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PHYLOGENETIC ANALYSIS AND SEQUENCING OF ISOLATED LUMPY SKIN DISEASE VIRUS (LSDV) NEETHLING STRAIN FROM VACCINATED ANIMALS

HANAFY M MADBOULY [1]; SABRY M TAMAM [1]; HUSSEIN A.S. [1]; HOSEIN I HOSEIN [2] AND LUTFI M BAKAR [3]

[1] Department of Virology, Faculty of Veterinary Medicine Beni-Suef University, Egypt
[2] Department of Animal Medicine, Faculty of Veterinary Medicine Beni-Suef University, Egypt
[3] Faculty of Veterinary Medicine, EL-Zawia University, Libya

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ABSTRACT

During 2017-2018, successive outbreaks of LSDV in cattle vaccinated with sheep pox virus (SPPV) vaccine were confirmed in Beni Suef Governorate, Egypt. Nucleotides and amino acids sequencing of the protein receptor gene (GPCR), homogeneity ratio between the Egyptian strain isolated in 2018, and the phylogenic tree of this strain with previous Egyptian , African, Asian, and European isolates of Capripox viruses has been done. The target sequence of this virus was submitted to the gene bank under the name (LSDV/ Egy-BSU / 2018) and with the accession number (MK552139.1). All Egyptian strains from 2014 to 2018 are homogeneous by 98-100%, while the homogeneity ratio with sheep pox or goat pox is within 92%. This Egyptian LSDV isolated during 2018 contains deleted amino acids in locus (30-33), but it does not deleted in the original strains from Kenya (LSD NI-2490 (AF325528.1) and (LSDV/Kenyan/SGP/O-240 (KJ818281.1). The deletion of these amino acids residues (30-33) may be due to the emergence of a recombinant strain of circulating field strains and the prototype strain of the live attenuated virus vaccine used. Therefore, it has become urgent to use the recently isolated specific Neethling strain of LSDV to prepare inactivated vaccines for controlling LSD and avoid using any other Capripox viruses' live attenuated vaccines due to the natural occurrence of genetic recombination among these viruses. We recommend the use of inactivated vaccines prepared from modern homogeneous strains for their specific hosts, to avoid: (1) virus shedding, (2) return to virulence, and (3) the emergence of recombinant vaccine strains. To provide sustained elevated antibodies and activate cellular immunity, each of the inactivated Capripox vaccines must be adjuvanted with nonspecific natural immune like *Nigella Sativa* oil. The most important issue, live Caprybox virus attenuated vaccines must be banned to avoid the reappearance of the disease in vaccinated animals.

Keywords: LSDV - nucleotides - amino acids - sequence - lineage – identity

INTRODUCTION

Lumpy skin disease virus (LSDV) of cattle, *sheeppox* virus (SPPV) and *goatpox* virus (GTPV) are members of genus Capripoxvirus, of family *Poxviridae* (Buller *et al.,* 2005). Members of this genus have double-stranded DNA genomes, with nucleotide sequence identities of 92% between them. Most SPPV and GTPV genes are present in LSDV (Tulman *et al.,* 2001). Both SPPV and GTPV have nine genes related to the virulence and host range functions. Otherwise, a gene unique to LSDV (LSDV132) and genes similar to those coding for interleukin-1 receptor, of *myxomavirus* and *vacciniavirus*, with the absence of these genes in SPPV and GTPV suggesting a significant role for them in the bovine host range (Tulman *et al.,* 2002).

Corresponding author: Hussein A.S.
E-mail address: ahmedrahini@yahoo.com*Present address:* Dept. of Virology, Faculty of Veterinary Medicine Beni-Suef University, Egypt

The LSD is widely spread in most countries of Africa and the Middle East, including (Egypt, Lebanon, Jordan, occupied Palastein), in northern Asia (Iran, Iraq, Turkey) and Central Asia (Azerbaijan). The disease was also reported in Cyprus, Greece, and the Russian Federation (FAO 2015).

In Egypt, the LSD first appeared in quarantine station in Suez Governorate 1988, with cattle imported from Somalia, then spread to other Egyptian Governorates, leading to severe economic losses (House *et al.,* 1990). The disease reappeared in 1995, 1998 and 1999 (Fahmy, 2000 and Abdel-Rahim *et al.,* 2002). Further outbreaks were reported in 2005 and 2006.

Laboratory diagnosis of LSD can be performed using serological and molecular techniques (group specific gene of a CaPV), G-protein-coupled chemokine receptor (GPCR), gene product 554 bps, and RNA polymerase 30 kDa subunit specific for LSDV (RPO30) amplified the RP030 gene product 172 bps from the extracted DNA of the isolated LSDV; virus isolation in cell cultures; and transmission electron microscopy [OIE, (2010); Stubbs, *et al.* (2012); Haegeman, *et al.* (2015); Tuppurainen (2005) and Balinsky, *et al.* (2008)].

In Egypt, two types of live attenuated vaccines, a Kenyan *sheeppox* vaccine (Michael *et al.,* 1994), and the Neethling strain that isolated by (Daoud *et al.,* 1998 and Abdelwahab, *et al.* 2016) were used for protection of cattle against LSD. The Romanian strain of sheeppox vaccine (Davies, 1991) that used before was discontinued since 2021. The appearance of the disease in cattle vaccinated with sheeppox, goatpox or Neethling strains is questionable?!

On the other hand, inactivated LSDV vaccines were prepared and evaluated (Saber *et al.,* (2000) and El-Desawy (2001) and produced good protection against LSD, but was not used also. Madbouly *et al.,* (2002) employed seven months old Frisian calves for vaccination with different programs for protection against LSDV. They noticed that the inactivated LSD of Ismailia (Neethling strain) vaccine adjuvanted with *Nigella sativa* oil (nonspecific immune stimulant), was safe and provided higher antibody titers and activated the cell mediated immunity. Therefore, to offer good protection against LSDV without virus shedding, they recommended using of the inactivated LSDV vaccine prepared from the recent strain adjuvanted with Nigella Sativa oil.

