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Original Research Article

Serological and molecular characterization of recent lumpy skin disease virus isolates from naturally infected previously vaccinated cattle in Egypt

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ABSTRACT

lumpy skin disease virus (LSDV) was isolated, from naturally infected cattle that have a history of previous vaccination with live attenuated sheep pox virus (SPV) vaccine. The virus was isolated on chorio-allantoic membranes (CAM) of embryonated chicken eggs (ECE) and Madin Darby Bovine Kidney Cells (MDBK) and identified by agar gel precipitation test (AGPT) and immunofluorescent antibody technique (IFA). Characteristic pock lesions and intracytoplasmic fluorescence granules are identified respectively. Molecular characterization using polymerase chain reaction (PCR) using specific primer for G-Protein Coupled Chemokine Receptor Gene of LSDV isolates specific amplified product 554 bp. Sequence analysis revealed two new isolates of LSDV.

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Introduction

Lumpy skin disease (LSD) is an infectious, eruptive and occasionally fatal disease of cattle characterized by nodules formation in the skin, mucosal membranes, lymphadenopathy and occasionally death [1].

LSD is caused by the strain of capripoxvirus which is genetically and antigenically close related to the strain of sheep and goat pox virus and the prototype strain is known as the Neethling Pox virus [2].

The disease is listed in the office international des Epizooties List "A" which identifies diseases with the potential rapid spread and severe economic losses [3]

In Egypt, LSDV was first isolated and identified from cattle during two outbreaks in Suez and Ismalia governorates in 1989 [4]. The possible introduction of new strains of LSDV by the uninterrupted movement of animals across borders is a major constant threat. In early 2006, a severe LSD outbreak struck foreign (imported from Ethiopia) and local cattle in different Egyptian governorates, causing enormous economic losses. Many confusing arguments have arisen about this invasive LSDV that needed to be unraveled.

Control of LSDV in Egypt depends on prophylactic vaccination programs using live attenuated cell culture adapted sheep pox virus vaccine [5].

This work aims to clarify the reasons for appearance of LSDV in vaccinated cattle in Egypt.

Materials and Methods

Samples: intracutaneous skin nodules were collected by biopsy from naturally infected cattle suspected to be infected with LSDV infection (fig. 1) with a history of previous vaccination with live attenuated sheep pox virus (SPV) vaccine located at different areas in Beni-Suef Governorate. Collected samples were ground in sterile phosphate buffer saline (PBS) containing antibiotics. The obtained supernatant was stored at -20 until used.

Embryonated chicken egg (ECE): 11-13 days old ECE were purchased from El-Azzab poultry production farm, Fayoum, Egypt and used for virus propagation and titration. ECE was inoculated via CAM rout and incubated at 37° C and 70% humidity .

Tissue culture cells: Madin Darby Bovine Kidney (MDBK) cells were obtained from Virology Department, Animal Health Research Institute, Dokki, Giza used for virus propagation.

Sera and antiserar: reference lumpy skin disease antisera were obtained from Virology Department, Animal Health Research Institute, Dokki, Giza.

Virus isolation: LSDV was isolated from collected samples on chorioallantoic membrane

(CAM) of ECE according to House et al., [6]. The infectivity titer of isolated virus was determined and egg infective dose 50 (EID₅₀) were calculated according to Reed and Munch [7]. Also propagation on MDBK cells according to OIE [8].

Agar gel precipitation test (AGPT): it was applied according to Davies [9].

Indirect fluorescence antibody technique (IFA): was applied according to Walid et al., [10].

Molecular characterization:-

Extraction of viral DNA: VIVANTIS GF-1 Tissue DNA Extraction Kit is used for virus isolation from skin biopsies according to manufacturer instructions.

Primers: it were designed depending on G-Protein Coupled Chemokine Receptor Gene of LSDV and manufactured by Invetrogen company (United Kingdom) with the following sequences: forward primer 5'-AGT ACA GTT AGT AGC GCA ACC- 3' and reverse primer 5'-GGG TGA ACT ACA GCT AGG TAT C- 3'. The amplicon size of polymerase chain reaction is 554 bp.

Polymerase chain reaction (PCR): according to according to Alaa,A-ElKholy [11].

Analysis of PCR amplification products (amplicons): the resulting PCR amplicons were analyzed using 1.5% agarose according to viljoen et al., [12].

