome of LSDV is roughly 151 kbp in size and encodes about 156 genes (Tulman et al., 2001; Tuppurainen et al., 2017). Over the past decades, the G protein–coupled chemokine receptor (GPCR), P32, and RPO30 genes have been widely used as the target genes for genetic analysis of LSDV worldwide (Tulman et al., 2001; Le Goff et al., 2009; Sprygin et al., 2018; Selim et al., 2021; Singhla et al., 2022). The GPCR protein encoded via the GPCR gene is associated with the structural features of the G–protein–coupled chemokine receptor subfamily and is related to the host antiviral response. Besides, the GPCR gene is popularly known as one of the most variable genes of LSDV (Le Goff et al., 2009; Tuppurainen et al., 2017; Ochwo et al., 2020). Therefore, the GPCR gene is a notable target for the molecular studies of LSDV in cattle around the world (Le Goff et al., 2009; Sprygin et al., 2018; Ochwo et al., 2020; Selim et al., 2021; Sudhakar et al., 2022). However, there was a limitation of studies on genetic analysis of the GPCR gene of LSDV in cattle in Vietnam, especially in the Mekong Delta (MD) region which is an important livestock region of Vietnam (Tran et al., 2021; Trinh et al., 2022; Tran et al., 2024).

In Vietnam, the first LSD outbreak in cattle was discovered in Huu Lung district, Lang Son province on 13 October 2020 and the molecular characteristics of field LSDV sequences were rapidly detected via sequencing the partial of P32, RP030, thymidine kinase, and ORF103 genes (Tran et al., 2021). Since the first outbreak in Lang Son province, LSD has spread to most of the provinces and cities of Vietnam, decreased the number of cattle, and partially affected the economy of the country (Trinh et al., 2022). Several studies focusing on the molecular characteristics of field LSDV sequences in Vietnam based on the P32, RP030, thymidine kinase, F, GPCR, and ORF103 genes were published (Tran et al., 2021; Trinh et al., 2022; Tran et al., 2024). However, almost all of the current studies on LSDV in Vietnam only focused on some provinces in the North region (Ha Giang, Lang Son, Bac Giang), and the North–Central region (Hue, Nghe An, Quang Binh, Thanh Hoa, Ha Tinh, Quang Tri) (Tran et al., 2021; Trinh et al., 2022; Tran et al., 2024). Reports of LSD outbreaks in the MD region which is a South–Western of Vietnam seem rare. The MD region importantly contributed to the number of cattle in the whole country, reaching 14.97% (951.3/6,353.1 thousand heads) in 2022. In

which, Ben Tre province has been considered as an important area of cattle in the MD region, reaching 22.58% (214.8/951.3 thousand heads) (General Statistics Office of Vietnam, 2022). Therefore, the present study was performed to determine the molecular features and phylogenetic analysis of LSDV collected from the field outbreaks in Ben Tre province of Vietnam.

MATERIALS AND METHODS

Ethics statement

The collected protocol was based on the approved guideline by the Ministry of Agriculture and Rural Development (MARD) Vietnam (QCVN 01–83:2011).

Sample collection in the field outbreaks

A cross–sectional study was performed on 70 households over the period from February to November 2023 to collect the samples from LSDV–suspected cattle in Ben Tre province of Vietnam (Figure 1). The information on the field of LSD outbreaks was based on the report of the Sub–Department of Animal Health of Ben Tre provinces. LSD was diagnostic via typical clinical features of the disease including skin nodules, fever, increased nasal secretion, salivation, and weakness. Prior to sample collection, all the information on clinical signs and some epidemiological features such as age, sex, breed, and vaccination status of individual cattle was carefully recorded in the structured questionnaire. In the outbreaks in individual districts of Ben Tre province, nasal swabs were collected from the nasopharyngeal area of 3 cattle per household and pooled to be one sample. Besides, individual samples were also collected from individual diseased cattle to classify them after the pooled samples were positive. The samples were then carefully labeled, stored in glycerol 10% in an individual sterile container, and submitted to the laboratory within 24 hours under a cool container (2–8°C). All the samples were kept at –20°C in the laboratory of the Faculty of Veterinary Medicine, College of Agriculture, Can Tho University for further steps.

