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Molecular identification and characterization of Lumpy skin disease virus emergence from cattle in the northeastern part of Thailand

Tossapol Seerintra [1], **Bhuripit Saraphol** [1], **Sitthichai Wankaew** [2], **Supawadee Piratae** [3,*]

[1]Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham 44000, Thailand
[2]Laboratory of Veterinary teaching hospital of Mahasarakham University, Maha Sarakham 44000, Thailand
[3]One Health Research Unit, Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham 44000, Thailand

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***Corresponding author:**
Supawadee Piratae
One Health Research Unit, Faculty of Veterinary Sciences, Mahasarakham University, Maha Sarakham 44000, Thailand.
Email: supawadee.p@msu.ac.th
https://orcid.org/0000-0003-4053-1909

ABSTRACT

Background: Lumpy skin disease (LSD), a disease transmitted by direct and indirect contact with infected cattle, is caused by the Lumpy skin disease virus (LSDV). The disease affects cattle herds in Africa, Europe, and Asia. The clinical signs of LSD range from mild to the appearance of nodules and lesions in the skin leading to severe symptoms that are sometimes fatal with significant livestock economic losses.
Objectives: This study aimed to characterize LSDV strains in the blood of infected cattle in Thailand based on the GPCR gene and determine the phylogenetic relationship of LSDV Thailand isolates with published sequences available in the database.
Methods: In total, the blood samples of 120 cattle were collected from different farms in four provinces in the northeastern part of Thailand, and the occurrence of LSDV was examined by PCR based on the P32 antigen gene. The genetic diversity of LSDV based on the GPCR gene was analyzed.
Results: Polymerase chain reaction assays based on the P32 antigen gene showed that 4.17% (5/120) were positive for LSDV. All positive blood samples were amplified successfully for the GPCR gene. Phylogenetic analysis showed that LSDV Thailand isolates clustered together with LSDVs from China and Russia.
Conclusions: The LSD outbreak in Thailand was confirmed, and a phylogenetic tree was constructed to infer the branching pattern of the GPCR gene from the presence of LSDV in Thailand. This is the first report on the molecular characterization of LSDV in cattle in Thailand.

Keywords: G protein-coupled chemokine receptor; lumpy skin disease virus; epidemiology; Southeast Asia; Thailand

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INTRODUCTION

Lumpy skin disease (LSD) is a transboundary viral disease caused by the Lumpy skin disease virus (LSDV). This virus belongs to the family *Poxviridae*, genus *Capripoxvirus*, which comprises LSDV, sheep pox virus (SPPV), and goat pox virus (GTPV) [1,2]. LSDV can cause infection in

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ORCID iDs
Tossapol Seerintra
https://orcid.org/0000-0001-5125-4797
Bhuripit Saraphol
https://orcid.org/0000-0001-6887-4757
Sitthichai Wankaew
https://orcid.org/0000-0003-2239-922X
Supawadee Piratae
https://orcid.org/0000-0003-4053-1909

Author Contributions
Conceptualization: Seerintra T, Piratae S; Data curation: Seerintra T, Wankaew S, Piratae S; Formal analysis: Piratae S; Funding acquisition: Piratae S; Investigation: Seerintra T, Saraphol B, Wankaew S, Piratae S; Methodology: Seerintra T, Saraphol B, Wankaew S, Piratae S; Resources: Seerintra T, Saraphol B; Writing - original draft: Piratae S; Writing - review & editing: Piratae S.

Conflict of Interest
The authors declare no conflicts of interest.

