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Epidemiological Characteristics and Phylogenetic Analysis of Lumpy Skin Disease in Cattle in the Mekong Delta of Vietnam

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Abstract | Lumpy skin disease (LSD) is an increasing threat to the global cattle industry, and Vietnam is among the countries recently affected. In response to this growing concern, a cross-sectional study was conducted to assess the infection status and risk factors associated with LSD outbreaks in the Mekong Delta. The study documented an LSD outbreak in the Mekong Delta provinces of Tien Giang, Tra Vinh, and Ben Tre between May 2023 and June 2024. A questionnaire-based survey was conducted through face-to-face interviews with farmers impacted by the disease. The collected epidemiological data were analysed, and the LSDV strains were genetically characterised. Out of 1,278 animals clinically examined across 180 farms, 80 tested positive via PCR, resulting in a morbidity rate of 6.26% and a herd prevalence of 37.22%. The morbidity rates in the 19 districts surveyed ranged from 1.45% to 17.95%. Risk factor analysis revealed that unvaccinated animals had the highest incidence rate at 17.94% (P<0.05), with an OR of 5.52 (95%CI: 3.62–8.44). Additionally, calves under six months old have a significantly higher risk of LSD compared to cows aged 6–24 months (OR 1.84, 95%CI: 1.14–3.00) and compared to animals older than 24 months (OR 4.82, 95% CI: 2.73-8.51). Phylogenetic analysis of the P32 gene of LSDV (PP934191-PP934205) indicated 100% similarity with other isolates from China, Russia, and Thailand. The findings of this study provide critical epidemiological insights into the risk factors associated with LSD in the Mekong Delta. This information is essential for government livestock regulators to develop effective prevention and control strategies, thereby mitigating the negative impact of LSD on cattle farming.

Keywords | Lumpy skin disease virus, Epidemiological, Characterization, Gene P32, Mekong Delta, Vietnam

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INTRODUCTION

The World Organisation for Animal Health (OIE) categorises Lumpy Skin Disease (LSD) as a transboundary disease because of its rapid dissemination and substantial economic consequences (Tuppurainen and Oura, 2012). First identified in Zambia in 1929, the Neethling strain of *Capripoxvirus* causes LSD. The disease is widespread throughout Africa and the Middle East. The recombinant lineages of LSDV, which originated from the Middle East and Africa, have disseminated throughout China, Mongolia, Vietnam, Cambodia, Laos, Thailand, Malaysia, and Indonesia (Li *et al*., 2023). The first instance of LSD use in Vietnam was documented in Lang Son province around October 2020. Since then, LSD has been reported in 55/63 provinces and cities, 457 districts, and 4,280 communes. From January to May 2024, 68 outbreaks were reported in 10 provinces, with 404 diseased cattle and 93 buffaloes and cows destroyed, marking a 9.67% increase compared to 2023 (DAH, 2024). LSD causes circular, slightly elevated papules on the neck, legs, tail, and back after the onset of fever (Şevik and Doğan, 2017). The disease can reduce milk production by 10% to 85% due to high body temperature and secondary mastitis, and it may cause lasting skin damage, impaired cattle development, infertility, miscarriage, and death (Şevik *et al*., 2016). Viral genomes are identified in nodules, ulcers, secretions, semen, and infected cattle blood (Bedeković *et al*., 2018). PCR targeting the P32 gene is commonly used for LSDV identification and analysis (Sameea Yousefi *et al*., 2018). The P32 protein is highly conserved across *Capripoxviruses*, enabling differentiation between SPPV (Sheep pox virus), GTPV (Goat pox virus), and LSDV (Koirala *et al*., 2022). The preservation of the P32 protein renders it a dependable indicator for genetic investigations on *Capripoxviruses*. There are currently no studies or publications on the LSD situation in the Mekong Delta; this study was done to investigate the prevalence of LSD in the Mekong Delta, Vietnam, in light of the rising number of LSDV cases reported to the Department of Animal Health Vietnam till May 2024. The inquiry included contemporaneous surveillance of the sickness and examination of historical data. Our objective was to assess the effectiveness of the PCR technique, which mainly focuses on the P32 gene for identification, and to characterise LSDV isolates using molecular methods.