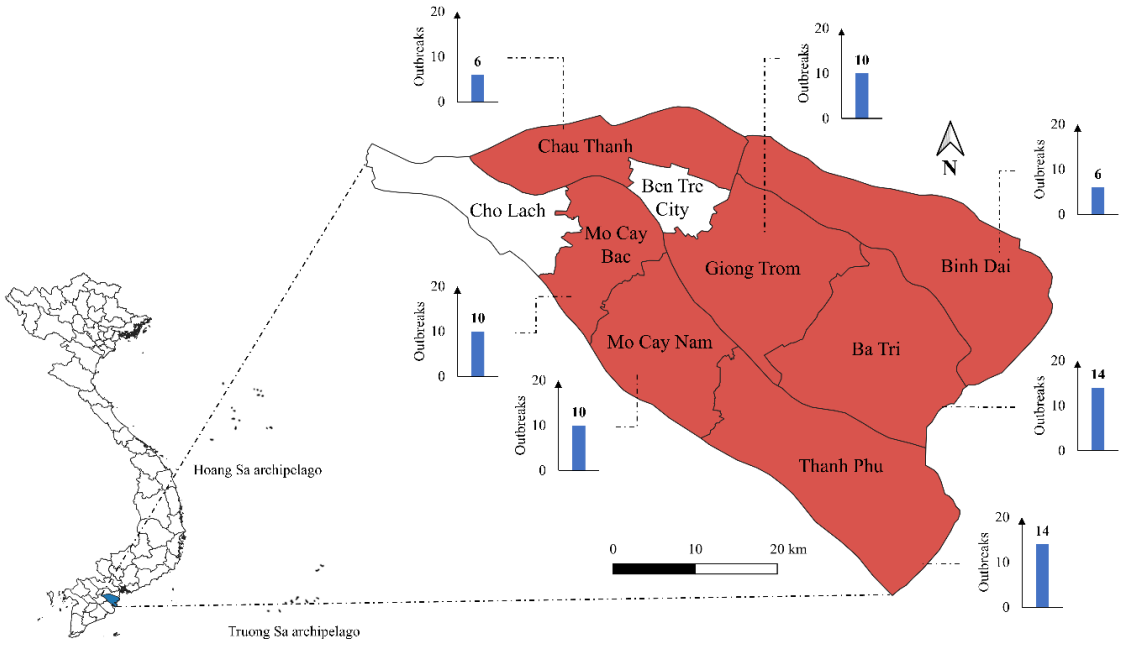

Figure 1 The geographical location of the study area. The districts investigated were shown in pale red for Ben Tre province. Bar charts represented the outbreaks of LSD during the investigated period of the current study.

Viral extraction

The samples were first homogenized in phosphate–buffered saline (PBS) at 3,500 rpm for 10 min at room temperature. The supernatants were then collected and extracted total DNA via the TopPURE viral DNA/RNA extraction (ABT, Vietnam) according to the manufacturer's guidelines. Total DNA productions subsequent to extraction were stored at –20°C in the laboratory for further analysis of the field LSDVs.

Real–time polymerase chain reaction (real–time PCR) to confirm LSDV outbreak in Ben Tre province

The pooled samples from the outbreaks were used for real–time PCR to confirm LSDV in cattle in Ben Tre province using a special primer pair to amplify the P32 gene of LSDV (Bowden et al., 2008). The forward primer 5'–AAAACGGTATATGGAATAGAGTTGGAA–3', reverse primer 5'–AAATGAAACCAATGGATGGGATA–3', and probe 5'–FAM–TGGCTCATAGATTTCCT–MGB/NFQ–3'. The reaction was run in a total of 25 µL volume including 12.5 µL of Toughmix (Quantabio, Cat No. 95132–500), 1.25 µL of forward and reverse primers each (18 µM), 1.25 µL of probe (5 µM), 5 µL of DNA template, and 3.75 µL of nuclease–free water, and the cycling conditions of 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 2 min, followed by 45 cycles at 95°C for 15s and 60°C for 45s.

Amplification and sequencing of the GPCR gene of LSDV in Ben Tre province

All the samples from LSDV–suspected cattle in Ben Tre province were amplified for the presence of the LSDV genome by a primer targeting 1,158 bp of the GPCR gene according to the prior study (Le Goff et al., 2009). The forward primer 5'–TTAAGTAAAGCATAACTCCAACAAAAATG–3' and reverse primer 5'–TTTTTTTATTTTTTATCCAATGCTAATACT–3' were amplified the region from nucleotide 6,916 to 8,119 of the LSDV genome. The PCR reaction was carried out in a total of 25 µL volume including 12 µL of GoTaq DNA Polymerase (Promega, USA), 1 µL of forward and reverse primers each (10 µM), 3 µL of DNA template, and 8 µL of nuclease–free water. PCR amplification was run with an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30s, annealing at 58°C for 30s, extension at 72°C for 30s, and final extension at 72°C for 5 min. Following, the PCR products were electrophoresed on a 1.5% agarose gel in 1X TAE buffer and visualized in an ultraviolet cabinet (UVP Transilluminator, USA). Subsequent to amplifying the GPCR gene via the PCR method in the pooled samples, individual samples were then performed one more time to classify the positive sample in the pooled samples. The data were recorded and used to calculate and interpret the results.

In the current study, only one representative sample per village in the district of Ben Tre province was chosen for sequencing of the GPCR gene of LSDV. Therefore, a total of eight PCR products were purified using the TopPURE PCR/Gel/DNA kit (ABT, Vietnam) and sent to a commercial company (Nam Khoa Biotek, Vietnam) for sequencing the GPCR gene of the LSDV via the Sanger method.

Molecular and phylogenetic analysis of the GPCR gene of LSDV in Ben Tre province

The obtained sequences were assembled using the BioEdit software package version 7.2.5 (Hall, 1999) and then deposited in the GenBank with the accession numbers from PP387459 to PP387466. The nucleotide and amino acid identities of the GPCR gene of LSDV were performed via the molecular evolutionary genetics analysis (MEGA) software package version 7.0.26. The phylogenetic

analysis was done via the MEGA software package version 7.0.26, using the maximum likelihood method with the Tamura 3–parameter model and 1,000 bootstrap replications (Kumar et al., 2016) and visualized by the iTOL package version 6.0 (Letunic and Bork, 2024). The reference sequences of LSDV, SPPV, and GTPV strains in the current study were based on the previous sequences available in the GenBank database. Furthermore, the BioEdit software package version 7.2.5 was utilized to compare an alignment of the deduced amino acid sequences between the field LSDV sequences and the reference sequence.

