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ISOLATION AND CHARACTERIZATION OF POX VIRUS CIRCULATING IN SHEEP AND GOAT FROM OUTBREAK CASES OF ADEA BERGA DISTRICT, WEST SHOA ZONE, CENTRAL ETHIOPIA

MVSc. Thesis

BY

ABDI ASSEFA CHALA

Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of Veterinary Clinical Studies, MSc Program in Veterinary Epidemiology

June, 2017

Bishoftu, Ethiopia

ISOLATION AND CHARACTERIZATION OF POX VIRUS CIRCULATING IN SHEEP AND GOAT FROM OUTBREAK CASES OF ADEA BERGA DISTRICT, WEST SHOA ZONE, CENTRAL ETHIOPIA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Epidemiology

BY

ABDI ASSEFA CHALA

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Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by **ABDI ASSEFA CHALA**

entitled: **ISOLATION AND CHARACTERIZATION OF POX VIRUS CIRCULATING IN SHEEP AND GOAT FROM OUTBREAK CASES OF ADEA BERGA DISTRICT, WEST SHOA ZONE, CENTRAL ETHIOPIA** and recommended that it be accepted as fulfilling the thesis requirement for the Degree of Master of Veterinary Science in Veterinary Epidemiology

Dinka Ayana (DVM, MSc, Asso. Prof.)	_____	_____
Chairman	Signature	Date
Rahamato Abebe (DVM, MSc, Asso. Prof.)	_____	_____
External Examiner	Signature	Date
Haileleul Nigussie (DVM, MSc, PhD Assi. Prof.)	_____	_____
Internal Examiner	Signature	Date
Fufa Abunna (DVM, MSc, Asso. Prof.)	_____	_____
Major Advisor	Signature	Date
BarbaraWeilnda (DVM, PhD)	_____	_____
Co-Advisor	Signature	Date
Fufa Abunna (DVM, MSc, Asso. Prof.)	_____	_____
Department Chairperson	Signature	Date

DEDICATION

This thesis manuscript is dedicated to my Wife, Sisay Bekele

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SIGNED DECLARATION SHEET

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

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Name: Abdi Assefa Chala Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

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LIST OF ABBREVIATIONS

ABWLDHO	Adea Berga Woreda Livestock Development and Health office
AGPT	Agar gel Precipitation Test
APHRD	Animal and Plant Health Regulatory Directorate
AU_IBAR	African Union-Interafrican Bureau for Animal Resources
CDC	Center for Disease Control
CEIP	Counter Immune Electrophoresis
DNA	Deoxyribonucleic Acid
EEV	Extracellular enveloped virus
ELISA	Enzyme Linked Immune Sorbent Assay
ESGPIP	Ethiopian sheep and goat productivity improvement program
FAT	Fluorescent Antibody Techniques
GPV	Goat Pox Virus
IgG	Immunoglobulin G
ILR	International Livestock research Institute
IMV	Intracellular Mature Virus
Kb	Kilo base
Kbp	Kilo base pairs
LAT	Latex Agglutination Test
LIVES	Livestock and Irrigation value chain for Ethiopian small holder
LSD	Lumpy Skin Disease
LSDV	Lumpy Skin Disease Virus
MAb	Monoclonal Antibody
OIE	Office of International des Epizootics
PCR	Polymerase Chain Reaction
RBCs	Red Blood Cells

LIST OF ABBREVIATIONS (Continued)

REA	Restriction Enzyme Analysis
SGP	Sheep and Goat Pox
SNT	Serum Neutralization Test
SPV	Sheep Pox Virus
SRHT	Single Radial Hemolysis Test
SRID	Single radial Immune Diffusion
VNT	Virus Neutralization Test

ABSTRACT

A cross-sectional study was conducted to isolate and characterize the pox viruses circulating in sheep and goats from outbreak cases of Adea Berga district from November 2015 to March 2017 using tissue culture and PCR. The study was employed questionnaire, outbreak search, virus isolation and molecular characterization. The questionnaire survey indicated that sheep and goat pox was the most common disease in all study areas and the disease was frequently seen during the long rainy (*Ganna*) and short rainy (*Afrasa*) seasons. A total of 600 sheep and goats (412 sheep and 188 goats) from ten Peasant Associations (in which the outbreaks were occurred) were clinically examined for the presence of pox lesions on their skin. Out of these, 137(33.3%) sheep and 51(27%) goats had pox lesions on their skin. The overall morbidity and mortality proportion of sheep and goat pox was 31.3% and 4.5%, respectively. High mortality rate was observed in young age groups with odd ratio 1.90 at 95% CI ($P < 0.05$) as compared to adult. From 27 tissue sample collected, the virus was isolated from 25 skin samples (13 sheep and 12 goats). The tissue culture showed a typical characteristic of pox virus: cytopathic effect of cell syncytia, ballooning, aggregation and detaching of cells on Vero cell culture. Similarly, the conventional PCR revealed that 25 out of 27 tested samples were positive by developing band size of 172bp (*goat pox virus*) whereas two of them could not produce any band size on gel electrophoresis. Even though the existing knowledge suggested that *Capri pox virus* is strictly host specific. The current study PCR result confirmed that sheep were affected by goat pox virus similarly to goats and hence classification of pox virus based on infected host in small ruminant has been found to be inconclusive. Thus, genotyping of the isolates should be conducted carefully instead of naming the virus genotype based on the name of animals from which the samples has been collected.

Key words: Adea Berga**,** Sheep and Goat, Pox virus, outbreak, PCR, Seasonal calendar

1. INTRODUCTION

Small ruminants have high reproductive capacity and growth rates and are ideally suited to production by resource-poor small holders (Tibbo, 2006). Indigenous goat and sheep are reported to be relatively resistant to some infectious diseases and parasites, good flocking instinct, ability to walk long distances in search of feed, high tolerance to adverse climatic conditions, and have endurance to droughts and low and fluctuating nutrient availability (OIE, 2008a). They require smaller investments, have shorter production cycles and greater environmental adaptability, and hence have a unique niche in smallholder agriculture. Ethiopia has about 25 million sheep and 23 million goats (CSA, 2015).

Sheep and goats supply more than 30% of the domestic meat consumption and generate cash and hard currency from export of meat, live animals and skin. Although the contribution of sheep and goats to the ethiopian agrarian economy is significant, small ruminant production in the country is not appropriate with the demand. This is because the sub-sector is constrained by various factors such as animal diseases, traditional system of management, inadequate feed supply and poor genetic potential (Alemayehu and Fletcher, 1991). Infectious diseases of small ruminants also cause substantial loss through morbidity and mortality. Sheep and goat pox (SGP) are among the commonest of the diseases that affect small ruminants entailing a huge economic loss. The two diseases are Office International des Epizooties (OIE) listed trans- boundary diseases affecting the economy of the country through limiting international trade of animals and animal products (OIE, 2008a, 2008b).

Sheep and goat pox (SGP) are viral diseases of sheep and goats characterized by fever, generalized papules or nodules, vesicles (rarely), internal lesions (particularly in the lungs), discharge from the eyes and nose, necrotic skin lesions, oedema of the limbs, swollen lymph nodes and death. Sheep pox and goat pox are caused by strains of *Capripoxvirus* in the family *Poxviridae*, which can infect both sheep and goats (OIE, 2008a and 2008b).

The viruses have immunological similarities and sometimes cross-protect against one another but are distinct in finer genetic and antigenic detail. The *Capripoxvirus* virion is enveloped, brick-shaped with dimensions of 300×270x200nm and covered in short tubular filaments. The double stranded DNA genome is linear in conformation and is approximately 154kb in length. There are two distinct virus particles that exist; the intracellular mature virus (IMV) and the extracellular enveloped virus (EEV). *Capripoxvirus*es can be found in saliva, conjunctival secretions and milk, as well as in skin lesions and their scabs (Carn and Kitching, 1995a).

Sheep pox and goat pox virus are mainly transmitted by the aerosol route following close contact with infected animals. Alternative routes of infection include through alternative mucus membranes or abraded skin and occasionally via biting insects or contact with contaminated implements, vehicles or objects. In endemic areas, the mortality rate is usually less than 10% (Chihota *et al*., 2001). However, fatality rates of nearly 100% can occur in young animals. Viral shedding occurs in nasal; oral and conjunctival secretions starting from the appearance of papules, with the quantity and duration of shedding dependent on the virus isolate and host species (Bowden *et al*., 2008). Viral DNA and infectious virions can be detected in some secretions for up to a month following resolution of acute disease (Bowden *et al*., 2008). The amount of viral shedding correlates with the severity of clinical disease, with sheep and goats displaying mild clinical signs shedding fewer viruses than sheep and goats that have more severe clinical disease (Mellor *et al*., 1987; Chihota *et al*., 2003, 2001).

Skin lesions as well as nasal and oral swabs are the most useful samples for virus isolation (Bowden *et al*., 2008). Sheep and goat poxviruses can be grown using a variety of sheep and goat cells (Binepal *et al*., 2001). Currently, primary lamb kidney or primary lamb testis cells are the most commonly used cells for isolation (Plowright and Ferris 1958; Kalra and Sharma, 1981; Zhou *et al*., 2004). They induce the formation of distinct plaques (Soman and Singh, 1980) with a cytopathic effect characterized by elongated cells (Jassim and Keshavamurthy, 1981). However, primary cells have several

disadvantages including the need to constantly establish new cultures, cell lot variation, and contamination with extraneous agents. A lamb testis secondary cell line has been evaluated as a replacement for primary cells (Babiuk *et al*., 2007). Sheep and goat poxvirus isolation can be confirmed by immune staining using anti-*capripoxvirus* serum (Gulbahar *et al*., 2006; Babiuk *et al*., 2007) but it is not yet possible to differentiate between SPV, GPV and LSDV, as there is only a single *capripoxvirus* serotype (Kitching, 1986).

Severity of the disease depends on breed, age, nutritional and immune status, virus strain, virulence, the nature of the secondary infection and organs involved. Generally, the disease and associated mortality are less commonly seen in indigenous breeds in endemic areas as compared with exotic breeds (Sileshi, 2009). These animals may exhibit mild form of the disease characterized by mild and few skin lesions on certain areas such as the ears and around the tail. However, indigenous animals are more likely to suffer from the disease in areas where it has been absent or dormant for a period of time.

Sheep and goat pox occur throughout Africa, the Middle East, the Indian sub-continent, and central and eastern Asia (Kitching and Taylor, 1985). In endemic areas, most cases are seen in young animals and outbreaks are sporadic. The disease is also endemic in all regions of Ethiopia (APHRD, 2010). For instance, the Animal and Plant Health Regulatory Directorate received 389 sheep pox and goat pox outbreak reports from all regions in 2009/2010. The report indicated that a total of 2,177,741 sheep and goats contracted the disease and 6,522 sheep and goats were at risk in areas where outbreaks occurred. 11.88% morbidity and 0.13% mortality was recorded based on one-year outbreak report and vaccination have been implemented for the control of the disease in the country. However, the fatality rate was as low as 4.63% of the sick goats and sheep (APHRD, 2010).

Polymerase chain reaction (PCR) does provide a rapid and sensitive diagnostic technique for capripoxvirus genome detection. Several groups have reported using conventional PCR (Heine *et al*., 1999; Mangana-Vougiouka *et al*., 2000) or real-time PCR (Balinsky *et*

al., 2008; Bowden *et al*., 2008) for detection of capripoxvirus genetic material. The strengths of real-time PCR are its speed, its quantitative nature and the ability to include controls for detection of reaction inhibitors. Despite these benefits, PCR results should be confirmed by at least one additional test. It has been possible to develop a single PCR based assay to identify all capripoxvirus isolates, and the assay could possibly be refined to be specific only for vaccine isolates (Orlova *et al*., 2006) or specific for only sheep, goat or cattle isolates if distinct signatures are found. There were no any work done on isolation and identification of Sheep and Goat pox in the district. Information on prevalence of sheep and goat in the study area is scant.

The control strategy of goat pox varies according to the disease status of the country. Pox free countries rely upon quarantine barriers to prevent entry of infected animals to maintain their disease free status of a country. Usually these quarantine barriers are coupled with a policy of "stamping out" whenever the disease occur, to rapidly re-establish disease-freed status. Live attenuated vaccines are considered more effective, providing immunity for 12 months or longer (Mondal *et al*., 2004). Ethiopian sheep and goat have been facing poxvirus infection for so many years. Updated information about sheep and goat pox has a paramount importance to minimize the occurrence of this disease so as to encourage intervention options. However, there were a few works conducted so far on the isolation and characterization of *Capri poxvirus* affecting the livestock population. Additionally, there is a little information currently exists about the cross reactivity of the capripoxvirus infections. Therefore, keeping in view the importance of this disease, the present study was designed to gain an insight into the situation of sheep and goat pox disease with the following objectives:

- To investigate sheep and goat pox outbreak in the study district and to determine its morbidity, mortality and case fatality.
- To isolate and characterize pox virus circulating in sheep and goat using cell culture method and conventional PCR method.
- To identify the seasonal occurrence of sheep and goat pox outbreaks and suggest appropriate vaccination schedules to prevent and control the disease.

2. LITERATURE REVIEW

2.1. Definition

Sheep and goat pox (SGP) is an acute to chronic disease of sheep and goats characterized by generalized pox lesions throughout the skin and mucous membranes, a persistent fever, lymphadenitis, and often a focal viral pneumonia with lesions distributed uniformly throughout the lungs (Davies, 1976).

2.2. Etiology

Sheep and Goat pox disease is caused by a virus in the family *Poxviridae* and genus *Capripoxvirus* and is highly contagious. Among the viruses it is the largest one with brick shaped morphology of 170 to 260 by 300 to 450-nm-diameter capsid. Its genome is double-stranded DNA, non-segmented, and linear of approximately 150 kilo bases. A false lipid envelope surrounds the genome (Kitching, 2004).

2.3. Epidemiology

The geographic range of sheep pox and goat pox has been restricted in the last 50 years mainly to Asia and Africa, extending from Africa north of the Equator (Asagba and Nawathe, 1981; Kitching *et al*., 1989; Mariner *et al*., 1991; Achour and Bouguedour, 1999), into the Middle East (Daoud, 1997), Turkey (Oğuzoğlu *et al*., 2006), and Asia including regions of the former Soviet Union (Orlova *et al*., 2006), India (Mondal *et al*., 2004; Bhanuprakash *et al*., 2005) and China (Zheng *et al*., 2007).

Sheep pox or goat pox extended their range into Bangladesh in 1984 (Kitching *et al*., 1987b) and more recently into Vietnam (2005 and 2008) and Mongolia (2006 and 2007) in the east, and repeated incursions have been reported in Greece in southern Europe 2007 (OIE, 2008). The spread of sheep and goat poxvirus into new areas is predominantly associated with the increase of illegal animal movement through trade

(Domenech *et al*., 2006) as well as inadequate or breakdown of veterinary services (Rweyemamu *et al*., 2000). Countries free of sheep and goat poxvirus usually have in place legislation based on OIE recommendations that attempt 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. Biting flies have also been implicated in the spread of *capripoxvirus*es (Yeruham *et al*., 1995). *Capripoxvirus*es are not present in north, central or south America, South East Asia (excluding Vietnam) or Australasia.

The impacts of global climate change on insect vectors, established as a route of transmission for sheep pox and goat pox viruses (because of very high viral loads in the skin), suggest that there are real risks of further spread of these diseases into other geographic regions (Domenech *et al*., 2006).

In Ethiopia, the disease is endemic in all regions. In 2007/2008, the Animal and Plant Health Regulatory Directorate received 893 SGP outbreak reports from all regions except Gambela, Harari and Dire Dawa. Out of the 57,638 sick sheep and goats, 6,401 (11.1%) died. The disease reporting rate in Ethiopia is only about 35-40%. The figures in terms of affected, vaccinated and dead animal is, therefore expected to be higher than the reported figures (Sileshi, 2009).

