2019-12-26

# OUTBREAK INVESTIGATION AND MOLECULAR DETECTIONS OF POX VIRUS CIRCULATING IN SHEEP AND GOATS IN SELECTED DISTRICTS OF WEST GOJJAM AND AWIE ZONES NORTHWET, ETHIOPIA

ALEMZEWUD, WONDIM


**BAHIR DAR UNIVERSITY**

**COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCE**

**SCHOOL OF ANIMAL SCIENCE AND VETERINARY MEDICINE**

**VETERINARY EPIDIMOLOGY AND ECONOMICS POSTGRADUATE PROGRAM**

# OUTBREAK INVESTIGATION AND MOLECULAR DETECTIONS OF POX VIRUS CIRCULATING IN SHEEP AND GOATS IN SELECTED DISTRICTS OF WEST GOJJAM AND AWIE ZONES NORTHWET, ETHIOPIA

**M. Sc Thesis**

**BY**

**ALEMZEWUD WONDIM**

**October 2019**

**Bahir Dar, Ethiopia**


# BAHIR DAR UNIVERSITY

## COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

## SCHOOL OF ANIMAL SCIENCE AND VETERINARY MEDICINE

## VETERINARY EPIDIMOLOGY AND ECONOMICS POSTGRADUATE PROGRAM

# OUTBREAK INVESTIGATION AND MOLECULAR DETECTIONS OF POX VIRUS CIRCULATING IN SHEEP AND GOATS IN SELECTED DISTRICTS OF WEST GOJJAM AND AWIE ZONES NORTHWET, ETHIOPIA

**M. Sc Thesis**

**BY**

**ALEMZEWUD WONDIM**

**Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science (MSc.) ''in Veterinary Epidemiology and Economics"**

**October 2019**

**Bahir Dar, Ethiopia**

# THESIS APPROVAL SHEET

As member of the Board of Examiners of the Master of Sciences (M.Sc.) thesis open defense examination, we have read and evaluated this thesis prepared by **Alemzewud Wondim Shiferaw** entitled "**Outbreak Investigation and Molecular Detections of Pox Virus Circulating in Sheep and Goats in Selected Districts of West Gojjam and Awi Zones Northwest, Ethiopia".** We hereby certify that, the thesis is accepted for fulfilling the requirements for the award of the degree of Master Sciences (M.Sc.) in **"Veterinary Epidemiology and Economics".**

**Board of examiners**

Dr. Takele Abayeneh (DVM, MSc, PhD, Associate Professor) _____ _____

**Name of External Examiner** Signature Date

Dr. Mussie Hailemelekot (DVM, MSc, Associate Professor) _____ _____

**Name of Internal Examiner** Signature Date

Dr. Birhan Agmas (DVM, MSc, Assistant professor) _____ _____

**Name of Chairpers**on Signature Date

# DECLARATION

This is to certify that this thesis entitled "**Outbreak Investigation and Molecular Detections of Pox Virus Circulating in Sheep and Goats in Selected Districts of West Gojjam and Awi Zones Northwest, Ethiopia"** submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in **Veterinary Epidemiology and Economics** to the Graduate Program of College of Agriculture and Environmental Sciences, Bahir Dar University by Alemzewud Wondim **(**ID. No. BDU1018692PR) is an authentic work carried out by him under our guidance. The matter embodied in this project work has not been submitted earlier for award of any degree or diploma to the best of our knowledge and belief.

**Name of the Student**

Alemzewud Wondim Shiferaw

Signature & date _____

**Name of the Supervisors**

1) Dr. Habtamu Tassew (DVM, MVSc, Associate Professor)
(Main Advisor) Signature & date_____

2) Dr. Esayas Gelaye (DVM, MSc, PhD)
(Co-advisor) Signature & date_____

# ACKNOWLEDGEMENTS

First and foremost, I thank the almighty GOD and his mother Saint Marry for granting me courage, strength and health to finalize my study and success in this very important stage of my life.

I am grateful to my major advisor Habtamu Tassew (DVM, MVSc, Asso.Prof.) for his willingness and tireless cooperation in correcting and giving constructive comments and suggestions for this thesis from the inception of study to the end.

I would like to express my deepest gratitude to my co-advisor Esayas Gelaye (DVM, MSc, PhD) for his permit to conduct tissue processing and molecular detection of sheep and goat pox virus at National Veterinary Institute (NVI), and provision of sample collection materials. I also extend to thank W/ro Hawa Mohammed for her tissue processing employee and Alebachew Belay and Getaw Deresse for their positive collaborations, guiding, and molecular detection of *Capripoxvirus.*

I would like to thank Ambaye Kenubeh (DVM, MSc, Asst. Prof.), Getahun Asebe (DVM, MSc, PhD Candidate) and Yechale Teshome (DVM, MSc, Asst. Prof.) for their unreserved comments, guidance, ideal and moral supports from the start of these thesis research and Yohans Hagos (DVM, MSc, Asso. Prof.) assisting the analysis of this data and his encouragements.

I would like to thank Bahir Dar University, College of Agriculture and Environmental Science for giving me the opportunity to learn this post graduate program.

I am also grateful to the staff of the Bahir Dar Regional Animal Health Diagnostic and Investigation Laboratory especially Elias Demis, Kumera and Sera for their kind and technical assistances, during transportation sample storages.

My appreciation also goes to district and rural kebele veterinary professionals specially our previous college students who helped in creating awareness to animal owners' for their giving time and permission to observe, examine and collection samples from their infected small ruminants is highly acknowledged. My appreciation also extended to sheep and goat owners

in the study districts for their willingness to skin scrapping collections and giving time for questionnaire.

I have no words for expressing my special gratitude for my mother, Workayehu Nigussie and my father Wondim Shiferaw and all my family members and relatives for their invaluable helps to grow up well and starting of my education up to these stages. They have always a special place on my heart.

All us everything shall belong to my precious wife, Asselef Amesalu, for your priceless love, trust and dense support in all aspects of my life. I would specially like to express deep appreciation to my wife's family members' including her sister Ambelenesh Amesalu and her husband Yirga Alem and her all other sisters and brothers for their moral and financial supports throughout my academic career GOD bless all.

At the last but not least special thanks for all Burie polytechnic college animal production and health department colleagues especially Ayana Wubie (DVM) and Hailehezeb Cheru (DVM) for their financial and unreserved encouragements and extend my appreciations to Aschenek Kassa for his tolerances and encouragements.

# DEDICATION

This MSc thesis work is dedicated to the memory of Ato Amesalu Dessie; a father of beloved and kind wife's father, passed away at May 06/ 2010 E.C. It is always surprising to me that his belief about education was so excited. He allowed me to have a new life led by education. More importantly, his far sighted visioning, honorable and disciplined personality, enhanced social relationship, positive attitude and hardworking spirit had shaped me and paved the way to my present situation. May GOD rest his soul in heaven!

## TABLE OF CONTENTS

PAGES

**Contents**

# LIST OF TABLES

PAGES

# LIST OF FIGURES

PAGES

# LIST OF APPENDICES

PAGES

# ABBREVIATIONS

| | |
|---|---|
| AGID | Agar Gel Immunodiffusion |
| CaPV | *Capripoxvirus* |
| CCPP | *Contageous Caprine Pleuropneumonia* |
| CFSPH | Center for Food Security Public Health |
| CSA | Centeral Stastical Agency |
| EDTA | Ethyline Diamine Tetraacetic Acid |
| ELISA | Enzyme linked immune sorbent assay |
| ESGPIP | Ethiopian Sheep &Goat Productivity Improvement Program |
| FAO | Food and Agriculture Organization |
| GTPV | Goat pox virus |
| ITR | Inverted Terminal Repeat |
| Kbp | Kilo base pair |
| KSG | Kenyan Sheep and Goat |
| LSDV | Lumpy Skin Diseases Viruses |
| NVI | National Veterinary Institute |
| OIE | World Organization for Animal Health |
| ORF | Open Reading Frame |
| PBS | Phosphate buffer saline |
| PCR | Polymerase Chain Reaction |
| PPR | *Pestei des Petites Ruminants* |
| REA | Restriction enzyme analysis |
| RKs | Rural Kebeles |
| RNA | Ribonucleic Acid |
| Rpm | revolution per minute |
| SGP | Sheep and Goat Pox |
| SGPV | Sheep Goat Pox Virus |
| SNNRP | South National Nationalities Regional People |
| SPPV | Sheep pox virus |

"**Outbreak Investigation and Molecular Detections of Pox Virus Circulating in Sheep and Goats in Selected Districts of West Gojjam and Awi Zones Northwest, Ethiopia".**


## *ABSTRACT*

*A cross sectional study was conducted from November 2018 to May 2019 in selected districts of West and Awi zones Northwest, Ethiopia with the aim of outbreak investigation and molecular detection of pox virus circulating in sheep and goats. The study was engaged different outbreak investigation approaches including clinical examinations and lesions, laboratory analysis and questionnaire survey. The districts and rural kebeles were selected randomly when an active outbreak of the diseases were reported and observed. From a total of 485 small ruminants (303 sheep and 182 goats) clinically examined for presence of specific skin lesions suspected of sheep and goat pox, 71 (14.64%) showed pox lesions of which 35 (11.55%) sheep and 36 (19.78%) goats manifested clinical signs and lesions suggestive for the diseases and 24 (*4.95*%) were dead during the outbreaks of sheep and goat pox. The study revealed that the highest morbidity of the disease was found in Jawie (*31.25%) *and Gunagua (14.89%) districts in goats and sheep respectively. The lowest was recorded in Dega Damot district both in sheep and goats which was 6.45% and 7.14% in sheep and goats respectively. However, the mortality rate was >1% in all districts except Dega Damot which was not found in both species. From a total of 38 skins tissue samples randomly collected from 71 sheep and goats showing typical pox lesions 19 samples were selected based on geographical distribution for molecular detection of the virus. All 19 samples (6 sheep and 13 goats) were found to be positive with goat pox virus. Even though the previous information suggested that Capripoxvirus is strictly host specific, however, sheep were found infected by goat pox virus in the current study suggesting that classification of pox virus based on infected host in small ruminant needs re-considerations. The significant potential risk factors were free animal movements, age of sheep and goats, flock size and composition, body conditions, vaccinations status and season of the year. The study showed that in the absence free movement of animals the diseases was less likely to occur with, (OR=0.05, CI 95%; 0.02, 0.15) and in the absence of vaccination small ruminants were more affected with, (OR=0.01, CI 95%; 0.00, 0.05).*



***Keywords**: Amhara, Epidemiology, Ethiopia, Outbreak, Pox virus, Sheep and Goat.*

# Chapter 1. INTRODUCTION

## 1.1.Background and Justification

Ethiopia is one of the countries with the largest number of livestock in Africa and livestock production plays a major role in the development of Ethiopia's agriculture. Ethiopian livestock population is estimated to be 59.49 million cattle, 30.697 million sheep, 30.200 million goats, 8 million donkey, 2.16 million horse, 1.20 million camels, 0.4 million mules and 59.495 million poultry (CSA, 2017).

This makes Ethiopia stands home to one of the largest heads of small ruminants in Africa next to Nigeria (Behnke and Fitaweke Metaferia, 2011; CSA, 2013). Small ruminants (sheep and goats) have a unique role in smallholder agriculture as they require small initial investments, faster growth rates, have shorter production cycles and greater adaptability to the environment as compared to large ruminants (Markos Tibbo *et al*., 2006). They are important protein sources in the diets of the poor societies and help to provide extra income and support the survival of many farmers in the tropics and sub-tropics (Markos Tibbo *et al*., 2006; Nottor, 2012; CSA, 2013).

Sheep and goats play an important economic role and make a significant contribution to both domestic and export markets through provision of food that is meat and milk and non-food which are manure for fertilizers, skin and wool products (Alvarez *et al.,* 2009; Gemeda Duguma *et al.,* 2012). Although sheep and goats play a significant role in the national economy of the country to date the benefit obtained from these small ruminants are hampered by different constraints. Small ruminant diseases are among the important top challenges that have slowed down the development of the livestock sector by decreasing production and hindering trade in animal and animal products (Jilo *et al.,* 2016; Nesradin Yune and Nejash Abdela, 2017). Among these infectious disease sheep and goat pox are the major and widely distributed diseases in all regions of Ethiopia (Dereje Tsegaye *et al.,* 2013).

Sheep and goat pox (SGP) are a systemic viral disease that causes high morbidity and mortality in sheep and goats (Nesradin Yune and Nejash Abdela, 2017). Characteristically, the disease is less commonly seen in indigenous sheep and goat breeds in areas where it is endemic as compared with exotic breeds (Takele Tesgera *et al*., 2018). Indigenous small ruminants are more likely infected by the disease in areas where it has not been establish or hidden for a period of time, when intensive husbandry systems are introduced or in association with other disease complications like *Peste des petits ruminants* (ESGPIP, 2009). Sheep and goat pox diseases are a serious problem in small ruminant production. SGP are important World Organization for Animal Health (OIE) trans-boundary and notifiable diseases of sheep and goats, respectively (Madhavan *et al*., 2016).

In general, *Capripoxviruses* (CaPV) are considered to be very host- specific because disease outbreaks or virus isolates may preferentially occur in one host species (Munz and Dumbell, 1994; Babiuk *et al*., 2009). This has been revealed specifically for Nigerian, Middle Eastern, and Indian strains of SPPV and GTPV and LSDV. However, some *Capripoxviruses* are not host-specific like Kenyan sheep and goat pox virus (KSGPV) and Yemen and Oman infect both sheep and goats (CFSPH, 2008; Yan *et al*., 2012).

Recent findings stated that some strains of SPPV in India and GTPV Africa, China and Yemen posed an outbreak in both species (Babiuk *et al*., 2009; Bahanuprakash *et al*., 2010; Yan *et al.,* 2012). However, in some outbreaks and experimental infections they are more severe in homologous host (Abu-Elzien *et al*., 2003; Babiuk *et al*., 2009). However, no evidence exists on *Capripoxviruses* infecting all the three species that are sheep, goats and cattle (Aberaham Damena, 2018).

*Capripoxviruses* are responsible for some of the most economically significant diseases of domestic ruminants in Africa and Asia (Bhanuprakash *et al*., 2006).Sheep pox and goat pox are enzootic in Africa, particularly to the North and West of the Sahara, in Middle and Far-East and Indian sub-continent. Transmission takes place through direct contact between infected and susceptible animals. Transmission also occurs via aerosol, insect vectors and contaminated inanimate objects. Transmission of SPPV and GTPV is thought to occur through exposure to aerosols or respiratory droplets produced by acutely infected animals or by direct or indirect contact with lesions or oro-nasal secretions (Balinsky *et al*., 2008).

These diseases are manifested by skin and internal organ and characterized by pox lesions, fever, conjunctivitis with oculonasal discharge and excess salivation. Occurrence of these diseases is associated with high morbidity, mortality and export restriction of sheep and goats and their by-products and hence it is economically an important disease (Babiuk *et al*., 2008). Morbidity and mortality vary with breed of small ruminants, its immunity to *Capripoxviruses*, and viral strain. Mortality in young animals can exceed 50% (Bhanuprakash *et al*., 2005). They also interfere with the import of new productive breeds because of the mortality reaches up to 50% in a fully susceptible flock and could be as high as 100% in young animals (OIE, 2012 Bhanuprakash *et al*., 2005).

Amplification methods for detection of the viral DNA genome are rapid, specific to the genus *Capripoxvirus* and sensitive for detection throughout the disease including before and after the emergence of antibody responses. These methods include conventional PCR, real-time PCR, and most recently loop-mediated isothermal amplification (OIE, 2017). Nucleic acid recognition methods can be used to detect the *Capripoxvirus* genome in biopsy, swab or tissue culture samples (OIE, 2017). Several PCR methods have been stated with varying specificity for *Capripoxviruses* in general, sheep pox, or goat pox virus (Zro *et al*., 2014a; OIE, 2017).

Conventional PCR methods are particularly useful for obtaining sufficient genetic material necessary for species identification by subsequent sequence and phylogenetic analysis (Le Goff *et al*., 2009). Several highly sensitive and specific fluorescent detection-based real-time PCR methods have been developed and validated (Balinsky *et al*., 2008; Bowden *et al*., 2008; Das *et al*., 2012; Stubbs *et al*., 2012). Each test detects a small conserved genetic locus within the *Capripoxvirus* genome. Real-time PCR methods for direct *Capripoxvirus* genotyping without the need for gene sequencing have been described (Lamien *et al*., 2011; Esayas Gelaye *et al*., 2013). Comparison between conventional gel-based PCR and real-time PCR techniques revealed that the later one is more sensitive, allowing detection of even low viral titers of *Capripoxvirus* (Tian *et al*., 2012).

The control strategy of sheep and goat pox varies according to the disease status of the country. Pox free countries depend on quarantine barriers together with test and slaughter techniques to prevent the entry of infected animals to maintain disease-free status of a country. Usually, these quarantine barriers are together with a stamping out policy whenever

the disease occurs; to prevent the reestablish disease. On the other hand, endemic countries such as Ethiopia focus on vaccination strategy to control or prevent the occurrences of small ruminant pox disease. Ring vaccination and restriction of animal movement are used to control outbreaks cases. For effective control of pox disease through vaccination, proper diagnosis of disease outbreaks and identification of the effective vaccine is essential. Though various kinds of vaccines are available; the live attenuated vaccine is the best choice (Bhanuprakash *et al*., 2006; Abdi Assefa, 2017). Live attenuated vaccines are considered more effective, providing immunity for one or longer years (Mondal *et al*., 2004).

In Ethiopia, wherever sheep, goats and cattle are affected, a live attenuated vaccine strain (KSGP-O-180) is used for immunization of both small ruminants and cattle. Although the existence of the disease in vaccinated small ruminants are frequently reported, information on the circulating isolates and their relation to the vaccine strain in use are still missing (Esayas Gelaye *et al*., 2015).

### 1.2.Statements of the Problem

Sheep and goats are important contributors to food production in Ethiopia, providing 35% meat consumption and 14% of milk consumption (Samuel Tefera, 2004; Solomon Gizaw, 2008). In central highlands where mixed crop- livestock production system has been practiced, small ruminants account for 40% of cash income and 19% of the household meat consumption (Samuel Tefera, 2004; Aberham Kebede *et al.,* 2018). Owing to their high fertility, short gestation interval and adaptation even in harsh environments, sheep and goats are considered as investments and insurance to provide income to purchase food during seasons of crop failure and to meet the needs of seasonal purchase of rural household such as improved seed, fertilizer and medicine (Samuel Tefera, 2004; Aberham Kebede *et al.,* 2018). Sheep and goat pox is one of the most important diseases of sheep and goats in Ethiopia following *Pesti des petits ruminants* (PPR) and *Contagious Caprine PleuroPneumonia* (CCPP) (Dereje Tsegaye *et al.,* 2013)

However, sheep and goat pox disease can result in heavy economic losses in the livestock sector and the most serious diseases among livestock pox infections. Sheep and goat pox (SGP) disease can result in a substantial loss in the production and productivity of sheep and

goats and it distributed in all regions of Ethiopia (Zinash Sileshi, 2009). Frequently, sheep and goat pox is a disease of considerable economic importance for Ethiopian sheep and goat farming (MORAD, 2010).

In Ethiopia very limited works has been done on sheep and goat pox virus but some researches have been made on participatory disease surveillance (PDS) in selected districts of Afar region (Chifira, Adaar, Amibra, Awash and Fenta) and Northeastern part of Ethiopia and central Ethiopia by Getachew Gari *et al.* (2015) and sero-prevalence, risk factors and distribution of sheep and goat pox virus in selected areas of Northwest Amhara region (North Gondar, South Gondar, East Gojjam, West Gojjam and Awi) administrative zone in Northwestern part of Ethiopia Tsegaw Fentie *et al.* (2017) and Furthermore isolation and characterization of pox virus was done by (Abdi Assefa, 2017; Aberaham Demena, 2018) in west Shoa and central Ethiopia respectively. A report on epidemiology and economic importance of sheep and goat pox: A review on past and current aspects indicated that the disease distributed in all regions of Ethiopia and economically important due to production loss and mortality (Dereje Tsegaye *et al.,* 2013; Nesradin Yune and Nejash Abdela, 2017).