Gene sequence and Sequence analysis:

For sequencing, PCR-products were excised from gel and purified with WizardR SV gel and PCR clean up system according to the Manufacturer's Instructions then the DNA was dried and shipped for target gene sequencing.

Sequencing was performed by **Macrogen Inc.** (908 World Meridian Venture Center #60-24, Gasan-dong Geumchun-gu, Seoul 153-781, Korea) in both forward and reverse directions using the same primer sets that have been used for amplification of each gene.

- The received sequences were imported into alignment windows with the downloaded highly similar sequences into BIOEDIT version 7.0.4.1 software.

- Multiple sequence alignment was conducted using Clustal 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

Isolation of lumpy skin disease virus on CAM of ECE: three successive passages were done on CAMs of ECEs producing characteristic pock lesion fig. (2).



Fig.(1): intracutaneous skin nodules CAM.



Fig.(2) characteristic pock lesion on

Results of virus titration: shown in table.(1).

Passage No	1 st	2 nd	3 rd
Virus titer log 10	6.	6.	7.
EID50	2	7	6

Propagation of LSDV on tissue culture: clear cytopathic effects appear on MDBK cells in the form of cell rounding, clustering, aggregation and plaque formation fig.(3)

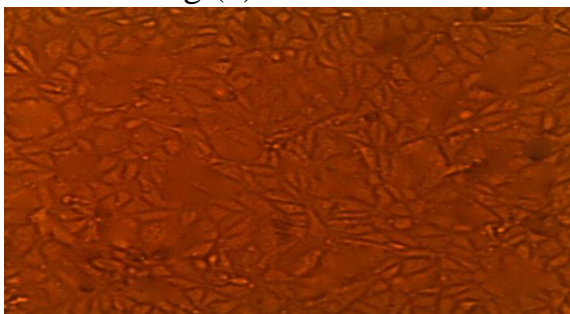


Fig.(3): CPE of LSDV on MDBK in the form of cell rounding, aggregations and cell detachment

Identification of isolated LSDV:

Technique	Results
AGPT	appearance of clear line of precipitation
IFA	Intracytoplasmic fluorescence (Fig.4)
Histopathology	Intracytoplasmic inclusion bodies (Fig 5)

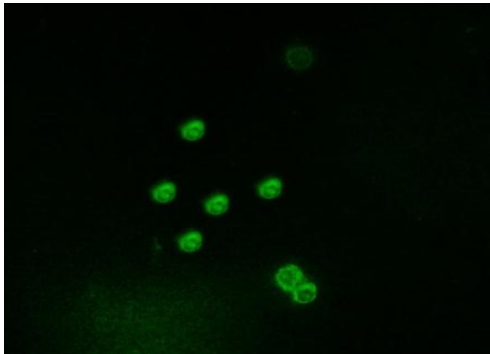
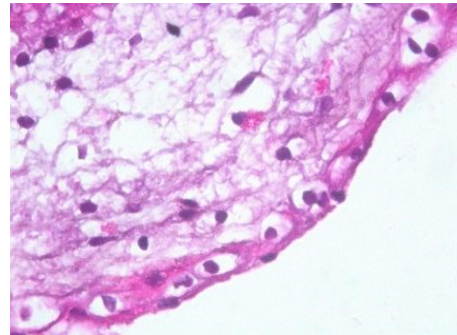


Fig.(4) The specific intracytoplasmic yellowish green fluorescent granules

molecular identification: The specific primers set amplified a DNA fragment of 554 bp equivalent to the expected amplification product (amplicon) size from LSDV. Subsequently, it was certain that these specimens contained DNA of LSDV fig.(6).

Sequence analysis: representative DNA amplicons (n = 2), resulted from specific direct PCR amplification, were purified and analyzed for their nucleotide sequences. Two new isolates named LSDV Egypt/BSU-1/2014 isolate and LSDV Egypt/BSU-



Fig(5) intracytoplasmic inclusion bodies

2/2014 isolate are identified with accession numbers KJ 561442 and KJ 561443 respectively on GenBank. Sequences in the alignment of the LSDV isolates were subjected to blast search versus the GenBank database. The closest sequences to the two new isolates of LSDVs were those of isolates/strains of LSDV, SPV and GPV. The phylogenetic tree produced confirmed the results obtained from both nucleotide sequence alignments and blast search as illustrated in figure (6).

