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EPIDEMIOLOGY OF PESTE DES PETITS RUMINANTS, ISOLATION AND MOLECULAR DETECTION OF THE VIRUS IN SELECTED DISTRITS OF AWI AND METEKEL ZONES, NORTH WEST ETHIOPIA

BY

YALEW ABIYU SENBETO

JUNE, 2022

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

DEPARTMENT OF CLNICAL STUDIES, MVSc IN VETERINARY EPIDEMIOLOGY

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: **Yalew Abiyu Senbeto** entitled '**Epidemiology of Peste Des Petits Ruminants, Isolation and Molecular Detection of the Virus in Selected Districts of Awi and Metekel Zones, North West Ethiopia**' and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary Science in Veterinary Epidemiology.

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I affirm that this research is solely my own work, and that all sources of materials used in this thesis work have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for Master of Veterinary Science in Veterinary Epidemiology degree at Addis Ababa University's College of Veterinary Medicine and Agriculture, and it has been deposited in the College library to be made available to borrowers in accordance with the Library's rules. I solemnly declare that I am not submitting my thesis to any other institution anywhere for the award of any academic reward.

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LIST OF ABBREVATIONS

AGID	Agar Gel Immune Diffusion
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AHI	Animal Health Institute
AU-PANVAC	African Union Pan African Veterinary Vaccine Center
b-ELISA	Blocking Enzyme Linked Immunosorent Assay
BLAST	Basic Local Alignment Search Tool
CCPP	Contagious Caprine Pleuropneumonia
cDNA	Complementary Deoxyribonucleic Acid
CFSPH	Center for Food security and Public Health
CIEP	Counter Immune Electrophoresis
CIEP	Counter Immuno Electrophoresis
CPE	Cytopathic Effect
CSA	Central Statistical Agency
Ct	Cycle threshold
DIVA	Differentiation of Infected from Vaccinated Animals
DMEM	Dulbeccos`s Minimum Essential Medium
dNTPs	Deoxyribonucleoside triphosphates
ESGPIP	Ethiopia Sheep and Goat Productivity Improvement Program
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
FMD	Foot and Mouth Disease
HA	Haemagglutination
HIT	Haemagglutination Inhibition Test
HRP	Horse Radish Peroxidase
Ic-ELISA	Immunocapture Enyme Linked Immunosorent Assay
IHC	Immunohistochemistry
LAMP	Loop-mediated isothermal Amplification
LFD	Lateral Flow Device
MAbs	Monoclonal Antibodies
m.a.s.l	meter above sea level
NVI	National Veterinary Institute

OD	Optical Density
OD_{NC}	Optical Density of Negative Control
OD_{PC}	Optical Density of Positive Control
OIE	World Organization for Animal Health
PBS	Phosphate Buffered Saline
PAs	Peasant Associations
PI	Percentage of Inhibition
PIT	Precipitinogen Inhibition Test
PPR	Peste Des Petits Ruminants
PPRV	Peste Des Petits Ruminants Virus
RdRp	RNA-dependent RNA- polymerase
RPV	Rinderpest Virus
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SLAM	Signaling Lymphocytic Activation Molecules
SOP	Standard Operating Procedures
$TCID_{50}$	Fifty Percent Tissue Culture Infective Dose
TMB	Tetramethylbenidine
VDS	Vero Dog SLAM cells
VN	Virus Neutralization
VNT	Virus Neutralization Test
VTM	Virus Transport Medium

ABSTRACT

Ethiopia possesses huge population of sheep and goats despite the country benefited little from it due to different factors including Peste des Petits Ruminants (PPR), highly contagious and economically important trans-boundary disease of small ruminants associated with high morbidity and mortality. Cross-sectional study involving questionnaire survey, retrospective outbreak data, seroprevalence, isolation and molecular data analysis were conducted from February 2020 to May 2021 in selected districts of Awi and Metekel zones with the objective of revealing epidemiological status, isolation and molecular detection of PPRV circulating in the area using c-ELISA and real time PCR. Of the total 714 sera samples tested, 467 (65.4%) were found positive for PPRV antibody. Significantly higher prevalence of 70.7% (236/334, P=0.007, CI=65.4-75.4) in Metekel than Awi zone (60.8%) was observed. Seroprevalence of 66.8% (243/364) in sheep and 64% (224/350) in goats with no statistical significant difference (P> 0.05) was also revealed. In this study, significantly higher seroprevalence was recorded in old animals (75.2%, OR=3.5) than adult (72.7%, OR=2.8) compared to young (52.1%) (CI=2.3-6.2, P=0.000). A prevalence of 64.9% and 54.4% was observed in female and male animals respectively (P=0.000, $\chi 2$=13.18). From 42 samples examined with the PCR, 38.1%; 15 (5.7%) goat and 1 (2.4%) sheep were detected positive. The virus was also successfully isolated from VDS cell cultured samples. The questionnaire survey revealed that 89.7% (n=78) of farmers know the disease though most of them are not familiar with prevention and control measures. About 64.1% of them also indicated that PPR outbreak occurs each year. The retrospective data (2016-2021) showed a total of 632 PPR outbreaks with high morbidity and mortality rate in Benishangul Gumuz the outbreaks being common in dry season. As evidenced from the questionnaire survey, retrospective, serology and molecular detection in addition to the field observation result of this study, PPR is found to be endemic in the study area. The higher PPRV circulation, the usual free animal movement and communal grazing system in the area indicates possibility of further spread within and to other areas. Therefore, it should have get priority attention from control and eradication campaign of the country. Creating awareness to farmers, restricting animal movement and targeted vaccination is necessary to prevent spread of the disease. Further study on sequencing and characterizing the circulating virus to a lineage level is also recommended.

Keywords: *Epidemiology, Isolation, Pest des petits ruminants, molecular detection, small ruminant, Northwest Ethiopia*

1. INTRODUCTION

Livestock systems in developing countries are characterized by rapid change resulting from influencing factors such as population growth, increases in the demand for livestock products and urbanization. It is the fast growing sub-sector of agriculture contributing about thirty percent of agricultural gross domestic product in developing countries (Yasin *et al*., 2017). Sheep and goats are widely distributed animals that play a major role in rural economies. They are found being managed under different production systems including feral, trans-human, extensive, and intensive systems. In the arid and semi-arid areas, small ruminants are the principal source of income and play a major role in livelihood of the farmers especially during drought and famine periods (Swai *et al*., 2009). Due to their low cost of production, high prolificacy, adaptive capacity to harsh environments through dynamic feeding behavior and fast growth rate, small ruminants are considered an important assets of poor farmer exploited for different purposes especially in developing countries (Hailegebreal, 2018).

Ethiopia possess the largest livestock population in Africa, with an estimated population of 2.16 million horses, 8.44 million donkeys, 0.41 million mules, 1.21 million camels, 59.5 million cattle, 56.53 million chickens, 30.7 million sheep, 30.2 million goats and 5.92 million bee hives (CSA, 2017). The livestock system currently contributing to the livelihoods of estimated 80% of the rural populations (Husen *et al*., 2018). Thus, the country has large population of small ruminant animals. However, Ethiopia has benefited far below expectations from this enormous resource due to different factors including diseases. Peste des Petits Ruminants (PPR) is one of the most important diseases that affect production and productivity of small ruminants in a wide range of agro-climatic zones (Swai *et al*., 2009; Afera *et al*., 2014).

