African Journal of Advanced Pure and Applied Sciences (AJAPAS)
Online ISSN: 2957-644X
Volume 3, Issue 4, October - December 2024
Page No: 9-16
Website: https://aaasjournals.com/index.php/ajapas/index
ISI 2024: 0.877 **SJIFactor 2024: 6.752** **معامل التأثير العربي: 1.55**

Genetic Characterization and Sequencing of Lumpy Skin Disease Virus Isolate Circulating in Beni-Suef Governorate, Egypt

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Received: August 13, 2024	Accepted: September 20, 2024	Published: October 03, 2024

Abstract:
Large outbreaks of skin lesions in cattle have been observed recently throughout the governorate. The LSDV was shown to be the causative factor. The local strain of LSDV was sequenced and deposited into GenBank, where it was assigned the accession number MK552139 and designated as LSDV/Egy-BSU/2018. The discovered strain resources were connected and to other LSDVs, however the phylogenetic tree clustered viruses of sheep pox, goat pox, and LSD viruses independently. The GPCR gene nucleotide and the LSDV's inferred amino acid sequences were examined in this work by comparing them to the corresponding reference sequences found in the GenBank.

Keywords: LSDV, Isolation, Sequencing, Poxviruses.

الملخص:
في الآونة الأخيرة، كانت هناك تقارير واسعة النطاق عن تفشي مرض جلدي في الأبقار في جميع أنحاء المحافظة .وقد ثبت أن الالتهاب الجلدي الليمفاوي كان العنصر المسبب في هذا الوباء .بعد إجراء التسلسل الجيني له، تم إدخال السلالة المحلية من الالتهاب الجلدي الليمفاوي في بنك الجينات وإعطائها رقم الوصول MK552139، وتحديدها باسم LSDV / Egy-BSU / 2018. قامت الشجرة التطورية بتجميع فيروسات جدري الأغنام وجدري الماعز وفيروسات الإلهاب الجلدي الليمفاوي بشكل منفصل، على الرغم من حقيقة أن موارد السلالة المحددة كانت مرتبطة ببعضها البعض وبفيروسات LSDV إضافية في هذا العمل، تم تحليل نوكليوتيد جين GPCR وتسلسلات الأحماض الأمينية المتوقعة من فيروس الإلتهاب الجلدي الليمفاوي من خلال مقارنتها بالتسلسلات المرجعية المناسبة من بنك الجينات.

الكلمات المفتاحية: فيروس الإلهاب الجلدي الليمفاوي، عزل، تسلسل جيني، مرض الجدري.

Introduction

A major loss in productivity is caused by the viral transboundary cattle illness known as lumpy skin disease (LSD). The lumpy skin disease virus (LSDV) prototype Neethling virus is the cause of the illness. The causal agent belongs to the subfamily Chordopoxvirinae of the Poxviridae family, specifically the Capripoxvirus genus [1]. With a genome composed of double-stranded DNA and 30 homologues of structural or nonstructural pox viral proteins, LSDV is genetically and antigenically closely related to goatpox virus (GTPV) and sheeppox virus (SPPV), with 96% nucleotide sequence identity between the two species, [2]**,** [3]**, &** [4]**.**

Up until 1986, LSD was only available in sub-Saharan Africa. After that, it began to expand to the Middle East, [5]**.** Kuwait reported using LSD for the first time outside of Africa in 1986–1988 [6]. Most of Africa, the Middle East, including Egypt, Lebanon, Jordan Iran, Iraq, Turkey, and Central Asia (Azerbaijan), have documented cases of LSD. The LSD initially surfaced in Egypt in the governorates of Suez and Ismailia in May and October of 1988, respectively. It was believed to have come from Somalia with livestock brought to a nearby quarantine station [7]**.** In 1995, 1998, and 1999, the illness resurfaced [8] and [9]. Finally, reports of epidemics surfaced in 2005 and 2006. Several epidemics have emerged in several Egyptian governorates this year (2018).

The Office International des Epizooties [10], serological and molecular methods, viral isolation in cell cultures, transmission electron microscopy, and other methods can all be used in the laboratory to diagnose LSD.