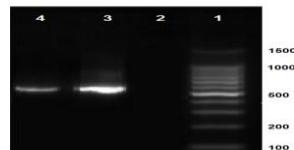


Fig.(6) Gel electrophoresis of PCR products using specific primer set (With 554 bp expected product size)

Lane 1: 100 bp DNA ladder, Lane 2: control negative sample,

Lanes 3-4: LSDV suspected samples.

Deduced amino acid sequence of GPCR gene of LSDV:

NC_003027 LSDV NI-2490/Kenya/58 ATGAATTATA CTCCTAGTAC AGTTAGTAGC GCAACCATGT ATAATAGTCAGTAATATT
ACCACTATAG CTACTACAAT TATTAGTACA [90]

FJ869377 LSDV Egypt/Ismailia/89 [90]
 KP071937 LSDV isolate Egy/11T..... [90]
 KJ561442 LSDV strain Egypt/BSU-1/12 -----A.... [90]
 KJ561443 LSDV strain Egypt/BSU-2/12 ----- [90]
 KP233217 LSDV strain Egypt/VRLCU/14 -----A.... [90]
 KJ818284 SPPV Jovivac(RM-65)/Romania/14A..... [90]
 KF495237 SPPV Roumanian Fanar/13A..... [90]
 FJ869378 SPPV Vaccine Morocco/09A..... [90]
 KP663700 GTPV Chagni/Ethiopia/12AC.....G..TACG.....T.....A.....C... [90]
 FJ869360 GTPV Saudi Arabia/93A..... [90]
 FJ869361 GTPV Sudan/09A..... [90]

NC_003027 LSDV NI-2490/Kenya/58 ATTCTCAGTA CAATTTCAAC AAATCAAAA AATGTTACAA CGCCTTCAAC TTATGAAAAT
ACAACAACGA TATCTAATTA TACAACCGCA [180]

FJ869377 LSDV Egypt/Ismailia/89 [180]
 KP071937 LSDV isolate Egy/11G... [180]
 KJ561442 LSDV strain Egypt/BSU-1/12T..... [180]
 KJ561443 LSDV strain Egypt/BSU-2/12 [180]
 KP233217 LSDV strain Egypt/VRLCU/14T..... [180]
 KJ818284 SPPV Jovivac(RM-65)/Romania/14G.....A.....G..G.A.....A..A... [180]
 KF495237 SPPV Roumanian Fanar/13G.....A.....G..G.A.....A..A... [180]
 FJ869378 SPPV Vaccine Morocco/09G.....A.....G..G.A.....A..A... [180]
 KP663700 GTPV Chagni/Ethiopia/12G T...T.....T..A.....A.....G..A..... [180]
 FJ869360 GTPV Saudi Arabia/93G.....A.....G..G.A.....A..A... [180]
 FJ869361 GTPV Sudan/09G.....A.....G..G.A.....A..A... [180]

NC_003027 LSDV NI-2490/Kenya/58 TATAATACAA CTTATTATAG CGATGATTAT GATGATTATG AAGTGAGCAT AGTCGATATC
CCACATTGTG ATGATGGTGT GGATACTACA [270]

FJ869377 LSDV Egypt/Ismailia/89 [270]
 KP071937 LSDV isolate Egy/11CC... [270]
 KJ561442 LSDV strain Egypt/BSU-1/12 [270]
 KJ561443 LSDV strain Egypt/BSU-2/12 [270]
 KP233217 LSDV strain Egypt/VRLCU/14 [270]
 KJ818284 SPPV Jovivac(RM-65)/Romania/14T..C...C.....A.....C.....T..... [270]
 KF495237 SPPV Roumanian Fanar/13T..C...C.....A.....C.....T..... [270]
 FJ869378 SPPV Vaccine Morocco/09T..C...C.....A.....C.....T..... [270]
 KP663700 GTPV Chagni/Ethiopia/12T.....T.T.....C.....A..... [270]
 FJ869360 GTPV Saudi Arabia/93T..C...C.....C.....A.....C.....T..... [270]
 FJ869361 GTPV Sudan/09T..C...C.....A.....C.....T..... [270]