Peste des Petits Ruminants is highly contagious, acute economically important notifiable trans-boundary viral disease of sheep and goats associated with high morbidity and mortality. It is caused by *Peste des petits ruminants virus* (PPRV) of genus genus *Morbillivirus* (Balamurugan *et al*., 2014). The virus has a single serotype with four distinct lineages (I-IV) grouped based on the virus nucleoprotein and fusion gene C-terminus sequence comparison (Clarke *et al*., 2018).

Clinically, PPR is characterized by signs such as pyrexia, conjunctivitis, ocular and nasal discharges, necrotizing and erosive stomatitis, diarrhea and bronchopneumonia followed by death or recovery from the disease. The disease can be tentatively diagnosed by observing the characteristic symptoms and postmortem lesions. However, it is confirmed by cultural isolation and various serological and molecular techniques (Balamurugan *et al*., 2014).

Peste des Petitis Ruminants disease is transmitted by direct contact with new infected animals introduced to the herd. Asymptomatically infected animals can shed the virus for up to 12 weeks or longer in recovered animals. Thus, quarantine and testing before introduction of new animals is very important to decrease the risk of infection with the disease (CFSPH, 2008). The disease is the most economically important disease of sheep and goats (Ishag *et al*., 2015).

It causes direct economic losses as a consequence of reduced weight gains, impairing growth, lowered milk and meat production, mortality, high veterinary costs and trade ban (Swai *et al*., 2009). Furthermore, the disease is characterized by high morbidity and mortality rates. Morbidity of 50%-100% and mortality ranging from 20% to100% has been reported (Ishag *et al*., 2015). Peste des Petits Ruminants can be controlled and even eradicated with a combination of quarantines, movement control, and euthanasia of infected and exposed animals, vaccination of high risk population, and cleaning and disinfection of infected premises (CFSPH, 2008).

In Ethiopia, PPR was clinically suspected to be present in 1977 in goat herds of Afar region. However, the presence of the disease was confirmed late in 1991 with cDNA probe from lymph node and spleen samples collected from an outbreak that occurred in a holding land near Addis Ababa. The disease in this outbreak was caused more than 60% death. Serological survey carried out in 1997 at Bishoftu abattoirs reported high PPR sero-prevalence. Since the first confirmed cases of PPR in Ethiopia, the disease is continuously affecting small ruminant production. It aggravate food insecurity posing remarkable economic impact on production and export of the animals particularly in vulnerable regions of the country (Abraham, 2005; Delil, 2007).

Several sero-prevalence studies (Waret-szkuta *et al*., 2008; Delil *et al*., 2012; Afera, 2014; Dejene, 2016; Bello, 2017; Gebre *et al*., 2018; Hailegereal, 2018; Agga *et al*., 2019; Yalew *et al*., 2019; Gelana *et al*., 2020) and few molecular detection studies (Abraham, 2005; Alemu, 2014; Muniraju *et al*., 2016; Alemu *et al*., 2019; Nwankpa *et al*., 2019) have been done on PPR in different parts of the country. However, there is scarce information in Awi and Metekel Zones where the disease is highly prevalent. There is only single PPR sero prevalence report both in Metekel (Woldemichael *et al*., 2018) and Awi zones (Fentie *et al*., 2018) yet, despite no any study is done in the current study area districts. Moreover, there is no report regarding molecular detection of PPRV from the current study area.

Currently, Ethiopia developed a strategy for the progressive control and eradication of PPR in collaboration with FAO and OIE. For effective control and eradication of the disease understanding epidemiology of the disease and confirming the circulating virus is very important. It helps to implement the program due to that repeated vaccination of all susceptible small ruminants found in the country is unaffordable to be implemented. However, the epidemiology of PPR disease is not well understood in Ethiopia and thus, there is scarce information on epidemiology of the disease especially in the Northwest part of the country including Metekel and Awi Zones in that there is only one sero-prevalence report from the zones and there is no molecular level report as indicated above. Therefore, additional epidemiological information is important to support the current Ethiopian initiative towards control and eradication of the disease.

General objective

- ✓ To reveal epidemiology, isolation and molecular detection of small ruminant peste des petits ruminants circulating in Awi and Metekel zones

Specific objectives

- ✓ To estimate small ruminant PPR sero prevalence in selected districts of Awi and Metekel zones
- ✓ To determine risk factors associated with PPR sero prevalence in the study area
- ✓ To access recent spatial and temporal distribution of PPRV in Ethiopia
- ✓ To access awareness of the farmers on PPR disease and associated risk factors
- ✓ To isolate and molecularly detect the PPRV circulating in the study area

2. LITERATURE REVIEW

2.1. PPR: The Disease

A Peste des petits ruminants (PPR) is a highly contagious disease of small ruminant domestic and wild animals. The disease was initially believed to be rinderpest due to their similar clinical signs manifestation. However, rinderpest was ruled out by observing the inability of the disease to infect cattle exposed to infected small ruminants. Peste des petits ruminants is also called pseudo-rinderpest, goat plague, kata, stomatitis pneumo-enteritis syndrome, and pneumo-enteritis complex (SOP, 2013).

Depending on the extent of predisposing factors and virulence of the virus, PPR severity can be classified as per-acute, acute, sub-acute, and subclinical of which the acute form is most common. Acute form of PPR disease is characterized by sudden depression, high fever, anorexia, nasal and ocular discharge, mouth erosive lesions, pneumonia and severe diarrhea. The disease mostly occurs in developing countries, particularly in areas where small ruminant farming is an important component of trade and food production (Megersa *et al*., 2011).

2.2. Etiology

Peste des petits ruminants is caused by PPRV of the family *Paramyxoviridae*, subfamily *Orthoparamyxovirinae*, genus *Morbilli virus*, and species, Small ruminant *morbilli virus* (Dundon *et al*., 2020). It has close antigenic relation to the *rinder pest virus* (RPV) of bovines and buffaloes, *distemper virus* of dogs and other wild carnivores, human *measles virus* and *Morbilli viruses* of marine mammals specifically phocid *distemper virus* and dolphin *distemper virus (*Khan *et al., 2008;* Yalew *et al*., 2019).

2.2.1. Structure and genome organization of the virus

PPR virus is enveloped, pleomorphic virus containing non-segmented single stranded RNA that is approximately 16 kb long with a negative polarity as a genome. The genome of PPR virus is the longest of all the *morbilli viruses* so far consisting about 15,948 nucleotides that encodes two non-structural and six structural proteins. The two non-structural proeins of the virus are V and C proteins while the structural proteins are nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin protein (H) and large polymerase protein (L). The proteins are arranged in the order of 3´-N-P(C/V)-M-F-H-L-5´ within the virus genome (Bello, 2013; Gebre *et al*., 2018; Dundon *et al*., 2020).