Although, there are no detailed studies on prevalence of SGP in Ethiopia, some reports indicate that it is one of the widely distributed and common small ruminant production problems in the country (Bhanuprakash *et al*., 2006). Morbidity and mortality from central highland of the country based on the clinical sign and histopathological lesions was found about 49.5% morbidity and 10.42% respectively (Mersha, 2011). In additions, 11.88% morbidity and 0.13% mortality was recorded based on one year outbreak report (APHRD, 2010). Prevalence of pox was 10.34% and 12.88% in sheep and goat in Adama town, Oromia regional state (Yakob *et al*., 2008). A clinical disease associated with pox was reported to be 22% in Sheep and 18% in Goats in Wollo, North east Ethiopia

(Weldemeskel and Mersha, 2009). It is one of the endemic OIE listed disease in the country (OIE, 2009).

According to the AU-IBAR the number of African countries affected by sheep pox and goat pox (SGPX) had, before 2011, shown an increasing trend for three consecutive years. The number of countries reportedly affected by SGPX in 2011 reduced remarkably from the previous year. In 2011, twelve countries reported occurrence of SGPX in their territories, which is a 46% reduction from the 26 countries affected by the disease in 2010 (AU-IBAR, 2011). There is no plausible explanation for this decrease in reporting as there is no ongoing continental program against SGP although there might be national interventions against the disease. The top three countries that recorded the highest number of outbreaks in 2011 include Ethiopia (223), Somalia (170) and Algeria (44). Overall, a total of 541 epidemiological units were affected on the continent involving 9932 cases and 1619 deaths, with a case fatality rate of 16.3% (AU-IBAR, 2011).

Table 1: Countries reporting sheep pox and goat pox to AU-IBAR in 2011

Country	Outbreaks	Cases	Deaths	Slaughtered	Destroyed
Algeria	44	306	14	0	0
Cameroon	2	15	0	0	
Ethiopia	223	4827	815	90	
Ghana	1	2	0	0	0
Kenya	2	9	0	0	0
Lesotho	1	5	0	0	0
Niger	41	945	235	NS	NS
Nigeria	2	33	7	9	5
Senegal	7	410	28	0	0
Somalia	170	2393	324	49	47
Sudan	29	859	185	5	0
Tunisia	19	128	11	5	15
Total (12)	541	9932	1619	158	67

NS: Not specified (Source, AU-IBAR, 2011)

2.4. Host range

*Capripoxvirus*es only infect some ruminant species and have a tropism for certain cell types (McFadden, 2005); they are not infectious to humans (Regnery, 2007). Sheep pox virus and GPV cause clinical disease in sheep and goats, respectively; however, there is a wide range of clinical disease seen with different field isolates (Davies, 1976). The nomenclature is largely made up of the location (country) and the species from which it has been isolated (sheep or goat or sheep and goat). This issue of naming strains sheep pox, goat pox or sheep and goat pox remains problematic since this has been based on field observation of the species affected whether sheep or goats (McFadden, 2005). There is little else to support the species strain designation except when the viruses are used to experimentally infect both hosts under controlled conditions. 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. Some isolates are uniformly pathogenic in both sheep and goats such as some *capripoxvirus* strains from Kenya (Davies, 1976) and the Middle East (Kitching *et al*., 1986). Most isolates, however, cause more severe disease in either sheep or goats and only mild or sub-clinical infection in the other species. It is unknown whether closely related North American species such as mountain goats and mountain sheep would be susceptible to GPV or SPV, but it is likely they would exhibit at least some degree of susceptibility (Kitching *et al*., 1986).

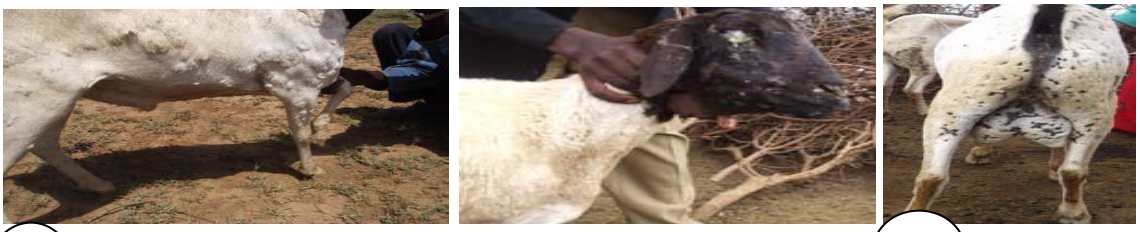
Typically, SPPV and GTPV infect and cause clinical disease in either sheep or goats, respectively, and most isolates induce more severe disease in either sheep or goats and only mild or sub-clinical infection in the other species (Babiuk *et al*., 2008). However, some isolates are pathogenic for sheep and goats, particularly some strains from central Africa (Davies, 1976) and the Middle East (Kitching, 1986).

There is, to date, no evidence of SPPV and GTPV viruses in wildlife, and it is assumed that wildlife do not play a relevant 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. In support of this fact, the lumpy skin disease virus, closely related to SPPV/GTPV, has been isolated from wild ruminants (Tuppurainen and Oura, 2012).

2.5. Clinical signs

The incubation period of the disease varies from 4 to 21 days with an initial rise in temperature, pulse and respiratory rates, edema of the eyelids, nasal discharge, in appetence, arched back, lacrimation, coughing, salivation and nasal discharge leading to crust formation, pneumonia, hypersensitivity, constipation and scanty urine (Singari *et al*., 1990). One to two days later, skin eruptions appear over the less wooly parts of the body. The lesions undergo macular, papular, vesicular, and pustular stages typical to any pox disease. Scabs persist for 3–4 weeks and after healing cicatrix may remain. A fatal septicemia and pyemia may develop and the virus itself may result in the death of the animal during the febrile eruptive phase of the disease in cases of hemorrhagic pocks. Mouth lesions constitute an important source of virus spread. Severity depends on breed, age, nutritional and immune status, virus strain, virulence, the nature of the secondary infection and organs involved. Septicemia/pyaemia due to secondary bacterial complications may lead to death. Aggravations of latent brucella, tendovaginitis, orchitis, abortion and peripheral paresis have also been reported after sheep and goat pox infection (Singh *et al*., 1984). Depending on the severity of the disease, the infection is characterized by different course: The slight course: *variola ovina sine exanthema* and/ or *variola ovina compressa*; The normal course: *variola ovina confluence*; The severe course: *variola ovina haemorrhagica-pastulosa* or *nigra* and *v.o. gangraenosa* (seifert, 1996)



A) Loss of eye sight B) Skin damage C) Skin damage
(Source ESGPIP, 2009)

Figure 1: Sheep and goats affected by pox virus

2.6. Routes of transmission

2.6.1. Direct transmission

The main mode of transmission of SPPV is direct contact between an infectious and a susceptible animal. Upon infection, animals develop pox lesions in the mucous membranes of the mouth, nasal cavities and conjunctiva, excreting the virus in saliva and ocular and nasal discharge. The virus is spread in droplets/aerosols via coughing, sneezing, head shaking, vocalizations and breathing. Naive animals acquire the virus via inhalation, orally or via skin abrasions (Babiuk *et al*., 2008). Infected animals also have high virus titers in skin lesions and scabs, and skin to skin contact may directly spread the virus via skin abrasion. Sucking lambs and kids may contract infection from the milk and the skin of the teats (Babiuk *et al*., 2008).

In the field setting, the first clinical signs of SPP observed in a flock are a high fever, nasal and ocular discharge, through which virus particles are shed, and skin lesions that appear on the face. Importantly, animals are already shedding an infectious virus when the first clinical signs of SPP are detected in the flock. Animals with mild clinical disease, with only a few pox lesions on the skin and mucous membranes, do not spread the virus as effectively as animals with severe signs, although they are still infectious. In endemic regions, infections may go unnoticed, and in that situation movement of animals from infected farms often months after recovery can often lead to introduction of the disease into naive flock (Bhanuprakash *et al*., 2006b).

The high concentrations of the virus in the skin may also contribute to the spread of SPP and GTP via insect vectors (Babiuk *et al*., 2008). There is evidence that stable flies (*Stomoxys calcitrans*) can act as an efficient mechanical vector of SPPV and GTPV (Mellor *et al*., 1987).

Flies transmit the virus to susceptible sheep and goats, and the virus can remain viable for four days in some flies. High virus titers and intrinsic resistance of the virus, vectors with large mouthparts and their frequent feeding habits are the basic factors favoring

mechanical transmission. No transmission was detected with biting (*Mallophaga* spp.) and sucking lice (*Damalinia* spp.), sheep head flies (*Hydrotaea irritans*) and midges (*Culicoides nubeculosus*), even though virus could be isolated from previously infected sheep, which was the host for feeding of the aforementioned flies. There is no evidence that SPPV can replicate in arthropod vectors (Mellor *et al* 1987).

2.6.2. Indirect transmission

Virus in saliva, ocular and nasal discharge, skin lesions and scabs, urine and faeces may 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; when scabs dissolve, the virus may be released into the environment and this may continue for several months after the outbreak (Rao and Bandyopadhyay, 2000). Unfortunately, no reports have been published on survival of SPPV in litter, fodder and feed. Untreated skins, hides and wool collected from infected animals may contain skin lesions and scabs with viable virus and, therefore, they may be a source of infection for naive sheep and goats (Rao and Bandyopadhyay, 2000).

The risk posed by untreated skins, hides and wool imported from endemic countries has recently been evaluated by the UK Department for Environment Food & Rural Affairs (DEFRA, 2014). In this qualitative assessment it is concluded that the risk of release of capripoxviruses into the UK, via the importation of one untreated animal skin/hide/wool bale from the EU, is low. However, this estimate is highly dependent on the probability that a flocks/flock is infected within the EU; should this increase, the overall risk of release will increase (DEFRA, 2014).

The *Capripoxvirus* prevalence is expanding, which is evident from outbreaks in Vietnam, Ethiopia, Mongolia, Egypt, Greece and Israel. Poor quarantine measures and trade across the border of live animals may lead to further spread of the disease (Babiuk *et al*., 2008). The grazing and migration pattern indicates that the disease was probably introduced to the farm in Jammu, India by local sheep (Mondal *et al.,* 2004). The occurrence and

spread of this skin disease are associated with poor management, climatic factors, feed scarcity and inadequate veterinary services. The increasing threat of skin diseases to the development of sheep production warranting an urgent control intervention is indicated. It was noted that sheep pox, goat pox and lumpy skin diseases differ from each other but their viruses may transmit through a similar way like mechanically by biting insects (Carn, 2002).

2.7. Postmortem lesions

The nodular lesion in intestine and lungs were recorded on necropsy. Typical pock lesions disperse over the body of the affected animals with nodular lesions observed in the lung tissue of the dead animals (Mondal *et al*., 2004). Postmortem of dead animal's shows vesicles and pock lesions on affected skins. The lungs are diffusely inflamed (bronchopneumonia) with dispersed Pock lesion (Dubaib, 2002).

2.8. Risk factor

2.8.1. Pathogen risk factor

The poxviruses are thought to have prolonged survival in environment and inactivated by drying, freezing, thawing, and remain viable for months in the lyophilized state. But it is sensitive to 1% of formalin and extreme PH. can remain infectious for up to six months in sheep pens, and may also be found on the wool or hair for as long as three months after infection (Sharma *et al*., 1988). Capripoxvirus are highly stable in normal environment condition and can survive for 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 (Davies, 1981).

2.8.2. Host risk factor

Group of sheep and goat 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 stock. Sheep and goat pox infect only sheep and goat and have no zoonosis. Wild ungulate is not reservoir for this disease (ESGPIP, 2009). Capripoxvirus can affect sheep, goat and cattle. Virus of goat pox is highly host-specific, infecting only goats, but from isolate to isolate host specificity varies. It is possible that the host preference shown by different strains is due to their adaptation to the presence of either sheep or goat alone in a limited geographical area. Isolates of capripoxvirus are not host-specific; cattle, goats, and sheep who have recovered from infection with capripoxvirus isolates from a heterologous host have immune to any challenge with a virulent homologous virus (Kitching *et al*., 1989).

There are two types of sheep pox virus (Singari *et al*., 1990), in which, one affects both sheep and goats (Kenyan sheep and goat (KSG) strain while the other is host specific. Recent records indicate that strains of sheep pox do pass between sheep and goats, although most cause more severe disease in sheep. Recombination also occurs between strains of SPV producing a spectrum, showing intermediate host preferences and a range of virulence (OIE, 2000).

2.8.3. Environment risk factor

Environmental determinants play a great role in the occurrence of sheep and goat pox. It had impact on the agent, host and vectors as well as interaction between them. These predisposing factors have a great role in maintenance of *stomoxys calcitrans* and the tsetse fly to susceptible animals which are the vectors for transmission of disease (Webbs, 1980).

2.9. Pathogenesis

Incubation period of sheep pox is 4-8 of that of goat pox is 4-15 days. After it inters, goat pox virus replicates locally in the tissues. Since the virus is epitheliotropic, it will infest the epithelium tissues of the organism. On the 7^{th} day post-inoculation, the virus titer reached to its peak. The virus spread to the regional lymph nodes, after 3-4 days of

primary viremia. The viremia spread in the body, and affected spleen, lungs and liver. The virus inhaled may also cause lungs lesions. In skin nodules from 7 to 14 days after inoculation, the virus titers persisted and decreased with the development of serum antibodies. Within 24 hours of the appearance of generalized papules, affected animals develop conjunctivitis, rhinitis and enlargement of all the superficial lymph nodes, in particular the prescapular lymph nodes. Excessive salivation can also occur after infection (OIE, 2012).

There are five stages in the development of pox infection. Roseola stage is stage in which skin lesions typically begin with small red spots within three days of infection which is followed by papules**.** The affected animals are febrile at this stage. The second stage of pox lesion is Papules which develops after 3 days of roseola stage. Nodular skin lesions that are developed from roseola stage (red spots) those are hard during palpation. Papules within 5-6 days are changed to vesicles and known as vesicular stage. Pustular stage develops after 3 days of vesicular stage. The last stage of pox lesion is scab. Quantitative analysis using real-time PCR and isolation of the pathogenesis of sheep pox virus and goat pox virus in their respective hosts revealed high viral loads in skin (Bowden *et al*., 2008).

2.10. Morbidity and mortality

Morbidity and mortality rates vary according to breed, previous exposure to the virus, and the strain of the virus. Mild infections are common among indigenous breeds in endemic areas, but more severe disease can occur in young or stressed animals, animals suffering from other infections, or animals introduced from places where SGP is not present. Morbidity rates in indigenous breeds can be 70-90% with mortality ranging from 5-10%. Mortality and morbidity rates in newly imported animals can reach 100% (ESGPIP, 2009).

2.11. Economic impact

Sheep pox and goat pox in endemic areas are associated with significant production losses because of reduced milk yield, decreased weight gain, increased abortion rates, damage to wool and hides, and increased susceptibility to pneumonia and fly strike, while also being a direct cause of mortality (Yeruham *et al*., 2007).

Presence of sheep pox in a country limits the trade of new breeds and development of intensive sheep production. The disease has major impact on the economy with average morbidity and mortality rates of 50 and 100%, respectively. The effect is such that it would take 6 years for a flock or flocks to recover from an outbreak with average income losses up to 30–43% of total annual revenue depending on flock type and owners actions. The level of impact varies from country-to-country both qualitatively and quantitatively. SGPV is one of the animal bioterrorist agents as it causes high morbidity and mortality, has potential for rapid spread, potential to cause serious socio-economic or public health consequences and is of major importance in the international trade of animals and animal products. Sheep pox virus is one of the 15 animal pathogens listed by Animal World Health Organization (OIE) and 23 by Animal and Plant Health Inspection Agency (USDA, 2002) which can be used as an animal biological warfare agent.

2.12. Diagnosis

Sheep and goat Pox can be diagnosed based on observable clinical sign like, fever, dyspnea and pox lesion in different parts of the un woolen skin. Clinical pathology and species of affected host are also important in the diagnosis of this disease. Epidemiology of the disease is also important in diagnoses of sheep and goat pox. As the virus of sheep and goat pox are very closely related it’s indistinguishable by serology. It appears that the host preference shown by these viruses with respect to 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. It is also known that heterologous diagnostic reagents tend to be less efficient than homologous reagents for confirmatory diagnosis (Yune and Abdella, 2017).