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Early outbreak detection, prompt laboratory confirmation of the preliminary clinical diagnosis, prompt stamping-out of all or only those animals exhibiting clinical signs of LSD, vaccination, stringent animal movement control, quarantine, disinfection, vector control, and preventive biosecurity measures at affected farms and regions are critical for the successful control and eradication of LSDV.

A Romanian sheep pox vaccination strain developed in lamb skin, as reported in [11] and a Kenyan sheep pox tissue culture vaccine strain produced in Vero cell line [12] were used in Egypt to protect cattle against LSD. The Ismailia strain of the live modified lumpy skin disease virus vaccine was created, and it has shown to be effective, safe, and capable of defending cattle against virulent LSDV challenge [13].

Even though animals received sheeppox vaccinations, the illness is still rapidly spreading throughout Egypt's governorates in spite of the control measures that have been put in place. Furthermore, under the supervision of the general organization of the veterinary authorities, cattle previously vaccinated with the sheep poxvirus vaccine (SPPV) in Beni-Suef Governorate, Egypt, experienced consecutive outbreaks of LSDV as well as occasional cases during the past few years (2017–2018). The primary goal of this study was to gather proof that the LSDV that is now circulating among cattle is different from that which was described in earlier LSD outbreaks in terms of severity and fatalities.

Thus, it was crucial to focus the current study's investigation on using phylogenetic analysis to estimate the degree of homology between the isolates recovered in this study and the previously recovered Egyptian isolates, as well as the phylogenetic relatedness with published reference Capripoxvirus genome sequences.

Material and Methods

In the Beni-Suef Governorate of Egypt, cases of lumpy skin disease (LSD) in cattle were reported in a number of locations between 2017 and 2018. Twenty cattle who were clinically suspicious were utilized to get viral samples from scabs and skin nodules across the body.

Viral samples:

Clinical samples were taken from cattle exhibiting the characteristic symptoms of lumpy skin disease. Samples consisted of scabs and/or skin sores. On embryonated chicken eggs, skin biopsies from cutaneous nodules were collected on 50% glycerol saline and utilized to isolate the Lumpy Skin Disease Virus (LSDV).

Gene-specific primers: Gene specific primers used in PCR were designed by Invitrogen, Analysis for life technologies (Cairo, Egypt).

Pair 1: GPCR based PCR was used to amplify viral G-protein Coupled chemokine Receptor Gene of LSDV.

AGT ACA GTT AGT AGC GCA ACC- 3''5 LSD F

LSD R 5’ GGG TGA ACT ACA GCT AGG TAT C- 3'

Pair 2: RPO30 based PCR was used to amplify the region containing a 21-nucleotide deletion in (SPPV) sequences

Table (1): Primers used in the amplification of viral G-protein Coupled chemokine Receptor Gene of LSDV.

LSD F 5’-TCTATGTCTTGATATGTGGTGGTAG-3’

LSD R 5’AGTGATTAGGtGGTGTATTATTTTCC-3’

Virus isolation on Chorioallantoic membrane (CAM) of embryonated chicken eggs (ECE):

Samples were inoculated on chorioallantoic membranes (CAMs) of ECE (3 eggs for each sample) according to **Van Rooyen** [14].

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PCR: The GF-1 Tissue DNA Extraction kit (QIAquick Gel Extraction kit) was used to extract the LSDV genome from the suspicious material (tissue homogenate). The target gene, LSDV, was amplified in a final volume of 25µl. The following steps were used in order to identify the PCR products using agarose gel electrophoresis [15]. Using an ultraviolet transilluminator, the amplified DNA products were found by comparing them to a DNA molecular weight marker.

Gene sequence and Sequence analysis:
Partial nucleotide sequencing of the G-protein-coupled chemokine receptor (GPCR) gene for LSDV isolated in 2018 from Beni-Suef Governorate was carried out in the Animal Health Research Institute, Dokki, Egypt.

Sequence was submitted to GenBank (LSDV/ Egy-BSU/2018) under accession number of (MK552139).The received sequence was imported into alignment windows with the downloaded highly similar sequences into BIOEDIT version 7.0.4.1 software. Multiple sequence alignment was conducted using Crustal W application embedded in BIOEDIT version 7.0.4.1 software. Sequence editing, correction, frame adjustment, amino acid alignment and allocation of antigenic sites were also conducted using different options of BIOEDIT version 7.0.4.1 software. All finely adjusted sequences were exported from BIOEDIT version 7.0.4.1 software as separate FASTA files. FASTA files were inserted into MEGA 5.05 DNA alignment tool and exported into MEGA format. MEGA files were used as a base for phylogenetic analysis using neighbor joining method. One thousand bootstrap replicates were conducted to assess the statistical support for the tree topology. The resultant trees were saved as photos.