During (2017-2018), successive outbreaks of LSDV, as well as sporadic cases were confirmed in cattle vaccinated with sheep poxvirus vaccine (SPPV) in Beni-Suef Governorate. The affected animals showed typical signs of LSD with losses of cattles that had severe signs. The present study was planned to investigate the identity of the current circulating LSDV strain with the phylogenetic relatedness of reference Egyptian isolates and other regional isolates.

MATERIALS AND METHODS

Animal samples: Skin nodules and scabs were collected from twenty clinically suspected cattle for LSD, located at Beni-Suef Governorate, Egypt. These samples were collected in sterile containers containing sterile phosphate buffer saline (pH 7.4) and also on 50% glycerol saline from the skin nodules of the infected animals. Then preserved in deep freeze – 20^0C, prepared for virus isolation, and were inoculated on chorio-allantoic membrane (CAMs) of embryonated chicken eggs (ECE)**.** This study was conducted before the formation of the Professional Ethics Committee at Beni Suef University at February 2022.

Virus strains: Tissue culture-adapted Neethling strain and SPPV were obtained from Veterinary Serum and Vaccine

Research Institute (VSVRI), Abbassia, Cairo, Egypt. The vaccine vial (contained 100 doses) has a titer of $10^{4.5}$ $TCID_{50}$/ml in Vero cells. Both strains were supplied in lyophilized state; and used as a positive control in PCR.

Gene-specific primers: Two types of paired primers were designed by Invitrogen, Analysis for Life Technologies (Cairo, Egypt) and used in PCR technique. The first primer was used to amplify the specific G-protein-coupled chemokine receptor (GPCR) gene product 554 bps, and the second primer RNA polymerase 30 kDa subunit specific for LSDV (RPO30) amplified the RP030 gene product 172 bps from the extracted DNA of the isolated LSDV.

Table 1: Gene-specific sequence primers for PCR

primer	Gene-specific sequence primers	amplify LSDV genes	Reference
Pair 1: GPCR	LSD F 5' AGT ACA GTT AGT AGC GCA ACC-3' LSD R 5' GGG TGA ACT ACA GCT AGG TAT C- 3'	G-protein CRG* 554 bp.	**Le Goff *et al.* (2009)**
Pair2: RPO30	LSD F5'TCTATGTCTTGATA TGTGGTGGTAG3' LSD R5'AGTGATTAGGtGGTG TATTATTTTCC-3'	172bp	**Lamien *et al.* (2011)**

*CRG: Coupled chemokine Receptor Gene

RESULTS

Virus isolation: the LSDV was isolated from the collected samples during 2018, on the CAM of embryonated chicken eggs (ECE), and the infected CAM were subjected to histopathological examination to ensure the intracytoplasmic inclusion bodies related to poxviruses (Fig. 1 a, b). The isolated virus was characterized by PCR and revealed LSDV (Figure 2).

Primers specific for GPCR gene amplified a 554 bp product from LSDV genome confirming positivity of the sample for, presence of LSD viral genome (Fig. 2), while primers set targeting RP030 gene amplified a 172 bp product from LSDV genome, confirming positivity of the sample for presence of LSD viral genome. Tissue culture adapted SPPV vaccinal strain amplified a (152 bp), which was easily distinguishable relative to the LSDV amplicons (Fig. 2).

Partial Sequencing of (GPCR) gene:
Partial nucleotide sequencing of (GPCR) gene for LSDV isolated during 2018 was carried out; and compared with other selected Egyptian strains, African strains, and Asian strains as depicted in Table (2). The amplified target genes (GPCR) amplified a 554 bps and RP030 gene amplified a 172 bps product of the extracted LSD viral genome, confirming positivity of the tested CAM pooled samples. Tissue culture adapted SPPV vaccine strain amplified a (152 bps) was easily distinguishable relative to the LSDV amplicons. The target sequence was submitted to GenBank (LSDV/Egy-BSU/2018) under accession number of (MK552139.1).

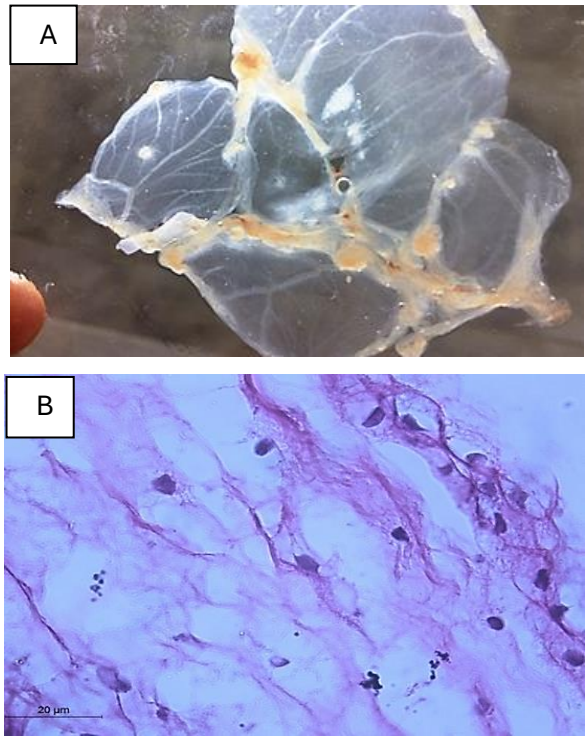

Fig. (1) A: Characteristic pock lesions of infected CAM of ECE. B: Stained inoculated CAM with suspected LSD viral samples showing eosinophilic intracytoplasmic inclusions (H and E 100X, oil emersion lens).