The H and F proteins are found on viral envelope and they are very important proteins for the induction of protective host immune response against the virus (Bello, 2013). Fusion protein enables the virus to penetrate the host cell by mediating the fusion of the viral and cellular membranes at neutral pH. The H protein enables the virus to bind to the cell receptors to signal the lymphocyte activation molecule called CD 150. It also cooperates with F protein for the fusion activity of the protein. The L protein is used to carry the activities necessary for genomic RNA replication and transcription into functional mRNA. Phosphoprotein is a multifunctional protein. It acts as a cofactor for the RNA-dependent RNA-polymerase (RdRp). Furthermore, it binds both the N and L proteins and acts as a chaperone to keep the N in a soluble form for binding to RNA. The M protein is located inside the viral envelope and is the most conserved protein within the *morbilli virus* group. The virus C protein acts as infectivity and virulence factor. It is also indicated to be interferon antagonist. The non-structural V protein highly inhibits interferon actions. Accordingly, it contributes to immune-suppression induced by *morbilli virus* infections (Rudra, 2019).

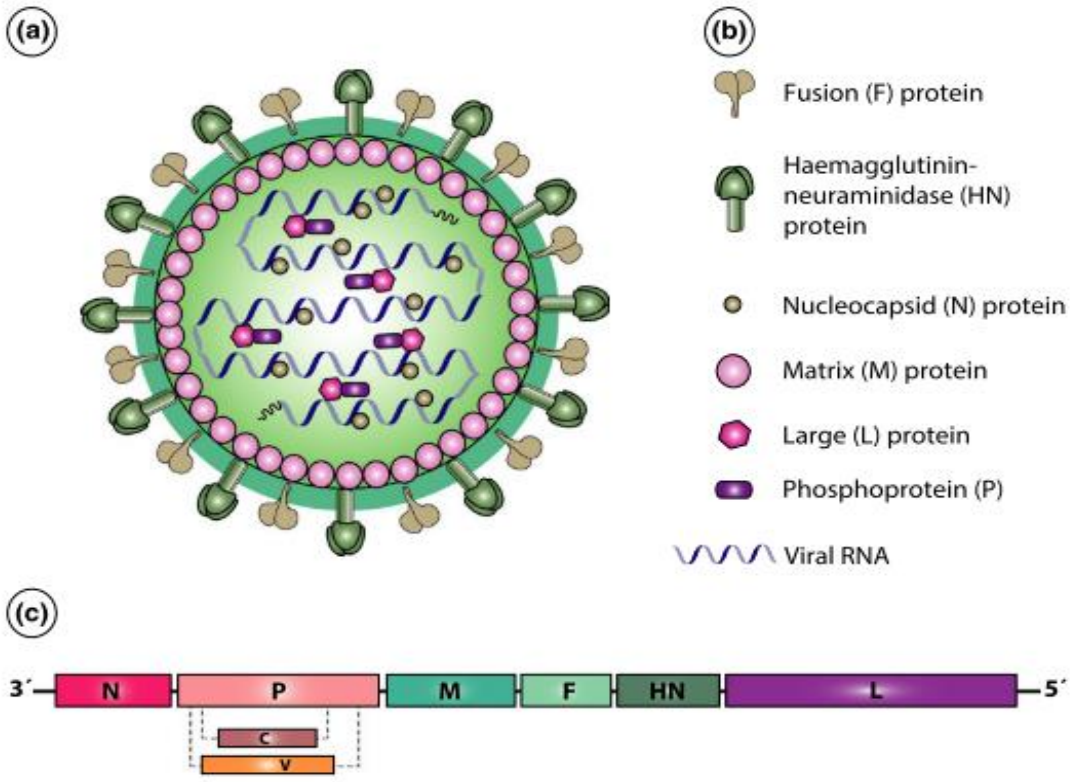


Figure 1: Structure and genome organization of PPR virus.
schematic structure (a), structural components (b) and (c)genome organization of PPRV (Munir, 2014).

2.2.2. Physiochemical property PPR virus

Molecular weight of the PPR virus genome is 5.8 x 106. Intact virion has a diameter of about 130-390nm with the thickness of the ribo-nucleoprotein measuring approximately 14-23nm. It is wrapped by a nuclear protein which is associated with P protein and the L protein (Bello, 2013). The virion is very sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde and oxidizing agents. The virus is usually destroyed at 50^{o}C for 60 minutes or 37^{o}C for 2 hours. However, it survives for long periods in chilled and frozen tissues (Dejene, 2016).

2.2.3. Lineage of the virus

Peste des petits ruminants virus has single serotype and four different lineages namely lineage I, II, III and IV. The lineages are classified based on phylogenetic analysis of the virus genes. Inter-lineage resolution is better when N gene is used compared to H or F gene. Each lineage was found to have specific geographic distribution pattern that has been changed in recent years (Dundon *et al*., 2020).

Lineage I is commonly found in West Africa and have recently been reported in central Africa where as lineage II isolates are found in western Africa countries. Lineage III is reported to be found in eastern Africa and some parts of the Middle East including Arabian countries such as Oman and Yemen. The lineage IV isolates are also found in the Arabian Peninsula, the Middle East and South Asian countries (Bello, 2013; Shahriari *et al*., 2019; Dundon *et al*., 2020).

Lineage IV viruses have also been regularly reported in different African countries including Central, East, South, North and Northeast Africa since 2008. Thus, it is becoming the predominant lineage on the continent (Roos, 2016; Clarke *et al*., 2018; Shahriari *et al*., 2019; Dundon *et al*., 2020).

Molecular characterization of PPRVs into lineages has no relationship to virulence of isolates rather it is a result of geographical speciation (Roos, 2016). Virulence of PPR virus is found to vary between the lineages. The virulence study conducted on West African goats indicated Lineage I to cause per-acute to acute disease and Lineage II to cause mild to in-apparent disease. The study also indicated Lineage III to cause acute to mild infection and Lineage IV to cause acute PPR disease. Due to its mildness, Linage II is used in the initial attenuation by multiple passages on Vero cells to produce the current PPR vaccine (Bello, 2013)

