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Molecular Detection and Phylogenetic Analysis of Lumpy Skin Disease Virus in Iran

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Abstract

BACKGROUND: Lumpy skin disease (LSD) is a significant viral disease of cattle sometimes found in Iran.

OBJECTIVES: The aim of this study was the molecular detection of LSD virus (LSDV) and the determination of their relationship with other Iranian isolates. Moreover, the origin and spread of these viruses were evaluated.

METHODS: The lymph node samples taken from clinically affected cattle from the Kurdistan province of Iran were tested for LSDV using the polymerase chain reaction (PCR).

RESULTS: The partial *P32* gene of LSDV was detected by PCR, sequenced, and phylogenetically analyzed. The LSDVs detected in the present study were 42.98%-100% similar to other LSDVs of Iran.

CONCLUSIONS: Iranian LSDV isolates in this research had the highest similarity to the isolates found in the Indian regions. However, they showed the lowest nucleotide identity with the countries located in the west and southwest of Iran, namely Turkey and Saudi Arabia LSDVs. It could be concluded that these viruses have entered Iran from the eastern borders. It seems that the monitoring of the country borders should be taken into consideration. Further studies should be carried out on LSDV pathogenesis and molecular epidemiology.

KEYWORDS: Cattle, Lumpy skin disease, PCR, *P32* gene, Phylogenetic analysis

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Introduction

Lumpy skin disease virus (LSDV) is a double-stranded DNA virus that belongs to the genus *Capripoxvirus* of the *Poxviridae* family. The LSDV is one of the major poxviral diseases that cause considerable economic damages because of reduced milk production, increased abortion rates, diminished weight gain, elevated susceptibility to secondary bacterial infections, and

high mortality (MacLachlan and Dubovi, 2017). Clinical signs of LSDV in cattle are fever and nodular skin lesions that can spread on the body. Generalized lymphadenitis and edema of the limbs may also occur.

The LSDV was first recognized in 1929 in diverse animals in Zambia and other African countries (Tuppurainen and Oura, 2012). The LSDV was observed in the Middle East in 1989, and since then, several outbreaks have occurred, and there is a risk of LSDV becoming endemic in some countries in the region (Oie, 2010). Before 2012, the disease was reported sporadically in the Middle East. However, the incidence of the disease has increased in many countries since 2012 (Al-Salihi and Hassan, 2015; Ben-Gera *et al.*, 2015; Kasem *et al.*, 2018; Mercier *et al.*, 2018; Sameea Yousefi *et al.*, 2017; Şevik and Doğan, 2017). The LSDV outbreaks were reported in the northwestern provinces of Iran in 2014. The disease leads to detrimental economic effects due to animal mortality, reduced milk production, and health costs (Sameea Yousefi *et al.*, 2017).

The results of another study showed the presence of LSDVs in the northwest of Iran, which were genetically related to each other with more than 99% identity (Yousefi *et al.*, 2018). Sameea Yousefi *et al.* studied the relationships between LSDVs isolated from different regions of Iran. Phylogenetic analysis revealed a high sequence similarity between LSDVs in Iran and African isolates. They suggested that LSDVs had entered Iran from Iraq (Yousefi *et al.*, 2018).

In the present study, the diagnosis of LSD was based on clinical signs that were confirmed by the polymerase chain reaction (PCR) detection of *Capripoxvirus* infection. In the clinical examination of the cattle with LSDV, skin nodules, superficial lymph node enlargement, and loss of appetite were the most frequent symptoms. Other signs included fever, edema in various body parts, and mucosal discharge. A large number of studies have documented the same symptoms in natural (Agag *et al.*, 1992; Body *et al.*, 2012; El-Neweshy *et al.*, 2013) or experimental infections (Osuagwuh *et al.*, 2007). PCR is the common diagnostic method for this disease (Zhou et al., 2012). The *P32* gene is a structural protein suitable for molecular detection and phylogenetic analysis (OIE, 2016; Tian *et al.*, 2010). Furthermore, the P32 antigen plays an essential role in disease pathogenesis and the production of antibodies against Capripoxviruses (El-Kholy *et al.*, 2008; Hosamani *et al.*, 2004; Mafirakureva *et al.*, 2017; Tian *et al.*, 2010; Zhao *et al.*, 2017). In a recent study, it was reported that tracing the origin of LSDV isolates using the *P32* gene could be reliable in phylogenetic studies (Mafirakureva *et al.*, 2017).

