

**ISOLATION AND MOLECULAR CHARACTERIZATION OF *GOAT POXVIRUS*
FROM ETHIOPIAN SHEEP AND GOAT**



A Thesis Submitted To The College Of Veterinary Medicine And Agriculture Of Addis Ababa University In Partial Fulfillment Of The Requirements For The Degree Of Master Of Science In Veterinary Microbiology

By
Teferi Degefa Negi

June, 2014
Bishoftu, Ethiopia

**Medicine and Agriculture, Department of Microbiology, Immunology and Veterinary
Public Health**

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: Teferi Degefa entitled Isolation And Molecular Characterization Of *Goat Poxvirus* From Ethiopian Sheep and Goat and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Veterinary Microbiology

Dr Fikedu Ragasa	_____	_____
Chairman	Signature	Date
Dr Gelagye Aylet	_____	_____
External Examiner	Signature	Date
<u>Dr Badaso Mamo</u>	_____	_____
Internal Examiner	Signature	Date
Dr <u>Gezahegne Mamo</u>	_____	_____
Major Advisor	Signature	Date
Dr <u>Esayas Gelaye</u>	_____	_____
Co-Advisor	Signature	Date
Dr <u>Badaso Mamo</u>	_____	_____
Department chairperson	Signature	Date

TABLE OF CONTENTS

Contents

TITLE PAGE	i
APPROVAL PAGE	ii
TABLE OF CONTENTS	iii
SIGNED DECLARATION SHEET	viii
ACKNOWLEDGEMENTS	ix
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF TABLES IN THE ANNEXES	xii
LIST OF ANNEXES	xiii
LIST OF ABBREVIATIONS	xiv
ABSTRACT	xv
1. INTRODUCTION	1
2. LITERATURE REVIEW	4
2.1. History and Occurrence	4
2.2. Epidemiology	4
2.3. Economic impact	5
2.4. Hosts	5
2.5. Transmission	6
2.6. Etiology	7
2.7. Pathogenesis	7
2.8. Diagnosis of Goat poxvirus	8
2.9. Clinical Signs	8
2.10. Postmortem Lesions	9

2.11. Histopathology.....	9
2.12. Virus Isolation and Propagation.....	10
2.12.1. <i>Sample collection and preparation</i>	10
2.12.2. <i>In vivo virus isolation</i>	10
2.12.3. <i>In vitro virus isolation</i>	10
2.13. Detection viral antibody.....	11
2.13.1. <i>Latex agglutination test (LAT)</i>	11
2.13.2. <i>Neutralization test</i>	12
2.14. Detection of Antigen.....	12
2.14.1. <i>Immunohistochemistry</i>	12
2.14.2. <i>Agar gel precipitation test (AGPT)</i>	13
2.14.3. <i>Counter immunoelectrophoresis (CIEP)</i>	13
2.14.4. <i>Fluorescent antibody technique (FAT)</i>	14
2.14.5. <i>Enzyme linked immunosorbent assay</i>	14
2.14.6. <i>Western blotting</i>	15
2.15. Molecular Diagnosis Techniques.....	15
2.15.1. <i>Conventional Polymerase chain reaction (PCR)</i>	15
2.15.2. <i>Real-time Polymerase Chain Reaction (RTPCR)</i>	16
2.16. Differential diagnosis.....	17
2.17. Prevention and Control.....	18
2.18. Vaccines.....	18
3. MATERIALS AND METHOD.....	20
3.1. Study Area.....	20

3.2.	Study Animals.....	22
3.3.	Sampling Strategy and Size	22
3.4.	Field Clinical Examination	22
3.5.	Questionnaire Survey.....	23
3.6.	Sample Collection.....	23
3.7.	Samples Processing.....	23
3.8.	Preparation of Vero Cell Monolayer.....	24
3.9.	Isolation of Virus	24
3.10.	Polymerase Chain Reaction (PCR).....	25
3.10.1.	<i>DNA Extraction</i>	25
3.10.2.	<i>Conventional PCR</i>	26
3.10.3.	<i>Agarose Gel Electrophoresis of PCR Product</i>	27
3.10.4.	<i>Real time PCR</i>	27
3.10.5.	<i>PCR for Sequencing and PCR product Purification</i>	28
3.11.	Data Analysis	29
4.	RESULT	30
4.1.	Questionnaire Survey.....	30
4.2.	Field Clinical Examination	31
4.3.	Virus Isolation.....	31
4.4.	Conventional Gel-Based PCR Result	32
4.5.	Real Time PCR Results	33
4.6.	PCR Product Purification Result	37
5.	DISCUSSION.....	39
6.	CONCLUSION AND RECOMMENDATIONS	43