Before collecting or sending any samples, the proper authority's samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease. Samples for virus isolation must be sent to the laboratory as soon as possible. They should be kept cold and shipped on gel packs (Davies, 1981). 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 into the centre of the tissue and destroy the virus. Sheep and goat antigen can be detected using routine laboratory procedure (Davies, 1978). Direct fluorescent antibody test using edema fluid, Agar gel immuno Diffusion (AGID) using biopsies of lymph nodes using specific immune sera and ELISA are used to detect sheep and goat pox antigens. Other laboratory diagnoses of sheep and goat pox include observation of the virus using electron microscope, virus isolation, and indirect fluorescent antibody and detection of antibody by virus neutralization test or both; and characteristic histopathologic lesions (Davies, 1981).

2.12.1. Agar gel precipitation test (AGPT)

It was Bechold who first demonstrated the AGPT, but application in serology and diagnosis of sheep pox was reported much later (Bhambani and Krishnamurthy, 1963) using either homologous or heterologous serum [Bhanuprakash *et al*., 2003, Kirubaharan *et al*., 1994, Soad *et al*., 1996). Unfortunately, AGPT does not distinguish between capripox and CPD due to antigen cross-reactivity (OIE 2000) and is also less sensitive (Mangana-Vougiouka *et al*., 2000). Of late, an efficient AGPT using soluble antigens has been reported (Rao *et al*., 1997). Earlier, workers had improved the sensitivity of AGPT using (35S) methionine labelled antigen (Kitching *et al*., 1986)

2.12.2. Counter immune electrophoresis (CIEP)

Counter immune electrophoresis has been found more sensitive and rapid than AGPT for the detection of SPV (Rao *et al*., 1997). Additionally, CIEP has been more effecting in detecting SPV specific antigen in infected tissues (skin, lymph nodes, lungs and liver) as well as cell cultures than AGPT because of its relatively more sensitivity (Uppal and

Nilakantan, 1967). Similarly, CIEP has also been reported to be more sensitive than AGPT for detection of vaccinia antigen in calf, rabbit, and human skin lesions as well as infected CAM (Uppal and Nilakantan, 1967).

2.12.3. Neutralization test

Although neutralization is very specific for almost all the viruses, the test is not very effective in diagnosing sheep pox mainly due to partial neutralization, low serum neutralization (SN) indices and virus breakthrough due to variable susceptibility of cell cultures (Plowright and Ferris, 1958). Further, neutralization has been considered to be unreliable in determining the immune status of an animal (Ramyar and Hessami, 1970). The use of Vero cells in neutralization has been reported to give more consistent results than the LT or LK cells. A constant virus-variable serum method using serum dilutions in the range of 1/5–1/500 and fetal calf muscle cells can overcome the virus breakthrough as these cells are least sensitive than LT or LK cells (OIE, 2000). Therefore, constant serum-variable virus method has been developed and included unheated guinea pigs serum to enhance the neutralization of SPV by serum antibodies from immune sheep (Martin *et al*., 1991).

The use of guinea pig serum in the constant-virus variable-serum method did not have this breakthrough. Despite all these drawbacks, many researchers have employed SNT to study the antigenic relationship between SPV, GPV and LSD (Davies and Otema, 1981), to confirm the disease or to assess the post-vaccinal immune status (Bhanuprakash *et al*., 2003, Fassi-Fehri *et al*., 1984).

2.12.4. Fluorescent antibody technique (FAT)

FAT has been widely accepted as one of the most important techniques in the study of viruses (Soman, 1986). An indirect FAT is more sensitive than direct FAT because of the availability of additional binding sites. The fluorescent antibody technique has been successfully applied: (i) to detect and locate the SPV antigen, (ii) epidemiological

investigations (Davies, 1976), (iii) pathogenesis (Gurel, 1979, (iv) sequential growth of SPV in vitro (Sarkar *et al*., 1980), (v) cross-reactivity of SPV, GPV and cowpox and antigenic relationship between capripox and orthopox viruses (Ramyar and Hessami, 1970), (vi) for providing rapid diagnosis (Mangana-Vougiouka *et al*., 2000), (vii) to study the replication site of the virus and timing of appearance of antigens in cell cultures (Soman, 1986), (vii) to assess the extent of cell-to-cell virus spread and synthesis in cell cultures and (viii) immune status of animal (Debnath, 1992). SPV antigens have been detected as early as 14–24 h of post infection in sheep embryo muscle cell cultures (Soman and Singh, 1980). Using this technique, it was possible to explore an unusual replication site of LT cell-adapted SPV in the nucleus rather than cytoplasm where poxvirus normally multiplies (Soman and Singh, 1980). Test is reliable and simple for the detection of SPV (Davies and Otema, 1978). It is possible to detect SPV antigen in infected edema fluid, skin, LK and LT cells (Soman, 1986), chicken embryo and bovine kidney fibroblast monolayer. However, development of monoclonal antibody (MAb) based indirect FAT will provide more sensitive and specific diagnosis (Soad *et al*., 1996).

2.12.5. Latex agglutination test (LAT)

Antigens can be coated very effectively on to latex beads (carriers), which can be applied in agglutination test. This test is economical, rapid, reliable, easy to perform and successful in the detection of antigen–antibody systems. LAT is more efficient than CIEP in the diagnosis of sheep pox (Rao *et al*., 1997).

2.12.6. Single radial hemolysis test

The principle of the single radial hemolysis (SRH) test is the lysis of erythrocytes when the antigen–antibody complexes using complement are formed. The test is very simple and used successfully for quantifying the antigen, antibody and post-vaccinal immune response (Kataria and Sharma, 1992) of sheep pox. The test has been reported to be positive only with sheep RBCs but not with that of chicken (Rao and Chandra, 1986).

2.12.7. Single radial immune diffusion (SRID)

The single radial immune diffusion test has been successfully used for the quantification of immune response against SPV. Immune response in terms of IgG in lambs vaccinated with SPV was estimated depending upon the diameter of the precipitation ring (Sharma and Sharma, 1990).

2.12.8. Enzyme linked immune sorbent assay (ELISA)

An indirect ELISA based on SPV P32 recombinant fusion protein expressed in Escherichia coli was found rapid, reliable, non-infectious and detected antibodies to *capripoxvirus* post-infection earlier than VNT (Carn *et al*., 1994) as the latter test is besotted with disadvantages as discussed elsewhere. Similarly, an anti-recombinant P32 antibody based ELISA has been developed (Heine *et al*., 1999). Unlike in immune fluorescence (Davies 1982) and AGID (Kitching *et al*., 1986), no cross-reactions have been reported with orthopox viruses and parapox viruses in P32 ELISA (Carn *et al*., 1994). An antigen-trapping ELISA has also been developed (Carn, 1995), which was compared well with virus isolation from skin biopsy samples of sheep, goats and cattle and reduced the reliance of diagnostic laboratories on tissue culture facilities. The test is most suitable to detect *Capripoxvirus* in tissue culture. Recently, a relatively simple immune capture ELISA for the detection of SPV antigens in scab suspensions has been developed (Rao *et al*., 1997). Post-vaccinal immune response has been efficiently monitored with different kinds of ELISAs (Debnath *et al*., 1992). Although, ELISA is advantageous, its application is limited at the field level because of the requirement of special reagents and technical expertise. Development of MAbs against SPV and GPV will definitely overlay the way for differentiation of SPV and GPV thereby providing specific diagnosis (Carn, 1995).

2.12.9. Western blotting

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. However, the test is expensive and difficult to carry out (OIE, 2000, Chand *et al*., 1994). Positive test samples and controls produce a consistent reaction with the major structural proteins of *capripoxvirus* of molecular weights 67, 32, 26, 19 and 17 kDa, whereas negative serum samples will not react in this pattern. P32 protein is specific for *capripoxvirus* (Heine *et al*., 1999, Carn, 1995), which has potential for use in dot-blot or ELISA assays.

2.12.10. Polymerase chain reaction (PCR)

Rapid laboratory confirmation of sheep and goat poxvirus based on clinical signs, electron microscopy and previously mentioned 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 CPV DNA in infected cell culture supernatants, biopsy samples (Heine *et al*., 1999, Ireland and Binepal 1998) and also to differentiate between 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 SPV and GPV from field samples when combined with restriction enzyme analysis (REA) of PCR-amplicons (Rao and Bandyopadhyay, 2000). Recently, SPV and GPV from infected cell culture supernatants and skin biopsy were clearly differentiated by REA of PCR amplified P32 gene products (Hosamani *et al*., 2004).

2.13. Treatment

Affected sheep and goats should be isolated and placed in hygienically maintained shed. Palliative treatment is indicated along with the feeding of balanced diet. Debilitated sheep

need to be administered with 10% glucose saline intravenously (Rao *et al*., 2000). Lesions should be washed with 1:100–1:10,000 KMNO4 lotion and application of 1:10 boric acid (Rao *et al*., 1994)/mild antiseptic (Singari *et al.,* 1990) or antibiotic ointment (Nandi *et al*., 1999) topically with parentral antibiotic injection to prevent secondary bacterial complications.

2.14. Control and prevention

2.14.1. Immunization and vaccines

SGP immunization has been practiced since the early attempts by Borrel in 1903. A generation of killed adjuvant vaccines (Singh *et al*., 1979) has been succeeded by a generation of many modified live virus vaccine strains, which have been developed in many parts of the world (Ramyar and Hessami, 1967; Davies, 1985). They have been derived from local pathogenic strains by passage in cell culture or embryos. Most have been effective although some strains have unacceptably high levels of residual pathogenicity and may cause local lesions and even abortion. For example, in different countries and sometimes within a country, various live, attenuated vaccines have been produced to prevent goat pox and these have had varying degrees of efficacy (El-Zein *et al*., 1983). The efficacy of such vaccines was judged upon the appearance of a local reaction at the site of inoculation, and this was taken as an indication of relative efficacy. Most live SGP vaccines produce the lifelong immunity. In enzootic areas, both live attenuated and inactivated vaccines are useful in the prevention and control of goat pox, but inactivated vaccines give only short-term immunity (Prasad and Datt, 1973; Yadav *et al*., 1986). Heterologous vaccines generally work well (Yadav *et al*., 1986).

A subunit vaccine also appears to be of some use in the control of disease as revealed by higher neutralization indices in immunized goats (Carn *et al*., 1994b). Moreover, a single vaccine prepared from a strain of *capripoxvirus* that infects sheep and goats equally is effective in controlling both goat pox and sheep pox for at least 12 months (Kitching *et al*., 1987b). Nevertheless, reports of cross-protection of sheep and goats against goat pox and sheep pox and other related diseases such as contagious ecthyma are often

contradictory and inconclusive attempts to protect either goats with sheep *capripoxvirus* vaccines or sheep with goat *capripoxvirus* vaccines are largely unsuccessful (Carn, 1993).

2.14.2. Husbandry methods and good practice

Vaccination is recommended for animals of all ages and thereafter lambs and kids should be vaccinated annually, at 12-16 weeks of age, when the maternal antibody has disappeared. Where enclosed farming systems exist, the use of vaccine for several years will eliminate the disease completely, as long as vaccination is maintained on an annual basis for all young stock and great care is taken to introduce only vaccinated stock from clean areas. Individual farms can maintain complete freedom from disease in this way and coordinated national programs can have a dramatic effect upon the disease. Coordinated control of movements from uninfected foci and complete restriction of movements from the infected areas will maintain the disease-free situation. Ring vaccination is frequently practiced during outbreaks in enzootic areas, but usually only the species that are clinically affected are vaccinated (Carn, 1993).

If national vaccination programrs are established with strict quarantine and movement controls, and if disease foci are identified, they will have a dramatic effect upon SGP in 3-5 years. A stage will be reached where a stamping out policy can be adopted for any new foci of disease. Absolute integrity and enforcement of movement controls is critical when the infected foci have been identified. If this can be achieved, SGP eradication programs can be successful in quite a short time frame (Ramyat *et al*., 1974).

In enzootic areas, both live attenuated and inactivated vaccines may be useful in the prevention and control of goat pox and sheep pox. However, the inactivated vaccines that are available for immunization against GPV and SPV infections give only short-term immunity (Prasad and Datt, 1973; Yadav *et al*., 1986). The live attenuated vaccines are highly immunogenic but their usefulness is limited because they stimulate a pock reaction and/or lead to the death of some of the vaccinated animals because of generalization of

the disease. Usually, homologous vaccination incorporating the locally prevalent strains of GPV or SPV is successful in protecting goats and sheep against goat pox and sheep pox. Therefore, in different countries and sometimes within a country, various live attenuated vaccines for sheep and goat pox have been available from time to time, with varying degrees of protective efficacy (Ramyat *et al*., 1974; El-Zein *et al*., 1983; Mahmood *et al*., 1982).

Similarly, live attenuated vaccines using different isolates of SPV serially passage in various cell culture systems have been available for the control of sheep pox (Singh *et al*., 1984; Mahmood *et al*., 1982).In India, a live attenuated vaccine prepared from the Romanian strain of SPV is currently used to produce immunity in all types of sheep against sheep pox and is quite safe and effective. Although it may not always be successful, a single vaccine prepared from a strain of *capripoxvirus* that infects sheep and goats equally can be effective in consoling both sheep pox and goat pox (Kitching *et al*., 1987). A subunit vaccine has proved effective in protecting goats' against capripox. On the whole, reports of cross-protection of sheep and goats against goat pox and sheep pox and other related diseases, such as contagious pustular dermatitis, are often contradictory and inconclusive because attempts to protect goats with SPV vaccines are usually unsuccessful (Prasad and Datt, 1973). Hence, it is recommended that homologous vaccines should be used to protect goats and sheep against goat pox and sheep pox (Carn *et al*., 1994b).

2.14.3. Local control

In the semi-arid zones of Africa, Eastern Europe and Asia, where SGP is endemic, the problems of control of the disease are great: the range is not enclosed, there is movement of animals in search of water and grazing, and mixing of different population groups is inevitable in these extensive husbandry systems. Trade is also continuous, extensive and largely beyond any institutional control, with little regards for national boundaries. In these situations the control of SGP is difficult and demanding. Individual animal owners practice vaccination and national schemes may achieve extensive vaccination cover over

very large areas. Drought, civil unrest or other similar events, can totally destroy all the progress, which has been made, however, and large regionally coordinated schemes may be required to have any lasting impact (Prasad and Datt, 1973).

In endemic areas, a regular cleaning program for winter housing is essential to eliminate any residual virus that may remain dormant. Poxviruses are capable of long intervals between animal to animal transmissions. Owners often report the appearance of cases when they house the animals for the winter period. Virus may persist for several months in organic matter and this is even more essential, if there have been cases of disease. Thorough cleaning and removal of the dung and subsequent treatment with phenol, alkali or other suitable disinfectants is advisable to eliminate any residual virus. Detergents will kill the virus by dissolving the outer lipid membrane (Nandi *et al*., 1999).

Capripox-free countries maintain their disease-free status by the restriction of imports of livestock and animal products from affected areas. In the case of countries remote from enzootic areas, the rapid implementation of a radical slaughter policy and severe movement restrictions, coupled with a ring vaccination of radius 25-50 km should result in elimination of disease (Carn, 1993).

3. MATERIALS AND METHODS

3.1 Description of study area

The present study was conducted in ten selected peasant association (PA) of Adea Berga district (Deku Kito, Maru Chobot, Bishan Dimo, Gatira Nebe, Ulagora, Sire Berga, Iteya, Haro Shobore Were elu and Reji Mokoda) which were purposively included for the isolation and characterization of the virus from pox outbreaks. Geographically, Adea Berga district is located at 64 km north west of Addis Ababa on the road to Mugher cement Enterprise and located at 9^0 12^1 to 9^0 37^1 N latitude and 38º 36¹ 69E longitude. Inchini is the capital city of the District (ILRI, 2013). Administratively, Adea Berga district consists of 37 kebeles (34 rural kebele and 3 urban kebele). Among the PAs on which the study was conducted, the four PAs (Deku Kitto, Sire Berga, Iteya and Maru Chobot) found south east of Inchini town, where as the four PAs (Bishan Dimo, Gatira Nebe, Ulagora and Reji Mokoda) found North West of Inchini town surrounding the compound of Dangote cement factory and two PAs (Haro shobore and Were elu) found east of Inchini town. According to the publication Bureau of planning and Economic development for Oromia regional state, West showa office (2014) the maximum and minimum temperature of the distric is $25c^0$-$10c^0$ respectively and an annual rain fall ranging from 918-1368 mm and an altitude ranges from 1400-3270m above sea level (ABLDHO, 2016).