Materials and Methods

Sample Collection

During the onset of LSD in two cattle herds in Kurdistan, Iran in January 2020, lymph node samples were collected from dead cows and transferred to the laboratory under cold chain conditions. Phosphate-buffered saline solution and sterile homogenizer were used to prepare a homogenate of the sampled tissues (100 mg). The suspensions were centrifuged and the supernatant was collected for viral DNA extraction.

DNA Extraction

Total DNA was extracted from samples according to the instructions of the manufact-urer of the commercial extraction Kit (Sina-Clon Co., Iran).

PCR

The PCR was performed using the primers described by Ireland and Binepal (Ireland and Binepal, 1998). The primers were designed to amplify a specific segment of 192 bp. The sequences of forward and reverse primers for PCR amplification were 5′-TTTCCTGATT-

TTTCTTACTAT-3′ and 5′-AAATTATATACG TAAATAAC-3′, respectively.

The PCR was carried out with a total volume of 25 µL containing 2.5 µL genomic DNA, 1 µL of each primer, 12.5 µL of Taq DNA Polymerase Master Mix RED (Amplicon, Denmark), and 8 µL of distilled water. The PCR reactions were conducted under the following thermal conditions: initial denat-uration for 2 min at 94°C, followed by 40 cycles of denaturation (50 s at 94°C), primer annealing (50 s at 50°C), and strand extension (60 s at 72°C), ending with a final strand extension step for 10 min at 72°C. The PCR products were visualized in 1.5% (w/v) agarose gel under a UV transilluminator.

DNA Sequencing and Phylogenetic Analysis

All samples were evaluated by PCR and the PCR products of positive samples were sequenced (Bioneer Co., Korea). Sequences were aligned using ClustalW pairwise alignment. Sequences of reference strains and other detected LSDVs were obtained from the NCBI database. Analysis was performed using the neighbor-joining statistical method with 1000 bootstrap replications based on the distance and phylogenetic tree of LSDVs isolates. Sequences were selected from the close strains of the virus in different countries based on location, time, and the results of genetic analysis. The sequences of identified LSDVs in this study were submitted in GenBank under the accession numbers MT050465 and MT050466.

Results

Molecular Detection

Viral DNAs specific for LSDV were found in all samples. In the current investigation, a 192 bp fragment of the *P32* gene was amplified and matched with the published articles on the *P32* gene.

Sequence Analysis

By sequencing the fragments of 192 bp of PCR products, the partial *P32* gene was identified, which encodes the antigenic structural protein. The nucleotide alignment of the sequences showed a similarity of 42.98%-100% between the two selected LSDV sequences and other Iranian strains of the LSDV isolates (Table 1). Phylogenetically, four distinct clusters were indicated in the constructed tree of the *P32* gene. In the present study, the identified strains of the LSDVs belonged to the second cluster, which contains other isolates of the virus from India (Figure 1).

Table 1. The similarity matrix calculated using Mega 7 for the LSDVs and other Iranian selected LSDVs based on the partial *P32* gene sequences.

		1	2	3	4	5	6	7	8	9	10	11
1	Lumpy_skin_disease_virus_strain_UT-Samira-Hamid1	## ##										
2	Lumpy_skin_disease_virus_strain_UT-Samira-Hamid_2	98. 77										
3	Sheeppox_virus_isolate_SPPV-GL(KT438551.1)	98. 63	98. 63									
4	Sheeppox_virus_strain_SPPV/SA5/2016(MG232386.1)	98. 63	98. 63	100 .00								
5	Sheeppox_virus_strain_204/14(MH924596.1)	98. 63	98. 63	100 .00	100 .00							
6	Lumpy_skin_disease_virus_isolate_LSDV-WA-1(KX960778)	45. 06	45. 96	48. 28	48. 28	48. 28						
7	Lumpy_skin_disease_virus_isolate_GPV-Vaccine-Gorgan(KX960782)	45. 06	45. 96	48. 28	48. 28	48. 28	99. 41					
8	Lumpy_skin_disease_virus_isolate_ShPV-Vaccine-RM65(KX960781)	43. 83	44. 72	46. 90	46. 90	46. 90	97. 65	97. 06				

