



## COMPETITIVE ELISA SEROLOGICAL INVESTIGATION OF *PESTE DES PETITS RUMINANTS VIRUS* AND ITS RISK FACTORS IN SELECTED LEKA DULECHA AND WAYU TUKA DISTRICT EAST WOLLEGA ZONE, OROMIA, ETHIOPIA

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### ABSTRACT

Small ruminant's production contributes significantly to food security across the globe. African communities but frequent attack by fatal diseases has brought a big challenge. Among these disease Peste des petits ruminants (PPR) diseases are capable of fast spreading to other areas. A cross sectional study was conducted from October 2019 to March 2020 in Leka Dulecha and Wayu Tuka districts of Oromia Regional State to determine the sero prevalence of PPR virus and its risk factor in sheep and goat as well as semistructure questionnaire survey was conducted. A total of 768 serum samples were collected from six (6) peasant association and the sera were tested for the presence of antibodies against PPRV using competitive Enzyme Linked Immuno sorbent Assay (C-ELISA). The total of sero prevalence PPR was found to be 7.2% (55/768). There is statistical significant difference in the two districts (OR=2.6 (1.427-4.74) and (P<0.05) and there were statistical significant difference between sex of sheep and goat (p<0.05), (OR=2.022) and regarding to body condition status, statistical significant was (p<0.05) and Semi structured questionnaire survey was administered to those sheep and goat owners showed their willingness to participate in the study in the selected PAs. From each districts about 25 to 34 people in each PAs were selected for the survey the owners responds that the clinical signs included fever, ocular and nasal discharge, oral ulceration, few abortions, respiratory distress and diarrhea and most of owners had know

about PPRV and more of them have not know about the PPRV. In Conclusion this study reveals a higher seroprevalence and subsequent endemic establishment of PPR in small ruminants in selected area. PPRV needs harmonization in the control and eradication of the disease between the study districts. Therefore it is recommended to plan strategic vaccination in the districts in order to control the PPR virus. These diseases must be reporting needs awareness, harmonization, and network of all partners (region, district and field professionals) to mitigate the risk factors.

**Keywords:** C- ELISA, East Wollega, Goat, PPR, Risk factors, Seroprevalence. Sheep.

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## 1. INTRODUCTION

Small ruminants are vital livestock for supporting food security because of their high reproductive capacity; faster growth rates, greater environmental adaptability and low initial investment and hence have a unique niche in smallholder agriculture. 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 (Tibbo, 2006). Small ruminant's production contributes significantly to food security across the globe. Peste des petits ruminants (PPR) are capable of fast spreading to other areas and are called transboundary animal diseases (TADs).The Southern Africa Development Community (SADC) region has not been spared of such diseases especially those attacking small ruminants like peste des petits ruminants (PPR). PPR can cause direct economic

losses to livestock keepers and affects the socio-economic status of African communities (Kihuet *et al.*, 2015).Livestock sector development for poverty reduction an economic and policy perspective Livestock's many virtues. Infectious disease is considered a major restriction causing direct losses, such as death and decreased production and indirect losses, such as export constraints (*Pradère*, 2014) The World Organization for Animal Health (WOAH-OIE) defines Peste des petits ruminants (PPR) as an acute, highly contagious, notify able and economically important transboundary viral disease affecting small ruminants. It is also called as 'Goat Plague' and clinically resembles with Rinder Pest (RP) in cattle, the later being characterized by pyrexia, conjunctivitis, oculonasal discharges, necrotizing and erosive stomatitis, diarrhea and bronchopneumonia followed by either death or recovery (*Gomes et al.*, 2016). Competitive ELISA (c-ELISA), agar gel precipitation test (AGPT) and virus

neutralization tests were employed to detect antibodies against PPR virus (PPRV). The c-ELISA assay based on the inhibition of binding of monoclonal antibody (MAb) to antigen in the presence of PPRV antibodies in test sample is considered highly sensitive and specific, thus employed in the present study to record seroprevalence of PPR among sheep and goat population (Santhamani *et al.*, 2016).

In Africa, PPR was first recognized as a contagious “rinder pest like” condition in goats in Nigeria in 1930 and it was first described in Côte d’Ivoire in 1942 and later in the Benin Republic during 1944 (Lebbie *et al.*, 1994). PPR is widespread in Africa, Arabia, and the Middle East and in some geographical areas of Asia, including much of the Indian subcontinent. Furthermore, because of outbreaks in Morocco and the existing commercial trade between Morocco and both Algeria and Spain, the situation raised huge concern owing to the increased risk of introduction of the disease into free zones in northern Africa and into Europe (FAO, 2009;Khalafalla *et al.*,2010).

The disease is mostly present in developing countries which often rely heavily on subsistence farming of small ruminants for trade and food supply (De Nardi *et al.*, 2012)-Since 2007, more than one billion small ruminants in Africa and Asia have

been considered at risk of being infected with the PPRV (FAO, 2009). Because of the dramatic clinical incidence and associated restrictions on animal and product movements, PPR is considered as a disease of major economic impact and has to be notified to the World Animal Health Organization (OIE) (Albina *et al.*, 2013).

The most effective way to control PPR is mass immunization of small ruminants as often, farmers in areas where the virus is endemic are unable to afford and implement the strict sanitary control measures, including the stamping out policy, required to contain the virus. Therefore, the control of PPR requires an effective vaccine and for this purposes several vaccines including both homologous and recombinant vaccines have been developed (Abubakar *et al.*, 2011a).The homologous vaccine, however, also has certain limitations, including requirement for cold-chain maintenance, and inability to differentiate vaccinated from infected animals (DIVA). Alternative thermo tolerant PPR-recombinant poxvirus vaccines have been engineered in the past (Jones *et al.*,1993; Diallo *et al.*, 2002, 2007; Berhe *et al.*, 2003; Chen *et al.*, 2010), which should provide simultaneous protection against both diseases, although none of them have yet been launched in the market and used in

the field. Development and technology transfer of an efficacious thermo-stable vaccine against PPR for (sheep and goat) in the short term remains an area for further research (FAO, 2013). Furthermore, a DIVA vaccine would facilitate surveillance for actual disease during an ongoing vaccination campaign.

It is also essential to fully understand the role of wildlife in the spread and potential maintenance of PPRV in the environment in order to be able to initiate successful control strategies (Baron *et al.*, 2011). Another major gap for the success of PPR control is the lack of economical assessment of control strategies, and even of PPR cost. Such information would be useful to help veterinary services in convincing governments and international organizations to support and fund PPR control (Albina *et al.*, 2013).

The development of trade relations, transport, tourism and migration of wild animals susceptible to PPR contributed to the spread of the disease beyond the boundaries of western Africa. Recent field and laboratory data show that PPR is spreading with recent incursions in China and Bhutan and that it is moving fast towards southern and eastern Africa where it affects a wide belt of countries South of the Equator from Gabon to Tanzania and the Democratic Republic of Congo (DRC)

(Libeau *et al.*, 2011).

PPR in Ethiopia was suspected on clinical grounds to be present in goat herds in Afar region of Eastern Ethiopia in 1977 (Pegram *et al.*, 1981). Moreover, serological and clinical evidences were reported by Taylor (1984). However, the presence of the virus was only confirmed in 1991 with cDNA probe in lymph nodes and spleen specimens collected from an outbreak in a holding land Near Addis Ababa. PPR was characterized by ocular and nasal discharges, mouth lesions, pneumonia, gastro enteritis and diarrhea. The disease in this outbreak caused more than 60% mortality. The disease probably was introduced into Ethiopia in 1989 in the Southern Omo river valley from where it moved eastward to Borena region and then northwards along the Rift valley to Awash (Gopilo *et al.*, 1991, Roeder *et al.*, 1994). The disease became endemic in goats

(Abraham and Berhan, 2001).