Deduced nucleotide and amino acid sequence revealed a deletion of 12-nucleotides (ORF position 88-99) that led to absence of amino acid residues at positions 30-33. All LSDV isolated from cattle used in sequence analysis support this deletion, except LSD NI-2490 isolate (AF325528.1) & LSDV/Kenyan/SGP/O-240 (KJ818281.1) do not possess these amino acids deletion. Multiple sequence alignment showed that isolated LSDV/Egy-BSU/2018 differ from LSDV isolated from Sharqia/Egypt (MF156211.1) and LSDV isolated from Beni-Suef/Egypt (KJ561442.1) during 2014 in one silent mutation (A111T) and one non-silent mutation (G86A) that lead to amino acid substitution (S29N) (Serine into Asparagine), while LSDV isolated from Mansoura/Egypt/2011 (KP071936.1) differs in two silent mutation (A156G) and (T246C) and two non-silent mutation (A82T) and (T247C) that led to amino acid substitution (I28F) (Isoleucine into Phenylalanine) and (C83R) (Cysteine into Arginine) respectively. Similarly, an amino acid substitution was observed in isolated LSDV/Egy/BSU/MEVAC/2016 (MH427386.1) at position (T59S) (Threonine into Serine) and in isolate LSDV/Sharqia/Egypt/2016 (MG970343.1) at position (C83R) (Cysteine into Arginine).

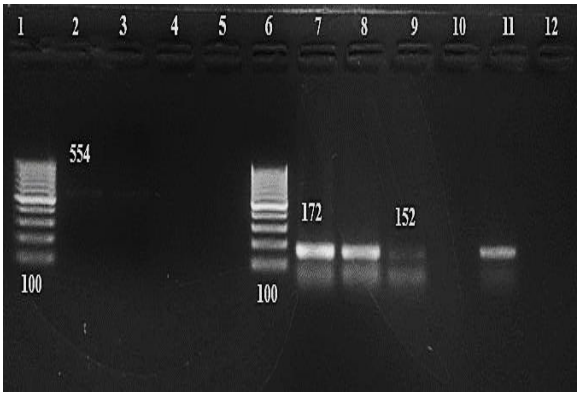

Fig. 2: Gel electrophoresis of PCR products using two LSDV specific primer sets. Lane 1 and 6: 100 bp DNA ladder, Lane 2 and 3: LSDV suspected samples (554 bp), Lane 4 and 5: Negative samples, Lane 7, 8 and 11: LSDV suspected sample (172 bp), Lane 9: Sheep pox vaccine (152), Lane 10: Control negative sample, Lane 11: Control positive sample.

Nucleotide and amino acid identities of GPCR gene:

This isolated LSDV shared 100 % nucleotide and amino acid identities with each of the following isolates: 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/ Greece/ 2015 (KY829023.3); LSDV/Egypt/BSU/MEVAC/2015(MH427384.1) as in Table (3).

Table 2: Data of selected GPCR gene sequences used in comparative analysis

Virus isolate	GenBank accession No.	Year of Isolation	Related 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-Suef
LSDV/Egypt/BSU-2	KJ561443.1	2014	Egypt, Beni-Suef
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	١٩٨٧	Kenya
LSDV/Egypt/2016-01	MG970343.1	2016	Egypt, Sharqia

In comparison of the currently isolated LSDV to the previously Egyptian strains, LSDV/Egypt/ Sharqia /2014 (MF156211.1) and LSDV/Egypt/ Beni-Suef -1/2014 (KJ561442.1), it shared 99.6% nucleotide and 99.4% amino acid identities (Table3). However, nucleotide and amino acids identities were found to be 99.2%, 98.7%, respectively, with LSDV/Egypt/Mansoura/2011 (KP071-936.1). On comparing this isolated strain with goatpox viruses isolated from China 2014 (China strain: KY020783.1_ Sheeppox_virus), it showed nucleotides identity 92.8% and amino acids identity 92.1%. ؛ with those isolated from Turkey (FJ869388.1_Sheeppox_virus_ Turkey/98) it showed nucleotides identity 92.6% and amino acids identity 91.5%; with (FJ869362.1_Goatpox_virus_Yemen/83) that was isolated from Yamen; and with (FJ869390.1_Sheeppox_virus_Oman/84_isolate) it showed nucleotides identity 92.8% and amino acids identity 92.1% that was isolated from Oman 1984 (Table 3).

Regarding lumpy skin disease virus strain (AF325528.1_LSD_NI-2490 LSDV 1958 Kenya Wild type) isolated from cattle in 1958 nucleotide and amino acids identities was found to be 97.5% with this isolated LSDV 2018 (Table 3). On the other hand, the identity to the available sheep and goat pox sequences was observed (91.5%: 93% nucleotide and amino acid identity) respectively (Table 3), and this denotes the heterogeneity between this strain that far from the LSDV strains.

Table 3: Nucleotide and amino acid identities of G-protein-coupled chemokine receptor gene of Egyptian LSDV strain compared with selected sequences