RESULTS

LSD outbreak in Ben Tre province and LSDV confirmation

In this study, we followed 70 outbreaks of LSD in seven districts of Ben Tre province over the period from February to November 2023 according to the report of the Sub–Department of Animal Health of Ben Tre provinces and typical clinical features of LSD (Figure 1). To confirm the pathogen–caused in cattle in Ben Tre province, the real–time PCR technique based on the P32–specific primer pair and PCR technique based on the GPCR–specific primer pair were utilized. In total, 70 pooled samples collected from LSDV–suspected cattle were positive with LSDV (100%) (data not shown). Eight representative samples in Ben Tre province were then chosen to sequence the GPCR gene of the field sequences via the Sanger method. The samples subsequent to sequencing were carefully checked and deposited in the GenBank with the accession numbers from PP387459 to PP387466 (Table 1).

Table 1 Information on the LSDV–positive samples collected in Ben Tre province.

No.	Village/District	Age (months)	Sex	Breed	Vaccination status	GenBank accession numbers
1	Huu Dinh/Chau Thanh	24	Male	Cross	No	PP387459
2	Binh Thanh/Giong Trom	8	Male	Cross	No	PP387460
3	Phuoc Ngai/Ba Tri	48	Female	Cross	No	PP387461
4	Tan Xuan/Ba Tri	6	Male	Cross	No	PP387462
5	Phu Long/Binh Dai	12	Female	Cross	No	PP387463
6	Hoa Loi/Thanh Phu	36	Female	Cross	No	PP387464
7	Tan Trung/Mo Cay Nam	24	Male	Cross	No	PP387465
8	Tan Binh/Mo Cay Bac	7	Male	Cross	No	PP387466

The nucleotide and amino acid identities of LSDV in Ben Tre province

The nucleotide and amino acid identities of LSDV between the field sequences in Ben Tre province and those from the prior studies in Vietnam, China, and Thailand were compared in the current study. The reference sequences were recruited from the GenBank database. In the present results, the nucleotide homology among the LSDV field sequences in Ben Tre province was 98.4–100%, between the LSDV field sequences in Ben Tre province and other Vietnamese sequences were 98.4–99.7, between the LSDV field sequences in Ben Tre province and other reference sequences in the world were 98.4–99.6%, and between the LSDV field sequences in Ben Tre province and vaccine sequence (LSDV–LumppyVac–GPCR–Thailand–2021) were 78.7–79.0%. Likewise, by amino acid identity, the field LSDV sequences exhibited a homology of 99.4–100% together, the homology between the LSDV field sequences in Ben Tre province and other

Vietnamese sequences were 99.7–100, between the LSDV field sequences in Ben Tre province and other reference sequences in the world were 99.7–100%, and between the LSDV field sequences in Ben Tre province and vaccine sequence (LSDV–LumppyVac–GPCR–Thailand–2021) were 78.0–78.2% (Table 2).

Phylogeny of LSDV in Ben Tre province

In the current study, the GPCR gene of LSDV was chosen to identify the phylogenetic analysis of the field sequences. To detect the genetic proximity between the field sequences in Ben Tre province and those circulating in the neighboring countries such as China, Thailand, and another country such as Russia, our study recruited the LSDV reference sequences from the GenBank database which included vaccine strains, GTPV, and SPPV. Phylogenetic analysis of the field sequences of LSDV in Ben Tre province based on the GPCR gene indicated very tight genetic proximity among the field sequences, other Vietnamese sequences, and LSDV reference sequences isolated in China, Thailand, and Russia. The field sequences in Ben Tre province were clustered into the same branch with the reference sequences from the prior studies in the North of Vietnam, China, Thailand, and Russia. In contrast, the field sequences in Ben Tre province were separately grouped from the LSDV vaccine strain (Neethling), GTPV, and SPPV. Interestingly, there was grouped into the same branch between the field sequences in Ben Tre province and the GPCR sequences of the vaccine strain namely LumppyVac in cattle in Thailand (LSDV–LumppyVac–GPCR–Thailand–2021) (Figure 2).

Alignment of the deduced amino acid sequences of LSDV in Ben Tre province

To explore further molecular characterization of LSDV in cattle in Ben Tre province, a deduced GPCR amino acid sequence analysis was performed. As shown in the present result, multiple sequence alignments of the field sequences in Ben Tre province and reference sequences in the North of Vietnam, China, and Thailand demonstrated no different alignment in the GPCR region except the position of Y3. In contrast, between the field sequences and vaccine strain (LSDV–LumppyVac–GPCR–Thailand–2021), there were seven different alignments at amino acid positions of T59, A60, S76, M127, T268, F362, and T363 (Figure 3).