7. REFERENCES.....	44
8. ANNEX.....	51

SIGNED DECLARATION SHEET

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate

Name: Teferi Degefa Negi Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: 09/06/2014

ACKNOWLEDGEMENTS

It is a matter of pleasure to glance back and recall the path one traverses during the days of hard work and perseverance. It is my bounden duty now to recall all the faces and spirits in the form of parents, teachers, and friends. First, I would like to thank the **Almighty** for guiding me throughout the course of my work.

I would like to express my hearty gratitude to **Dr. Gezahegn mamo, (DVM, M.Sc, Ph.D.)** AAU CVMA for his guidance and for help in thesis writing and Guidance

I would like to thank **Dr. Esayas Gelaye (DVM, MSC, Ph.D.)**. Senior researcher officer in National Veterinary Institute, for his constant encouragement, his inspiring guidance, support and for facilitating the completion of the project work.

I take this chance to express my sincere thanks to Dr. Berecha Bayisa DVM, MSC senior vaccine production in National Veterinary Institute for his intensive encouragement and for his timely help, ideas and suggestions.

I am very much thankful to Mr. Alebachew Belay (Diploma AH, BSC) senior laboratory technology in National Veterinary Institute for generously providing the lab facilities.

I am immensely grateful to Dr. Shiferaw Jenberie (DVM, MSC) senior researcher officer in National Veterinary Institute, for encouragement and thought provoking suggestions rendered throughout the work of my study

I record my sincere and heartfelt gratitude to Hawa Muhamd (Diplom AH, BSC) and Ejagayu Assefew for their valuable advice in extending and facilities in virology section laboratory

Deep from my inner soul I would like to express my eternal gratitude to my family (Helina Alemu), who guided me in mental to monetary strength so as to make this project a success and meet the timeliness in my life.

The authority of National Veterinary Institute is highly indebted to my work for the financial support and for hosting and providing all the necessary facilities for smooth and effective execution of this work.

LIST OF TABLES

	PAGE
Table 1. Sheep and Goats Clinically Examined For Pox Lesion and Sampled.....	30
Table 2. Sample GTPV Positive And Show CPE From Different Town.....	32
Table 3. Purified PCR Product Concentration and Purity.....	37

LIST OF FIGURES

Page

Figure 1. Map Of Ethiopia Showing Pox Outbreak Areas Where Pox Samples Were Collected From Clinically Diseased Sheep and Goat	21
Figure 2. Pox Lesions In Clinically Diseased Sheep: A) Face And Neck Region And B) Perennial Region	31
Figure 3. Picture Taken Using Camera Fitted Inverted Microscope: 1) Cells Developed Characteristics Cpe Of Pox Virus As Shown By Arrow, And 2) Normal Vero Cell Monolayer.	32
Figure 4 . Goatpox Virus PCR Positive Bands On Gel Electrophoresis	33
Figure 5. PCR Amplification:	34
Figure 6. The Fluorescence Melting Curve Analysis Of The PCR Products Shows Two Melting Peaks For Each Of The Capv Three Genotypes (GTPV, SPPV And LSDV).	35
Figure 7. Melting Peaks For Each Tested Samples With <i>Capripox Virus</i> Three Genotype Positive Controls. NTC: No-Template Control	36
Figure 8. Melting Curve Of Capripoxviruses Showing Two Melting Regions For A Single Tested Samples.	37

LIST OF TABLES IN THE ANNEXES

	Page
Table 1. Components of the Real-Time PCR Reaction Mix.....	56
Table 2. Real-Time PCR protocol.....	57
Table 3. Melting peaks of samples and positive capripox virus (SPPV, GTPV and LSDV).....	57
Table 4. Goat and sheep pox diseases survey assessment questionnaires form.....	58

