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Isolation and Molecular Characterization of LSDV From Some Egyptian Governorates in Year 2017

A Thesis Submitted by

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**For The Master Degree in Veterinary Medical Sciences
(Virology)**

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**Isolation and Molecular Characterization of LSDV from some
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Abstract

In this study LSDV was isolated from cattle with history of vaccination showing LSD signs to confirm LSD viral infection. Nodular samples were collected from cattle showing signs of LSD and Molecular detection of LSDV with conventional PCR was applied using G protein-coupled receptor (G-PCR) gene primer which revealed 93 positive samples out of 130 samples results for LSDV. Modern Isolation of LSDV was started on (CAM) and MDBK Cell culture was applied; Morphological identification was confirmed by using Transmission Electron Microscopy (TEM) also antigenic identification by using Virus Neutralization Test (VNT), and also serological identification was carried out by SNT using reference antisera. Genetic characterization was conducted on (G-PCR) gene segment of these isolates, ensuing that 4 samples were positive for LSDV. Sequencing of those 4 positive isolates revealed identity of LSDV by targeting (GPCR) gene which confirm that there is no genetic changes in LSDV, and phylogenetic analysis of LSDV at summer 2017 revealed that recent isolates has no genetic difference compared by other isolates from Egypt or Africa during previous few years. The study reports the circulation of LSD in cattle population in year 2017.

Key words: LSDV, MDBK, CAM, EM, SNT, SPV, PCR, Egypt.

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LIST OF ABBREVIATIONS

° C	Degree Celsius
AGPT	Agar Gel Precipitation Test
BLAST	Basic local alignment search tool
bp.	Base pair
CAM	Chorio allantoic membrane
CaPVs	Capripox viruses
Cat. No	Catalogue number
Cm	Centimeter
CO ₂	Carbon dioxide
CPE	Cytopathic Effect
D.D H ₂ O	Double Distalied water
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTPs	Deoxy nucleotide tri-phosphate
dsDNA	Double strand Deoxyribonucleic acid
ECE	Embryonated chicken egg
EDTA	Ethylene diamine tetra acetic acid
EEV	extracellular enveloped virus
EGF	Epidermal growth factor
ELISA	Enzyme linked immuno sorbent assay
FAO	Food and Agriculture Organization
GAGs	Glycosaminoglycan's
GIT	Gastro intestinal tract
GPCR	G protein-coupled β -Chemokine receptor
GPV	Goat pox virus
HBSS	Hanks Balanced Salt Solution
HCL	Hydro chloric acid
IFN	Interferon
IL	Interleukin
IMV	intracellular mature virus
Taq	Thermus aquaticus
ITR	inverted terminal repeat
Kbp	Kilo base pair
KCL	Potassium Chloride
LSD	Lumpy Skin Disease
LSDV	Lumpy Skin Disease Virus
LT	Lamb Testis

MDBK	Madin Derby Bovine Kidney
MEM	Minimal Essential Medium
TEM	Transmission Electron Microscopy
mg	Milligram
Min	Minute
ml	Milliliter
μl	Micro liter
mRNA	Messenger Ribonucleic acid
NaHCO ₃	Sodium bicarbonate
NaH ₂ PO ₄	Sodium dihydrogen phosphate
NCBI	National Center for Biotechnology Information
nm	Nano meter
No	Number
O.D.	Optical Density
OIE	World Organization for Animal Health
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pmol	picomole
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Round per minute
Sec	Second
SNT	Serum neutralization test
SPF	Specific pathogen free
SPV	Sheep pox virus
TBE	Tris base EDTA
USA	United states of America
UV	Ultra violet
VI	Viral inhibition
VNT	Virus neutralization test
NaCl	Sodium Chloride