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Genetic relatedness between recent field isolate strain of LSDV and other field and vaccinal strains based on GPCR, ORF095 and ORF103 genes in Egypt 2020

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ABSTRACT

Lumpy skin disease (LSD) is a financially significant Capripoxvirus-induced disease of cattle. Despite Egyptian veterinary officials' comprehensive immunization program, LSDV is still prevalent and widespread throughout the country. Understanding molecular epidemiology is critical for developing better LSD eradication and control measures. The present research was designed to assess the genetic relatedness between newly strain from Menofia governorate 2020 and previously isolated field and vaccinal strains through molecular characterization and phylogenetic analysis. During the summer of 2020, ten skin tissue samples were taken from LSD-infected cattle that were involved in the epidemic. LSDVs were detected in clinical samples using PCR targeting the GPCR gene. By PCR, all ten samples tested positive for LSDV. One of these PCR-positive samples and the vaccinal strain were then amplified and sequenced for molecular characterization utilizing a G-protein coupled chemokine receptor (GPCR) gene, ORF95 and ORF103. Phylogenetic analysis indicated that LSDV strain circulating in Egypt 2020 was closely related with sequences previously isolated from Egypt and that from neighboring countries. A comparison of the GPCR, ORF095, and ORF103 genes revealed that outbreak isolate dissimilar from strain included in vaccine. This information is essential for comprehending LSDV molecular epidemiology and to contribute to the Egyptian government's development strategies of control methods.

1. INTRODUCTION

Lumpy skin disease (LSD) is a severe, notifiable, transboundary viral disease of cattle caused by the Neethling LSD virus, which is a member of the Capripoxvirus genus, subfamily Chordopoxvirinae, family Poxviridae. (Buller et al., 2005). LSDV is a 151 kb double-stranded DNA genome that contains 156 putative genes and a core coding region surrounded by 2.4 kb identical inverted terminal repeats. (Tulman et al., 2001).

Although LSDV, sheep pox virus (SPPV) and goat pox virus (GTPV) have high antigenic similarity and genetic identity (>96%), nine genes are known to occur in LSDV but are disrupted in GTPV and SPPV, and host specificity is most likely genetically determined (Tulman et al., 2001; Tulman et. al., 2002). Molecular epidemiological studies of LSDV primarily depend on the examination of several key regions of the genome, including the GPCR, RPO30, P32, and EEV genes. (Erster et al., 2019).

LSD is attracting considerable attention due to its significant economic losses as it disrupts cattle trade and their byproducts, causing permanent hide damage, decrease weight gain, reduce milk yield, temporary or permanent infertility may occur in cows and bulls, and sometimes death with one % mortality rate (Babiuk et al., 2008).

All cattle breeds are susceptible to infection with different clinical forms (Gari et al., 2011). The clinical disease is characterized by pyrexia, enlarged superficial lymph nodes, nasal discharge, watery eyes, and generalized skin nodules all over the body. The typical clinical symptoms are a tentative diagnosis of LSD (El-Kholy et al., 2008), followed by laboratory confirmation tests such as virus isolation (VI), serological, and molecular assays. (Awad et al., 2010). PCR depending on LSDV-specific primers targeting genome termini, the virus's most variable region, demonstrated their capacity to distinguish LSDV from other CaPVs. (Stram et al., 2008). Previous studies on LSDV in Egypt confirms the conserved nature of the LSDV genome even with the circulation of the virus in different districts in country (Abdallah et al., 2018; Rouby et al.,2019; selim et al., 2021). So, this study aimed to determine the genetic relatedness between newly isolated strain from Menofia governorate 2020 and previously isolated field and vaccinal strains.

2. MATERIAL AND METHODS

Ethical approval

The Ethics Review Committee of Benha University's Faculty of Veterinary Medicine accepted this work with ethical approval number (BUFVTM 09-01-2023). All of the

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experimental procedures were performed in the Virology Research Laboratory, Animal Health and Research Institute (AHRI), Giza and Department of Virology, Faculty of Veterinary Medicine, Benha University, Egypt.

2.1. Sample's collection and preparation

During the LSD outbreaks in different areas of Menofia governorate, Egypt in 2020, from clinically infected cattle; showing fever, salivation, nasal and ocular discharges, swelling of superficial lymph nodes and skin nodules covering different parts of the body and, in certain cases, the entire body a total of Ten skin lesions were obtained under a septic condition by surgical excision while cattle under local anesthesia and preserved in PBS with 10% antibiotic and sent to the laboratory. Each tissue sample was processed in accordance with (Burleson et al., 1997) and stored at 20 °C for subsequent molecular analysis.