MATERIALS AND METHODS

Specimen Collection

The LSD epidemic was investigated in 180 houses in Tien Giang, Ben Tre, and Tra Vinh in Vietnam's Mekong Delta from May 2023 to June 2024. In the Mekong Delta, known for its large cow population (Figure 1, Figure 2), farmer complaints and proactive case identification by LSD led to investigations. Veterinary inspections and farmer reports on afflicted cattle ranches identified outbreaks as farms with at least one LSD case. LSD cases were identified by clinical symptoms such as raised, spherical, and solid nodules measuring 2-7 cm in diameter (Şevik and Doğan, 2017). The study included 1,278 bovines assessed for LSD symptoms. Nasal swab samples were collected aseptically, preserved on ice, and sent to the Faculty of Veterinary Medicine at Can Tho University. Samples were stored at -20°C until processing, following TCCS 04:2020/TY–DT (MARD, 2020) and (OIE, 2017) guidelines.

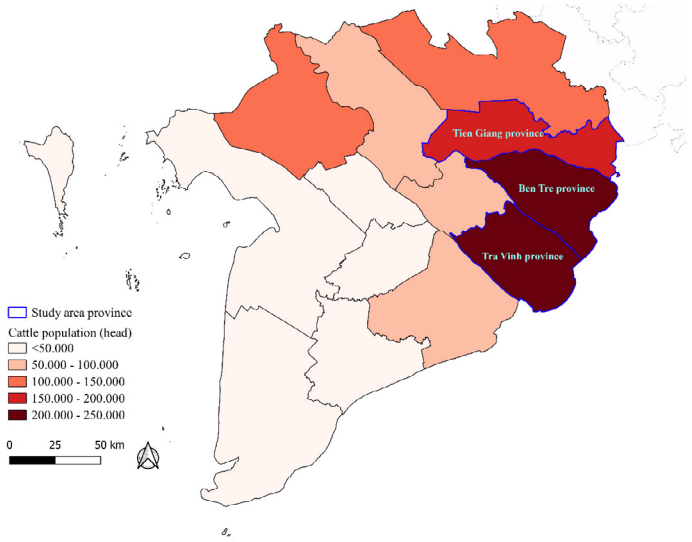

Figure 1: The cattle farming situation in the Mekong Delta during the period of 2022-2023.

Figure 2: Study implementation and sample collection site.

DNA Extraction and PCR Detection of Virus

Viral DNA was isolated from nasal swab samples using a TopPURE® RNA/DNA Viral Extraction Kit (ABT, Vietnam). LSDV presence was confirmed with a PCR assay targeting the P32 gene, using the primers forward 5′-TTTCCTGATTTTTCTTACTAT-3′ and reverse

5′-AAATTATATACGTAAATAAC-3′, producing a 192 bp amplicon (OIE, 2017). The PCR was performed in a 25 µL reaction mixture with 12 µL GoTaq® DNA Polymerase (Promega, USA), one µL of each ten µM primer, eight µL deionised water, and three µL DNA template. Cycling conditions were: 5 minutes at 94˚C, 35 cycles at 94˚C for 60 seconds, 50˚C for 30 seconds, and 72˚C for 60 seconds, with a final 5 minutes at 72˚C. PCR products were analysed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualised using a BIO-Rad UV transilluminator.

Fifteen representative samples from Tien Giang, Ben Tre, and Tra Vinh in Vietnam's Mekong Delta were selected for PCR assay targeting the P32 gene for phylogenetic analysis, using the primers P32-F (5′-TCGTTGGTCGCGAAATTTCAG-3′) and P32-R (5′-GAGCCATCCATTTTCCAACTCT-3′). This produced a 759 bp fragment between nucleotides 65163-65921 (Sameea Yousefi *et al*., 2018). The PCR reaction was prepared similarly, with cycling conditions of 5 minutes at 94˚C, 35 cycles of 95˚C for 45 seconds, 56˚C for 45 seconds, and 72˚C for 65 seconds, followed by a final 5 minutes at 72˚C. PCR products were visualised on a 1.5% agarose gel.