This could be one of the possible reasons for higher frequency of PPR outbreaks between March to June which also correspond to lean period of kidding. Although seasonal occurrence of PPR virus outbreaks is disputed, disease transmission is certainly affected by animal movement for which socioeconomic factors and variations in agro climatic conditions

are responsible. Large group of animals move to large areas and even between different districts. With the start of rains, the movement of animals is restricted due to the easy availability of local fodder. Nutritional status of the animals also gets improved during the rains.

This may reduce disease transmission after the start of rains and during the period of easy availability of fodder. Similar observations were also recorded in tropical humid zone of Southern Nigeria during a period of 5 years of observations (Taylor, 1984).

Viral diseases of farm animals, rather than being a diminishing problem across the world, are now appearing with regularity in areas where they have never been seen before. Across the developing world, viral pathogens such as peste des petits ruminants virus (PPRV) place a huge disease burden on agriculture, in particular affecting small ruminant production and in turn increasing poverty in some of the poorest parts of the world. PPRV is currently considered as one of the main animal transboundary diseases that constitutes a threat to livestock production in many developing countries, particularly in western Africa (Kaukarbayevich, 2009).

Developments of small ruminants are under the influence of several constraints in the

study area, one of the main limiting factors is the presence of infectious animal diseases such as Peste des Petits Ruminants which has high economic impact (PPR). The potential of animal production remains low valued and one of the main limiting factors is the presence of PPR. The socioeconomic impact of PPR results in heavy losses of production and its impact on market access of small livestock, affecting all players in the sector. Development of small ruminants in the country is under the influence of several constraints: the rearing facilities are not very adequate they remain dominated by the extensive method (FAO *et al.*, 2003).

Transboundary animal diseases have devastating impact on the survival of small ruminants and also economic, food security and livelihoods of poor people across the globe (Banyard *et al.*, 2010). A disease like PPR has a potential of spreading rapidly irrespective of continental, regional or national borders. PPR is considered one of the damaging animal diseases in Africa, the Middle East and Asia (OIE, 2015). The global distribution of the disease, although expanding relentlessly, is fairly known. Quantification of disease status is one of ways to ensure effective surveillance and control to keep the disease from spreading. Application of diagnostic techniques such

as serology for screening coupled with the knowledge of risk factors associated with the distribution of the PPR can be used to sensitize the public and other practitioners on possible ways to manage the disease (FAO-EMPRESS, 2012).

The Oromia region is under threat of the spread of PPR introduction with possibility of reaching other countries within the region. Lack of interest paid to the disease since its discovery is largely responsible for its spread (Libeau *et al.*, 2011). Seroprevalence and risk factors associated with possible spread of PPR in parts of East Wollega zone from Leka Dulecha and Wayu Tuka districts have not been researched to provide evidence of the absence or present of PPRV. Therefore, the present study to be used for effective control and prevent measures for the disease. Therefore the objectives of present study were To assess the seroprevalence and identify the risk factors of PPR in the Leka Dulecha and Wayu Tuka districts. To assess knowledge, attitude, practice of the farmer through the questionnaire survey.

## **2. MATERIALS AND METHODS**

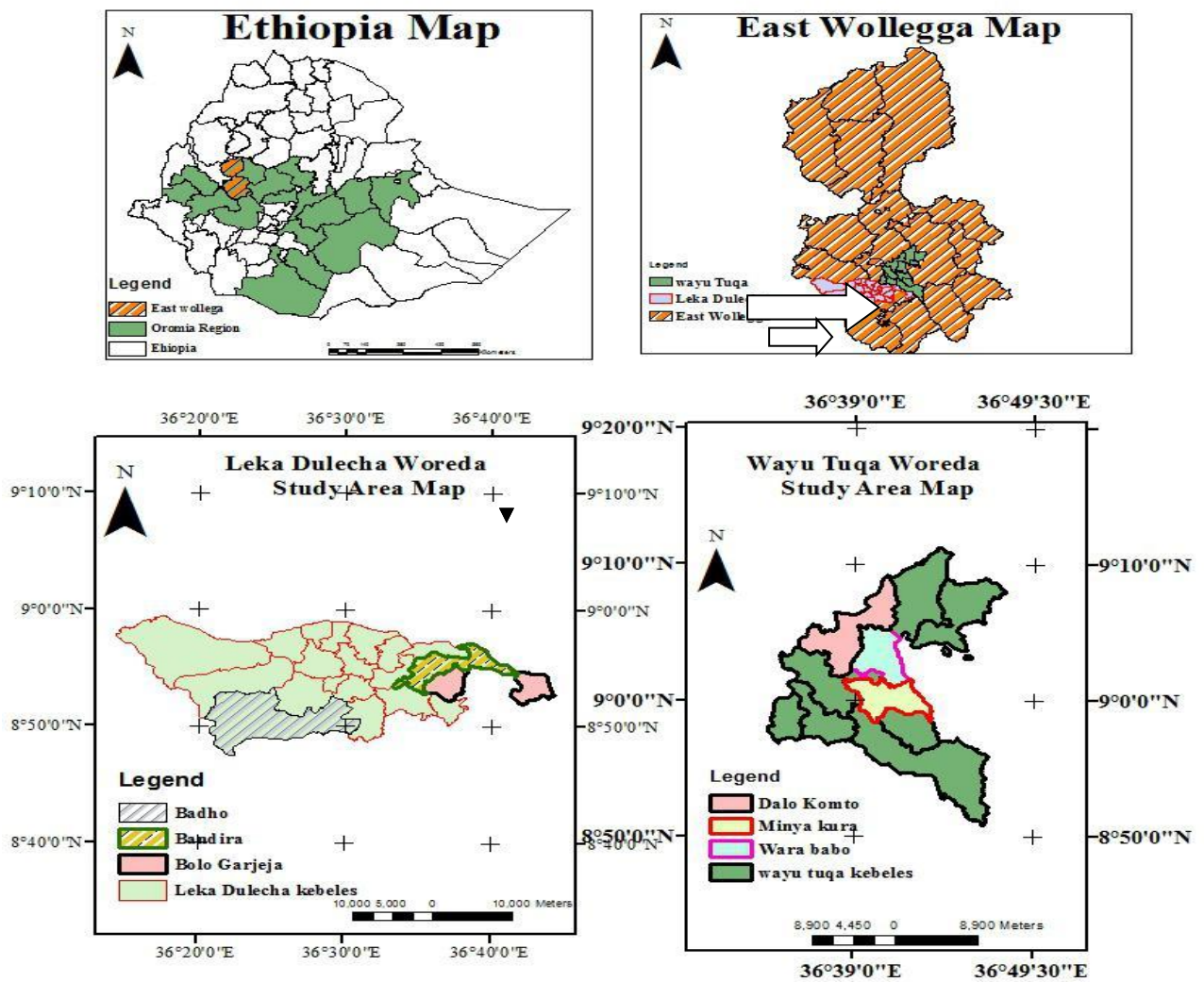
### **2.1 Study Area**

A cross sectional study was conducted from October 2019 to March 2020 in two districts selected from East Wollega Zone of Oromia Regional State; Western