2.2. Reference Neethling LSDV vaccine, Egypt

It was obtained from Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt as the vaccinal strain used in Egypt. It was used as the center in PCR reaction based on GPCR, ORF095 and ORF103 also for sequencing and phylogenetic study.

2.3. DNA extraction

The DNA extraction from tissue homogenates was done according to manufacturer's instructions of Thermo Scientific Gene JET Genomic DNA Purification Kit.

2.4. PCR assay

The partial open reading frames of the GPCR, ORF 095, and ORF 103 genes were amplified via PCR from DNA extracted from field nodular samples and reference vaccinal strain currently used in Egypt. The 2 primer sets were specific for the GPCR gene LSDVF (5′-AGT ACA GTT AGT AGC GCA ACC-3′) and LSDVR (5′-GGG TGA ACT ACA GCT AGG TAT C-3′) according to Mashaly et al. (2020), ORF 095 gene (forward primer: 5'-ATGGACTTCATGAAAAAATATACT-3'; reverse primer: 5'-TTTGCTGTTATTATCATCCAG3') and ORF 103 gene (forward primer: 5'-ATGTCTGATAAAAAATTATCTCG-3'; reverse primer: 5'-ATCCATACCATCGTCGATAG-3') according to Zhu et al. (2013).

The PCR and cycling procedures were carried out in accordance with (Amin et al., 2021) using the AmpliTaq GoldTM 360 Master Mix kit manufacture instructions, supplied by Applied Biosystems™, Thermo Fisher Scientific, Waltham, MA, USA and the amplified PCR products were analyzed using 1.5 agarose gel electrophoresis also in accordance with Amin et al. (2021).

2.5. Sequencing and phylogenetic analysis

Amplified PCR product of two samples (nodule and vaccine) by different three primers (GPCR, ORF095 and ORF103) were subjected to purification using QIAquick genomic PCR purification kit in accordance with the manufacturer's recommendations. The quantity of DNA determined by SPECTRO stars ^{Nano} (BMG LABTECH) and dispatched to 3500 Genetic Analyzers, USA using BigDye® Terminator v3.1 Cycle Sequencing Kit for DNA sequencing. Comparative alignment of the GPCR, ORF095 and ORF103 gene sequences was conducted using the ClustalW Multiple alignment of BioEdit Version 7.0 software, and sequence identities and divergences were estimated (Hall, 1999). A phylogenetic tree was constructed using MEGA Version 7 software. The tree was built using he neighbor-joining method based on 1000 bootstrapped data sets (Saitou and Nei, 1987)

3. RESULTS

Polymerase chain reaction

Using primer sequences that specifically target the GPCR gene, a fragment of 554 bp has been obtained from all 10 samples under study and reference vaccinal strain. Additionally, one of these samples and reference vaccinal strain were amplified using other two primer sequences; ORF 95 and ORF 103 and a fragment of 483 bp and 570 bp were obtained respectively (Fig 1).

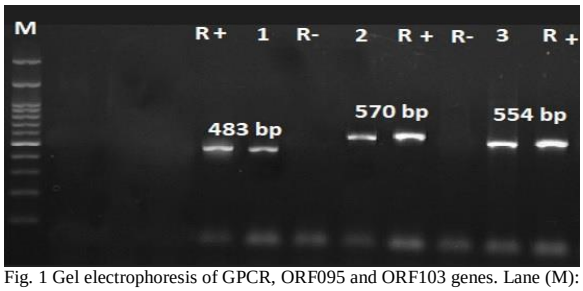

Fig. 1 Gel electrophoresis of GPCR, ORF095 and ORF103 genes. Lane (M): 100bp DNA marker. Lane (R+): control positive. Lane (R-): control negative. Lane (1): positive reaction using ORF095 gene. Lane (2): positive reaction using ORF103 gene. Lane (3): positive reaction using GPCR gene.