Nucleotide Sequencing and Phylogenetic Analysis

The P32 amplicons, which were 759 bp in length, were purified from the PCR products using the TopPURE® PCR/Gel DNA purification kit from ABT, Vietnam. The purified products were sequenced on both forward and reverse strands by NamKhoa-Biotech, Vietnam. Sanger sequencing was executed using the ABI Prism BigDye™ Terminator v1.1 cycle sequencing kit on an ABI PRISM 3500×1 Genetic Analyzer.

The LSDV P32 sequences were aligned multiple times using Geneious Prime® version 2024.0.2, with manual corrections. The NCBI BLAST tool assessed the sequences' similarity to GenBank sequences (https://www.ncbi.nlm.nih.gov/). The objective was to determine the taxonomic categorisation of the sequences within the CaPV genus. Nucleotide sequences were aligned and compared with P32 gene sequences of other LSDV, SPPV, and GPV viruses from GenBank using Clustal W. A phylogenetic tree was constructed using the maximum-likelihood technique with the Tamura-Nei model in MEGA v.11.0 software. The reliability of the tree's branching patterns was evaluated by bootstrap analysis with 1000 replications. The trees were created and labelled using the Interactive Tree of Life (iTOL) program (https://itol.embl.de). They were analysing nucleotide similarity ratios using STD v.1.3 software. The nucleotide sequences of the P32 gene for all 15 LSDV isolates were uploaded to the GenBank database and assigned accession numbers PP934191-PP934205.

Data Analysis

Every shepherd participated in a survey aimed at analysing risk factors for LSD, with epidemiological data analysed using Microsoft Excel 2010. Descriptive analysis was conducted to assess the frequency, central tendency, and dispersion of quantitative variables and the percentage of qualitative variables. Predictor factors included gender, age (< 6 months, 6-24 months, > 24 months), breed (crossbreeds or native breeds), grazing pattern (communal or zero-grazing), and vaccination status. The response variable was PCR-detected LSD. A generalised linear model was employed to compare epidemiological factors by breed, sex, vaccination status, and age. Model coefficients were converted to odds ratios (ORs) using exponential transformation, with statistical significance set at 0.05. Favourable rates were analysed using Chi-square and Fisher's exact tests ($P < 0.05$) via WinEpi (http://www.winepi.net). Direct odds ratios were estimated using the Mantel-Haenszel technique, providing a precise 95% binomial confidence interval.

RESULTS AND DISCUSSION

Infection Situation and Risk Factors for LSD Infection in the Study

The research utilised PCR to screen 1,278 cattle from 180 households for LSDV, identifying 80 positive cases and determining a morbidity rate of 6.26%. The epidemic's impact varied across the 19 districts, with morbidity rates ranging from 1.45% to 17.95% (Table 1, Figure 3). Notably, the outbreak did not result in any fatalities. Results show that the highest infection rate was in Ben Tre at 6.27%, varying between 2.27% and 14.9% across different districts. Tien Giang followed this at 6.04% and Tra Vinh at 5.82%, with no significant difference between the provinces ($P>0.05$). Differences in disease incidence compared to previous studies may be attributed to geographic factors, farm practices, insect activity, circulating LSDV strains, and variations in animal populations and immune status (Tuppurainen and Oura, 2012; Bianchini *et al*., 2023; Tran *et al*., 2024).

The severity of LSD symptoms in cattle varied. Common clinical signs included depression, decreased appetite, elevated body temperature, and discharge from the nose and eyes. Additionally, swollen lymph nodes and localised skin nodules measuring 2–7 cm were observed in various body areas. These nodules sometimes spread throughout the body (Figure 4). This observation is consistent with findings by Li *et al*. (2023), which reported that severe LSD can cause significant nodules, leading to discomfort and stiffness. Necrotic nodules, prone to secondary bacterial infections, underscore the need for vigilant monitoring and treatment (El-Neweshy *et al*., 2013). The study offers valuable insights into LSD outbreaks in small backyard cattle, with clinical signs aligning with previous reports (Şevik and Doğan, 2017; Wolff *et al*., 2021). Notably, the sloughing

Table 1: Epidemiological characteristics of LSD outbreaks in cattle.