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large ruminants, mainly in domestic cattle (*Bos* sp.) and water buffaloes (*Bubalus* sp.) [3,4]. This virus is transmitted by direct contact with the secretions of other infected animals, indirect contact from contaminants, and arthropods comprising mosquitoes, ticks, and flies [5,6]. The clinical signs of LSD are fever, lacrimation, nasal discharge, hypersalivation, lethargy, anorexia, and weakness, followed by the development of nodular lesions in the skin and mucous membranes of the whole body that become open wounds, leading to secondary bacterial infections that cause severe clinical symptoms [7-11]. This virus is endemic in many African countries and spread to Europe, the Middle East, and Asia [12]. LSD is a newly emerging disease in Southeast Asia, including Thailand and neighboring countries [13,14]. In Thailand, the disease was first reported in cattle farms in March 2021 [15] and caused economic loss for cattle farmers and government payments.

LSDV is a double-strand DNA virus. The genome of this virus is easy to detect in a variety of specimens, including nodules, ulceration, secretions, semen, and blood of infected cattle [8,16,17]. Polymerase chain reaction (PCR) and modified PCR methods targeting p32 and GPCR genes are used widely to detect and characterize LSDV [18,19]. The G protein-coupled chemokine receptor (GPCR) gene is prominent on the dsDNA of the LSDV genome and has been reported as an appropriate target for a genetic distinction between Capripoxviruses owing to its variability among Capripoxviruses [20]. Recently, a PCR assay was optimized to characterize LSDV and has been widely used for the phylogenetic grouping of the LSDV [9,21-23]. Although previous studies have explored the presence of LSDV in Thailand [15], there are no data on the molecular characterization of circulating LSDV viruses. These data are essential for understanding the molecular epidemiology and vaccine design for disease control. Hence, this study molecularly characterized LSDV strains in the blood of infected cattle that have been emerging in Thailand based on the GPCR gene and determined the phylogenetic relationship of LSDV Thailand isolates with published sequences available in the database.

MATERIALS AND METHODS

Study area and sample collection

This study examined the presence of LSDV in cattle in Thailand. Blood samples of 120 suspected infected cattle were collected from four provinces in the northeastern part of Thailand (Udonthani, Maha Sarakham, Roi Et, and Ubon Ratchathani) between June to November 2021. Information on the clinical signs of the suspected LSD-affected animals was recorded and evaluated by veterinarians. Blood was collected (approximately 1–3 mL) from the jugular vein, coccygeal vein, or lateral ear vein into sterile tubes containing ethylenediaminetetraacetic acid anticoagulant and kept on ice during transport to the laboratory of the faculty of Veterinary Sciences of Mahasarakham University for further molecular analysis. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee, Mahasarakham University IACUC-MSU-27/2021.

Sample processing and DNA extraction

The blood samples were frozen at −20°C until DNA extraction. The aliquots (200 µL) of each anticoagulated whole blood were used for DNA extraction using GF-1 Viral Nucleic Acid Extraction Kit (Vivantis, Malaysia) according to the manufacturer's instructions. The DNA was eluted in 50 µl of elution buffer and stored at −20°C until further use. The total DNA concentrations were determined by exposing the DNA to 260 nm ultraviolet light with a NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA).

Molecular detection

Each extracted DNA sample was examined for an LSDV infection by PCR using the specific primers targeting of P32 antigen gene; the genetic diversity of the LSDV was determined based on the GPCR gene. The primer sets of the P32 antigen gene were used from a previous study (forward primer, 5′-CGATTTCCATAAACTAAAG-3′ and reverse primer, 5′-CTAAAATTAGAGAGCTATACTTCTT-3′) [24]. DNA fragment results are visualized and considered positives at the appropriate size of 390 base pairs. A second PCR was carried out on all positive samples to amplify the GPCR gene for phylogenetic analysis. This was done using the primers that were designed to amplify a fragment of approximately 1,190 base pairs with the following sequences 5′-TTAAGTAAAGCATAACTCCAACAAAAATG-3′ and 5′-TTTTTTTATTTTTTATCCAATGCTAATACT-3′ [9]. The PCR reactions were conducted in a final volume of 25 µL consisting of 40 pmol of each primer, 5 µL of 5X SuperFi Buffer including 7.5 mM $MgCl_2$, 5 µL of 5X SuperFi GC Enhancer, 0.2 mM dNTPs, 0.5 U of the Platinum Superfi DNA Polymerase (Invitrogen, USA), and 5 µL of template DNA. The PCR program consisted of an initial denaturation at 98°C for 30 sec, followed by 45 cycles at 98°C for 10 sec, 50°C for 20 sec, and 72°C for 45 sec using a PCR machine (Biometra GmbH, Germany). PCR master mixes containing only the primers with no DNA template served as negative controls. After amplification, the expected PCR product of approximately 1200 base pairs was analyzed by 1% gel electrophoresis stained with ViSafe Red Gel Stain (Vivantis, Malaysia) and visualized under ultraviolet light on a Gel Documentation System (Bio-Rad, USA).