Table 2 The nucleotide (below diagonal) and amino acid (above diagonal) identities of LSDV in cattle in Ben Tre province and reference sequences in GenBank.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1		100	100	100	99.7	100	100	100	78.2	100	100	100	100	100	100	100	100
2	100		100	100	99.7	100	100	100	78.2	100	100	100	100	100	100	100	100
3	100	100		100	99.7	100	100	100	78.2	100	100	100	100	100	100	100	100
4	100	100	100		99.7	100	100	100	78.2	100	100	100	100	100	100	100	100
5	99.9	99.9	99.9	99.9		99.7	99.7	99.7	78.2	99.7	99.7	99.7	99.7	99.7	99.4	99.7	99.7
6	100	100	100	100	99.9		100	100	78.2	100	100	100	100	100	100	100	100
7	100	100	100	100	99.9	100		100	78.2	100	100	100	100	100	100	100	100
8	100	100	100	100	99.9	100	100		78.2	100	100	100	100	100	100	100	100
9	78.7	78.7	78.7	78.7	78.7	78.7	78.7	78.7		78.2	78.2	78.2	78.2	78.2	78.0	78.2	78.2
10	99.4	99.4	99.4	99.4	99.3	99.4	99.4	99.4	79.0		100	100	100	100	99.4	100	100
11	99.4	99.4	99.4	99.4	99.3	99.4	99.4	99.4	79.0	100		100	100	100	99.4	100	100
12	98.4	99.4	99.4	99.4	99.3	99.4	99.4	99.4	79.0	100	100		100	100	99.4	100	100
13	98.4	98.4	98.4	98.4	98.4	98.4	98.4	98.4	79.0	98.4	98.4	98.4		100	99.4	100	100
14	99.4	99.4	99.4	99.4	99.3	99.4	99.4	99.4	79.0	100	100	100	100		99.4	100	100
15	99.6	99.6	99.6	99.6	99.7	99.6	99.6	99.6	78.7	99.6	99.6	99.6	99.6	99.6		99.4	99.4
16	99.4	99.4	99.4	99.4	99.3	99.4	99.4	99.4	79.0	100	100	100	100	100	99.6		100
17	99.4	99.4	99.4	99.4	99.3	99.4	99.4	99.4	79.0	100	100	100	100	100	99.6	100	

(1) MN508357.1–LSDV–China–2019; (2) MN598006.1–LSDV–China–2019; (3) OK323151.1–LSDV–Thailand–2021; (4) OK323152.1–LSDV–Thailand–2021; (5) OR137810.1–LSDV–Vietnam–2020; (6) OR137811.1–LSDV–Vietnam–2020; (7) OK258129.1–LSDV–Vietnam–2021; (8) OK258133.1–LSDV–Vietnam–2021; (9) ON024930.1 –LSDV–Vaccine–LumppyVac–Thailand–2021; (10) PP387459.1–LSDV–Vietnam–Ben Tre–2023; (11) PP387460.1–LSDV–Vietnam–Ben Tre–2023; (12) PP387461.1–LSDV–Vietnam–Ben Tre–2023; (13) PP387462.1–LSDV–Vietnam–Ben Tre–2023; (14) PP387463.1–LSDV–Vietnam–Ben Tre–2023; (15) PP387464.1–LSDV–Vietnam–Ben Tre–2023; (16) PP387465.1–LSDV–Vietnam–Ben Tre–2023; (17) PP387466.1–LSDV–Vietnam–Ben Tre–2023