Province	Study area	Farms			Animals		
		No.	positive	Morbidity rate (%)	No.	positive	Morbidity rate (%)
Tien Giang	My Tho city	10	4	40.00	65	6	9.23
	Cho Gao district	10	4	40.00	117	6	5.13
	Chau Thanh district	10	1	10.00	69	1	1.45
	Go Cong town	7	4	57.14	39	7	17.95
	Go Cong Tay district	8	2	25.00	26	3	11.54
	Go Cong Dong district	6	1	16.67	33	1	3.03
	Tan Phu Dong district	9	1	11.11	65	1	1.54
Ben Tre	Ba Tri district	9	6	66.67	67	10	14.93
	Giong Trom district	11	3	27.27	132	3	2.27
	Thanh Phu district	11	2	18.18	67	2	2.99
	Mo Cay Nam district	11	9	81.82	118	9	7.63
	Mo Cay Bac district	11	8	72.73	77	8	10.39
	Chau Thanh district	7	1	14.29	25	1	4.00
Tra Vinh	Cang Long district	10	3	30.00	57	3	5.26
	Chau Thanh district	10	4	40.00	65	5	7.69
	Tieu Can district	10	2	20.00	60	2	3.33
	Cau Ngang district	10	2	20.00	70	2	2.86
	Tra Cu district	10	4	40.00	59	4	6.78
	Cau Ke district	10	6	60.00	67	6	8.96
	Total	180	67	37.22	1,278	80	6.26

of lesions resulted in a "reverse cone" appearance, facilitating worm infestation and bacterial invasion, potentially worsening sepsis.

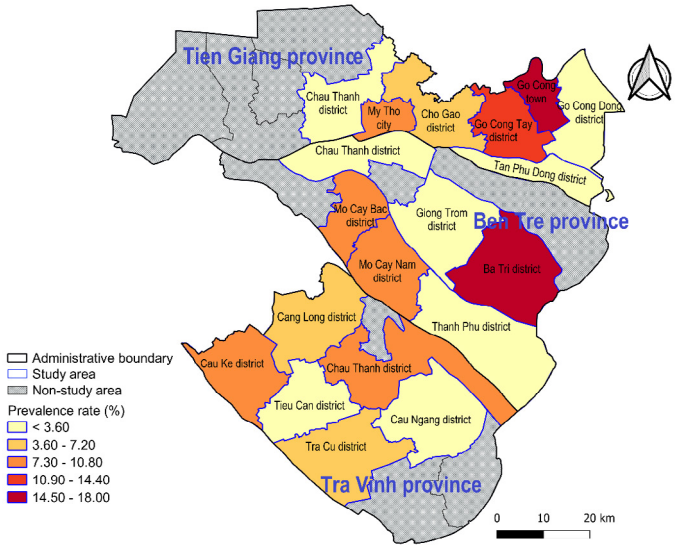

Figure 3: The prevalence rate of LSD at the animal distribution level across districts/towns/cities for research sampling.

The analysis results (Table 2) revealed that factors such as province, sex, breed, and age did not show a statistically significant relationship with the incidence of LSD.

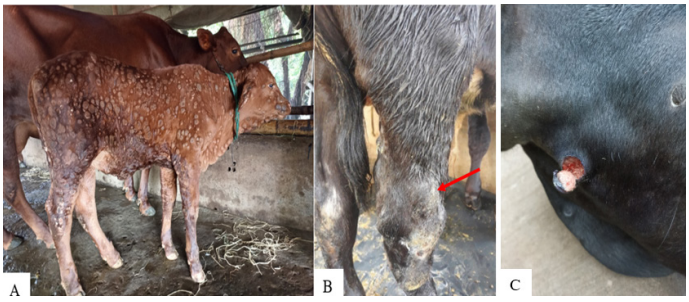

Figure 4: Some symptoms of cattle infected with LSD; **A:** Inflammatory nodules appear all over the body; **B:** Cattle suffering from joint inflammation; **C:** Skin ulceration with a "reverse cone" shape.

However, the study found that both the animal's age and its vaccination status had significant associations with LSD status. Specifically, the incidence of LSD was significantly higher in non-vaccinated animals compared to those that had been vaccinated, with an OR of 5.52 (95%CI: 3.62–8.44; P<0.05). This indicates that animals without vaccination were much more likely to develop LSD. Although there is a clear difference in disease incidence between vaccinated (3.25%) and unvaccinated cattle (17.52%), the infection rate among vaccinated cattle is still higher than expected. Several factors may explain this: vaccination coverage is suboptimal due to some cattle being too young for the LSD vaccine and delays in administering booster doses. Tuppurainen *et al.* (2021), emphasise that cattle aged six

Table 2: Summary of associated risk factors related to lumpy skin disease in cattle.