Based on the altitude, the agro-climatic regions of the district are Highland (Badda) 29%, midlatitude (Badda daree) 34% and lowland (Gammojji) 37%. The soil types in the area are black (44%), red (39%) and brown (mixed) (17%). The rain is bimodal with short rainy season, February to March and long rainy season from June to September. Agriculture is the main occupation of the population and the agriculture is mixed type with cattle rearing and crop production under taken side by side. The major annual crops include Teff, Wheat, Barley, Pea, Bean, Maize and Sorghum. The major livestock reared are cattle, sheep, goat and equines (ILRI, 2013).

According to the information obtained from Adea Berga district veterinary clinic report, 2015/2016, the total livestock population of this district is estimated to be 163,730 cattle, 51,917 sheep, 29,192goats, 64,243 poultry and 14,378 equines. The total human population is estimated to be 16,335 urban population (7,899 male, 8,436 female) and rural population 103,842 (52,249 male and 51, 593 female) (ILRI, 2013)

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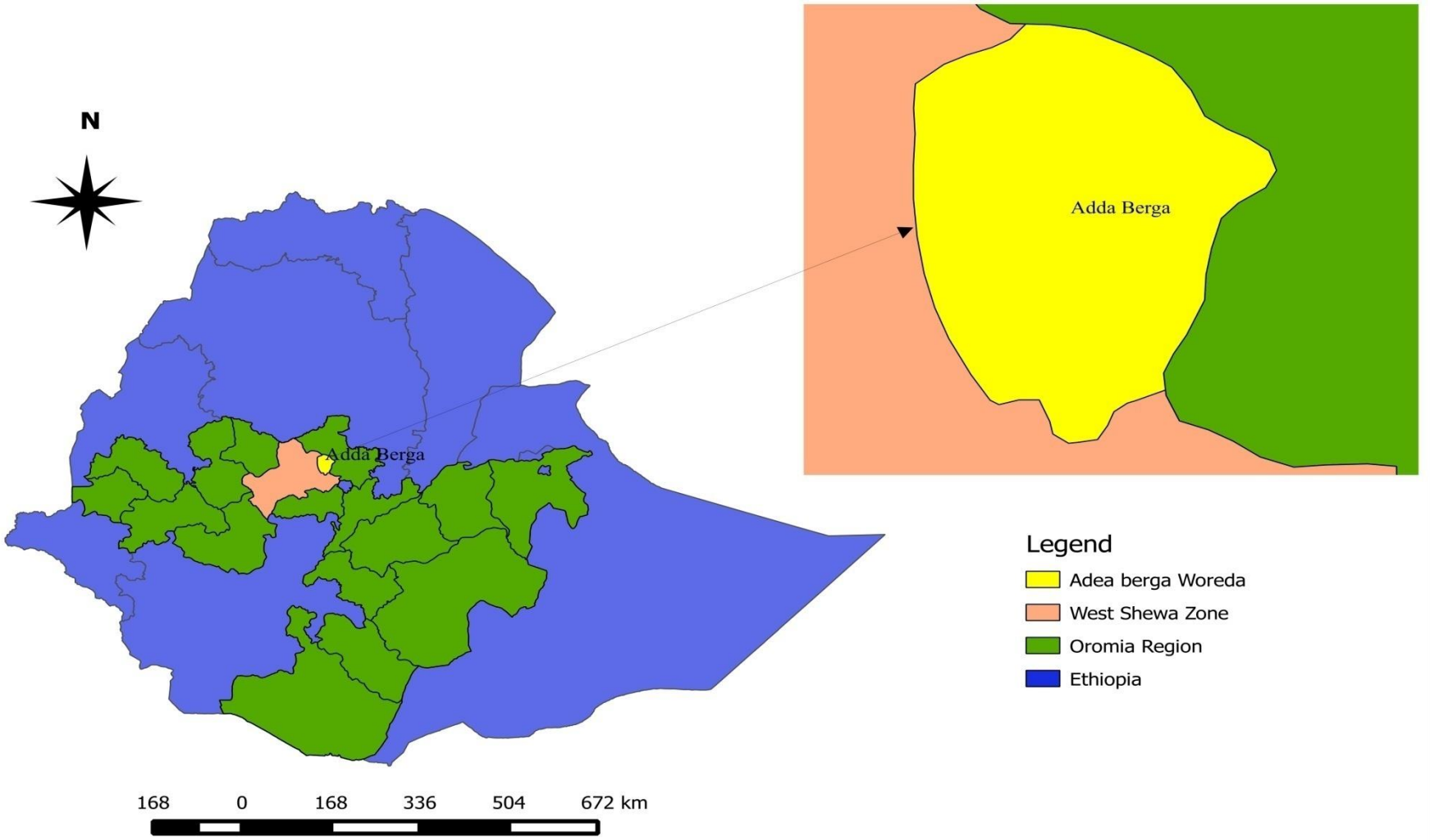


Figure 2: Map showing the study areas

3.2. Study animals

The current study was conducted in sheep and goat that had experienced outbreaks of disease and clinically infected and manifested clinical signs of sheep and goat pox on their body and those in close contact with sheep and goat pox outbreaks. Animals with clinical symptoms of sheep and goat pox in different PAs were under consideration in respective of age groups, sex and species during the entire period of investigation. Almost all animals were indigenous breeds, but there were very few numbers of sheep and goats having exotic blood. All the study breeds were local breed with both sexes. The indigenous breeds included were Arsi-Bale and Horo breeds of sheep and goats. The study sheep and goat were managed under small holder farming system; female animals were predominant in flockss, though some households own only male for fattening.

3.3. Study design

The study was conducted from November 2015 to March 2017 using a case study design (Dohoo *et al.,* 2003; Thrusfield, 2005) to examine and sample a particular animal for virus isolation and molecular characterization of pox virus circulating in sheep and goat. The PA's were purposively selected based on the occurrence of suspected sheep and goat pox disease and accessibility of the PA's for transportation. From selected PAs, the flocks were also purposively selected based on the occurrence of suspected cases of sheep and goat pox and based on the lists of outbreak reports obtained from the livestock and fishery office of Adea Berga district. When an active outbreak of sheep and goats were encountered or reported, a field level investigation was conducted purposively at a particular site of outbreaks and a tissue sample were taken from flocks using simple random sampling technique. In addition, a participatory epidemiological study method was used to identify the seasonal occurrence of sheep and goat pox (Catley *et al.,* 2012). The 95% CI for proportions were calculated using the formula given by Petrie and Watson (2006). Logistic regression analysis was employed to determine the associations of hypothetical risk factors with the morbidity and mortality of sheep and goats. Odd ratio (OR) was used to point out the degree of

risk factors association with the disease occurrence indicated by 95% confidence interval.

3.4. Sampling strategy and size

In outbreak areas, physical inspection of clinically sick animals was conducted to record clinical observations and date of disease appearance in the flocks. The flocks were purposively selected based on the occurrence of sheep and goat pox. Four flocks each from Gatira Nebe, and Haro Shobore, three flockss each from Deku Kito, Bishan Dimo and Reji Mokoda, two flockss each from Maru Chobot, Sire Berga, Iteya, Wera elu and Ula gora, totally 27 flockss were selected and included for detailed clinical examination based on the presence of sheep and goat pox disease with typical pox lesions in the flocks and owner's willingness to cooperate with the study was also considered. A total of 412 sheep and 188 goats from ten PAs were purposively selected and clinically examined. Accordingly, animals with clear signs, symptoms and suspected of infected with pox virus were purposively selected. Sequentially detailed clinical examinations of suspected cases were performed. From each pox infected flocks one tissue sample, totally 27 tissue samples were collected using simple random selection (lottery system) and fresh (unfixed) samples were submitted to the NVI, virology laboratory, for virus isolation and molecular characterization.

3.5. Field clinical examination

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

3.6. Questionnaire and participatory epidemiological (PE) survey

Questionnaire was prepared regarding general information on livestock ownership, importance of sheep and goat rearing, the common diseases of sheep and goats, awareness on sheep and goat pox and its effect on sheep and goat production. This part of the study was conducted in two phases in all PAs. The first phase was a questionnaire survey to get baseline information about the common sheep and goat diseases in the study area. Hundred respondents from all PAs were interviewed and they disclosed that the major sheep and goat diseases by their local names, their clinical symptoms and ranked them based on the frequent occurrence of each listed disease using open-ended questions (SSI). The top 5 ranked diseases were selected to be studied in detail using participatory epidemiology (phase 2 below). Sheep and goat pox was one of the 5 top ranked priority diseases to be scored during the subsequent participatory epidemiological studies. The second phase was the actual participatory epidemiology of sheep and goat pox using 12 independent groups whereby each group composed of 5-10 respondents. Explicitly, 2 groups were interviewed per PA and informants included those people whose sheep and goats were being sampled. During the selection of FGD, model farmers, elders, religious leaders, other people present nearby with good sheep and goat flocksing experience and rich indigenous knowledge related to sheep and goat diseases and health care were also invited to join the discussion by the research team.

The investigation was carried using selected tools including seasonal calendars and semi-structured interviews (SSI) according to the objectives and context of the study (Catley *et al.*, 2012; FAO, 2000). All PAs selected for tissue sample collections were included and the method was practiced (pre-tested) on some animal health workers and sheep and goat owners before using it for the actual field work in order to make sure that the method was understood and the questions were clear. The survey team composed of three interviewers comprising of the team leader (researcher), a community mobilizer /extension agent and a translator. The community extension agent

made prior arrangements and preparations with the farmers in each village and ensured time and place for the interview.

3.7. Seasonal calendar

Seasonal calendars, a time-related data source, were used to describe the seasonal occurrence of the five important sheep and goat diseases selected using simple ranking (ILRI, 2009). To construct a seasonal calendar, four seasons by their local names (Annex 10) were represented as: *''Ganna''* (July-August), followed by *''Birra''* (September-February), *''Bona''* (March-Apr) and *'Afrasa'* (May-June) on the X-axis. Pieces of papers with pictures and local names of the diseases printed on them placed along the Y-axis. These were placed on the flip chart and explained to the informant group after they were arranged to sit in convenient places. The informants were then requested to explain the meaning of each symbol to know whether they have understood what it represented. The informants were then given 30 stones and asked to show the relative occurrence of each disease in each season. When placing of the stones for one disease against the season was complete, the group was requested to thoroughly check the scores and if they wanted, rearrange the scores until they were contented with the result. The seasons, diseases and number of stones were kept constant across all informant groups to make the technique more reproducible.

3.8. Semi-structured interviews (SSI)

Following scoring of the seasonal calendar, the results were discussed with the participants using open and probing questions through the use of SSIs. The informant groups were specifically probed more on the disease of interest (sheep and goat pox) with regard to the seasonal occurrence, impact, age group affected and predisposing factors.

3.9. Laboratory investigation

3.9.1. Sample collection

A total of 137 sheep and 51 goats suspected for pox virus infection were carefully examined for the presence of clinical lesion on their skin. The diagnosis of sheep and goat pox disease was done on the basis of clinical observation of pox lesions. Tissue samples of skin biopsies were collected from the outbreak areas. About 3 gm of tissue samples was taken from goats and sheep showing typical pox lesions. The samples were placed in sterile universal bottle containing 50% phosphate buffer saline (PBSA) at a pH of 7.2 with 1% Gentamycine. Species, sample code, sex, age and village was labeled on the bottles. The samples were transported using cold box to the National Veterinary Institute (NVI), Bishoftu and after arrival they were kept at -20ºC until processed.

3.9.2. Samples processing

The biopsy samples were thawed at room temperature and washed three times using sterile PBSA containing antibiotics and antifungal at a pH of 7.2 in Bio-safety cabinet Class II. About 1 gm of the samples was ground using sterile mortar and pestle by adding 9 ml of sterile PBSA. The tissue suspension was centrifuged at 3,500 rpm for 10 min at 4ºC. The supernatant was collected, filtered through 0.45 µm membrane filter and preserved at -80ºC until use.

3.9.3. Preparation of glassware

For cultivation and maintenance of Vero cell line, glassware, reagents and media were prepared and sterilized according to the standard operating procedures. The used glass wares were dipped in surface detergent, brushed thoroughly and washed in running tap water for 20 minutes and washed ten times with deionized water. The washed glass wares were kept inverted on a clean surface tabletop to drain out the water content and

to dry. The glass wares were wrapped in wrapping papers and aluminum foils. All the glass wares including fresh were placed in hot air oven at 180 0C or 30 min.

3.9.4. Preparation of Vero cell monolayer

African Green Monkey kidney Cell line (Vero) (AU-PANVAC, Ethiopia) was used for isolation of virus. Dulbecco's Modified Eagle's Medium (DMEM) was prepared (Annex1) according to the Manufacture instruction for cell line propagation and virus isolation (HiMedia, India). The DMEM solution was supplemented with 10% inactivated calf serum, 10% tryptose phosphate Broth (TPB) (Oxoid, England), 1% gentamycin solution prior to use. Vero cell line was grown in Roux flask in the facilities of Virology Laboratory of Research and Development Section, NVI. Cells were observed daily for their confluent monolayer under inverted microscope.

This cell line was processed for harvesting and transferring to new 25 cm^2 plastic tissue culture flasks. The growth medium overlaying the cell monolayer was pour off in a sterile beaker under sterile conditions. The monolayer was rinsed, washed twice with 10 ml sterile PBSA and covered with 5 ml of sterile 0.25% trypsin for about 5 minutes in an incubator at 37ºC. The trypsin was removed quickly to avoid wastage of detached cells. 10 ml of the complete media was added and rolled with jerking and hitting avoiding damage to cells. The cells detached from the flasks was collected and mixed to form homogenous cell suspension. Equal volume of the cell suspension added to each of the three flasks already containing 10 ml growth medium with 10% fetal calf serum. The whole process was carried out under aseptic and sterile conditions under Bio-safety cabinet Class II. These flasks were placed horizontally in the incubator at 37ºC. After three days, all the flasks had developed a confluent monolayer with typical cell sheet with light frosted glass appearance, having clearly visible fibroblastic whirls. The cells in that phase was considered fit for sub-culturing and virus infection.

3.9.5. Isolation of virus

Isolation of pox virus was carried out according to a previously described protocol (OIE, 2012). Briefly, inoculation of the field virus was made on two days after sub-culturing when monolayer reached 80% confluence. The processed supernatants kept at -80ºC were thawed in advance of inoculation. The exhausted medium from the flask having confluent monolayer was discarded and the monolayer was washed with sterile PBSA. Three flasks were used. Monolayer of Vero cells grown in 25 cm^2 tissue culture flasks were inoculated with 1 ml of sample supernatant using two flasks per sample. Following incubation at 37°C for 2 hours for virus adsorption the inoculate were discarded, the flasks were washed three times in the medium, followed by the addition of maintenance medium containing 2% calf serum, penicillin 10,000 UI/ml, streptomycin 100 µg/ml, kanamycin 50 µg/ml and amphotericin B 2.5 µg/ml. The third flask containing confluent monolayer cells was filled with media only and kept as control flask.

Three flasks per sample were incubated at 37ºC and each flask was observed daily for 7 – 10 days under the inverted microscope for any cytopathic effect (CPE) development. The medium having 2% calf serum was changed every 48 hours. When 80% CPE was observed, the flasks were frozen at -20ºC (after pH adjustment). The virus was harvested after two freeze-thaw cycles. When no CPE is visible until day 14, the culture was freeze–thawed three times, and clarified supernatant inoculated on to fresh Vero cell culture. In general, two more blind passages were carried out for samples that were initially negative for CPE.

3.9.6. Polymerase chain reaction (PCR)

The confirmatory diagnosis of the cell culture positive sample was made by conventional PCR using primers that amplify RNA polymerase subunit 30 kDa (RPO30) gene which could enable to differentiate goat pox virus from other Capri poxviruses.