		1	2	3	4	5	6	7	8	9	10	11
9	Lumpy_skin_disease_virus_isolate_LSDV-EA-4(KX960772)	43.83	44.72	46.90	46.90	46.90	98.24	97.65	95.88			
10	Lumpy_skin_disease_virus_strain_Kubash/KAZ/16(MN642592.1)	43.48	42.98	43.93	43.93	43.93	46.34	47.15	46.34	46.34		
11	Lumpy_skin_disease_virus_isolate_Kenya(MN072619.1)	43.48	42.98	43.93	43.93	43.93	46.34	47.15	46.34	46.34	100.00	

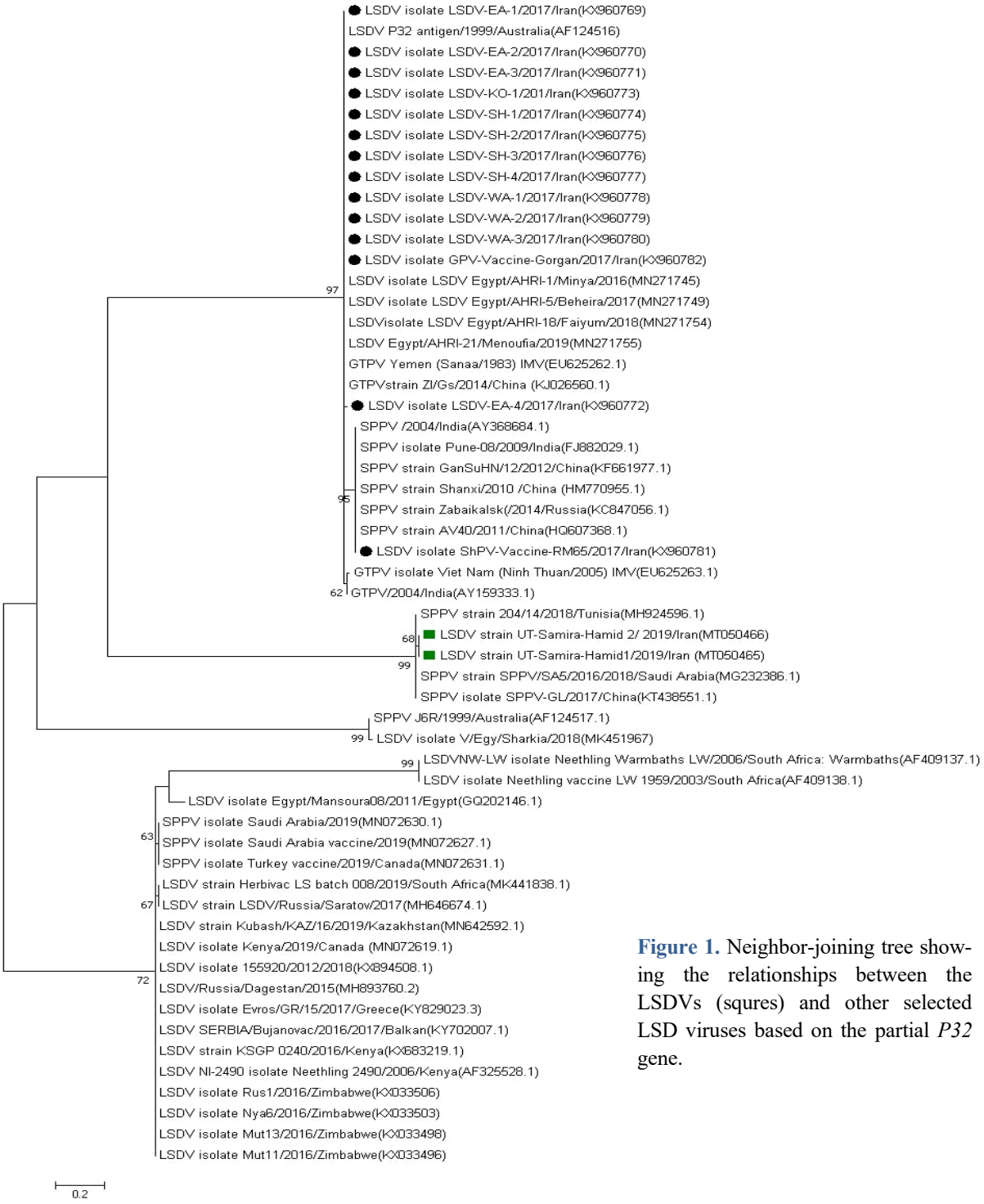

Figure 1. Neighbor-joining tree showing the relationships between the LSDVs (squres) and other selected LSD viruses based on the partial *P32* gene.

Discussion

The LSDV is a contagious viral disease that infects cattle. In this study, two herds of cattle were sampled to assess the LSDV. Dead cattle were also studied for the presence of LSDVs by PCR. Among the studied animals, two LSDV cases were identified based on the *P32* gene, were examined for phylogeny, and the phylogenetic tree was drawn.

The phylogenetic tree shows four groups as follow: Group 1 is related to the Iranian isolates that were reported in the past years (circles), Group 2 belongs to the LSDVs detected in the current study (squares), and Groups 3 and 4 refer to other isolates from other countries, especially in the west of Iran (Figure 1). Nucleotide sequence analysis of these isolates showed 99.98% similarity with LSDV strains from different regions of India. However, phylogenetic trees of LSDVs from Turkey, Saudi Arabia, Russia, Serbia, and Kenya had separate clusters from LSDVs in this study (Table 1).

The LSDVs found in the present study had fewer similarities to other Iranian isolates in other investigations. It could be concluded that ,probably the source of Iranian isolates is India and the LSDVs entered Iran from India. Illegal transport of livestock from widespread