Variables	Category	The number of examined animals	Number of clinically affected animals	Morbidity rate (%)	OR	95%CI	P-value
Province	Ben Tre	486	33	6.79	Ref.		
	Tien Giang	414	25	6.04	1.12	0.68-1.86	0.647
	Tra Vinh	378	22	5.82	1.17	0.69-1.97	0.562
Sex	Male	619	39	6.30	Ref.		
	Female	659	41	6.22	1.01	0.66-1.55	0.954
Breed	Crossbreeds	829	55	6.63	Ref.		
	Native breeds	449	25	5.57	1.19	0.75-1.88	0.452
Grazing	Communal	149	12	8.05	Ref.		
	Zero-grazing	1,129	68	6.02	1.34	0.74-2.41	0.336
Vaccine	No	262	47	17.94	Ref.		
	Yes	1,016	33	3.25	5.52	3.62-8.44	0.000
Age	<6 months	164	24	14.63	Ref.		
	6-24 months	455	36	7.91	1.84	1.14-3.00	0.013
	>24 months	659	20	3.03	4.82	2.73-8.51	0.000

CI: confidence interval; **OR:** odds ratio; **Ref:** reference.

months and older need vaccination for disease prevention. Kumar *et al.* (2023), note that poor hygiene during immunisation can lead to virus transmission through needles. Additionally, improper vaccine storage or insufficient time for protective antibody development can reduce vaccine efficacy. LSD in vaccinated animals may result from inadequate vaccination protocols, ineffective vaccines, or infection before immunity develops (Kasem *et al.*, 2018). These factors underscore the need for effective vaccination strategies and ongoing monitoring to manage LSD outbreaks.

Furthermore, age also played a crucial role in the likelihood of contracting LSD. Calves under six months old were at a significantly higher risk than cows aged 6–24 months, with an OR of 1.84 (95% CI: 1.14–3.00; P<0.05). Additionally, the incidence of LSD was markedly greater in calves under six months old compared to animals older than 24 months, with an OR of 4.82 (95% CI: 2.73-8.51; P<0.05). The high disease incidence in cattle under six months of age can be explained by their underdeveloped immune system and the lack of protective immunity from vaccines or their mothers. During the survey, many mother cows were not fully vaccinated or had little time to develop an effective immune response. Furthermore, research by Leliso *et al.* (2021) in Ethiopia, Bianchini *et al.* (2023), Faris *et al.* (2021) in Egypt, and Tran *et al.* (2024) also indicate that younger and weaker cattle are at a higher risk of disease than mature and healthy cattle. These findings underscore the importance of vaccination as a preventive measure against LSD and highlight the increased vulnerability of younger animals, particularly those under six months old, to the disease.

Phylogenetic Analysis and Estimated Identity Between P32 Gene Sequences of CaPVs

The *Capripoxvirus* (CaPVs) genus includes closely related viruses that infect domestic ruminants, such as cattle, sheep, and goats, and cause significant economic losses in livestock production. This study utilised nucleotide sequences of the P32 gene, an essential immunogenic protein in all *Capripoxvirus* species phylogenetic analysis, to explore genetic diversity within the genus. A phylogenetic tree was constructed using the P32 gene's nucleotide sequences (759 bp) from various CaPVs, revealing distinct and unique clusters for LSDV, SPPV, and GTPV, highlighting genotypic diversity despite genomic similarities.