Currently, at the national and regional level, there is no governmental strategy in control of SGP through vaccination and movement restrictions. Lack of vaccination strategies that is; vaccination coverage and timing of vaccination problems, presence of free animal movement without certifications and poorly established and implementations quarantine stations in the country that increase the spread of SGP along the small ruminant market chain. For the development of adequate SGP control and prevention of SGP outbreaks investigation and molecular characterization of the, virus is currently needed to mitigate the problem in the study areas. There is no recent study on the outbreak investigation and molecular characterization of sheep and goat pox virus infection in small ruminants in outbreak cases of Northwest Amhara. Circulating sheep and goat pox virus strain isolation and characterization had not been done in the study areas. Therefore, this research is initiated to produce informations on, outbreak investigation and molecular detection of sheep and goat pox diseases in Northwest Amhara National Regional State. Therefore, the study was initiated with the following objectives;

## 1.3. Objectives

1. 3.1.General Objective

- ❖ The general objective of this study was to investigate the outbreaks and molecular detections of pox virus circulating in sheep and goats in selected districts of West Gojjam and Awi zone Northwest, Ethiopia.

1.3.2. Specific Objectives

- ➢ To investigate sheep and goat pox outbreaks in selected districts of West Gojjam and Awi zone Northwest Ethiopia.
- ➢ To determine the morbidity, mortality and case fatality rates of SGP diseases.
- ➢ Molecular detection of pox viruses circulating in sheep and goats from outbreak cases.
- ➢ To determine the potential risk factors for the occurrences of sheep and goat pox diseases in the study area.

## 1.4. Research Questions

1. What does the outbreaks of sheep and goat pox currently looks like in selected districts of West Gojjam and Awi zone Northwest, Ethiopia?

2. What is the morbidity, mortality and case fatality of sheep and goat pox diseases in the study areas?

3. What are the molecular features *Capripoxvirus*?

4. Does the non-animal factors: season, geographical location significant risk factors for pox infection in sheep and goats in the study areas?

5. Does animal factors: age, sex, body conditions, vaccination status significantly associated with pox infection in sheep and goats in the study areas?

# Chapter 2. LITERATURE REVIEW

## 2.1. Sheep and Goat Pox

*Capripoxvirus* (CaPV) are considered as reportable agents to the World Organization for Animal Health (OIE) due to its potential for significant economic impact on the livestock industry and its rapid spread in wider areas. Sheep and goat pox (SGP) are serious, fatal viral systemic diseases (OIE, 2017). The diseases are characterized mostly by skin lesions extending all over the skin, but most obvious on face regions, eyelids and ears, perineum and under the tail and internal organs (Bhanuprakash *et al*., 2005).

Sheep and goat pox diseases in endemic areas are associated with significant production losses because of reduced milk yield, decreased reproduction, abortion, reduce the quality of wool, hides and meat posing nontariff barriers on international trade and increased susceptibility to other disease, while also being directly by causing high morbidity and mortality (Yeruham *et al*., 2007).

Generally, SPPV and GTPV infect and cause clinical disease in either sheep or goats, respectively, and most isolates induce more severe disease in either sheep or goats and only mild or sub-clinical infection in the other species (Babiuk *et al*., 2008). *Capripoxviruses* only infect some ruminant species like sheep, goats and cattle and have a tropism for certain epithelial cell types (McFadden, 2005). However; some isolates are pathogenic for sheep and goats, particularly some strains from central Africa like strains from Kenyan sheep virus (Madhavan *et al*., 2016).

Surprisingly, there is no evidence reservoir of wild ruminants for SPPV and GTPV viruses and assumed that wild life do not play a significant role in the epidemiology of SPP and GTP (Babiuk *et al*., 2008), although it cannot be excluded that wild sheep and wild goats can be infected with SPPV or GTPV. In support of this fact, lumpy skin disease virus, closely related to SPPV/GTPV, has been isolated from wild ruminants (Tuppurainen and Oura, 2012).

Human infections during the handling of infected animals are rarely seen (Rao and Bandyopadhyay, 2000). Mild lesions of small red papules followed by vesicles on the hands and arms have been reported in humans working with *Capripoxvirus* in Sweden and India. No general infections have occurred. These isolated incidents and humans are generally regarded as has not been susceptible by *Capripoxvirus* (Regnery, 2007). No pathogenicity for humans has been recorded for most sheep pox strains (Madhavan *et al*., 2016).

Usually, nomenclature of SPPV, GTPV and LSDV has been based on the country and the animal species from which the virus was first isolated, but cross-species transmission may complicate the situation since this has been based on field observation of the species affected whether sheep or goats or both (McFadden, 2005). Given the large size of the viruses and the complexity of encoded factors likely to determine host specificity, currently there are no molecular criteria upon which to base strain (sheep or goat or sheep and goat) designation.

### 2.1.1. Taxonomy

Sheep and goat pox diseases have resulted from infection caused by sheep pox virus (SPPV) or goat pox virus (GTPV) of the *Poxviridae* family, *Chordopoxvirinae* sub-family and *Capripoxvirus* genus (Buller *et al*., 2005; Gitao *et al*., 2017). It is one of the largest (170 to 260 nm by 300 to 450 nm) capsid, enveloped double-stranded DNA viruses (Matthews, 1982; Tulman *et al*., 2002). The genus *Capripoxvirus* comprises of sheep pox virus (SPPV), goat pox virus (GTPV), lumpy skin disease virus (LSDV) which causes disease in sheep, goats and cattle, respectively (OIE, 2014). Various strains of *Capripoxvirus* are responsible for the small ruminant pox disease and these are antigenically and serologically indistinguishable from strains causing sheep pox and goat pox but different at the genetic level (Babiuk *et al*., 2008). There are no serotypes of *Capripoxvirus* as SPPV, and GTPV and LSDV are antigenically indistinguishable (Kitching, 1986).

### 2.1.2. Characteristics of *Capripoxvirus*

*Capripoxviruses* have prolonged survival in the environment, resistant to drying and freezing, thawing and remain viable for months in the lyophilized tissues. But it is sensitive to ether, chloroform, 1% formalin and extremely high and low pH (Fulzele *et al*., 2006; OIE, 2014). They are inactivated by sun light and heat, but can survive in cool dark environment for up to

six months (Davies, 1981). Virus can be destroyed at 56°C for 2 hours or 65°C for 30 minutes. High alkaline and acidic pH is detrimental to SPPV and GTPV. Infectivity of the virus gets affected by repeated freezing and thawing (Garnar *et al*., 2000).

### 2.1.3. Virus Morphology and Genome Structure

Among the viruses, *Capripoxvirus* is the largest one with a brick-shaped morphology of 170 to 260 by 300 to 450 nm in diameter capsid (Tulman *et al*., 2002; Karapinar1 *et al*., 2017). Virions are brick-shaped, enveloped with round ends, have a complex symmetry and about 300×270×200 nm in size (Dashprakash *et al*., 2015). Its genome is double-stranded DNA, non-segmented and linear of approximately 154 kb in size (King *et al*., 2011). Within the sub-family Chrodopoxvirinae, CaPVs have the highest A-T content that is 73 to75%. An extensive DNA cross-hybridization between species of the genus *Capripoxvirus* is reported (Black *et al*., 1986). Sheep pox and goat pox viruses share 147 orthologous genes that encode proteins of 53 to 2,027 amino acids in size likely involved in viral replication, structure, virulence and host range functions (Tulman *et al*., 2001). The SPPV, GTPV and LSDV exhibit 96% nucleotide and amino acid identity over their entire length (Tulman *et al*., 2002; Zeng *et al*., 2014).

The central genomic region bounded by two identical inverted terminal repeats (ITR) at the ends of SGPV (ORFs 024 to 123) contains homologues of conserved pox virus genes involved in basic replication mechanisms, including viral DNA replication, viral transcription, RNA modification, and structure and assembly of intracellular mature and extracellular enveloped virions (Moss, 2001; Tulman *et al*., 2001, 2002; Kara *et al*., 2003) whereas, the terminal genomic regions (ORFs 001 to 023 and 124 to156) contain genes involved with putative virulence, host immune evasion and host range functions (Tulman *et al*., 2001; Madhavan, 2016). P32 is one of the structural proteins shared by all *Capripoxviruses*. Protein 32 contains major antigenic determinants (Chand, 1992; Gu *et al*., 2018) which are important for the pathogenicity, diagnosis, prevention, and control of *Capripoxvirus* infections. The fourth membrane protein, which exists in all pox viruses and encoded by the L5R gene, is essential for cell entry, cell to cell fusion, plaque formation, and infectious virion production (Townsley *et al*., 2005). A false lipid envelope surrounds the genome (Kitching, 2004; Tulman *et al*., 2002; Gitao *et al*., 2017).

All genes of SPPV and GTPV are present in LSDV, however, LSDV has an additional nine genes including a gene unique to LSDV (LSDV132) that are non-functional in sheep pox and goat pox viruses, some of which are likely responsible for their ability of virulence and to infect cattle which are disrupted in SPPV and GTPV (Tulman *et al*., 2001). Both sheep pox virus and goat pox virus are likely derived from LSDV like ancestor but they possess specific nucleotide differences suggesting that both are phylogenetically distinct. Sheep pox virus and goat pox virus genomes sequences are phylogenetically distinct from each other and LSDV, and they contain species-specific nucleotide variations that may be associated with features of host range. Relatively few genomic changes in SPPV and GTPV vaccine viruses reason for viral attenuation (Tulman *et al*., 2002).

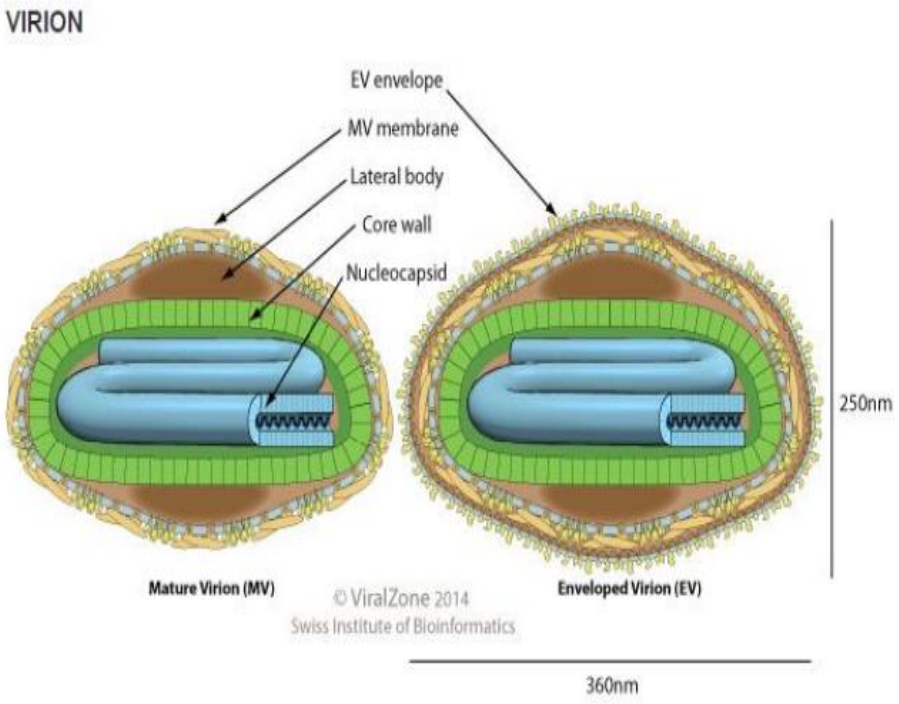



Figure 2. 1 Morphology of *Capripoxvirus* (Viral zone, 2014)

**2.2. Epidemiology**

2.2.1. Global Distribution of Sheep and Goat Pox

There are distinct differences between the geographic distribution of sheep pox, goat pox and lump skin diseases viruses. Geographical distribution of the SGP has been relatively stable

(Mirzaie1 *et al*., 2015). Goat and sheep pox was first reported in 1879 in Norway and later observed in Macedonia during the First World War, where it became enzootic in the year 1926, with a mortality rate of 15% (Rao and Bandyopadhyay, 2000). Sheep and goat pox has been seen in north and central Africa excluding South Africa, Middle Eastern countries, central Asia as indicated in Figure.2.2 (Orlova *et al*., 2006). These diseases are endemic in Nepal, China, Bangladesh, Equator, Iran, Turkey, Pakistan, Iraq, Afghanistan, Indian subcontinent. Sporadic outbreaks occur in southern Europe and other parts of the world (Rao and Bandyopadhyay, 2000; OIE, 2010). Sheep and goat pox extended their range into Bangladesh in 1984 (Kitching *et al*., 1987).

Recent outbreaks have occurred in Vietnam in 2005 and 2008, in Mongolia in the years 2006 and 2007 in the east areas, Kazakhstan and Azerbaijan in 2008 and 2009 and Bulgaria, Greece and Turkey in 2013, in Israel in 2014, and Russia and Mongolia in 2015 and repeated incursions have been reported in Greece in southern Europe 2007 (OIE, 2008). *Capripoxviruses* are not present in north, central or south America, southeast, Asia excluding Vietnam or Australia. The *Capripoxvirus* prevalence is going up, which is evident from outbreaks in Vietnam, Ethiopia, Mongolia, Egypt, Greece and Israel (Takele *et al*., 2018).

The spread of sheep and goat pox virus in to new areas is predominantly associated with the increase of illegal animal movements and their products through trade (Domenech *et al*., 2006) as well as inadequate quarantine services (Rweyemamu *et al*., 2000). Poor quarantine measures and trade across the boundary of live animals may lead to further spread of the disease (Babiuk *et al*., 2008). Countries free of sheep and goat pox virus usually have in place legislation based on World Organizations for Animal Health (OIE, 2008) recommendations that challenge to prevent the trans-boundary spread of production limiting diseases, but increasingly these are becoming more difficult to enforce, including on the border of the European Union (EU). Biting flies have also been implicated in the spread of *Capripoxviruses* (Babiuk *et al*., 2008*).* The impacts of global climate change on insect vectors, established as a route of transmission for sheep and goat pox viruses because of very high viral loads in the skin, suggest that there are real risks of further spread of pox diseases in to other geographic regions (Domenech *et al*., 2006).

According to the study carried out by Bhanuprakash *et al. (*2005) outbreaks were recorded during all months of the year, but mostly occur between November and May, and the peak outbreaks occurred in March.The seasonality of SGP observed could be explained either by the capability of the viruses to survive for many months in wet and cold weather in association with the lambing and kidding season, or by the poor physiological condition of flocks in the autumn (Bhanuprakash, 2005; Yeruham *et al.*, 2007; Zangana and Abdullah, 2013).

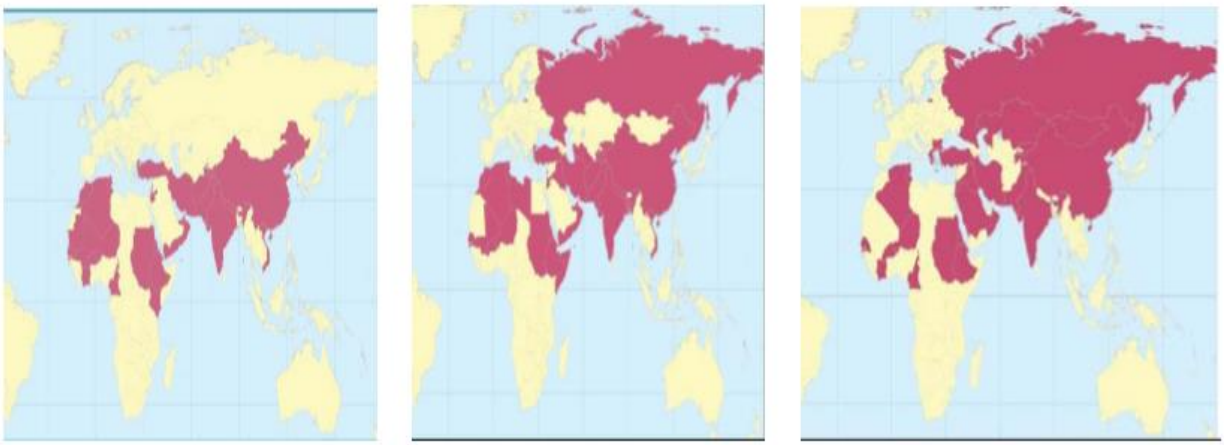



Figure 2. 2 Global occurrences of SPP and GTP (OIE, 2010, 2013, 2015)

### 2.3. Source of Infection and Mode of Transmission

Sheep pox and goat pox viruses are highly contagious and spread through aerosols and close contact with infected animals and by indirect means as contamination of cuts and abrasions (Kitching and Carn, 2004). Transmission of SPPV and GTPV occurs by direct and indirect contact to aerosols, respiratory droplets or contact with oronasal secretions produced by acutely infected animals (Bowden *et al*., 2008; Verma *et al*., 2011).

Transmission of sheep and goat pox between and among flocks occurs from the movement of sheep and goats (Bhanuprakash *et al*., 2005; OIE, 2008; Gitao *et al*., 2017). Sheep pox virus and goat pox virus appear to be transmitted mainly during close contact, but also occur in contaminated environments. These viruses enter via the respiratory tract and the body through

mucous membranes or abrasion skins. Sheep and goat pox virus can shed in saliva, nasal and conjunctival secretions. The viruses are also abundant in skin lesions and their scabs, and also it has been detected in milk, urine, feces and semen. Animals are most contagious during the first week of the diseases after the onset of clinical signs, but some experimentally infected sheep and goats continued to shed smaller amounts of virus in nasal, conjunctiva and oral secretions for one to two months. Sheep and goats do not develop chronically infected carriers for sheep and goat pox diseases. Spread of the diseases also happen from contact with contaminated materials and through skin abrasions produced iatrogenically or by insects (Gitao *et al*., 2017). Movement of infected small animals is the main cause of spreading SGP viruses (Kitching, 2004; Radostits *et al*., 2006).

### 2.3.1. Direct Transmission

The main route of transmissions for SGPV is direct contact between an infected and a susceptible sheep and goats (Nesradin Yune and Nejash Abdela 2017). Upon infection, small ruminants develop pox lesions in the mucous membranes of the mouth, nasal cavities and conjunctiva, perianal regions then excreting the virus in saliva and ocular and nasal discharges (Bhanuprakash *et al*., 2005). The virus spread in droplets or aerosols via coughing, sneezing, head shaking, vocalizations and breathing. Naive animals acquire the virus through inhalation, orally or skin abrasions (Babiuk *et al*., 2008). Infected animals have high virus titers in skin lesions and scabs, and skin to skin contact can directly spread the virus through skin abrasion (Bowden *et al*., 2008). Suckling lambs and kids may contract infection from the milk and the skin of the teats (Babiuk *et al*., 2008).

Significantly, animals are already shedding an infectious virus when the first clinical signs of SPPV and GTPV are detected in the flock which includes a high fever; nasal and ocular discharge (Bowden *et al*., 2008). Animals with mild clinical disease, with only a few pox lesions on the skin and mucous membranes, do not spread the virus as successfully as animals with severe signs, although they are still infectious (Carn and Kitching, 1995).In endemic regions, infections may go unnoticed, and in that situation movement of animals from infected farms entirely for months after recovery commonly lead to the introduction of the disease in to naive flock (Bhanuprakash *et al*., 2006).