Amino acid identities																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	AF325528.1_LSD_NI-2490 LSDV 1958 Kenya Wild type		97.5	97.5	97.5	97.5	96.9	96.3	96.9	97.5	97.5	97.5	97.5	94.1	94.1	94.1	94.7	94.1	93.4	93.4	93.4	94.1	
2	AF409137.1LSDV_NW-LW_isolate	97.5		100	100	100	99.4	98.7	99.4	100	100	100	100	92.1	92.1	92.1	92.8	92.1	91.5	91.5	91.5	91.5	92.1
3	FJ869369.1LSDV_Sudan/06_Obied	97.5	100		100	100	99.4	98.7	99.4	100	100	100	100	92.1	92.1	92.1	92.8	92.1	91.5	91.5	91.5	91.5	92.1
4	KP663691.1_LSDV/Ethiopia/2011	97.5	100	100		100	99.4	98.7	99.4	100	100	100	100	92.1	92.1	92.1	92.8	92.1	91.5	91.5	91.5	91.5	92.1
5	MK552139.1 LSDV/Egy-BSU/2018	97.5	100	100	100		99.4	98.7	99.4	100	100	100	100	92.1	92.1	92.1	92.8	92.1	91.5	91.5	91.5	91.5	92.1
6	MF156211.1_LSDV_Egypt_VRLCU_2014.	97.1	99.6	99.6	99.6	99.6		98.1	100	99.4	99.4	99.4	99.6	91.5	91.5	91.5	92.1	91.5	90.8	90.8	90.8	90.8	91.5
7	KP071936.1LSDV_Egy_2011	96.7	99.2	99.2	99.2	99.2	98.7		98.1	98.7	98.7	98.7	99.2	90.8	90.8	90.8	91.5	90.8	90.1	90.1	90.1	90.1	90.8
8	KJ561442.1_LSDV/Egypt/BSU-1	97.1	99.6	99.6	99.6	99.6	100	98.7		99.4	99.4	99.4	99.6	91.5	91.5	91.5	92.1	91.5	90.8	90.8	90.8	90.8	91.5
9	KJ561443.1_LSDV/Egypt/BSU-2_	97.5	100	100	100	100	99.6	99.2	99.6		100	100	100	92.1	92.1	92.1	92.8	92.1	91.5	91.5	91.5	91.5	92.1
10	KY595106.1_LSDV/RNOA-15_.Russia	97.5	100	100	100	100	99.6	99.2	99.6	100		100	100	92.1	92.1	92.1	92.8	92.1	91.5	91.5	91.5	91.5	92.1
11	KY829023.3__LSDV/Evros/GR/15	97.5	100	100	100	100	99.6	99.2	99.6	100	100		100	92.1	92.1	92.1	92.8	92.1	91.5	91.5	91.5	91.5	92.1
12	MH427384.1LSDV_Egy-BSU/MEVAC/2015	97.5	100	100	100	100	99.6	99.2	99.6	100	100	100		92.1	92.1	92.1	92.8	92.1	91.5	91.5	91.5	91.5	92.1
13	AY077836.1_Goatpox_virus_G20-LKV	95.2	93.0	93.0	93.0	93.0	92.6	92.6	92.6	93.0	93.0	93.0	93.0		100	100	96.1	100	92.8	92.8	92.8	92.8	100
14	AY077835.1_Goatpox_virus_Pellor	95.2	93.0	93.0	93.0	93.0	92.6	92.6	92.6	93.0	93.0	93.0	93.0	100		100	96.1	100	92.8	92.8	92.8	92.8	100
15	KX576657.1_Goatpox_virus_strain_Gorgan	95.2	93.0	93.0	93.0	93.0	92.6	92.6	92.6	93.0	93.0	93.0	93.0	100	100		96.1	100	92.8	92.8	92.8	92.8	100
16	FJ869362.1_Goatpox_virus_Yemen/83_isolate.	94.5	92.4	92.4	92.4	92.4	91.9	91.9	91.9	92.4	92.4	92.4	92.4	96.9	96.9	96.9		96.1	94.1	94.1	94.1	94.1	96.1
17	KY020783.1_Sheeppox_virus_china.	95.0	92.8	92.8	92.8	92.8	92.4	92.4	92.4	92.8	92.8	92.8	92.8	99.8	99.8	99.8	96.7		92.8	92.8	92.8	92.8	100
18	FJ869388.1_Sheeppox_virus_Turkey/98_	95.0	92.6	92.6	92.6	92.6	92.1	92.1	92.1	92.6	92.6	92.6	92.6	95.0	95.0	95.0	94.3	94.8		100	100	100	92.8
19	MG232393.1_Sheeppox_virus_strain-SA6/2016	95.0	92.6	92.6	92.6	92.6	92.1	92.1	92.1	92.6	92.6	92.6	92.6	95.0	95.0	95.0	94.3	94.8	100		100	100	92.8
20	KY020783.1_Sheeppox_virus_.	95.0	92.6	92.6	92.6	92.6	92.1	92.1	92.1	92.6	92.6	92.6	92.6	95.0	95.0	95.0	94.3	94.8	100	100		100	92.8
21	FJ869391.1_Sheeppox_virus_HSL_isolate.	95.0	92.6	92.6	92.6	92.6	92.1	92.1	92.1	92.6	92.6	92.6	92.6	95.0	95.0	95.0	94.3	94.8	100	100	100		92.8
22	FJ869390.1_Sheeppox_virus_Oman/84_isolate.	95.0	92.8	92.8	92.8	92.8	92.4	92.4	92.4	92.8	92.8	92.8	92.8	99.8	99.8	99.8	96.7	100	94.8	94.8	94.8	94.8	
Nucleotides identities																							

Phylogenetic analysis

Phylogenetic analysis of 45 members of *Capripoxvirus* was delineated into three species-specific distinct genetic clusters consisting of LSDV; GTPV and SPPV based on the GPCR gene. The goatpox viruses isolated during the 21st century were nearly related to LSDV, and both lineage shows noticeable diversity from SPPV lineage. The strains of LSDVs fall into two subgroups; the first one includes (LSDV/ Egy-BSU/2018, (MK552139). that 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/2 015(KY829023.3) LSDV/ Egypt/BSU/ MEVAC/2015 (MH427384.1); LSDV/ Russia/ Dagestan/2015, (MH893760.2); LSDV/SERBIA/Bujanovac/2016(KY70200 7.1);LSDV/TURKEY/2014 (KR024774.1); LSDV/Kenyan/SGP/O-240 (KJ818281.1) and LSD/NI-2490 (AF325528.1). The isolated strains of LSDV/Samara/2017/ Russia (MH753583.1) and LSDV/Russia/ Saratov/2017 (MH646674.1) are clustered separately as a second subgroup (Fig.3)