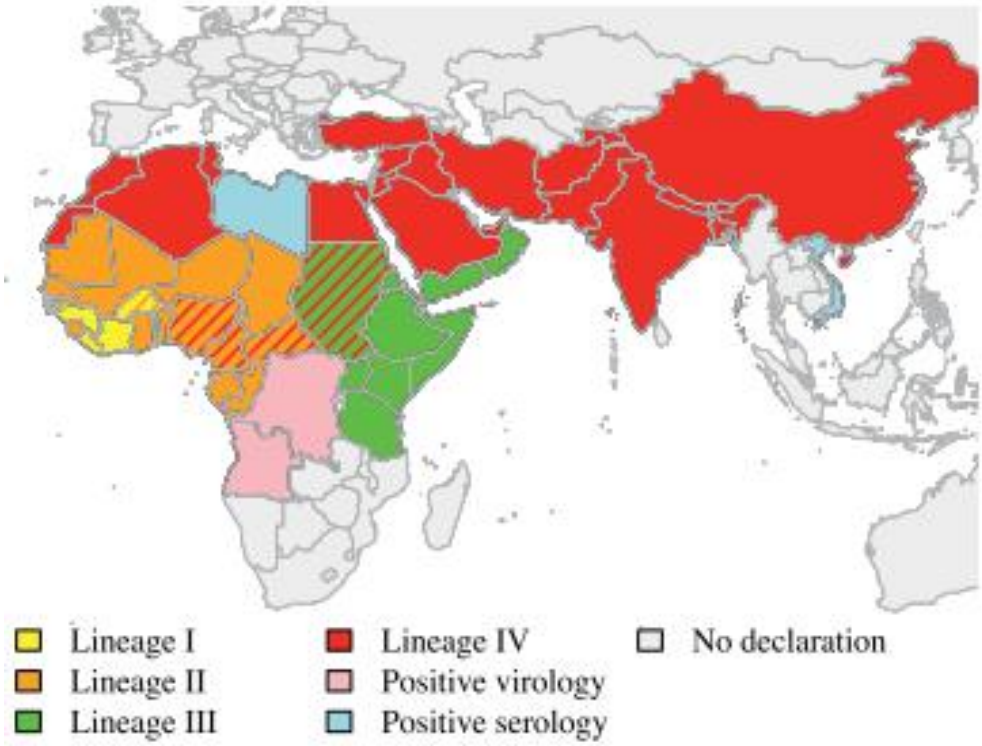


Figure 2: Worldwide cumulative distribution of PPR virus lineages
hatched bars represent the last identified lineage in the country; the pink color indicates evidence of PPR infection with isolation of the virus; the blue color indicates serological evidence of PPR infection with no virus isolation and the grey color indicates missing information or absence of the disease report (Albina *et al.*, 2013*;* Alemu, 2014).

2.3. Epidemiology of the Disease

2.3.1. Origin and geographic distribution

The disease was first reported in Cote d'Ivoire, west Africa in 1942 by Gargadennec and Lalanne and at a present it is spreading across the Sub-Saharan Africa, Morocco, Arabian Peninsula, Middle East, Turkey, Iran, Iraq, Pakistan, India, Bangladesh, Nepal, Tajikistan and Kazakhstan, Tibet, and China (Luna, 2012; Gari *et al*., 2017; Clarke *et al*., 2018). Initially, it was assumed that PPRV was a West African strain of rinderpest that had lost its ability to infect cattle. Before the advent of PPR specific diagnostics tools that could differentiate between RPV and PPRV, such as cDNA clones, most cases of PPR in sheep and goats were misdiagnosed as rinderpest and PPRV was circulating in RPV endemic areas for decades before it was identified (Hodgson, 2018).

With the notable exception of most southern African countries including South Africa, Botswana, Namibia, Zimbabwe, Mozambique and Malawi, PPR is now recognized to be endemic throughout Africa as well as the Middle East, Central, East and south Asia (Clarke *etal.*, 2018).

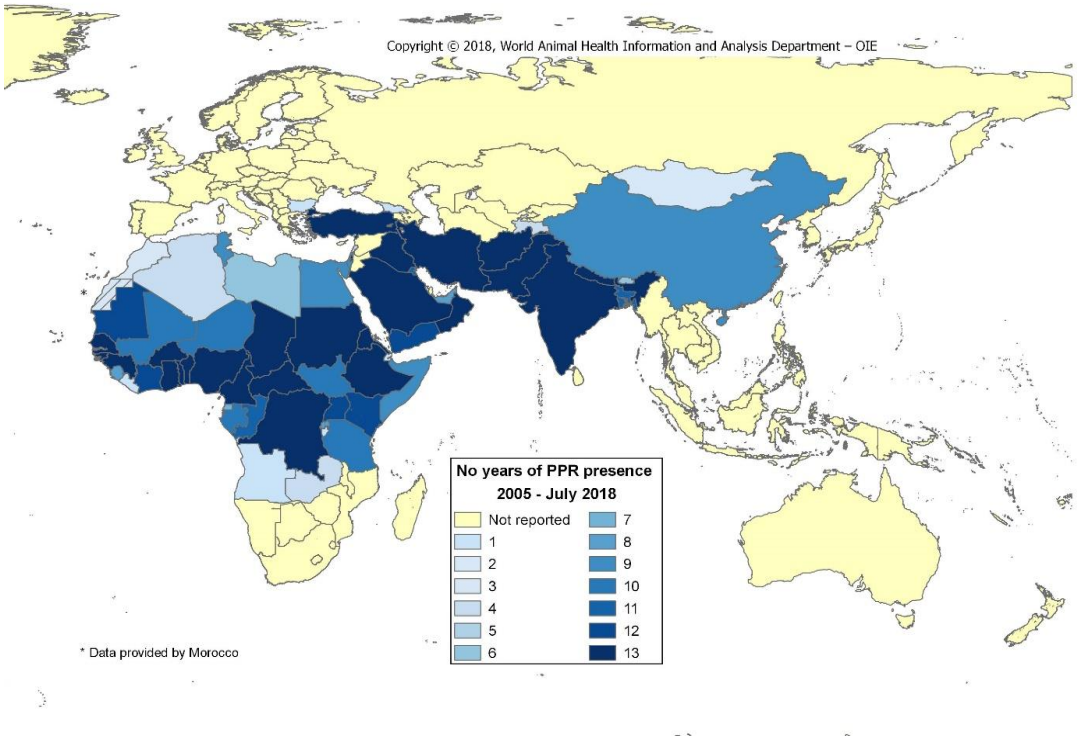


Figure 3: Map of number of years PPR reported in different countries from 2005 to 2018
Source: (OIE, 2020a*).*

Currently, around 70 countries have either reported infection to the OIE or are suspected of being infected. Of these, more than 60% of the countries are from Africa including North Africa the other infected countries being from Asia (South-East Asia, China, South Asia and Central Asia/West Eurasia including Turkey) and the Middle East. Another 50 countries are considered to be at risk for PPR disease. As of May 2014, 48 countries in the world were also officially recognized with PPR free status (OIE and FAO, 2015b). However, about 59 countries including Namibia and South Africa are currently declared to have the disease free status, Namibia being the only country with zonal PPR free status (seven free zones).