3.9.7. DNA extraction

DNA extraction was conducted in the Molecular Biology laboratory of the National Veterinary Institute. Extraction of DNA from 10% (w/v) tissue sample suspension and/or cell culture homogenate was carried out using DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's instruction. Accordingly, 200µl cell culture suspension was transferred into a labelled 1.5 ml eppendorf tube. 20 µl proteinase K and 200 µl Buffer AL was added for each tube and mixed by vortexing and incubated at 56ºC for 30 minutes (until completely lysed). 200µl 96% ethanol was added per tube and mixed thoroughly gently by vortexing. The mixture was transferred to a labelled DNeasy mini spin column placed in a 2ml collection tube and centrifuged for 1 minute at 12000rpm. The collection tube was changed by new one and 500µl Buffer AW1 was added into the spin column and centrifuged for 1 minute at 12000rpm. The collection tube was again changed by new tube and 500µl Buffer AW2 was added and centrifuged for 3minutes at 20000rpm.

Finally, the spin column was transferred into a labelled 1.5ml eppendorf tube and 40 µL 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 10,000 rpm to elude the DNA into the eppendorf tube. The nucleic acid bound to the silica membrane was eluted and the tube was labeled properly and kept at -20ºC until analysis.

3.9.8. Conventional PCR

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. The method is able to differentiate goat poxvirus from sheep poxvirus since the gene harbor a well conserved sequence signature for the differentiation and genotyping of

the two poxviruses. Accordingly, PCR was conducted to amplify small fragment of the RPO30 gene using the primers and protocol described by Lamien *et al*. (2011).

The primers used were SpGpRNApolF (5'-TCTATGTCTTGATATGTGGTGGTAG-3') and SpGpRNApolR (5'-AGTGATTAGGTGGTGTATTATTTTCC-3') and synthesized by VBC Biotech (Vienna, Austria) and purified by reverse phase high-performance liquid chromatography.

The strategy was the primers flanking the region containing a 21-nucleotide deletion in SPPV sequences so that the PCR amplification products from SPPV isolates would be shorter in comparison to those from GTPV isolates (151bp for sheep poxvirus and 172bp for goat poxvirus). A total of 27 samples representing from different geographical areas and animal species were analyzed by PCR.

PCR was carried out in a volume of 20µL containing 2µL forward primer, 2µL reverse primer, 10µL iQ supermix (BioRad, Germany), 4µL DNase free water and 2µL viral DNA. No-template, positive and negative controls for each genotype were included.
The PCR tubes containing 20 µl final volume were transferred into the thermal cycler (2720, Applied BioSystems). The thermal cycling protocol was first initial denaturation for 5 min at 95ºC followed by 40 cycles of 95ºC for 30 sec, 55ºC for 30 sec and 72ºC for 30 sec; and final extension at 72ºC for 2 min.

3.9.9. Agarose gel electrophoresis of PCR product

PCR products were analyzed by 3% agarose gel electrophoresis as described by Lamien *et al*. (2011). Briefly, 3 gm 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. 5 µl GelRed nucleic acid stain (Biotium, Germany) was added. 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 gel was placed in the

electrophoresis tank containing 1X TAE running buffer and the comb was removed carefully.

In the first lane 10 µl 50 bp DNA ladder (Fermentas, Lithuania) was loaded, while in remaining lanes 10 µl sample PCR products, Positive control of sheep pox and non-template and mixed with 2 µl DNA 6x loading dye were loaded in each wells by using micropipettes. Micropipette tips were changed for each sample. The gel-running tank was connected to the power supply. The voltage was adjusted to 100 volt and run for 1 hour. The gel was then observed under the UV trans-illuminator gel documentation system and gel picture was captured using a Polaroid photograph camera. Virus genotyping was determined and recorded based on the band size of the PCR product.

3.10. Ethical clearance

The study was approved by Addis Ababa University, College of Veterinary Medicine animal research ethical review committee ref. no. VM/ERC/13/06/09/2017(Annex12).

3.11. Data analysis

The collected data during sampling and laboratory analysis was entered and stored into Microsoft office Excel spread sheet 2007. The data were thoroughly screened before subjecting to statistical analysis. The data were then imported to STATA 13 (Stata Corp, 2013). Descriptive statistics was used to summarize data of lesion, questionnaire and laboratory findings. PCR product of 151 bp for SPPV and 172 bp for GPV band size on agarose gel electrophoresis was used for genotyping CaPV using conventional PCR. Univariable logistic regression analysis was employed to determine the associations of hypothetical risk factors with the mortality and morbidity of sheep and goat. Odd ratio (OR) was used to point out the degree of risk factors association with the disease occurrence indicated by 95% confidence intervals. A significance level ($P < 0.05$) and confidence level (95%) was set to determine the presence or absence of statistically significant difference between the given parameters. Differences were

considered significant and highly significant when P-values were less than 0.05 and 0.01, respectively, using logistic regression and Chi-square test. Data collected by seasonal calendar was analyzed using statistical package for social sciences software version 20.0 (SPSS, v. 20.0). Scores were summarized using median scores, minimum and maximum scores and 95% confidence intervals. The Kendall's coefficient of concordance (W) was used to assess agreement between informant groups.

4. RESULTS

4.1. Questionnaire survey

The result of the questionnaire survey indicated that sheep and goat pox was a common disease where 96% (n=100) of the respondents reported the frequent occurrence of pox disease in their sheep and goat flockss. Similarly, with regard to seasonal occurrence, 88% (n=100) of the respondents informed that the disease was more prevalent during summer followed by spring season and the remaining respondents did not associate the disease occurrence with the season. According to their explanation, the disease is often observed throughout summer and the beginning of the spring season. All respondents informed that the disease equally affected both sheep and goat; with higher severity in sheep. They also explained that young age groups are more susceptible than adult age groups of both species and reported high mortality in young age groups (kids and lambs < 1 year). The incursion of the disease in Maru Chobot and Bishan Dimo followed the recurrence of SGP in Gatira Nebe and Ulagora and a particular increase in the number of outbreaks in the same area after August of the same year_ particularly in PAs bordering to Meta Robi and Ejere District which are connected with the main road of Holeta to Mugher through which a large number of animal movement to the capital city of the district was reported. They also reported that source of the disease was new purchased sheep from the market of Meta Robi district.

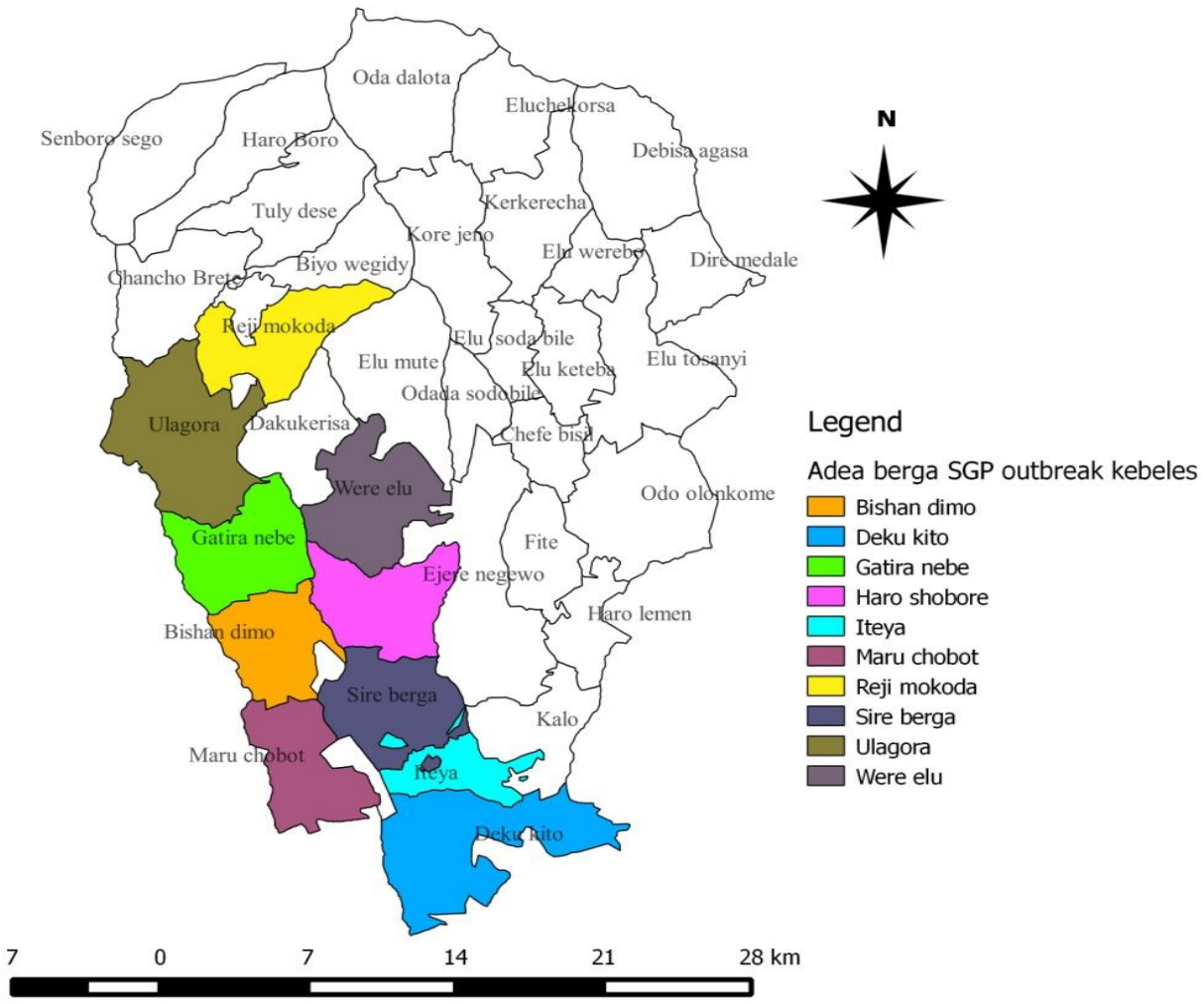


Figure3. Map showing pox outbreak area and samples collected

4.2. Field clinical examination

Out of 600 sheep and goats examined, 137(33.3%) sheep showed pox lesions on their skin where as 51 (27%) goats were found to be positive for pox lesion on their skin. The highest number of pox lesion on sheep and goat was observed in Maru Cobot (41.2%) and Ulagora (40%), respectively and the lowest pox lesion was observed in Deku Kito (20.3%) and in Iteya (25%) in sheep and goat, respectively. Accordingly, 27 tissue samples (15 sheep and 12 goats) with severe pox lesion on their body were sampled (Table2).

Table 2 Number of Sheep and goats clinically showed pox lesion and sampled

PAs	No. of animals examined		Proportion of animals with pox lesion (%)		No. Sampled	
	Goat	Sheep	Goat	Sheep	Goat	Sheep
Bishan Dimo	12	40	4(33.3)	10(25)	1	1
Deku Kito	29	40	3(10.3)	11(27.5)	1	1
Gatira Nebe	12	58	2(16.7)	25(43.1)	1	2
Haro Shobore	38	13	8(21)	4(30.8)	1	1
Iteya	6	50	1(16.7)	13(26)	1	1
Maru Chobot	14	54	9(64)	19(35.2)	1	2
Reji Mokoda	1	59	1(100)	17(28.8)	1	2
Sire Berga	18	46	4(22.2)	18(39.1)	1	2
Ulagora	11	39	3(27.2)	17(43.6)	1	2
Were elu	47	13	20((42.5)	3(23.1)	3	1
Total	188	412	51(27)	137(33.3)	12	15
Over all total	**600**		**188(31.3)**		**27**	

During the field clinical examination, few sheep and goats showed pox lesion all over the skin but were mostly confined to the areas with little or no hair, such as the face, ears, groin, and perennial region, and under the tail (Figure 4). Accordingly, there were erythematous macules, papules and nodules (varying in size from 0.5 to 2 cm) on the skin, udder, vulva, testicles, foreskin, and the inner side of the thigh and in the whole free

part of the skin. These nodules could confluence and form lesions that look like skin tumors (Figure4. A, B, C& D), the nodular lesions were increased in number and when palpated felt hard. No vesicular changes were observed during the progression of the lesions. Moreover, the surviving animals showed skin lesions and scabs toward the end of disease evolution (Figure 4).

Figure 4: Pox lesions showed on clinically diseased sheep

A) Face and neck region B) perennial region C) Face D) face and perennial region

The morbidity proportion of sheep and goat pox within species in the outbreak PAs was 32.1% and 29.4%, respectively. Within different age groups, the morbidity was 28.1% in adult and 38.2% in young age groups. The overall morbidity (based on clinical signs) of sheep and goat pox was 31.3% which was largely observed in young age groups (73 cases/ 191 observations). Statistically, there were no significant differences between

species and sex groups (P > 0.05) but there was a statistical significant difference among age groups (P < 0.05) (Table3).

Table 3: The morbidity proportion of sheep and goat pox within species, age group and sex groups

Risk factors	Category	No. of examined	No. of Sick	Morbidity (%)	chi-square	P. value
Species	Goat	188	50	29.4	4.07	0.296
	Sheep	412	138	32.1		
Age	Adult	409	115	28.1	6.176	0.009
	Young	191	73	38.2		
Sex	Male	321	98	30.5	0.207	0.357
	Female	279	90	32.3		
Over all proportion		**600**	**188**	**31.33**		

The mortality proportion (based on clinical signs) within species was 6.5% in goat, 4.7% in sheep and within different age groups, 2.9% in adult and 9.9% in young age groups. This reveals that the average mortality of sheep and goat pox was 5.2%. From this observation we can conclude that there is higher mortality in sheep than in goat, in young age groups than in adult age groups and in female than in male. There was a significance difference between age groups (P < 0.05) but there was statistically no significant difference between species and sex (P > 0.05) at 95% CI (Table4).

Table 4: The mortality proportion of sheep and goat pox within species, sex and age groups

Risk factors	Category	No. of examined	No. of Died	Mortality (%)	chi-square	P. value
Species	Goat	188	11	6.5	0.823	0. 237
	Sheep	412	20	4.7		
Age	Adult	409	12	2.9	13.07	0.001
	Young	191	19	9.9		
Sex	Male	321	15	5.4	0.047	0.486
	Female	279	16	5.0		
Over all proportion		**600**	**31**	**5.2**		

The morbidity proportion per-season indicate that, the highest morbidity was observed in July (38.3%) followed by August (36.6%) which was the long rainy season and the lowest morbidity was observed in April (19.2%) followed by November (22.1%), which is the short rainy season and the short dry season, respectively. The overall seasonal morbidity was 31.3%. This shows that there was the highest morbidity of sheep and goat pox in the rainy season followed by the lowest morbidity in the dry season. There was statistically a significant difference between seasonal outbreaks (P< 0.05) (Figure 5).