Sequence and phylogenetic analysis

The products were sequenced using both forward and reverse strands at the commercial sequencing company (ATGC, Thailand). The obtained GPCR sequences of LSDV were multiple aligned using ClustalW in the BioEdit program [25] with final adjustment manually and compared for similarity with the sequences deposited in GenBank, using the BLAST program hosted by NCBI (https://www.ncbi.nlm.nih.gov/). The nucleotide sequences were deposited in the GenBank database. For phylogenetic analysis, the Maximum Likelihood method in MEGA X [26] was used to construct the phylogenetic tree. Bootstrap analysis with 1000 replications was used to estimate the confidence of the branching patterns of the trees. GPCR Sequences of GTPV (MT153178.1, KF495249.1) and SPPV (KF495251.1, KJ818285.1) were used as the outgroup for phylogenetic analysis.

RESULTS

One hundred and twenty cattle suspected of being infected with the LSDV from four provinces in the northeastern part of Thailand were sampled: Maha Sarakham, Roi Et, Ubon Ratchathani, and Udonthani (**Fig. 1**). All sampled animals were not previously vaccinated against the LSDV. From the 120 suspected samples, 70 animals showed LSD-like symptoms with different grading lesions on their body parts (**Fig. 2**), while 50 were asymptomatic.

The LSDV was detected in five out of 120 blood samples using PCR based on the P32 antigen gene. All five positive samples showed the clinical signs of LSD. Furthermore, GPCR partial sequences of approximately 1,087–1,111 base pairs were amplified in all positive samples. After the quality was checked and edited, the GPCR sequences of the Thailand LSDV in beef cattle were deposited in GenBank under the accession numbers ON184045–ON184059. All five LSDV samples in Thailand were closely related to the LSDV in China (accession no. MN598006.1, MN508357.1) and Russia (accession no. MK358808.1) with 99%–100%

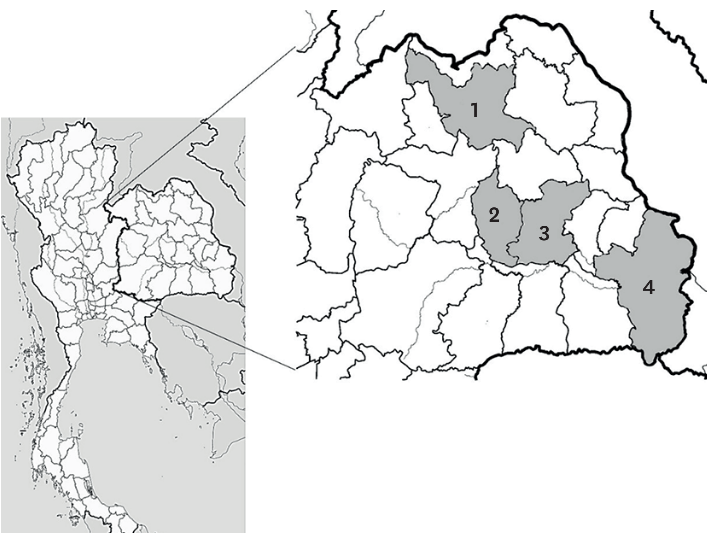

Fig. 1. Map of the study area, showing four provinces in the northeastern part of Thailand where the blood samples were collected. Codes for sampling localities are as follows: 1 = Udonthani; 2 = Maha Sarakham; 3 = Roi Et; 4 = Ubon Ratchathani.