NC_003027 LSDV NI-2490/Kenya/58 AGTTTTGGAC TGATTACTIT ATATTGCGACT ATATTCTTTC TTGGATTATT TGGAAATATA
ATTGTGTAA CTGTTCTTCG TAAATATAAG [360]

FJ869377 LSDV Egypt/Ismailia/89 [360]
 KP071937 LSDV isolate Egy/11 [360]
 KJ561442 LSDV strain Egypt/BSU-1/12 [360]
 KJ561443 LSDV strain Egypt/BSU-2/12 [360]
 KP233217 LSDV strain Egypt/VRLCU/14 [360]
 KJ818284 SPPV Jovivac(RM-65)/Romania/14 ...C....T.....TA... [360]

KF495237 SPPV Roumanian Fanar/13C.....T.....TA..... [360]
 FJ869378 SPPV Vaccine Morocco/09C.....T.....TA..... [360]
 KP663700 GTPV Chagni/Ethiopia/12A.....TT.....A [360]
 FJ869360 GTPV Saudi Arabia/93T.....TA..... [360]
 FJ869361 GTPV Sudan/09T.....G.TA..... [360]

NC_003027 LSDV NI-2490/Kenya/58 ATAAAAACAA TACAGGATAT GTTTTGCTT AATTGACAC TGCTGATT AATTTTCGTG
 TTGGTGTTC CTTTAATTT ATACGATAGT [450]

FJ869377 LSDV Egypt/Ismailia/89 [450]
 KP071937 LSDV isolate Egy/11 [450]
 KJ561442 LSDV strain Egypt/BSU-1/12 [450]
 KJ561443 LSDV strain Egypt/BSU-2/12 [450]
 KP233217 LSDV strain Egypt/VRLCU/14 [450]
 KJ818284 SPPV Jovivac(RM-65)/Romania/14G.....A.....T.....A..... [450]
 KF495237 SPPV Roumanian Fanar/13G.....A.....T.....A..... [450]
 FJ869378 SPPV Vaccine Morocco/09G.....A.....T.....A..... [450]
 KP663700 GTPV Chagni/Ethiopia/12A.....T.....A.....A..... [450]
 FJ869360 GTPV Saudi Arabia/93G.....A.....T.....T.....A..... [450]
 FJ869361 GTPV Sudan/09G.....A.....T.....A..... [450]

NC_003027 LSDV NI-2490/Kenya/58 ATCGCTAAAC AATGGAGTTT AGGAGATTGT TTGTGTAAT TAAAGCTAT GTTTACTTT
 GTTGGTTTTT ACAATAGCAT GTCATTATA [540]

FJ869377 LSDV Egypt/Ismailia/89 [540]
 KP071937 LSDV isolate Egy/11 [540]
 KJ561442 LSDV strain Egypt/BSU-1/12 [540]
 KJ561443 LSDV strain Egypt/BSU-2/12 [540]
 KP233217 LSDV strain Egypt/VRLCU/14 [540]
 KJ818284 SPPV Jovivac(RM-65)/Romania/14 ..A.....G.....C..... [540]
 KF495237 SPPV Roumanian Fanar/13 ..A.....G.....C..... [540]
 FJ869378 SPPV Vaccine Morocco/09 ..A.....G.....C..... [540]
 KP663700 GTPV Chagni/Ethiopia/12G..... [540]
 FJ869360 GTPV Saudi Arabia/93 ..A.....G.....C..... [540]
 FJ869361 GTPV Sudan/09 ..A.....G.....C..... [540]

NC_003027 LSDV NI-2490/Kenya/58 ACATTGATGA GTATTGATAG ATACCTAGCT GTAGTTCACC CA [582]

FJ869377 LSDV Egypt/Ismailia/89 [582]
 KP071937 LSDV isolate Egy/11 [582]
 KJ561442 LSDV strain Egypt/BSU-1/12 [582]
 KJ561443 LSDV strain Egypt/BSU-2/12 [582]
 KP233217 LSDV strain Egypt/VRLCU/14 [582]
 KJ818284 SPPV Jovivac(RM-65)/Romania/14A.....T..... [582]
 KF495237 SPPV Roumanian Fanar/13A.....T..... [582]
 FJ869378 SPPV Vaccine Morocco/09A.....T..... [582]
 KP663700 GTPV Chagni/Ethiopia/12 [582]
 FJ869360 GTPV Saudi Arabia/93A.....T..... [582]
 FJ869361 GTPV Sudan/09A.....T..... [582]

