JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

SERO-PREVALENCE AND RISK FACTORS OF PESTE DES PETITS RUMINANTS (PPR) IN SELECTED DISTRICTS OF BUNO-BEDELE ZONE,SOUTHWEST ETHIOPIA

M.Sc. THESIS

BY:

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JIMMA, ETHIOPIA

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A Thesis

Submitted to Jimma University College of Agriculture and Veterinary Medicine, School of Veterinary Medicine in partial fulfillment of Masters of Science (M.Sc) in Veterinary Epidemiology

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Jimma, Ethiopia

DEDICATION

I dedicate my M.Sc thesis work to my mother and Bedele Regional laboratory staff those supported me during sample collection and laboratory analysis.

BIOGRAPHICAL SKETCH

The author was born in Alge Sachi Woreda, Ilu Ababor Zone of Oromia regional State on January 21, 1989 from his Mother Tadalu Tadasa and from his father Seyoum Gulilat. He attended his lower primary school (1-4 grades) at Bogo and Caca Primary school at Alge 01 kebele, Junior primary School (5-8 grade) and secondary school (9-12 grades) at Algie Sachi secondary and preparatory school in Algie Town. In 2008, the author joined Hawassa University and graduated with Doctor of Veterinary Medicine in July 2012. Soon after graduation, he was employed in government agricultural office as Animal Health expert and served for about four years. Meanwhile, He joined the school of graduate studies of Jimma University college of Agriculture and veterinary Medicine October 2018 to pursue Master of Science Degree (M.Sc) in Veterinary Epidemology.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my 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 Masters (M.Sc) degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University library/college to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Name: Yitagesu SeyoumSignature_____

Jimma University; College of Agriculture andVeterinary Medicine

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LIST OF ABBREVATIONS

AGID	Agar Gel Immuno diffusion
AUSTVETPLAN	Australian Veterinary Emergency Plan
CFSPH	Center for Food Security and Public Health
CIEP	Counter Immuno Electrophoresis
DIVA	Differentiation of Infected from Vaccinated Animals
EDI	ELISA Data Interchange
ELISA	Enzyme Linked Immuno Sorbent Assay
ELMP	Ethiopia Livestock Master Plan
ESGPIP	Ethiopia Sheep and Goat Productivity Improvement Program
GDP	Gross Domestic Product
IGAD	Inter Governmental Authority on Development
IHC	Immuno Histochemestry
MAb	Monoclonal Antibodies
MeV	Measles Virus
NAHDIC	National Animal Health Diagnostic and Investigation Center
OD	Optical Density
PA	Peasant Association
PANVAC	Pan African Vaccine Center
PI	Percentage of Inhibition
PVE	Post Vaccination Evaluation
TCRV	Tissue culture rinderpest vaccine
VNT	Viral Neutralization Test
WAHIS	World Animal Health Information System

ABSTRACT

Peste des Petits Ruminants (PPR) is a highly infectious epizootic disease of small ruminants that cause high mortality and has become an increasingly important trans-boundary disease. PPR is endemic in Africa, Middle East and Asian countries and causes an economic and social disaster, and ultimately is a threat to the national food security. Therefore, cross-sectional study was conductedto determine the seroprevalence and associated risk factors of peste des petits ruminants' virus infection (PPRV) in the selected districts of Buno Bedele zone of Oromia from October 2018 to August 2019. For the purpose a total of 680 (414 Goat and 266 sheep) serum samples were collected. These samples were subjected for PPR competitive ELISA test. In addition, questionnaires were administered to 124 households to collect information on risk factors associated with the presence of the disease. Univariable and multivariable logistic regression were performed in SPSS at 95% confidence level to determine the presence disease. The study revealed that animal and flock level sero-prevalence of PPR were 4.7% and 24.2% respectively. The overall seroprevalences of PPR recorded were 5.9%, 4.2% and 3.6% for Bedele, Borecha and Chewakadistricts, respectively. Based on the result of multivariable logistic regression analysis,introduction of newly purchased goat into a flock were 3.75 times more likely getting risk of PPR infection when compared with households which keep goats born at home (OR=3.75, P= 0.034), similarly goats which were kept in communal land for grazing were found to be 2.65 times more likely to be affected when compared with those goats kept under private (tethered) grazers (OR= 2.65, P=0.033).When we combine the result of sheep and goats all together, the odds of caprine were 2.37**(**95%CI=1.16-6.60, P=0.046) times more likelyto be seropositive than ovine species and grazing system(P=0.010), the odds of getting the risk of PPR infection from animals sampled from the communal grazing system was 3.34 times more than animals sampled from private (tethered) grazer (OR 3.34(95%CI=1.49-7.6). It was concluded that, the establishment of early warning system and proper implementation of control measures are neededto improve sheep and goat production.

Keywords*:Buno Bedele zone, cELISA, seroprevalence, Sheep and Goats, peste des petits ruminants virus, Risk factors*

1. INTRODUCTION

Ethiopia has a huge number of small ruminants with 30.7 and 30.2 million populations of goat and sheep, respectively (CSA, 2017). Sheep and goats represent an important segment of the Ethiopian livestock system. They are important sources of income for the agricultural communities and are important sources of animal protein, providing 35% of meat and 14% of milk consumption (MOA, 2010). Owing to their high fertility, short gestation interval and adaptation even in harsh environments, sheep and goats are considered as investments and insurance to provide income to purchase food during seasons of crop failure and to meet seasonal purchase such as improved seed, fertilizer, and medicine for rural household (Tibbo,2006;Tulu, 2018).The contribution of sheep and goats to the national economy with regard to foreign currency earnings is through the sale of live animal, meat, and skin. Among the export products, skin has the largest share of exports followed by live animal (Jemere *et al*., 2011).

There is an immense opportunity for increased livestock production in Ethiopia with growing human population, urbanization, economic development, domestic and export markets. However, prevalence of different diseases is found to be a major constraint of the sector (Biruk, 2014). One of the major factors restricting maximum efficient small ruminant production is the presence of high negative impact of infectious animal diseases Such as Peste des Petits Ruminants (Biruk, 2014).

The name peste des petits ruminants (plague of small ruminants) reflects two things about this disease. First, that it was initially described from Francophone West Africa and second, that it is a disease that kills a large number of sheep and goats. Many authors prefer the name “Ovine Rinderpest”. But, official agencies such as FAO and OIE use the French name “Peste des Petits Ruminants” (Tewodros and Melese, 2012). PPR was first described in Ivory Coast, West Africa in 1942 and subsequently spread to other regions. In the late 1970s, sub-Saharan Africa, then the Middle East and Asia faced severe epidemics. The infection has long been considered as caused by a variant of rinderpest virus, adapted to small ruminants but recognition of PPR virus as a novel member of the Morbillivirus genus occurred only in the late 1970s by using more sensitive laboratory techniques (Kula, 2016).

Peste des petits ruminant (PPR) is an acute, highly contagious and economically important transboundary viral disease of goats and sheep, which is listed by the World Organization for Animal Health (OIE) as notifiable disease (Yadav*et al*., 2009). The disease is characterized clinically by severe pyrexia, oculo-nasal discharge, necrotizing and erosive stomatitis, enteritis, and pneumonia (Balamurugan *et al*., 2011).PPR is caused by Peste des petits ruminants virus (PPRV). The virus is a member of the genus *Morbillivirus* in the family *Paramyxoviridae*. Virions are pleomorphic in nature, varying between 130-390 nm in diameter (Bailey *et al*., 2005).

The PPR virus has a widespread distribution spanning West and Central Africa, Arabia, the Middle East and southern Asia. This area encompasses much of the developing world that relies heavily on subsistence farming to supply food or goods for trade, and small ruminants provide an excellent supply of both. With its associated high morbidity and mortality, Peste des petits ruminants virus constitutes one of the major obstacles to subsistence farming (Banyard *et al*., 2010). Peste des petits ruminant was clinically suspected for the first time in Ethiopia in 1977 in a goat herd in the Afar region. Later clinical and serological evidence of its presence has been reported and confirmed in 1991 with complementary DNA (cDNA) probe in lymph nodes and spleen specimens collected from an outbreak in a holding near Addis Ababa (Abraham, 2005).

When we look the epidemiology of the disease in flocks of small ruminants it may be associated with several factors, such as: history of recent movement or gathering of sheep and/or goats of different ages, introduction of recently purchased animals into flocks can be mentioned. Other factors include contact of a village flock with those that had been sent to market but returned unsold, change in weather condition, contact with trade or nomadic animals through shared grazing, change in husbandry practices and trading practices (Abraham, 2005).

- *Statement of the Problems*

Livestock keeping is the main source of livelihood for most households found in East Africa countries including Ethiopia(Gitao *et al*., 2010). These areas are characterized by extreme climatic features of drought, flooding, low investments, fragile ecosystems and poverty

levels approaching 65%.Major losses of sheep and goats in these regions have been incurred from peste des petits ruminants (Gitao *et al*., 2010).

The contribution of livestock to the country's' agricultural GDP was estimated to reach 47% (IGAD, 2013). The Ethiopia Livestock Master Plan (ELMP) calls for establishment of a robust animal health information system; reduced production losses by controlling prioritized diseases; increased export earnings by reinforcing the quarantine, inspection, and certification system; decreased impact of zoonotic diseases on public health by controlling them and ensuring safety of animal products, improved infrastructure, and addressing policy issues (ELMP, 2015).