3.2. Genetic analysis and relationship of LSDV strain

3.2.1. GPCR gene

Menofia LSDV strain Egypt 2020 sequenced and submitted in GenBank under Accession number OQ679868. A neighbor-joining (NJ) phylogenetic tree categorized LSDV strains into two subgroups field strains and vaccination strains. Menofia LSDV strain Egypt 2020 (OQ679868) was discovered to be grouped with virulent LSDV field strains identified in African, the Middle East, and European countries and clustered separately from vaccine strains (Figure 2). For GPCR gene, Menofia LSDV strain Egypt 2020 shared highest sequence similarity ranging from 99% to 100% with previously field strains from Egypt like (KJ561443, MK736888) and with LSDV field strains from Africa, Middle East, and Europe. Among study Menofia LSDV strain Egypt 2020 and vaccinal strains including SERVAC LSDV Neethling Vaccine, Egypt shared similarity percentage 95% except for vaccinal strain from Kenya (KX683219) were 97%. GPCR nucleotide sequence alignment (Fig. 3) indicated the presence of a 12-nucleotide deletion in Menofia LSDV strain Egypt 2020, which is shared by all LSDV field strains now circulating in Africa, the Middle East, Central Asia, and East Europe. In contrast, such a loss was not discovered in any of the live attenuated LSDV vaccine strains including the SERVAC LSDV Neethling Vaccine from Egypt. GPCR amino acid sequence analysis revealed presence of 4 amino acid (STIL) deletion at positions (14–17) in Menofia LSDV strain Egypt 2020 and other field strain from GenBank including that previously isolated from Egypt (KJ561443 and MK736888) and show no substitution between them. Such deletion not found in vaccinal strain However, there are five amino acid substitutions (V/G1, T/N7, A/N9, S/N61 and M/I112), where Menofia LSDV strain Egypt 2020 differed from SERVAC LSDV Neethling Vaccine, Egypt (Fig 4).

3.2.2. ORF 95 gene

The NJ tree based on ORF 95 gene sequences showed three clades; one clade comprised the LSDV wild strains which currently circulated in Africa, Middle East, and Europe and second clade comprised vaccinal strain and third one for field sheep pox strains (Fig 5). It is noted that current study Menofia LSDV strain Egypt 2020 (OQ701618) closely related to all three clades but very nearest to clade comprised Africa, Middle East, and Europe strains. For

ORF 95 gene, the nucleotide sequence homology between Menofia LSDV strain Egypt 2020 (OQ701618) and LSDV field strains from other countries was in the range of 97%–99% and 97% with vaccinal strain from GenBank and 98% with SERVAC LSDV Neethling Vaccine, Egypt. The ORF95 amino acid sequence alignment revealed that Menofia LSDV strain Egypt 2020 differ from other field strain from GenBank only in one amino acid substitution (S/F24) and differ from SERVAC vaccine in two amino acids substitution (E/D84 and S/A128). (Fig 6).

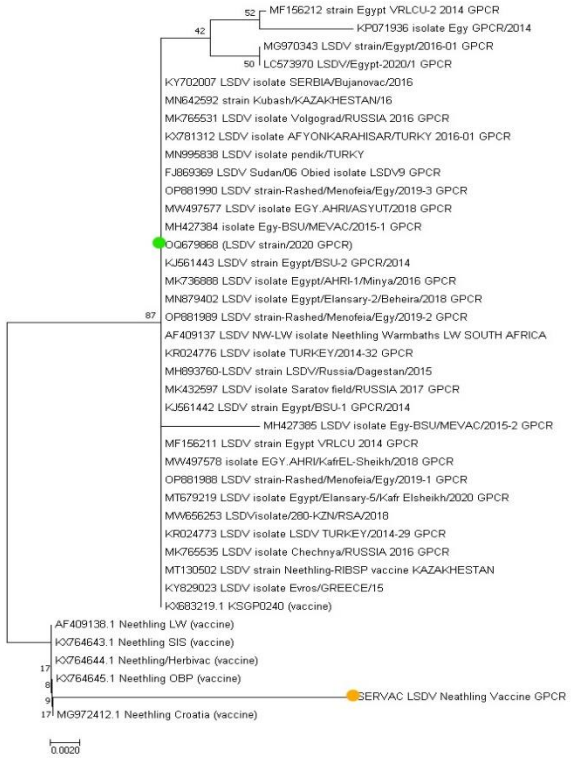

Fig. 2 Neighbor-joining (N-J) tree depicting phylogenetic relationships of the lumpy skin disease viruses isolated in this study and other Capripoxvirus isolates based on G-protein-coupled chemokine receptor nucleotides sequences. The tree was analyzed by N-J analysis with 1000 bootstrap replicates.