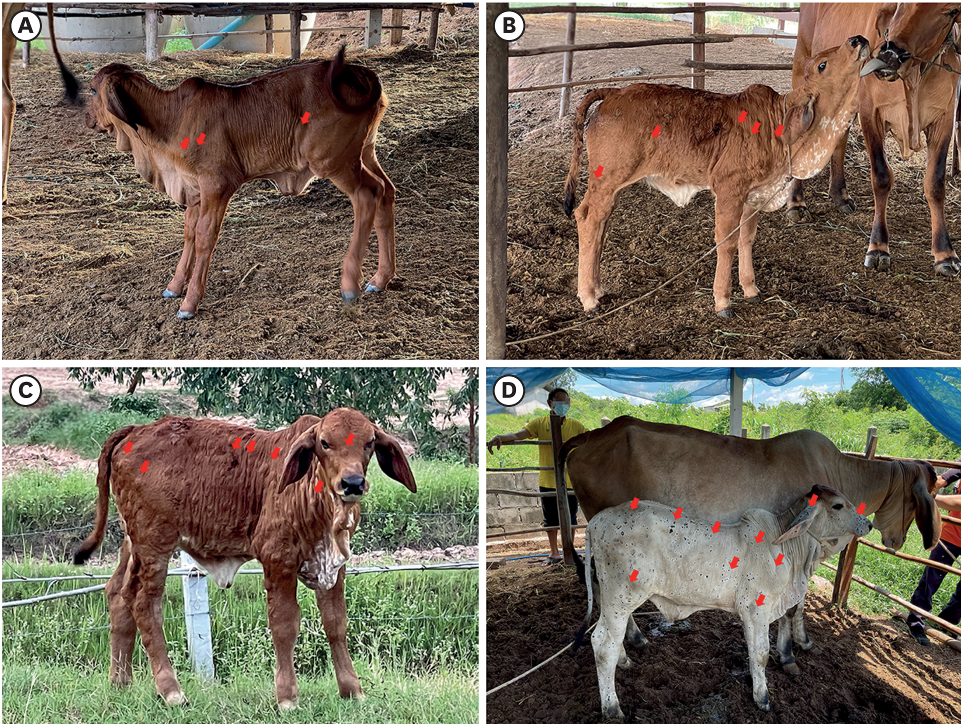

Fig. 2. Observed clinical signs of lumpy skin disease in cattle in this study. The severity of nodular skin lesions (indicated by arrows) was classified based on a visual assessment as follows: mild (A), moderate (B), severe (C), and recovery ulcer (D).

similarity. Moreover, they were similar to the LSDV in Iran (accession no. MT015606.1), Kenya (accession no. KJ818281.1), and Namibia (accession no. MW115948.1) with 98%–99% similarity. Phylogenetic relationships among five isolates of LSDV in Thailand and all 21 LSDVs GPCR gene available from the GenBank database were conducted. In addition, two sequences of the GTPV (MT153178.1, KF495249.1) and two sequences of the SPPV