Sequencing of 15 LSD viruses from cattle in the Tien Giang, Ben Tre, and Tra Vinh provinces in the Mekong Delta of Vietnam showed close clustering with LSDV strains from other regions globally. The phylogenetic tree grouped LSDVs into two subclusters: sub-cluster I-a included strains from Kenya, Serbia, Egypt, Saudi Arabia, Kazakhstan, Kurdistan, and South Africa, while sub-cluster I-b encompassed strains from North Vietnam, China, Thailand, Hong Kong, Taiwan, Indonesia, Russia, and LSDV vaccine strains. The LSDVs from this study are closely aligned with sub-cluster I-b (Figure 5). The P32 gene's high conservation makes it a valuable tool for molecular diagnosis and genetic characterisation of CaPVs, enabling differentiation between virus members (Sprygin *et al.*, 2018). The study confirmed that LSDV sequences from the Mekong Delta were highly similar to those from North Vietnam, China, Russia, and Thailand, with 100% nucleotide sequence identity. This finding supports prior research

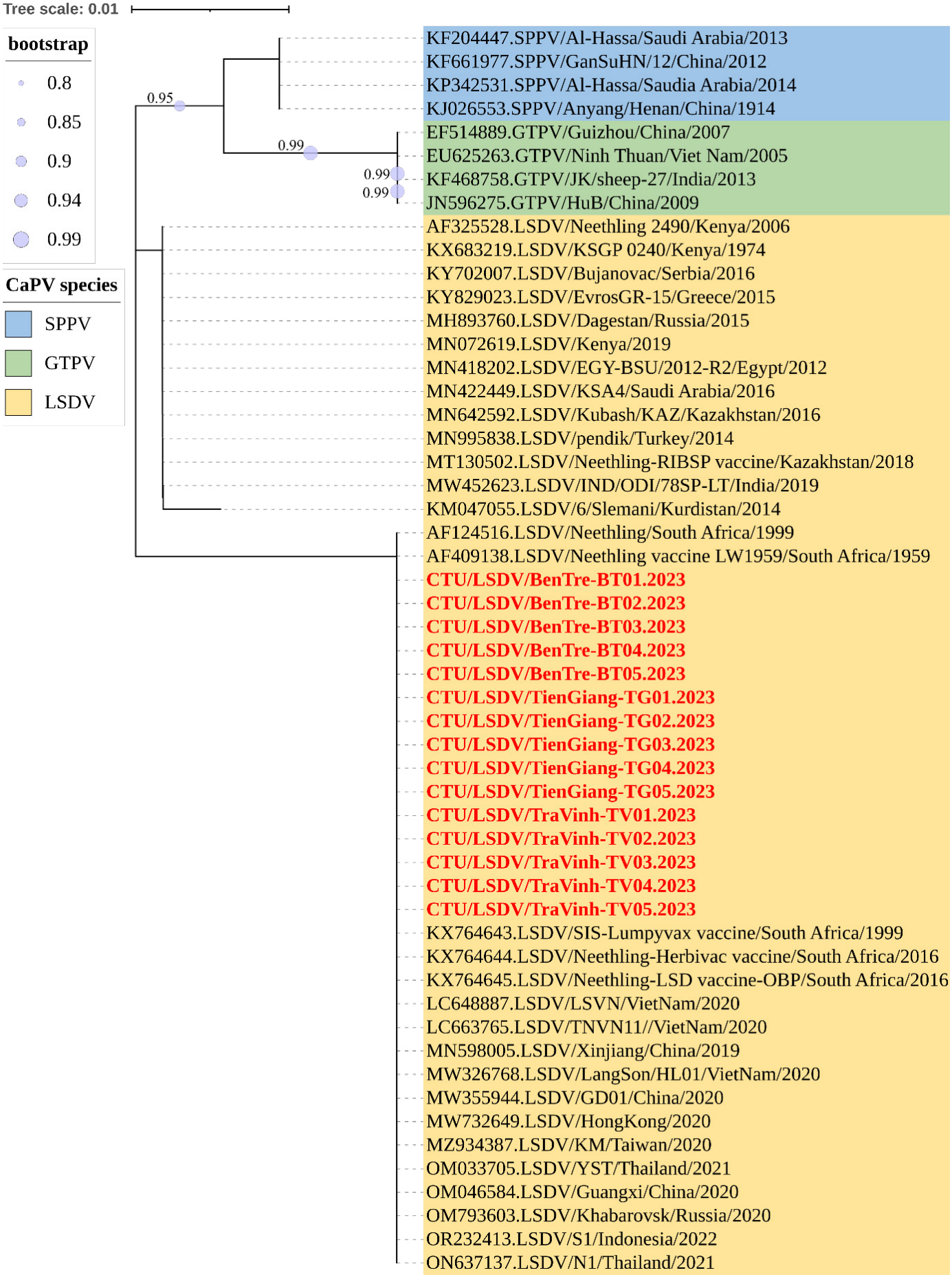

Figure 5: Phylogenetic tree demonstrating the relationship between P32 gene sequences obtained from the Mekong Delta in Vietnam (shown in red and bold) and other *Capripoxvirus* P32 gene sequences acquired from GenBank.