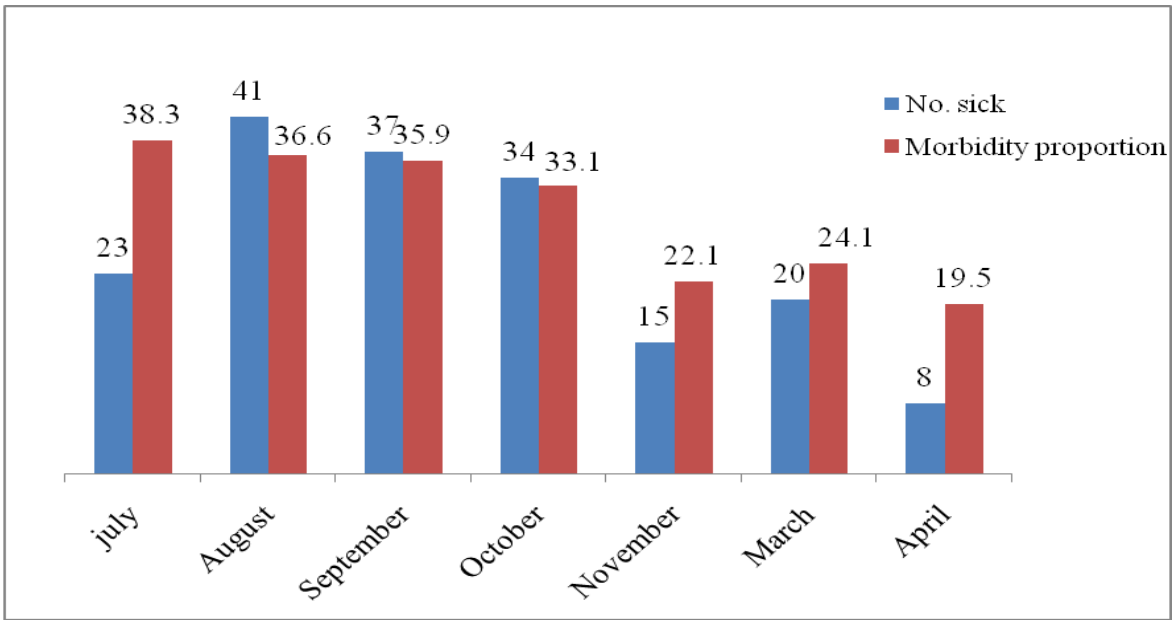


Figure 5: The Morbidity proportion of sheep and goat pox within the study period

The mortality proportion within season indicate that, the highest mortality was observed in August (the long rainy season) 9.8% followed by September (6.8%) the short dry season and the lowest mortality was observed in April (2.4 %) followed by November (2.9%), which is the long dry season and the short dry season, respectively. The overall seasonal mortality rate was 5.2%. This shows that there is a highest mortality of sheep and goat pox in the rainy season followed by the lowest mortality in the dry season with Chi-square = 8.5 and P = 0.004. Statically there was a significant difference in the seasonal occurrence of sheep and goat pox ($P < 0.05$) (Table5).

Table 5: The mortality proportion of sheep and goat pox per seasons

Risk factors	Category	No. of examined	No. of Died	Mortality (%)	95% CI
Season	April	41	1	2.4	0.06 –2.90
	August	112	11	9.8	5.0 – 16.92
	July	60	3	5	1.0 – 13.9
	March	83	3	3.6	0.75 – 10.20
	November	68	2	2.9	0.35 – 10.22
	October	133	4	3	0.83 – 7.52
	September	103	7	6.8	2.7 – 13.5
Over all proportion		**600**	**31**	**5.2**	**3.54 -7.25**

The morbidity proportion, mortality proportion and case fatality proportion in young age group of sheep and goat was 38.2%, 9.9% and 26.03% respectively. The morbidity proportion, mortality proportion and case fatality proportion in adult age group of sheep and goat was 28.10%, 2.9% and 10.44%, respectively. There were high morbidity proportion, high mortality proportion and high case fatality proportion in young age groups than adult age groups (Figure 6).

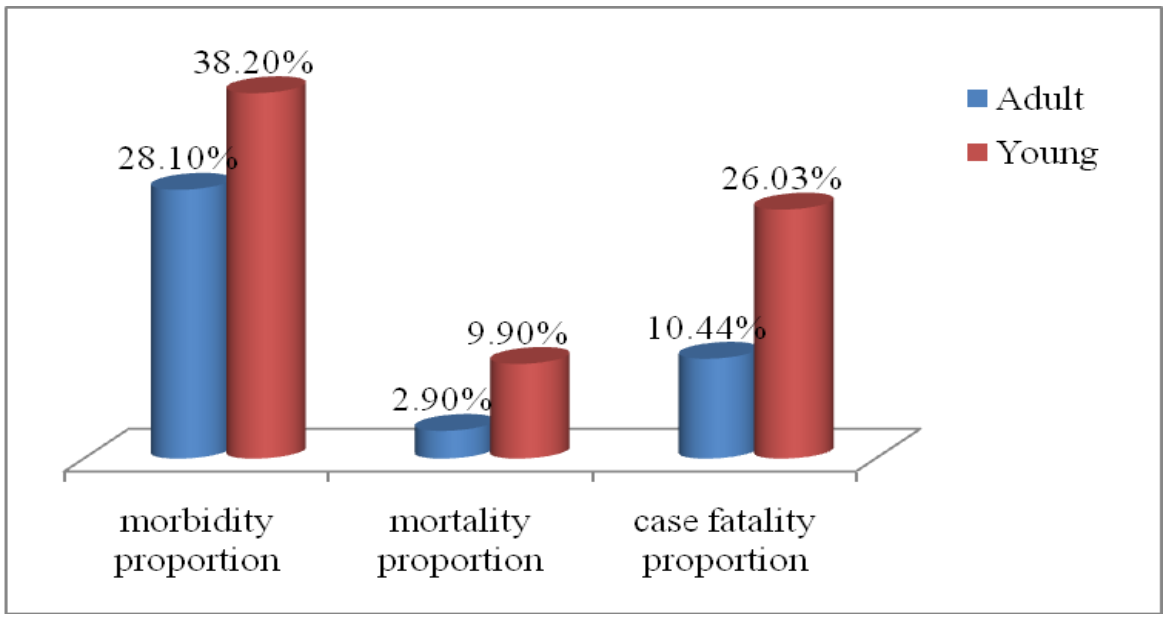


Figure 6: Morbidity proportion, mortality proportion and case fatality proportion with in age group of sheep and goats

The morbidity proportion, mortality proportion and case fatality proportion in sheep were 32.1%, 4.7% and 14.5%, respectively and in goat were 29.4%, 6.5% and 22% respectively. There were high morbidity proportion in sheep, high mortality proportion and high case fatality proportion in goat, respectively (Figure7).

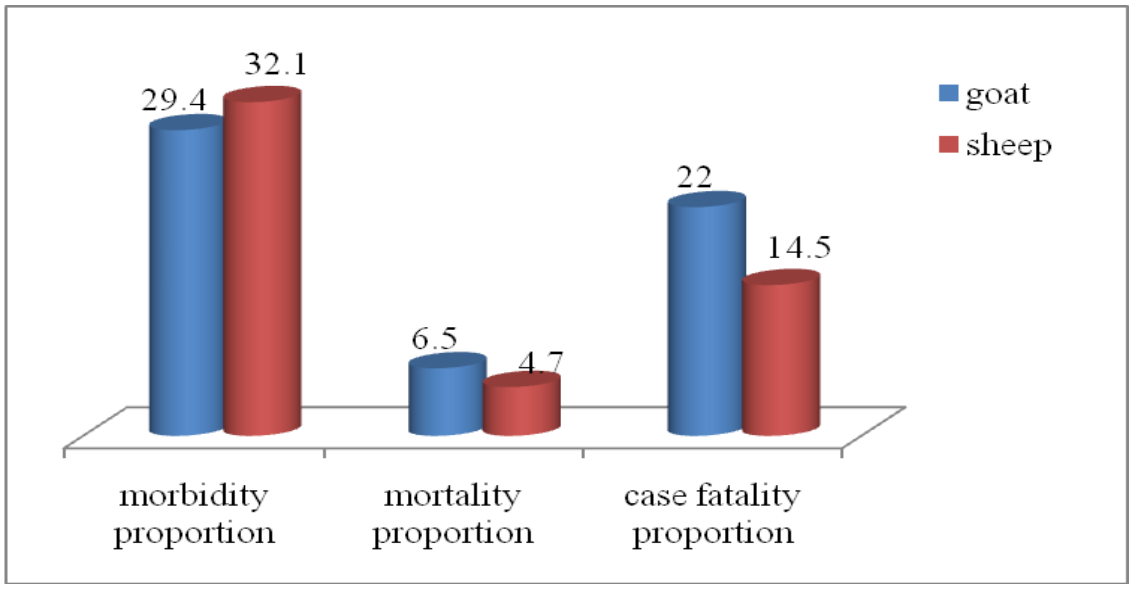


Figure 7: Morbidity, mortality, and case fatality proportion with in species

The multivariate logistic regression analysis showed that the highest occurrence of sheep and goat pox within study PAs was recorded in Were elu followed by Gatira Nebe as compered to Bishan Dimoo with OR= 6.33 and 4.57 at 95% CI respectively. That means sheep and goats found in Were elu had 6.3 times greater chance of being infected with pox virus than those of sheep and goat found in Bishan Dimoo. The lowest occurrence of sheep and goat pox within study PAs were recorded in Deku Kito followed by Maru Chobot as compered to Bishan Dimoo with OR= 0.92 and 1.63 at 95% CI respectively (Table6). Even though the highest and the lowest occurrence of sheep and goat pox were recorded, there was statistically no significance difference within the study PAs ($P > 0.05$). The highest seasonal occurrence of sheep and goat pox was recorded in July followed by August with OR = 2.56% and 2.38% respectively at 95% CI and the lowest seasonal occurrence of sheep and goat pox was recorded in November followed by March as compared to Appril with OR = 1.17% and 1.30% respectively at 95% CI. Statically there was a significant difference between age groups and seasonal occurrence ($P < 0.05$) (Table6).

Table 6: Multivariate logistic regression analysis of the occurrence of pox virus within different study PAs, seasons, age groups, species and sex.

Health status	Odds Ratio	Std. Err.	z	P>|z|	95%CI
PAs					
Bishan Dimo*	Ref	NA	NA	NA	NA
Deku Kito	0.92	1.65	-0.05	.961	.027 - 31.04
Gatira Nebe	4.57	4.95	1.37	0.172	.520 - 38.92
Haro Shobare	2.35	4.46	0.45	0.653	.058 - 97.37
Iteya	3.01	4.37	0.76	0.449	.174 - 52.00
Maru Chobot	1.63	.68	1.18	0.239	.720 - 3.670
Reji Mokoda	1.99	2.49	0.55	0.583	.17 -23.23
Sire Berga	2.15	2.71	0.61	0.545	.18 - 25.39
Ulagora	4.07	4.69	1.22	0.223	.42 -38.99
Were elu	6.33	12.42	0.94	0.347	.135 - 29.62
Month					
April*	Ref	NA	NA	NA	NA
August	2.38	1.05	1.97	0.049	1.00 –5.645
Julay	2.56	1.22	1.98	0.048	1.01 – 6.508
March	1.30	0.62	0.57	0.566	0.52 -3.292
November	1.17	0.60	0.32	0.752	0.45 – 3.055
October	2.04	0.89	0.64	0.101	0.87 – 4.784
September	2.31	1.03	1.89	0.059	0.98 – 5.525
Species					
Goat*	Ref	NA	NA	NA	NA
sheep	1.22	.31	0.81	0.420	.75 - 1.99
Sex					
Female*	Ref	NA	NA	NA	NA
Male	1.26	.28	1.03	0.303	.812 -1.95
Age					
Adult*	Ref	NA	NA	NA	NA
Young	1.90	.45	2.71	0.007	1.2 -3.03

Key: * = Constant; Ref = Reference, NA = Not applicable

4.3. Virus isolation

Out of 27 tissue samples processed, 25(92.6%) produced cyptopathic effect (CPE) on Vero cell line at the first passage. However, Gatira Nebe-1 and Haro Shobore-4 samples was evident even at no any CPE on Vero cell line was evident even at second blind passage incubated for 10 days (Table 7).

Table 7: Number of samples developed characteristic pox virus CPE with area of collection

PAs	No. of sample processed	Result	
		With CPE	Without CPE
Maru Cobot	2	2	-
Gatira Nabe	4	3	1
Daku Kito	3	3	-
Bishan Dimo	3	3	-
Ula Gora	2	2	-
Sire Barga	2	2	-
Itaya	2	2	-
Haro Shobore	4	3	1
Wara Ilu	2	2	-
Reji Mokoda	3	3	-
Total	**27**	**25**	**2**

The result of the CPE effect was characterized by small syncytia, cell ballooning, rounding, aggregation and detachment (sloughing) of the cell sheet. The infected cell lines were showed the early CPE at an average of 6 days post inoculation. The virus was harvested within 7–10 day of inoculation for further diagnosis (Figure 8).

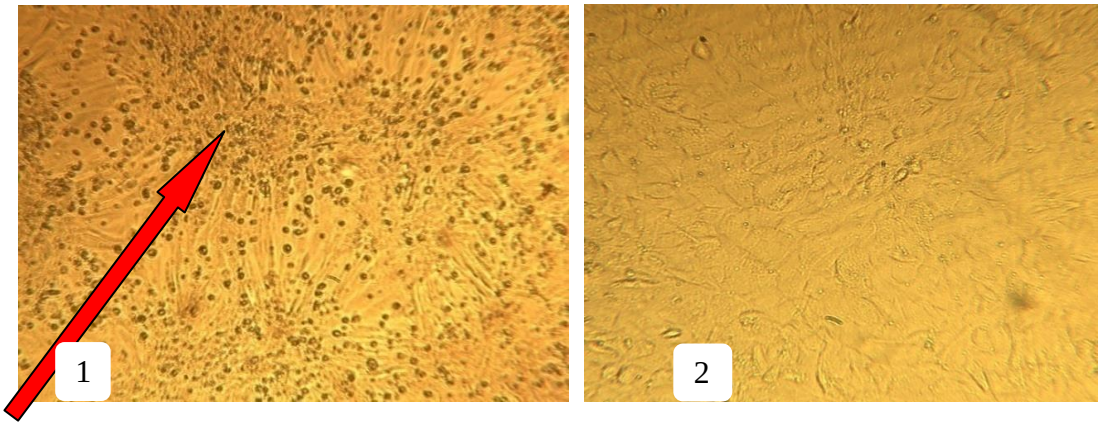
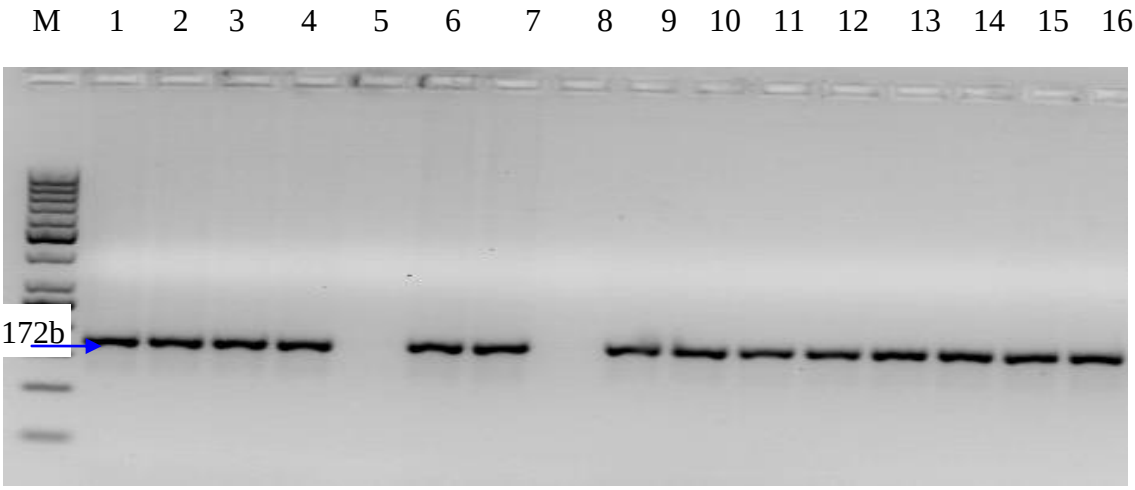


Figure 8: CPE picture taken using camera fitted inverted microscope

1) Cells developed characteristics CPE of pox virus as shown by arrow, and 2) Normal Vero cell monolayer.

4.4. Conventional gel-based PCR result

All 27 skin samples were analyzed by conventional genotyping PCR. Similarly, out of those, 25(87.5%) samples were positive for *goat pox virus*. With respective to PAs, 25 samples produced band size of 172 bp on agarose gel electrophoresis. However, two samples from Gatira Nebe and Haro Shobore, i.e., Gatira Nebe -3 on lane 5 and Haro Shobore -2 on lane 8 were again negative by conventional PCR since they could not produce any band on gel electrophoresis (Figure 9).