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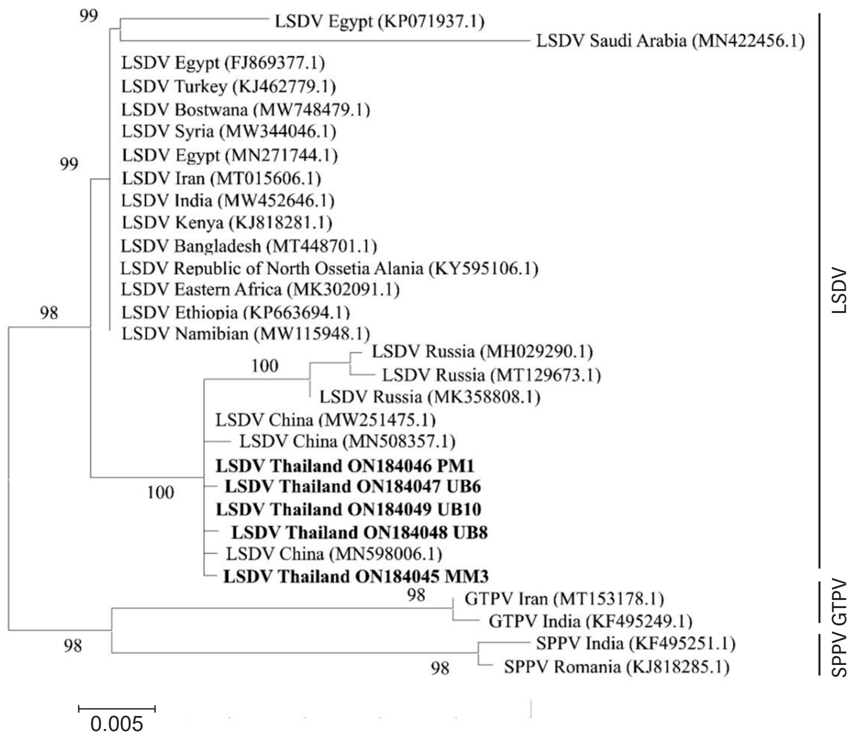

Fig. 3. Phylogenetic analysis using the maximum-likelihood method based on the GPCR gene from five LSDVs Thailand isolates collected in this study (bold type) and 21 GPCR complete sequences in GenBank.
LSDV, lumpy skin disease virus; GTPV, goat pox virus; SPPV, sheep pox virus.

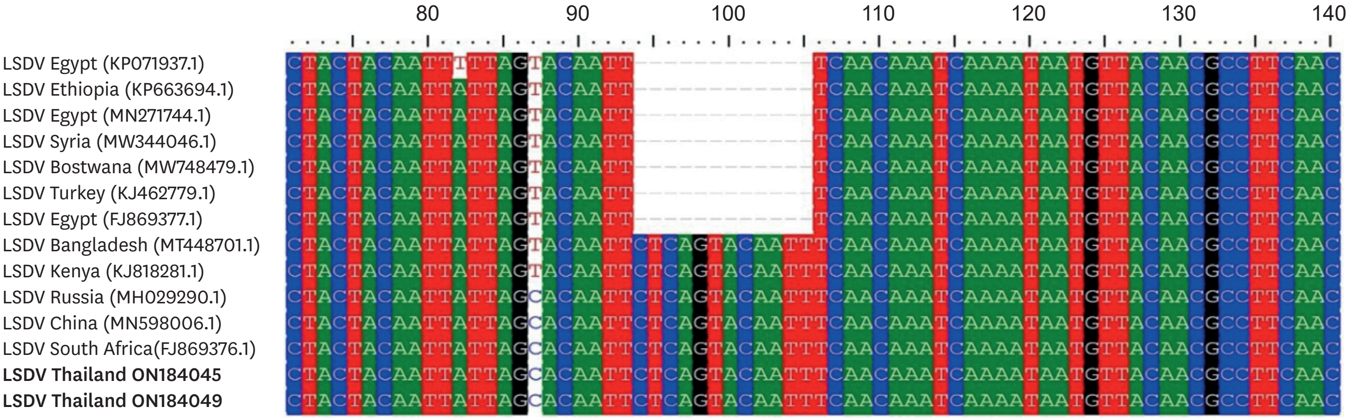

Fig. 4. Multiple sequences alignment of GPCR gene showing 12 base pairs nucleotide insertion (positions 94 to 105) in the GPCR gene from cattle in Thailand (bold type).
LSDV, lumpy skin disease virus.