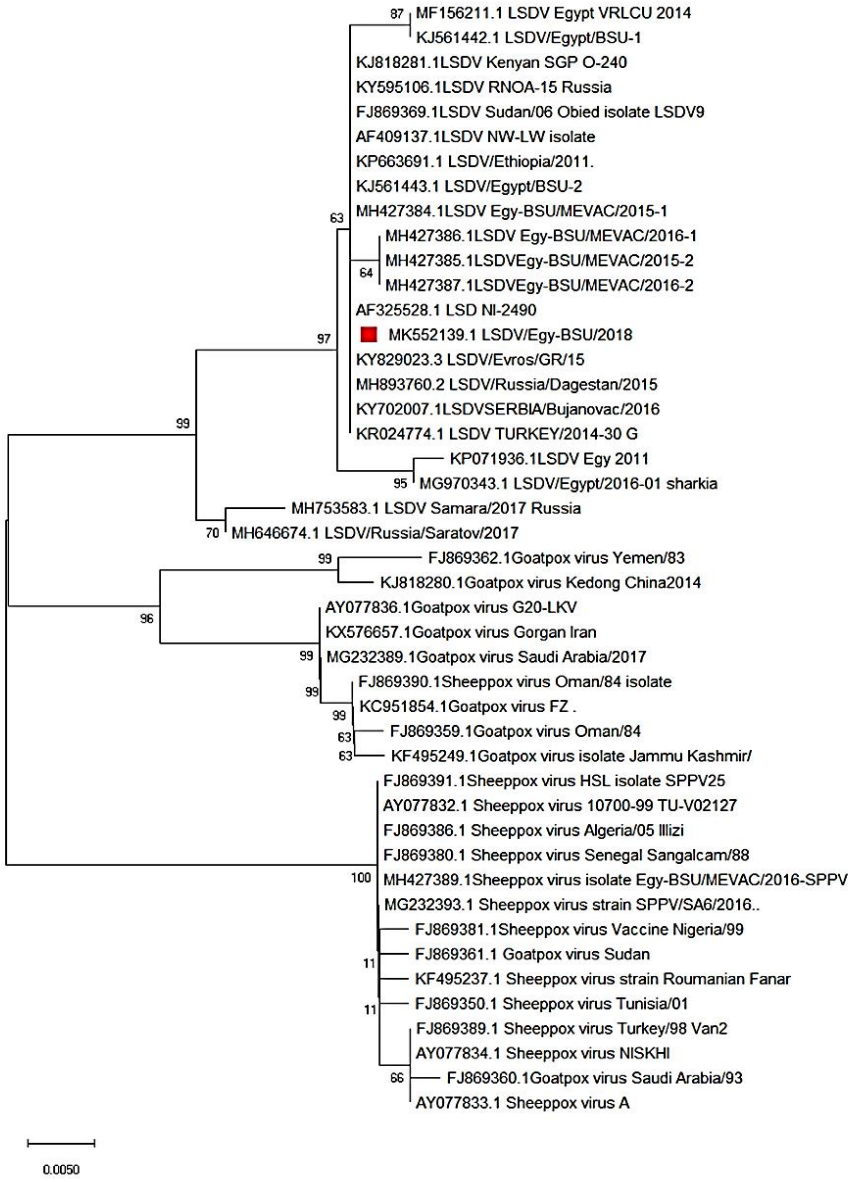

Fig 3: Phylogenetic analysis of GPCR gene of recent Egyptian. African. Asian and Europe strains of the genus Capripoxvirus

From Figure (3), it is very clear that, all LSDV strains were clustered in a definite clade (clade 3), while Goatpox virus strains clustered in (clade 2), and Sheepox viruses clustered in (clade 1). All LSDV strains that isolated from Egypt during the period between 2014-2018 are clustered in clade-3 and showed great similarities. Goatpox viruses were mainly isolated from Asian countries (Kashmir, India, Oman, Yemen, China, Russia and Saudi Arabia) were clustered in clade - 2. Otherwise, Sheeppox virus strains that were isolated mainly from African countries (Tunisia, Algeria, Sudan, Nigeria, Senegal and Egypt) were clustered in clade- 1, as shown in (Fig.3).