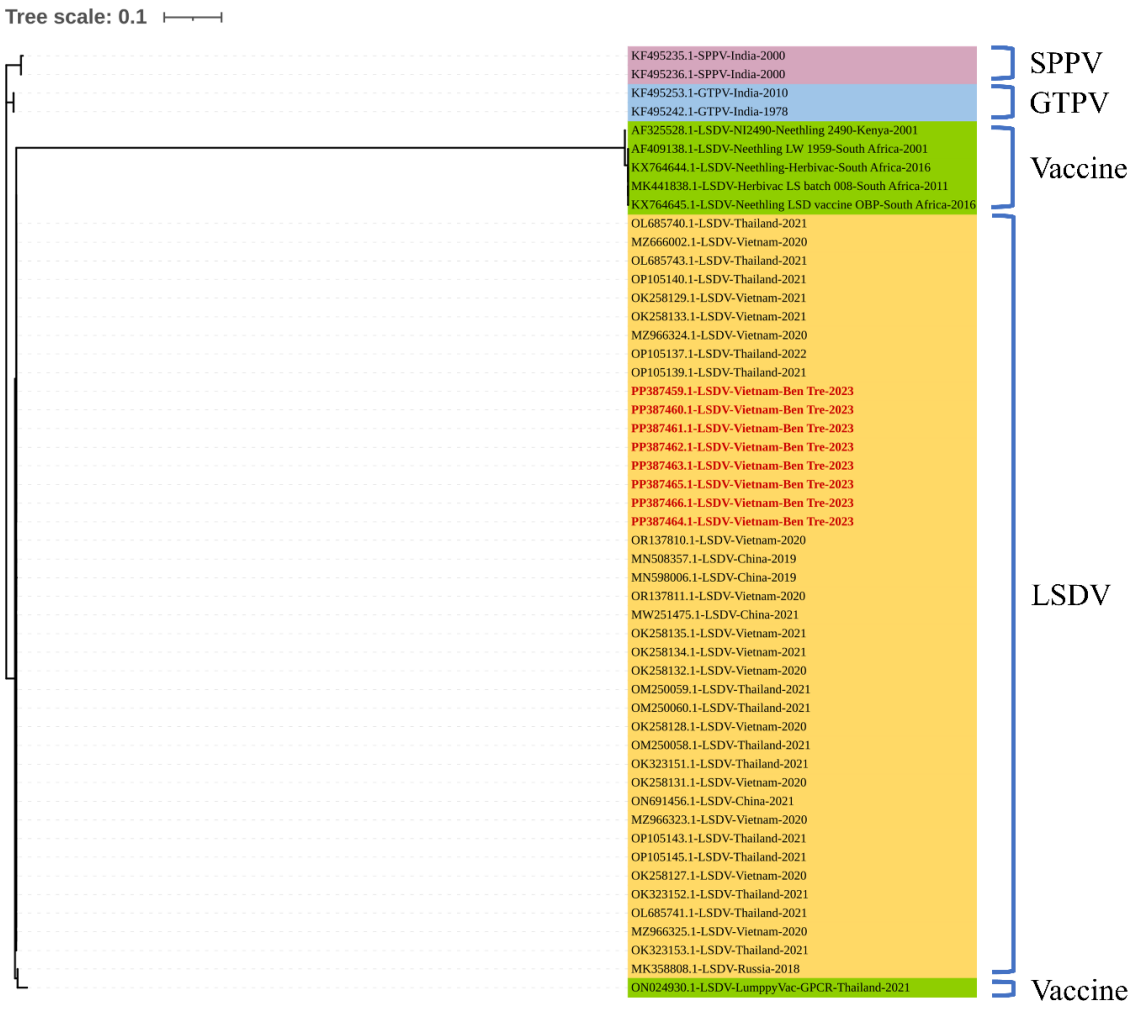

Figure 2 The maximum likelihood phylogenetic tree of LSDV in cattle in Ben Tre province and reference sequences were done based on the nucleotide sequences of the GPCR gene. Other LSDV, GTPV, SPPV, and vaccine sequences were utilized from the GenBank database. The field sequences obtained from this study were bold in red color.

DISCUSSION

Recently, LSD has been an infectious viral disease in domestic cattle in several districts of Vietnam. The present study documents the molecular features and phylogenetic analysis of LSDV in Ben Tre districts of Vietnam according to the GPCR gene. The outcomes of this study expanded the understanding of basic knowledge on the molecular characteristics of LSDV in Ben Tre province in particular and the whole country in general, and potentially contribute to constructing strategies for effectively controlling this disease in Vietnam.

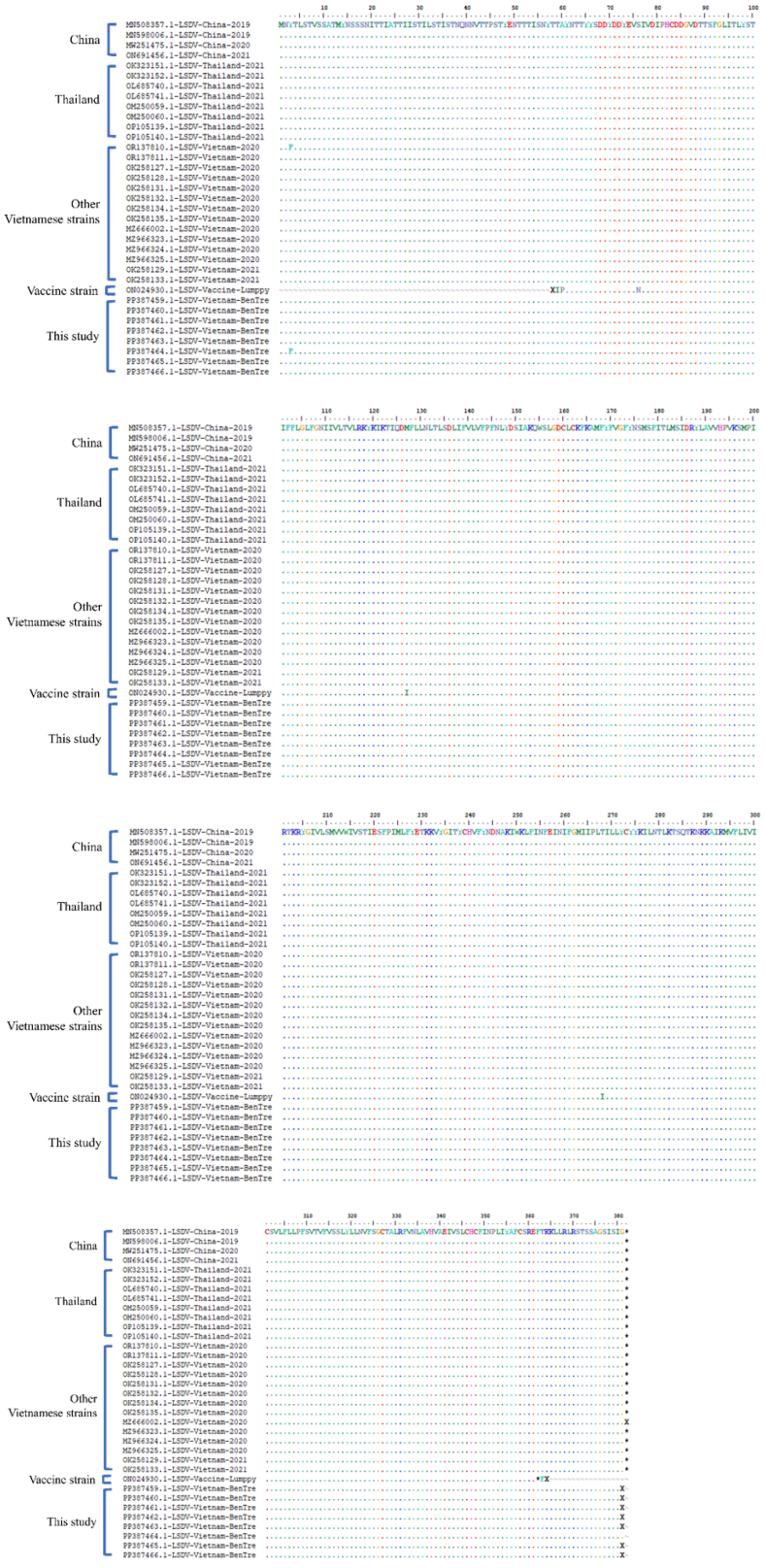

Figure 3 Multiple sequence alignment of the field LSDV sequences in Ben Tre province and reference sequences from the previous studies in China, Thailand, and Vietnam. Letters represented the differences, meanwhile, dots represented the identical residues among the sequences.