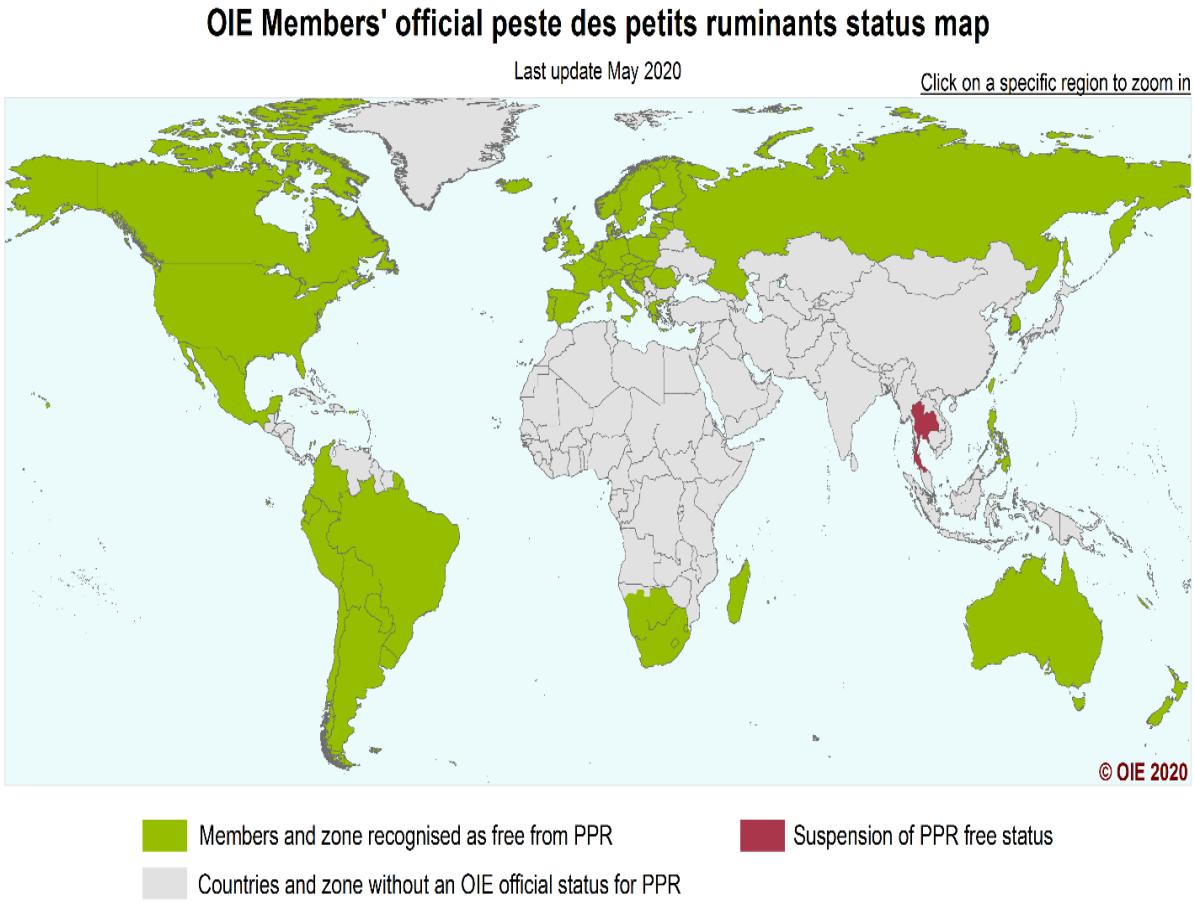


Figure 4: Official PPR status map of OIE member countries.

Source: (OIE, 2020a)

2.3.2. Host range and reservoirs

There is no carrier status for PPRV and the virus relies on a constant supply of new susceptible hosts for its maintenance (Kihu, 2014). Peste des petits ruminants primarily affect sheep and goats but goats are more susceptible than sheep. Goats and sheep show typical clinical disease and transmit the disease after infection with the PPRV. Thus, they are recognized as reservoir hosts (Schulz *et al*., 2019). Other domestic animals such as cattle, camels, buffalo and pigs are also known to be susceptible to the PPR experiencing subclinical infection to the disease. However, they do not contribute to the epidemiology of the disease as they are unable to excrete the virus. Cattle are considered dead end hosts (Luna, 2012; Robi, 2019).

Similarly, camels were previously considered as dead end host for PPRV. However, the transmission of the virus from infected pigs to contact pig and goat in addition to the excretion of infectious PPRV by pigs and wild boar that observed recently after experimental intranasal infection with the virulent PPRV lineage IV strain, showed that suids can indeed act as a potential source of PPRV infection (Schulz *et al*., 2019). The PPR disease is also found to infect captive or free wild ruminant animals including wild goats, ibex, blue sheep, gazelles, springbuck, saiga, buffalos, bushbuck, nilgai, kobs, waterbucks, Oryx, duikers, hartebeests, and impalas. The disease severity in wild animals varies from asymptomatic to the one with severe clinical signs (Ruget *et al*., 2019). The PPRV was also detected in Argali sheep, Goitered gazelle, and Siberian ibex in both China and Mongolia (Figure 5) (Fine *et al*., 2020).

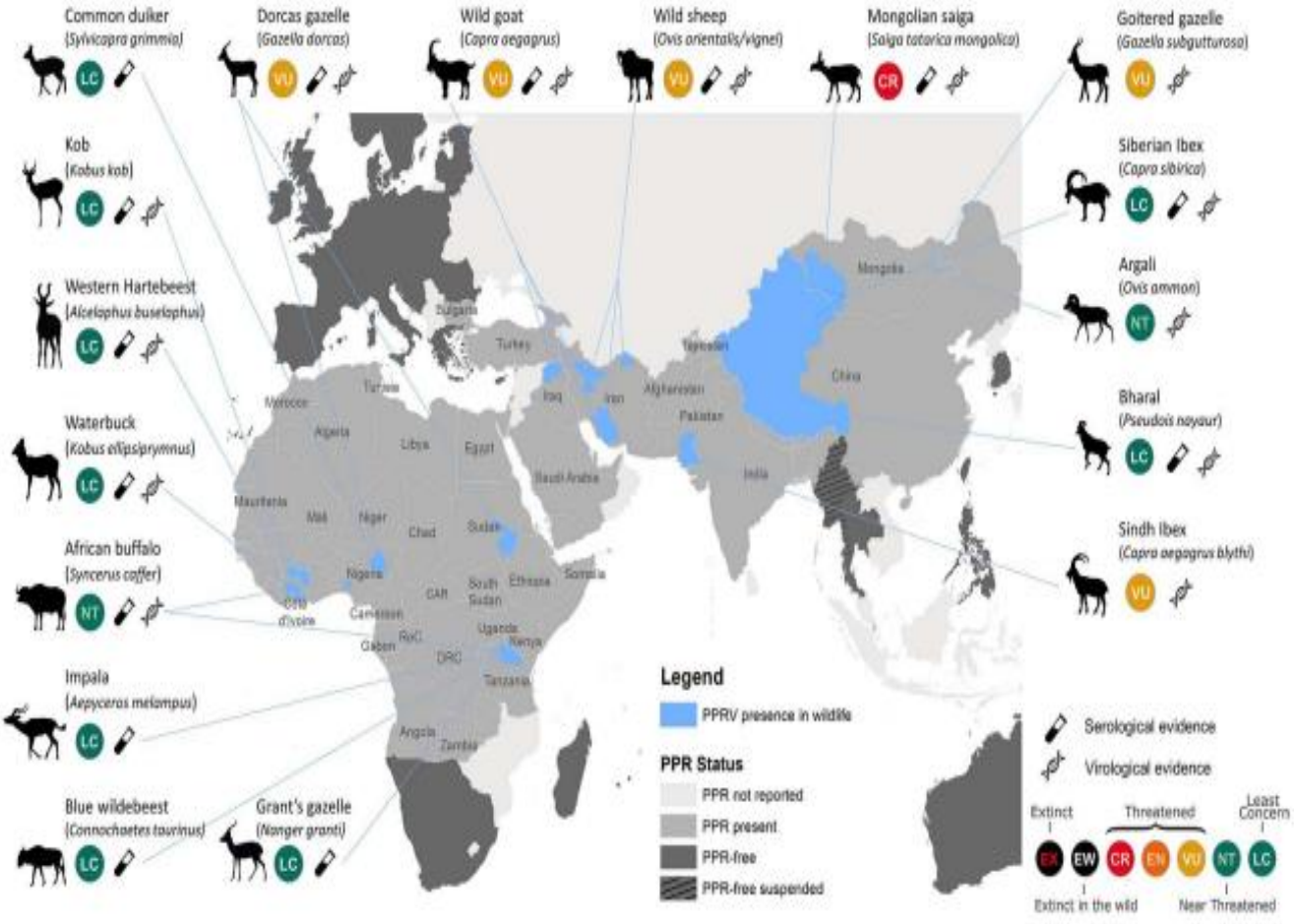


Figure 5: Map illustrating published reports of PPR virus detection in free-ranging wildlife species.