Ethiopia and East Wollega is one of the 18 Administrative Zones of Oromia National Regional State. Administratively, the zone has 17 districts, of which 17 are rural districts which are again subdivided into 286 kebeles (peasant associations). Nekemte Town, which is located at a distance of 331km from Addis Ababa, is the capital of the zone. It is located between 9°5' N latitudes and 36°10' minute East longitudes. It is located in the western part of Oromia Region, bordered with Benishangul Gumuz Regional State in the western; west Wollega Zone in the West, Horo Guduru Wollega Zone in the East, and Buno Bedele Zone in the Southwest, Jimma zone in the south. The land area of the Zone is estimated to be 1315894.65hectar. High land 7.18% wyeina degas 51.08% and kola 41.74%. It experiences tropical climate because of the relatively high angular position of the sun. The mean annual temperature is fairly high. Generally, minimum and maximum annual temperature of the Zone varies from 10-36°C. As reported by East Wollega Zone Finance and Economic Development Office (2011), the annual rainfall pattern in the Zone generally decreases from East to West following the physiographic nature of the Zone (Socio-economic Abstract of zone of East Wollega Zone, 2011).The

minimum and maximum annual rainfall of the Eastern high lands range from 1800-2400mm, while in the central plateaus range between 800- 1800mm and in the remaining parts of the Zone it becomes between 800-1600 mm and becomes less than 1200 mm in the Southwestern parts of the Zone. The farming system East wollega zone are mixing farming system and artificial and natural production system are used. Livestock population of East Wollega Zone is 2282100 Bovines, 533267 Ovine, 35230 caprine, 28403 horse, 158635 donkey, 16466 mule and 2073732 poultry (East Wollega Zonal Livestock development & Health Agency Office CSA, 2011). From the seventeen (17) rural districts of the Zone, the Leka dulecha and Wayu Tuka districts was selected purposively. Leka Dulecha : having an area of 61745 hectare, is located in the western part East Wollega zone at a district of 27km from Nekemte town .It is situated along Nekemte to Jima main road. It shares common boundaries with Nekemte, Leka Dulecha, Jima Arjo and Nunu qumba district, and Buno bedelle zone. Getema town city is its capital which is about 27km far away from Capital city of the Zone

(Nekemte). Altitudinally, it stretches between 1300 and 1400 m.a.s.l. Annual rain fall is 1400ml. Annual temperature 20°C. The district has high livestock potential with 110066 cattle, 27668 Ovine, 15723 Caprine, 10575 Equine, 62070 Poultry. The district is classified into kola (42% and woinadega (43%) dega 15%, agro climatic zones (Socio Economic Profile of Leka dulecha District, 2011). The second study was conducted in Wayu Tuka 'woreda 'in East Wollega Zone, Oromia Region state, western Ethiopia. Wayu Tuka was separated from Guto Wayu districts. It is bounded by Sibule in the north and east, Leka Dulecha in the south, and Guto Gida in west. It is located 324 km from the capital Addis Ababa as well as located 12 km from Nekemte town at an altitude of 1700–2200 m above sea level and has an average annual rainfall of 2400 mm. the temperature range of the woreda minimum 12°C and maximum 32°C. During survey is conducted, Bovine 99416, sheep 31679, goat 16578, mule 1072, horse 7756, donkey 6213 and poultry 137854 (information from (Socio-Economic Profile of Wayu Tuka District, 2011).



**Figure 1:**A map indicating of study area( Arc Map GIS)



## 2.2. Study Design

Cross sectional study design was conducted from October 2019 to March 2020 to determine the seroprevalence and associated risk factors for PPRV occurrence in the study area. To select the study districts and house hold owners by non-probability sampling (purposive sampling) technique was used and random sampling was followed to select PAs and simple random sample technique was used (sheep and goat) were selected to be included in the study area. In selected districts (L/Dulecha and W/Tuka) which selected from East Wollega zones in western Ethiopia, Oromia regional state. Before the beginning of the survey, the local government and owners of animas was consulted on the purpose of the study and permission as well as support should be obtained. Epidemiological data related to risk factors associated with PPR occurrence such as sex and age Body condions species of sheep and goats were collected by using a checklist.

## 2.3. Study Animals

The study animals include small ruminants shoat according to the listed age group that 6month-1years,>1-2years, and >2years (Ozkul *et al.*, 2002;Singh *et al.*, 2004) which kept in extensive management system in L/Dulecha and

W/Tuka EWZ, Oromia Regional State. The districts and house holds were purposively selected based on the accessibility, presence of livestock markets activity, production and management system, history of contact with different animals and ,there were report to the BLR about occurance of disease to check whether PPRV or not.

## 2.4. Sampling and Sample Size Determination

### 2.4.1. Sample size determination

The sampling frame consisted of a list of PAs from associated sheep and goat population and a total of 384 samples were selected from Leka dulecha and 384 from Wayu Tuka districts based on small ruminant populations and PAs size of the district. Since the prevalence of the disease in this area is not determined or not known. Hence, the approximate prevalence of the disease in the area taken as 50% and 5% absolute level of precision was considered to calculate the number of animals to be sampled (Thrusfield, 1995).Therefore, a sample size of 768 was considered for this study. The limits of the associated interval indicate the specified bounds within which the estimate will lie with the defined level of confidence. Generally, based on the consultation with the respective experts of Livestock

Resource, Development and Health Agency Offices of both districts and flock owners whether they introduced new animal (which might vaccinated or not) into the flock, samples were collected from non-vaccinated small ruminants population in 768 sheep and goat distributed in 6 PAs of 2 (Two) districts of the Zone. During sample collection, the estimated age of each sampled animal was determined by consulting the owners of the sheep and goat.

$$n = \frac{(1.96)^2 (P_{exp}) (1 - P_{exp})}{d^2}$$

Where; n=Total number of sample size, Pexp = Expected prevalence, d = Absolute precision (0.05).

#### 2.4.2. Sampling Techniques

To select the study districts and zone non-probability sampling (purposive sampling) technique was used. The two districts (Leka dulecha and Wayutuka) which selected from East Wollega zones. Purposively based on: nearby to the center, easily accessible and densely populated area of small ruminants. as well as there were report to the Bedele Regional Veterinary Laboratory about information of disease to check whether PPRV or not . After made of a brief discussion with the selected districts of livestock and fishery

resource development office and health agencies, peasant associations (PAs) which mean the smallest administrative unit of the districts was purposive selected from the two districts.

The simple random sampling technique was followed, to select individual animals to be used for the study in the study area. To select households (flock) and individual animal two stages sampling methods was employed. The primary sampling unit from each PA, households that having at least one sheep or goat was recruited purposively secondary sampling unit individual animals of households List the name of animals (sheep and goat) selected by simple hover system (by lottery system), animals that owned by the sampled households).

To select households (flock) and individual animal two stages sampling methods was employed. The primary sampling unit household that having at least one sheep or goat in each selected PA and defined as flock. From each PA, households that having at least one sheep or goat was recruited purposively based on households that found nearby to PA level veterinary clinics (type C, type D and Others clinics) and the secondary sampling unit individual animals of households, List the name of animals (sheep and goat). Since between-cluster variance of PPR disease of

the area was unknown a simple random sampling method applied to calculate the number of small ruminant to be included from each flock in study (Tadeg *et al.*,2015). Households (owners) of the study area recognizes each Goat and sheep owned by name, thus, animals was randomly sampled using the name ,making mark on the back of animal and blood samples collections was conducted. On each study household, questionnaire survey of sheep and goat owners whose voluntary have been conducted and the knowledge, attitude and practice (KAP) of small ruminants of owners or respondents“ had towards clinical sign of disease in general and PPR disease in particular was assessed.From each interviewed household, animals “blood samples collections was performed and the overall small ruminants and flock level seroprevalence of PPR was estimated as well as the associated potential risk factors was identified.