LIST OF ANNEXES

	Page
Annex 1. Dulbecco's Modification of Eagle's Minimum Essential preparation.....	51
Annex 2. Phosphate Buffered Saline A (PBSA).....	51
Annex 3. Trypsin 2.5 % (W/V) Solution (10x Stock Solution).....	52
Annex 4. Penicillin And Streptomycin Stock Solution.....	53
Annex 5. DNA Extraction Kits, Master Mix, Gel, TAE Buffer, GelRed, Loading Dye, Ladder.....	54
Annex 6. Materials Real Time PCR Master Mix And Reaction	56
Annex 7. Melting Peaks Temperature Of Samples And Positive Capripox Virus.....	57
Annex 8. Goat And Sheep Pox Diseases Survey Assessment Questionnaires Form.....	58

LIST OF ABBREVIATIONS

AGPT	Agar Gel Precipitation Test
AU-PANVAC	African Union-Pan African Veterinary Vaccine Center
CAM	Chorioallantoic Membrane
CaPV	Capripoxvirus
CIEP	Counter Immunoelectrophoresis
CK	Calf Kidney
CPE	Cytopathic Effect
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FAT	Fluorescent Antibody Test
GEP	Gel-electrophoresis
DMEM	Dulbecco's Modification of Eagle's Minimum Essential
GTPV	Goat Pox Virus
KSG	Kenyan Sheep and Goat Pox Strain
LAT	Latex Agglutination Test
LK	Lamb Kidney
LSDV	Lumpy Skin Disease Virus
LT	Lamb Testis
MAb	Monoclonal Antibodies
PBSA	Phosphate Buffer Solution A
PCR	Polymerase Chain Reaction
REA	Restriction Enzyme Analysis
RNA	Ribonucleic Acid
RPO30	RNA polymerase subunit 30 kDa
SGP	Sheep and Goat Pox
SPPV	Sheep Pox Virus
TCID ₅₀	Tissue Culture Infective Dose 50%

ABSTRACT

The present study was conducted from November 2013 to May 2014 in areas where suspected pox disease outbreaks occurred in sheep and goats population. Skin lesion samples were collected from six different geographical areas with the aim of isolation and molecular characterization of the virus responsible for causing pox lesion in sheep and goats. The study was employed questionnaire, outbreak search, virus isolation, and genotyping methods. The questionnaire survey indicated that sheep and goat pox is the most common disease in all study areas and the disease is more frequent during rainy season followed by spring and autumn seasons. Purposive sampling method was used to get samples for laboratory investigation. A total of six pox suspected outbreaks were assessed for virus isolation and genotyping during the study period. Out of 102 sheep examined, 35 sheep (34.3%) developed pox lesions on their skins where as from 50 goats examined eight goats (16%) were found to be positive for pox. Virus was isolated from 14 skin samples (n=16; 14 sheep and 2 goat). The virus developed characteristic pox virus cytopathic effect of cell syncytia, ballooning, aggregation and detaching of cells on Vero cell culture. Similarly, the conventional genotyping PCR revealed 14 out of 16 tested samples were positive by developing band of 172 bp (*Goat poxvirus*) whereas two of them could not produce any band on gel electrophoresis. The real-time genotyping PCR analysis also indicated that 14 samples were properly amplified and genotyped as *goat poxvirus* only while 2 samples not. Even though the existing knowledge suggested that *Capri pox virus* is strictly host specific, this genotyping assay confirmed that sheep are affected by goat pox virus similar to goats. Classification of *poxvirus* based on infected host in small ruminants has been found to be inconclusive. Thus, genotyping of the isolates should be conducted carefully instead of naming the virus genotype based on the name of animals from which the samples has been collected. Hence, further genotyping studies are needed to differentiate sheep pox virus and goat pox virus with respect to their host specificity in order to select appropriate vaccine candidate and challenge virus for the control of pox in the small ruminants.

Key words: Ethiopia, genotyping, goat, goat poxvirus, sheep, virus isolation