CR=Critically Endangered; EN=Endangered; VU=Vulnerable. The blue color indicates areas where PPR is detected from wild animals (Fine *et al*., 2020).

2.3.3. Host determinants and other risk factors

Host determinants that affect morbidity of PPR include age, sex, breed and species of the animals. Regarding the age of the animals, young sheep and goats are more susceptible to PPRV infection because, they are less likely to have developed protective antibody titers (Dejene, 2016). Small ruminants aged 3 to 18 months are more severely affected than adults or un-weaned young animals. In addition, kids over four months and under one year of age are indicated to be most susceptible to the disease. Female small ruminants are also indicated to be more affected by the disease (Alemu, 2014).

Severity of PPR varies with species of the animals infected. It is higher in goats than sheep though recovery rate is higher in sheep. *Peste des petits ruminants virus* is considered to be non-pathogenic to cattle and African buffaloes (domestic and wild) even though they are sub-clinically infected (Alemu, 2014). With respect to 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 (Kumar *et al*., 2014; Bedore *et al*., 2019; Robi, 2019).

The presence of mixed population flocks, introduction of new animals into a flock and the return of non-sold animals from market to source population are major risk factors for further spread of PPR disease (CFSPH, 2008). Livestock trade, nomadic herding and aggregation of susceptible populations of the animals at watering points and in livestock markets also play an important role in distribution of the disease especially in nomadic areas. Stress factors such as change of diet, habitat, and intensification of the farms also lead to increased risk of infection with PPR. Furthermore, nutritional deficiency due to insufficient availability of fodder can leads to increased susceptibility of and subsequent infection of large numbers animals (Alemu, 2014).

Climatic factors can also affect PPR occurrence. In rainy season, the disease outbreaks are minimized due to decreased movement of animals as fodder availability increases. As a result of the increased fodder availability, nutritional and health status of the animals is improved leading to improvement in immune status of the animals (Abubakar *et al*., 2015).

2.3.4. Disease pattern and seasonal occurrences

There are considerable differences in PPR epidemiologic pattern in different agro-ecological systems and geographical areas. In the humid areas where the disease occurs in an epizootic form, high morbidity and a mortality rates are reported. However, in arid and semi-arid areas, PPR is found to be rarely fatal and usually occurs as a subclinical infection (Abraham, 2005). It is also suspected that PPRV can circulate silently in the population occasionally causing sporadic epidemics when the host animal population's immunity are low to resist the infection (Alemu, 2014).

In endemic areas, PPR also exhibits a seasonal pattern with an increased number of outbreaks at the beginning of the cooler wet season (Ruget *et al*., 2019). The seasonal epidemiologic patterns of the disease differ in different agro-ecological systems depending on the culture and livelihood patterns of the small holder farmers (Bedore *et al*., 2019). However, the disease seasonality in all geographic zones is not clearly apparent. In subtropical areas, the occurrence of PPR disease is reported to be more common during winter and rainy seasons. Confinement and restricted movement of the animals in tropical countries in rainy seasons may also affect nutritional status of the animals and can predispose them to PPRV infection. In most endemic areas, PPR is reported to be most common during the cool dry season (Abubakar *et al*., 2009; Munir 2013). High morbidity rate is also observed with unfavorable weather conditions and poor fodder (Alemu, 2014).

2.3.5. Transmission

Transmission of PPRV can occurs by different methods including direct contact with infected animals, inhalation of aerosol, or contacts with ocular and nasal secretions, saliva and feces. *Peste de Petitis Ruminants virus* is mainly transmitted by the aerosol route. But, it requires close contact with the infected animals. Infected animals in febrile stage of the disease are important source of infection. The virus can be shed during subclinical cases or during incubation periods (Alemu, 2014). The infectious period of PPR is short and infected animals can either die or recover with a lifelong immunity. Within herd PPRV transmission occurs between animals in close contact and between herds transmission occurs as a result of contact at communal pastures, watering areas and at live animal markets (Ruget *et al*., 2019).

Asymptomatically infected animals can shed the virus for up to 12 weeks. However, shedding of the virus can be longer in recovered animals (CFSPH, 2008). Thus, the virus can contaminate water, feed troughs and bedding to act as additional routes of transmission. Fortunately, the virus does not survive for long period of time outside the host, and therefore, most transmission occurs by contact during the febrile stage of the disease (Abubakar *et al*., 2015)

2.3.6. Morbidity and mortality

Morbidity and mortality due to PPR disease is very high and it can vary with stage of the disease, the species and age of the animals affected. In severe cases, morbidity and mortality can be as high to 100 and 90 percent respectively (Fentie *et al*., 2018). Morbidity is also higher in goats than sheep and the small ruminant animals with age from 3 months to 2 years are more severely affected compared to younger or older animals. In susceptible goat populations, mortality rates of 50-100 percent can be also expected. In endemic areas low levels of infection are constantly circulating and periodic outbreaks can occur when a naive population is introduced or grows in number. Usually such periodic outbreaks are characterized by almost 100 percent mortality both in sheep and goats. High mortality rates have been also reported among captive animals (SOP, 2013).

2.4. Pathogenesis

Peste des petits ruminants virus has significant affinity to lymphoid and epithelial tissue of respiratory and gastrointestinal tracts. After the entry of the virus through respiratory system, it replicates itself in lymph nodes (pharyngeal and mandibular lymph nodes) and tonsil (Bello, 2013). Subsequently, the virus enters the retropharyngeal mucosa and spreads to local lymphatic tissue for further replication. Consequently, it produces primary viremia within 2 to 3 days. The viremia facilitates spread of the virus to other lymphoid tissues and organs such as spleen, bone marrow and mucosae of gastrointestinal and respiratory tract where it cause severe damage replicating in endothelial, epithelial and monocyte cells (Abubakar *et al*, 2011; Rudra, 2019).

Destruction of the lymphoid tissues causes lymphopenia and significant immuno-suppression on the host leading to secondary opportunistic infection by increasing susceptibility of the animals to other microorganisms (Alemu, 2014; Ebissa, 2020).

