Benha University
Faculty of Veterinary Medicine
Department of Virology

Molecular and serological detection of sheep pox disease virus in kalubyia governorate

A Thesis

Submitted To Faculty of Veterinary Medicine

Benha University

Presented by

Shimaa Nassef Abd – Elhafeiz

(B. V. Sc., Benha University, 2011)
(M.V.S., Benha University,2016)

For the degree of Ph.D.
(Virology)

Under supervision of

Prof. Dr. Ehab Mostafa- El nahas

Professor of Virology
Faculty of Veterinary Medicine,
Benha University

Prof. Dr. Ayman Said Emam El-Habbaa

Professor of Virology
Faculty of Veterinary Medicine
Benha University

Prof. Dr. Lamya Attia Mohamed Fathi

Chief Researcher, Virology
Animal Health Research Institute,
Benha branch

(2021)

List of contents

List of Abbreviations

°C	Degree Celsius
Ag	Antigen
Abs	Antibodies
AGPT	Agar Gel precipitation test
AHA	Animal Health Australia
Blast	Basic local alignment search tool
bp	Base pair
CAM	Chorio allantoic membrane
CaPV	Capripox virus
Cat. No	Catalogue number
CFSPH	The center for food security and public health
CIE	counter-immuno-electrophoresis
Cm	Centimeter
CO2	Carbon dioxide
CPE	Cytopathic effect
D.D H2O	Deionized. Distilled water
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	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
GPCR	G protein-coupled β-Chemokine receptor
GPV	Goat pox virus
HBSS	Hanks Balanced Salt Solution
HCL	Hydro chloric acid
IFN	Interferon
IFAT	Indirect fluorescent antibody technique
IL	Interleukin
IMV	intracellular mature virus
i-Taq	Taq polymerase
ITR	inverted terminal repeat
Kbp	Kilo base pair
KCL	Potassium Chloride
KSA	Kingdom Saudi Arabia
LSD	Lumpy skin disease
LSDV	Lumpy skin disease virus
LT	Lamb testis
MA104	Monkey African cell line
MDBK	Madin derby bovine kidney

MEM	Minimal essential medium
Mg	Milligram
Min	Minute
Ml	Milliliter
µl	Micro liter
mRNA	Messenger Ribonucleic acid
NaCl	Sodium Chloride
NaHCO3	Sodium bicarbonate
NaH2PO4	Sodium dihydrogen phosphate
NCBI	National Center for Biotechnology Information
Nm	Nano meter
No	Number
O.D.	Optical denisty
OIE	Office International Des Epizooties
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
pmol	picomole
RBO30	30 KDa DNA-dependent RNA polymerase
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Round per minute

Sec.	Second
SGP	Sheep Goat Pox
SNT	Serum neutralization test
SPF	Specific pathogen free
SPV	Sheep pox virus
TBE	Tris base EDTA
TEM	Transmission Electron Microscopy
USA	United states of America
UV	Ultra vilot
VI	Viral inhibition
VNT	Virus neutralization test

List of Figures

List of Photos

LIST OF TABLES

7-SUMMARY

SPV is a serious infectious skin disease which considered one of the most economically important viral diseases that infect sheep. The disease is endemic in Egypt and further isolation and characterization of the SPV virus is required to provide more reliable methods for detection and control of outbreak of the virus.

Consequently, the key objective of this work was to study the genetic identity between skin field isolates of SPPV and skin field isolates of LSDV from clinically suspected diseased sheep and cattle during occurrence of LSDV in Kalubyia governorate, Egypt in 2018. And also trials for viral isolation and molecular basis to improve novel insights into the biology and aid the development of a new method for Capripoxviruses control.

The applied experiments revealed that:

1) The prevalence of serum samples from unvaccinated sheep were examined by ELISA for detection of antibodies against Capripoxviruses in Kalubyia governorate, Egypt with total percent of positive sera were 30% (30/100) of the total examined sheep sera.

2) The prevalence of serum samples from unvaccinated cattle were examined by ELISA for detection of antibodies against Capripoxviruses in Kalubyia governorate, Egypt with total percent of positive sera were 60% (60/100) of the total examined cattle sera.