3.2.3. ORF 103 gene

The NJ phylogenetic tree categorized LSDV as field strains and vaccine strains, with LSDV field strains further classified into two clades., our study Menofia LSDV strain Egypt 2020 (OQ701619) was belonging to clade comprises all the currently circulating field strains from Africa, Middle East, and Europe (Fig 7). For ORF 103 gene, the nucleotide sequence identities between Menofia LSDV strain Egypt 2020 and LSDV field strains from other countries was ranging from 98% to 100%, 98% with vaccinal strain from GenBank and 97% with SERVAC LSDV Neethling Vaccine, Egypt. The deduced ORF103 amino acid revealed that Menofia LSDV strain Egypt 2020 show no difference with other field strain from GenBank including that previously isolated from Egypt (MW357660) and differ from SERVAC LSDV Neethling Vaccinal strain, Egypt in eight amino acid substitution (L/F11, L/F25, T/N32, P/T54, G/S71, S/F75, S/L76 and A/G 138) fig (8).

Fig 3 Alignments of the first 100 nucleotides of the G-protein-coupled chemokine receptor gene of lumpy skin disease virus (LSDV) strain and those of other recovered from GeneBank; deletion of 12 are evident in the sequences of the study new LSDV strains when compared with those of vaccinal strain.

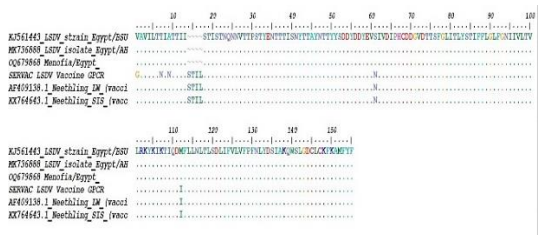

Fig. 4 Differences in amino acid motifs between LSDV field and vaccine strains in G protein-coupled receptors gene

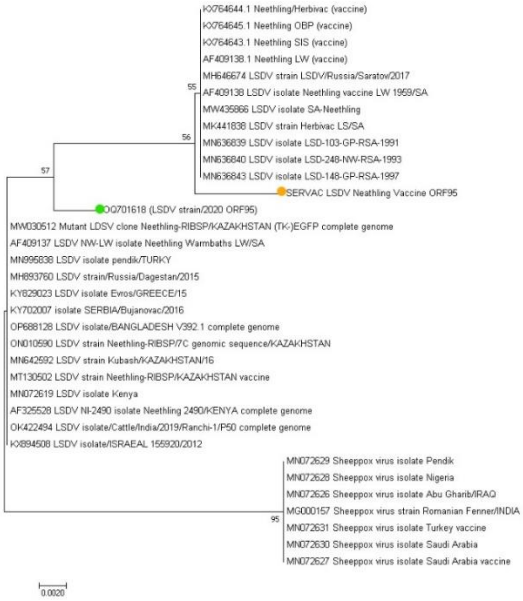

Fig 5 Neighbor-joining (N-J) tree depicting phylogenetic relationships of the lumpy skin disease viruses isolated in this study and other Capripoxvirus isolates based on ORF 095 nucleotides sequences. The tree was analyzed by N-J analysis with 1000 bootstrap replicates.

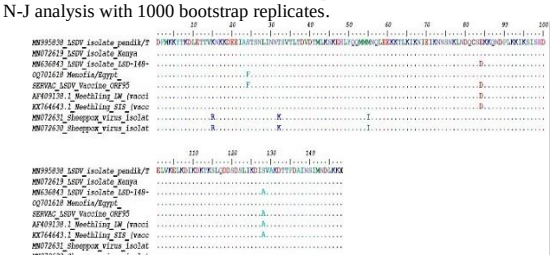

Fig 6 Differences in amino acid motifs between LSDV field and vaccine strains in ORF 095 gene.

Fig 7 Neighbor-joining (N-J) tree depicting phylogenetic relationships of the lumpy skin disease viruses isolated in this study and other Capripoxvirus isolates based on ORF 103 nucleotides sequences. The tree was analyzed by N-J analysis with 1000 bootstrap replicates.

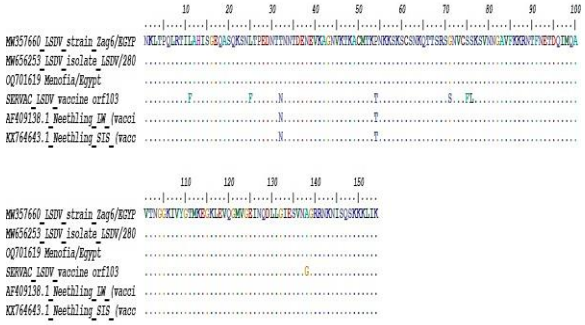

Fig 8 Differences in amino acid motifs between LSDV field and vaccine strains in ORF 103gene.