The epidemiology of PPR is not well understood in East Africa and more so in Ethiopia(Wondimagegn, 2016). In fact, in Ethiopia the first clinical and serological case of PPR was reported in 1977 in a goat herd in the Afar region (Abraham, 2005). And a risk based surveillance was conducted by NAHDIC during 2009 to 2011 in Afar, Oromia and Somali regions and the recorded prevalence for PPR was 60% (n=442), 59% (n=765) and 60% (n=465), respectively. An overall seroprevalence of 6.4% (n=13,651) was recorded during 1999national survey in seven regions of Ethiopia (Waret-Szkuta *et al*., 2008). The above mentioned study shows PPR has been extensively circulating across Ethiopia. The disease threatens food security and the livelihoods of smallholders and prevents animal husbandry sectors from achieving their economic potential. Reducing the number of PPR endemic countries is therefore a shared interest and should be considered a global public good (OIE and FAO, 2015). Therefore, this disease contributes to impeding safe trade and depriving poor farmers of access to lucrative global markets in livestock and livestock products.

Buno Bedele zone is immense zones of oromia Regional state of the Ethiopia in small ruminant numbers. However, a number of diseases are widespread that hinder production and productivity of sheeps and Goats. There is not enough data on the prevalence of many diseases including PPR in these areas. This disease is the most important disease of small ruminants that needs seroepidemiological investigation in these areas. For the purposes of control and prevention. Therefore, the objectives of the study area:

- ✓ To determine sero-prevalence of PPR in the study areas at individual animal and flock level.
- ✓ To assess associated risk factors for the occurrence of PPR in the study areas.

Hypotheses tested

- ✓ PPR is not an endemic disease in Buno Bedelezone.
- ✓ The seroprevalence of PPR in Buno Bedelezoneis not the same as other localities in Ethiopia.
- ✓ The seroprevalence of PPR in sheep is the same as that of goats in the study areas.
- ✓ Risk factors such as flock size, species, age, etc. do not contribute for the occurrence of PPR in sheep and goats.

2. LITERATURE REVIEW

2.1. Etiology

Peste des petits ruminants (PPR) is an acute, highly contagious and devastating disease of small ruminants which leads to high morbidity and case fatality thereby resulting in withering economic consequences to the livestock industry (CFSPH,2008; Kumar *et al*., 2017). Peste des petits ruminants virus is classified in the *order Mononegavirale family Paramyxoviridae, subfamily Paramyxovirinae, genus Morbillivirus*(Tahir *et al*., 2017) genetically similar to other members of the Morbillivirus genus such as measles virus, rinderpest (RP) virus, canine distemper virus and other viruses affecting aquatic mammals. The non-segmented genome encodes eight proteins: the nucleocapsid protein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin protein (H), the large polymerase protein (L) and the two non-structural proteins, C and V (Fig.1) (Banyard *et al*., 2010;Tahir *et al*., 2017).

Peste des petits ruminants virus is an enveloped virion which contains a genome of single stranded RNA with negative polarity. It has a genome length of 15,948 nucleotides which is the second longest genome among morbilliviruses after a newly identified feline morbilliviruses (Munir, 2013). The PPRV genes arranged from 3' to 5' on the genome is in an order of N-P-M-F-H-L separated by intergenic region. At the 3' and 5' end of the genome there is a leader (52 nucleotides) and trailer (37 nucleotides) region contain promoter functions (Muthuchelvan *et al*., 2015). The virus exists as single serotype, which is grouped into four distinct lineages (I, II, III, and IV) based on sequence comparison of small sequences of F gene or N gene. All four lineages have been detected recently in Africa whilst only lineage IV is circulating across Asia (Ugochukwu *et al*., 2019).

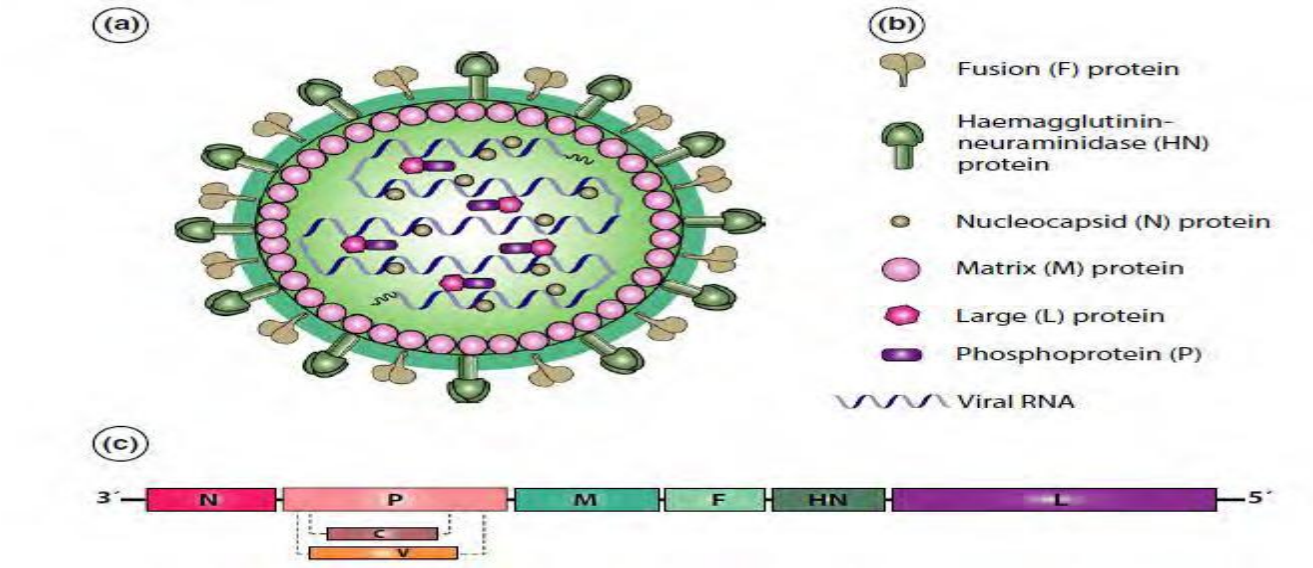

Figure 1: Schematic structure of a typical morbillivirus (PPRV). (b) The structural components of PPR. (c) The genome organization of all known genes of PPRV

Source **: (**Wondimagegn, 2016)

2.2. Epidemiological Situation

2.2.1.Geographical Distribution

The current Peste des petits ruminants (PPR) situation is that around 70 countries have either reported infection to the OIE or are suspected of being infected. Of these, more than 60% are in Africa the other infected countries being in Asia (South-East Asia, China, South Asia and Central Asia/West Eurasia including Turkey) and the Middle East. Another many countries are considered to be at risk for Peste des petits ruminants. As of May 2014, 48 countries in the world were officially recognized by the OIE as PPR free (OIE and FAO, 2015). Sporadic outbreaks of PPR have been reported in both the Anatolia (Asian Turkey) and Thrace (European Turkey) parts of Turkey since it was first officially reported in 1999 (Sevik, 2014).

The lineages are defined on the basis of sequence comparison of a fragment of either the nucleocapsid (N) or fusion (F) protein genes of the virus (Baron*et al*., 2017). Of note, the N gene fragment sequences were more related to those of viruses from Egypt, Eritrea,

Ethiopia, and Sudan and the F gene fragment sequences clustered with viruses from Egypt, Ethiopia, and Sudan. Unexpectedly, the N and F gene fragment sequences for viruses isolated from countries close to Georgia (e.g., Turkey, Iran, and Iraq) were less similar to the Georgia viruses than to the ones from Africa (Marina, 2018).

Peste des petits ruminants virus (PPRV) lineage IV is found predominantly in Asia and the Middle East, whereas all IV lineages have been reported in Africa. The different PPR viruses (PPRV) that have been isolated so far in all these areas were classified into four lineages (I - IV) based on partial sequence analysis of the F gene. Lineage I is represented by viruses isolated in Africa in 1970s. Lineage II which includes viruses isolated in the late 1980s in West Africa (Ivory Coast and Guinea) is the only African lineage that did not cross the Red Sea to the Asian countries. Lineage III is a combination of isolates from eastern Africa (Sudan and Ethiopia). Lineage IV of PPR virus isolates which includes the Asian isolates from Israel, Iran, Nepal, Bangladesh, Turkey and India, is confined to Asia (Abraham, 2005).

Until 2000, lineage IV was confined to Asia and the Middle East. However, this lineage had recently been identified in Africa (in Sudan in mid-2000s and in Morocco in 2008). PPRV infection had also been identified in both Tunisia and Algeria. This situation, together with the first discovery of the disease in Uganda, Kenya and Tanzania during 2006–2007, indicated a shift in disease dynamics on the continent (OIE, 2011).

Recently, lineage IV has been identified in Ethiopia. The full genome sequence data of PPRV (Ethiopia/2010) clusters genetically with lineage IV isolates. This isolate was derived from the intestine of a goat suffering from severe clinical disease during the 2010 outbreak in Debre Zeit, Ethiopia (Muniraju *et al*., 2016). The distribution of PPR has steadily expanded, covering large regions in Africa, the Middle East and Asia. By 1989, PPR has spread to Egypt and by 1992 spread further south to Sudan. Between 1996 and 2001, PPR spread rapidly across Africa, the Middle East and Asia, and by 2006, the most southerly affected African country was the Democratic Republic of Congo (Sunelle, 2012).