2.3.2. Indirect Transmission

Virus in saliva, ocular secretions and nasal discharge, skin lesions and scabs, urine and feces have the chance to contaminate feed, water, wool and the environment, leading to an indirect transmission of the virus either orally or via skin abrasions. Infectious virus is well protected inside scabs, which are shed by infected animals; after scabs dissolve, the virus may be released in to the environment and this may continue for several months after the outbreaks (Rao and Bandyopadhyay, 2000). Untreated skins, hides and wool collected from infected animals may contain skin lesions and scabs with viable virus consequently; it may be a source of infection for naive sheep and goats (Rao and Bandyopadhyay, 2000).

The high concentrations of the virus in the skin may also contribute to the spread of sheep and goat pox virus through insect vectors (Babiuk *et al*., 2008). High virus titers and intrinsic resistance of the virus and vectors with large mouth parts and their frequent feeding habits are the basic factors favoring mechanical transmission by insect bites. There is no evidence that SPPV and GTPV can replicate in arthropod vectors (Kitching and Mellor, 1986).

## 2.4. Associated Risk Factors

2.4.1. Host Related Risk Factors

Sheep and goats of all age, breed and sex are susceptible to sheep and goat pox. In areas where sheep pox is enzootic, imported breeds such as Merinos or some European breeds may show greater susceptibility than the native flocks. *Capripoxviruses* are generally considered to be host specific even though some strains found to infect both sheep and goats while, most SPPV and GTPV isolates show distinct host preferences with more severe disease evident in the homologous host (Bhanuprakash *et al*., 2006; Babiuk *et al*., 2009).There are two types of sheep pox virus (Singari *et al*., 1990), one affects both sheep and goats which is Kenyan sheep and goat pox (KSGP) strain while the other is host-specific like Nigerian, Indian and Middle East strains. Recent findings indicate that strains of sheep pox virus cross infect between sheep and goats, although most cause more severe disease in sheep.

2.4.2. Environmental Risk Factors

Environmental factors play a great role in the occurrence of sheep and goat pox. It had an impact on the agent, host and vectors as well as interaction between them (Nesradin Yune and Nejash Abdela, 2017). These influencing factors have a great role in the maintenance of *Stomoxys calcitrans* vector and tsetse fly to susceptible animals which are the vectors for mechanical transmission of pox disease (Webbs, 1980; Mellor *et al*., 1987).

The *Stomoxys calcitrans* (stable fly) and Musca species flies have been implicated in mechanically transmitting the virus to a susceptible sheep and goat in 24 hours once feeding on exudate from lesion which was contaminated (Bhanuprakash *et al*., 2006). Insects act as mechanical vectors of the virus rather than biological. There is no evidence of the virus persisting longer than 4 days in insects. No transmission was detected with biting flies that are Mallophaga species, Damalinia species, *Hydrotaea irritans* (sheep head flies) and *Culicoides nubeculosus* (midges) even though virus could be isolated from previously infected sheep, which was the host for feeding of the above mentioned flies (Kitching and Mellor, 1986).

2.4.3. Pathogen Risk Factors

The *Capripoxviruses* have prolonged survival in the environment and inactivated by drying, freezing, thawing, and remain viable for months in the lyophilized state. It can remain infectious for up to 6 months in sheep pens, and may also be found on the wool or hair for 3 months after infection (Nesradin Yune and Nejash Abdela, 2017)). But it is sensitive to 1% formalin and extreme pH. *Capripoxvirus* are highly stable in normal environment condition and can survive for a prolonged time, with or without susceptible animal. They are inactivated by sun light and heat, but can survive in cool dark environment for up to 6 month (Nesradin Yune and Nejash Abdela, 2017).

**2.5. Pathogenesis**

Sheep and goat pox is acute febrile and highly contagious trans-boundary viral disease of sheep and goats (Mangana-Vougiouka *et al*., 2000; Babiuk *et al*., 2008; Madhavan *et al*., 2016). In natural cases, the diseases have an incubation period of sheep pox is 4 to 8 days and

that of goat pox is 4 to 14 days. The pathogenesis of the sheep and goat pox depends on both the strains of *Capripoxvirus* and breed of host (Madhavan *et al*., 2016).

*Capripoxviruses* have a predilection sites for epithelial cells of the skin and lungs (Bowden *et al.*, 2008*;* Embury-hyatt *et al*., 2012). After *Capripoxvirus* inters it, replicates locally in the tissues. Since the virus is epitheliotropic, it will invade the epithelium tissues of the animals. Also besides, virus replication is correlated with the severity of clinical disease that is the highest level of virus replication occurs in association with severe disease. On the $7^{th}$ day post-inoculation, the virus titer reached to its peak. The virus spread to the regional lymph nodes, after 3 to 4 days of primary viraemia. The viraemia spread in the body, and then affect spleen, lungs and liver. The inhaled virus may also cause lung lesions. The skin nodules developing from 7 to 14 days after inoculation, the virus titers persisted and decreased with the development of serum antibodies. Within 24 hours of the manifestations of generalized papules, affected animals develop conjunctivitis, rhinitis and enlargement of all the superficial lymph nodes, in particular the pre-scapular lymph nodes. Excessive salivation can also occur after infection (CFSPH, 2008; OIE, 2012).

There are five stages in the development of pox infection usually observed and characterized by the appearance of skin lesions on the entire body surface evolving from macules, papules, vesicles or vesiculo- pustules and scabs at the end of disease development (Yan *et al*., 2012). The roseola stage is when the skin lesions typically begin with small red spots within 3 days of infection which followed by papules**.** The second stage of the pox lesion is papules which develop after 3 days of the roseola stage. Papule within 5 to 6 days changed to vesicles and leads vesicular stage then after 3 days of vesicular stage pustular stage developed lastly scab stage formed (Bowden *et al*., 2008).

### 2.6. Replication of Pox Virus

The pox viruses replicate entirely in the cytoplasm. The assembly of progeny virions is located in specific cytoplasmic places called viroplasm or viral factories, which can be separated from other cellular structures in the form of virosomes. The cytoplasmic mode of replication implies that the machinery necessary for RNA synthesis and modification has to be carried out by the incoming particles for the early viral genes to be expressed. Consequently among the active enzymes found in proteins included in the capsid, including

RNA polymerase, capping and methylating enzymes, and a ploy A. polymerase. These enzymes upon un-coating of the virus, start the transcription of a series of early genes, among which are those responsible for DNA synthesis, such as DNA polymerase, ligase, thymidine kinase and thymidine synthases. After the DNA synthesis has been taken place, the mode of transcription changes to a discontinuous one to transcribe the so-called late genes like structural genes. The life cycle is divided into several steps which include; penetration and un-coating within 30 minutes, early transcription it lasts 1 to 2 hours, DNA synthesis it takes 2 to 4 hours, late transcription and assembly ends within 4 to 6 hours, and release (budding) of the virions. During the late transcription period many early genes cease to be transcript. In the late transcription phase of the life cycle, the structural viral proteins are expressed. An early effect of pox virus infection on the host cell is the inhibition of cellular protein and DNA synthesis (Talavera and Rodriguez, 1991; Aberham Damena, 2018).

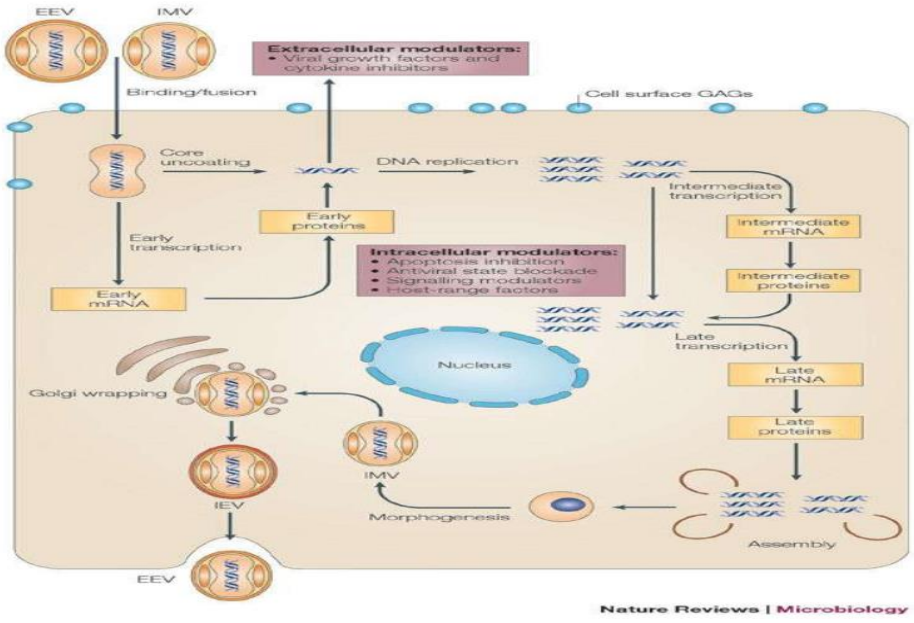



Figure 2.3 Replication cycle of poxvirus (Viral zone, 20117)

**2.7. Clinical Signs**

Clinically, both sheep pox and goat pox have similar clinical signs (Bowden *et al*., 2008). The incubation period of the disease varies from 4 to 15 days succeeding contact between infected and susceptible animals. The clinical sign of sheep and goat pox can be either malignant or benign. The malignant form of sheep and goat pox is mostly common in lamb and kids and

the benign form is frequently observed in adult sheep and goats. In field condition there was an initial rise in rectal temperature (40°C to 42°C), rise pulse and respiratory rates, edema of the eyelids, loss of appetite, arched back, lacrimation, coughing, salivation and nasal discharge leading to crust formation, pneumonia, hypersensitivity, constipation and scanty urine (Radostits *et al*., 2006; Mirzaie1 *et al*., 2015).

After 1 to 2 days, skin eruptions appear over the less wooly parts of the body. The lesions undergo macular, papular, vesicular, pustular and scabular stages typical to any pox disease. Scabs persist for 3 to 4 weeks and after healing cicatrix may remain. Septicemia or viraemia due to secondary bacterial complications may result in the death of animal during the febrile eruptive phase of the disease in cases of hemorrhagic pox. Aggravations of latent brucella, tendovaginitis, orchitis, abortion and peripheral paresis have also been reported after sheep and goat pox infections (Radostits *et al*., 2006; Mirzaie1 *et al*., 2015).

### 2.8. Diagnosis

Diagnosis of *Capripoxvirus* has been done clinically on the basis of signs and lesions, species of the host affected and post-mortem findings. Clinical pathology and epidemiology of the diseases are also important in diagnosis of sheep and goat pox. However, sheep and goat pox should be differentiated from contagious ecthyma, blue tongue, Pesti des petits ruminants, foot and mouth disease, dermatophilosis, mange mites, photosensitization, parasitic pneumonia, multiple insect bites and caeous lymphadenitis as they cause similar form of skin lesions in affected hosts (Rao and Bandyopadhyay, 2000; OIE, 2014; Madhavan *et al*., 2016).

As the virus of sheep and goat pox are very closely related it's indistinguishable by serologically and antigenically (Babiuk *et al*., 2008; Venkatesan *et al*., 2012). It appears that the host preference shown by these viruses concerning either sheep or goats, accompanied by the case history, may be regarded as partially affirmative for either sheep pox or goat pox, but confirmatory diagnosis requires laboratory studies similar to molecular technique is necessary to conclude the outbreaks of the diseases (Oguzoglu *et al*., 2006). It is also known that heterologous diagnostic reagents tend to be less efficient than homologous reagents for confirmatory diagnosis (Nesradin Yune and Nejash Abdela, 2017).

### 2.8.1. Clinical Signs

Clinically, both the diseases exhibit fever and generalized pock lesions, coughing and salivation, edema of eyelids, diarrhea, emaciation and arched back followed by progressive development of erythematous macules, papules, vesicles, pustules and scabs on skin (Venkatesan *et al*., 2012). Lesions may also develop on the mucous membrane and internal organs. Small ruminants developing lung lesions may have respiratory signs including coughing, nasal discharge and dyspnea. However, the lesions are restricted to a few nodules under the tail and are thus only identified on close examination particularly in enzootic countries (Bhanuprakash *et al*., 2006).

### 2.8.2. Post Mortem Lesions

Lesions of sheep pox and goat pox can be developed in lungs, kidneys, spleen, lymph node and other internal organs (Mondal *et al*., 2004). Lesion may also present in the mouth, nasal cavities, eyes, and eyelids and on the carcass. Affected mucous membranes may become ulcerated or slough and necrotized. Nodules may occur in the digestive, respiratory and urogenital system. Skin lesions have congestion, hemorrhage, edema, vasculitis and necrosis involving all layers of the epidermis, dermis, and, in severe cases, extend in to the adjacent musculature. Characteristic pox lesions disperse all over the body of the affected animals with nodular lesions observed in the lung tissue of the dead animals (Mondal *et al*., 2004). Postmortem finding of dead animals shows vesicles and pox lesions on affected skins. The lungs are diffusely inflamed that is bronchopneumonia with diffused pox lesion (Dubaib, 2002).

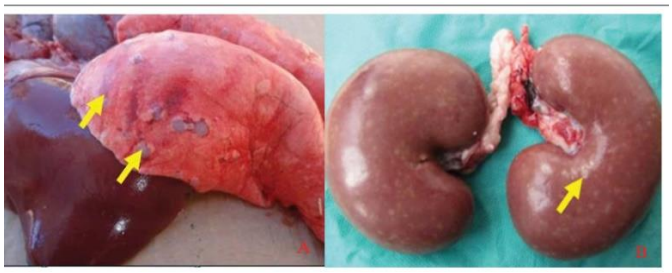


Figure 2.4 Post mortem lesions of SGP on lung (A) and kidney (B) from left to right **(**Tesgera *et al*.,2018

### 2.8.3. Histopathology

Microscopically, epidermal thickening, hyperplasia, acanthosis, hydropic degeneration of prickle cell layer, micro-vesiculation and necrotizing vasculitis observed. Characteristic large intra-cytoplasmic eosinophilic inclusion bodies noticed in the dermal cells of the epithelial (Singh *et al*., 2007; Pawaiya *et al*., 2008). The microscopic lesions supported by the gross lesions and characterized by the entry of sheep pox cells and lymphocytes in lung, skin, heart and spleen while skin scabs revealed hydropic changes, acanthosis and necrosis in epithelial layers (OIE, 2017). Microscopic examination revealed that marked proliferative change especially in the ectodermal layers with the presence of intra-cytoplasmic acidophilic inclusion bodies (Joshi *et al*., 1995).

*Capripoxvirus* can be seen under an electron microscope and can be readily differentiated from the virions of *Parapoxvirus,* which cause contagious pustular dermatitis however; indistinguishable from those of *Orthopoxvirus,* cause diseases in humans but, *Orthopoxvirus* did not show lesions in sheep and goats (OIE, 2017).

### 2.8.4. Laboratory Diagnosis

Even though skin and visceral pox lesions caused by *Capripoxviruses* are strongly suggestive of the diseases in question, a definitive diagnosis requires laboratory confirmation. Before collecting and sending any samples, the proper authority's samples should be under secure conditions and to authorized laboratories to prevent the spread of the disease. Samples for *Capripoxvirus* molecular characterizations must be sent to the laboratory as soon as possible. The sample should be kept in cold chain and shipped on an icebox with ice packs. If these samples must be shipped long distances without refrigeration, glycerol (10%) can be added; tissue samples must be large enough that glycerol does not penetrate the center of the tissue and destroy the virus (OIE, 2014).

Initially, agar gel diffusion test (AGPT) was the principal test for identifying these viruses. With the advancement of time, the uses of soluble antigen fractions from the viruses have been integrated and several test and their modifications have been developed (Rao and Bandyopadhyay, 2000). Virus neutralization is the most specific serological test but not adequately sensitive since immunity to *Capripoxvirus* infection is predominantly cell-

mediated, infected animals may only produce undetectable low levels of neutralizing antibody. Neutralizing antibodies can interfere with the isolation of virus and some antigen detection tests; samples for these tests must be collected during the first week of illness. Enzyme-linked immune sorbent Assay (ELISA) using P32 antigen or another appropriate antigen expressed by a suitable vector could be used to develop an acceptable and standardized serological test. An antigen detection ELISA is also available (Radostits and Hinchcliff, 2006; Animal Health Australia, 2011). For the detection of antibodies specific for CaPVs in sheep, goats and cattle sera, an indirect ELISA has also been developed (Babiuk *et al*., 2009).

Western blotting of test sera against *Capripoxvirus* infected cell lysate provides a sensitive and specific system for the detection of antibody to *Capripoxvirus* structural proteins, although the test is expensive and difficult to carry out (Chand *et al*., 1994; OIE, 2000).

Using electron microscopy, large numbers of characteristic pox cells containing inclusion bodies and typical *Capripoxvirus* virions have been seen in biopsies of the skin. Virus detection can be done before the development of neutralizing antibodies. Direct fluorescent antibody test is used to detect the presence of pox virus in the edema fluid and the antigen can be detected in biopsies of lymph glands by AGID using specific immune serum.

Virus isolation is also considered a gold standard for the diagnosis of viral diseases, but its use in the detection of *Capripoxvirus* is limited due to the long incubation period that is 2 to 4 weeks typically needed to obtain results (Bowden *et al* ., 2008). The virus can be cultured in tissue culture but virus isolation as a method of rapid diagnosis is limited and it takes time for a virus to develop cytopathic effects and also it needs with some strains for several blind passages.

Sheep and goat pox require an urgent and precise laboratory confirmation as the diseases are severely contagious. Rapid laboratory confirmation of sheep and goat pox virus based on clinical signs, electron microscopy and different serological tests are not always reliable. Even, virus isolation in cell culture and ELISA fail to detect virus particles that are bound to neutralizing antibody (Ireland and Binepal, 1998). To overcome these limitations, simple, rapid and specific PCR techniques have been developed to detect CaPV DNA in infected cell

culture supernatants and skin biopsy samples (Ireland and Binepal, 1998; Heine *et al*., 1999) and also used to differentiate SGPV and LSDV on the basis of unique restriction sites in the corresponding PCR fragments (Heine *et al*., 1999).

The PCR technique becomes more effective for the diagnosis of SPPV and GTPV from field samples when combined with restriction enzyme analysis (REA) of PCR amplicons (Rao and Bandyopadhyay, 2000). Recently, SPPV and GTPV from infected cell culture supernatants and skin biopsy were differentiated by REA of PCR amplified P32 gene products (Hosamani *et al*., 2004).

Molecular diagnosis via amplification of genetic material by using DNA by PCR product became a "gold standard" for rapid diagnosis of viral diseases, including CaPV. Several PCR based assays have been reported for rapid diagnosis of CaPVs, including conventional PCR and real-time PCR or quantitative PCR (Balinsky *et al.*, 2008; Bowden *et al*., 2008).

### 2.9. Economic Importance

Sheep pox and goat pox are highly devastating systemic viral disease of sheep and goats and are the most common cause of economic loss in Ethiopia. The disease is fatal in newly introduced animals, however, mild in indigenous breeds of small ruminants from the endemic region. The outbreak of sheep pox and goat pox may cause serious problems and economic loss in sheep and goat industries (Garner and Lack, 1995). In endemic areas the disease is economically important due to losses of production, decreased weight gain, loss of live weight, reduction of milk yield, damage to hide and skin, cause abortion and increase susceptibility to fly strike and other diseases while also cause a direct death of animals

Sheep and goat pox (SGP) is one of the animal bioterrorist diseases as it causes high morbidity and mortality, has the potential to rapidly spread, and cause serious socio-economic losses of major importance in the international trade of animals and animal products (OIE, 2008). Sheep and goat pox virus is one of the 15 listed A diseases by the World Organization for Animal Health (OIE, 2004) and by Animal and Plant Health Inspection Agency (USDA, 2002) which can be used as an animal biological wale fare agent.

Flock size, number of adult small ruminants and number of days of illness play significantly influencing the economic losses due to sheep and goat pox (Senthilkumar and Thirunavukkarasu, 2010).