NC_003027 LSDV NI-2490/Kenya/58 MNYTLSTVSS ATMYNSSSNI TTIATTIIST ILSTISTNQN NVTTPSTYEN TTTISNYTTA
 YNTTYYSDDY DDYEVSIVDI [80]

FJ869377 LSDV Egypt/Ismailia/89 [80]
 KP071937 LSDV isolate Egy/11F..... [80]
 KJ561442 LSDV strain Egypt/BSU-1/12N..... [80]
 KJ561443 LSDV strain Egypt/BSU-2/12 [80]
 KP233217 LSDV strain Egypt/VRLCU/14N..... [80]

KJ818284 SPPV Jovivac(RM-65)/Romania/14R.... -----.R...K.A...K. ..I.....N.... [80]
 KF495237 SPPV Roumanian Fanar/13R.... -----.R...K.A...K. ..I.....N.... [80]
 FJ869378 SPPV Vaccine Morocco/09R.... -----.R...K.A...K. ..I.....N.... [80]
 KP663700 GTPV Chagni/Ethiopia/12N...G YA.....V.L.....KI..... [80]
 FJ869360 GTPV Saudi Arabia/93R.... -----.R...K.A...K. ..I.....N.... [80]
 FJ869361 GTPV Sudan/09R.... -----.R...K.A...K. ..I.....N.... [80]

NC_003027 LSDV NI-2490/Kenya/58 PHCDDGVDTT SFGLITLYST IFFLGLFGNI IVLTVLRKYK IKTIQDMFLL NLTLSDLIFV
 LVFPFNLYDS IAKQWSLGDC [160]
 FJ869377 LSDV Egypt/Ismailia/89 [160]
 KP071937 LSDV isolate Egy/11R..... [160]
 KJ561442 LSDV strain Egypt/BSU-1/12 [160]
 KJ561443 LSDV strain Egypt/BSU-2/12 [160]
 KP233217 LSDV strain Egypt/VRLCU/14 [160]
 KJ818284 SPPV Jovivac(RM-65)/Romania/14Y.. .S....LI.....N [160]
 KF495237 SPPV Roumanian Fanar/13Y.. .S....LI.....N [160]
 FJ869378 SPPV Vaccine Morocco/09Y.. .S....LI.....N [160]
 KP663700 GTPV Chagni/Ethiopia/12N.....F.....I.....N..... [160]
 FJ869360 GTPV Saudi Arabia/93Y.. .L.....I.....N..... [160]
 FJ869361 GTPV Sudan/09Y.. .CL.....I.....N..... [160]

NC_003027 LSDV NI-2490/Kenya/58 LCKFKAMFYF VGFYNSMSFI TLMSIDRYLA VVH [193]
 FJ869377 LSDV Egypt/Ismailia/89 [193]
 KP071937 LSDV isolate Egy/11 [193]
 KJ561442 LSDV strain Egypt/BSU-1/12 [193]
 KJ561443 LSDV strain Egypt/BSU-2/12 [193]
 KP233217 LSDV strain Egypt/VRLCU/14 [193]
 KJ818284 SPPV Jovivac(RM-65)/Romania/14 I.Y [193]
 KF495237 SPPV Roumanian Fanar/13 I.Y [193]
 FJ869378 SPPV Vaccine Morocco/09 I.Y [193]
 KP663700 GTPV Chagni/Ethiopia/12 [193]
 FJ869360 GTPV Saudi Arabia/93 I.Y [193]
 FJ869361 GTPV Sudan/09 I.Y [193]