### **2.5. Questionnaire Survey**

The semi structured questionnaire was designed to record the risk factors by direct contact with the flock’s owners. The particulars gathered included locality, species, age, breed, sex, and husbandry system, housing, animal movement, sharing pasture and water, and newly introduced animals. Also questionnaire such as name of villages, general status of flocks, mixing

of sheep and goats, separation of different age groups in flock, any health problems observed by owners, and observed clinical sings like lacrimation, nasal discharge, diarrhea, oral ulceration, dullness, fever, abortion, availability of veterinary services, impression about veterinary services, and other observations. A questionnaire survey was based on the formula recommended by Arsham (2002).

Where N=sample size, SE=standard error assuming the standard error of 5% at a precision level of 0.05 and the confidence interval of 95%. Accordingly, 200 volunteer individuals were selected and interviewed considering different age, sex and working conditions.

### **2.6. Blood Sample Collection and Transportation**

The field work was carried out every five days in the selected sites for blood sampling. Sampling was by puncturing the jugular vein of ovine and caprine animals aged at least six months and above were not vaccinated. All collected samples were making the mark on the back of sheep and goat, date of sampling on the list of paper record and sample type. A total of 768 serum samples was collected by jugular vein puncturing from goats and sheep in selected district.The samples were collected in sterilized containers (tube) and were

brought in ice box to make the centrifuge to obtain the serum. The sera was shipped in a cool box with ice packs to and upon arrival they was stored Bedele Regional Laboratory Research until testing. Sera was decanted into cryovials, identified and stored at -20°C until screened for antibodies against natural PPR virus exposure using serological study. At the end, I had used t o the C-ELISA Kit for the detection of anti NP antibodies of the PPR in the serum. It is a universally recognized method as simple, fast and reliable. It has a high sensitivity and high specificity. The biological material (blood) collected in dry sterile tubes of 10 ml (type Venoject ND) was processed on the field and in the laboratory to obtain the serum.

### **2.7. Data Collection**

Animal health and production professionals working in the study areas and district agricultural offices provided relevant information. Data on housing system, whether animal owners recently introduced newly purchased animals into a flock during the last 6 months and flock size was collected. Additionally, data on other factors such as species, age, sex, availability of nearby veterinary clinics in the PA, altitude and grazing management system was collected during sample collection.

### **2.8. Laboratory Procedure**

The microwell plates was read on ELISA reader (Erba Lisa Scan II™, Mannheim, Germany) to give optical density (OD) of each well at a wavelength of 492 nm. The obtained OD values was used for calculating percentage inhibition (PI) values of the control and test sera by using the formula  $PI \text{ of serum} = 100 - (\text{average OD of serum} / \text{OD of control serum}) \times 100$ . The reliability of the assay performance was decided based on the OD values for the monoclonal antibody control (Cm), and PI values for the positive control (C+), negative control (C-) and conjugate control (Cc). All acceptance criteria was according to the manufacturer's instructions, where by the upper and lower limits of OD values for the C+ had to read 1.3 and 0.3 respectively, the PI value for the C+ at least 50%, the PI value for the C- not less than 25% and for the Cc the PI value had to range from 95% to 5%. In addition, the PI values for the Cm had to be at least 50%. The test sera were replicated on the same micro-well plate. For a test serum to be considered as positive, each of the two replicates had to produce a PI value of at least 50%, where as test sera giving mean PI values less than 50% was regarded as negative. The optical density (OD) was read and recorded at 450 nm. For each sample, the competition percentage of per

negative control.(N)(S/N)%; S/N%=OD Sample was calculated OD Negative control

Interpretation of samples using an S/N (%) was as follows;

An S/N less than or equal to 50% are considered positive

An S/N greater than 50% and less than or equal to 60 are considered doubtful.

An S/N greater than 60% are considered negative.

### 2.9. Statistical Analysis

Statistical methods data was entered in to excel and transport to SPSS. Analysis was performed by SPSS version20 statistical software package. Frequency and percentage were calculated for the study variable. Odds ratio and p-value an explanatory variables or independent predictors includes all the risk factors those contributed the disease occurrence and dependent or response variables includes the test result of the study. Cross tabulation was used to analyze the risk factors and its association with exposure to the disease. In all the analyses, confidence levels at 95%

were calculated, and a P<0.05 was used for statistical significance level. The risk of association such as Odd Ratio (OR) was analyzed for the risk factors and sero positivity of the disease to determine the degree of association risk factors and the disease. Descriptive statistics like prevalence was used to calculate sero-positivity by dividing the number of PPR positive animals by the total number of animals tested.

### 3. RESULTS

A total of 768 serum samples were collected from two selected districts, Leka Dulecha and Wayu Tuka districts .Out of which 7.2% (55/768) were found to be seropositive for PPR antibody. In both districts, the distribution of PPR virus was observed with the prevalence ranging from 5.1% to 2.1% and significance difference was seen statistically (p<0.05). Out of total 768 samples, 210 serum samples were from male and 558 serum samples from female with prevalence of 8.5% (18/212) and 6.5% (36/556), respectively.

**Table1:** Seroprevalence of PPRV of Sheep and Goats in study Leka Dulecha and WayuTuka Districts

Districts	Tested sample	Positive sample	Prevalence%	OR(95%CI	X <sup>2</sup> p-value
Le/Dulecha	384	39	10.2%		
Wayutuka	384	16	4.2%	2.6(1.43-4.74)	10.36(.001)

<b>Total</b>	<b>768</b>	<b>55</b>	<b>7.2%</b>
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The total seroprevalence of PAs, Bado 12.5 % (16/128), Bandira 10.2%,(13/128), Bologarjeja 7.8 % ( 10/128) all three PAs listed above were sampled from Leka Dulecha districts and total of sample 384 collected. from 384 sample 39 sample were positive which 10.2% (39/384) and from Wayu Tuka districts Dalokomto 4.5% (7/155), Migna Kura 2.4% (3/128) and Warababo 5.9% (6/101) and total of sample from Wayu Tuka 384 from these positive sample were 16 sample that 4.2% (16/384) and all which were statically significant (P<0.05) from above result. The PPRV were different circulation in both districts and in all six PAs. where sample collected show in (Table 1)

**Table 2:** Seroprevalence of PPRV of Sheep and Goats based on the peasant association PAs

PAs	Tested sample	Positive sample	Prevalence%	X <sup>2</sup> (p-value)
Bado	128	16	12.5%	
Bandira	128	13	10.2%	
Bologarjeja	128	10	7.8%	13.64(.001)
Dalokomto	155	7	4.5%	
MignaKura	128	6	4.7%	
Warababo	101	3	3%	
<b>Total</b>	<b>768</b>	<b>55</b>	<b>7.2%</b>	

The seroprevalence in both sexes were 3 % in males and 9.84% in females were tested positive which show in (Table3) in this case when I compere both female of Sheep and Goat the seroprevalence within the male of sheep and goat both female were more affected than male sheep and goat.