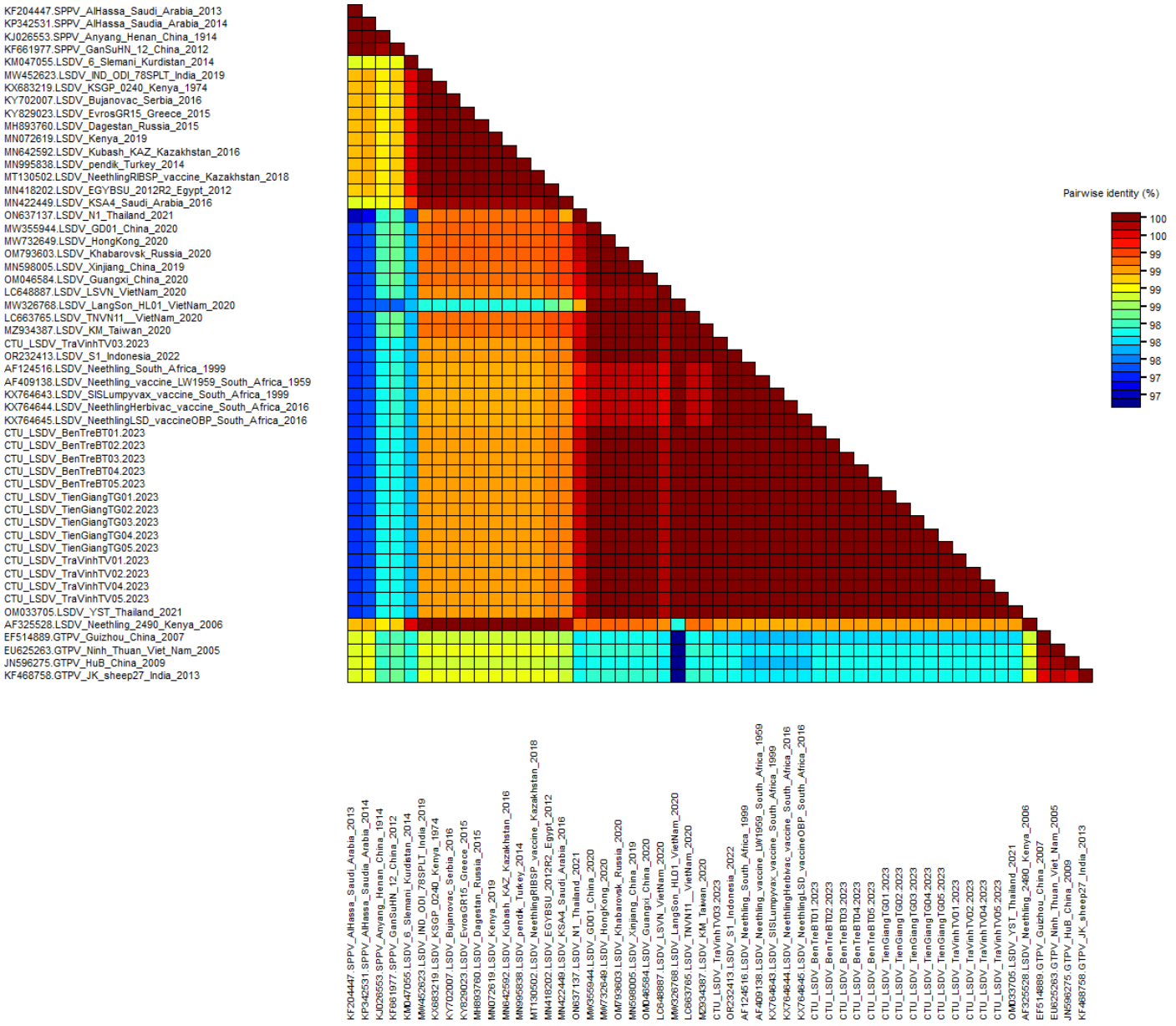

Figure 6: Nucleotide sequence identity distances for the P32 gene of local LSDV isolates compared to reference LSDV, SPPV and GTPV virus published in GenBank.

on the adequate amplification and sequencing of the P32 gene (Trinh *et al*., 2022).