3) The seroprevalance of SPV antibodies in un vaccinated sheep sera based on animal age revealed that the higher percent were detected in 6 months old 36.6% (11/30) and 30% (9/30) within 1year old followed by 20% (6/30) in 1 .5-2.5 years old age, but lower percent were detected in older stock 3years animal with 13.3% (4/30) from total positive samples.

4) The seroprevalance of LSDV antibodies in unvaccinated cattle sera based on animal age revealed that the higher percent induced in 1 year age 36.6% (22/60) and 25% (17/60) within 6 month old followed by 21.6% (13/60) in 1.5-2.5 1year old while low percent obtained in 3 years old age with 16.6% (10/60) from total positive samples.

5) Trials for viral isolation from suspected skin lesion samples from sheep and cattle by three blind passages on CAM of SPF-ECE. The induced characteristic lesion on CAM which appeared in the form of congestion and clotting of blood in CAM Blood vessels, presence of pock lesion in the form of small, scattered, numerous white foci and turbidity of the membrane observed in 40% (10/25) for sheep while the observed CAM lesions were induced in 28% (7/25) for cattle from of total isolated skin lesion samples.

6) Molecular detection of SPV isolate using PCR targeting ORF103 gene were induced in 8% (2/25) milk samples of clinically suspected infected and apparent healthy sheep and 50%(5/10) for sheep, 42.9%(3/7) for cattle from harvested CAM of the nodular samples of sheep and cattle respectively in addition to the sheeppox vaccine were positive revealed the presence of specific PCR product at the correct expected size (570 bp).

7) For further confirmation, the extracted genomic DNA products of 6 selected positive isolated samples of harvested CAM of skin lesion samples of sheep and cattle were subjected to conventional PCR for the amplification of the fusion protein p32 encoding gene revealed the presence of specific PCR product at the correct expected size (390 bp).

8) The 570-bp-DNA sequenced data of ORF103 amplicons from positive milk sample analyzed and showed identity to SPV. Moreover, BLAST program analysis showing that sequence alignment of the amplicons attained from 97.1 % nucleotide homology of detected Kazakhstan strain Sheeppox virus NISKHI, complete genome. In addition to 95-96 % homology to the nucleotide sequence of the

detected SPPV isolate in Minufiya governorate in Egypt, Chinese strains (Sheeppox virus isolate SPPV-GH, complete genome ; Sheeppox virus isolate SPPV-GL, complete genome and Kazakhstan strain (Sheeppox virus A, complete genome).

9) The multiple alignment of nucleotide sequences of 5 ORF103 amplicons revealed that LSDV isolates were closely identical (94.29 %). whereas our capripox virus isolate from sheep were identical (94.7%). On comparing with other sequences of capripoxviruses ,the field skin isolate from cattle and sheep were showed to have more than 97-98% identity at the nucleotide level with Egyptian LSDV isolates from neighboring governorates in Egypt (Lumpy skin disease virus isolate LSD/Egy/BeniSuif, Lumpy skin disease virus strain LSD/AHRI/ALWadi Elgidid/Egypt/saad/2018 and LSD/EGY/Menufia/2017.Unlike ,the aligned sequences show lower than 95% homology to the nucleotide sequence of the detected SPV isolate in Minufiya governorate in Egypt and Sheeppox virus strain SPPVE1 Menya al-qamh orf103.

10) Two positive samples for fusion protein P32 coding gene were selected and aligned together and then they were aligned with other strains of LSDV, SPV and GPV available in the GeneBank. Comparison of the two sequences showed that they share 92.33% similarity with each other. On the same pattern of ORF103 gene sequence analysis, P32 gene sequence analysis showed skin disease virus isolate P32 envelop protein P32 gene, partial cds . That our field skin isolates of LSDV from cattle was more closely related 96.75 % to LSDV as Lumpy skin disease virus isolate LSD/Egy/BeniSuif. While our field skin capripox virus isolate from sheep from sheep shares 96% nucleotide identity with Lumpy skin disease virus.

11) Phylogenetic tree constructed to calculate, examine the relationships of the sequence and show the relationship between isolated strains of the study and LSDV, SPV strains available in gene bank database. The phylogenetic analysis shows that our strains are closely related to LSDV group than to SPV &GPV.