2.4. Clinical Signs

Sheep and goats are the primary hosts for PPRV. However, goats are highly susceptible to the disease than sheep. Incubation period of the virus can range from 3 and 10 days even though the typical period is 4 to 6 days. At acute stage of PPR disease, the animals usually exhibit clinical signs such as fever (up to 41°C) lasting for 3 to 5 days, depression, anorexia and muzzle dryness (Ebissa, 2020). They can also show excessive salivation, watery nasal and lachrymal discharges that gradually changes to mucopurulent. Erosive lesions are also formed in oral cavity and may become necrotic as the disease stage progress. Subsequently, the animals develop diarrhea and cough with labored abdominal breathing in the later stage of infection. The disease condition may last for 14 days before recovery from infection or leads to death during the acute stage of infection. In general, the clinical signs considerably depending on the virulence of virus (Muniraju, 2015).

2.5. Differential Diagnosis

As to differential diagnosis, PPR is usually confused with other sheep and goat diseases that have similar clinical signs with it. These diseases include rinderpest, foot and mouth disease (FMD), bluetongue, contagious ecthyma (Orf), pneumonic pasteurellosis, contagious caprine pleuropneumonia (CCPP), and gastro-intestinal helminths infestations (Robi, 2019).

Foot and mouth disease is distinguished from PPR by the absence of respiratory problems and diarrhea, and the presence of marked lameness in infected animals. The foul smelling exudes from the mouth of PPR infected animals is also usually not present in FMD cases. Bluetongue disease can cause fever, nasal discharge and oral lesions similar to PPR. However, it is characterized by edema of the head region and bluish discoloration of the oral cavity. Clinically, CCPP can be also differentiated by absence of mouth lesion and diarrhea from PPR. In addition, adhesion of the lungs to the chest cavity and fibrin deposits on the lung are found at postmortem examination in CCPP infected animals.

Orf is confused with PPR in that the nodules it forms resemble the thick scabs observed on the lips of the animals in PPR infection. However, pneumonia, diarrhea and sometimes oral lesions are not usually observed in uncomplicated Orf. In animals infected with pneumonic pasteurellosis, there is no diarrhea and oral lesion found the disease being purely a respiratory disease. However, in cases where PPR presents without oral lesions, the diagnosis can only be confirmed by detecting the PPRV (Bello, 2013).

2.6. Current Diagnostic Techniques

The routine diagnosis of PPR depends on clinical examination, gross pathology, histo-pathological findings and laboratory confirmation by PPR antigen and antibody detection, viral isolation, viral nucleic acid hybridization and polymerase chain reaction (Luna, 2012). For diagnosis, swab samples (conjunctival, nasal and oral mucosa) can be collected from live animals. Whole blood in early stage of the disease can also be collected in anticoagulant for virus isolation, polymerase chain reaction (PCR) and hematology. At necropsy, tissue samples from mesenteric and bronchial nodes, lungs, spleen and intestinal mucosa should be collected and transported being chilled in ice. Samples collected for histopathology have to be kept in 10% formalin. Blood can also be collected for serological diagnosis using plain vacutainer tubes (OIE, 2004).

2.6.1. Isolation of the virus

For PPRV isolation in cell culture different cell lines have been used. These includes marmoset-B-lymphoblastoid (B95a) cell lines, primary lamb kidney cells (ovine kidney cells), caprine kidney cells, bovine kidney cells, African green monkey kidney (Vero) cells and transformed monkey cells. Previously, B95a was primarily used for culturing eventhough primary lamb kidney and Vero cell cultures have been successful (Alemu, 2014).

Vero cells have been the cells of choice for isolation and propagation of PPRV for a long time. However, some isolates may not grow well in these cells. Recently, transformed monkey cells expressing sheep/goat signaling lymphocytic activation molecules (SLAM or CD150) has been observed to have an increased sensitivity for isolation of the PPRV (Alemu, 2014).

For successful isolation of the virus, a samples must be collected during hyper-thermic phase and transported to laboratory in cold ice. The samples that can be used for the virus isolation include blood, swabs (ocular, nasal, oral and rectal), tonsil, mesenteric lymph nodes, spleen, section of colon and lung (Rudra, 2019). However, blood collected in EDTA/heparin, ocular and nasal swabs collected at early stage of the disease are the samples of choice (Santhamani *et al*., 2016).

In culture and isolation technique, monolayer cell cultures are inoculated with buffy coat, swab material or 10% tissue suspensions and daily examined for evidence of cytopathic effect (CPE). The CPE produced by PPRV can develop within 5 days of inoculation and consists of cell rounding and aggregation that culminate in syncytia formation in lamb kidney cells (OIE, 2008).

In Vero cells, it is sometimes difficult to see the syncytia and they are very small if they exist. However, small syncytia are always observed in stained infected Vero cells. Syncytia are recognized by a circular arrangement of nuclei giving a 'clock face' appearance. Cover-slip cultures may give a CPE earlier than day 5. There are also intra-cytoplasmic and intra-nuclear inclusions. Some cells are vacuolated. Similar cellular changes can also be seen in stained histopathological sections of infected tissues. After 5-6 days, blind passages should always be carried out as CPE may take time to appear (Bello, 2013; OIE, 2019).

2.6.2. Antigen detection methods

The antigen of PPRV can be detected by using a diagnostic tests such as immune capture ELISA (Ic-ELISA), counter immune electrophoresis (CIEP), agar gel immune diffusion (AGID), hem-agglutination test (HA), latex agglutination tests and immune histochemistry (IHC). The Ic-ELISA and CIEP tests can differentiate between PPRV and RPV. Although AGID test is simple and cheap method, it cannot differentiate PPRV and RPV due to its less sensitivity. But, Ic-ELISA is a rapid, sensitive and virus specific test for PPRV antigen detection and it can differentiate between PPRV and RPV. Moreover, it is more sensitive than AGID (Delil, 2007; Bedore *et al*., 2019).

The very simple and inexpensive test that can be performed in any laboratory and even in a field, AGID is used to diagnose PPR from swab and tissue samples using PPRV specific antibodies (Santhamani *et al*., 2016). Results of AGID test are obtained in one day, but the test is not sensitive enough to detect mild forms of PPR due to the low quantity of viral antigen that is excreted. Standard PPR viral antigen for the test is prepared from lymph nodes (mesenteric or bronchial lymph nodes), spleen or lung material and ground up as 1/3 suspensions in buffered saline. The suspension is centrifuged at 500 g for 10-20 minutes, the supernatant fluid is collected and stored in aliquots at -20°C (OIE, 2020b).

The cotton material from the cotton bud used to collect swab samples is removed using a scalpel and inserted into a 1ml syringe. Then, 0.2 ml phosphate buffered saline (PBS) is drawn with the syringe and the sample is extracted by repeatedly expelling and filling the PBS into an Eppendorf tube. The resulting swab extract can be refrigerated at -20°C until used. Negative control antigen is also prepared similarly from normal tissues. Standard antiserum is made by hyper-immunizing sheep with 1ml 104 fifty percent tissue culture infective dose (104 $TCID_{50}$) titer PPRV per ml given at weekly intervals for 4 weeks. Subsequently, the animals are bled 5-7 days after the last injection for the standard antiserum collection. Standard rinderpest rabbit hyper-immune antiserum can also be used being effective in detecting PPR antigen (OIE, 2008).