Results
LSDV was found using PCR utilizing viral DNA that was isolated from pooled ECE CAMs. While primers designed targeting the RP030 gene amplified a 172 bp product from the LSDV genome, primers targeted for the GPCR gene amplified a 554 bp product from the LSDV genome, confirming the sample's positive status for the presence of the LSD viral genome. A (152 bp) amplified by a tissue culture-adapted SPPV vaccine strain was discernible from the LSDV amplicons.
The G-protein-coupled chemokine receptor (GPCR) gene for the latest local LSD virus has been partially sequenced and submitted to GenBank.

Partial nucleotide sequencing of G-protein-coupled chemokine receptor (GPCR) gene for LSDV isolated during 2018 from Beni-Suef Governorate was carried out. Sequence was submitted to GenBank (LSDV/ Egy-BSU/2018) under accession number of (MK552139).
A 12-nucleotide deletion (ORF position 88–99) that resulted in the lack of amino acid residues at positions 30-33 was discovered by deducing the nucleotide and amino acid sequence. With the exception of LSD NI-2490 isolate (AF325528.1) and LSDV/Kenyan/SGP/O-240 (KJ818281.1), which do not have this aa deletion, all cattle isolates examined in the sequencing study support this deletion.
The isolate LSDV/Egy-BSU/2018 was found to differ from LSDV isolated from Sharqia/Egypt (MF156211.1) and LSDV isolated from Beni-Suef/Egypt (KJ561442.1) in 2014 with respect to one silent mutation (A111T) and one non-silent mutation (G86A) that resulted in amino acid substitution (S29N) (serine into asparagine). Meanwhile, the isolate LSDV isolated from Mansoura/Egypt/2011 (KP071936.1) differs from both silent mutations (A156G) and (T246C) and two non-silent mutations (A82T) and (T247C) that led to amino acid substitution (I28F) (Isoleucine into Phenylalanine) and (C83R) (Cysteine into Arginine), respectively. The isolates LSDV/Egy/BSU/MEVAC/2016 (MH427386.1) and LSDV/Sharqia/Egypt/2016 (MG970343.1) both showed an amino acid substitution at position (T59S) (Threonine into Serine) and (C83R) (Cysteine into Arginine).

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Table 1 Data of selected GPCR gene sequences used in comparative analysis.

Virus isolate	GenBank accession No.	Isolation year	Isolation country
LSDV/Egy-BSU/2018	MK552139.1	2018	Egypt
LSDV-NW-LW/1999	AF409137.1	1999	South Africa
LSD_NI-2490_isolate	AF325528.1	1958	Kenya
LSDV_isolate/Ethiopia 2011	KP663691.1	2011	Ethiopia
LSDV_Sudan/06_Obied_isolate	FJ869369.1	2006	Sudan
LSDV_Egypt_VRLCU	MF156211.1	2014	Egypt, Sharqia
LSDV_isolate_Egy	KP071936.1	2011	Egypt, Mansoura
LSDV/Egypt/BSU-1	KJ561442.1	2014	Egypt, Beni-suief
LSDV/Egypt/BSU-2	KJ561443.1	2014	Egypt, Beni-suief
LSDV/RNOA-15_Russia	KY595106.1	2015	Russia
LSDV/Evros/GR/15	KY829023.3	2015	Greece
LSDV_Egy-BSU/MEVAC/2015	MH427384.1	2015	Egypt
LSDV_Egy-BSU/MEVAC/2016	MH427386.1	2016	Egypt
LSDV_Kenyan_SGP_O-240	KJ818281.1		Kenya
LSDV/Egypt/2016-01	MG970343.1	2016	Egypt, Sharqia

Nucleotide and amino acid identities of G-protein-coupled chemokine receptor gene

The LSDV isolate used in this study was 100% identical in terms of nucleotide and amino acid identities to the following isolates: NW-LW/LSDV/ South Africa /1999 (AF409137.1), LSDV/Sudan/Obied /2006 (FJ869369.1), LSDV/Ethiopia /2011 (KP6636691.1), LSDV/Egypt/Beni-suef-2/ 2014 (KJ561443.1), LSDV/RNOA/Russia /2015 (KY595106.1), LSDV/Evros/Greece /2015 (KY829023.3), and LSDV/Egypt/BSU/MEVAC/2015 (MH427384.1).