**Table 3:** seroprevalence of sheep and goat respect to sex

Sex	Animal tested	Positive animal	Prevalence	OR(95%CI	X <sup>2</sup> (p-Value)
Male	210	23	11%	.21 (1.16-3.544)	
Female	558	32	5.7%		6.25(.001)
<b>Total</b>	<b>768</b>	<b>55</b>	<b>7.2%</b>		

Regarding species the seroprevalence in Sheep was 2.1% and in Goat 5.1% was tested positive respectively. In general, depend up on species goat are more affected than sheep (**Table 3**).

**Table 4:** Seroprevalence of PPRV based on the species

Species	Tested sample	Positive sample	Prevalence%	OR(95%CI)	X <sup>2</sup> (p-value)
Sheep	443	16	3.6%	2.6(1.427-4.74)	
Goat	325	39	12%		19.84(.001)
<b>Total</b>	<b>768</b>	<b>55</b>	<b>7.2%</b>		

In different age groups the seroprevalence in animals from 6month-1years 8.5% (18/212) ,>1-2years 7.8% (36/462), >2years 1.1% (1/94) shows the result respectively. In the present study also over all sero prevalence were results showed that among the host related potential risk factors that considered age, sex, and body condition assessed with PPRV sero status of the animal show the risk factors significantly explain (p <0.05) the

occurrence of PPRV. As the result of the study described sero positivity was higher in >1-2year (4.7%) than in 6month-1year (2.3 %) and >2year was 0.2% with the statically Significant, (P<0.05) Where as highest in poor body condition (2.3 %) than in medium (2.2 %) and good body condition (1.8%).The overall body condition are statically significant with,(p<0.05) show in (**Table 4**)

**Table 5:** Risk factors of PPRV analysis by cross tabulation respect to sheep and goat

Category	Animal tested	+Ve animals	Prevalence	OR(95%CI)	P-Value
Districts	768	55	7.2%	2.66(1.44-4.89)	.002
Species	768	55	7.2%	.271(.148-.498)	.000
Sex	768	55	7.2%	1.814(1.019-3.299)	.043
PAs	768	55	7.2%	1.362(1.144-1.64)	.001

Age	768	55	7.2%	1.67(1.0123-2.73)	.020
Body Condition	768	55	7.2%	2.76(1.714-4.454)	.000

### 3.1 Questionnaire survey outcomes:

The questionnaire survey was done in six PAs of localities of both Districts (Leka Dulecha and Wayu Tuka), localities based on the willingness of owners to respond. The questionnaire was distributed to in the different localities to come up with information about the situation of PPR in the study area and the extent of the farmer's knowledge and Socio-demographic characteristics and Attitude of the respondents on PPRV disease District; L/Dulecha (50%) and W/Tuka (50%) Gender of respondent: male (68%), female

(32%), Maritalstatus: single (74%), married (20%), Widowed (3%) and Divorced (2%), Educational Background: Primary (50%), Secondary (20%).College or university (6%), Vocational school (3%) and No formal education (21%) Flock structure: sheep (58.5%) and goat (41.5%), 35.5% male and (63.5%), female respectively. respect to the small ruminant age 0.6-1years (26%),>1-2years (57.5%) >2years (36.5%) and (Agro-ecology: lowland (32%) midland,(17.5%) and Highland (51%).the result show in the (Table 5)

**Table 6:** summarized the questionnaire survey responses among the small ruminants' owners.

Parameter		Frequency	Proportion (%)
District	Leka Dulecha	100	50%
	Wayu Tuka	100	50%
Gender of respondent	Male	136	68%
	Female	64	32%
Marital status	Single	40	20%
	Married	150	75%
	Widowed	6	3%
	Divorced	4	2%
Educational Background	Primary	60	50%
	Secondary	42	20%
	College or university	10	6%



	Vocational school	24	3%
	No formal education	64	21%
Flock structure	Sheep	1823	58.5%
	Goat	1285	41.5%
Sex	Male	1125	36.5%
	Female	1983	63.5%
	Total	3108	100%
Age	( 0.6-1 years)	810	26%
	(>1-2 years)	1789	57.5%
	(>2year)	509	16.5%
Agro-ecology	Lowland	973	32%
	Midland	550	17.5%
	Highland	1585	51%

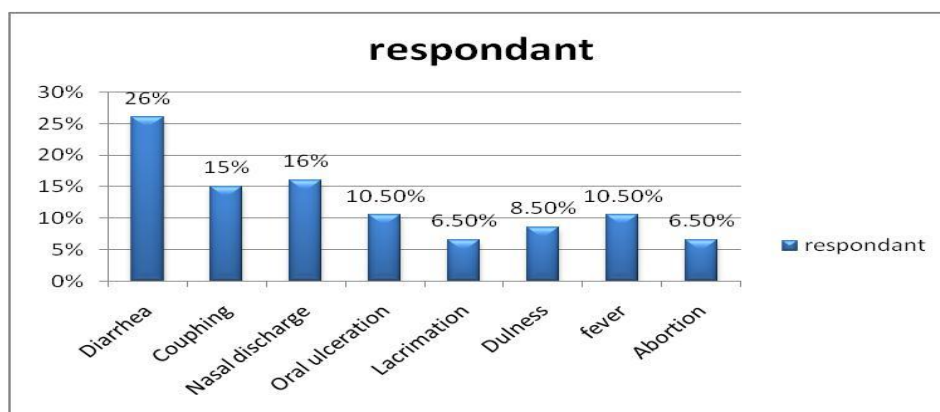
Sero prevalence and its associated Risk Factors of PPRV disease occurrence in the study area and questionnaire surveys were carried out simultaneously and it has been tried to identify risk factors for PPRV occurrence.

The sheep and goat owners were asked about the condition of their animals. A total of 200 sheep and goat owners who provided their animals for sampling were interviewed. Out of the respondents, Diarrhea 26%, nasal discharge 16.5% coughing, 15%, oral ulceration 10.5% and lacrimation, 6.5%, dullness 8.5%, fever 10.5% and abortion 6.5%.replied that their small ruminants were affected by the PPRV disease (**Fig:5**). From the

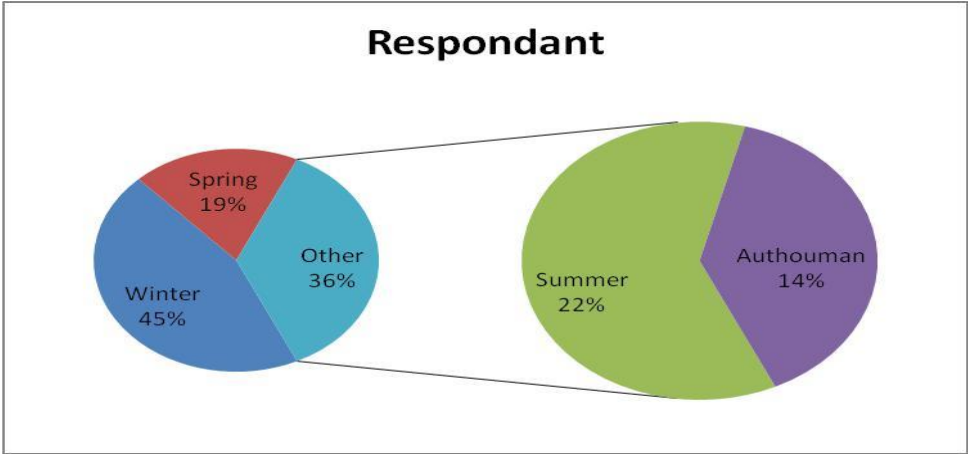
respondents of owners started from clinical sign of enteritis stomatitis of PPRV 39%, 43.5% and 18% of respondents had understands the disease, no understand the clinical sign of disease and do not know about the disease respectively and the seasons occurrence of disease were compared by farmer about 60%,50%, 40%,10% of respondents informed that Winter, summer, Authouman and Spring was the season at which the disease occurred in the area respectively (**Fig 6**).According to the finding of the questionnaire survey, when animals become sick, to prevent the PPRV measure taken by the owners was traditional treatment (5%), modern treatments (75%) and vaccination