Nucleotide identities

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
		99.9	99.6	100	99.7	100	98.9	96.9	95.1	93.5	93.5	93.8	93.6	93.6	93.5	93.6	93.5	1	FJ869377.1 LSDV Egypt/06
99.7			99.5	99.8	99.5	99.9	98.9	96.9	95	93.4	93.4	93.8	93.5	93.5	93.5	93.5	93.5	2	FJ869371 LSDV Springbok isolate/S.Africa/06
98.9	98.4			99.7	100	99.7	97.6	95.3	92.3	89.2	89.1	90	89.5	89.3	89.3	89.3	89.3	3	KJ561442 LSDV Egypt/BSU-1/12
99.5	98.9	98.9			99.6	100	97.8	95.6	92.6	89.6	89.5	90.5	89.6	89.6	89.6	89.6	89.6	4	KJ561443 LSDV Egypt/BSU-1/12
98.51	98.4	99.5	98.9			99.6	97.6	95.3	92.3	89.3	89.1	90.2	89.5	98.3	98.3	89.3	89.3	5	KP233217 LSDV strain Egypt/BSU-1/12
100	99.7	98.9	98.9	98.9			98.9	96.9	95.1	93.5	93.5	93.6	93.6	93.6	93.5	93.6	93.5	6	KP663694 LSDV/Andassa/B04/Ethiopia/12
98.9	98.7	96.9	98.9	96.9	98.9			97.9	95.9	94.4	94.4	94.8	94.6	94.6	94.5	94.6	94.5	7	NC 003027 LSDV NI-2490/Kenya/58
97.9	97.7	95.8	99	95.8	97.9	98.9			95.6	93.9	93.9	94.5	94.1	94.1	94	94.1	94	8	AF409138 LSDV Neeth.Vacc.LW/S.Africa/59
95	94.8	91	97.4	91	95	95.8	95.8			93.5	93.4	95.9	93.5	93.5	93.5	93.5	93.5	9	KP663700 GTPV Chagni/Ethiopia/09
91	90.8	86.3	96.3	86.3	91	91.9	92.7	92.4		99.5	95.6	99.6	99.6	99.6	99.6	99.6	99.6	10	FJ869361 GTPV Sudan/09
91.3	91	86.9	91.6	86.9	91.4	92.1	92.9	92.7	99.2		95.4	99.6	99.6	99.7	99.7	99.7	99.7	11	FJ869360 GTPV Saudi Arabia/09
93.5	93.2	88.9	86.9	88.9	93.5	94.2	94.2	95.3	93.6	93.9		95.6	95.6	95.5	95.6	95.5	95.5	12	FJ869390 SPPV Oman isolate/84
91.6	91.4	86.9	87.4	86.9	91.6	92.4	93.2	92.7	99.5	99.7	94.1		99.8	99.7	99.9	99.7	99.7	13	FJ869386 SPPV Algeria Illizi isolate/05
91.4	91	86.3	86.9	86.3	91.4	92.1	92.9	92.7	99.2	99.5	93.9	99.7		99.9	100	99.9	100	14	FJ869378 SPPV Vaccine Morocco/09
91	90.8	86.3	86.9	86.3	91	91.9	92.7	92.4	98.9	99.7	93.6	99.5	99.7		99.9	100	100	15	KF495237 SPPV Roum. Fanar P-36/13
91.4	91	86.3	86.9	86.3	91.3	92.1	92.9	92.7	99.2	99.5	93.9	99.7	100	99.7		99.9	99.9	16	KJ818285 SPPV Saudi Ar. vaccine/14
91	90.8	86.3	86.9	86.3	91	91.9	92.7	92.4	98.9	99.7	93.6	99.5	99.7	100	99.7		99.9	17	KJ818284 SPPV Jovivac (RM-65)/romania/14