### 2.10. Treatments

There is no specific treatment for sheep pox and goat pox infection so, the treatment should include use of broad spectrum antibiotics and use of supportive treatments directed to control secondary bacterial complications (OIE, 2017). Clean, ventilate enclosure and provide a balanced diet as supportive therapy recommended. Wash and clean the nostril with a weak solution of potassium permanganate (1:10000) to relieve respiratory-related sign. Topically apply antibiotic ointment is important for skin lesion (Senthilkumar and Thirunavukkarasu, 2010).

### 2.11. Control and Prevention

Socio-economic and political stability, availability of veterinary services and adequate infrastructure and logistic supports are essential for implementing effective control programs (Bhanuprakash *et al*., 2011).

Countries that are considered free from *Capripoxvirus* they maintain their diseases free status by restriction on import of livestock and animal products from enzootic areas. Infected animal products should be decontaminated before entry to non-enzootic status. In the case of disease-free countries, the implementation of a radical slaughter policy, restricted animal movement coupled with ring vaccination with live attenuated vaccine will help in the elimination of the disease (Carn, 2000).

Control of sheep and goat pox, once the disease has been entered, is usually by early detection and notification; prompt movement restriction of animals, culling infected and contact animals, and ring vaccination with a live attenuated vaccine. Control of *Capripoxvirus* infections is possible by application of appropriate diagnostics, use of effective vaccine(s) and animal management to restrict movement of animal and their products to prevent introduction in to naive areas (Bhanuprakash *et al*., 2011). After specific diagnosis, active mass immunization is the main approach to control *Capripoxvirus* infection.

Routine control measures of sheep and goat pox include cleaning and disinfection of depopulated premises and establishment of protection and surveillance zones, with a radius of 3 and 10 km, respectively, around the outbreak areas as recommended by the EU council directive (Mangana *et al*., 2008). Sheep and goats introduced to endemic areas should be quarantined for 21 days. Quarantine of areas and premises containing infected or exposed animals is required to prevent the disease spread. Infected herds and sick animals should be isolated from susceptible ones for at least 45 days after recovery ( OIE, 2017).

Movement of sheep and goats from infected to non-infected areas and movement of small ruminant products containing meat, wool, hair and skin from infected areas should also be controlled. The virus may persist for up to 6 months in shaded, unclean shelters and for 3 months in dry scabs on the skin, tools and equipment that have been in contact with infected animals must be cleaned and disinfected with disinfectants like ether, formalin, sodium hypochlorite, 2% hydrochloric acid or phenol. It helps to remove part of the top dirt and burn it. When sheep and goat pox outbreak occurred, affected animals should be isolated immediately. Shelters should be cleaned and disinfected. Carcasses and contaminated materials should be buried or burned.

Sheep and goats around the outbreak areas should be vaccinated as soon as possible. In areas of frequent SGP occurrence, the most effective means of controlling is annual vaccination with a live attenuated vaccine. Massive vaccination leading followed by animal movement control can be an effective strategy to control SGP if the disease has spread extensively in an area. Vaccination with commercially available live attenuated vaccines has been applied as the main control measure for SPP or GTP in endemic regions. Annual vaccinations using live attenuated KSP vaccines provide good protection and can control the outbreaks when the minimal coverage of 75 % is reached and maintained. Experience obtained from the FAO Regional Animal Disease Surveillance and Control Network for SPP eradication program in 2000 within Maghreb countries demonstrated that a considerable reduction in SPP and GTP cases had been achieved when the goal for vaccination coverage was set between 75% and 90 % (FAO, 2001).

The main factors that favor control of SGPV in enzootic countries include easy detection of the disease, high economic impact of the disease, and absence of reservoir hosts other than

domestic ruminants, induction of solid immunity after vaccination and a relatively low annual turnover rate of animals in flocks. In contrast, the factors which may hinder control of the diseases are prolonged stability of the virus on wool, long incubation period of the disease, and unrestricted introduction of new animals (Bhanuprakash, 2011; Gitao *et al.*, 2017).

### 2.12. Status in Ethiopia

Sheep and goat pox (SGP) disease can results in a substantial loss in the production and productivity of sheep and goats in Ethiopia. Regarding the status of the disease in Ethiopia sheep and goat pox was found almost all regions of Ethiopia (Zinash Sileshi, 2009). This disease is comparably more serious in lowland arid areas than in midland and highland agro-ecologies (Zinash Sileshi, 2009).

In Ethiopia, a total of 57,638 sheep and goats contracted the disease and 4,853,347 sheep and goats were at risk in areas where outbreaks occurred. Out of the 57,638 sick sheep and goats, 6,401 animals died with the case fatality rate of 11.11%. The disease reporting status in Ethiopia is only about 35-40%. The actual figures in terms of affected, vaccinated and dead animals are, therefore expected to be higher than the reported numbers (ESGPEP, 2009). Although, there were no detailed studies on prevalence of SGP in Ethiopia, some reports indicate that it is one of the widely distributed and it is the common small ruminant production problems in Ethiopia (Bhanuprakash *et al*., 2006).

The prevalence of pox was 10.34% and 12.88% in sheep and goats, respectively in Adama town, Oromia Regional State (Yakob Hailu *et al.,* 2008). According to Moges Woldemeskel and Gashaw Marsha in 2010, the prevalence of pox was 22% in sheep and 18% in goats in Wollo, Northeast Ethiopia. The sero-prevalence of sheep and goat pox was 17% and 15.5% respectively in Northwest Amhara Region (Tsegaw Fentie *et al.,* 2016). In recent study based on Daniel Teshome (2016) findings the prevalence was 40% in sheep and 8.12% in goats in Gondar University veterinary clinic. According to Bereket Molla *et al*. (2017) the prevalence was 31.96% in sheep and 35.28 % in goats in Gam Gofa zone of SNNRP. According to Aberham Kebede *et al*. (2018) the overall prevalence of small ruminant pox was 11.23%, out of which 12.9 % were goats and 9.5 % were sheep (Table 2.1).

Table 2. 1 Sero-prevalence of sheep and goat pox in different areas of Ethiopia

| **Area** | **Prevalence in sheep** | **prevalence in goats** | **References** |
|---|---|---|---|
| Adama | 10.34% | 12.88% | Yakob Hailu *et al.(*2008) |
| Wollo | 22% | 18% | Moges Woldemeskel and Gashaw Mersha (2010) |
| Ethiopia | 49.5% | - | Mersha Chanie (2011) |
| Western Amhara | 17% | 15.5% | Tegaw Fentie *et al*.(2016) |
| Gam Gofa zone | 31.96% | 35.28% | Bereket Molla *et al*.(2017) |
| Dibate District | 9.5% | 12.9% | Aberham Kebede *et al*.(2018) |

# Chapter 3. MATERIALS AND METHODS

## 3.1. Description of the Study Area

The study was carried out in selected districts of West Gojjam and Awi zones of Northwest, Ethiopia as shown in (Figure 3. 1). The sub-region is situated between 10.00 ° to 14.00° North latitude and 35.10° to 38.35° East longitude with the total annual rainfall ranging from 878 mm to 2100 mm and the annual average minimum and maximum temperature of 22°C and 30.7°C, respectively. The study area encompasses three distinct agro ecologies namely highlands, midlands and lowlands. Western Amhara region is characterized by subsistence crop-livestock production (Aynalem Adugna, 2014) and ranked first in its small ruminant population in the Amhara National Regional State of, Ethiopia. An estimated 6.6 million sheep and goats are reared in western Amhara region (CSA, 2013).

Specifically the present study areas were selected randomly when SGPV diseases outbreaks were reported in five districts Burie, Mecha, Yilmana Densa, Dega Damot and Sekela of West Gojjam zone and three districts of Awi zone namely Banja Shikudad, Gunagua and Jawie districts of the Northwest Amhara region from November, 2018 to May, 2019 to investigate outbreaks and detection of pox virus circulating in sheep and goats.

Burie district is located between $10^{o}15'$ to $10^{o}42'$ North latitude and between $36^{o}52'$ to $37^{o}7'$ East longitude. It is located 400 km north of Addis Ababa and 148 km southwest of the Amhara National Regional State capital city Bahir Dar. It has an altitude which range from 713 to 2604 meters above sea level (IPMS, 2007). The annual rainfall varies from 1386 mm to 1757 mm (IPMS, 2007). According to IPMS (2007), the long term annual temperature ranges from 14 °C to 24 °C. Burie have different livestock composition like 90475 cattle, 52304 sheep, 13523 goats, 6716 donkeys, 620 horses and 228 mules.

Mecha district is located, between 11°10’ and 11°25’ North latitude and 37°2’ and 37°17’ East longitude. The district is located about 525 km Northwest of Addis Ababa and about 40

km south of Bahir Dar capital city. It receives an average annual rainfall that ranges from about 820 mm to 1250 mm (DOA, 2015). The temperature ranges between 24 º C to 27º C and altitude ranges from 1800 to 2500 meters above sea level. The livestock populations were accounted as bovine 351, 844, sheep 110, 834, goats 61,883, equine 39,214 and poultry 230,286 (DOA, 2015).

Yilmana Densa is located at about 40 km in Southeast of Bahir Dar city between geographical coordinates of 11°10′ to 11°15′ North latitude and 37°30′ to 37°40′ East longitude and an average altitude of about 2220 meters above sea level. The area receives a mean annual rainfall of about 1270 mm and it ranges from 1051 to 1488 mm. The areas have a mean temperature value of 18.6$^{o}$c, ranging from 11.54$^{o}$c to 32.3$^{o}$c (Bahir Dar Metrology Office Report, 2015). The livestock population of the area is estimated to be cattle 123,440, sheep 79,217, goats 11,471, equine 24,904 and poultry 88,439 (DOA, 2015).

Sekela is located at 10 °55'0" North latitude and 37 °31'60" East longitude and found an elevation of 3062 meters above sea level. It is located at 459 km in Northwest of Addis Ababa; 160 km in Southeast the capital city of Bahir Dar. The average annual rainfall of the area ranges from 1600 mm to 1800 mm with an average temperature of 18 ◦ C. Dega Damot is located 399 km far from capital Addis Ababa. The woreda town Feres Bet is located at about 3400 meters above sea level. The district receives with annual rain fall between 900 mm and 1200 mm.

Banja Shikudad district lies in the latitude range of 10.052’00” to 11.02’ 44”North and 36° 38’ 26” to 37 ° 7’ 8” East longitudes. The woreda town is located 120 km south of the regional capital city to Bahir Dar and about 448 km west of Addis Ababa along the main road from Addis Ababa to Bahir Dar. The altitude ranges from 1850 to 2870 meters above sea level. The annual rainfall of the area ranges between 2200 mm and 2400 mm. The annual temperature ranges between 19 $^{0}$c and 11$^{0}$c (BWADO, 2012). The district has 55,543 bovine, 59,510 sheep and goat and 23,523 equine (BWAOR, 2012).

Gunagua woreda town Chagnie is located 189 km southwest of the regional capital city to Bahir Dar and 505 km away from Addis Ababa situated in the southwestern part of Awi zone. The district has a longitude and latitude of 10.950°North latitude and 36.500°E longitude with

an elevation that ranges from 800 to 2300 meters above sea level. The temperature range from 22 $^{0}$c to 31$^{0}$c and has an average rainfall 1550 mm annually (ANRS, Bureau of Finance and Economic Development, 2005).

Jawie is found within the geographical location of 10$^{0}$ 38` to 11$^{0}$ 30` North latitude and 36$^{0}$ to 37$^{0}$ East longitude. It has an average altitude of 983.5 meters above sea levels with a range of 965 to 1002 meter above sea level. The temperature is hot most of the year with an average ranging between 30 $^{0}$ C to 35$^{0}$ C, in *Bega* season from (December to February) and 35$^{0}_{C}$ to 40 $^{0}_{C}$ during *Belg* season (March to May). The district receives very little amount of rainfall that that ranges between 400 mm to 800 mm.

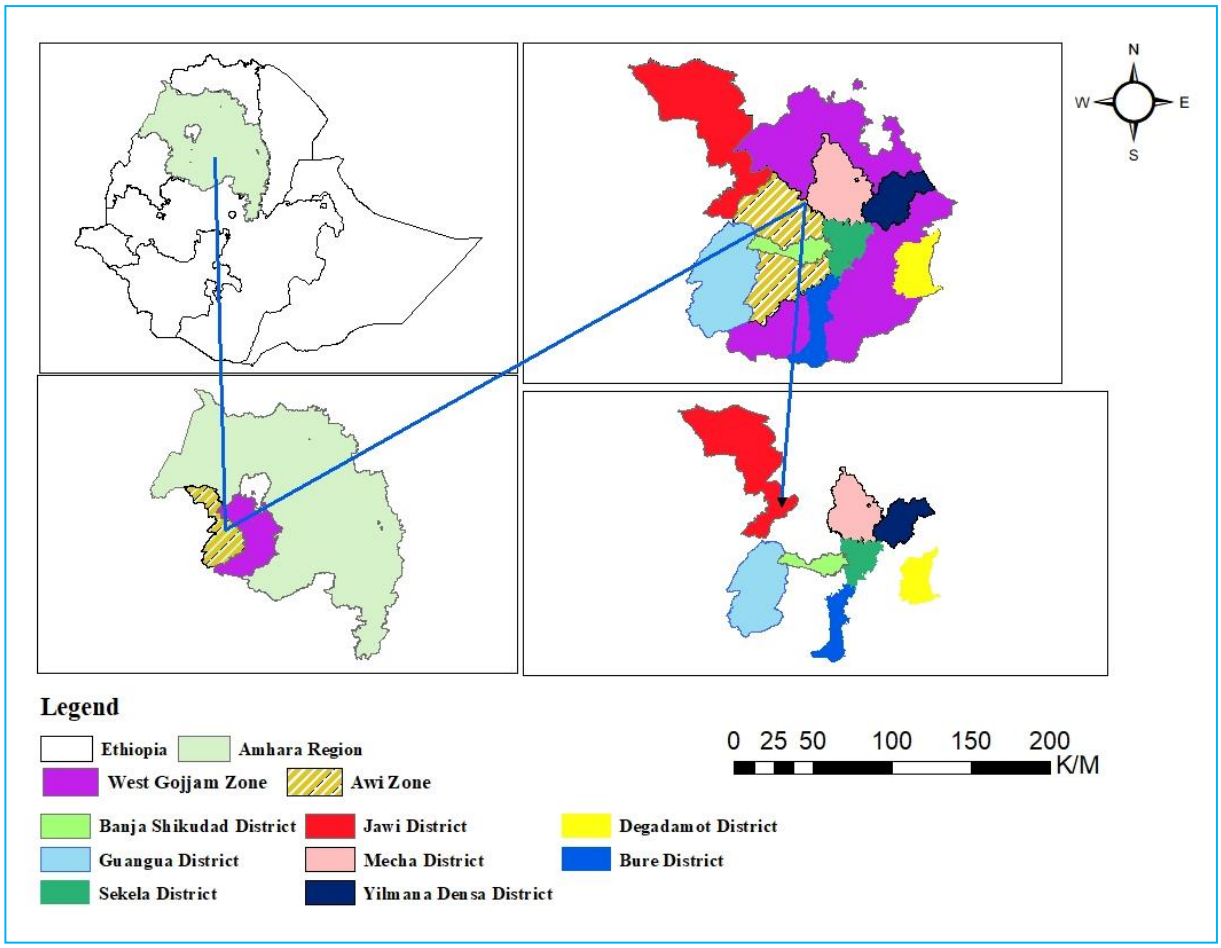



Figure 3. 1. Map of study areas (Geographical information system, 2019)

### 3.2. Study Populations and Management System

The study population consists of small ruminants that were found in eight selected districts and nine PAs that manifests clinical signs of SGPV and those sheep and goats which have close contact with clinically infected small ruminants. The sampling units for the study were local sheep and goat breeds of all ages and both sex groups under extensive management system by small holder farmers. The age of the animals was determined based on the information obtained from the owners and also by looking the dentition pattern of small ruminants (Cocquyt *et al*., 2005) as shown Appendix 7.2. Small ruminants were divided into two age groups according to their dentitions, as young (≤ 2 years old) and adult animals (>2 years old) (Aiello and Mays, 1998).

### 3.3. Study Design and Sampling Methods

An outbreak investigation was done using cross sectional study design (Dohoo *et al*., 2003; Thrusfield, 2005) from November 2018 to May 2019 in sheep and goats showing clinical signs of pox infection.

The districts and rural kebele were randomly selected based on the occurrence of suspected sheep and goat pox disease and accessibility for transportation. From selected districts and rural kebeles, the flocks and individual small ruminants for examinations were also selected purposively based on the occurrence of suspected cases of sheep and goat pox and based on communicating zonal head offices, districts and kebele animal health professionals who are working in veterinary clinics. When an active outbreak of sheep and goat pox were encountered or reported, a field level investigation was carried out at a particular site of outbreak animals were closely examined both physically and clinically. The detailed physical and clinical examination of the diseased animal was documented. Samples for skin biopsies were randomly collected in outbreak cases from clinically sick animals (11 sheep and 27 goats) showing suspected *Capripoxvirus* lesions.

### 3.4. Sample Size Determination

The sample size was determined based on the reports of sheep and goat pox outbreaks in the study areas. During the study period a total of 485 small ruminants (303 sheep and 182 goats) from nine rular kebeles were clinically examined.

Because of unwillingness of the owners to take samples from all infected sheep and goats randomly a total of (38) tissue samples (11 sheep and 27 goats) were collected from eight different outbreak districts clinically suspected field cases of sheep and goat pox and those small ruminants with typical signs of the pox lesion for molecular detection of sheep and goat pox outbreaks

### 3.5. Data Collection Methods

The study engaged different outbreak investigation approaches including clinical examinations, molecular detection and questionnaire survey.

#### 3.5.1. Active Outbreak Investigation and Field Clinical Examinations

As soon as a particular outbreak areas were reported, assessed and identified, sheep and goats included in this study were carefully examined for the presence and appearance of the clinical signs and lesions of sheep and goat pox. In each outbreak, physical examination of all parts of the body including the mucous membranes, mouth, the ears, under the tail, less wool covered body parts, scrotal and teat areas were carried out. Rectal temperature was also taken. Visual inspection and palpation of the skin were utilized to detect nodular skin lesions.

#### 3.5.2. Laboratory Analysis

*Sample Collections and Processing*

According to the procedure of OIE (2017), viral samples to confirm outbreak investigation and molecular detections were collected from clinically sick sheep and goats skin lesions. Samples for skin biopsies were collected randomly in outbreak cases from clinically sick animals (11 sheep and 27 goats) showing suspected *Capripoxvirus* lesions. A minimum of 1 gram of tissue samples were collected aseptically by washing and cleaning the area and removing the hairs with the help of sterile scalpel blade and simply under the tail. The collected samples were placed in a caped universal bottle with a 50% phosphate buffer saline

(PBS) at a pH of 7.2 to 7.6 by adding penstrep and statin to inhibit bacterial and fungal growth respectively.

Species, identification number, sex, age, districts and rural kebeles were leveled and immediately placed in a cold icebox and transported to Bahir Dar Regional Animal Health Diagnostic and Investigation Laboratory then placed in -$20^0$c until shipping to NVI, Debre Zeit/Bishoftu Ethiopia. The collected specimens then transported and submitted to the NVI, then placed at-$20^0$c until it is processed in virology and molecular biology laboratory for molecular detection of sheep and goat pox virus.

The collected skin scraping samples were thawed with scissors at room temperature and washed three times using sterile 50% PBS containing antibiotics like penstrep and antifungal at a pH of 7.2 in Class II biosafety cabinet. About 1gram of the thawed samples was taken from sterilized petridish using thumb forceps then grounded using sterile mortar and pestle by adding 9 ml of sterile 50% PBS. The grounded specimen put in to the sterilized vacutainer tube then the tissue suspension was centrifuged at 3500 rpm for 10 minutes at 4ºC. The 1000µl supernatant was collected after centrifuge by micropipettes then put in to cryovial tubes, then capped and wrapped by aluminum foils then labeled with date of processing and tentative diagnosis finally preserved at -20ºC until sending to molecular biology laboratory.