(20%) their sheep and goat (Fig 7). Among the respondents, there was a practice to keep sheep and goats owners replied that their small ruminants were, Sheep and goat grazing separately (39%), Sheep and goat grazing together (60%) Sheep and goat grazing with other livestock (30%) Sheep and goat tethered feeding at home (40%) (Fig 8) and From the two hundred respondents they know name of PPRV by local language. 30%, 60% no local language and 10% do not know about the PPRV by local language. Respondents seen out break since >1year,3year and >year 40%,10% and 50% and watering managements was communal (60%),private (22.5%) and both private and communal (17.5%) and housing managements was

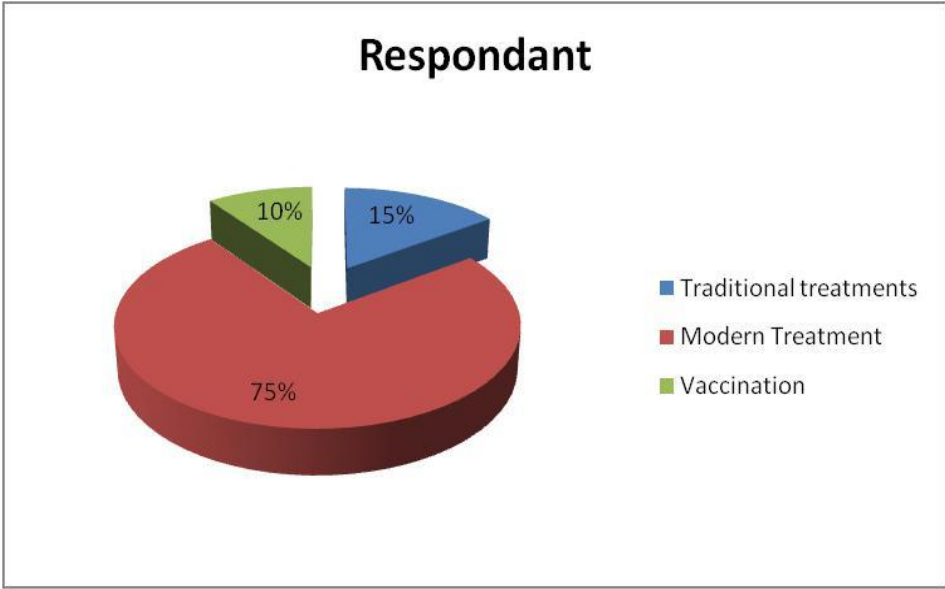
sedentary and house barn 100% as well as common season of kidding and lambing June (30%),October (20%),February (10%),march (50%) and may (40%) and seasonal where critical feed shortage where April (10%),May (50%),march (10%) and may (50%).and all of above information gather from respondents of sheep and owners.Respected on the Knowledge of PPRV from respondents, Most of respondent know the information of PPRV .Among these respondents. Male 35.5%, Female 64.5% and single 25%, married 65%.College or University 16%, primary school 50%, secondary school 20% vocational school 3%,no formal education 31%.



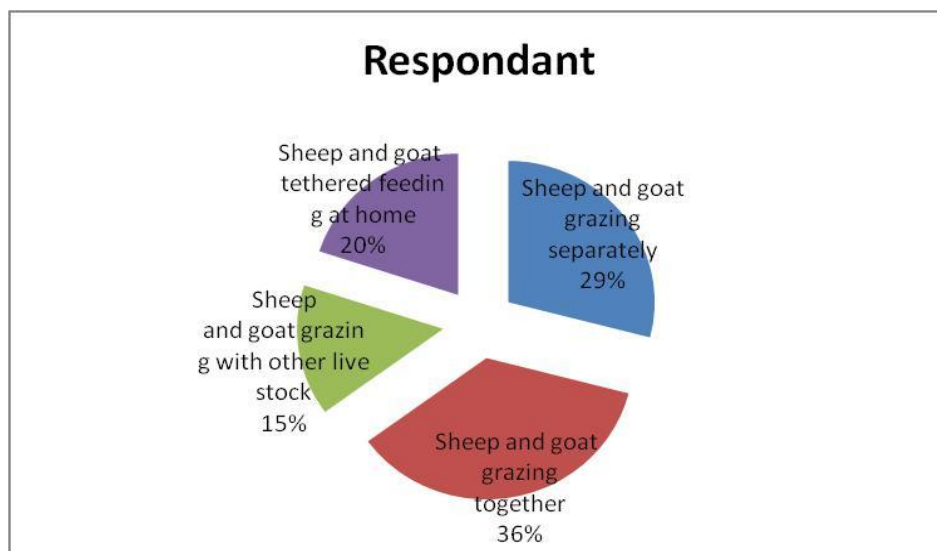
**Figure 2:** Knowledge of Suspected clinical symptoms of PPR as reported by respondents (N=200)



**Figure 3:** The attitude of farmer on the Seasonal occurrence of PPRV disease reported.



**Figure 4:** Over all proportion of the measure taken to prevent the PPRV health problems respond from owners



**Figure 5:** Level of the raising type of sheep and goat from the study area among the farmer practice.

#### 4. DISCUSSION

The cross sectional study conducted on sheep and goat population of the present study revealed an overall seroprevalence of 7.2% (55/768), indicating the spread of PPR virus throughout the study areas. This result indicated that the PPR virus is circulating in the study areas and needs particular attention since it is one of the most economically important disease affecting both production and productivity of small ruminant. The overall seroprevalence of 7.2% observed in the current study which agrees with the seroprevalence of 6.8% by Abraham *et al.* (2005) and 6.4% by Waret-Szkuta *et*

*al.*,(2008).as well as agrees result found in Ghana from 2005 to 2013 was 6.84% reported by Kaukarbayevich.,*et al.*,(2009) and Abubakar *et.al* (2011) who concluded that control of PPRV may be attained using measures including movement control, quarantine infected animals, removal of potentialyl infected fomites and restriction on the importation of sheep and goats from infected areas. However, it is lower than the report of Kifle, and Tsegaw,2012 in Metema district of Amhara region (26.3%), (Megersa *et al.*,2011).in Gambella region (27.3%),(Biruk *et al.*,2014).in Amhara region (28.1%) and (Wondemagegn *et al.*,2016) in Somali region (41%) In

contrast to this result, higher overall seroprevalence was recorded in other African and Asian countries like 43.3% in Pakistan (khan *et al.*, 2007) 45.8% in Tanzania (Swai *et al.*,2009) 45.0% in Republic of Niger (Farougou *et al.*,2013) 54.9% in Nigeria (EL-Yoguda *et al.*, 2013) 67.9% in India ( Saritha and Sreedevi 2014) 45.6% in Sudan ( Waret-Szkuta *et al.*,2008) 23.2% in Nigeria (Woma *et al.*,2013) and 38.2% in Saudi Arabia (Abdellatif *et al.*, 2016).