Amino acid Identities

Tabl (2): Nucleotide and Amino acid identities

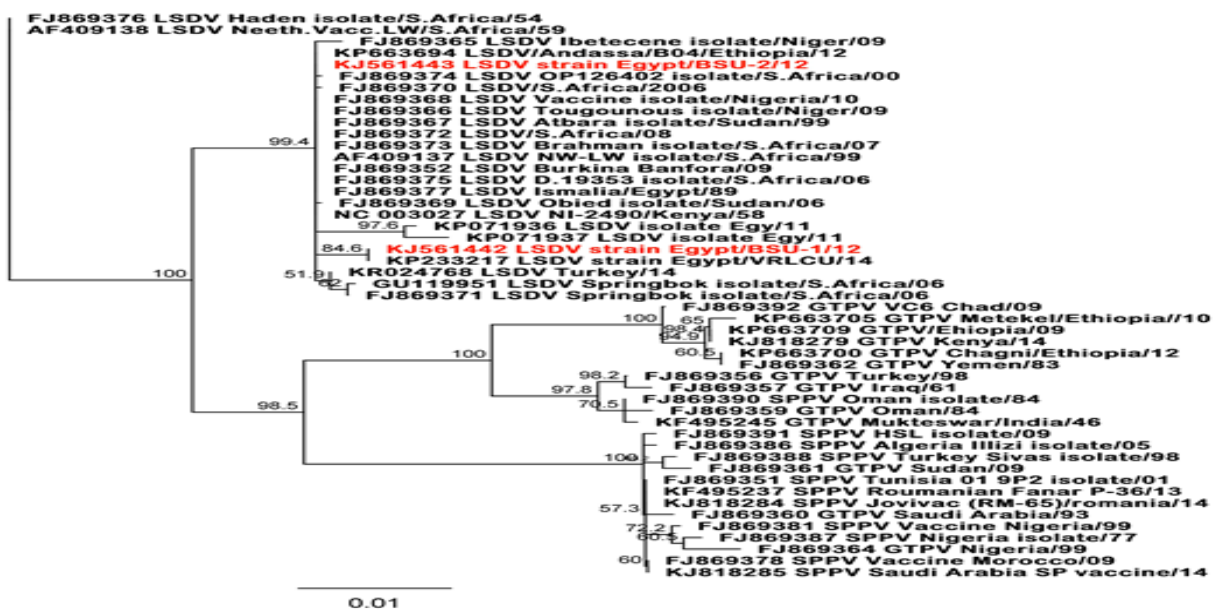


Fig. (7):Phylogenetic analysis of GPCR gene of LSDV.

Discussion

Lumpy skin disease (LSD) is a serious disease of cattle caused by a capripoxvirus. It is characterized by nodular cutaneous eruptions, lymphadenitis, oedema in one or more legs [13]. The present study concerned with trials for isolation of LSD V from skin nodule samples from infected cattle on embryonated chicken eggs and tissue culture with further identification by means of serological tests as AGPT and IFAT of LSDV as well as advanced molecular characterization of virus isolates using PCR.LSDV was isolated from samples collected from naturally infected cattle by inoculation on CAM of ECE. Characteristic pock lesions were observed after 1st passage and become clear after 3rd passage (Fig. 2). These findings come in complete agreement with those of House *et al* [14]. and Tamam [15] who successfully cultivated LSDV on CAM of ECE and detected the characteristic pock lesions. Also MDBK cell culture showed characteristic cytopathic effect as shown in fig (3). CPE was characterized by cell rounding, cell aggregation, coalesce together to form clusters that scattered all over the Passage sample monolayer within 72 hr post inoculation and gradually sample increased till 70-80 % of sheet was completely detached. These findings agree with those of Ibrahim [16] and Fahmy [17]. Isolated LSDV was identified by serological tests. Clear precipitation lines were appeared in AGPT using reference LSDV antisera and Characteristic specific Intracytoplasmic yellowish green fluorescent granules were appeared in

IFAT (Fig. 4) as observed by Davies [1]. Also characteristic intracytoplasmic inclusion bodies were seen in infected CAM stained by H&E fig (5).

Isolation of LSDV from naturally infected cattle with history of previous vaccination with sheep pox virus vaccine may attributed to vaccination failure either due to evolution of mutant strain of LSDV or defects in vaccine manipulation as storage and vaccine administration .

Serological methods are useful for confirming LSD but are too time consuming to be used as rapid diagnostic methods Davies [1] and Heine et al., [18]. Therefore, PCR was the test of choice for rapid detection and identification of the LSD outbreak causative agent. The PCR assay used in this work showed high specificity as a unique band of the expected size (~ 554 bp) was obtained for DNA samples derived from skin biopsies fig.(6). Multiple sequence alignments showed high nucleotide sequence identity and close branch distance of phylogenetic tree between our recent isolates of LSDV and not only other local isolates but also other capripox viruses, these results coincide with the theory that capripox viruses are genetically related and originated from one ancestor lineage [19] . these results comes in agreement with Boshra et al [20] who found that novel knockout strain of LSDV has potential as a vaccine

to protect livestock against sheep pox and goat pox.

In conclusion, (1) diagnosis of LSDV using PCR assay should be applied besides conventional techniques for any cases with skin lesions as early as possible to diagnosis and apply adequate control measures. (2) Appearance of LSDV in previously vaccinated cattle may attributed to defects in vaccine manipulation, storage, transportation and administration

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