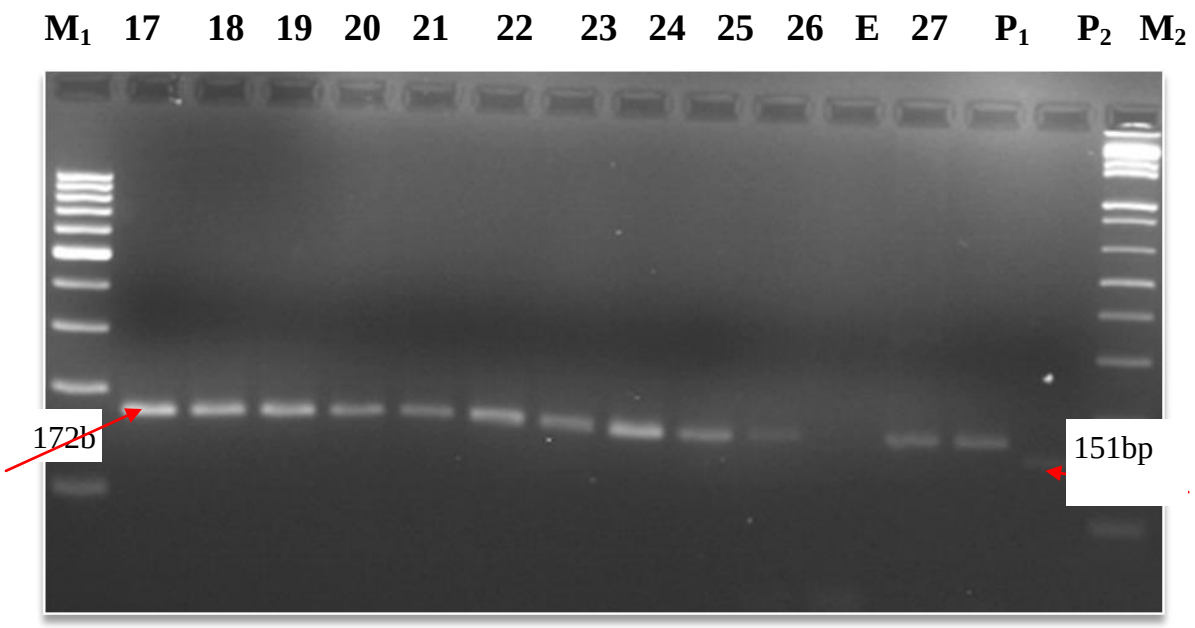


Figure 9: Classical PCR for differentiating GTPV from SPPV

The PCR products were separated by electrophoresis on a 3% high resolution agarose gel. This gel picture shows the PCR results of different pox samples. Where lane 1-2 = Maru Cobot; 3- 6 = Gatira Nebe; 7-10 = Haro Shobore; 11-13 = Bishan Dimo; 14-16 = Ula gora; 17-18 = Reji Mokoda; 19-21 = Deku Kito; 22-23 = Sire Berga; 24-25 Were elu; 26 -27 = Iteya E = RNase free water extraction control, no amplification; P2 = Sheep poxvirus (positive control, 151bp); P1 = Goat poxvirus (positive control, 172bp); and M = Molecular marker 50bp (Fermentas).

4.5. Questionnaire and participatory epidemiological (PE) survey

During the initial questionnaire survey, the main constraints for livestock production were discussed in respective PAs. Major infectious and non-infectious disease problems within the knowledge scope of the informant were assessed through the interview questions. Types of diseases occurring in the area were listed as declared by the informants with the clinical definitions and local name (vernacular name) of the diseases. Vernacular name of the some disease slightly vary from one PA to another and all the different name were recorded for cross-checking purposes. The corresponding scientific

name of these diseases was also given based on the clinical manifestation declared by the informants.

The community informant's unanimously defined four seasons in a year mainly marking a reference point at the rainy time. Accordingly, they classify the four seasons by vernacular name as Ganna (June - August) which is a long rainy season, Birra (September – November) short dry season, Bona (December – February) long dry season and Afrasa (March – May) short rainy season.

Various sheep and goat diseases with their symptoms were mentioned by the farmers based on their common occurrences (Annex 8). Among which five diseases, including sheep and goat pox (Annex9) were selected by simple ranking method (ILRI, 2009) for the seasonal calendar study. In addition to these, occurrence of orf was included intentionally for the seasonal calendar as reference control.

Results of the 12 seasonal calendars are summarized in Table8. Agreement between informant groups was quantified using the Kendal coefficient of concordance and were categorized according to critical values for W as weak, moderate and good if W-values were less than 0.26 ($p>0.05$), between 0.26 and 0.38 ($p<0.05$) and greater than 0.38 ($p<0.01$ to <0.001), respectively. This was by assuming ranking of four objects (seasons) by 12 judges (groups of informants) (Seigel and Castellan, 1994, cited from Catley *et al,* 2002).

Good agreement was evident among the 12 informant groups concerning seasonal patterns of the selected sheep and goat diseases except for *Abba gorba* (blackleg) and *Handara* (Orf) which scores weak agreement (W= 0.1540) and (W= 0.1395) respectively. Sheep and goat pox (*Finno Hoola /Fentata*) incidence peaked during long *(*Ganna*)* and short (*Afrasa)* rainy seasons with a median score of 8.25 (0-30) and 8.0 (0-19), respectively (Table8).

According to the explanation of informant groups, the high incidence of sheep and goat pox during the rainy seasons was due to the occurrence of the rain and congregation in

small areas that favor transmission of the disease. Increasing humidity (rain) during *ganna* (long rainy) season was also explained as a predisposing factor and the reason for the highest occurrence of the disease in the season. The disease was reported mainly to affect young sheep and goat that allowed grazing during the rainy seasons. Increasing frequency of contact with other sheep and goats and grazing on green pasture and bushes which are abundant during rainy seasons were mentioned as predisposing factors of the disease in young sheep and goat.

On the other hand, occurrences of the other selected sheep and goat diseases including anthrax, blackleg and fasciolosis, and Pasteurollosis were associated with the long dry season *Bona* (December-February), short dry season *Birra* (September-November) and *Afrasa* (March-May) of the year respectively **(**Table8**).** This was mainly due to shortage of feed, starvation and flooding (long rain) during these seasons.

Table 8: Summarized seasonal calendar on the occurrence of selected sheep and goat diseases in Adea Berga district.

Disease	Season			
	Ganna (June - August)	Birra (Sep - Nov)	Bona(Dec-Feb)	Afrasa(Mar-May
Ramoo Tiru/Dodo'o (Fasciolosis)** (W = 0.3563)	●●●●●●●	●●●●●●●	●●●●●●●	●●●●●
		●●●	●	4.50(0-16)
	7.25 (2-16)	10.16(2-19)	8.33(0-24)	
Abba Gorba (Blackleg)* (W= 0.1540)	●●●●●●●	●●●●●●●	●●●●	●●●
	●●●●●	●●●●●		
	11.33(2-22)	11.58(0-28)	4.08(0-23)	3.17(0-14)
Abba Sanga (Anthrax) *** (W=0.3963)	●●●	●●●●	●●●●●●●	●●●●●
			●●●●●●●	
			●●●●	
	2.5(0-12)	3.92(0-6)	18.33(4-28)	5.25(0-18)
Finnoo Holaa (fentata) (SGP) *** (W= 0.7643)	●●●●●●●	●●●●●●●	●●●●●●	●●●●●●●
	●			●
	8.25 (0-30)	7.0 (0-15)	6.25(0-16)	8.0 (0-19)
Gororsaa Holaa (Pasteurellosis)*** (W=0.5163)	●	●●●	●●●●●●●	●●●●●●●
	1.33(0-8)	3.17(3.16)	●	●●●●●●●
			8.25(0-22)	●●●●
				17.75(7-30)
Handara (Orf)* (W=0.1395)	●●	●●●●●	●●●●●	●●●●●●●
	1.58(0-7)	●●●	●●●	●●●●●
		7.8(0-20	7.75(0-19)	12.41(0-20)

Key: N= 12; SGP = Sheep and Goat pox, W= Kendall's coefficient of concordance ($^{ns}P > 0.05$ (non - significant); **p < 0.01; ***p < 0.001). W values vary from 0 to 1.0; the higher the value, the higher the level of agreement between the informant groups. The black dots represent the median scores (number of stones) that were used during construction of the seasonal calendars. The minimum and maximum limits are shown in parentheses.

5. DISCUSSIONS

Pox infection is a very common disease of sheep and goats in Ethiopia and causes huge economic losses to the farming community, leather industry and national GDP. This disease causes high morbidity and mortality in small ruminants and it is one of the endemic OIE listed disease in the country (OIE, 2009). Although, there are no detailed studies on prevalence of SGP in Ethiopia, some reports indicate that it is one of the widely distributed and common small ruminant production problems in the country (Mersha, 2011).

The present study was undertaken to provide adequate information regarding the occurrence of sheep and goat pox outbreak cases in Adea Berga district. The study was based on a cross sectional survey in various PAs of the district. Different approaches were adopted to diagnose the disease. 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.

With respect to some recorded epidemiological criteria, in this study, the result of the questionnaire survey indicated that sheep and goat pox was a common disease in the study areas in which 96% (n=100) of the respondents reported the frequent occurrence of pox disease in their sheep and goat flockss. The survey revealed that majority of the respondents had previously experienced disease in their flockss and familiar with the clinical sign of the disease, which they locally called ‘’*Fentata* in Amharic *and Finno hoolaa* in Afan Oromo”. This stement was also supported by Mersha (2011) and Teferi (2014) who reported that the disease was named with similar local name in other part of the country. Similarly, with regard to seasonal occurrence, 84% (n=100) of the respondents informed that the disease was more prevalent during summer followed by spring season of the year and the remaining respondent did not associate the disease occurrence with the season. According to their explanation, the disease is often observed throughout summer and the beginning of the spring season. All respondents informed that the disease equally affected both sheep and goat; with more morbidity in sheep

population. They also explained young age groups are more susceptible than adult age groups of both species and high mortality in young age groups. These might be due to climatic stress or influence, frequency of occurrence or the severity of various diseases and the immune status of the animals. This study was consistence to the finding of Schwabe *et al*. (1977) who reported the morbidity and mortality of sheep and goat pox was high in young age groups than adult age groups. Also Radostits *et al*., 1994 reported that climatic stress affects the immune status of the animals resulting in increasing of susceptibility to diseases. It is also likely that sever cold weather and the shortage in feed supplies predisposed sheep and goat to pox infection. These finding was correlated with the findings of Hailat *et al*. **(**1994). Significant association of sheep and goat pox occurrence with various types of ecosystem, physiography, soil types, rainfall, relative humidity and temperatures has been studied and all these factors have strong influence on disease occurrence (Murray *et al.,* 2003). Similarly, the influence of various biometeriological factors on SPGV occurrence has also been reported in Algeria (Achour, and Bouguedour, 1999).

The incursion of the disease in Maru Chobot and Bishan Dimo followed the recurrence of SGP in Gatira Nebe and Ulagora and a particular increase in the number of outbreaks in the same area after August of the same year particularly in PAs bordering to Meta Robi and Ejere district which are connected with the main road of Holeta to Mugher through which a large number of animal movement to the capital city of the district is reported. These might be due to the illegal movement of large number of animals from Meta Robi and Ejere district to the capital city of district (Inchini), non- vaccination, poor management practice and the grazing and migration pattern of sheep and goat in the district which was extensive system. They also reported that source of the disease was new purchased sheep from the market of Meta Robi district. This observation was in agreement with the work of 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.

The present data collected have provided a reliable indication of the extent and severity of goat pox disease in Adea Berga district. In the survey of sheep and goat pox outbreak from different PAs of Adea berga district, it was observed that animals suffering from a clinical disease showed classical signs including hyperthermia with marked depression, weakness, decrease of appetite and discharges from eyes and nostrils. After 1-3 days typical pox lesions were appeared on skin and mucous membrane. These findings were correlated with previous studies Kitching *et al*. (1986, 1987b, 1989) Kitching and Taylor (1985a) and Chaudhary *et al. (*2009). The lesions were found under the surface of tail, udder, perineum, medial aspect of thigh, around external genitalia, head and neck. Moreover focal papular lesions were pronounced on eye lids, lips and nasal mucosa. Diarrhea was also observed in some animals and was more pronounced in kids and lambs. These observations were similar to that stated in other literatures Singh *et al*. (1979); Davies (1981) Sharma *et al*. (1986), Mersha, (2011) and Radostits *et al*. (1994). Nodular form of pox was observed as round firm flat surface nodules on lateral aspect of abdomen and thoracic and face in some cases. These nodules were similar to that observed in cattle infected with lumpy skin disease. These findings were also described by Afshar *et al*. (1986), Jan, *et al*. (1987) and Hungerford (1990).

Out of 600 sheep and goat examined, 137(33.3%) sheep developed pox lesions on their skin where as 51 (27%) goats from the total of 188 were developed pox lesion on their skin. Multivariable logistic regression analysis of pox virus showed that the morbidity proportion within species were high in sheep with OR = 1.22 at 95% CI. The highest number of pox lesion was observed in Maru Chobot 28 (41.2%) and Gatira Nebe 27(38.6%) and the lowest pox lesion was observed in Haro Shobore 12(23.5%) and Deku Kito 14(20.3%). These might be due to the illegal movement of large number of animals from Meta Robi and Ejere district to the capital city of district (Inchini) through Maru Cobot, climatic factors, the grazing and migration pattern of sheep and goat in the PA which was extensive system of grazing. This result was in agreement with the findings of

Mersha (2011) who reported the morbidity and mortality of sheep and goat pox based on the clinical sign and histopathological lesions as 49.5% and 10.42%, respectively, from central highland of the country. Both sheep and goats have been affected, but most of animals having the pox lesion were young age groups (kids and lambs < 1 year) and high prevalence of pox lesion is observed on sheep (33.3%) than goat (27%). This finding was similar with the report of Bhanuprakash *et al*. (2006). In additions, 11.88% morbidity and 0.13% mortality was recorded based on one year outbreak report by APHRD (2010). In Adama town, Oromia regional state the prevalence of pox virus in sheep and goat was reported as 10.34% and 12.88%, respectively (Yakob *et al*., 2008). A clinical disease associated with pox was reported to be 22% in sheep and 18% in goats in Wollo, North east Ethiopia (Weldemeskel and Mersha, 2009). These findings were also correlated with previous clinical findings of Mersha (2011); Woldemeskel and Ashanafi (2003); Kitching *et al*. (1986; 1987b, 1989); Kitching and Taylor (1985a) and Chaudhary *et al. (*2009).

The morbidity and mortality of sheep and goat pox within species was 32.1% and 29.4%, respectively. This was due to the large number of sheep population found in the study areas. CaPVs have traditionally been considered host-specific, causing outbreaks in a preferred host. However, recent records have indicated that some CaPV strains infect both sheep and goats (Bhanuprakash *et al*., 2006, 2010). Even though the morbidity rate was high in sheep than goat there were no significant differences between species and sex groups (P> 0.05). Within different age groups, the morbidity was 28.1% in adult and 38.2% in young age groups. Statistically, there were significant differences among age groups (P < 0.05). This might be due to high susceptibility (immune stress) in young age groups, environmental stress and agent factor (secondary complication). This result was in agreement with the result of Bhanuprakash *et al*. (2005) who reported that all age groups can be affected, however the disease is more severe in young animals than adults.

The mortality proportion (based on clinical signs) within species was 6.5% in goat and 4.7% in sheep. This was due to the severity of the pox virus strain circulating in the infected population of sheep and goat in the study areas which was a GPV. It was confirmed that the severity of pox virus was more severe in a homologues species. This

result was in general agreement with the result of Bhanuprakash *et al*. (2006b) and Babiuk *et al*. (2009) who reported that the majority of SPPV and GTPV strains showed a host preference, but some strains do cause diseases in both sheep and goats but goats may have mild clinical disease when infected with SPPV compared to severe disease in sheep. Likewise, sheep may have mild clinical disease when infected with GTPV compared to severe disease in goats. OIE, 2008 reported that strains of *capripoxvirus* do pass between sheep and goats, although most cause more severe clinical diseases in only one species; recombination also occurs between these strains, producing a spectrum showing intermediate host preferences and a range of virulence.