In present study statistically significant variation was observed between the two study districts that the high seroprevalence of 5.1% and relatively lower 2.1% was registered in Leka Dulecha and Wayu Tuka districts respectively. This could possibly be explained as same in geographical location, various forms of stress factors as predisposing factors, management and/or infectious factors and bordering of Diga district and Nekemte town with areas which have high movement of animals.

With respect to the species seroprevalence of PPR in goat show that it was higher (12%) as compared to sheep (3.6%), which statically significance ( $p < 0.05$ ) Show in the (Table 4 and 5) was agreement with another study done by (Gelagay, 1996, Ozkul *et al.*,2002, Al-

Majali *et al.*, 2008, Waret Szkuta *et al.*, 2008) these difference are of highly movement animals during shortage of grazing, outbreak of disease, trade, tourism and migration of wild animals. reported a higher seroprevalence in goats than in sheep linked to higher fecundity in goats compared to sheep. The difference in prevalence could be due to the difference in the proportion of sampled animals and Besides, since goats were used for meat and selling compared with sheep that were less considered for economical purpose, in return pastorals were intense to keep more goats than sheep. The pathophysiology of species-wise difference in seroprevalence remains unclear. Higher recovery rate (lower case fatality rate) and/or a greater longevity of sheep compared with goats have been observed (Abdellatif *et al.*, 2016).

In other studies, goats showed greater susceptibility to infection with PPRV while recovery rate of goats to infection is considerably less than that of sheep (Couacy-Hymann,*et al.*,2015).However, this finding was. disagreed with the report of Abraham *et al.*, 2005 and Mehmood *et al.*, 2009 had reported a higher PPR prevalence in sheep than goats. In a PPR outbreak in Turkey of PPR, goats remained unaffected (Yesilbag, *et al.*, 2005).Breed susceptibility differences in sheep and

goats in different parts of infected areas were reported (Salih, *et al.*, 2014.).In one experimental study conducted by ( Diop *et al.*, 2005) different breeds of goats have been shown to respond differently to infection with the same virus strain. Sex has also been reported as a risk factor for susceptibility/resistance to the disease. Since the off-take of males, in a farm, is higher and at an early age compared to females, which end up staying in the herds for longer periods females are more likely to demonstrate higher antibody titers than the males (Singh *et al.*,2004).

In the present study also revealed the higher prevalence of 4.2% (32/768) in female shoat than male 3% (23/768) which statically significance was ( $p<0.05$ ),OR=2.022 which 95%CI (1.153-3.544) which agreed with previous report of (EL-Yoguda *et al.*,2013,Afera *et al.*,2014, Nizamani *et al.*,2015, Bello *et al.*, 2016). In agreed to this finding, sex with sero prevalence of PPR also reported higher in female than male (Thakor *et al.*, 2016).The higher prevalence in female than male in current study may be due to physiological difference where female reveal some degree of predominance infection as a result of production and reproduction stress which female more prone to infection and that females are

subject to more stressing factors like pregnancy and lactation; in addition, the productive life span of females is longer than that of males. The proportionally higher number of females in herds in comparison to males could be another explanation, why, statistically, females were found to be of increased risk of attaining a seropositive status in this study.(Megersa *et al.*, 2011).

The age wise sero prevalence was (2.4%),(4.9%) and (0.2%) in from 6month-1year, > 1-2 year and greater than 2 year of age group respectively. which are statically signifiacne ( $p<0.05$ ) This finding was agreed with the report of (EL- Yoguda *et al.*,2013 and Rahman *et al.*,2017) have been reported higher sero prevalence in adult than young shoats and these results are consistent with those by Sow *et al.*,(2008),who noted a prevalence of 33.4% in adults compared with 28.0% in young animals.

However, Tounkara *et al.*,(1996) further noted that the PPR seroprevalence was higher in older small ruminants because in an enzootic area they are more prone to exposure to the virus. This finding is not supported by Singh *et al.*,(2004b) who reported different observations. Age appears to be a risk factor for sero-positive

status and its linear effect suggests that PPRV is highly immunogenic, naturally infected animals remaining positive for a long time (Roger *et al.*, 2008). The young, having been in the flock for a shorter period, are less likely to have been in contact with virus. However this finding was disagreed with the report of (Mahajam *et al.*, 2013 and Afera *et al.*, 2014) which said that young sheep and goat more affected than adults. which said that young sheep and goat more affected than adults.

My data suggested that animals younger than 6month-1 year and older than 2 years had lower chances of being sero-positive to PPR. These findings are in agreement with previous reports by (Abubakar *et al.*, 2009; Ozkul *et al.*, 2002; Singh *et al.*, 2004) who found that animals >1years-2years were more susceptible to PPRV. It has been documented that sheep and goats exposed to PPRV at a very young age may carry antibodies for 1-2 year following exposure (Dhar *et al.*, 2002; Ozkul *et al.*, 2002;)

In the study area, the demographics showed that the majority of the animals tested were in the age bracket of 2 years. It is most probable that my findings are related to the practice of selling older animals and leaving the adult ones as replacement for

production. The risk factor of sheep and goat are age risk factor, species risk factor, sex risk factors, PAs risk factors, Districts risk factors are statically significant which are  $p < 0.05$  during sero prevalence of questionnaire survey out come.

## 5. CONCLUSION AND RECOMMENDATIONS

The findings of this study confirmed that the circulation of PPR virus among populations of small ruminants (sheep and goats) in the study areas and prevalence in actual outbreaks situation, which should be kept in mind while deciding the vaccination strategy for the control of the disease. The overall sero-prevalence of PPR in shoats in the selected districts of Leka Dulecha and Wayu Tuka was 7.2% while the PAs level prevalence was Bado (2.1%), Bandira (1.7%), Bologarjeja (1.3%), Da lokomto (0.9%), MignaKura (0.8%) and Warababo (0.4%) respectively. From this seroprevalance at each of PAs at least above three positive animal was considered a positive shoat for PPR. The shows the transmissibility of the virus within flocks is slowly circulation when compared between the PAs. The fact that antibodies of PPR virus were detected in the some peasant associations and districts suggests the endemicity of the disease in the studied districts. Because of the economic impact, morbidity and mortality

increments, attention was given towards the disease regionally as well as nationally through time. Disease must be reporting needs awareness, harmonization, and network of all partners (region, district and field professionals) to mitigate the potential risk factors.

Therefore, based on the above conclusion the following recommendations are forwarded:

- It is necessary to plan out strategic vaccination not only in the studied district but also in the regions with a history of recurrent disease outbreaks in order to prevent the circulation of the virus.
- It needs harmonization in the control and eradication of the disease between the study districts, Regions and the neighboring countries specially Sudan where there is active movement of livestock across the border.
- In addition, strict sero prevalence and monitoring of PPR is recommended, together with uninterrupted vaccination of migratory flocks at the borders between districts or provinces or regions, for effective control of the disease.
- Further research should be undertaken on the development of differentiating infection from vaccinated animal's vaccine which is the most important measure for prevention and also identify the gene

sequences and lineage of the PPR virus isolated in this study area.

