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

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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

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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

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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 **o**f 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