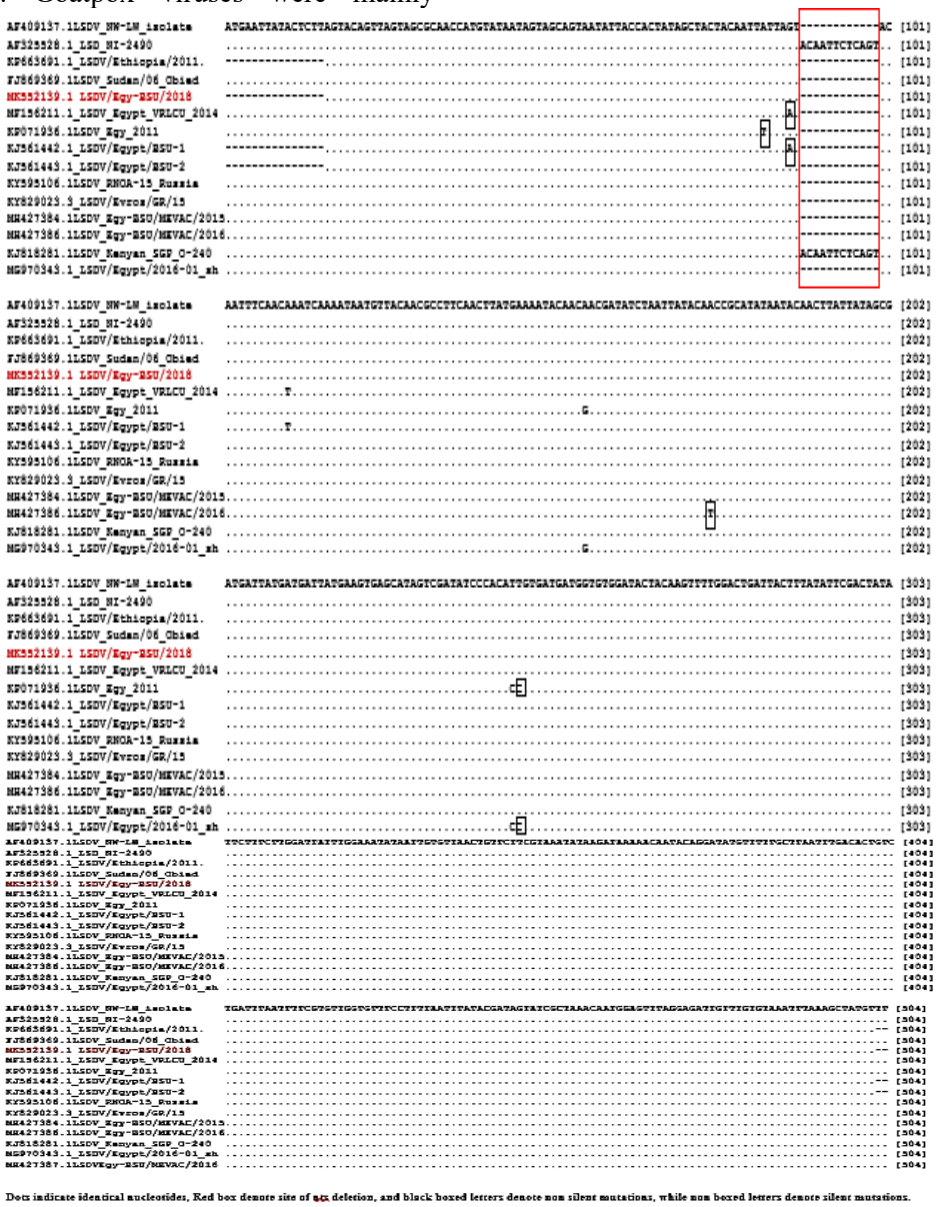

Fig (4): Deduced nucleotide sequence of G-protein-coupled chemokine receptor gene

From Fig. (4) it is very clear that all Egyptian strain isolated during period 2014 -2018 dots showed were identical.

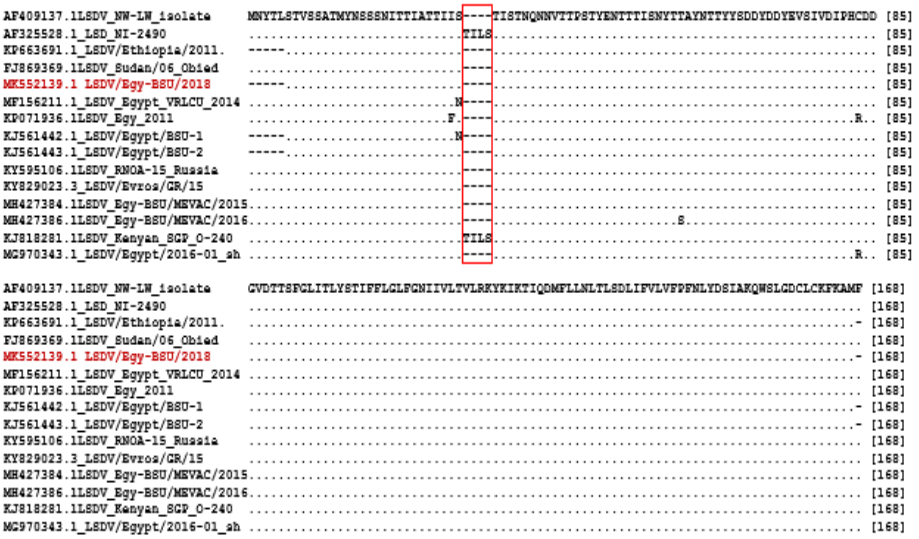

Fig. (5): Deduced amino acid sequence of G-protein-coupled chemokine receptor gene**.**

From Fig. (4) and (5) it is very clear that, the sequence of G-protein-coupled chemokine receptor gene informed deduced nucleotide and amino acid sequence revealed a deletion of 12-nucleotides (ORF position 88–99) that led to absence of amino acid residues at positions 30-33. All LSDV isolated from cattle used in sequence analysis support this deletion, except LSD NI-2490 isolate (AF325528.1) and LSDV/Kenyan/SGP/O-240 (KJ818281.1). They also informed one silent mutation (A111T) and one non-silent mutation (G86A). Otherwise, the nucleotide and amino acids identities with LSDV/Mansoura/Egypt/2011 KP071936.1) were 99.2%, 98.7%, respectively, and maybe 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), respectively (Figure 5).

DISCUSSION

In 1988, the first report of LSD in Egypt was declared, from where it spread in 1989 to the Middle East (House *et al.,* 1990). During the period 2014–2018 Intercontinental transmission occurred again, and it was expanded into Greece; (Tasioudi, 2016); Eastern Europe and Russia (Saltykov, YV, *et al.,* 2021)); India (Sudhakar *et al.,* 2019) and Egypt. The isolated strains revealed close proximity with unique signatures of historical Kenyan NI-2490/ Kenya/KSGP-like field strains. The disease was reported in China (Lu *et al.,* 2021) and neighboring countries of India and Vietnam during 2019–2020 (Mathijs *et al.,* 2021). Live attenuated LSD vaccines with the strain of the "Neethling" as a prototype has been used in Africa for over 50 years and are now widely used in most affected countries (Calistri *et al.,* 2019 and Hunter *et al.,* 2001). A novel LSDV strain, known as Saratov/2017, from diseased cattle in Southern Russia in 2017 revealed similarities with the Neethling and KSGPO-like vaccine strains (Sprygin *et al.,* 2018).

In this study, the isolated LSDV strain was sequenced and submitted to GenBank with their designation as; (LSDV/ Egy-BSU/2018) under accession number of (MK552139). Phylogenetic tree grouped viruses of genus Capripoxvirus into LSD, sheeppox and goatpox viruses separately. These Capripoxvirus strains in our resources were related to each other with low special deviation from each of them (Fig. 3).