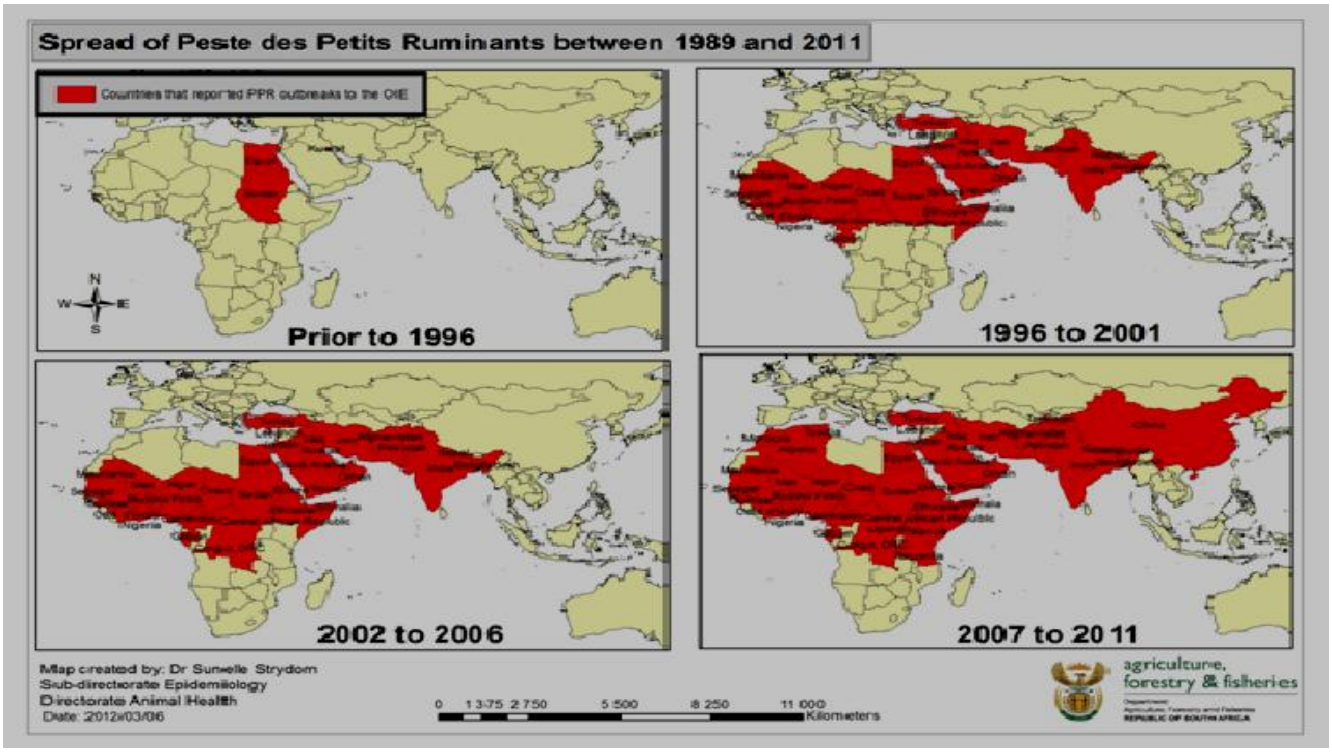

Figure 2:- Distribution of PPR in different times.

Source: (Sunelle, 2012)

2.2.2. Host range and Reservoirs

Peste des petits ruminants virus primarily infects sheep and goats, although both cattle, pigs and camel are susceptible to infection, but do not contribute to the epidemiology as they are unable to excrete the virus. The existence of sylvatic reservoirs for PPRV has been reported with infections and deaths in captive wild ungulates from several species having been described previously (Abu-Elzein *et al*., 2004; Kinne *et al*., 2010). Clinically, PPR is seen in both sheep and goats however, goats are more susceptible than sheep. Breed of goats play important role in susceptibility as Guinean breeds of West African dwarf goats such as Lagoon, Kirdi and Djallonké breeds are considerably more susceptible than the major Sahelian breeds (Abu-Elzein *et al*., 2004)

A Peste des petits ruminant isnow recognized as an emerging disease in camelids causing respiratory syndrome in Sudan (Khalafalla *et al*., 2010). According to Abraham *et al*., (2005), a 3% antibody seroprevalence was recorded in camels in Ethiopia. A serological

evidence of camel exposure to PPRV was confirmed in Tanzania with overall seroprevalence of 2.6% (Swai *et al*., 2011). It is believed that PPR virus circulates in domestic ruminants and acts as a potential source of virus for wildlife. It is quite possible that in cases of pastures exchange between domestic and wild animals, the spread of PPR is facilitated between the two populations (Hamed *et al*., 2016). PPR was detected from different wild ruminants (Table 1).

Table 1. Detection of PPRV in Wildlife Species.

Species	*Scientific name*
Laristan sheep	*Ovis gmelini laristanica*
Gemsbok	*Oryx gazelle*
Dorcas gazelles	*Gazella dorcas*
Thompson's gazelle	*Eudorcas thomsonii*
Nubian Ibex	*Capra nubiana*
Indian buffalo	*Bubalus bubalus*
African Grey dukier	*Sylvicapra grimma*
Arabian oryx	*Oryx leukoryx*
Bubal hartebeests	*Alcelaphus buselaphus*
Buffaloes	*Syncerus caffer*
Defassa waterbuck	*Kobus defassa*
Kobs	*Kobus kob*
Arabian mountain gazelles	*Gazella gazelle cora*
Springbuck	*Antidorcas marsupialis*
Arabian gazelles	*Gazella gazelle*
Barbary sheep	*Ammotragus lervia*
Bushbucks	*Tragelaphus scriptus*
Impala	*Aepyceros melampus*
Rheem gazelles	*Gazella subguttorosa marica*
Afghan Markhor goat	*Capra falconeri*

Source:-(Banyard *et al*., 2010; CFSPH, 2015)

2.2.3. Transmission

Infection usually takes place through direct contact between susceptible and infected animals. Inhalation is thought to be an important route of spread or Coughing and sneezing project virulent aerosols into the air. This virus can be shed during the incubation period, before the appearance of the first clinical signs, and can last up to over 2 months following recovery, has been found in nasal and ocular secretions, saliva, urine ,diarrheic fecesand all bodily secretions and excretions are highly contaminated (Radostits *et al*., 2007). These periods of

silent virus presence, increase the risk of disease spread to other small ruminants, and dromedaries. The airborne transmission of the disease is horizontal**.** It is rapid within herds of animals living close together. But, no vertical transmission of PPR through the placenta (EURL, 2016).

Contamination also is possible by the ingestion of infected food or drink. Feeding and drinking troughs and soiled bedding also can be indirect sources of infection, but only for short periods because PPRV, like all of the morbilliviruses, there is very little information on the survival of PPRV in the environment; however, this virus is very similar to rinderpest virus, which is inactivated by ultraviolet light and desiccation within 3-4 days or less, and normally survives for very short periods in carcasses. Temperatures above 70°C, as well as PH less than 5.6 or greater than 9.6, are also expected to inactivate PPRV (CFSCH, 2015).

2.2.4. Risk Factors

Host determinant factors of PPR spread have been reported in various studies, highlighting age, sex, breed and animal species (Munir *et al.*, 2013).

Breed:-Sahelian breeds of sheep and goats are believed to be more resistant than the dwarf breeds in the humid and sub-humid zones of West Africa. In a particular flock, the risk of an outbreak is greatly increased when a new stock is introduced or when animals are returned unsold from livestock markets. Recovered animals have lifetime immunity (Radostits *et al.*, 2007).

S*pecies*:-The disease causes more severe lesions in goats than sheep. Although, the reason forthis host specificity is not fully understood, difference in genetic makeup and/or receptor distributions of the host might have a role. Different levels of SLAM mRNA could influence the virus replication in different species (Pawar *et al*., 2008).

Age:-Young animals are more severely affected than adults or unweaned young (Kinne *et al*., 2010) and less likely to have developed protective antibody titers and therefore are more susceptible to PPRV (Luka *et al*., 2011). This high susceptibility in the young has been reported in Ethiopia (Waret-Szkuta *et al.,* 2008), Kenya (Abubakar *et al.,* 2009); thus, age of small ruminants is a key risk factor for susceptibility/resistance to the disease.

Sex:-Females are more likely to demonstrate antibody titers than the males. The recruited young males, having been in the herds for a shorter period, are less likely to have been in contact with virus (Sarker *et al.,* 2011).And also, the off-take of male small stock for social economic activities is higher and at an early age compared to females which end up staying in the herds for longer periods for productive purposes females (Singh *et al.,* 2004).

Season:-Climatic condition is also a risk factor and outbreaks are most frequent during the rainy season or the cold dry season (Kinne *et al*., 2010).Seasonality of PPR in Ethiopia has been attributed to seasonal movement of small stock in search for water and pasture resources during dry seasons, social exchange of animals and livestock marketing which exhibit seasonal patterns with pick outbreaks being experienced in March-June and October-November (Waret-Szkuta *et al.,* 2008).

Livestock rearing and herd management:- In endemic areas, livestock rearing and herd management practices increase the risk of PPRV circulating. This is the case when herds are mixed, combining animals with different levels of viral susceptibility. Large herds also are environments with a high risk of PPRV. It also is the case when the movement and gathering of animals promote frequent and repeated contact between animals with unknown disease status. When the usual herding routes (cross-border seasonal transhumance, nomadism) are changed the risk of spreading the virus also is increased (EURL, 2016).

Trade movements:-The increased trade movements of live animals, without sanitary controls, between livestock producing countries and areas to consumer countries and areas is contributing to the spread of the virus over long distances.*Social*:-In pastoral societies, local social and cultural practices and human behavior may increase the risk of introducing the disease into as yet disease-free areas (EURL, 2016).