*DNA Extraction*

Deoxyribonucleic acid (DNA) extraction was carried out in the molecular biology laboratory at the NVI. Extraction of DNA from 10% (w/v) tissue sample suspension homogenate was carried out using DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer‟s instruction. Accordingly, 200µl processed tissues supernatant was transferred into a leveled 1.5ml eppendorf tube. 20µl proteinase K and 200µl Buffer AL was added for each tube to expose the DNA and mixed by vortexed and incubated at 56ºC for 30 minutes for enzyme activations and until completely lysed. 200µl 96% ethanol was added per tube after removed from the water bath and homogenize carefully by vortexed to facilitate the precipitations of the extracted DNA. The 620µl homogenized mixture was transferred to a leveled DNeasy mini spin column placed in a 2 ml collection tube and centrifuged for 1 minute at 12500 rpm. The collection tubes were changed by the new one and 500µl Buffer AW1 was added into the spin column and centrifuged for 1 minute at 12500 rpm. About

500µl Buffer AW2 was added to new eppendorf tube and centrifuged for 3 minutes at 13400 rpm. Lastly, the spin column was transferred into a labeled 1.5ml eppendorf tube and 50µl Buffer AE (elution buffer) was added to the center bottom of the column and the content was incubated for 3 minutes at room temperature and centrifuged for 1 minute at 13400 rpm to elude the DNA into the eppendorf tube. The nucleic acid bound to the silica membrane was eluted and the tube was leveled properly and kept at -20ºC until analysis. All steps carried out within in a microbiological safety cabinet IIA.

*Polymerase Chain Reaction (PCR) Protocol*

Polymerase Chain Reaction (PCR) protocol described by Mangana-Vougiouka *et al*., (2000) was followed. Conventional PCR was performed aiming to amplify a small fragment of the 30KDa RNA polymerase subunit (RPO30) gene of *Capripoxviruses*. By using *Capripoxvirus* specific primers of SGP RNA Polymerase forward 5pm/µl and primer RNA Polymerase reverse 5pm/µl synthesized by VBC biotech (Vienna, Austria). The method is able to differentiate goat pox virus from sheep pox virus since the gene harbor a well conserved sequence signature for the differentiation and genotyping of the two pox viruses. Accordingly, PCR was conducted to amplify small fragment of the RPO30 gene using the primers and protocol described by Lamien *et al.* (2011).

Table 3. 1 Specific primer used for detection of *Capripoxvirus*

| **Gene** | **Primer** | **Primer Sequences** | **Length** | **References** |
|---|---|---|---|---|
| RP030 | Forward | 5'TCTATGTTCTTGATATGTGGTGGTAG3' | 26 | Lamien *et a*l. (2011) |
| | Reverse | 5'AGTGATTAGGTGGTGTATTATTTTCC3' | 26 | |

The procedure was the primers flanking the region containing a 26-nucleotide deletion in SPPV sequences so that the PCR amplification products from SPPV (151bp for sheep pox virus) strains would be shorter in comparison to those from GTPV (172bp for goat pox virus).

Conventional PCR was done in a total reaction volume of 20 µl containing RNase free water 3µl, forward primer 2µl, reverse primer 2µl, IQ super mix 10µl, and template DNA sample 3µl. The PCR eppendorf tube with all the components were transferred to a thermal cycler

and amplification was conducted (Applied Bio Systems). The PCR protocol at NVI was performed with an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing 55°C for 30 seconds and extension 72°C for 30 seconds, and final extension at 72°C for 7 minutes by 1cycle followed by 4°C for storage. The PCR products were loaded and separated using agarose gel electrophoresis apparatus (BIORAD) at 120 volts for 1:20 hours in 3% Tris/Acetate /EDTA stained with gel red.

*Agarose Gel Electrophoresis of PCR products*

Agarose gel electrophoresis provides a means of analyzing amplified DNA products by separating molecules based on size of base pairs as described by Mangana-Vougiouka *et al*. (1999). Briefly, 3 gram agarose was added in to a flask containing 100 ml of 1X TAE (Tris-acetate-EDTA) buffer then shake and homogenize it. The mixture was boiled using micro-oven at 880 watts for 3 minutes and a gelling temperature of 55ºC.The gel was poured on gel caster placed horizontally and the comb was placed in the caster. When the gel was completely solidified after 20 minutes, the comb was removed carefully then, the amplified DNA was added in pours after removing the combs. The presence of DNA was checked in the prepared agarose gels 3% Tris-Acetate-EDTA.

In the first lane, 10µl 100 bp DNA ladder was loaded, while in remaining lanes 10µl amplified PCR products, positive control of sheep pox and negative template were loaded in each well by using micropipettes. The amplified DNA products were analyzed with a component 4µl gel red loading dye then gel running tank was connected to the power supply at 120 volts for 1:20 hours. Parallel with DNA molecular weight marker in electrophoresis apparatus until the DNA samples have migrated to the positive charge anodes a sufficient distance through the gel. DNA amplicon sizes were visualized using UV trans-illuminator at a wavelength of 590 nm and positive results were confirmed according to the size of the amplicon formed on agarose gel (NVI). The PCR results were considered to be positive for sheep pox virus when 151bp and goat pox virus 172 bp (Lamien *et al*., 2011).

3.5. 3. Questionnaire Survey

A questionnaire survey was used to assess the perception and attitudes of sheep and goat owners, about the occurrence and frequency of sheep and goat pox diseases. Additionally, the

questionnaire survey includes; on the risk factors of SGP including watering and feeding system, marketing system, sex and age of animals, affected herd composition and number, vaccination history. These data were collected using a format listing important variables as indicated in (Appendix 7.1). The questionnaire was filled by face to face interview with one representative sheep and goat owners. To determine the sample size of participants included in the interview the formula ($n = 0.25/SE^2$) given by Arsham. (2002), the standard error (SE) of 0.05 with 95% confidence interval (CI) was used.

$n = 0.25/SE^2 = 0.25/ (0.05)^2 = 100$

Where: n = the required interviewed sample size

SE = Standard Error

### 3.7. Ethics Statements

Samples were collected from infected sheep and goats during the outbreaks for the disease confirmation. All efforts were made to minimize animal suffering during sample collections in pox suspected outbreak areas and disinfecting with 2% iodine to the sample taking areas. Willingness was obtained from the animal owners during the collection of tissue samples. Sample collection, shipping of specimens and their use were according to NVI laboratory animal use ethics to minimize the spread of the disease.

### 3.8. Data Management and Stastical Analysis

The collected data during questionnaire survey was entered and stored into Microsoft Office Excel spread sheet 2007. The data were thoroughly screened and coded before subjecting to statistical analysis. The data was then imported to STATA version 12 (Stata, 2012). Descriptive statistics like percentages were used to determine morbidity, mortality, case fatality rates and the frequency of diseases occurrences. Amplified PCR product of 151 bp for SPPV and 172 bp for GTPV band size on agarose gel electrophoresis was used for genotyping CaPV using conventional PCR. Univariable logistic regression analysis was carried out to identify the potential risk factors. The results of analysis were reported as statistically significant if p-value is less than 5% ($P < 0.05$) and 1 is not included in 95% confidence interval. Multiple logistic regressions used to quantify the risk factors and to identify the confounding factors.

# Chapter 4. RESULTS AND DISCUSSION

## 4.1. Active Outbreak Investigation and Field Clinical Examinations

In this study in eight districts outbreak cases were investigated between November, 2018 and May, 2019. The first outbreak was reported from rural kebeles (RKs), followed by Sentom, Senkegna, Lijnegus and Lijambera RKs respectively in west Gojjam Zone. Similarly, in Awi zone there were reported outbreaks in Menta weha, then Jahemala and lastly in Basena and Chaba Gessa RKs.

During field clinical examination, the animals were closely examined both physically and clinically. The detailed physical and clinical examination of the diseased animal was documented. Most affected sheep and goats become weak with no appetite. The common clinical signs observed in sheep and goat during outbreak investigations pox virus were fever, depression, loss of appetite, loss of body condition; different size skin nodules, necrotic nodule under the tail, perineum, udder and testicles, lacrimation, nasal discharge in lambs and kids, cutaneous papules and nodules in areas of skin with less hair were prominent signs of the disease.

A total of 485 small ruminates (303 sheep and 182 goats) were examined, from which 35 sheep and 36 goats have showed pox lesions as indicated in (Table 4.1 and 4.2).

Table 4. 1 Morbidity, Mortality and Case fatality rates in goats in West Gojjam and Awi zone

| **District** | **No. of individual goats examined** | **No. of goats affected** | **Number of death** | **Morbidity** | **Mortality** | **Case fatality Rate** |
|---|---|---|---|---|---|---|
| Burie | 12 | 3 | 1 | 25% | 8.33% | 33.33% |
| Mecha | 21 | 5 | 2 | 23.80% | 9.52% | 40% |
| Yilmana Densa | 12 | 3 | 1 | 25% | 8.33% | 33.33% |
| Sekela | 15 | 2 | 1 | 13.33% | 6.66% | 50% |
| Dega Damot | 28 | 2 | 0 | 7.14% | 0% | 0% |
| Banja | 46 | 7 | 4 | 15.21% | 8.69% | 57.12% |
| Guangua | 32 | 9 | 2 | 28.13% | 6.25 % | 22.22% |
| Jawie | 16 | 5 | 2 | 31.25% | 12.5% | 40% |
| **Total** | **182** | **36** | **13** | **19.78%** | **7.4%** | **36.11%** |

Table 4.2 Morbidity, Mortality and Case fatality rates in sheep in West Gojjam and Awi zone

| District | No. of individual sheep examined | No. of affected sheep | Number of death | Morbidity | Mortality | Case fatality Rate |
|---|---|---|---|---|---|---|
| Yilmana Densa | 21 | 2 | 1 | 9.52% | 4.76% | 50% |
| Dega Damot | 31 | 2 | 0 | 6.45% | 0% | 0% |
| Banja | 204 | 24 | 8 | 11.76% | 3.92% | 33.33% |
| Guangua | 47 | 7 | 2 | 14.89% | 7.14% | 28.56% |
| **Total** | **303** | **35** | **11** | **11.55%** | **3.63%** | **31.43%** |

During the current outbreak investigation, most sheep and goats showed pox lesion all over the skin however, few confined to the areas with little or no hair, such as the face, ears, as indicated in (Fig. 4.1). Accordingly, there were erythematous macules, papules and nodules under the tail, groin, perennial region, and the inner side of the thigh as shown (Appendix 8).

In the current study, the observed clinical signs in infected sheep and goats were fever with marked depression, weakness, enlarged lymph nodes, discharges from eyes and nostrils in lambs and kids, scabs on head, face, ears, nostrils, multiple cutaneous macula, and papules in inner aspect of thigh and under the tail and the whole body were observed and considered as first indicator for pox virus infection. These clinical findings were consistent with the findings Daoud (1997) in Jordan, Davies and Otema (1981) in Kenya, Achour *et al*. (1999) in Algeria, Singh *et al.* (2007) in India, Mersha Chanie (2011) in sheep in Ethiopia, Sharawi *et al*. (2011) in Egypt, Zangana and Abdullah (2013) in Iraq and Hamouda *et al*. (2017) in Saudi Arabia.

The present study revealed that from the total of 485 small ruminants (303 sheep and 182 goats) were clinically examined (35 sheep and 36 goats) had showed typical pox lesions. Overall 71/485 (14.64%), 24/485 (4.95%) and 24/71 (33.80%) morbidity, mortality and case fatality rate were found respectively. From the affected small ruminants, 11 sheep and 13 goats died. In sheep the morbidity, mortality and case fatality were 11.55%, 3.63% and 31.43% whereas in goats 19.78%, 7.14% and 36.11% morbidity, mortality and case fatality rate of pox lesion on their skin respectively (Table 4.1 and 4.2).

The study revealed the overall morbidity during outbreaks of SGP was higher than the findings of Yakob Hailu *et al. (*2008) and Aberham Kebede *et al*. (2018) which were 10.34% in sheep and 12.88% in goats in Adama and 9.5% in sheep and 12.9% in goats in Beneshangul Gumuz Dibate district respectively. This might be free grazing and watering of small ruminants in agreement with (Kitching, 1994; Sheikh *et al*., 2004) who stated traditional forms of animal husbandry practices increases disease transmission.

However, the morbidity was lower than Bereket Molla *et al*. (2017) in Gam Gofa zone both in sheep and goats and Mersha Chanie (2011) sheep in Ethiopia as indicated in (Table 2.1). This might be contributed animals in endemic areas developed lifelong immunity after recovery in line with (Castro and Huschele, 1992) who stated that sheep and goats in endemic areas more resistant due to possessing protective antibodies from previous infection or vaccination.

The present result also indicated that the morbidity and mortality was lower than Babiuk *et al*. (2009) characterized by 60% morbidity and approximately 45% mortality rate in Vietnam in goats this might be breeds of sheep and goats originated from Europe are very susceptible to *Capripoxvirus*, and mortality may reach up to 100% (Radostits *et al*., 2006; Mirzaie1*et el*., 2015 ).

Based on the clinical signs highest morbidity (14.89%) affected sheep was found in Gunagua district and the lowest morbidity (6.45%) affected sheep was recorded in Dega Damot district. The highest number (31.25%) of goats affected by pox virus was in Jawie district and the lowest number (7.14%) affected goats was observed in Dega Damot district.

The increase in morbidity in Jawie and Guangua district might be due to the geographical location of the districts, number of illegal animal movements by local traders from nearby market of Dibate, Gelgel Beles and Manbuk districts in Metekel zone, climatic factors, the grazing and migration pattern of sheep and goat in the districts and extensive grazing system of sheep and goat vary the number of affected sheep and goat between districts.

This work was in agreement with the work of Domenech *et al*. (2006) who stated that the spread of *Capripoxvirus* into new areas is predominantly associated with the increase of illegal animal movement through trade and also Babiuk *et al*. (2008), who reported that poor quarantine measures and trade of live animals across the border may lead to further spread of

the disease and Mondal *et al*. (2004) who said that the grazing and migration pattern of sheep and goats, poor management, climatic factors, feed scarcity and inadequate veterinary services probably increase the spread or transmission of sheep and goat pox to the free area. Additionally the high morbidity in both districts might be also likely that the shortage in feed supplies in the dry season predisposed sheep and goat to pox infection. These finding was correlated with the findings of Hailat *et al*. (1994).

The spread of the disease in Banja Shikudad, Guangua and Jawie districts followed the recurrence of SGP in the nearby districts of Dibate district was reported by Aberham Kebede *et al*. (2018) which are connected with the main road of Guangua and Banja Shikudad districts through which a large number of small ruminants from the areas to the districts were reported. These might be due to the illegal movement of large number of animals from the nearby Beneshangul Gumuz Regional State to different nearby districts of Awi zones, the grazing and migration pattern of sheep and goats in the districts which was extensive system and related to traditional forms of husbandry practices (Kitching, 1994; Sheikh *et al*., 2004).

The highest morbidity of pox in sheep and goats in Guangua and Jawi districts respectively might be associated with lack of strategic vaccinations as informations collected from rural kebele veterinary professions, woreda and zonal head offices there was no vaccination practices in the two districts. Additionally, in both districts the might be presences of biting flies that favors the transmission of sheep and goat pox.

The lowest mortality in Dega Damot district both in sheep and goats might be associated with use of strategic vaccinations as informations gathered from the rural kebele and woreda, and zonal head offices and the presences of biting flies not expected in areas since there is no trpanosomasis in the district.

Figure4.1 Characteristic pox signs observed during the study

### 4.2. Results of Molecular Identifications

The conventional PCR indicated that samples collected from those sheep and goats showed typical pox signs, all 19 samples, processed in NVI for molecular detections, were positive for goat pox viruses. These indicated that the clinical signs were suggestive for sheep and goat pox by producing an amplicon size of 172 bp (Fig. 4.2 and 4.3). The outbreak might be lack of strategic vaccination to susceptible animals, communal feeding and watering at common points, free movement of small ruminants in markets from other nearby districts.

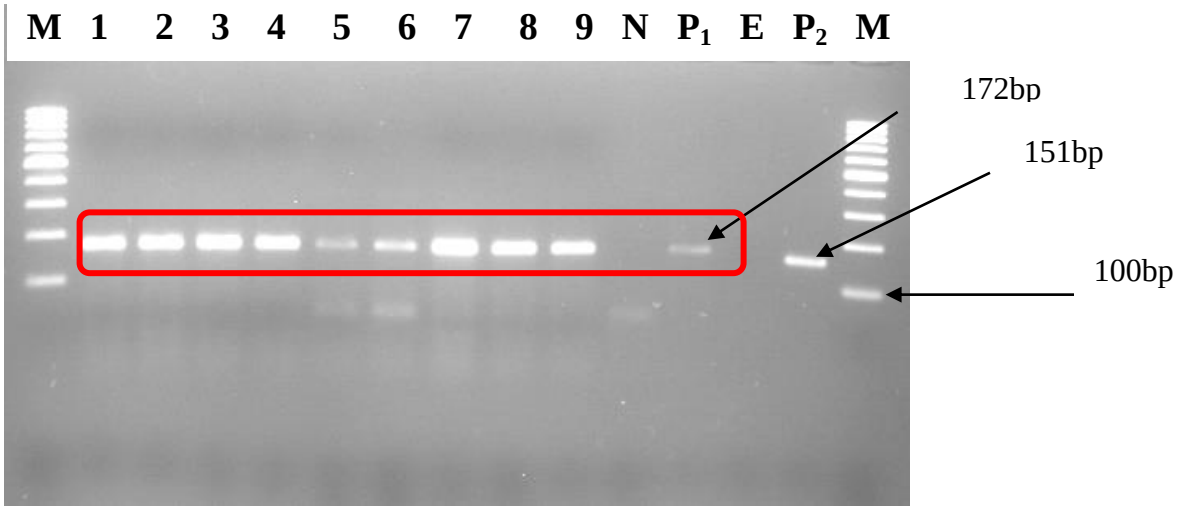



Figure 4. 2 Conventional PCR for differentiating GTPV from SPPV

Lan M: DNA ladder (100bp)
Lane1-9: positive sample of goat pox
Lane N: negative template control for goat pox
Lane $P_1$: goat pox positive control (172 bp)
Lane E: Extraction control – Negative;
Lane $P_2$: SPP positive control (151bp)
.