Its nucleotide and amino acid identities were 99.6% and 99.4% alike with the Egyptian strains LSDV/Egypt/ Sharqia /2014 (MF156211.1) and LSDV/Egypt/ Beni-Suef -1/2014 (KJ561442.1). However, using LSDV/Egypt/Mansoura/2011 (KP071936.1), nucleotide and amino acid identities were determined to be 99.2% and 98.7%, respectively.

The nucleotide and amino acid identities of the newly identified LSDV in the current investigation and the 1958 lumpy skin disease virus NI-2490 (AF325528.1) were determined to be 97.5% similar.

There was extremely little commonality (91.5%: 93% nucleotide and amino acid identity, respectively) found between the sequences of sheeppox and goatpox that were already accessible. KP071936.1, /Mansoura/2011.

Phylogenetic analysis

Phylogenetic analysis of 45 Capripoxviruses showed that members of Capripoxvirus were delineated into three species-specific distinct genetic clusters consisting of lumpy skin disease virus, goatpox virus and sheeppox virus based on the GPCR gene with goatpox viruses being more related to LSDV.

The LSDV and the GTPVs lineages show more diversity than in the SPPV group. LSDVs fall into two subgroups. Recent LSDV isolate obtained in this study are in the same clade and essentially identical to NW-LW/LSDV/ South

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Africa /1999 (AF409137.1), LSDV/Sudan/Obied /2006 (FJ869369.1), LSDV/Ethiopia/2011 (KP663691.1), LSDV/Egypt/Beni-suef-2/2014 (KJ561443.1), LSDV/RNOA/Russia /2015 (KY595106.1), LSDV/Evros/Greece/2015(KY829023.3), LSDV/ Egypt/BSU/MEVAC/2015 (MH427384.1), LSDV/Russia/Dagestan/2015 (MH893760.2) and LSDV/SERBIA/Bujanovac/2016 (KY702007.1) LSDV/TURKEY/2014 (KR024774.1), LSDV/Kenyan/SGP/O-240 (KJ818281.1) and LSD/NI-2490 (AF325528.1). Only LSDV/Samara/2017/Russia (MH753583.1) and LSDV/Russia/Saratov/ 2017 (MH646674.1) are clustered separately.

