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Isolation and Characterization of Pox Virus Circulating in Sheep and Goats from Outbreak Cases of Adea Berga District, West Shoa Zone, Oromia, Ethiopia

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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 survey, outbreak investigation, virus isolation and molecular characterization. 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 questionnaire survey indicated that sheep and goat pox was the most common disease and frequently observed during the long rainy (*Ganna*) and short rainy (*Afrasa*) seasons. 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 ($p < 0.05$). 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 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 have been collected.

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Adea Berga; Sheep and Goat; Pox virus; Outbreak; PCR

Introduction

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). Capripoxviruses can be found in saliva, conjunctival secretions and milk, as well as in skin lesions and their scabs (Carn and Kitching, 1995a).

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. 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 about the cross reactivity of the capripoxvirus infections. Therefore, the present study was designed to isolate and characterize pox virus circulating in sheep and goat using cell culture method and conventional PCR method and to identify the seasonal occurrence of sheep and goat pox outbreaks.

Materials and Methods

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*) of Oromia regional state. Geographically, it is located at 64 km north west of Addis Ababa and located at 9^0 12' to 9^0 37' N latitude and 38º 36' 69E longitude. The maximum and minimum temperature of the distric is 25°C-10 °C, respectively and an annual rain fall rangs from 918-1368 mm and an altitude ranges from 1400-3270 meter above sea level (ABLDHO, 2016). The agro-climatic regions of the district are Highland (*Badda*) 29%, midlatitude (*Badda daree*) 34% and lowland (*Gammojji*) 37%. The soil types

is 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 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)

Study animals: The study animals (indigenous breeds, but there were very few numbers of sheep and goats having exotic blood) were sheep and goats that had an outbreak cases of pox disease and clinically infected and manifesting clinical signs of sheep and goat pox and those in close contact with sheep and goat residing in pox outbreak areas. The indigenous breeds included were Arsi-Bale and Horo breeds of sheep and goats managed under small holder farming system; female animals were predominant in flocks, though some households own only male for fattening.

Study design: The study was carried out from November 2015 to March 2017 using a case study design (Dohoo *et al.,* 2003; Thrusfield, 2005) 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. A participatory epidemiological study method was also 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.

Sampling strategy and size determination: In outbreak areas, physical inspection of clinically sick animals was employed to record clinical observations and date of disease manifestation 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 flocks were selected and included for detailed clinical examination based on the presence of sheep and goat pox disease typical 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 sampling (lottery system) and fresh (unfixed) samples were submitted to the National Veterinary Institute virology laboratory for virus isolation and molecular characterization.

Field clinical examination: Sheep and goats 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 were carried out. Rectal temperature was also taken and 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.

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.

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 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.

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**.**

Sample collection and processing: 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. 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. 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. 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 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.

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. 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. 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. 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. 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.

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.

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.

Results and Discussions

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.

Out of 600 sheep and goats examined, 137(33.3%) sheep showed pox lesions on their skin whereas 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). 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. 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 (Figure 4A, 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.

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 ($P < 0.05$) (Table 3).

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).

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 1).

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).

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. 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. 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 compared 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 compared 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 April with OR = 1.17% and 1.30% respectively at 95% CI. Statically there was a significant difference between age groups and seasonal occurrence ($P < 0.05$) (Table 6).

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).

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 2).

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).

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. Among which five diseases, including sheep and goat pox 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.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**	