2.3.Pathogenesis

Peste des petits ruminants is an acute or sub-acute and highly contagious viral disease of small ruminants. Because of the respiratory signs, PPR can be confused with contagious caprine pleura-pneumonia (CCPP) or pasteurellosis. In many cases, pasteurellosis is secondary infection of PPR, a consequence of immuno-suppression that is induced by PPRV.

Peste des petits ruminants virus is transmitted mainly by aerosols between animals living in close contact (Abraham, 2005). The nucleocapsid (N), phosphoprotein (P) and large polymerase protein (L), in tandem with the viral RNA, form the ribonucleoprotein complex (RNP). The matrix (M) protein forms a link between the RNP and the host cell derived plasma membrane, covered evenly with distinctive spikes of the viral glycoproteins, the haemagglutinin (H) and fusion proteins (F). The interaction between the H and F proteins regulates the virus entry into a host cell (Buczkowski *et al*., 2014). In the pathogenicity of this virus, Lymph nodes, other lymphoid tissues and digestive tract organs were the predominant sites of virus replication (Troung *et al*., 2014).

Peste des petits ruminants virus penetrates the retropharyngeal mucosa, sets up a viremia and specifically damages the alimentary, respiratory and lymphoid systems. Infected cells undergo necrosis, and in the respiratory system, also proliferation. Death may occur from severe diarrhea and dehydration, before respiratory lesions become severe, or is hastened by concurrent diseases such as pneumonic pasteurellosis, coccidiosis or coliform enteritis (Radostits *et al*., 2007).

2.4. Clinical findings

Morbidity and mortality rates can be as high as 80-100% in herds. Nonetheless, in endemic areas, morbidity and mortality range between 10 and 100% where previous immunity, age and species of infected animal define the severity of the resultant outcome (Torrson *et al*., 2016). Peste des petits ruminants has an incubation period of 2-10 days, followed by severe pyrexia(40-41 °C), followed by mucopurulent nasal and ocular discharges, cough, dyspnoea, erosive and necrotic stomatitis with sores in the mouth, gastroenteritis, this ultimately leading to diarrhoea, conjunctivitis, constipation. These painful sores in the oral mucous membranes prevent the animal from eating and, in synergy with the watery diarrhoea, leads to severe dehydration. This can result in the death of the animal within 10-12 days after the onset of pyrexia (Berguido *et al*., 2016; Ugochukwu *et al*., 2017). Crusts may form on the nose, resulting in the blocking of the nostrils and respiratory distress, while matting together of the eyelids may also result. One to two days following the onset of the pyrexia, the oral and ocular mucous membranes become hyperaemic. This then is progressing to multifocal pin-point necrosis of the epithelium of the gingiva, dental pad, palate, lips, inner aspects of the

cheeks, and the upper surface of the tongue. These necrotic areas extend and may even coalesce (Sunelle, 2012).

Diarrhea commonly appears about two to three days after the onset of fever although, in early or mild cases, it may not be obvious. The faeces are initially soft and then watery, foul smelling and may contain blood streaks and pieces of dead gut tissue. Where diarrhea is not an obvious presenting sign, the insertion of a cotton wool swab into the rectum may reveal evidence of soft faeces which may be stained with blood (FAO Animal Health Manual No.5).

2.5. Differential Diagnosis

Frequently, PPR is confused with other diseases which have grossly similar clinical signs. These diseases include rinderpest, foot and mouth disease (FMD), bluetongue, contagious ecthyma (Orf), pneumonic pasteurellosis, contagious caprine pleuropneumonia (CCPP) and gastro-intestinal helminth infestations. The most frequent sources of confusion are: the mouth lesions, which could be due to rinderpest, FMD, bluetongue or orf; difficult breathing, which could be due to pneumonic pasteurellosis or CCPPor diarrhea which could be due to coccidiosis or gastro-intestinal helminth infestations (Dilli *et al*., 2011).

2.6.Diagnostic Methods

2.6.1.Clinical diagnosis

Clinical diagnosis of PPR in the field is based on the symptoms such as pyrexia, lachrymation, nasal discharges, oral erosion, pneumonia, diarrhea and death. Historic epidemiological information of PPR in the region or farms can help field personnel to report a suspicious case. A differential clinical diagnosis should be made with other syndromic diseases. However, it is recommended to sample sick animals for a confirmatory diagnosis (Couacy-Hymann, 2013).

2.6.2. Post mortem diagnosis

Erosive lesions may be extensive, extending from the oral cavity to the reticulo-rumen junction. There is an apical pneumonia, with enlarged, edematous and congested lymph nodes. Pleuritis and hydrothorax may be present. The spleen is congested and enlarged, and

necrotic lesions may be present. Necrotic or hemorrhagic enteritis is usually present and linear hemorrhages or zebra stripes may be located in the colon and caecum (Sunelle, 2012).

2.6.3. Laboratory diagnosis

Laboratory confirmation of the disease is usually done through virus isolation and virus neutralization assay, which are time consuming and laborious. Several assays have been described to detect virus-specific antibodies or viral antigens.

Detection of Antibodies

A monoclonal antibody based competitive ELISA (cELISA) for detection of antibodies against PPRV has been developed in laboratory (Singh *et al*., 2004). The assay was compared with VNT and found to be 90.2% and 98.84%, of specificity and sensitivity, respectively. Although, this test is suitable for mass screening, it uses live virus as positive antigen, which could be a disadvantage for use in PPR free countries/regions. A polyclonal antibody based indirect ELISA was developed for detection of antibodies against PPRV in the serum samples (Balamurugan *et al*., 2011).

Detection of Antigen

Counter-Immuno-Electrophoresis(CIEP)and Agar Gel Immuno Difusion (AGID) tests using hyper-immune serum. Agar Gel Immuno Diffusion test is simple and can be performed in a basic laboratory but remains relatively insensitive. Moreover, it cannot distinguish PPRV from RPV. Counter-Immuno-Electrophoresis is sensitive and specific and able to differentiate PPRV from RPV sample.Immuno-histochemestry (IHC) on tissue samples.It allows the localization of specific PPRV antigens in pathological tissue sample (CouaCy-Hymann, 2013).

Virus Isolation

This technique needs cell culture facilities which are not common in many laboratories in the developing countries (CouaCy-Hymann, 2013). Virus isolation remains the "*gold standard*" for the diagnosis of PPR. Blood collected at the height of the temperature is the best material for this purpose. The nasal or ocular swabs or 10% tissue suspension can also be used. The PPRV can be propagated *in vitro* in several primary bovine and sheep cells, as

well as established cell lines like Vero (African green monkey kidney cells) and B95a (Marmoset B-lymphoblastoid cells) (Malama, 2015). In most of these cells, the PPRV manifests morbillivirus-specific cytopathic effect (CPE) by 3-5 days post infection. In some cases, up to five blind passages are needed for isolating the virus (Muthuchelvan *et al*.,2015).

Sandwich ELISA

The sandwich-ELISA for the detection of PPRV antigen in the clinical samples was developed in laboratory. This assay uses a monoclonal antibody directed against N protein. The test is compared with IC-ELISA and found to have 89% and 93%, sensitivity and specificity, respectively (Singh *et al*., 2004). That is easy to perform and is routinely adopted by many diagnostic laboratories. Like c-ELISA, in this assay also live attenuated PPRV used as positive antigen. Attempts were made to develop a recombinant N protein based ELISA (Yadav *et al*., 2009).

Detection of Nucleic Acid

Real-time Polymerase Chain Reaction (RT-PCR) is an accurate, rapid and reliable method that can be used for the detection and also for the quantization of specific DNA molecules (Vinayagamurthy *et al*., 2012). The conventional RT-PCR has been developed for the specific amplification of the NP gene or for the amplification of the fusion (F) gene and is established in various laboratories. The Real-Time RT-PCR assay specific for PPRV and the loop-mediated isothermal amplification technique which has such an intrinsic potential for point of care diagnosis have been recently extensively used for laboratory diagnosis of PPRV infections are also available for the genome detection of PPRV (CouaCy-Hymann, 2013; Ashraf *et al*., 2017). Reverse Transcription-Polymerase Chain Reaction is the method of choice for detecting nucleic acids of PPRV in clinical samples. RT-PCRs have been reported for detection and differential diagnosis of RP and PPR viruses targeting the N and F gene (Balamurugan *et al*., 2011). The test can detect viral RNA in infected tissue culture fluid with a titre as low as 0.01 TCID50 and is used to evaluate only critical samples (Saravanan *et al*., 2010).

2.7.Treatment

There is no treatment for PPR but it helps to give broad spectrum antibiotics to stop secondary bacterial complications (Bharath *et al*., 2016). Post-exposure therapeutic approaches for PPR infections are not mentioned much in the literature. However, treatment of affected animals by administration of antibiotics (long acting oxytetracycline, chlortetracycline) to prevent secondary bacterial infections and anti-diarrhoeal medicines has been practiced with supportive therapy (B complex and Dextrose saline) for 5-7 days, which may be useful to reduce the severity of the disease. Treatment and management of clinical cases of PPR or in the event of outbreaks in sheep and goats is necessary in order to minimize the economic losses to farmers (Ugochukwu *et al*., 2019).