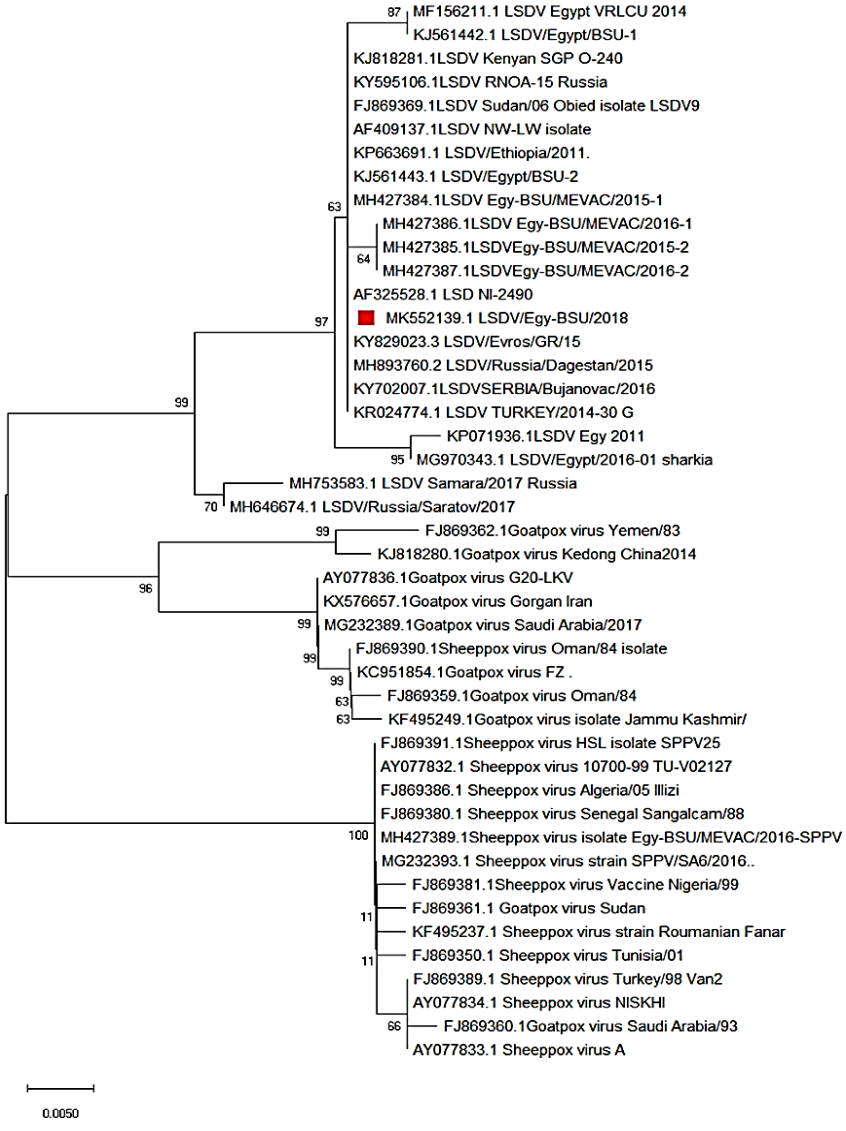

Figure 1 Phylogenetic analysis of the G-protein-coupled chemokine receptor gene. The tree was generated using Mega 7.1 program by the neighbour-joining analysis. Bootstrap confidence values were calculated on 1000 replicates according to the maximum-likelihood approach.

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Discussion

LSDV was found by PCR using isolated viral DNA from pooled CAMs of ECE for molecular characterization.

Two different kinds of oligonucleotide primer pairs were employed in this investigation to identify the local LSDV strain affected CAM. The first primer was designed specifically to amplify the 554 bp unique product, and it was specific to the GPCR gene. Targeted by the second primer, the LSDV RP030 gene is anticipated to produce a particular product of 172 bp when amplified using PCR from the extracted DNA products.

PCR was used to increase the amount of isolated viral DNA from the combined CAMs of ECE. A 554 bp product from the LSDV genome was amplified using primers specific for the GPCR gene, indicating that the sample tested positive for the presence of the LSD viral genome. As the sample tested positive for the presence of the LSD virus genome, primers designed targeting the RP030 gene amplified a 172 bp product from the LSDV genome. A (152 bp) amplified by a tissue culture-adapted SPPV vaccine strain was clearly discernible from the LSDV amplicons.

The local strain of LSDV was sequenced and deposited into GenBank, where it was assigned the accession number MK552139 and designated as LSDV/Egy-BSU/2018. Even though the strain resources we used were linked to one another and to the other LSDVs, phylogenetic trees clustered viruses associated to LSD, sheep pox, and goat pox independently.

The present investigation examined the GPCR gene nucleotide and the resulting inferred amino acid sequences of LSDV by comparison with corresponding reference sequences sourced from GenBank (Fig 1, 2). The most recent Egyptian isolate of LSDV, MK552139.1 LSDV/Egy-BSU/2018, was found to have 100% nucleotide and amino acid identities with the following close relatives: NW-LW/LSDV/ South Africa /1999 (AF409137.1), LSDV/Sudan/Obied /2006 (FJ869369.1), LSDV/Ethiopia /2011 (KP663691.1), LSDV/Egypt/Beni-suef-2/ 2014 (KJ561443.1), LSDV/RNOA/Russia /2015 (KY595106.1), LSDV/Evros/Greece /2015 (KY829023.3), and LSDV/ Egypt/BSU/MEVAC/2015 (MH427384.1).