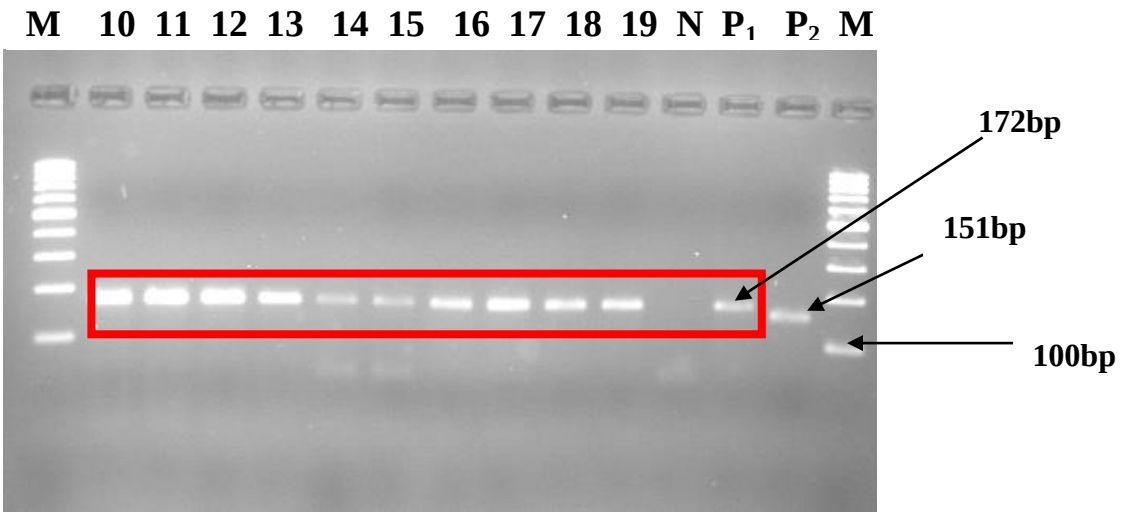



Figure 4. 3 Conventional PCR for differentiating GTPV from SPPV

LaneM: DNA ladder /Molecular marker (100bp)
Lane10-19: positive sample of goat pox and sheep pox
Lane N: negative template control for goat pox
Lane $P_1$: Got pox positive control (172 bp)
Lane $P_2$: sheep pox

Where lane **1**, **2** and **4** = Dega Damot district goat species; Lane **3**, **5** and **6** = Jawie district goat species; Lane **7** and **8**=Yilmana Densa district goat species; Lane **9**=Sekela district goat species; Lane **10** to **12**= Banja district goat species; Lane **13** = Burie district goat species; Lane **14** to **17** =Guangua district sheep species; Lane **18** and 19 = Banja Shikudad district sheep species.

Based on the PCR findings, the present samples collected and processed from sheep and goat populations in different districts showed that the pox virus circulating in sheep and goats were detected as goat pox virus having amplicon size of 172 bp; whereas sheep pox virus were not identified even from a single specimens. Even though the strain isolated by conventional PCR in both species was goat pox virus which was in line with (Kitching, 2003; Lamien *et*

*al*., 2011;Aberaham Damena, 2018 and Abdi Assefa *et al*., 2019) the strains of *Capripoxvirus* causing disease in sheep and goats are not host specific and may either affect both or one species and disagreed with (Babiuk *et al*., 2008; Bhanuprakash *et al*., 2006; 2010; Beard *et al*., 2010) who stated the GTPV or SPPV strains isolated from outbreaks were usually classified and designated based on the host from which the virus was isolated and sheep and goat pox host specific, leading outbreak in one host.

### 4.3. Questionnaire Survey

From a total of 100 individuals subjected to questionnaires, 83% (n=100) of them responded that the disease usually occurred as an epidemic and frequently in every year, 11% it occurs every two years and the remaining 6% responded the diseases occurs every three years and locally named as ‘’Fenetata”. Furthermore, (100%) of them said that animals shared communal watering points, and free grazing. The survey of this study indicated that majority of the respondents (83%) (n=100) had previously experienced sheep and goat pox disease in their herds that was lower than Abdi Assefa (2017) 96% and Aberham Damena (2018) which was 95% and familiar with the clinical sign of the disease, which they locally called ‘’Fenetata”. Similarly, Mersha Chanie (2011); and Tsegaw Fentie *et al.* (2016) also stated that the disease was named with similar local name in Northwest Ethiopia.

### 4.4. Risk Factors Associated with the Disease

100 sheep and goat owners (10 in the seven districts and 30 in Banja district) were responded about their risk factors for the occurrences of SGP diseases. A total of 11 related potential risk factors were analyzed for their significance with the occurrence of sheep and goat pox. Age of sheep and goats, vaccination status, herd size and composition, free movement of sheep and goats, and season were found statistically significant ($P < 0.05$) and 1 is not included in the CI.

Table 4. 3 Analyses related risk factors for the occurrence of sheep and goat pox in univariable logistic regression

| Risk factors | No. Respondents | OR 95% CI | P-value |
|---|---|---|---|
| Species | | | |
| Sheep* | 48(48%) | 1 | - |
| Goat | 52(52%) | 1.02 (0.43, 2.41) | 0.972 |
| Sex | | | |
| Male* | 43(43%) | 1 | - |
| Female | 5757%) | 1.11 (0.47,2.65) | 0.814 |
| Age | | | |
| Young* | 64(64%) | 3.87 (1.5, 9.60) | 0.003** |
| Adult | 36(36%) | 1 | - |
| Body condition | | | |
| Poor* | 83(83%) | 1 | - |
| Medium | 9(9%) | 0.11 (0.02,0.49) | 0.004** |
| Good | 8(8%) | 1 | - |
| Vaccination status | | | |
| Not vaccinated* | 74(74%) | 1 | - |
| Vaccinated | 26(26%) | 0.01 (0.00, 0.05) | 0.000** |
| Flock size | | | |
| <10* | 9(9%) | 1 | - |
| 10-19 | 38(38%) | 11.08 (1.2, 101.68) | 0.033** |
| 20-30 | 53(53%) | 35.7 (3.95, 322.90) | 0.001** |
| Flock composition | | | |
| Sheep only* | 24(24%) | 1 | - |
| Goat only | 33(33%) | 58.90 (10.38, 334.37) | 0.000** |
| Sheep and goats | 43(43%) | 16.63 (4.77, 57.98) | 0.000** |
| Sources of outbreak | | | |
| Common point* | 21(21%) | 1 | - |
| Introduction of new animals | 79(79%) | 1 | - |
| Free movement | | | |
| Present* | 75(75%) | 1 | - |
| Absent | 25(25%) | 0.05 (0.02, 0.15) | 0.000** |
| Agro ecology | | | |
| High land* | 55(55%) | 1 | - |
| Mid land | 33(33%) | 1.52 (0.55, 4.21) | 0.417 |
| Low land | 12(12%) | 0.41 (0.11, 1.46) | 0.083 |
| Outbreak seasons | | | |
| Dry* | 72(72%) | 3.8 (1.56, 9.60) | 0.003** |
| Rainy | 28(28%) | 1 | - |

*Risk factors considered significant when CI did not include 1 and P-value<5%*

Based on the findings of the analysis of risk factors the result clearly explain that both sheep and goats were equally susceptible to goat pox virus and it was only goat pox virus circulating and causing pox disease both in sheep and goat population with higher severity in goats agreed with Bidjeh *et al.* (1991) who reported that difference of sensitivity between sheep and goats was statistically insignificant but disagreed with Abdi Assefa (2017) who stated that the disease is more important in sheep due to genetic differences. Infection of GTPV in sheep might be communal herding of sheep and goats from the outbreaks study areas it causes clinical disease in sheep and goats. Additionally, the results agreed with Heine *et al.* (1999) who mentioned sheep and goat pox virus are not considered as host specific and although the majority of strain shows a host preference, a single strain may cause disease in both sheep and goat.

In the investigated current outbreaks, all age groups were affected. It also explained that young age groups are OR (3.87) times more susceptible than adult age groups of both species with CI (1.5-9.60) and P- value 0.003. These might be due to climatic stress or recovered animals developed lifelong immunity and the immune status of the animals consistent with Abereham Damena, (2018) who stated climatic stress affects the immune status of the animals resulting in increasing of susceptibility to diseases.

The respondents informed that the disease equally affected both sheep and goat; with more morbidity in goat population and both sex were equally susceptible to the diseases but the diseases were more severe in female sheep and goats this might be related to female small ruminants physiology is related with lactating and pregnancy which could result poor immunity system than male shoats agreed with (Aberham Damena, 2018).

The current study revealed that all body condition sheep and goats were affected by the diseases however, medium body condition sheep and goat was less likely affected with the diseases, (OR=0.05, CI 95%; 0.02, 0.15).

The present study showed that in the absence free movement of animals the diseases was less likely to occur with, (OR=0.05;CI, 0.02, 0.15 and P-value 0.000) and in the absence of vaccination small ruminants were more affected with, (OR=0.01, CI 95%; 0.00, 0.05) as indicated in Table 4.4 that were in line with (Hussein *et al.,* 1989; Sheikh *et al*., 2004; Mahmoud *et al.,* 2016) who stated illegal animal movement and irregular uses of vaccination

appeared to be the main cause of distribution and susceptibility to infection with the pox virus, increased.

This study showed the diseases in dry seasons were found OR (3.8) times higher with CI (1.56-9.60 ) and P-value 0.003 in dry season from December to February (*Bega*) and from February to May (*Belg*) this might be associated with free movement of small ruminants in the market from other infected areas by illegal local traders which favor transmission of the disease agreed with the study conducted by Bhanuprakash *et al.* (2005); Takele Tesgera *et al.(*2018) who described outbreaks were recorded mostly occur between November and May, and the peak outbreaks occurred in March but disagreed with the reports of Mersha Chanie, 2011, who demonstrated higher occurrence of sheep and goat pox outbreaks during rainy seasons with the appearance of more severe forms.

Additionally, the diseases in dry seasons might be associated with shortage in feed supplies that may cause stress predisposing sheep and goats to pox infection. These finding was correlated with the findings of Hailat *et al*. (1994).

In general, the present study revealed that the disease was higher OR (11.08) with CI (1.2-101.68) and P-value 0.033 when the flock size increased from 10 to 19 and 35.7 times higher with CI (3.97-322.900 and P-value 0.001 when the flock size greater than 20 individual animals which is in agreement with Aberham Damena (2018) who revealed that large flock size and herding of sheep and goat together are predisposing factors for the occurrence of the pox disease.

The study also indicated that the diseases was found (OR=58.90) times much higher in goat herds and ( OR=16.63) than in sheep and goat herds With CI (4.77-57.98 ) and P-value 0.000 in comparison to sheep herds only this might be the virus isolated was goat pox virus that preferred the homologous hosts.

Table 4.4. Analysis of host and management related risk factors for the occurrence of sheep and goat pox in multiple logistic regression

| Risk factors | No. of small ruminant | OR 95% CI | P-value |
|---|---|---|---|
| Species | | | |
| Sheep* | 48(48%) | - | - |
| Goat | 52(52%) | - | - |
| Sex | | | |
| Male* | 43(43%) | - | - |
| Female | 57(57%) | - | - |
| Age | | | |
| Young* | 64(64%) | 2.4(0.81,7.32) | 0.114 |
| Adult | 36(36%) | 1 | - |
| Body condition | | | |
| Poor* | 83(83%) | - | - |
| Medium | 9(9%) | - | - |
| Good | 8(8%) | - | - |
| Vaccination status | | | |
| Not vaccinated* | 74(74%) | 1 | - |
| Vaccinated | 26(26%) | 86.8(20.091,375.761) | 0.000 |
| Flock size | | | |
| <10* | 9(9%) | 1 | - |
| 10-19 | 38(38%) | 10.5(1.03,107.26) | 0.047 |
| 20-30 | 53(53%) | 80.5(6.66,974.42) | 0.001 |
| Flock composition | | | |
| Sheep only* | 24(24%) | - | - |
| Goat only | 33(33%) | - | - |
| Sheep and goats | 43(43%) | | |
| Sources of outbreak | | | |
| Common point* | 21(21%) | - | - |
| Introduction of new animals | 79(79%) | - | - |
| Free movement | | | |
| Present* | 75(75%) | - | - |
| Absent | 25(25%) | - | - |
| Agro ecology | | | |
| High land* | 55(55%) | - | - |
| Mid land | 33(33%) | - | - |
| Low land | 12(12%) | - | - |
| Outbreak seasons | | | |
| Dry* | 72(72%) | 11.6(2.97,45.82) | 0.000 |
| Rainy | 28(28%) | 1 | - |

*Risk factors considered significant when CI did not include 1 and P-value<5%*

The current findings of multivariable logistic regression analysis indicated of the seven significant factors in univariable logistic regressions only three were significant which were vaccination status, flock size and season.

The diseases were 10.5% when the flock size ranged from 10 to 19 and 80.5% as the flock size increased beyond 20 as compared to the flock size lower than 10.The study revealed that highest seasonal occurrence of sheep and goat pox was 11.6% much higher when compared to the rainy seasons as indicated in (Table 4.4)

The multiple logistic regressions revealed that vaccinated sheep and goats were 86.8 % more protected than the non-vaccinated small ruminants.

# Chapter 5. CONCLUSION AND RECOMMENDATIONS

Pox infection is a very common disease of sheep and goats in the study areas with outbreak reports causing huge economic losses to small ruminant farming societies', due to high morbidity and mortality. The study indicated that there was a wide spread distribution of sheep and goat pox in Northwest Amhara National Regional State. The spread might be occur through illegal trade of infected animals and as well as through the movement of insect vectors. Based on conventional PCR targeting RPO30 genes to detect the pox virus circulating in sheep and goats showed that only goat pox virus was found in sheep and goats disproving the host specificity classification of CaPV. The overall (14.64%), (4.95%) and (33.80%) morbidity, mortality and case fatality rate were found respectively. The morbidity, mortality and case fatality rate of sheep and goat pox within species were 11.55%, 3.63% and 31.43% in sheep and 19.78%, 7.14% and 36.11% in goats respectively. According to the current results, pox disease is more prevalent in dry and short rainy seasons and the disease was reported mainly to affect young sheep and goats. The morbidity; mortality and case fatality rates were high in goat species when compared to sheep and the disease was also commonly seen in unvaccinated sheep and goats, young age groups are more susceptible than adult age of both species and high case fatality rates in young age groups and highly important in large herd size and mixed herd size of sheep and goats. Therefore, based on the above conclusions the following recommendations were suggested:-

- The national and regional governments should strictly establish and implement quarantine stations to control illegal animal movements.
- Continuous tracing and surveillance strategies of the diseases need to be established to determine the source and extent of infection.
- Sheep and goat pox vaccination programs should be carried out regularly before the end of rainy season since the disease frequently occurs from November to May in the study areas.
- Further comprehensive studies should be conducted on the prevalence of the diseases, epidemiology the diseases outbreaks, and host specificity of CaPV.

# 6. REFERENCES


Abdi Assefa, Barbara W. and Fufa Abunna. 2019. Isolation and characterization of pox virus circulating in sheep and goats from outbreak cases of Adea Berga district, West Shoa zone, Oromia, Ethiopia. *Int. J. Curr. Res. Aca. Rev.* 7**: (6)** 1-7

Abereham Damena. 2018. *Outbreak investigation, isolation and molecular characterization of sheep and goat pox virus in central Ethiopia.* MSC Thesis, College of Veterinary Medicine and Agriculture, Addis Ababa University, Debre Zeit, Ethiopia.Pp 9-10.

Aberham Kebede, Eteneh Haile Mariam and Jirgena Dugassa. 2018. Prevalence of common skin diseases of small ruminants in Dibate district Metekel zone Beneshangul Gumuz Regional State Northwestern Ethiopia. *Multidisciplinary J. Adv. Vet. Sci*.**2:** 2-3.

Abu-Elzein E., Housawi F., Ramadan O. 2003. Observations on natural and experimental infection of sheep and goats with a virulent field *Capripoxvirus* with high affinity to goat. *Veterinarski Arhiv*. **73**: 119-131.

Achour H.A. and Bouguedour R. 1999. Epidemiology of sheep pox in Algeria. *Rev. Sci. Tech. Off. Int. Epiz.* **18 (3):** 606-617.

Aiello S. and Mays A. 1998. *The Merck veterinary manual* 7th ed. Whitehouse Station, N.J., Merck and Co. in cooperation with Merial Ltd.

Alvarez I., Traore A., Tamboura H.H., Kabore A., Royo L.J., Fernandez I., Ouedraogo-Sanou G., Sawadogo L. and Goyache F. 2009. Microsatellite analysis characterizes Burkinafaso as a genetic contact zone between Sahelian and Djallonke sheep. *Anim. Biotechnol*. **20**: 47-57.

Animal Health Australia. 2011. Disease strategy sheep pox and goat pox.

Arsham H. 2002. Descriptive sampling data analysis. *Statistical thinking for managerial decision making; accessed*: *http://home.ubalt.edu/ntsbarsh/Business-stat*. Retrieved in 2002.

Aynalem Adugna. 2014. Amhara Demography and Health, Bulletin, http://www.ethiodemographyandhealth.org/ [Accessed 11 August, 2019]

Babiuk S., Bowden T.R., Boyle D.B., Wallace D.B., Kitching R.P. 2008. *Capripoxviruses:* An emerging worldwide threat to sheep, goats and cattle. *Transbound. and Emerg. Dis*.**55**:263–272.

Babiuk S., Bowden T.R., Parkyn G., Dalman B., Hoa D.M., Long N.T., Vu P.P., Bieu D.X., Copps J. and Boyle D.B. 2009. Yemen and Vietnam *Capripoxviruses* demonstrate a distinct host preference for goats compared with sheep. *J. Genes. Virol*. **90**: 105–114.

Balinsky C.A., Delhon G., Simoliga G., Prarat M., French R.A., Geary S.J., Rock D.L. and Rodriguez L.L. 2008. Rapid preclinical detection of sheep pox virus by real –time PCR assay. *J. Clin. Microbiol*. **46**: 438-442.

Beard P.M., Suga S., Bazarragchaa E., Gerelmaa U., Tserendorj S., Tuppurainen E. and Sodnomdarjaa R. 2010. A description of two outbreaks of *Capripoxvirus* disease in Mongolia. *Vet. Microbiol*. **142**: 427-431.

Behnke R. and Fitaweke Metaferia. 2011. The contribution of livestock to the Ethiopian economy part II. IGAD LPI working paper 02-11. Addis Ababa, Ethiopia: IGAD Livestock Policy Initiative.

Bereket Molla, Haba Haile and Sefinew Alemu. 2017. Prevalence and risk factors associated to skin diseases in small ruminants in Gamo Gofa zone, Southwestern Ethiopia *J. Vet. Med. and Anim. Heal.* **9(8):** 228-234.

Bhanuprakash V. 2011. Prospects of control and eradication of *Capripoxvirus* from the Indian subcontinent: A perspective. *Antiviral Res*. **91**: 225-232.

Bhanuprakash V., Indrani B.K., Hosamani M., Singh R.K. 2006. The current status of sheep pox disease. *Comp. Immunol.Microbiol. Infect. Dis*. **29**: 27-60.

Bhanuprakash V., Indrani B.K., Moorthy A.R.S. and Krishnappa G. 2003. Isolation, purification and comparison of protein profiles of sheep pox viruses. *Indian J. Comp. Microbiol. Immunol. Infect. Dis*. **24**: 15-20.

Bhanuprakash V., Moorthy A. R., Krishnappa G., Gowda R. N. and Indrani B. K. 2005. An epidemiological study of sheep pox infection in Karnataka State, India. *Rev. Sci. Tech*. **24:** 909-920.

Bhanuprakash V., Venkatesan G. and Balamurugan V. 2010. Pox outbreaks in sheep and goats at Makhdoom (Uttar Pradesh), India: Evidence of sheep pox virus infection in goats. *Transbound. and Emer. Dis*. **57**: 375-382.

Bidjeh K. K., Ganda C., Diguimbaye I. 1991. Goat smallpox in Chad: Study of the pathogeny of the virus in sheep and goats. *Rev. Elev. Med. Vet. Pays Trop.* **44(1)**: 33-36

Black D.N., Hammond J.M. and Kitching R.P. 1986. Genomic relationship between *Capripoxviruses*. *Virus Res*. **5**: 277-292.

BOFED. 2008. The Amhara National Regional State Bureau of Finance and Economic Development 2006/ 2007 Budget Year Annual Statistical Bulletin. Bureau of Finance and Economic Development, Bahir Dar.

Bowden T.R., Babiuk S.L., Parkyn G.R., Copps J.S. and Boyle D.B. 2008. *Capripoxvirus* tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats. *Virol.* **371**: 380-393.

Buller R., Arif B. and Black D. 2005. *Poxviridae in virus taxonomy:* 8th ed. report of the international committee on the taxonomy of viruses. Elsevier Academic Press. Oxford. 117.

BWADO. 2012. Banja woreda agricultural development office.

Carn V.M. 2000. Control of *Capripoxvirus* infections. Vaccine, **11**: 1275-1279.