The GPCR gene nucleotide and their deduced amino acid sequences of LSDV were analyzed and compared with respective reference sequences obtained from the Gene Bank (Fig. 4 and 5). Multiple sequence alignment showed that, the recent Egyptian LSDV isolate (MK552139.1 LSDV/Egy-BSU/2018) is closely related 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) and LSDV/ Egypt/ BSU/MEVAC/2015 (MH427384.1) with 100% nucleotide and amino acids identities (Fig. 3,4,5 and Table 3). The currently strain of LSDV (MK552139.1 LSDV/Egy-BSU/2018) is most related to strains (MF156211.1_LSDV_Egypt_VRLCU and to KJ561442.1_LSDV/Egypt/BSU-1) that were isolated during 2014; to (KJ561443.1_LSDV/Egypt/BSU-2) that was isolated 2015; and to the MEVAC strains that were isolated during 2015 and 2016 (MH427384.1LSDV_Egy-BSU/MEVAC/2015-1, MH427385.1LSDV_Egy-BSU/MEVAC/2015-2, MH427386.1LSDV_Egy-BSU/MEVAC/2016-1, and MH427387.1LSDV_Egy-BSU/MEVAC/2016-2). All these strains clustered with LSDV strain isolated from Kenya (KJ818281.1LSDV Kenyan SGP 0-240/87; J869369.1LSDV_Sudan/06_Obied; KP663691.1_LSDV/Ethiopia/2011; KY595106.1_LSDV/RNOA-15_Russia. All these strains showed nucleotides identity ranged from (99.6-100%) and amino acids identity ranged from (99.4-100%) (Table 4). Data depicted in Fig. (3) showed that the isolated LSDV strains during 2014 -2018 are closely related to each others and also related to the LSDV strain isolated from Kenya 1987; to LSDV_Sudan/ 06_Obied; LSDV/ Ethiopia/2011;_LSDV/RNOA-15_. Russia), and this denotes the genetic stability of LSDV strains with minor variation.

Our sequenced LSDV 2018 strain showed nucleotide and amino acids identities 99.6%, 99.4%, respectively, with LSDV/ Egypt/ Sharqia/2014 (MF156211.1) and LSDV/Egypt/Beni-Suef-1/2014 (KJ561442.1) (Table 3) with the presence of one silent mutation (A111T) and one non-silent mutation (G86A) that led to amino acid substitution (S29N-Serine into Asparagine). Otherwise, the nucleotide and amino acids identities with LSDV/ Mansoura/Egypt/2011(KP071936.1) were 99.2%, 98.7%, respectively (Table 3), and may be 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), respectively (Figure 5).

Deduced amino acids sequence analyses revealed that LSDV/Egy BSU/2018 isolate contains an amino acid deletion located at positions 30-33 (Fig. 5). The absence of amino acid residues at positions 30–33 was observed exclusively, but not systematically in cattle isolates located in the database. LSD NI-2490 isolate (AF325528.1) and LSDV/Kenyan/SGP/O-240 (KJ818281.1) do not possess this amino acid deletion even with (97.5% nucleotide and amino acids identities). The overall identities of this gene and the encoded amino acids ranged from 91.5% to 93% with the available sheep and goat pox sequences (Table 4).

To study the relationship between the resources of Capripox viruses, a representative phylogenetic analysis using the neighbor-joining method was performed on nucleic acid sequences. The phylogenetic tree grouped viruses of LSD,

sheeppox and goatpox viruses separately (Fig. 3). Goatpox viruses were more related to LSDV than the Sheeppox viruses, as proposed by researchers who carried out phylogenetic studies on different genome segments (Hosamani *et al.,* 2004 for (P32 gene) and Rouby and Aboulsoud, (2016) for two hypothetical proteins: (LSDV001 and LSDV002). The LSDV and the GTPVs lineages showed the nearest diversity than for the SPPV group (Le Goff *et al.* (2005).

Our 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 Kara *et al.* (2003), who stated that lumpy skin disease viruses are genetically stable. In spite, these genetic stability between Capripox viruses, revealed the appearance of three clads of SPPV, GTPV and LSDV with specific two subgroups of LSDVs clusters that indicated host species-specific groups (Fig. 3), and these results denote a specific consideration when targeted for LSDV classification, and or as a candidate for selection of vaccine strain for controlling LSD. These currently obtained results denotes that either Sheeppox viruses or Goatpox viruses are poorly recommended to use for vaccinating cattle against lumpy skin disease virus. The deleted amino acid at positions 30-33 (Fig. 5). in the recent Egyptian LSDV isolate 2018 did not find in LSD NI-2490 isolate (AF325528.1) and LSDV/Kenyan/SGP/O-240 (KJ818281.1). These deleted 12 nucleotides and their resamples amino acids position (30-33) may be explained as laboratory synthetic mutant for vaccine production; or by escaped mutant due to using of Kenyan strain as a vaccine strain against LSDV during this time; or emergence of recombinant vaccine strain from the circulated field strains and live attenuated used vaccine prototype strain.

In Egypt, before 2021, the massive vaccination campaigns based on the use of a heterologous vaccine (sheeppox or goatpox vaccines), provided insufficient levels of protection. The appearance of the disease in cattle vaccinated with these heterologous vaccines becomes questionable? Although the prepared live attenuated vaccine by using Neethling strain (Daoud *et al.,* 1998) was not used as a massive production vaccine for protection of cattle against LSD until that time. The strain of sheeppox vaccine prepared by Michael *et al.* (199٤) that had been emergency used during the first LSDV outbreaks 1988-1989 was still used until 2020, and then was discontinued and replaced by live attenuated Neethling prototype strain vaccine since 2021. In China 2020 a new isolated LSDV strain named LSDV/NMG/ 2020, with a genomic characterization and phylogenetic analysis demonstrated that the LSDV/NMG/2020 isolate was a vaccine-like recombinant strain (Xiaohui Zan *et al.,* 2022).

In our opinion, the main problem with the emergence of LSDV strains in vaccinated animals and its expansion in-between the Africa, Asia and Europe intercontinental is occurred by using the live attenuated vaccines either as heterogeneous vaccines (sheepox, goatpox) or homogenous for vaccinating cattle against LSDV. In our spot light, the phenomenon of recombination within the family Poxviridae (Aleksandr *et al.,* 2020), should be assigned in our consideration when we look about the selection of vaccine strain of LSDV.

