

Suez Canal University
Faculty of Veterinary Medicine
Department Of Virology



Comparative Studies on Pox Disease Virus in Sheep and Goats in Suez Canal Area

By

Mona Rawy Fares Abd El-mgeed

B.V.SCS - Faculty of Veterinary Medicine - Suez Canal University
(2011)

M.V.SCS of virology (2015)

Under Supervision

Prof. Dr. Mohamed Saed Mohamed El-shahidy

Professor of Virology, Faculty of Veterinary Medicine, Suez Canal University

Prof. Dr. Momtaz Abd El-hady Afifi Shahin

Professor of Virology, Director of Animal Health Research Institute, Dokki, Giza

Dr. Mohamed Fawzy Ibrahim Mandour

Assistant professor of virology, Faculty of Veterinary Medicine, Suez Canal University

Thesis Submitted

To

Faculty of Veterinary Medicine – Suez Canal University

For the Degree of PHD of Veterinary Science

(Virology)

Department of Virology

(2019)

Name	Mona Rawy Fares Abd El-mgeed
Title	Comparative Studies on Pox Disease Virus in Sheep and Goats in Suez Canal Area
Faculty	Veterinary Medicine
Department	Virology
Location	Suez Canal University
Degree	PHD
Date	
Language	English
Supervision Committee	Prof. Dr. Mohamed Said Mohamed El-Shaheidy Prof. Dr. Momtaz Abd El-hady Afifi Shahin Dr. Mohamed Fawzy Ibrahem Mandour
Abstract	
<p>This study was designed for isolation, identification and differentiation of Capripoxviruses (sheep pox virus and goat pox virus) by egg inoculation, AGID test, 30 kDa RNA polymerase subunit (RPO30) gene, P32 gene-based polymerase chain reaction (PCR) and real time PCR in clinically affected animals in Suez canal area, then sequencing and comparing strains with national and international SGPV strains recorded in gene bank. The collected samples were 68 skin scabs mixed in 13 pools and 265 serum samples collected from Suez canal area in the period of 2017-2019.</p> <p>Typical pox lesion were observed in 10/13(76.92%) of pooled samples, while the rest of the sample 3/13 (23.07%) showed no pathological changes on the CAM.</p> <p>A total 43 out of 265 examined serum samples collected from sheep and goats were recorded positive by AGID test with a total percentage of 16.22%. Sheep pox antibody titer was high in vaccinated than non-vaccinated animals.</p> <p>Egyptian sheep pox (Ismailia 2019) isolate for RPO30 gene was closely related to other sheep pox viruses in gene bank and shared high identity percentages ranged between 98% and 98.7% indicating that the outbreak was caused by a SPPV. Sheep pox Ismailia 2019 was more distant from goat pox virus and lumpy skin disease virus with identity 93% & 93.7 %.</p> <p>Egyptian sheep pox (Ismailia 2019) and (Suez 2019) isolate for P32 gene was closely related, shared high identity percentages about 100% with other sheep pox viruses in gene bank indicating that the outbreak was caused by a SPPV. Sheep pox Ismailia/Suez 2019, was also closely related to goat pox virus and lumpy skin disease virus with identity 99.2 % & 100 %.The homology of Partial amino acid sequences of P32 protein between Ismailia 2019 and Suez 2019 strains of Capripoxvirus was 100%. The results showed 11 variable amino acids from the first 87amino acids.</p>	
Key words. Sheep and goat pox virus in Suez canal area	

List of content

Chapter	page
Acknowledgment-----	I
Dedication-----	II
List of Tables-----	VI
List of Figures-----	VII
List of abbreviation-----	IX
List of a.a abbreviation-----	XII
Chapter one: 1. Introduction-----	1
Chapter two: 2. Review of literature-----	5
2.1. History and Geographical distribution-----	5
2.1.1. History -----	5
2.1.2. Geographical distribution -----	6
2.2. Distribution of sheep and goat pox in Egypt-----	8
2.3. Host range and seasonal variation of sheep and goat pox-----	9
2.3.1. Seasonal variation-----	9
2.3.2. Host range-----	9
2.4. Economic Importance-----	11
2.5. Morbidity and Mortality -----	12
2.6. Transmission of Sheep and goat pox-----	12
2.6.1. Direct transmission-----	12
2.6.2. Indirect transmission-----	14
2.7. Virus morphology, genome organization and stability-----	15
2.7.1. Virus structure -----	15
2.7.2. Genome organization -----	16
2.7.3. Physico chemical properties -----	17
2.8. Genetic and Antigenic relationship-----	18

2.8.1.SPPV and GTPV genomes-----	18
2. 8.2. Comparison of SPPV and GTPV-----	21
2.8.3.Comparison of SGPV and LSDV -----	22
2.8.4. RPO30 gene in sheep pox viral genome-----	29
2.8.5.GPCR gene in sheep pox viral genome-----	30
2.8.6.P32 gene in sheep and goat pox viral genome-----	31
2.9.Pathogenesis of sheep and goat pox viruses-----	33
2.10.Diagnosis of sheep and goat pox-----	34
2.10.1.Clinical signs-----	34
2.10.2.Post mortem Lesions(P.M)-----	35
2.10.3.Electron microscopy-----	37
2.10.4.Experimental infection-----	37
2.10.5.Virus isolation and propagation-----	37
2.10.5.1. In vivo methods-----	37
2.10.5.2.Cell cultures of ovine, caprine and bovine origin	38
2.10.6.Serological tests-----	40
2. 10.6.1.Agar gel precipitation test (AGPT)-----	40
2.10.6.2. Enzyme linked immunosorbent assay(ELISA)	41
2.10.6. 3. Complement fixation test-----	41
2. 10.6.4. Latex agglutination test (LAT)-----	42
2.10.6.5. Single radial hemolysis test-----	42
2.10.6.6.Neutralization test -----	42
2. 10.6.7.Haemagglutination (Aserologicalbehaviour)	43
2.10.6.8. Cross-reaction of SPPV and GTPV-----	43
2. 10.6.9.Polymerase chain reaction (PCR)-----	43
2.10.6.10.Differential diagnosis-----	44
2.11.Prevention and control of sheep and goat pox-----	44