Carn V.M., Kitching R.P., Hammond J.M. and Chand P. 1995. Use of a recombinant antigen in an indirect ELISA for detecting bovine antibody to *Capripoxvirus*. *J. Virol. Methods* **49**: 285-294.

Castro A. E. and Heuschelc W. P. 1992. Veterinary diagnostic virology Mosby- year book, Inc .I.J.S.A.

CFSPH. 2008. The Center for food security public health, Iowa State University, college of veterinary medicine and institution of international cooperation in Animal Biologics, an OIE collaborating center. Pp 294-4778

Chand P. 1992. *Molecular and immunological characterization of a major envelope protein of Capripoxvirus*. PhD. Thesis University Surrey UK. **227**: 187-196.

Chand P., Kitching R.P. and Black D.N. 1994. Evaluation of the western blot analysis of virus specific antibody responses to *Capripox* and contagious pustular dermatitis infections in sheep. *Epidemiol. Infect*. **24**:214-36.

Cocquyt G., Driessen B. and Simoens P. 2005. Variability in the eruption of the permanent incisor teeth in sheep. The Veterinary record **157(20)**:619-23.

CSA. 2012. Ethiopia agricultural sample enumeration, statistical report on livestock population, part 4, Addis Ababa, Ethiopia.

CSA. 2013. Central statistical agency federal democratic republic of Ethiopia agricultural sample survey report on livestock and livestock characteristics Addis Ababa, Ethiopia. **(2)**: 2012-2013.

CSA. 2015. Agricultural sample survey, report on livestock and livestock characteristics. Central statistical agency of Ethiopia.

Daniel Teshome. 2016. Prevalence of major skin diseases in ruminants and its associated risk factors at University of Gondar Veterinary Clinic, North West Ethiopia. *Austin J. Vet. Sci. Anim. Husbond*. **3**: 1019.

Daoud J. A. 1997. Sheep pox among Australian sheep in Jordan. *Trop. Anim. Heal. Prod*. **29**: 251-252.

Das A., Babiuk S. and Mcintosh M.T. 2012. Development of a loop-mediated isothermal amplification assay for rapid detection of *Capripoxviruses*. *J. Clin. Microbiol*. **50**: 1613–1620.

Dashprakash M., Venkatesan G., Ramakrishnan M.A., Muthuchelvan D., Sanka. Mr. M., Pandey A.B. and Mondal B. 2015. Genetic diversity of fusion gene (ORF 117), an analogue of vaccinia virus A27L gene of *Capripoxvirus* isolates. *Virus genes* **50**: 325-328.

Davies F. G. 1976. Characteristics of a virus causing a pox disease in sheep and goats in Kenya, with observation on the epidemiology and control. *J. Hyg.***76**: 163-171.

Dereje Tsegaye, Berhanu Belay and Aynalem Haile. 2013. Prevalence of major goat diseases and mortality of goat in Daro-Labu district of West Harrarghe, Eastern Ethiopia. *J. Sci. and Inn. Res.* **2**: 665-672.

DOA. 2015. Development of agriculture: Baseline line report. Mecha, Bahir Dar Zuria and Yilmana Densa district of development of agricultural office, Ethiopia.

Dohoo I., Martin W. and Stryhn H. 2003. *Veterinary epidemiologic research*, Edward Island, Canada, Pp. 144-149.

Domenech J., Lubroth J., Eddi C., Martin V. and Roger F. 2006. Regional and international approaches on prevention and control of animal transbound. Emerg. Diseases. *Ann. NY Acad. Sci*. **1081**: 90-107.

Dubaib M. 2002. A highly disfiguring sheep pox outbreak. *Egyptian J .Virol*. No. 29.

Embury-hyatt C., Babiuk S., Manning L., Ganske S., Bowden T.R., Boyle D.B. and Copps J. 2012. Pathology and viral antigen distribution following experimental infection of sheep and goats with *capripoxvirus*. *J. Comp. Pathol*. **146**: 106-115.

Esayas Gelaye, Alebachew Belay, Gelagay Ayelet, Shiferaw Jenberie and Marta Yami. 2015. *Capripox* disease in Ethiopia: Genetic differences between field isolates and vaccine strain and implications for vaccination failure. *Antiviral Res*. **119**: 28-35.

Esayas Gelaye, Lamien C.E., Silber R., Tuppurainen E.S., Grabherr R. and Diallo A. 2013. Development of a cost-effective method for capripoxvirus genotyping using snapback primer and dsDNA intercalating dye. Plos One, **8**:75971.

ESGPIP. 2009. Ethiopian sheep and goat productivity improvement program. Technical bulletin No.29. Sheep and goat pox: causes, prevention and treatment; Addis Ababa, Ethiopia. Retrieved from http:/www.esgpip.org. [Accessed 25 June, 2019]

Fulzele S., Singh R.K., Hosamani M., Mondal B.and Yadav M.B. 2006. Evaluation of duplex PCR and PCR-RFLP for diagnosis of sheep pox and goat pox. *Int. J. Trop. Med*. **1**: 66-70.

Garner M.G and Lack M.B. 1995. Modeling the potential impact of exotic diseases on regional Australia. *Australian Vet. J.* **72:** 81-87.

Garner M.G., Sawarkar S.D. and Brett E.K. 2000. The extent and impact of sheep pox in the state of Marahashtra, India. *Trop. Anim. Heal. Prod*. **31**: 205-223.

Gemeda Duguma, Kebede Degefa, Temesgen Jembere, Wudu Temesgen, Aynalem Haile and Getachew Legese. 2012. Value chain analysis of sheep in Horro district of Oromia Region, Ethiopia. Nairobi, Kenya: International Livestock Research Institute.

Getachew Gari, Gedlu Mekonen, Demeke Sibhat, Ashebir Abebe, Mesfin Sahle and Getnet Abie. 2015. Participatory Disease surveillance (PDS) of sheep and goat disease in selected district of Afar regional state particular focus on *Pestei des petits ruminants* (PPR) and sheep and goat pox disease (SGP); *Ethiopia Vet. J.* **19 (1)**:83-105.

Gitao C.G., Mbindyo C., Omani R. and Chemweno V. 2017. Review of sheep pox disease in sheep. Department of veterinary pathology and microbiology, University of Nairobi, Kenya *J. Vet. Med. and Res*. **4(1)**: 1068.

Gu S., Shi X., Liu X., Wang Z., Zheng M., Li Y., Huo N., Tang F., Bai R., Shi Z. and Yuan J. 2018. Identification and phylogenetic analysis of the sheep pox virus Shanxi isolate. *Biomed. Res.* **29 (3)**: 523-529

Gulbahar M. Y., Davis W. C., Yuksel H. and Cabalar M. 2006. Immunohistochemical evaluation of inflammatory infiltrate in the skin and lung of lambs naturally infected with sheep pox virus. *Vet. Pathol*. **43**: 67-75.

Hailat N. O., Rawashdelt A.L., Lafi S. and AI-Batcinch Z. 1994. An outbreak of sheep pox associated with unusual Winter conditions in Jordan. Anim. Heal. Pro. **23**:79-80.

Heine H.G., Stevens M.P., Foord A.J. and Boyle D.B. 1999. A *Capripoxvirus* detection PCR and antibody ELISA based on the major antigen P32 the homolog of the vaccinia virus H3L gene. *J. Immunol. Methods,* **227**: 187-96.

Hosamani M., Mondal P. A., Tembhurne S. K., Bandyopadhyay R., Singh K. and Rasool T. J. 2004. Differentiation of sheep pox and goat poxviruses by sequence analysis and PCR-RFLP of P32 gene. *Virus Genes,* **29**: 73-80.

Hussein M., El-Sadi H. I., Zangana I. K., El-Attar M. Y. and Al-Bana A. S. 1989. Clinical, pathological and virological studies on goat pox in Mosul, Iraq. *Vet. Med. J. Giza*, **37**: 65–76

IPMS (Improving Productivity and Market Success). 2007. Burie pilot learning woreda diagnosis and program design: Final draft. Pp. 95.

Joshi R.K., Garg S.K., Chandra R. and Sharma.V.D. 1995. Growth and cytopathogenicity of goat pox virus in MDBK cell line. *Indian J. Virol*. **11**: 31-33.

Kara P. D., Afonso C. L., Wallace D. B., Kutish G. F., Abolnik C., Lu Z., Vreede F. T., Taljaar L. C. and Zsak A. 2003. Comparative sequence analysis of the South African vaccine strain and two virulent field isolates of lumpy skin disease virus. *Arch. Virol*. **148**: 1335–1356.

King A.M.Q., Adams M. J., Carstens E.B. and Lefkowitz E. J. 2011. *Virus taxonomy: Classification and nomenclature of viruses.* 9 th ed. Report of the International Committee on Taxonomy of Viruses. Elsevier, academic press. London, UK. Pp. 291-309.

Kitching R. P. 1986. Passive protection of sheep against *Capripoxvirus*. *Res. Vet. Sci*. **41**:247-250.

Kitching R. P. and Carn V. M. 2004. Sheep pox and goat pox. Office International des Epizooties manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). OIE, Paris.

Kitching R. P., Bhat P. P. and Black D. N. 1989. The characterization of African strains of *Capripoxvirus*. *Epidemiol. Infect. Dis*. **102**: 335-343.

Kitching R. P., McGrane J. J. and Taylor W. P. 1986. *Capripoxvirus* in the Yemen Arab Republic and the Sultanate of Oman. *Trop. Anim. Heal. Prod*. **18**: 115-122.

Kitching R. P., McGrane J. J., Hammond J. M., Miah A. H., Mustafa A. H. and Majumder J. R. 1987. *Capripox* in Bangladesh. *Trop. Anim. Heal. Prod*. **19**: 203-208.

Kitching R.P. 2004. *Sheep pox and goat pox. In Coetzer JAW, Tustin RC (eds). Infectious Diseases of Livestock.* 2nd ed .Oxford University Press Southern Africa, Capetown. **2:** Pp. 1277-1281

Kitching R.P. and Mellor P.S. 1986. Insect transmission of *Capripoxvirus*. *Res.Vet. Sci*. **40**: 255-258.

Kitching R.P., Hammond J.M. and Taylor, W.P. 1987. A single vaccine for the control of *Capripox* infection in sheep and goats. *Res. Vet. Sci.* **42**: 53-60.

Lamien C.E., Le –Goff C., Silber R., Wallace D.B., Gulyaz V., Tuppurainen E., Madani H., Caufour P., Adam T., El Harrak M., Luckins A.G., Albina E. and Diallo A. 2011. Use of the *Capripoxvirus* homologue of vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: development of a classical PCR method to differentiate goat poxvirus from sheep poxvirus. *Vet. Microbiol*. **21**: 30-39.

Le Goff C., Lamien C.E., Fakhfafh E., Chadeyras A., Abu-Adulugbad E., Libeau G., Tuppurainen E., Wallace D., Adam T., Silber R., Gulyaz V., Madani H., Caufour P., Hamammi S., Diallo A. and Albina E. 2009. *Capripoxvirus* G-protein-coupled chemokine receptor, a host-range gene suitable for virus-animal origin discrimination. *J. Gen. Virol*. **90**: 67–77.

Madhavan A., Venkatesan G. and Kumar A. 2016. *Capripoxviruses* of small ruminants: Current updates and future perspectives. Asian *J. Anim. Vet. Adv.* **11**. 3-5

Mahmoud M A. and Khafagi M .H. 2016. Detection, identification, and differentiation of sheep pox virus and goat pox virus from clinical cases in Giza Governorate, Egypt. *J. Vet. World*, **9 (12)**: 1445-1449.

Mangana O., Kottaridi C. and Nomikou K. 2008. The epidemiology of sheep pox Greece from 1987-2007. *Rev. Sci. Tech. Off. Int. Epiz*. **27 (3)**: 899-901.

Mangana-Vougiouka O., Markoulatos P., Koptopoulos G., Nomikou K., Bakandritsos N. and Papadopoulos P. 2000. Sheep pox virus identification from clinical specimens by PCR cell culture, immunofluorescence and agar gel immunoprecipitation assay. *Mol. Cell. Probes*. **14**: 305-310.

Markos Tibbo, Philipsson J. and Workneh Ayalew. 2006. Sustainable sheep breeding programmes in the Tropics: Framework for Ethiopia. Conference on International Agricultural Research for Development, At Bonn, Germany.

Matthews R. E. 1982. Classification and nomenclature of viruses. *Inter. Virol*. **17**: 1-99.

McFadden G. 2005. Poxvirus tropism. *Natur. Rev. Microbiol.* **3**: 201–213.

Mellor P. S., Kitching R. P. and Wilkinson P. J. 1987. Mechanical transmission of *Capripox virus* and *African swine fever virus* by *Stomoxys calcitrans*. *Res. Vet. Sci*. **43**: 109-112.

Mersha Chanie. 2011. Clinical and histopathological study of sheep pox in Ethiopia. *Inter. J. Natur. Sci*. **1**: 8-92.

Mirzaie1 K., Mohammad S. B. and Bokaie S. 2015. A review of sheep pox and goat pox: perspective of their control and eradication in Iran *J. Adv. Vet. Anim. Res.* **2(4)**: 373-381.

Moges Woldemeskel and Gashaw Mersha. 2010. Study on caprine and ovine dermatophilosis in Wollo, North east Ethiopia. *Trop. Anim. Prod*. **42**: 41-44.

Mondal B., Hosamani M., Dutta T. K., Senthilkumar V. S., Rathore R. and Singh R. K. 2004. An outbreak of sheep pox on a sheep breeding farm in Jammu, India. *Rev. Sci. Tech*. **23**: 943-949.

MORAD. 2010. Sheep pox disease outbreak report Ministry of Agriculture and Rural Development (MORAD) Addis Ababa, Ethiopia.

Moss B. 2001. *Poxviridae: The viruses and their replication. In: Fields, B.N. Knipe D.M., Howley P.M., Chanock R.M., Melnick J.L., Monathy T.P., Roizman B., Straus S.E. (Eds.), Fields Virology.* 4 th ed. Lippincott, Williams and Wilkins, Philadelphia, Pa. Pp. 2849–2883.

Moss B. and Shisler J.L. 2001. Immunology 101 at poxvirus U: Immune evasion genes. Semin. Immunol., **13**: 59-66.

Munz E. and Dumbell K. 1994. Sheep pox and goat pox. In J. A. Coetzer, G. R. Thomson, and R. C. Tustin (ed.), Infectious diseases of livestock, vol. 1. Oxford University Press, Cape Town, South Africa. Pp 613–615.

Nesradin Yune and Nejash Abdela. 2017. Epidemiology and economic importance of sheep and goat pox: A review on past and current aspects. School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, *J. Vet. Sci. Technol*., **8**: 2157-7579.

Nottor D.R. 2012. Genetic improvement of reproductive efficiency of sheep and goat. *Anim. Reprod. Sci.,* **130**: 147-151.

Oguzagulu T. C., Alkan F., Ozkul A., Vural S.A.,Gungor A.B. and Burgu I. 2006. A sheep pox virus outbreak in central Turkey isolation and identification of *Capripoxvirus ovis*. *Vet. Res. Commun.* **30**: 965–971.

OIE. 2010. Manual of diagnostic tests and vaccines for terrestrial animals, lumpy skin disease, Paris: OIE. Chapter **2**.4-14,

OIE. 2012. Sheep pox and goat pox. Terrestrial Manual Chapter 2.7-14.

OIE. 2014. Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees). Biological standards commission, International Office of Epizootics. The international committee of the OIE. Paris. http://www.oie.int.

OIE. 2017. Sheep pox and goat pox Terrestrial Manual,Chapter 2.7.13. Pp1-12.

Orlova E. S., Shcherbakova A. V., Diev V. I. and Zakharov V. M. 2006. Differentiation of *Capripoxvirus* species and strains by polymerase chain reaction. *Mol. Biol*., **40**: 158-164.

Pawaiya R.V.S., Bhagwan P.S.K. and Dubey S.C. 2008. Histopathological study of goat pox in a natural outbreak the *Indian J. Small Ruminants*.**14**: 117-121.

Radostits O.M., Gay C.C and Hinchcliff K.W. 2006. *Veterinary Medicine*. 10th ed. Saunders, 1430-1431.

Rao T.V.S. and Bandyopadhyay S.K. 2000. A comprehensive review of goat pox and sheep pox and their diagnosis. *Anim. Heal. Res. Rev*., **1**: 127-136.

Rao T.V.S., Nandi S. and Sreenivasa B.P. 2001. Diagnosis of sheep pox and goat pox by PCR technique. *Indian J. Anim. Sci*. **2**:1-3

Regnery, R. L. 2007. Pox viruses and the passive quest for novel hosts. Curr. Top. Microbiol. Immunol. **315**: 345–361.

Rweyemamu M., Paskin R., Benkirane A., Martin V., Roeder P. and Wojciechowski K. 2000. Emerging diseases of Africa and the Middle East. *Ann. NY Acad. Sci*., **916**: 61-70.

Samuel Tefera. 2004. *Investigation in ectoparasites of small ruminants in selected sites of Amhara Regional state and their impact on tanning industry*. MSc Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Senthilkumar V. and Thirunavukkarasu M. 2010. Economic losses due to sheep pox in sheep farms in Tamil Nadu. Tamil Nadu *J. Vet. Anim. Sci*. **6**: 88-94.

Sharawi S.S.A. and Abd El-Rahim I.H.A. 2011. The utility of polymerase chain reaction for diagnosis of lumpy skin disease in cattle and water buffaloes in Egypt. *Rev.Sci. Tech. Off. Int. Epiz.*,**30 (3):** 821-830.

Sharma A. and Sharma K.N. 1990. Application of SRID and micro ELISA for the detection of sheep pox vaccination response in crossbred lambs. Indian *J. Virol*. **6**: 80-82.

Sheikh A. M., Mohammed E. H., Babiker H. A. and Abdel-Wahid S. A. 2004. Alteration in some epidemiological patterns and virus heterogeneity recently observed in sheep pox outbreaks in Sudan. *Veterinaski Arhiv*. 7**4**: 341–350.

Singh I.P., Rao V.D.P., Chandra R. and Garg S.K. 1984. Comparative evaluation of sheep pox vaccines. *Indian J. Anim. Sci*. **54**: 650-453.

Singh R., Chandra D., Singh K.P., Hosamani M., Singh R.K. and Chauhan R.S. 2007. Epidemiological investigation of sheep pox outbreaks in Rajasthan Indian. *J.Vet. Patholo.* **31:** 712-715.

Solomon Gizaw, Van Arendonk J.A.M., Komen H., Windig J.J. and Hanott O. 2007. Population structure, genetic variation and morphological diversity in indigenous sheep of Ethiopia. *Anim. Gene*s, **38**: 621-628.

Soman J. P. and Singh I.P. 1980. Plaque formation by sheep pox virus adapted to lamb kidney cell culture. *Indian J. Exp. Biol*., **18**: 313-314.

Stata. (2012) Statistical data analysis. Stata version 12.0 especial Edition. Stata corp 4905 Lakeway Drive, College station, Texas 77845 USA. 800 STAYA - PC

Stubbs S., Oura C.A., Henstocka M., Bowden T.R., King D.P. and Tuppurainen E.S. 2012. Validation of a high through put real-time polymerase chain reaction assay for the detection of *Capripox* viral DNA. *J. Virol. Methods*, **179**: 419–422.

Takele Tesgera, Jing Z., Jia H., Chen G., He X.B. 2018. A Review on sheep pox and goat pox: Insight of epidemiology, diagnosis, treatment and control measures in Ethiopia. *J. Infect. Dis Epidemiol.,* **4**:4-5.

Talavera A. and Javier M.R. 1991. Isolation and handling of recombinant vaccinia virus methods in molecular biology. Practical molecular virology. Edited by M. Collins. The Humana Press Inc. Cliton. *N.J*. **8**:235-249

Thrusfield M. 2005. *Veterinary Epidemiology*, 3rd Ed. Blackwell science Ltd, UK, Pp. 229-267.