4. DISCUSSION

The LSDV virus is a highly infectious viral illness that causes enormous economic losses in the cattle sector. LSD has been found in Egypt, Israel, Iraq, Iran, Lebanon, and Jordan, among other African and Middle Eastern countries (Abdallah et al., 2018). Since 1988, when LSD was first officially registered in Ismailia province, Egypt has had sporadic LSD epidemics. The goal of this study was to compare the genetic relatedness of Menofia LSDV strain Egypt 2020, which caused a recent outbreak among cattle in Menofia governorates in Egypt during the summer of 2020 to other CaPVs whose sequences are available in GenBank and SERVAC LSDV Neethling Vaccine, Egypt. In this study, conventional PCR assay targeting the GPCR gene was used for confirmation the presence of LSDV in collected skin nodules from suspected clinical cases. The PCR assay findings verified the presence of LSDV in all skin nodule samples collected as previous research by (Zeynalova et al., 2016) that indicate the studied cutaneous nodules generated more positive results for LSDV molecular detection. One positive sample for GPCR and currently used SERVAC LSDV Neethling Vaccine, Egypt strain also conducted to conventional PCR assay targeting another two genes ORF 95 and ORF 103 which also confirm presence of LSDV. The GPCR, ORF 95, and ORF 103 genes were used in a phylogenetic analysis of LSDV to evaluate the phenetic connection between Menofia LSDV strain Egypt 2020 and other Capripoxviruses whose sequences were acquired from GenBank. Phylogenetic analysis separated LSDV wild strains and LSDV vaccination strains into distinct clades within the Capripoxvirus family. Sheeppox virus strains and goatpox virus strains also clustered in separate clades. Phylogenetic analysis further showed that Menofia LSDV strain Egypt 2020 under study grouped with LSDV from Africa including Egyptian strains and isolates, Asian and Europe with homology percentage ranging from 97% to 100% and few or no amino acids substitutions indicating that the same LSDVs are responsible for outbreaks across borders and demonstrating LSDVs' remarkable stability as previously reported (Kara et al., 2003) The sequences obtained from the GPCR ,ORF 95 and ORF 103 gene amplicons for Menofia LSDV strain Egypt 2020 and SERVAC LSDV Neethling Vaccine, Egypt were translated into corresponding amino acid sequence and show four amino acids deletion at positions 14–17 in the GPCR gene of Menofia LSDV strain Egypt 2020 while no such deletion was observed in the SERVAC LSDV Neethling Vaccine, Egypt, this results agree with previous report (Rouby et al., 2019). Also, we noted some amino acids substitution between Menofia LSDV strain Egypt 2020 and SERVAC LSDV Neethling Vaccine, Egypt as follow five (V/G1, T/N7, A/N9, S/N 61 and M/I112),two (E/D84 and S/A128) and eight (L/F11, L/F25, T/N32, P/T54, G/S71, S/F75, S/L76 and A/G138) amino acids substitutions for GPCR, ORF 95 and ORF 103 respectively. inferences for Egypt are that the current LSDV strain producing outbreaks is genetically distinct from the LSDV in the country's Neethling immunizations. So that, additional diagnostic testing, as well as the sequencing of multiple LSDV genes are required to confirm the strain differences between the vaccination and the wild type of virus, as this study compared only a three partial gene sequences this finding agreed with previous obtained results (Ochwo et al., 2020). Also, A differential diagnostic approach based on this sequence difference between vaccine and wild type virus can potentially be developed and this can be used as a tool to monitor immunization (Agianniotaki et al., 2017).

Vaccination is stated to be the most effective method of LSDV control, and it can be performed using attenuated LSDV, sheeppox, and goatpox viruses (Klement et al., 2020) and recent studies have advised the use of homologous LSDV vaccines prepared from recently isolated Neethling virus for the control of LSDV illnesses, in conjunction with adequate immunization coverage and appropriate control methods. (Tuppurainen and Galon, 2016). However, before mass vaccination can be implemented in Egypt, the efficacy of the presently approved LSDV vaccines must be evaluated and tested in the field.

5. CONCLUSION

Based on clinical observations, sequencing, and phylogenetic analyses of LSDV strains, the current study establishes the presence of LSDV among cattle in Egypt. Furthermore, we find that newly virus isolated from last LSD outbreak in Menofia differed from vaccinal strain based on three gene sequence comparisons. To fully understand the genetic epidemiology of LSD in Egypt, more characterization utilizing whole-genome sequencing is required.

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