Table.1 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**		

Table.2 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**		

Table.3 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**

Table.4 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

Table.5 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**

Figure.1 The Morbidity proportion of sheep and goat pox within the study period

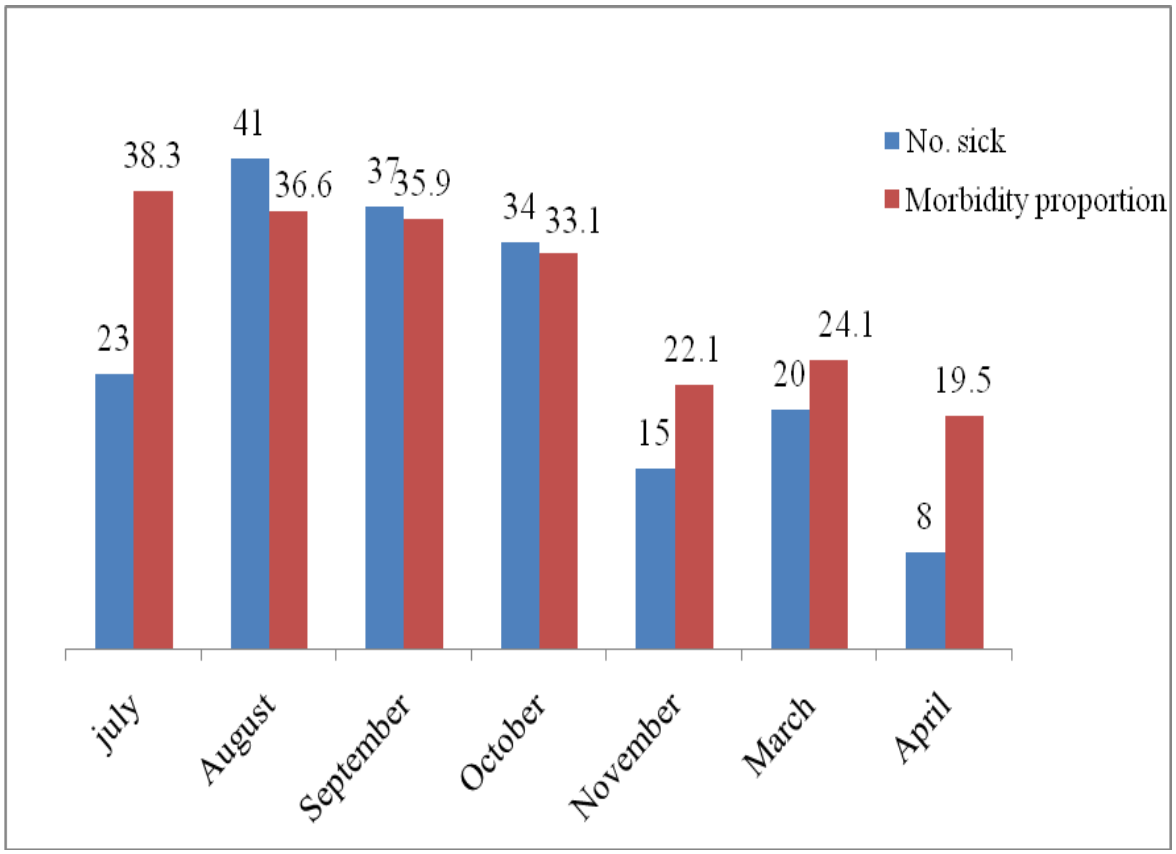

Figure.2 Classical PCR for differentiating GTPV from SPPV

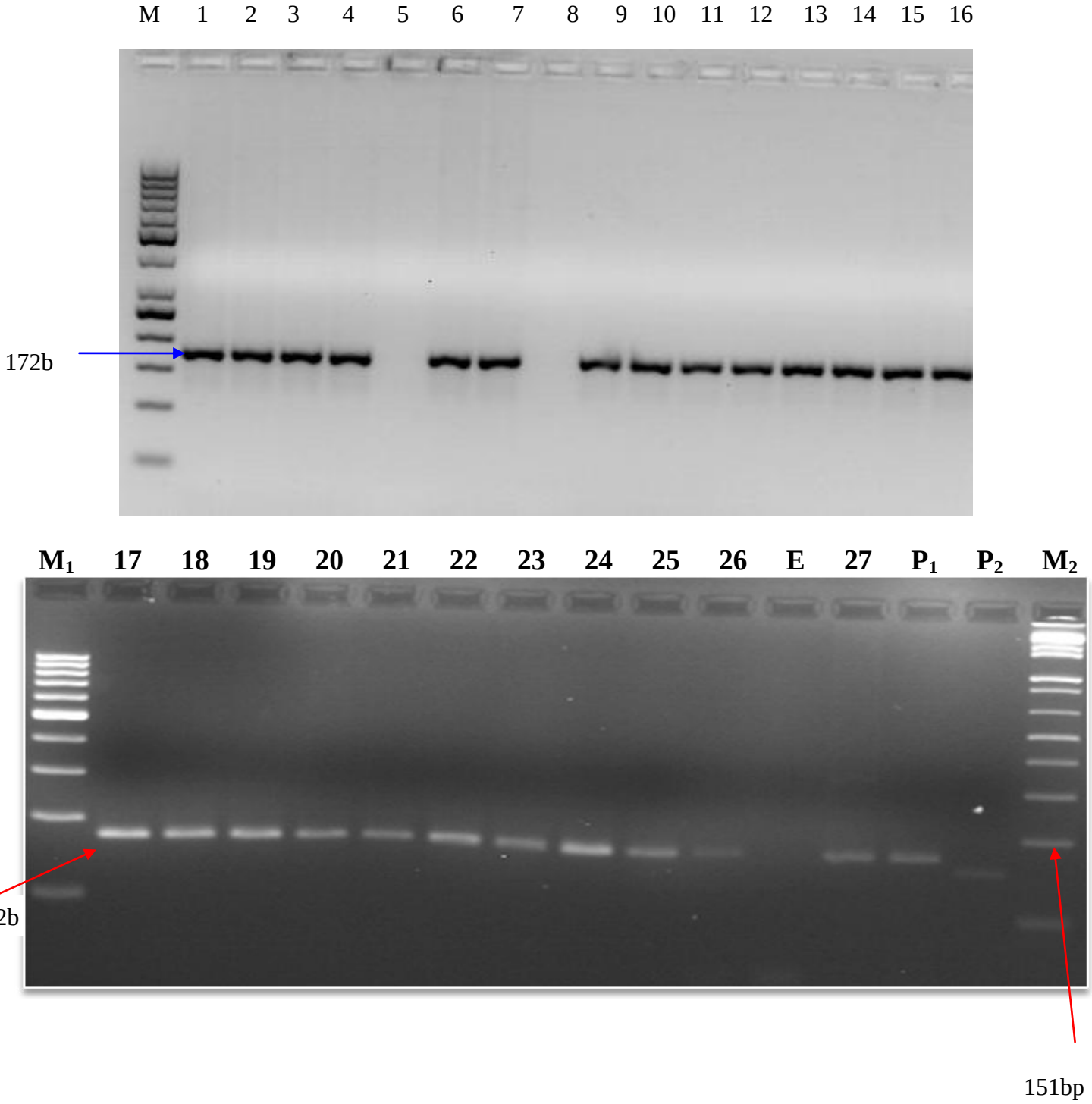

Table.6 Summarized seasonal calendar on the occurrence of selected sheep and goat diseases in Adea Berga district

Disease	Season			
	Ganna (June - August)	Birra (Sep - Nov)	Bona(Dec-Feb)	Afrasa(Mar-May
Ramoo Tiru/Dodo'o (Fasciolosis)** (W = 0.3563)	●●●●●●● 7.25 (2-16)	●●●●●●● ●●● 10.16(2-19)	●●●●●●● ● 8.33(0-24)	●●●●● 4.50(0-16)
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.

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 statement 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 districts 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.

Conclusion and Recommendations are as follows:

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 flocks. 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, there should be strict quarantine measures (illegal animal movement control) from outbreak area and a ring vaccination should be given to the health animals is recommended.

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