2.8.Prevention

The impact of morbilliviruses on both human and animal populations is well documented in the history of mankind. Indeed, prior to the development of vaccines for these diseases, morbilliviruses plagued both humans and their livestock (Buczkowski *et al*., 2014). For many years the tissue culture rinderpest vaccine (TCRV) was used effectively to protect sheep and goats from PPRV with the cross-neutralising antibody response affording protection for at least 12 months. However, the need to stop vaccinating animals with the TCRV during the rinderpest eradication meant that a homologous PPRV vaccine, Nigeria 75/1, was required. This vaccine was generated by serial passages of a virulent PPRV strain in cell culture (Buczkowski *et al*., 2014) and was reported to be able to protect goats and sheep from challenge with wild-type PPRV isolates for at least 3 years post-vaccination (Saravanan *et al*., 2010). The existence of only one serotype of PPRV means that this vaccine protects against challenge with viruses from all four PPRV lineages (Saravanan *et al*., 2010).Other preventive actions include public awareness creation, quick report, surveillance, and treatment of products and by-products (AUSVETPLAN, 1996).

2.9.Control

Control measures include strict quarantine and control of animal movements. Quarantine of newly purchased or newly arriving goats/sheep for at least 2-3 weeks to ascertain their health status and the source of any new animal(s) brought into the flock. Migratory flocks are threat to local sheep and goat therefore contact should be avoided (Ugochukwu *et al*.,

2017). Effective cleaning and disinfection of contaminated areas of all premises with disinfectants including fences, equipment and clothing is necessary. Dead animals/carcases should be burnt/ buried deeply. Monitor animals closely and frequently for any developing clinical signs of disease and isolate sick animals from the flock. Veterinarians should be contacted immediately to examined sick animals in the herd/flock. Administration of a live-attenuated vaccine should be done to confer strong immunity to the herd (Sen *et al*., 2010). It is important to use separate facilities and staff to handle isolated animals, educate and train the employees about PPR and its clinical signs and the monitoring of wild and captive animals, especially those in contact with sheep and goats (Ugochukwu *et al*., 2017).

Current controls of PPR require an effective vaccine and for this purpose several vaccines including both homologous and recombinant vaccines have been developed (Abubakar *et al.,* 2011). Nowadays, efficient live attenuated PPR vaccines are available that can induce lifelong protective immunity in vaccinated animals (OIE and FAO, 2015). The challenges in control activities arise it is not possible to distinguish vaccinated animals from those that have recovered from natural infection. A differentiation of infected from vaccinated animals (DIVA) vaccine/test would improve epidemiological data by allowing tracking of infection in areas where there has been partial vaccination. Animals that have been infected are detected by the presence of antibodies to the N protein, while vaccination coverage can be assessed by the presence of antibodies to the H protein in the absence of antibodies to the N protein (FAO, 2015).

2.10. Current Status of PPR in Ethiopia

In Ethiopia, a number of outbreaks have been reported for the federal ministry of agriculture at different times. Lineage III and IV are found in Ethiopia. Lineage III has been found to be circulating in East Africa countries such as Kenya, Sudan, Uganda and Tanzania (Banyard *et al*., 2010). In 1999, a serological survey on PPR was conducted in Ethiopia with the aim of informing a subsequent vaccination campaign which would be the first large scale vaccination campaign against PPR in the country. District level prevalence estimates ranged from 0% for Guba in Benishangul region or Abala in Afar region to 52.5% for Dolo Odo in Somali region (Waret-Szkuta *et al*., 2008). District with the higher prevalence levels seem to be mainly those in areas of low altitudes where pastoral management systems prevail over

sedentary ones. Reasons for this may be related to different production systems with exchanges and movements in areas of lowlands being more frequent and involving larger numbers of animals. This is particularly important during the dry season and in low altitude areas where resources are scarce. In addition, animals are exchanged between households and flocks as a result of social practices and changes in economic conditions that exhibit seasonal patterns. The seasonality of animal movements could partly explain the occurrence of the disease in Ethiopia mainly between the months of March and June (Waret-Szkuta *et al*., 2008).

According to Biruk (2014), the overall seroprevalence of PPR in sheep and goats in Eastern Amhara region in unvaccinated flock was 26.9% (n=133) and 28.6% (n=196), respectively. A cross-sectional seroprevalence study conducted in southern region of Tigray revealed 47.5% prevalence in goats (Berihun *et al*., 2014). According to Abraham *et al*., (2005), PPR antibody seroprevalence in unvaccinated herd/flock was 3% (n=628) in camels, 9% (n=910) in cattle, 9% (n=442) in goats and 13% (n=835) in sheep in Ethiopia.

Ethiopia has launched progressive PPR control strategy. The strategy will adopt geographic approaches. The initial area of operation will include at least the epidemiologically interconnected pastoral areas of the country, where a progressive control (ultimately leading to eradication) program will be implemented in strategically defined epidemiologically important (sub)-ecosystems. These areas include Somali and Afar regional states, pastoral districts of Oromia and South Omo zone of SNNPR. The strategy of the highland lowland interface will be similar to that of the pastoral areas. It includes districts immediately adjacent to the pastoral areas of the country and epidemiologically closely linked to the pastoral areas through seasonal grazing and marketing (FAO, 2012).

2.11. Global PPR Control Strategy

In 2013, the OIE and FAO jointly decided to embark upon the control of PPR on a global scale and develop a 'PPR Global Control and Eradication Strategy' with a strong willingness to address the animal health problems in a systematic way through approaching horizontal as well as more disease-specific (vertical) issues for 5 year action plan (i.e., 2013-2017). The task of eradicating PPR can benefit from a series of favorable elements. These

include the experience gained from eradicating rinderpest (RP), several favorable technical aspects in terms of diagnosis and surveillance, effective and inexpensive vaccines that covers all known strains/lineages of the virus, no long-term virus carriers and no significant role of wild life, a growing social and political commitment from various decision-makers at national, regional and global levels. The underlying objective of this strategy is that through the control and eradication of PPR and other major diseases and through reinforced Veterinary Services and global animal health systems, the improvement of animal health will reduce the impact of these diseases. This in turn strengthens the contribution made by the small ruminant sector to global food security and economic growth while at the same time improving the livelihoods of smallholders and poor farmers (OIE and FAO, 2015).

There are various tools to implement the global PPR control strategy. These include WAHIS (World Animal Health Information System), PPR monitoring and assessment tool (PMAT), post vaccination evaluation (PVE), vaccines, surveillance, diagnosis, regional and international laboratory networks, OIE standards and the performance of veterinary services (PVS) pathway and others (OIE and FAO, 2015).

3.MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted from October 2018 to August 2019 in three selected (Bedele, Borecha and Chewaka) districts of Buno Bedele zone of southwest, Ethiopia.

Bedele is one of the districts of Buno Bedele Zone and 483km from Addis Ababa,atlatitude of 8^o 26' N and 36^o 20'E longitudes and with an altitude of 1400 to 2010 meters above the sea level. The annual mean temperature ranges from $12.5C^0$ to 27.5 C^0 and the area receives annual rainfall greater than 1400mm. The small ruminant populations have been estimated to be 25,006 heads of sheep and 44,546 heads of goat (BWLFRDO, 2017).

Borecha district is located about 502 Km west of Addis Ababa, at an altitude of 1300 to 2400 meters above sea level and Latitude 8°16'N Longitude 36° 34'E. The climatic condition of the area is sub-humid with the mean annual rain fall of 1825 mm and annual minimum and maximum temperatures of 13°C and 28°C respectively. The estimated small ruminant population has 20,289 heads of sheep and 34,950 heads of sheep (BWLFRDO, 2018).

Chewaka district is located about 550 Km from Addis Ababa, at Latitude 8° 56'N Longitude 36° 08'E. The climate condition with long summer rain fall and winter dry season with mean annual rain fall 800mm and the altitude range between 1000 to 1800 meter above sea level with daily temperature of 37-42°C.The small ruminants population has been estimated to be 23,103 heads of sheep and 27,124 heads of goats(CHWLFRDO, 2017).