Tian H., Wu J. and Zhang K. 2012. Development of a SYBR green real-time PCR method for rapid detection of sheep pox virus. *J.Virolo*. **9**: 1-4.

Townsley A.C., Senkevich T.G. and Moss B. 2005. The product of the vaccinia virus L5R gene is a fourth membrane protein encoded by all poxviruses that is required for cell entry and cell-cell fusion. *J. Virol*. **79**: 10988-10998.

Tsegaw Fentie, Niguse Fenta, Samson Leta, Wassie Molla, Birahanu Ayelet,Yechale Teshome, Selesh Nigatu and Ashenafi Assefa. 2017. Sero- prevalence, risk factor and distribution of sheep and goat pox in Amhara region, Ethiopia. *J. BMC. Vet. Rese*.**13:** 385.

Tulman E. R., Afonso C. L., Lu, Z., Zsak L., Sur J. H., Sandybaev N. T., Kerembekova U. Z., Zaitsev V., Kutish G. F. and Rock D. L. 2002. The genomes of sheep pox and goat pox viruses. *J. Virol*. **76**: 6054-6061.

Tulman E. R., Afonso C. L., Zsak Z., Lu L., Kutish G. F. and Rock D. L. 2001.Genome of lumpy skin disease virus. *J. Virol*. **75**: 7122–7130.

Tuppurainen E.S. and Oura C.A. 2012. Review: lumpy skin disease: An emerging threat to Europe, the Middle East and Asia. Tranbound. Emerg. Dis. **59**: 40-48.

USDA. 2002. Agricultural bioterrorism act of. *Fedl. Regist*. **67**: 52383-9.

Van Regenmortel M. H.V., Fauquet M., Bishop D. H. L. 2000.*Virus taxonomy* 1st Ed. Seventh report of the international committee on taxonomy of viruses, San Diego: Academic Press. USA Pp 1162

Venkatesan G., Balamurugan V. and Bhanuprakash V. 2014. Multiplex PCR for simultaneous detection and differentiation of sheep pox, goat pox and Orf viruses from clinical samples of sheep and goats. *J. Virol. Methods*, **195**: 1-8.

Venkatesan G., Balamurugan V., Yogisharadhya R., Kumar A. and Bhanuprakash V. 2012. Differentiation of sheep pox and goat pox viruses by polymerase chain reaction-restriction fragment length polymorphism. *Wuhan Inst. Virolo*. 27 **(6)**: 353-359.

Verma S., Verma L.K., Gupta V. K., Katoch V. C., Dogra V., Pal B. and Sharma M. 2011. Emerging *Capripoxvirus* disease outbreaks in Himachal Pradesh, a Northern state of India. *Transbound. Emerg. Dis*.**58**:79–85.

Verma S., Verma L.K., Gupta V.K., Katoch V.C., Dogra V., Pal B. and Sharma M. 2011. Emerging *Capripoxvirus* disease outbreaks in Himachal Pradesh, a Northern state of India. Transbound. Emerg. Dis. **58**:79–85.

Webbs G. 1980. Sheep and goat pox, transmission of *Capripoxviruses* by various flies indicated the need for a reassessment of the methods of controlling this disease. Ann. Rep. Insti. Anim. Heal. Pirbright. UK.

Yakob Hailu, Nesenat Beriha and Dinka Ayana. 2008. Prevalence of major skin diseases in cattle, sheep and goats at Adama veterinary clinic, Oromia Regional State. *Revue. Med. Vet*., **159**: 455- 461.

Yan X.M., Chu Y.F., Wu G.H., Zhao Z.X., Li J., Zhu H.X. and Zhang Q. 2012. An outbreak of sheep pox associated with goat poxvirus in Gansu province of China. *Vet. Microbiol.* **156**:425–428.

Yeruham I., Nir O., Braverman Y., Davidson M., Grinstein H., Haymovitch M. and Zamir O. 1995. Spread of lumpy skin disease in Israeli dairy herds. *Vet. Rec*., **137**: 91-93.

Yeruham I., Yadin H., Van Ham M., Bumbarov V., Soham A. and Perl S. 2007. Economic and epidemiological aspects of an outbreak of sheep pox in a dairy sheep flock. *Vet. Rec*., **160**: 236-237.

Zangana K. and Abdullah M.A. 2013. Epidemiological, clinical and histopathological studies of lamb and kid pox in Duhok, Iraq. Bulgarian *J. Vet. Med.* **16**: 133-138.

Zeng X., Chi X., Li W., Hao W., Li M., Huang X., Huang Y ., Rock D.L ., Luo S. and Wang S. 2014. Complete genome sequence analysis of goat pox virus isolated from China shows high variation. *Vet. Microbiol*. **173**: 38-4.

Zheng M., Jin N., Liu Q., Huo X., Li Y., Hu B., Ma H., Zhu Z., Cong Y., Li X., Jin M. and Zhu G. 2009. Immunogenicity and protective efficacy of Semliki forest virus replicon-based DNA vaccines encoding goat pox virus structural proteins. *Virology* **391**:33–43.

Zinash Sileshi. 2009. Sheep and goat pox: Causes, prevention and treatment.Technical Bulletin; No.29.

Zro K., Azelmat S., Bendouro Y., Kuhn J.H., El Fahime E. and Ennaji M.M. 2014a. PCR-based assay to detect sheep pox virus in ocular, nasal, and rectal swabs from infected Moroccan sheep. *J. Virol. Methods*, **204**: 38-43.

# 7. APPENDICES

Appendix7.1 Questionnaire for epidemiology and associated risk factors for occurrences of sheep and goat pox in the study areas

The purpose of this questionnaire is to gather information regarding sheep and goat pox virus. The information will be kept confidential and be only applied for the study. Your right information helps to reach goals of the study. Thank you for investing your time and honesty completing this questionnaire. Please remember that there are neither rights nor wrong answers. Put "X" mark on the box OR Circle it.

SGP virus outbreak epidemiological Investigation data collection and questioner on risk factors format

**I. General information**: Owner Name----------

.Area (spatial) information: Region---------zone----------districts-------PAs--------

Specific location of the outbreak: latitude------longitude----------Altitude----------

Spp--------- Age ---------- Sex------ Breed ---------

1.1. Level of education Illiterate ☐ Elementary ☐ Above ☐

1.2. Marital Status:- Married ☐ Not Married ☐ Divorced ☐ Windowed ☐

**II. Question on risk factor of SGP**

1.3 Way of life A. mixed farming system B. modern production system C. pastoral

1.4 Feed system for animals A. free grazing B. rotational grazing C. zero grazing

1.5 Watering system for animals' A. communal B. individual

1.6. Production system of animal's A. extensive B. Semi-intensive C. intensive

1.7 Vaccination practice A. Present B. Absent

1.8. Free movement of animals A. Present B. Absent

1.9. Animal marketing system A. free contact of animals at market B. no contact of animal at market

1.10. Body condition of the animal affected A. Good B. Medium C. Poor

1.11. Total no sheep and goats in the herd A. < 10 B. 10-19 C.20-30

1.12. Herd states of sheep and goat affected A. Presence of sheep and goat B. Presence sheep only C. Presence goat only

1.13. General clinical examination

A. Temp_____ Heart rate _____Respiratory rate_____

1.14. Laboratory screening of animals when introduced to their stock

A. Present B. Absent

**III. Sheep and Goat pox Outbreak history**

2.1 Outbreak occurrence A. Every year----- B. Every two year ------C. Every three years---

2.2 Local name: ---------- meaning ----------

2.3. Season of occurrence: A. dry season -----B. rainy season ------

2.4. The last outbreak of SGP in the village occurred in:

A. Before 2000-------B. 2000-20005 ------C. 2006-2010----------

2.5 effect on shoats A. Absent B present

2.6 The likely sources of outbreaks:

A. Introduction of infected animal/animals--------

B. Contact of communal points------

2.7 First date of outbreak occurrence----------

2.8. Date of reported----------

2.9 Total days since outbreaks--------

2.10 livestock production in the farm -------- village-------

Table 7.1.1 1Total number of animals affected

| **Species** | **Sex** | **Age** | | **Total** |
|---|---|---|---|---|
| | | ≤2years | >2years | |
| Sheep | | | | |
| Goats | | | | |

Table 7.1. 2 Total number of animals dead

| **Species** | **Sex** | **Age** | | **Total** |
|---|---|---|---|---|
| | | ≤2years | 2> years | |
| Sheep | | | | |
| Goats | | | | |

Appendix7. 2 Age determination of small ruminants by teeth eruptions

| **Type of teeth** | **Age at eruptions** | **References** |
|---|---|---|
| central incisors | between 12 and 18 months | Cocquyt *et al*.,2013 |
| middle incisors | between 18 and 26 months | |
| permanent lateral incisors | between 24 and 36 months | |
| permanent corner teeth | between 32 and 44 months | |

Appendix7. 3 Procedures of tissue processing and DNA extraction in NVI

1. Wash the tissue sample by adding PBSA in petri dishes.
2. Cut washed skin tissue sample in to pieces by using scissors in sterilized petri dishes.
3. Thaw the biopsy samples at room temperature in safety cabinet II.
4. Take approximately 1gm washed tissue sample mix with 9 ml sterile PBS containing antibiotic (0.1% gentamicin) and ground using a sterile mortar and pestle.
5. Wash the grinded tissue by using 9 ml sterile phosphate-buffered saline (PBSA, pH 7.2) three times.
6. Transfer the mixed tissue samples from the mortar in to the sterilized test tubes the put in the plastic rack finally level it.
7. Centrifuge the mixed tissue samples at 3500 rpm for 10 minutes
8. After centrifugation collect the1ml (1000 µl) supernatant in to new cryovial tubes then level the samples
9. Take 200µl of supernatant put in to eppendorf tube and add 20 µl of proteinase K and mix by vortex. To ensure efficiency of lysis add 200µl of AL buffer (lysis buffer) and mix it with pulse vortexed for 15 sec.
10. Incubate it at 56°C for 30 minutes then briefly centrifuge to activate enzymatic activity.
11. Add 200µl ethanol (96%) and mix thoroughly for 1 minute by vortex mixer and briefly centrifuge it to facilitate precipitation of the DNA.
12. Apply this mixture to the QIAamp mini spin column and centrifuge at 620µl x (12500 rpm) for 1 minute.
13. Transfers the spin column in to 2 ml eppendorf tube and add 500µl buffer AW1and centrifuge it at 12500 rpm for 1 min.
14. Discard the collection tube, transfer the spin column in to new 2 ml eppendorf tube and add 500µlbuffer AW2 and centrifuge at full speed 13400 rpm for 3 min.
15. Carefully transfer the spin column in to a new 2 ml eppendorf tube and discard it.
16. Centrifuge the old collection tube with the filtrate for 1 min and add 200µl of buffer AE and incubate at room temperature for 1 minute and continue with centrifugation at 13400 rpm for 1 min and this step was repeated to get the finale extract.
17. Collect the extracted DNA and store at -20°C until use.

Appendix7. 4 Extraction kit Master Mix, Gel, TAE buffer, Gel Red, Loading Dye, Molecular Ladder.

- DNA Extraction kit Composition
  - QIAamp spin columns
  - Cryovial tubes
  - Buffer AL
  - Buffer AW1
  - Buffer AW2
  - Buffer AE1
  - Proteinase K
- Master mix for pox virus DNA convectional for one reaction
  - RNA free water 3µl
  - Primer SPGP RNA pol forward 2 µl
  - Primer SPGP RNA pol reverse 2µl
  - IQ supper mix SPGP l0 µl
  - Template DNA 3 µl

Appendix7. 5 Reference NO MB /19 Conventional PCR for Capripoxvirus differentiation procedure

Table 7.5. 1 Master Mix preparation

| **Ser.no** | **Type of reagent** | **For one reaction** | **Total reaction** | **Remark** |
|---|---|---|---|---|
| 1 | RNase free water | 3µl | 3µl*19=57 µl | |
| 2 | primerSPGPRNApol-Fow-5pm/ µl-5'-TCTATGTTCTTGATATGTGGTGGTAG-3' | 2 µl | 2 µl*19=36 µl | |
| 3 | Primer-SPGPRNApol-REV-5pm/ µL-5'-AGTGATTAGGTGGTGTATTATTTTCC-3' | 2 µl | 2 µl*19=36 µl | |
| 4 | IQ Super mix | 10 µl | 10 µl*19=190 µl | |
| 5 | Add Template (DN A) | 3 µl | 3µl*19=57 µl | |
| | Total volume | 20 µl | 20 µl*19=360 µl | |

Table 7.5. 2 Run PCR Reaction

| | **Temperature** | **Time** | **Cycle** | **Remark** |
|---|---|---|---|---|
| Initial Denaturation | 95$^{o}$c | 5 mints | 1-Cycle | |
| Denaturation | 95$^{o}$c | 30 sec | | |
| Annealing | 50$^{o}$c | 30 sec | 40 cycles | |
| Elongation | 72$^{o}$c | 30 Sec | | |
| Final Elongation | 72$^{o}$c | 7 mints | 1-Cycle | |
| Put at | 4$^{o}$c | Until machine off | | |

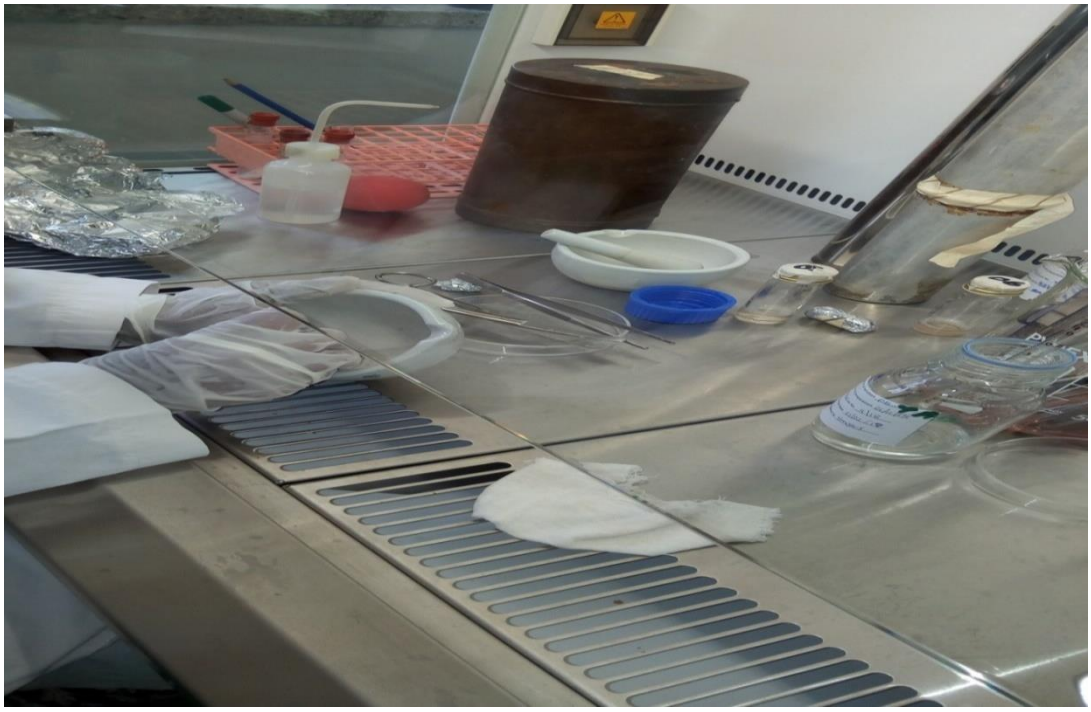
Appendix7. 6 Agarose Gel Preparation Procedures

- 3 gram agarose was added into a flask containing 100 ml of 1X TAE (Tris-acetate-EDTA) buffer.
- The mixture was boiled to dissolve and cooled to 55ºC.
- The gel was poured on gel caster placed horizontally
- The comb was placed in the caster.
- When the gel was completely solidified after 20 minutes, the gel was placed in the electrophoresis tank containing 1X TAE running buffer
- The comb was removed carefully.

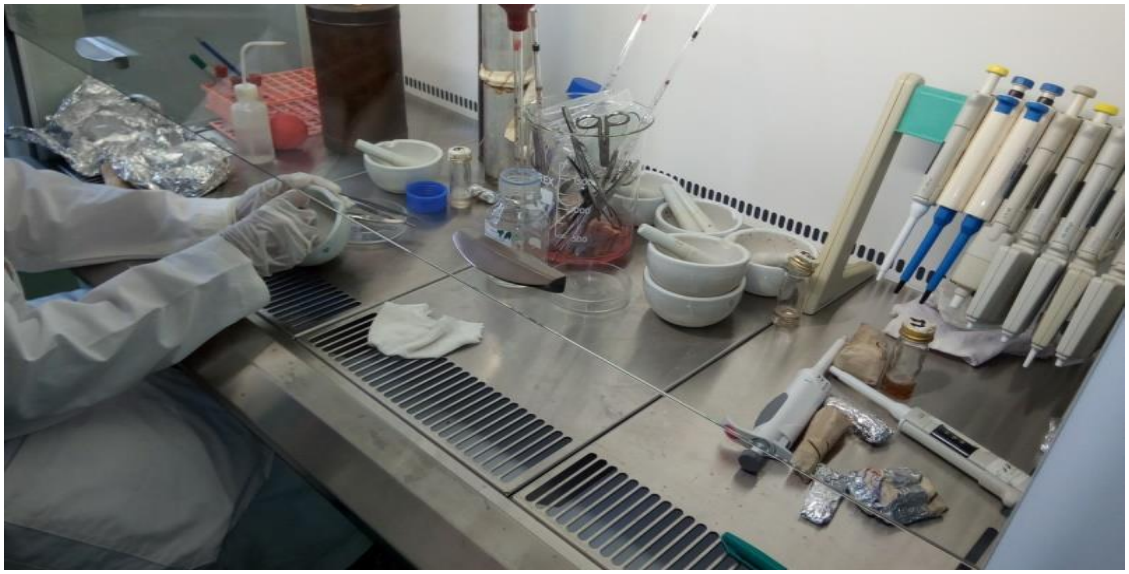
Appendix7. 7 Agrose Gel electrophoresis Procedures

- Add 4µl gel red with loading dye.
- Loading 10µl amplified PCR product and 10µl Marker (ladder)
- Run electrophoresis for 1:20 hour at 120V.
- Read the result by using UV-light.
- Sheep pox virus-around 151bp positive result
- Goat pox virus- around 172bp positive result.
- LSD around 172 bp positive result

Appendix7. 8  Collection of specimen in the inner side of the thigh muscles



Appendix 7. 9 Tissue Processing Materials



Appendix 7. 10 Tissue processing at NVI virology laboratory

# BIOGRAPHICAL SKETCH

The author was born in Guagusa Shikudad district, Awi zone of Amhara National Regional State of Ethiopia, on January, 1973 E.C from his mother W/ro Workayehu Nigussie and his father M/r Wondim Shiferaw. He attended his elementary education and junior secondary education in Tillili elementary school and junior secondary school respectively. Then, he attended his secondary education in Damot Senior secondary school in Fenote Selam town and he completed his secondary education in 1993 E.C and promoted to higher educations. In 1994, the author had joined Addis Ababa University Arat kilo Campus. After attending his common course he joined Addis Ababa University College of Veterinary Medicine in 1995, after his graduation, he was employing in Fageta Lekoma district as Animal Health Extension expert. After one year the author joined Burie ATVET College as Animal Health Instructor and he worked in Brooke Ethiopia as Equine Welfare Project Coordinator. In October 2017 he joined Bahir Dar University, College of Agricultural and Environmental Sciences, School of Animal Science and Veterinary Medicine for his post graduate study and he attended Master of Veterinary Epidemiology and Economics Program. Now he is working in Bahir Dar Poly technic college senior animal health instructor.