Morbidity, mortality and case fatality were 31.1 %. 5.2 % and 16.5 % for sheep under 6 months and 15.92 %, 1.55 % and 9.76 % for sheep above 6 months. These values were lower than that recorded by Chamoiseau (1985), Mariner *et al*. (1991), Tiwari and Negi (1994) and Radostits, *et al*. (1994). This might be contributed to sheep in endemic areas more resistant due to possessing protective antibodies from previous infection or vaccination (Castro and Huschele, 1992). However these results revealed that morbidity, mortality and case fatality were higher in sheep and goats less than 6 months. In this respect Button and Fraser (1977) demonstrated that sheep pox occurs in all breeds, sexes and ages of sheep and goat but lambs and kids suffer a higher disease incidence and often more sever lesions than adult animals.

Out of 27 tissue sample taken from sheep and goat, 25 (92.6%) samples showed typical CPE lesions to sheep and goat pox virus. In present study, the goat pox virus induced CPE such as small syncitia, cell ballooning, rounding, aggregation and detachment was observed within 7-10 days of incubation. Out of 27 skin biopsy samples, goat pox was isolated from 25 samples using Vero cell line while two samples could not develop any CPE in two passages. These findings were in agreement with Sajid *et al*. (2013) and Teferi (2014) reports who reported CPE development within 7-10 days.

In the present study, 25 samples out of 27 samples yielded a product size of 172bp on agarose gel electrophoresis. Therefore, the virus isolated from both sheep and goats were

not SPPV since the gel electrophoresis is greater than 151bp. This finding was in agreement with the pervious finding of Lamien *et al*. (2011) who reported, the genotyping result of SPPV was 151bp and of goat was 172bp.

Based on the findings of the PCR result, the present samples collected from sheep and goat population of different PAs of Adea Berga district showed that the pox virus circulating in sheep and goat were characterized as goat poxvirus; whereas sheep poxvirus were not identified from a single sample. This result clearly explain that both sheep and goats were equally susceptible to goat pox virus and it was only goat poxvirus circulating and causing pox disease in both sheep and goat population. The present molecular finding was in agreement with the previous report of Le Goff *et al*. (2009); Lamien *et al*. (2011) and Gelaye *et al*. (2013) who reported that goat poxvirus was identified from pox lesion collected from clinically diseased sheep from different countries of the world.

Seasonal calendar study was conducted to complement the diagnostic investigation of sheep and goat pox. The information could be useful for improving sheep and goat pox mitigation strategies such as timing of prophylactic (vaccination) or therapeutic interventions. Sheep and goat pox was reported to occur during the long and short rainy seasons by informant groups. This is in general agreement with the reports of Teferi (2014) and Mersha (2011) who demonstrated higher occurrence of sheep and goat pox outbreaks during rainy seasons with the appearance of more severe forms.

Good agreement was evident among the 12 informant groups concerning seasonal patterns of the selected sheep and goat diseases except for *Abba gorba* (blackleg) and *Handara* (Orf) which scores weak agreement (W= 0.1540) and (W=0.1395) respectively. Sheep and goat pox (*Finno Hoola fi Re'ee*) incidence peaked during long *(*Ganna*)* and short (*Afrasa)* rainy seasons with a median score of 8.25 (0-30) and 8.0 (0-19), respectively. According to the explanation of informant groups, the high incidence of sheep and goat pox during the rainy seasons was due to the occurrence of the rain and congregation in small areas that favour transmission of the disease. Increasing humidity

(rain) during *ganna* (long rainy) season was also explained as a predisposing factor and the reason for the highest occurrence of the disease in the season. The disease was reported mainly to affect young sheep and goat that allowed grazing during the rainy seasons. Increasing frequency of contact with other sheep and goats and grazing on green pasture and bushes which are abundant during rainy seasons were mentioned as predisposing factors of the disease in young sheep and goat.

On the other hand, occurrences of the other selected sheep and goat diseases including anthrax, blackleg and fasciolosis, and Pasteurollosis were associated with the long dry season *Bona* (December-February), short dry season *Birra* (September-November) and *Afrasa* (March-May) of the year respectively**.** This was mainly due to shortage of feed, starvation and flooding (long rain) during these seasons. Specifically, the relatively higher incidence report in *Ganna* (long rainy season) than *bona* (dry season) of this study was consistent with finding of Teferi (2014) who reported the highest occurrence of the diseases during *Ganna* followed by *Afrasa* seasons. The author suggested moisture as enhancing mechanism of virus stability in the environment and increase subsequent transmission to susceptible animal in rainy seasons. On the other hand, Wernery *et al*. (1997a) associated occurrence of the disease with the increased density of the stable flies' population during the rainy season.

6. CONCLUSION AND RECOMMENDATIONS

Sheep and goat pox infection is a very common disease of sheep and goats in Ethiopia causing huge economic losses to the farming community, skin damage and as consequence reduce national Gross Domestic Product. The current study revealed that goat pox infection is one of the endemic diseases of sheep and goat in the study area and implicated loss of production and productivity associated to high morbidity and mortality of diseases in small ruminants. Conventional PCR using RPO30 gene based genotyping confirmed that goat pox virus could cause pox outbreaks in both sheep and goat flockss. This study approved that host specificity classification of CaPV is inaccurate at least for GTPV. This finding may provide new information on the epidemiology of sheep pox and goat pox in Ethiopia. It has also important implication in the control of the disease of sheep and goats by the viruses of genus of CaPVs.The present participatory survey study provides evidence that pox disease is more prevalent during long rainy seasons (*Ganna*) followed by short rainy season (*Afrasa*) and short dry season (*Birra*),respectively. The disease was reported mainly to affect young sheep and goat that allowed grazing during the rainy seasons. Increasing frequency of contact with other sheep and goats and grazing on green pasture and bushes which were abundant during rainy seasons were mentioned as predisposing factors of the disease in young sheep and goat. Illegal animal movement was the major risk factor for the transmission of pox virus to the free area.

The seasonal calendar of sheep and goat pox was outlined by informant groups and it was claimed to occur during the long and short rainy seasons of a year. Furthermore, sheep and goat pox was listed to be one of the most common five sheep and goat diseases in the area by the farmers. Generally, the disease and associated morbidity and mortality were less commonly seen in adult age groups as compared to young age groups.

Therefore, based on these study findings the following recommendations are suggested:

- There should be strict quarantine measures (illegal animal movement control) from outbreak area and a ring vaccination should be given to the health animals

- Study should be undertaken on the susceptibility of sheep and goats for sheep pox virus
- Further studies should be conducted on the investigation of outbreaks and identification of the circulating Capri pox viruses
- Awareness should be given to animal health professionals and concerned bodies on inaccurateness of previous assumption of CaPVs host specificity
- According to the baseline information collected from informant groups, sheep and goat pox vaccination programs should be carried out before the start of rainy season. However, this should be validated by longitudinal observational studies.

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8. ANNEXES

Annex 1: Dulbecco's modification of Eagle's Minimum essential

Composition

- (DMEM) powder 12.5g
- Deionised water 1000ml

Preparation

- Dissolve, than make up volumetrically to desired volume
- Check if PH = 7.2
- Dispense into 200ml aliquots
- Sterilized by filtration by ESKS2
- Check sterility by broth culture
- Store at room temperature

Dulbecco's Minimum essential Medium 10% composition

- DMEM 80ml
- TPB 10ml
- Foetal calf serum 10ml

Preparation

- DMEM is dispensed into 100-200 ml amounts in screw cap bottles
- Store the bottles at 4^0C after sampling sterility testing
- If antibiotics are to be used add the antibiotic stock solution (Penicillin 5X 106IU vial + streptomycin 5 X 1g vial in 100ml of PBSA) to have it at a final concentrationof 0.2%

Annex 2: PBSA (Dulbecco's Phosphate buffered Saline)

Composition

- NaCl 8.0g
- KCl 0.2g
- $KH_2 PO_4$ 0.2g
- $Na_2HPO_{4.2}H_2O$ 1.44g
- Make up with distilled water to 1000ml

Preparation

- Dissolve, than make up volumetrically to desired volume
- Mark liquid level before autoclaving
- Adjust the PH to 7.2 at 20 ^{0}C or 7.4 at 36.5 ^{0}C with sterile 1M Na OH
- Dispense into 200ml bottles
- Sterilize by autoclaving at 121 ^{0}C at 15min.
- Store the solution bottles at +4 ^{0}C

Annex 3: Trypsin 2.5 % (W/V) solution (10 X stock solution preparation)

Composition

- NaCl 8.0g
- KCl 0.4g
- Na_2HPO_4 0.0475g
- $KH_2\ PO_4$ 0.06g
- $NaHCO_3$ 0.35g
- Trypsin (1:25) 25g
- Deionized and distilled water to 1000ml

Preparation

- Dissolve by string overnight at +4 ^{0}C
- Sterilize by filtration though a Seitz EK pad or a µm membrane filter
- Distribute aseptically into 100ml volumes
- Take sample for sterility tests
- Store at -20 ^{0}C
- For use add 100ml to 900ml sterile PBSA and adjust PH to 7.8 by the addition of sterile 1M NaOH

Trypsin 0.05% EDTA 0.02% solution for sub culturing

Composition

- Trypsin 2.5 % solution 20ml
- PBSA 970ml
- EDTA* 2% stock solution 10ml

EDTA (Versene) 2% stock solution

Composition

- NaCl 8.0g
- KCl 0.20g

- Na_2HPO_4 1.15g
- $KH_2\ PO_4$ 0.2g
- EDTA di-sodium salt.$_2H_2O$ 22.14g
- Phenol red 1.0 g

Preparation

- Dissolve and make up 1000ml with deionized distilled water
- Distribute in 100ml volume in screw cap bottles
- Autoclave at 121 ^{0}C for 15 min.
- Take sample for sterility tests
- Store at -20 ^{0}C

Annex 4: Penicillin and Streptomycin stock solution

Composition

- Sodium penicillin 5×10^6 i.u vials
- Streptomycin sulphate 5 x 1g vials
- PBSA 100ml

Preparation

- Add aseptically 5 ml PBSA to each vial
- Leave for a few minutes to dissolve
- Remove the PBSA from the vials and back to the PBSA 100ml bottle
- Mix well
- Dispense 10ml into sterile containers and stor at -20^{o}C

Annex 5: Extraction kit master mix, gel, TAE buffer, GelRed, Loading dye, Molecular ladder

DNA Extraction kit

- QIAamp spin columns 25 samples
- Collection tubes 750ml
- Buffer AL 54ml
- Buffer AW1 95ml
- Buffer AW1 66ml
- Buffer AE 110ml
- Proteinase K 6ml

Master mix for pox virus DNA convectional reaction

- RNA free water 4µl
- Primer SPGP RNA pol forward µl
- Primer SPGP RNA pol reverse µl
- IQ supper mix SPGP µl0
  Then mix and agitate divided into PCR tube

Gel electrophoreses

Composition

- Agarose gel 3g
- Distilled water 100ml

Preparation of Gel electrophoreses

- Mix very well and boiled with micro- oven at 180 ^{o}C temperature for 3 min. until melting
- Cool at 54 ^{o}C and pour into gel tank
- Insert the gel comb into melting agarose gel
- Dry for 20 minute

Preparation of TAE buffer

Reagents (per liter of 50 x solution)

- Tris base 242g
- Glacial acetic acid 57.1 ml
- 0.5 M EDTA 100ml

Preparation

- Measure 600 ml bi distilled water using the graduate cylinder and pour into Pyrex beaker
- Add stir bar, place on stir plate and begin stirring
- Add 242g tris, 57.1 ml acetic acid and 100ml 0.5 M EDTA
- Allow to stir until the tris goes into solution
- Adjust the PH to 8.0 if needed
- Bring the volume to 1L
- Transfer to a clean glass bottle
- Store at room temperature
- Dilute to 1 x before use (2 ml 50 x TEA per 98 ml waer)

GelRed is a non mutagen but must be handled with care. To make a 10mg/ml stock weight 1.0g and add it to 100ml distilled water, add a stir bar and let it dissolve several hours to overnight.

Store in a brown bottle

Gel loading buffer dye (good for just all applications)

- Glycerol 7.5ml
- Bromophenol blue 2mg
- Xylene cyanol 2mg
- 1M tris 100µL
- 0.5M EDTA 20µL
- 10 SDS 200 µL
- H_2O 2.2ml
- Total 10ml

The general rule of thumb is to use 1µL of gel loading dye per 5µL of sample

PCR ladder

The PCR 50 bp molecular ladder (fermentas) has been used for size determination of PCR generated DNA fragments. The recommended agarose gel concentration was 3.0%. The ladder contains 13 bands, ranking from 50-1000bp.

DNA size:	base pairs
1000	300
900	250
800	200
700	150
600	100
500	50
400	

The ladder is supplied as a solution in 10 mM tris HCL, PH 7.5-8.0, with 1.0 mM EDTA.

Annex 6: Sheep and Goat pox disease survey assessment questioner format

Region ----------

Zone----------

District----------

PA----------

Disease type----------

Date----------

Sheep and Goat pox disease survey assessment questioner format

PAS	Species		Age		sex		Clinical Sign	Disease seasonal
	Ovine	Caprine	Adult	Young	Male	Female		

Annex 7. Outbreak investigation format

S/n	Date	Place	Species	sickness	death	samples	comment
1.							
2.							
3.							
4.							
5.							
6.							

Annex 8: sheep and goat Diseases Listed by farmers (informant groups) in Adea Berga district based on their economic importance and Frequent Occurrence

Local Name (Adea Berga)	*Scientific Name
Rammo Tiru/Dodo'o	Fasciolosis
Abba Sanga	Anthrax/sudden death
Finno Holaa/Fentata	Sheep and Goat Pox
Abba Gorba/Arraba Mura	Blackleg
Gororsaa	Pasterollosis
Handera	Orf

Gatechisa	Abortion
Sombessa	Pneumonia
Ukaa	Respiratory Diseases (Coughing)
Bokoka	Bloating

*Scientific Name= Interpretation of local names to vernacular name is based on clinical symptoms mentioned by pastoralists and observation of some clinical cases

Annex 9: Seasonal calendar data recording sheet

Sheep and goat diseases	Adea Berga Season				Reason (Probing)	Remark
	Ganna	Birra	Bona	Afrasa		
Rammo Tiru/Dodo'o						
Abba Senga						
Finno Holaa/Fentata						
Abba Gorba/Arraba Mura						
Gororsaa						
Handera						

Annex 10: Seasons of the year in Adea Berga district

Adea Berga Season	Adea Berga Months	Ethiopian Calendar Months	Rainfall Pattern
Birra	Fulbana Onkololessa Sadaasa	September October November	Short dry
Bona	Mudde Amajji Gurandhala	December January February	Long dry
Afrasa	Bitootesa Eebila Camsa	March Appril May	Short rain
Ganna	Waxabajji Adolessa Hagaya	June July August	Long rain

Annex 11. Selected pictures taken during seasonal calendar data collection






Annex12. Ethical clearance certificate

አዲስ አበባ ዩኒቨርሲቲ
የ ንስሳት ሕክምናና
ግብርና ኮሌጅ
ቢሾፍቱ/ደብረ ዘይት

ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/13/06/09/2017

Name of Applicant: Abdi Assefa **(DVM,** MSc fellow**)**

Address: College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: Isolation and characterization of pox viruses circulating in sheep and goats from outbreak cases of Adea Berga District, West Showa Zone, Central Ethiopia

Date of application:	**02/06//2017**
Nature of the project:	**Mildly-invasive**
Target animal species:	**Sheep and goats**
Number of animals involved:	**600**
Study area:	West Showa Zone, **Ethiopia**

Minutes No. and date of review: **VM/ERC/06/09/017, 06/06/2017**

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is allowed to be executed provided that:

9. All procedures and conditions stipulated in the proposal are respected and any deviation or changes be reported to the committee
10. The project activities be open for occasional supervision by the committee whenever this is deemed necessary

Dr Getachew Terefe
Chairman

Signature

Federal Democratic Republic of Ethiopia
Dr. Dinka Ayana
Dean
College of Veterinary Medicine and Agriculture

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Please quote Our Ref. No. When replying

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Fax 251-11-4339933 Tel. +251 114338450 P.o.x. Box}34 Bishoftu/Debre Zeit, Ethiopia