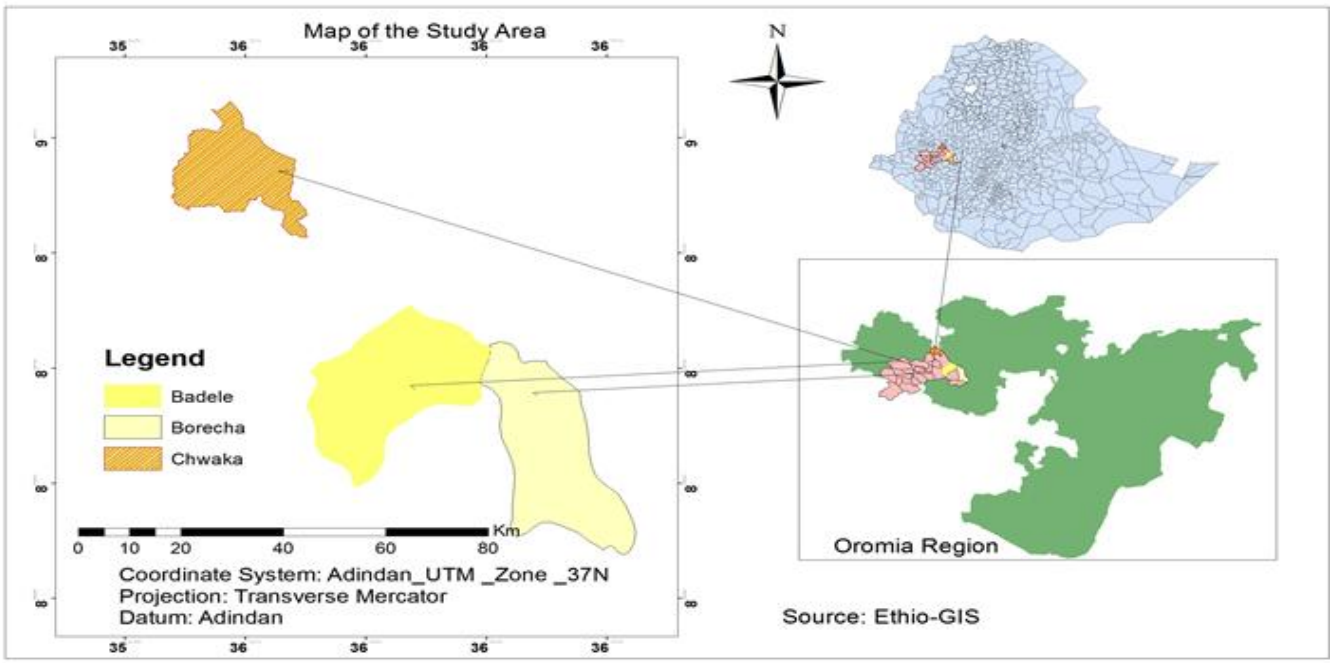

Figure 3: Map of study Area

3.2. Study Design and Sampling Techniques

A cross-sectional study design was conducted with purposively selected Zone based on lack of previous seroprevalence information; presence of livestock market activity and the three study districts were also selected based on representation of respective zone and lack of prior information of vaccinations of sheep and goat populations. Then, a list of peasant associations (PAs) within the district was obtained from the districts agricultural office and sampling three PAs per districts were selected on the basis of prior information of logistics and accessibility.Households selected by conveniently from willing farmers who keep sheep and goats. Finally, sheep/goats were selected randomly from selected households and allocated proportionally per each population size of districts.

3.3. Study animals

The study population was sheep and goats that are found in three selected study districts and all unvaccinated sheep and goat with more than six months of age were included in the sample. Age was determined using dentition (ESGPIP, 2009).

3.4. Sample size determination

The sample size was determined using Thrusfield (2007) formula. Since there was no prior similar study conducted in the area, expected seroprevalence of 50% was assumed to get the maximum number of samples sizes required. The absolute precisions were decided to be 5% and 95% confidence level. Thus, sample size estimation formula is shown below:

$$n = \frac{Z^2 P_{exp} (1 - P_{exp})}{d^2}$$

Where:

n = Sample size of the study population P_{exp} = Expected Prevalence

d = Absolute Desired Precision CI = Confidence Interval

Z= 1.96

The calculated sample size was 384; However, the sample size was increasedto increase the statistical precisions, in which a total of 680 (266 sheep and 414 goat) animals were sampled. Due to the difference in population size in the study districts and even within species in the same district, sample size was allocated proportionally based on the existing sheep and goat population per district(Table2).

Table 2.Respective number of small ruminants (sheep and goats) sampled per districts**.**

Districts	Districts populations			Expected Sample size per districts.			Actual Sample size taken per districts		
	Sheep	Goat	Total	Sheep	Goat	Total	Sheep	Goat	Total
Bedele	25006	44546	69552	55	97	152	97	173	270
Borecha	20286	34950	55239	44	78	122	79	135	214
Chewaka	27124	50227	50227	50	60	110	90	106	196
Total			175018	**149**	**235**	**384**	**266**	**414**	**680**

3.5. Sample Collection and examinations

Blood sample (5ml) was collected directly from jugular vein of sheep and goats by venipuncture using plain vacutainer tubes and sterile needles. Then, an identification code was given to each sample and labeled appropriately. The blood samples were allowed to stand in slant position overnight at room temperature to allow serum separation. Clear straw colored serum was decanted into 2ml cryovials andlabeled accordingly and kept in ice box

until arrival toBedele Regional Veterinary Laboratory Center and stored at -20°C deep freeze. Serum samples were analyzed there using competitive enzyme linkedimmuno-sorbent assay (C-ELISA) (collectively producedby ID. vet innovative diagnostics, France) according to instructions of the manufacturer.

3.6. Serological Test

The PPR competitive ELISA kit was used for ELISA test. Competitive ELISA has sensitivity of 94.4% and specificity of 99.8% (Abubakar *et al.*, 2011). The kit is based on a standard competitive enzyme linked immuno sorbent assay (cELISA) to determine the presence of anti-PPR antibodies in the serum. The test is based on the competition between the anti-H protein of PPR virus monoclonal antibodies (MAb) and the serum samples for binding the PPR antigen. The presence of antibodies to PPR virus in the serum samples blocks reactivity of the monoclonal antibodies which causes reduction in the expected color following the addition of enzyme labeled anti-mouse conjugate and chromogen solution. The negative and positive cut-off values were used from the controls of the test procedure (Annex I). The competitive ELISA kit (collectively produced by ID.vet innovative diagnostics France) was used for the test using an immunosunkan reader with an inference filter of 450nm. The reader was connected to a computer loaded with ELISA Data Interchange (EDI) software, which used to automate the reading and calculation of percentage of inhibition (PI) values. The optical density (OD) values were converted to percentage inhibition by using the following formula:

$$PI = [100 - -\frac{\text{OD control /test serum}}{\text{OD monoclonal control}}] \times 100$$

The samples with PI >50% (cut-off) was considered as positive (Waret-Szkuta *et al.*, 2008).

3.7. Questionnaire survey

A questionnaire surveys was conducted in each PAs during sampling of study animals (sheep and goat). Questionnaire survey was used to collect information on different risk factors. A total of 124 households (owner of flocks) were participated in this conversation. Semi-structure questions were used (Annex II). Data on management system, grazing system, presence of trade route (market activity), housing system, contact of flock with other group

of flock, whether animal owners recently introduced newly purchased animals into a flock during the last 6 months and flock size was collected. The number of sheep and goats farmers own was categorized as small size (≤6 sheep/goats), Medium size (7-12 sheep/goats) and Large size(>12 sheep/goats). Additionally, data on other factors such as species, age, sex, and availability of nearby veterinary clinics in the PA, altitude and watering system was collected during sample collection. Age of sheep and goats was recorded using dentition and asking the owners. The age was then categorized as young (6-12 months), adult (13-36months) and old(> 36months)(Annex III).

3.8. Data Management and Analysis

Data collected from study areas were entered into MS excel spread sheet program to create data base and it was coded and recorded before analyzed by using Statistical Package for Social Sciences (SPSS) version 20. The apparent seroprevalence was calculated by dividing the number of seropositive samples to the total animal sampled. Finally, for each species (variables) and flock level, the apparent prevalence (AP) was estimated as: Ap = y/n, where y denoted the total number of animals positive for PPRV antibodies out of the sample size (n). Confidence interval for proportions was calculated using Epi-Info online software (version 3.5.1., CDC). After adjusting to the specificity and sensitivity of the ELISA kit the True prevalence (TP) was calculated as:

$\text{True prevalence } (\%) = \frac{AP+SP-1}{Se+sp-1} \times 100$ and the flock level prevalence was calculated as follow

$$\text{Flock level prevalence}(\%) = \frac{\text{Number of positive flocks}}{\text{Total number of flocks sampled}} \times 100$$

Per each risk factor, proportions of seropositivity were calculated. Chi-square was used to determine the association of the disease with districts and peasant associations. Important variables were selected backward by univariable analysis. This was further tested by multivariable logistic regression model for final conclusion. A probability predictive limit of less than 5% ($P<0.05$) were set to indicate significant level. Odds ratio (OR) was used to associate the statistical strength ofPPR seropositivity with different potential risk factors. The effect of potential confounding factors and possible interactions was controlled using

adjustment method of multivariable analysis. Model fitness was assessed using Hosmer-Lemeshow goodness of test (P- value >0.05).

3.9. Ethical Issues

Owners were convinced that the study was not harm their animals. Proper cautions were followed while sample collection and restraining procedures are taking place. So, all data collection activities were depend on voluntary responses from the society. In addition, appropriate sample size was used to determine this seroprevalence study. This research was reviewed and approved by JUCAVM research and ethical review board.

4. RESULTS

4.1. Seroprevalence of PPR

Out of 680 serum samples tested by cELISA 32 (4.7%, 95% CI= 3.1-6.3%) of them were found to be positive for PPR antibody. In this result, 414 goat and 266 sheeps serum samples tested 25 (6%, 95% CI= 3.7-8.3%) and 7(2.6%, 0.7-4.6%) of them were found to be positive for PPR antibody respectively. The result showed Caprine were 2.37 times more likely to have risk of getting PPR infection (OR=2.37., 95%CI =1.01-5.58%) when compared to that of ovine and statistically significant(P=0.046) (Table 3).

Table 3. Species wise seroprevalence of PPR in selected (Bedele, Borecha and Chewaka) districts of Buno Bedele zone, Ethiopia.

Species	Sample tested	Positive sample	AP(95%CI)	TP (%)	Odds Ratio (95% CI)	P-value
Ovine*	266	7	2.6% (0.7-4.6%)	2.1%	1	
Caprine	414	25	6% (3.7-8.3%)	5.21%	2.37(1.01-5.58)	0.046
Total	680	32	4.7% (3.11-6.3%)	4.32		

TP = True prevalence, AP= Apparent prevalence, *=referance

The apparent seroprevalenceof small ruminants are 5.9%, 4.2% and 3.6% and true prevalence5.53%, 3.82% and 3.22% calculated at Bedele, Borecha and ChewakaRESPECTIVELYand the result showed statistically insignificant association between sero-positivity of PPRat studied districts with (P=0.457)(Table 4).