With LSDV/Egypt/Sharqia/2014 (MF156211.1) and LSDV/Egypt/Beni-suef-1/2014 (KJ561442.1), nucleotide and amino acid identities were found to be 99.6, 99.4 respectively due to the presence of one silent mutation (A111T) and one non-silent mutation (G86A) that led to amino acid substitution (S29N) (Serine into Asparagine). Conversely, with LSDV/Mansoura/Egypt/2011 (KP071936.1), nucleotide and amino acid identities were found to be 99.2, 98.7 respectively due to the presence of two silent mutations (A156G) and (T246C) and two non-silent mutations (A82T) and (T247C) that led to amino acid substitution (I28F) (Isoleucine into Phenylalanine) and (C83R) (Cysteine into Arginine).

Positions 30-33 of isolate LSDV/Egy-BSU/2018 have an amino acid deletion, according to deduced amino acid sequence analysis. Only two of the cattle isolates in the database, LSD NI-2490 isolate (AF325528.1) and LSDV/Kenyan/SGP/O-240 (KJ818281.1), showed a systematic lack of amino acid residues at positions 30-33. These isolates do not have this aa deletion (97.5% nucleotide and amino acid identities). Using the known sequences for sheep and goatpox, the overall identities of this gene and the encoded amino acids varied from 91.5% to 93%.

Nucleic acid sequences were subjected to a representative phylogenetic analysis utilizing the neighbor-joining technique in order to investigate the relationships among the CaPVs. The LSD, sheep pox, and goat pox viruses were grouped separately in the phylogenetic tree (Fig. 1), with the gopox viruses being more related to LSDV than the sheeppox viruses, as suggested by other researchers who conducted phylogenetic studies on different genome segments, such as [15] for the P32 gene and [16] for two hypothetical proteins: LSDV001 and LSDV002. Compared to the SPPV group, there is greater variability in the LSDV and GTPV lineages. This result is consistent with that found in [17]. There are two subgroups of LSDVs.

Conclusively the recent LSDV isolate obtained in this study is in the same clade and essentially identical to: NW-LW/LSDV/ South Africa /1999 (AF409137.1), LSDV/Sudan/Obied /2006 (FJ869369.1), LSDV/Ethiopia/2011 (KP663691.1), LSDV/Egypt/Beni-suef 2/2014 (KJ561443.1), LSDV/RNOA/Russia/2015(KY595106.1),LSDV/Evros/Greece/2015(KY829023.3),LSDV/Egypt/BSU/MEVAC/2015(MH427384.1),LSDV/Russia/Dagestan/2015(MH893760.2) LSDV/SERBIA/Bujanovac/2016 (KY702007.1), LSDV/TURKEY/2014 (KR024774.1) LSDV/Kenyan/SGP/O-240 (KJ818281.1) and LSD/NI2490 (AF325528.1).

Results of phylogenetic and multi-sequence analyses revealed a high degree of similarity between LSDV isolates from different locations with minimal genetic variation. The obtained results agree with [18] who stated that despite

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the geographic distance and time separation between origins of isolates, minimal genetic variation was observed thereby suggesting that lumpy skin disease is genetically stable. This finding is in accordance with that of [19] who reported that nucleotide sequence data revealed that SSPV, GTPV and LSDV clustered into host species-specific groups.

Positions 30-33 of the LSDV/Egy-BSU/2018 have an amino acid deletion. The LSDV/Kenyan/SGP/O-240 (KJ818281.1) and LSD NI-2490 isolate (AF325528.1) did not have the deleted amino acid present in the Egyptian LSDV isolate from 2018. This deletion might be attributed to an escape mutation that resulted from utilizing the Kenyan strain as a vaccine against LSDV. As a result of these findings, we advise against using Kenyan sheep or goat pox strains in favor of the original Neethling LSDV strain of cattle as a necessary strain for managing LSD.

In conclusion, as the recently found isolate is more closely linked to LSDVs than to the presently employed sheep poxvirus vaccines, it is crucial and advised to use the original Neethling LSDV strain of cattle as a key strain for controlling LSD in Egypt.

Conclusion

History of investigated animals indicated that these animals were infected with a LSDV before the reoccurrence of the disease with variable periods of 4-6 months. These animals suffered from severe signs differed from that reported in the previous LSD outbreaks, in terms of severity and deaths, so the general objective of this study was to obtain a proof of concept about the currently circulating LSDV among cattle to decide the stain to be used as a vaccine.

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