country borders without proper monitoring and control could be the reason for this subject. The isolates of Iran were less similar to the ,countries in the west and southwest of Iran such as Turkey and Saudi Arabia. This indicates that the viruses entered Iran from the east of the country.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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شناسایی مولکولی و آنالیز فیلوژنتیکی ویروس بیماری لامپی اسکین (LSDV) در ایران

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زمینه مطالعه: بیماری لامپی اسکین (LSD) یک بیماری مهم ویروسی در گاوها است که گاهی اوقات در ایران دیده می‌شود.

هدف: هدف از این مطالعه شناسایی مولکولی ویروس‌های بیماری لامپی اسکین و تعیین ارتباط آنها با سایر سویه‌های ایران، مبدا و محل گسترش آنها است.

روش کار: در این مطالعه نمونه‌های غده‌های لمفاوی از گاوهایی که در استان کردستان به صورت بالینی درگیر بیماری بودند، اخذ شد. نمونه‌ها از نظر مولکولی و با روش PCR مورد بررسی قرار گرفتند. نمونه‌های مثبت مولکولی از نظر ژن *P32* مورد مطالعه قرار گرفتند. سپس برای این نمونه‌ها سکانس و تعیین توالی صورت گرفت و آنالیزهای فیلوژنتیک انجام پذیرفت.

نتایج: ویروس‌های LSD این مطالعه شباهت ۴۲.۹۸ تا ۱۰۰ درصدی را با سایر جدایه‌های ایران نشان دادند. سویه‌های LSD این مطالعه بیشترین میزان شباهت را به سویه‌های هند داشتند. همچنین آنها کمترین شباهت را به سویه‌های کشورهای همسایه غرب و جنوب غرب ایران مانند ترکیه و عربستان سعودی داشتند.

نتیجه‌گیری نهایی: این یافته نشان می‌دهد که احتمالا سویه‌های مورد مطالعه در این پژوهش از مرزهای شرقی وارد کشور شده‌اند. کنترل و مانتیورینگ منظم مرزها بایستی مورد تاکید قرار بگیرد. مطالعات بیشتری پیرامون پاتوژنز و اپیدمیولوژی مولکولی ویروس‌های LSD بایستی صورت پذیرد.

واژه‌های کلیدی: گاو، بیماری لامپی اسکین، آنالیز فیلوژنتیک، ژن *P32*، PCR

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