Table 4. Seroprevalence of PPR in sheep and goats at Bedele, Borecha and Chewaka Districts of Buno Bedele zone, Ethiopia.

Districts	Sample tested	Positives	AP(95%CI)	T p (%)	Chi-square(X^2)	P-value
Bedele	270	16	5.9%(3.1-8.7%)	5.53%		
Borecha	214	9	4.2% (1.5-6.9%)	3.82%	1.566	0.457
Chewaka	196	7	3.6% (1-6.2%)	3.22%		

TP = true prevalence, AP= apparent prevalence,CI= confidence interval

Theoverall flock level apparent sero-prevalence was 24.2% (95% CI=16.7-31.7%) and the true prevalence was 23.94% assuming that antibody was detected at least from one flock (in this study antibody was detected from 30 flocks out of 124)(Table 5).

Table 5.Flock Level Prevalence of PPR in Sheep and Goats from selected (Bedele, Borecha Chewaka) Districts of Buno Bedele zone, Ethiopia.

Districts	Tested flock sample	Number positives	AP(95%CI)	TP%
Bedele	54	15	27.8% (15.8-39.7%)	27.17%
Borecha	42	8	19% (7.2-30.9%)	18.71%
Chewaka	28	7	25% (9-41%)	24.25%
Total	124	30	24.2% (16.7-31.7%)	23.94

CI: confidence interval; Ap= Apparent Prevalence, TP = true prevalence

4.2. Risk Factors for Sero-positivity

4.2.2. Univariable analysis for identification of potential risk factors for both sheep and goats.

The non-important hypothesized potential risk factor was reduced by univariable logistic regression. Factors excluded from the model using univariable logistic regression analysis were flock size, availability of nearby veterinary clinics. Overall species, sex, grazing management and introduction of newly purchased sheep/goat into the flock during the last 6 months were factors which had P-value less than the critical p-value and moved backward for multivariable logistic regression analysis (Table 6). In the analysis, all the significant risk factors were initially included in the model. Model building used backward elimination method to decide on the factors to exclude from the model using the likelihood ratio test ($P < 0.05$).

Table 6.Univariable logistic regression analysis of risk factors to both sheep and goats for PPR seropositivity.

Variables	Category	Tested sample	Positives	Prevalence %(95% CI)	Odds Ratio (95% CI)	P-value
Species	Ovine*	266	7	2.6(0.7-4.6)		
	caprine	414	25	6.0(3.7-8.3)	2.37(1.01-5.58)	0.040**
Sex	Male*	242	7	2.8(0.8-5)		
	Female	438	25	5.7(3.5-7.9)	2.03(0.86-4.7)	0.103
Flock size	Small size*	104	5	4.8(1.6-10.9)		
	Medium size	378	19	5.1(3.1-7.8)	1.05(0.38-2.87)	0.92
	Large size	198	8	4.0(0.18-7.8)	0.83(0.26-2.61)	0.75
Age	Young	251	10	4.0(2.1- 8.2)	1.33(0.29-6.12)	0.70
	Adult	374	18	4.8(2.6-7.0)	1.34(0.30-5.94)	0.71
	old*	55	2	3.6(0-8.6)		
Intr.new animal	No*	207	27	2.4(0.3-4.5)		
	Yes	473	5	5.7(3.6-7.8)	2.53(0.91-6.67)	0.06
Avail.vet. Clinic	Present*	370	15	4.1(2-6.1)		
	Absent	310	17	4.6(2.9-8)	1.37(0.67-2.73)	0.38
Grazing system	Private*	291	6	2.1(0.4-3.7)		
	Communal	389	26	6.7(4.2-9.2)	2.96(1.28-6.5)	0.010 **

*: referances, ** : significant; CI: confidence interval

4.2.3. Multivariable logistic Regression model analysis for each species and combined data of sheep and goats.

In multivariable logistic regression introduction of new animals and grazing type were risk factors for sero-positivity in goats shown as (Table 7). Introduction of new purchased goats into a flock were 3.75 times more likelygetting risk of PPR infection when compared with animals (goats) born in the home (OR=3.75, P= 0.034) and goats on communal land grazer have 2.65 times more likely getting risk of PPR infection when compared with those animals on private grazers(OR= 2.65, P=0.033) while in sheep population no significant risk factors for the occurrence of the disease when those factors analyzed backward multivariable logistic regression.

Table 7.Multivariable logistic regression model analysis for PPR seropositivity of goats and sheep separably.

		Goats		Sheeps	
Variables	Category	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	P-value
Intr.new animal	No*				
	Yes	3.75 (1.10-12.76)	0.034**	----	----
Grazing system	Private*				
	Communal	2.65 (1.08-6.49)	0.033**	5.26(0.62-19.56)	0.12

*:Referance ; ** :significant; CI: confidence interval

After running in the univariable logistic regression for both sheep and goats together, those variables less than critical p-value found significant (P ≤0.25) were subjected into multivariable logistic regression model. In the final multivariable logistic regression species and grazing type were risk factors for sero-positivity for both sheep and goats(Table 8).

Table 8:Overall multivariable logistic regression analysis of risk factors for both sheep and Goats.

Variables	Category	Tested sample	Positives	Odds Ratio (95% CI)	Std. Err	P-value
Species	Ovine*	266	7			
	caprine	414	25	2.37(1.16-6.60)	0.43	0.046**
Grazingsystem	Private*	291	6			
	Communal	389	26	3.34(1.49-7.6)	0.42	0.010**
Intr.new animal	No*	207	27			
	Yes	473	5	2.53(0.91-6.67)	0.42	0.060

*: references; ** :significant; Std. Err :standard error; CI: confidence interval

5.DISCUSSION

The current finding revealed that the overall seroprevalence 4.7%(95%CI=3.1-6.3%).This result indicated that the disease is important which needs particular attention in the south western part of the Oromia region particularly Buno Bedele zone as it is one of the most economically important disease affecting both production and productivity. The overall seroprevalence of 4.7% observed in the current study was agrees with report of Amhara and Somalia region 4.6% and approaches to 8.0% respectively. But, higher than the report of 1.8% in Southern Nations Nationalities and Peoples Region (SNNPR) and 2.1% of the finding at Kaffa and Bench Maji zone which was reported by (Waret-Szkuta *et al*., 2008; Tsegaye *et al*., 2018).However, it is lower than the report of Hailegebreal,(2018) in Silti and Meskan districts of SNNPR region 29.2%; Wondimagegn, (2016) in Somali region 41%; Gari *et al.,* (2017) in East Shewa and Arsi Zones of Oromia region 48.43%; Kifle and Tsegaw, (2012) in Metema district of Amhara region 26.3%;Megersa *et al.,* (2011) in Gambella region 27.3% and Biruk, (2014) in Amhara region 28.1%. The difference between the current report and the previous reports could be the reason for the variations in sample size, different production systems, host population density, agroclimatic conditions and the social environment that can influence the contact rates.

In the current study, seroprevalence of the disease among the study districts was observed. The apparent prevalence of the study area were 5.9% (95%CI=3.11-8.7%), 4.2% (95%CI=1.5-6.9%), 3.6% (95%CI=1-6.2%) in Bedele, Borecha and Chewaka respectively. Accordingly, the prevalence among districts was not statistically significantly different(p=0.457).This suggests remarkable contagious nature of the disease on wide geographic areas and infecting perhaps most of the susceptible animals in affected villages.

The overall flock level seroprevalence of 24.2% (95% CI=16.7-31.7%**)** was recorded assuming that antibody against PPRV was detected at least in one flock. The current overall flock level prevalence is higher than 18.8% (18/96) in kaffa and Bench Maji zone of SNNPR were reported by Tsegaye *et al*., 2018. But, lower than the prevalence of 96.9% (22/23) and 100% (9/9) which was found by Megersa *et al.,* 2011 in Gambella and Afar regions of Ethiopia, respectively. An overall true prevalence at individual animal and flock level was 4.32% and 23.94% at flock level respectively. This finding was more than 1.6% at animal

and 19.4% at flock level but very low compared to true prevalence of 43% at animal and 104% at flock level in Somali region reported by (Wondimagegn, 2016; Tsegaye *et al*., 2018) respectively.

The study indicated that highest seroprevalence in Caprine species 6% (95%CI=3.7-8.3%) was different from that of ovine 2.6% (95%CI=0.7-4.6%). Multivariable logistic regression showed species was statistically significant (P=0.046).Caprine species were 2.37 times more likely to have risk of getting PPR infection (OR=2.37., 95%CI =1.16-6.60%) when compared to that of ovine species.The difference in prevalence could be due to the difference in the proportion of sampled animals. Besides, goats are affected more severely to PPR virus exposure compared to sheep and they exhibit striking clinical sign while sheep undergo mild form of the disease (Taylor, 1984). Similar findings were reported showing a higher prevalence of PPR in goats than sheep (Abubakar *et al*., 2011; Tsegaye *et al*., 2018). However, Singh *et al*., 2004; Abraham *et al*., 2005; Mehmood *et al*., 2009) had reported a higher PPR prevalence in sheep than goats which disagrees with our finding.