In the current study, the GPCR gene was used to classify the LSDV collected in suspected cattle in Ben Tre province and differentiate among LSDV, GTPV, and SPPV via phylogenetic analysis. Currently, the GPCR gene is widely accepted as a notable gene for differentiation among LSDV, GTPV, and SPPV (Le Goff et al., 2009). Numerous studies globally utilized the GPCR gene as a target gene for the detection of LSDV from the field outbreaks and differentiation among three capripoxviruses (LSDV, GTPV, and SPPV) (Tulman et al., 2001; Le Goff et al., 2009; Ochwo et al., 2020; Selim et al., 2021; Singhla et al., 2022). In Vietnam, the GPCR gene was found in some studies on the molecular characteristics and phylogeny of LSDV in cattle in the North and the North–Central region (Trinh et al., 2022; Tran et al., 2024). Therefore, this study considered the GPCR gene as a target gene to demonstrate the molecular characteristics and phylogeny of LSDV collected from the LSDV–suspected cattle in seven districts of Ben Tre province via PCR technique. However, the full–length genome of LSDV in Ben Tre province was not performed in this study. Further investigations on the phylogeny of LSDV in Vietnam should be encouraged this limitation to clearly provide the phylogenetic characteristics of LSDV in the whole country.

The nucleotide and amino acid identities of LSDV sequences in Ben Tre province and other reference sequences collected in Vietnam, China, and Thailand showed a very high homology (up to 100%). The results of this study revealed a high homology between the field sequences and the reference sequences in neighboring countries such as China and Thailand. This finding was similar to the past studies in Vietnam, the nucleotide and amino acid identities were up to 100% when compared to the LSDV sequences in cattle in China (Tran et al., 2021; Trinh et al., 2022; Tran et al., 2024). Likewise, LSDV obtained from cattle in China displayed nucleotide homology between 99.7% and 99.8% when compared to the reference sequences in Vietnam (Wang et al., 2022). Moreover, LSDV collected from cattle in the Northern region of Thailand shared high homology with the Vietnamese strains, ranging from 98–100% (Singhla et al., 2022). In other geographical areas, a very high homology in nucleotide and amino acid of LSDV among neighboring countries was additionally exhibited (Sameea Yousefi et al., 2018; Ochwo et al., 2020; Selim et al., 2021; Maw et al., 2022; Sudhakar et al., 2022). However, the findings of this study showed that the nucleotide and amino acid identities of LSDV sequences in Ben Tre province were low homology with vaccine strain (LSDV–LumppyVac–GPCR–Thailand–2021), accounting for 78.7–79.0% and 78.0–78.2%, respectively. These results again confirm a high conservation of the LSDV genome in nature regardless of the distance among geographical areas.

Herein, the phylogenetic analysis indicated that all the field sequences were clustered into the same group with the LSDV collected from cattle in other provinces in Vietnam and those in the neighboring countries including China and Thailand. However, the field sequences were distinguished from the vaccine strain (Neethling), GTPV, and SPPV recruited from the GenBank database. Interestingly, the field sequences in cattle in Ben Tre province were grouped into the same branch as the LumppyVac sequence in cattle in Thailand (LSDV–LumppyVac–GPCR–Thailand–2021). Additionally, multiple sequence alignments of the field LSDV sequences in Ben Tre province and reference sequences from the previous studies in China, Thailand, and Vietnam revealed a tight homology in the GPCR region. Meanwhile, the field sequences shared seven different alignments in the GPCR region compared to the vaccine LSDV strain (LSDV–LumppyVac–GPCR–Thailand–2021). According to the current study, the field sequences in Ben Tre province showed a tight relationship among border countries such as China and Thailand. Especially, Vietnam and China share a long border, and the first outbreak of LSD in Vietnam seems to be related to the prior outbreaks in China (Tran et al., 2021; Trinh et al., 2022; Tran et al., 2024). The findings of the current study imply

that the LSD outbreaks are probably a genetic proximity to the prior outbreaks in countries sharing border attractions. This observation is in agreement with the fact that the previous LSD outbreaks in China were closely related to those in Russia and Vietnam (Tran et al., 2021; Trinh et al., 2022; Wang et al., 2022). Furthermore, studies on the LSD outbreak in Thailand additionally provided information that there was a tight genetic proximity among the field LSDV strain in Thailand, Vietnam, Hong Kong, and China (Sariya et al., 2022; Singhla et al., 2022). In the present decade, several reports among geographical areas globally confirmed that LSD outbreaks showed close genetic proximity to those in neighboring countries (Sameea Yousefi et al., 2018; Ochwo et al., 2020; Selim et al., 2021; Maw et al., 2022; Sudhakar et al., 2022). Blood–biting vectors and informal livestock trade were considered as a potential transboundary movement way of LSDV among countries across borders (Sameea Yousefi et al., 2018; Ochwo et al., 2020; Singhla et al., 2022; Sudhakar et al., 2022; Trinh et al., 2022). To date, several vaccines against LSDV in cattle are widely used worldwide and also in Vietnam to control LSD in the livestock industry (Klement et al., 2020; Bamouh et al., 2021; Bazid et al., 2023). Besides, the evidence of natural recombination between the field LSDVs and vaccine strains was reported (Sprygin et al., 2018). The present study did not analyze the recombination between the field LSDV strains and vaccine strains in Ben Tre province. Further investigations on the natural recombination between the field LSDV strains and vaccine strains in Vietnam should be promoted for a better understanding.