2. 11.1. Control strategy -----	45
2. 11.2. Cross-protection-----	46
2.11.3.Immunity and vaccination-----	47
Chapter three: 3.Materials and Methods-----	50
3.1.Materials -----	50
3.1.1. Samples -----	50
3.1.2. Preparation of Samples -----	51
3.1.3. Isolation of SGPV using E.C.E -----	51
3.1. 4. Detction and Identification of SGPV using AGID test-----	52
3.1.5. DNA extraction of SPPV and GTPV -----	52
3.1.6. Molecular detection of SGPV by Real time PCR-----	53
3.1.7. Molecular detection of SGPV by Conventional PCR -----	54
3.1.8. Agarose gel electrophoresis -----	56
3.1.9. Sequencing and phylogenetic analysis of RPO30 and P32 genes of SPPV -----	57
3.1.10.Equipments-----	58
3.2.Methods -----	63
3.2.1. Preparation of Clinical Samples -----	63
3.2.2. Isolation of SGPV using E.C.E -----	63
3.2.3. Detction and Identification of SGPV using AGID test-----	63
3.2.4. Molecular detection and identification of SGPV by PCR---	64
3.2. 5. Sequence analysis of P32 and RPO30 gene of SPPV -----	68
Chapter four: Results-----	70
Chapter five : Discussion -----	99
Chapter six: English Summary-----	113
Chapter seven: References-----	118
Chapter eight: Arabic summary	

List of Tables

Table	Title	Page
1	SPV and GPV ORFs	26
2	Distribution of skin and serum samples in sheep and goats	50
3	Primers used to amplify P32 gene of sheep and goat pox virus	53
4	Pox virus strains obtained from NCBI and used for phylogenetic analysis (RPO30 Gene)	60
5	Pox virus strains obtained from NCBI and used for phylogenetic analysis (P32 Gene)	61
6	The optimized condition for real time PCR	67
7	Epidemiological data of sheep and goats clinically showed pox lesion	73
8	Screening of pox antibodies in SGPV serum by AGID test	76
9	Extraction process and measurement of Nanodrop of 6 pooled sheep pox virus isolates	77
10	Ct value of 5 positive sheep pox isolates	78
11	Identity and similarity percentages of RPO30 gene of sheep pox virus Ismailia /Egypt 2019 strains with other Capripox virus strains	83
12	Identity and similarity percentages of SPPV Ismailia /Egypt 2019, SPPV Suez /Egypt 2019 strains with other Sheep pox virus strains	87
13	Identity and similarity percentages of sheep pox virus Ismailia /Egypt 2019 ,Suez /Egypt 2019 strains with LSDV and GTPV strains	88
14	Nucleotide and Amino acid change of p32 gene of Capripox virus strains compared with Ismailia 2019 and Suez 2019 strains	89

List of Figures

Figure	Title	Page
1	Distribution of SPPV and GTPV in Africa, Middle East countries and Europe	7
2	Distribution of sheep and goat pox virus in different Egypt governorates	8
3	Structure of pox virus	16
4	Small erthematus nodules, under tail of sheep.	70
5	Pox nodules in face, eye, lips and nostrils of sheep affected by SPPV	71
6	Typical cutaneous lesions of sheep pox virus in the chest of sheep	71
7	Emaciation and poor wool quality of animal affected with sheep pox virus	72
8	Thickening, oedema of CAM of eggs inoculated with sheep pox virus	74
9	Rounded white pock lesion in CAM and hemorrhages due to sheep pox virus	74
10	Haemorrhages and oedema of CAM with minute pock lesion	75
11	White necrotic foci with wide and large pock lesion in CAM	75
12	Amplification plot of real time PCR for 6 sheep pox isolates	78
13	Detection of sheep pox P32 gene by gel electrophoresis at (89 bp) using primer 1	79
14	Detection of sheep pox P32 gene by gel electrophoresis at (390 bp) using primer 2	79
15	Detection of sheep pox RPO30 gene by gel electrophoresis at (151bp)	80

List of Figures

16	Nucleotide sequence tree of RPO30 gene of sheep pox virus Ismailia 2019 compared to other sheep pox, goat pox and lumpy skin virus strains in gene bank	82
17	Nucleotide sequence tree of P32 gene of SPPV Ismailia/Egypt 2019 and SPPV Suez/Egypt 2019 compared to other sheep pox, goat pox and lumpy skin virus strains in gene bank	86
18	Nucleotide alignment of RPO30 gene of sheep pox virus Ismailia strain 2019 with other sheep pox strains in gene bank	90
19	Nucleotide alignment of RPO30 gene of sheep pox virus Ismailia strain 2019 with LSDV strains in gene bank	91
20	Nucleotide alignment of RPO30 gene of sheep pox virus Ismailia strain 2019 with goat pox virus strains in gene bank	92
21	Nucleotide alignment of P32 gene of SPPV Ismailia strain 2019 SPPV Suez strain 2019 and with other Capri pox strains in gene bank	93
22	Amino acid alignment of P32 gene of SPPV Ismailia strain 2019 SPPV Suez strain 2019 and with other Capri pox strains in gene bank	97