The apparent seroprevalence of PPR between sex groups showed that it was 5.7% (95%CI=3.5-7.9%) in female and 2.8%(95%CI=0.8-5%) in male but there was no statistically significant variation between female and male groups but, the chance of female 2.03 times more likely to have risk of getting PPR disease as compared to male (OR=2.03, P=0.103). This result is in agreed with previous finding (Waret-Szkuta *et al.*, 2008; Megersa *et al*., 2011; Tsegaye *et al*., 2018).This may associated with the physiological variation between female and male, whenever, females reveal a point of infection ensuring from stress because milk production and pregnancies the immunity status becomes lowered as a result their ability to resist the challenge of the infection will be low. Also females are more susceptible to PPR may be the fact that they are usually kept longer in a flock for the breeding purposes while males are either sold out or slaughtered for meat purposes (Munir *et al*., 2013).

With respect to the age of sheep and goats, adult age category showed the highest prevalence with 4.8%(95% CI=2.6-7%) than 4% (95% CI=2.1-8.2%)young ageand 3.6% (95% CI=0-8.6%) old agecategory. There was no statistically significant variation between age groups.

But, odds of adult animal category 1.34 times more likely getting risk of PPR infection(OR=1.34.,95% CI=0.30-7.0%) andyoung age 1.33 times more likely getting risk of PPR infection (OR=1.33., 95% CI=0.29-6.12%) when compared to old age groups. Higher seroprevalence in adult age groups may be the reason for a proportion of sampled small ruminants which exposed to PPR infection during young age but survived and seroconverted.This finding was agrees with the report of Singh *et al.,* 2004; Nizamani *et al.*,2015;Wondimagegn, 2016)who found more seropositivity in adult age groups. To the contrary, is in line with (Waret-Szkuta *et al*., 2008; Tsegaye *et al*., 2018).

This study revealed that introduction of newly purchased sheep/goat into a flock during the last 6 months revealed higher seroprevalence of 5.7% (95%CI=3.6-7.8%). The final multivariable logistic regression analysis in combined data of sheep and goats showed that the likelihood ratio of being seropositive for PPR for newly introduced sheep/goats had 2.53 times more likely at risk getting of PPR infection than home-borne (OR=2.53; 95% CI=0.96-6.67) and this practice was insignificant risk factor for the occurrence of PPR (P=0.060). But, the introduction of newly purchased goats into flock of goats were 3.75 times at a higher chance of being seropositive compared with animals (goats) born in the home (OR=3.75, P= 0.034)and significant for the occurrence of PPR in goats (P=0.034) when those individual caprine data analyzed in final multivariable logistic regression. These results are in agreement with the report of Biruk, 2014; Wondimagegn, 2016 and Tsegaye *et al*., 2018. This might be due to farmers do not seek veterinary support before mixing newly purchased sheep and goat into the existing flock (Radostits *et al.,* 2007). After buying in animals, owners do not practice separating new comers from existing flock to prevent the spread of animal diseases they give more attention for more number of their flock rather than the health status of their animals. Animals including sheep and goats are taken to market and brought to home. This may be explained by the fact that when sheep and goats from different origins come together in one market place and there might be contact to each other and spread of the virus. Consequently, they come home carrying the virus and play a role in the disease transmission (Abubakar *et al.,* 2009; Munir *et al.,* 2013).

This study showed higher PPR prevalence in communal grazing 6.7% (95%CI=4.2-9.2%) than private land(tethered)grazing 2.1% (95%CI=0.4-3.7%). Multivariable logistic regression

showed grazing land was highly statistically significant (p=0.010).Small ruminants which grazes on communal landwere 3.34 times more likely at risk of getting PPR infection (OR=3.34., 95% CI=1.49-7.6) when compared private land(tethered) grazer. This finding agrees with Salih *et al*., 2014; Tsegaye *et al*., 2018). This can be due to vulnerability of small ruminant flocks for infection since open communal grazing systems infect pastures and watering points (Salih *et al*., 2014). Although PPRV survives in the environment for short period of time, regular and continuous use of grazing pastures might have facilitated the exchange/transmission of the virus among small ruminants (OIE, 2013).

6. CONCLUSION AND RECOMMENDATIONS

The study provides the evidence that PPRV is widely circulating in small ruminant populations across the three selected districts of Buno Bedele zone.The current overall sero-prevalence of PPR in sheep and goats in the selected districts of Buno Bedele zone was 4.7% while the flock level prevalence was 24.2%. A flock with at least one positive animal was considered a positive flock for PPR. Factors like introduction of new animaland communal grazer land weresignificant risk factors forthedisease predictor in goat population.When we combine the result of sheep and goats all together the result indicated that high seroprevalence in caprine speciesandcommunal grazer were contributing risk factors for the disease. Based on the finding this particular study of disease needs great attention for zonal level control program.

Based on above conclusions,the following recommendations are forwarded.

- It is necessary to plan out strategic vaccination not only in the studied zones but also in other zones bordering study zones in order to prevent the circulation of the virus. .
- Further test should be conducted (e.g RT-PCR rather than c-ELISA) after this study to determine the exact prevalence of the virus in the study area.
- To confirm the effect of mixing different species (caprine and ovine) on PPRV transmission further study need to be conducted whether large ruminants like Cattles play a role in the epidemiology of PPR or they are only dead end host for the disease.

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8. ANNEXES

Annexs 1:- cELISA Test Procedure.

-All reagents were allowed to come to room temperature (21°C ±5°C) before use.

-All reagents were homogenized by vortex/inversion. 25µl dilutions were homogenized by vortex/inversion.

-25µl dilution buffer 13 was added to each well. 25µl of positive control (PC) was added to A1 and B1. 25µl of negative control (NC) was added to wells C1 and D1.

-25µl of each sample to be tested was added to the remaining wells. The prepared plate was incubated for 45min ±4min at 37°C ±3°C.

-Each well was washed 3 times with approximately 300µl of the wash solution by avoiding drying of wells between each washing.

-Conjugate 1x was prepared by diluting the conjugate 10x to 1/10 in dilution buffer 4.

-Then 100µl of conjugate 1x was added to each well. The prepared plate was incubated for 30min±3min at 21°C± 5°C.

-Each well was washed 3 times with approximately 300µl of the wash solution by avoiding drying of wells between each washing.

-100µl of substrate solution was added to each well.

-The prepared plate was incubated for 15min±2min at 21°C±5°C in the dark.

-100µl of stop solution was added to each well in order to stop the reaction.

-The optical density (OD) was read and recorded at 450 nm.

-For each sample, the competition percentage of sample (S) per negative control

$N = \frac{S}{N}\% = \frac{ODsample}{OD\ Negative\ \ sample}$ was calculated

-Then, <50% was considered as Positive, 50% < S/N <60%as Doubtful and S/N % 60% Negative.

Annexs 2:- Questionnaire survey.

A).Animal Information Sheet during Serum Sample Collection

Geo Ref: - Latitude_____ Longitude _____Altitude _____.

Date of collection _____

District_____

Kebele_____

No.	Owners' name	Breed	Species	Age	Sex	Flock	Origin of animal		Serology result
							Born	Bought	

B).Questionnaire survey sheet **Code_____**

Owner's name _____ Sex: M------ F--------

District _____ Date of collection_____

Kebele _____

General health status of the flock

List important health problems and signs that cause sheep and goat mortality in your area?

Sheep	Goats

- ✓ Have you had enteritis-stomatitis syndrome in your flock?
  Sheep: yes____ No____ Goat: Yes _____ No _____
- ✓ If yes, mention signs observed:

Sheep: _____

Goats: _____

- ✓ When did this disease commence in the area? DD _____Mon____ Year____
- ✓ Seasonal occurrence of PPR(seasonal occurrence)_____
- ✓ Frequency of occurrence(in time line)_____
- ✓ Have you seen such outbreak in the area before this time? Yes_____ No_____
- ✓ If Yes, In which age group?._____
- ✓ How frequent such disease occurs in your area? Don't Know ____Every 1yr____ Every frequently per a year_____
- ✓ Origin of such outbreak in the neighboring Kebele or district to your flock _____.
- ✓ What measures are taken to prevent the above listed health problems?

Traditional treatment ___ Modern treatment ___ Vaccination ___ Selling___

- ✓ After treatment/vaccination; Become healthy_____ Died____
- ✓ **Constraints:**
- ✓ Lack of modern services/clinics? Yes_____ No_____

- ✓ Lack of drugs and vaccines? Yes_____ No_____
- ✓ Others _____
- ✓ How many animals had got sick and/or died due to stomatitis-enteritis syndrome among your flocks?

Clinically sick	Sex	lamb/kid	Young	Adult
Died/slaughtered	Sex	lamb/kid	Young	Adult

Do you move your shoats to other places for grazing seasonally? Yes_____ No____

- ✓ If yes,

When (season)? _____

Where? _____

How long did you keep them there?_____

Grazing types:-

Communal_____

Private(tethered) _____

Both_____

Raising types:-

Sheep and goat grazing separately _____

Sheep and goat grazing together _____

Sheep and goat grazing with other livestock_____

Sheep and goat tethered feeding at home_____

Housing types: Fenced stable _____House barn_____

- ✓ Name of shoats market area _____
- ✓ Trade route around the area _____
- ✓ Are wild small ruminants available in the area? Yes _____ No _____

Annexs 3:- Description of dentition with corresponding Age estimates

Number of pairs of permanent incisors	Sheep	Goat
0	Less than 12 months	Less than 12 months
1	12 to 18 months	12 to 24 months
2	18 to 24 months	24 to 36 months
3	24 to 36 months	36 to 48 months
4	More than 36 months	More than 48 months
Broken mouth (teeth missing or worn down)	Aged	Aged

Source: ESGPIP (2009)