CONCLUSIONS

In conclusion, the present study highlighted information on the molecular characterization and phylogenetic analysis of LSDV from LSDV–suspected cattle in Ben Tre province of Vietnam using the GPCR gene. The findings of this study revealed that the field sequences in the outbreaks in Ben Tre province shared close genetic proximity with the reference LSDVs circulated in neighboring countries and once again emphasized the transboundary of LSDV in nature. The outcomes of the current study may beneficially contribute to constructing strategies for effectively controlling LSDV in Vietnam in the future.

ACKNOWLEDGEMENTS

The authors would like to display our gratitude to the Sub-Department of Animal Health and the Department of Science & Technology of Ben Tre province for their contribution to sample collection and funding for this study (No. 2136/HD–SKHCN).

AUTHOR CONTRIBUTIONS

Conceptualization: H.V.T., Q.T.K.N., U.D., B.N.T., C.T.P.N.
Investigation: H.V.T., Q.T.K.N., U.D., B.N.T., C.T.P.N.
Methodology: H.V.T., B.N.T., C.T.P.N., T.L.Q.
Project administration: H.V.T., B.N.T.
Supervision: H.V.T., B.N.T.
Writing-original draft: H.V.T., B.N.T., T.L.Q.
Writing-review & editing: H.V.T., B.N.T., T.L.Q.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Anwar, A., Na–Lampang, K., Preyavichyapugdee, N., Punyapornwithaya, V., 2022. Lumpy skin disease outbreaks in Africa, Europe, and Asia (2005–2022): multiple change point analysis and time series forecast. Viruses. 14(10), 2203.

Bamouh, Z., Hamdi, J., Fellahi, S., Khayi, S., Jazouli, M., Tadlaoui, K.O., Fihri, O.F., Tuppurainen, E., Elharrak, M., 2021. Investigation of post vaccination reactions of two live attenuated vaccines against lumpy skin disease of cattle. Vaccines. 9(6), 621.

Bazid, A.H., Wasfy, M., Fawzy, M., Nayel, M., Abdelmegeid, M., Thabet, R.Y., Yong, H.S., El–Sayed, M.M., Magouz, A., Badr, Y., 2023. Emergency vaccination of cattle against lumpy skin disease: Evaluation of safety, efficacy, and potency of MEVAC® LSD vaccine containing Neethling strain. Vet. Res. Commun. 47(2), 767–777.

Bowden, T.R., Babiuk, S.L., Parkyn, G.R., Copps, J.S., Boyle, D.B., 2008. Capripoxvirus tissue tropism and shedding: a quantitative study in experimentally infected sheep and goats. Virology. 371(2), 380–393.

Chouhan, C.S., Parvin, M.S., Ali, M.Y., Sadekuzzaman, M., Chowdhury, M.G.A., Ehsan, M.A., Islam, M.T., 2022. Epidemiology and economic impact of lumpy skin disease of cattle in Mymensingh and Gaibandha districts of Bangladesh. Transbound Emerg. Dis. 69(6), 3405–3418.

Gupta, T., Patial, V., Bali, D., Angaria, S., Sharma, M., Chahota, R., 2020. A review: Lumpy skin disease and its emergence in India. Vet. Res. Commun. 44, 111–118.

Hall, T.A., 1999. BioEdit: a user–friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic. Acids. Symp. Ser. 95–98.

Klement, E., Broglia, A., Antoniou, S.E., Tsiamadis, V., Plevraki, E., Petrović, T., Polaček, V., Debeljak, Z., Miteva, A., Alexandrov, T., 2020. Neethling vaccine proved highly effective in controlling lumpy skin disease epidemics in the Balkans. Prev. Vet. Med. 181, 104595.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33(7), 1870–1874.

Le Goff, C., Lamien, C.E., Fakhfakh, E., Chadeyras, A., Aba–Adulugba, E., Libeau, G., Tuppurainen, E., Wallace, D.B., Adam, T., Silber, R., 2009. Capripoxvirus G–protein–coupled chemokine receptor: a host–range gene suitable for virus animal origin discrimination. J. Gen. Virol. 90(8), 1967–1977.

Letunic, I., Bork, P., 2024. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic. Acids. Res. 52, W78–W82.

Maw, M.T., Khin, M.M., Hadrill, D., Meki, I.K., Settypalli, T.B.K., Kyin, M.M., Myint, W.W., Thein, W.Z., Aye, O., Palamara, E., 2022. First report of lumpy skin disease in Myanmar and molecular analysis of the field virus isolates. Microorganisms. 10(5), 897.