On the other hand, two studies by (Saber *et al.* (2000) and Madbouly *et al.* (2002) preferred and recommended the use of the inactivated LSDV vaccine prepared from LSDV Neethling strain. Madbouly *et al.* (2002) concluded that the use of natural immunostimulant adjuvant that activate the cellular immunity non-specifically, like *Nigella sativa* oil, instead of any mineral oil become essential and urgent to avoid

granuloma formation and to activate the cellular immunity, that is poor with using mineral oils adjuvants, and to substitute the advantage of live attenuated vaccine in triggering the cell mediated immunity.

Recommendation: we recommend using the inactivated vaccines against any type of Capripox viruses strains: sheepox vaccines to vaccinate sheep against sheeppox viruses, goatpox vaccines for vaccinating goats against goatpox viruses and inactivated LSDV vaccines against LSD. These inactiv-ated Capripox vaccines should be adjuvanted with natural non-specific immunostimulant. All these inactivated vaccines of Capripox viruses must be prepared from the recent isolated homogeneous strains of their specific hosts, to avoid: (1) virus shedding, (2) returns to virulence, and (3) emergence of recombinant vaccine strains.

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تحليل النشوء والتسلسل الجيني لسلالة NEETHLING لفيروس مرض الجلد العقدي (LSDV)المعزولة من الحيوانات المحصنة

حنفي محمود مدبولى[١]، صبري محمد تمام [١]، أحمد سعد حسين [١]، حسين إبراهيم حسين[٢]، لطفي محمد بكار[٣]

[١]قسم الفيرولوجيا - كلية الطب البيطري – جامعة بني سويف- مصر
[٢]قسم طب الحيوان - كلية الطب البيطري – جامعة بني سويف- مصر
[٣] كلية الطب البيطري جامعة الزاوية - ليبيا
Email: ahmedrahini@yahoo.com Assiut University web-site: www.aun.edu.eg

خلال (٢٠١٧-٢٠١٨) ، تم تأكيد تكرار تفشي فيروس الجلد العقدي LSDV في الماشية المحصنة بلقاح فيروس جدري الأغنام (SPPV) في محافظة بني سويف ، مصر. وقد ظهرت عليها علامات مرض الجلد العقدي (LSD).
تم إجراء تسلسل النيوكليوتيدات الجزيئي لجين مستقبلات البروتين (GPCR) والأحماض الأمينية التابعة له ، ونسبة التجانس بين السلالة المصرية المعزولة في ٢٠١٨ ، وتحديد شجرة العائلة لهذه السلالة مع السلالات المصرية السابقة ومع معزولات أخري أفريقية وأسيوية وأوروبية من فيروسات Capripox. كشفت نتائج تحليلات النشوء ومتعدد التسلسلات عن درجة عالية من التشابه بين عزلات LSDV من مواقع أخرى مع الحد الأدنى من التباين الجيني. تم تقديم التسلسل المستهدف لهذا الفيروس إلى بنك الجينات تحت مسمى (LSDV/ Egy-BSU / 2018) وبرقم الانضمام (MK552139) . كل العترات المصرية المعزولة في الفترة من ٢٠١٤-٢٠١٨ متجانسة بنسبة ٩٩ -١٠٠٪ ، بينما نسبة التجانس لهذه العترة المعزولة في ٢٠١٨ مع عترات جدري الأغنام أو جدري الماعز فهي في حدود ٩٢٪
تحتوي هذه المعزولة المصرية الحديثة LSDV خلال عام ٢٠١٨ على ١٢ نيوكليوتيد محذوفة والتي تمثل موضع الأحماض الأمينية (٣٠-٣٣). بينما كانت موجودة، ولم يتم حذفها في المعزولات الأصلية من معزولات كينيا LSD NI-2490 (AF325528.1) و LSDV / Kenyan / SGP / O-240 (KJ818281.1).
يمكن تفسير حذف هذه النيوكليوتيدات وما يتابعها من الأحماض الأمينية في مواقعها (٣٠-٣٣) على أنه متحولة معملية اصطناعية لإنتاج لقاح ضد هذا الفيروس؛ أو عن طريق الطفرة الهاربة بسبب الاستخدام المكثف للقاحات الحية المستضعفة ضد LSDV خلال هذا الوقت, أو ظهور سلالة لقاح مؤتلفة تتكون من سلالات ميدانية منتشرة وسلالة نموذجية للقاح الفيروس الحي المستضعف المستخدم. لذلك ، أصبح من الضروري والملح استخدام سلالة Neethling المحددة المعزولة مؤخرا من LSDV لإعداد لقاح معطل للسيطرة على LSD وتجنب استخدام أي لقاحات حية أخرى من فيروسات Capripox المستضعفة لتجنب الحدوث الطبيعي لإعادة التركيب الجيني بين هذه الفيروسات.
توصي الدراسة باستخدام اللقاحات المعطلة ضد أي نوع من سلالات فيروسات Capripox: لقاحات الغنم لتطعيم الأغنام ضد فيروسات جدري الأغنام ، ولقاحات جدري الماعز لتطعيم الماعز ضد فيروسات جدري الماعز، ولقاحات LSDV المعطلة ضد LSD. يجب تحضير كل هذه اللقاحات المعطلة لفيروسات Capripox من السلالات المتجانسة المعزولة الحديثة لمضيفيها المحددين ، لتجنب: (١) ذرف الفيروس ، (٢) العودة إلى الضراوة و (٣) ظهور سلالات اللقاح المؤتلفة.
ولتوفير أجسام مضادة مرتفعة مستدامة وتنشيط المناعة الخلوية ، يجب أن يكون كلٌ من لقاحات Capripox المعطلة مدعوما بمحفزات مناعية طبيعية مثل زيت حبة البركة. أخيرا وأهم قضية ، يجب حظر لقاحات فيروسات الكابريبوكس الحية الموهنة لتجنب ظهور المرض مرة أخرى في الحيوانات المحصنة.

الكلمات المفتاحية: LSDV - النيوكليوتيدات - الأحماض الأمينية - التسلسل - السلالة – الهوية