The analysis revealed a clear distinction between LSDV field strains affecting cattle and those causing disease in sheep and goats. LSDV strains from the Mekong Delta were found to be closely related to Neethling vaccine strains, such as SIS/Lumpyvac, Neethling/Herbivac, and Neethling/OBP, indicating that the circulating virus is of the Neethling strain. This supports the effectiveness of vaccines in controlling LSD. However, recent outbreaks of recombinant strains similar to vaccines have been reported in Russia, Kazakhstan, China, and Vietnam (Sprygin *et al*., 2018; Issimov *et al*., 2022; Wang et al., 2022; Tran *et al*., 2021), highlighting the need for ongoing surveillance to detect and identify new recombinant virus strains promptly.

Estimates of Sequence Identity Between *Capripoxvirus* P32 Gene Sequences

The calculated sequence identity across P32 sequences of CaPVs showed a nucleotide similarity ranging from 97.3% to 100% among these viruses. In this study, the nucleotide sequences of the P32 gene from the LSDV isolates and two other viruses, SPPV and GTPV, showed a 97.3–98.0% similarity. The P32 nucleotide similarity percentage between LSDVs from the Mekong Delta in Vietnam and those from other countries ranged from 99.1% to 100%. The P32 antigen sequences of the LSDV strains studied in the Mekong Delta of Vietnam, with accession numbers PP934191-PP934205, exhibit 100% similarity. These sequences are also 100% identical to the Neethling strain of the LSDV vaccine (accession numbers AF409138, KX764643, KX764644, and KX764645),

as well as to strains from North Vietnam (LC648887, MW326768, LC663765), China (MW355944, MN598005, OM046584), Thailand (OM033705), Hong Kong (MW732649), Taiwan (MZ934387), Indonesia (OR232413), and Russia (OM793603). The nucleotide sequence similarity was lower, at 99.2%, compared to strains India (MW452623), Kurdistan (KM047055), Kenya (MN072619), and Kazakhstan (MN642592) identified in countries of South Asia, West Asia, and Africa (Figure 6).

Since its initial discovery in 1929 in Zambia, *Capripoxvirus* has spread extensively across Africa and the Middle East. Over time, the virus's movement across continents in its hosts has led to changes in nucleotide sequences. Despite these changes, they are relatively minor. According to Tulman *et al*. (2001) and Rashid *et al*. (2017), this stability is attributed to the Poxviridae family's large and complex genome, which consists of a single, linear double-stranded DNA (dsDNA) molecule encoding approximately 200 proteins. The continuous nature of the DNA molecule, with no free ends, results in a low mutation rate. As a result, the genetic differences observed in the P32 genes between strains studied in Vietnam and the original African strains are minimal. This indicates the virus's genetic stability and highlights the importance of ongoing monitoring and research to understand the virus's evolution and spread better.

CONCLUSIONS AND RECOMMENDATIONS

Additional investigation is required to study the widespread dissemination of LSD throughout a vast geographical area. In the Mekong Delta of Vietnam, the prevalence of LSD has primarily impacted calves less than six months old, with a major contributing factor being the absence of immunisation. The incidence of LSD among individual animals is 6.26%, whereas the occurrence of LSD among herds is 37.22%. The PCR test focusing on the P32 gene is a fast and reliable technique for identifying LSDV field isolates. Utilising the nucleotide sequence of the P32 gene for phylogenetic research might provide practical assistance in identifying the source and management of LSD.

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AUTHORS' CONTRIBUTIONS

This research was conducted with the contributions of all the authors. The authors all participated in the study design, analysing the results, interpreting the results, and preparing the manuscript. All authors read and approved the final manuscript.

Ethics Statement

This study was carried out on naturally infected animals. The samples used for this study were diagnostic, and no experimental procedures were carried out in any animal. Written informed consent was obtained from the owners for the participation of their animals in this study.

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Conflict of Interest

We certify that there is no conflict of interest.

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