Ochwo, S., VanderWaal, K., Ndekezi, C., Nkamwesiga, J., Munsey, A., Witto, S.G., Nantima, N., Mayanja, F., Okurut, A.R.A., Atuhaire, D.K., 2020. Molecular detection and phylogenetic analysis of lumpy skin disease virus from outbreaks in Uganda 2017–2018. BMC Vet. Res. 16, 1–10.

Rafe–Ush–Shan, S.M., Begum, R., Roy, M., Islam, M.M., Hossain, H., Chowdhury, M.S.R., Uddin, M.B., Rahman, M.M., Hossain, M.M., 2025. Sero–prevalence and PCR identification of lumpy skin disease virus in cattle at Mymensingh district of Bangladesh. Vet. Integr. Sci. 23(2), 1–13.

Sameea, Yousefi, P., Dalir–Naghadeh, B., Mardani, K., Jalilzadeh–Amin, G., 2018. Phylogenetic analysis of the lumpy skin disease viruses in northwest of Iran. Trop. Anim. Health. Prod. 50, 1851–1858.

Sariya, L., Paungpin, W., Chaiwattanarungruengpaisan, S., Thongdee, M., Nakthong, C., Jitwongwai, A., Taksinoros, S., Sutummaporn, K., Boonmasawai, S., Kornmatitsuk, B., 2022. Molecular detection and characterization of lumpy skin disease viruses from outbreaks in Thailand in 2021. Transbound. Emerg. Dis. 69(5), e2145–e2152.

Selim, A., Manaa, E., Khater, H., 2021. Molecular characterization and phylogenetic analysis of lumpy skin disease in Egypt. Comp. Immunol. Microbiol. Infect. Dis. 79, 101699.

Singhla, T., Boonsri, K., Kreausukon, K., Modethed, W., Pringproa, K., Sthitmatee, N., Punyapornwithaya, V., Vinitchaikul, P., 2022. Molecular characterization and phylogenetic analysis of lumpy skin disease virus collected from outbreaks in northern Thailand in 2021. Vet. Sci. 9(4), 194.

Sprygin, A., Babin, Y., Pestova, Y., Kononova, S., Wallace, D.B., Van Schalkwyk, A., Byadovskaya, O., Diev, V., Lozovoy, D., Kononov, A., 2018. Analysis and insights into recombination signals in lumpy skin disease virus recovered in the field. PLoS One. 13(12), e0207480.

Sudhakar, S.B., Mishra, N., Kalaiyarasu, S., Jhade, S.K., Singh, V.P., 2022. Genetic and phylogenetic analysis of lumpy skin disease viruses (LSDV) isolated from the first and subsequent field outbreaks in India during 2019 reveals close proximity with unique signatures of historical Kenyan NI-2490/Kenya/KSGP-like field strains. Transbound. Emerg. Dis. 69(4), e451–e462.

Tulman, E., Afonso, C., Lu, Z., Zsak, L., Kutish, G., Rock, D., 2001. Genome of lumpy skin disease virus. J. Virol. 75(15), 7122–7130.

Tuppurainen, E., Oura, C., 2012. Lumpy skin disease: an emerging threat to Europe, the Middle East and Asia. Transbound. Emerg. Dis. 59(1), 40–48.

Tuppurainen, E., Venter, E.H., Shisler, J., Gari, G., Mekonnen, G., Juleff, N., Lyons, N., De Clercq, K., Upton, C., Bowden, T., 2017. Capripoxvirus diseases: current status and opportunities for control. Transbound. Emerg. Dis. 64(3), 729–745.

Tuppurainen, E.S., Venter, E., Coetzer, J., 2005. The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. Onderstepoort. J. Vet. Res. 72(2), 153–164.

Tran, A.T., Tran, H.T.T., Truong, A.D., Dang, A.K., Chu, N.T., Phan, L., Phan, H.T., Nguyen, H.T., To, N.B.T., Dang, H.V., 2024. Molecular characterization of lumpy skin disease virus in North Central Vietnam during 2021 and early 2022. Vet. Ital. 60(1).

Tran, H.T.T., Truong, A.D., Dang, A.K., Ly, D.V., Nguyen, C.T., Chu, N.T., Hoang, T.V., Nguyen, H.T., Nguyen, V.T., Dang, H.V., 2021. Lumpy skin disease outbreaks in Vietnam, 2020. Transbound Emerg. Dis. 68(3), 977–980.

Trinh, T.B.N., Nguyen, V.T., Nguyen, T.T.H., Mai, N.T.A., Le, P.N., Lai, T.N.H., Phan, T.H., Tran, D.H., Pham, N.T., Dam, V.P., 2022. Molecular and histopathological characterization of lumpy skin disease in cattle in northern Vietnam during the 2020–2021 outbreaks. Arch. Virol. 167(11), 2143–2149.

Wang, J., Xu, Z., Wang, Z., Li, Q., Liang, X., Ye, S., Cheng, K., Xu, L., Mao, J., Wang, Z., 2022. Isolation, identification and phylogenetic analysis of lumpy skin disease virus strain of outbreak in Guangdong, China. Transbound Emerg. Dis. 69(5), e2291–e2301.

How to cite this article;

Hieu Van Truong, Quyen Thi Kim Nguyen, Ut Danh, Chien Tran Phuoc Nguyen, Trung Quang Le and Bich Ngoc Tran. Molecular characterization and phylogenetic analysis of lumpy skin disease virus from the field outbreaks in Ben Tre province of Vietnam in 2023. Veterinary Integrative Sciences. 2025; 23(3): e2025081-1-13.