(KF495251.1, KJ818285.1) were included in the phylogeny. Phylogenetic analysis indicated that the LSDVs could be classified into two subgroups. The LSDV Thailand isolates were found in subgroup one with other LSDV isolates from China (MN508357.1, MN598006.1, and MW251475.1) and Russia (MH029290.1, MK358808.1, and MT129673.1) (**Fig. 3**). Moreover, the analysis of the GPCR gene showed 12 base pair nucleotide insertion in the GPCR gene from cattle in Thailand (**Fig. 4**).

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DISCUSSION

LSD was first reported in Zambia in 1929 and is endemic in most African countries, the Middle East region, and Asia [12]. Since 2020, LSD has been described in many countries in Southeast Asia, such as Vietnam [13], Laos, Cambodia, Myanmar [27,28], Malaysia [14], and Thailand. In Thailand, LSD is a newly emerging infectious disease with an incidence not previously described in cattle until March 2021 [15]. LSDV DNA has been extracted from nodules, lymph nodes, ulceration, milk, secretions, semen, and blood of infected cattle [8,16,17, 29]. In the present study, the LSDV was detected in the blood of infected cattle by PCR targeting P32 antigen gene. The positive samples were then amplified, targeting the GPCR gene for identifying and characterizing the LSDV outbreaks in Thailand. Although samples from suspected animals were collected, the positive rate varied because the LSDV DNA in the blood samples was intermittently positive [30]. The viral DNA was detected in five out of 120 samples; all the positive samples were derived from cattle showing clinical signs of LSD and are characterized into three groups: two, one, and two samples grouped as mild, moderate, and severe, respectively. This finding correlates with previous reports that PCR was the sensitive method for detecting viral DNA in blood in a short period of viremia from 4-11 days after infection. The length of the viremia period did not correlate with the severity of clinical signs [31]. In addition, blood is less susceptible to virus amplification than a skin biopsy [8,9] or nasal swab [32].

The G protein-coupled chemokine receptor (GPCR) gene is used widely to demonstrate nucleotide polymorphism in the LSDV genome. The finding showed that the GPCR gene could be a suitable target for constructing the phylogenetic relationship between members of the Capripoxvirus genus [5,20] and LSDV from wild field strains and vaccine strains [16,21,33]. The complete DNA sequences of GPCR genes are approximately 1,134 base pairs in the 12 nucleotide deletion group or 1,146 base pairs in the 12 nucleotide insertion group (data from GenBank). In this study, LSDV targeting the GPCR sequences was amplified by primers from a previous study [9]. From an analysis of the obtained sequences, 12 base pairs nucleotide insertion between nucleotide positions 94 and 105 in the GPCR gene from cattle in Thailand was also present in the LSDV isolates from South Africa (FJ869376.1), Kenya (KJ818281.1), Bangladesh (MT448701.1), India (MW452646.1), Russia (MT129673.1), China (MN598006.1), and the LSDV Neethling vaccine [34]. From phylogenetic analysis, the LSDV DNA of Thailand was identical to previously identified LSDVs from Russia and China. The LSDV in Thailand may share a common origin. Hence, further molecular epidemiological studies will be needed to confirm LSD outbreaks across the border to monitor the disease spread.

The LSDV remains veterinary relevant in Thailand. Nevertheless, there is a lack of knowledge about disease transmission and insufficient prevention from vectors. In addition, there are no effective treatments that remove the parasites remaining in the circulating blood of the reservoir host for an extended period before parasite clearance. Although the LSDV is widespread in Asia, it is a newly emerging disease in cattle in Thailand and neighboring countries. This study describes the presence of LSDV in the northeastern part of Thailand. This study constitutes the first molecular characterization of the GPCR gene from the LSDV isolates circulating in Thailand. Sequence analysis showed that the Thailand LSDVs resemble the Chinese and Russian isolates. This highlights the importance of tightening border control and laboratory preparedness to prevent disease spread and allows the rapid detection of disease.

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