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Date of collection \_\_\_\_\_

Code No \_\_\_\_\_

## 7.APPENDICES

### APPENDICES 1: Questionnaire survey format.

I study Location and Interviewee detail

1.Regional \_\_\_\_\_ Zoone \_\_\_\_\_ districts \_\_\_\_\_ Kebele \_\_\_\_\_ spe cific  
name of place \_\_\_\_\_

Total No of shoat in District \_\_\_\_\_ Sheep \_\_\_\_\_ goat \_\_\_\_\_

Total \_\_\_\_\_

Exotic sheep \_\_\_\_\_ goat \_\_\_\_\_

Total No of shoat in kebele \_\_\_\_\_ Sheep \_\_\_\_\_ goat \_\_\_\_\_

Total \_\_\_\_\_

Exotic sheep \_\_\_\_\_ goat \_\_\_\_\_

#### Section 1: Socio-demographic Characteristics of Respondents

1. Name of respondent: \_\_\_\_\_

1.1. Gender of the respondent: 1. Male 2. Female

1.2. Age (years): \_\_\_\_\_

1.3. Marital status: 1. Single 2. Married 3. Widowed 4. Divorced

1.4. Respondent's educational background:

1. Primary school 2. Secondary school 3. Vocational school

4. College / University 5. No formal Education

1.5. Respondent's position in the household (with respect to the head):

a. Husband b. Wife c. Daughter d. Son e. Relative living in a house f. Farm

laborer

1.6. Household size: a. 1-3 b. 4-6 c. Greater than



Cattle	Equine		Mule	Sheep	Goat	Others	Total	
	Donkey	Horse						

## II-The History of PPR occurrences

1. List important health problems and symptoms that cause Sheep and goat mortality in your area?

/It could be in local language/

2. Have you had enteritis-stomatitis syndrome in shoats of your flock? Yes \_\_\_\_\_ No \_\_\_

3. Have you had PPR (Local name??) in your shoats? Yes \_\_\_\_No\_\_\_\_\_

4. When did the disease commence in the area (Kebele)? Season\_\_\_\_ Mon \_\_year \_\_\_\_

5. Have you seen such outbreak in the area before this time, < 1yr\_\_

3Yrs\_\_\_\_>3Years\_\_\_\_\_

6.How frequent PPR reoccurs in the area? Don't Know\_\_\_Every 1yr\_\_\_Every 2yrs

>3yrs\_\_\_

7. What measures are taken to prevent the above listed health problems?

Traditional treatment [ ] Modern treatment [ ] Vaccination [ ] No treatment [ ] Other

8. What problems do you face when treating or vaccinating sheep in your area (rank them)?

Lack of modern services/clinics [ ] Lack of drugs and vaccines [ ] Transport/distance [ ]

Other\_\_\_\_\_

9. How many animals had got sick and died due to PPR among the flock?

1 one            2 Two            3 five            4 three            5 seven

10 Agro-ecology Lowland Midland Highland

## III. Flock Management

1. Do you move your shoats to other place for grazing seasonally? Yes /No

2. Grazing and watering resource managements

Grazing/watering mgt Communal\_\_\_\_\_Private\_\_\_\_\_Both\_\_\_\_\_

Farming system Sedentary\_\_\_\_\_Pastoral\_\_\_\_\_Transhumant\_\_\_\_\_

3. How do you raise your sheep and goat?

Sheep and goat grazing separately [ ] Sheep and goat grazing together [ ]

Sheep and goat grazing with other livestock [ ] Sheep and goat tethered feeding at home [ ] other

4. Housing: Fenced stable; House barn

5. Have you bought new shoats or introduced new shoats since 3 months before the onset of the

Outbreak? Yes/No

6. Name and distance (in km) of livestock market frequently used and the known shoats trade route around their area\_\_\_\_\_.

7. Did you vaccinate your shoats for PPR? Yes\_\_\_\_\_No\_\_\_\_\_

8. What is the common lambing/kidding season in which most of the animals born?

June – September [ ] October – January [ ] February – May [ ]

9. Did you encounter any critical season of feed shortage? Yes [ ] No [ ] If yes in which season

## **APPENDICES 2: C- ELISA principles.**

### **Materials required**

Precision micropipettes or multi- dispensing micropipettes Dispensable pipette tips.

Graduated cylinder for wash solution. 96-well micro plate reader (equipped with 450 nm filter)

Micro plate washer (manual, semi- automatic system) Use only distilled or

deionizer water for preparation of the reagents used in the test Micro plate covers

(lid, aluminum foil or adhesive) Centrifuge (200xg) Vortex or equivalent

Microplate shaker

Agitator-Incubator capable of maintaining temperature of + 37<sup>0</sup>C (+3<sup>0</sup>c).

Uncoated micro plate for sample preparation

### **Wash Solution**

The wash concentrate (20x) must be diluted 1:20 with distilled/demonized water before use (e.g. 15 ml of wash concentrate (20x) in 285 ml of distilled water).

This solution is here after called “wash solution”

**Note:** the wash concentrate (20x) should be brought to 18-26<sup>0</sup>c ] and well mixed to ensure dissolution of any precipitated salts. Wash solution is stable for up to 10-15 days when stored at 2-8<sup>0</sup>c.

### **Conjugate**

The conjugate concentrate (100x) must be diluted in dilution buffer N. 24

**Note:** - diluted conjugate solution is stable for up to 8 hours at 18-26<sup>0</sup>c

## Preparation of Samples

Samples and controls are pre-diluted on the prelate (uncoated) (see test procedure).

**Note:**-samples should not be de-complemented prior to the analysis.

## Kit components

Reagents
Microplate coated with PPR recombinant nucleoprotein
Anti NP-HRP concentrated conjugate(10x)
Positive control
Negative control
Dilution Buffer 13
Dilution Buffer 4
Wash concentrate(20x)
Substrate solution

Stop solution(0.5m)

## APPENDICES 3:Testing procedure.

Allow all the Reagents to come to room temperature ( $21^{\circ} \text{c} \pm 5^{\circ} \text{c}$ ) before use.

homogenize all Reagents by inversion or vortex

Add:

25 $\mu$ l of **Dilution Buffer13** to each well.

25 $\mu$ l of the **positive control** to wells **A1** and **B1**.

25 $\mu$ l of the **negative control** to wells **C1** and **D1**

25 $\mu$ l of each sample to be tested to the remaining wells.

2 incubate **45min  $\pm$ 4min** at  $37^{\circ} \text{c}$  ( $\pm 3^{\circ} \text{c}$ ).

3 wash each wells 3times with approximately300µl of the **wash solution**.avoid drying of the wells between washings.

4. Prepare the **cojugate1x** by diluting the **conjugate 10x** to 1/10 in **dilution Buffer4**.

5 Add 100 µl of the **conjugate 1x** to each well.

6 incubate **30min ±3min** at 21°c (±5°c).

7 wash each wells 3times with approximately300µl of the **wash solution**.avoid drying of the wells between washings.

8 Add 100 µl of the **substrate solution** to each well.

9. Incubate **15min ±2min at 21°c** (±5°c) in the dark

10 Add 100 µl of the **stop solution** to each well in order stop the reaction

11 Read and record the O.D.at 450nm.

### **Interpretation:**

Negative

Positive

S PI < 50 %

S PI 50 %

**Note:** for this test, the positivity threshold is set at 50% of inhibition.

However, every measurement has a certain uncertainty which depends from the kit itself and of the

capabilities of the testing laboratory. Sera with PI values within the range 50 % ± uncertainty of measurement

should be consider with care and distinguished from the others that are positive or negative with certainty. It is advisable to perform this ELISA testing under quality assurance and, whenever possible, with an accreditation (i.e. ISO 17025).

**Note:** IDEXX has instrument and software systems available which calculate results and provide data summaries.

**APPENDICES 4:** letter written by Bedele Veterinary Regional Laboratory after result process.