The most rapid test, CIEP is also used for viral antigen detection. In this test, the antigen to be detected and the specific antibody used to detect the antigen move in opposite direction in the electric field to form the line of precipitation at the point of interaction. Compared to AGID, CIEP is more quick and sensitive. However, both of the tests are less sensitive at early stages of infection and with mild form of the disease. Moreover, they do not differentiate between PPRV and RPV due to presence of cross reacting epitopes in the viruses (OIE, 2004; Santhamani *et al*., 2016).

Counter immune electrophoresis test is performed on ahorizontal surface with a suitable electrophoresis bath that consists of two compartments connected by a bridge. To do the test, the apparatus is connected to a high voltage source and 3ml of 1-2 percent agar or agarose dissolved in 0.025 M barbitone acetate buffer is dispensed onto microscope slides. Six to nine pairs of wells are punched in the solidified agar and the electrophoresis bath is filled with 0.1 M barbitone acetate buffer (OIE, 2019).

Subsequently, the nodal and cathodal wells are filled with sera and antigen respectively. Following this, the slide is placed on the connecting bridge and the ends are connected to the buffer in the troughs by wetted porous paper. Finally, the apparatus is covered and a current of 10-12 milliamps per slide is applied to the apparatus for 30-60 minutes and the slides are viewed by intense light. Accordingly, the presence of 1-3 precipitation lines between pairs of wells is considered as indicative of a positive reaction. There should be no reactions between wells containing the negative controls (OIE, 2019).

Hem-agglutination test is easy, cheap and effective test for PPRV diagnosis in that it does not need sterility or sophisticated instruments to perform. Indeed, it is also used to differentiate PPR infection from that of RPV (Kihu, 2014). The test can use human 'O' blood group, piglet or chicken red blood cells to detect PPRV antigen from clinical specimens. Even though they are not sensitive enough to detect early stages of disease and mild forms of disease, HA can be used to confirm CPE during virus infectivity titrations (Santhamani *et al*., 2016).

Pen side tests are the tests used at field to diagnose the disease using conjunctival, nasal and oral swabs. They are rapid tests that can give a result within 20 minutes and do not require additional equipment. However, they are not suitable to use on blood and tissue samples. Penside tests have been validated against PPRV isolates from all four lineages and show sensitivity similar to Ic-ELISA and 100% specificity in laboratory testing. In these test, the swab samples are rinsed with buffer and the buffer is applied to one end of a chromatographic strip so that the sample mixes with colored beads coated with a specific MAb that recognizes PPRV antigen. Accordingly, the buffer flow moves the beads along the chromatographic strip. If the sample contains PPRV antigen, it binds to the beads and form antigen-beads complex which is then captured by a line of anti-PPRV MAb part-way along the strip, making a colored line that indicate a positive result. If PPRV antigen is absent in the sample, no beads are bound by the test line (OIE, 2019).

Enzyme-linked immunosorbent assay (ELISA) employs enzyme-substrate reaction to detect antigen-antibody interactions. They are user-friendly and suitable for handling large sample sizes with a desirable level of sensitivity and specificity. Currently, different types of ELISA are available based on sensitivity and specificity requirements (Santhamani *et al*., 2016).

Monoclonal antibody based Ic-ELISA with a diagnostic sensitivity of $10^{0.6}$ $TCID_{50}$ was developed against the N protein of PPR and is commercially available. A sandwich ELISA was also developed in India using polyclonal sera for antigen capture and MAb (4G6) raised to the N protein of PPRV as detection antibody. It has 88.9 % relative diagnostic sensitivity and 92.8 % diagnostic specificity compared to Ic-ELISA (Santhamani *et al*., 2016).

Since the MAbs used in Ic-ELISA and sandwich ELISA are raised against non-overlapping domains of the N protein of PPR and RP viruses, the tests can be used to differentiate PPRV from RPV infected animals (Dejene, 2016).

Dot-ELISA has also been developed and described for PPRV antigen detection from clinical samples using anti-M protein and anti-N MAbs. This test can be used as diagnostic tools in laboratories where resources are limited. However, it has low sensitivity. The test is also prone to false positive results. Therefore, using known positive and negative specimens is advisable. Furthermore, an indirect ELISA called cell-ELISA was developed in micro-titer plate format for PPRV antigen detection in infected cells using the anti-N Protein MAb 4G6. This diagnostic test has more than 97 % a relative sensitivity and specificity in comparison to the virus infectivity titrations scored from CPE by visual observation. Cell ELISA can be used as a vaccine quality control tool in that both infectivity tests and specific detection of PPRV can be simultaneously performed with this assay (Santhamani *et al*., 2016).

The other very simple and precise PPRV antigen detection method is IHC. This assay is an informative and reproducible method used to determine cells or parts of cells in which a particular protein or other macromolecules are located by using antibodies to bind to specific antigens in a tissue section. It is performed on formalin-fixed paraffin-embedded tissue. Both polyclonal and monoclonal antibodies can be used in this test even though monoclonal antibodies exhibit greater specificity. Moreover, IHC is used for PPR diagnosis where fresh or frozen tissues are no longer available. It is also useful in retrospective examination of preserved specimens to verify past histological diagnosis (Rudra, 2019).

2.6.3. Antibody detection methods

For PPRV antibody detection in samples from infected animals, different diagnostic methods indicated below have been used. The competitive ELISA (c-ELISA) based on MAbs against the H or N proteins and virus neutralization test (VNT) are the most important diagnostic techniques used for the virus antibody detection (Bedore *et al*., 2019). Currently, c-ELISA is the most commonly used diagnostic technique for PPRV antibody detection. It has an overall specificity of 98.4% and sensitivity of 92.2% compared to VNT (Bello, 2013).

Virus neutralization test is a gold-standard antibody detection test prescribed for international trade. In the VNT, 100-1000 $TCID_{50}$ of PPRV is mixed with 100µL of twofold dilutions of serum and incubated at 37^{o}C prior to inoculation to cell culture in a 96-well micro-plate. The development of CPE in the microplate wells containing specific dilution of antibody indicates the absence of virus neutralization. Virus neutralization titer of a serum is expressed as the highest dilution that results in 50 % inhibition of CPE. This test detects virus-neutralizing antibodies, which could be an indication of in vivo protection in the case of *morbilli viruses*. Due to the requirements for cell culture facilities and sterile serum, it is difficult to use VNT for routine serosurveillance or seromonitoring activities, particularly when a large number of samples need to be screened. For this reason, it has been replaced by competitive and blocking ELISA techniques for a long period of time (Santhamani *et al*., 2016).

Compititive ELISA is considered suitable for large scale testing due to its simplicity and availability of the recombinant antigen. Several c-ELISAs have been described based on MAbs specific for H or N protein for detection of PPRV antibodies in serum samples from infected animals. The N protein based c-ELISA kits use purified recombinant N protein produced in *baculovirus* as a detection antigen and where as those based on H protein MAb use an antigen consisting of purified or part-purified PPRV. However, all the assays work on the principle that antibodies to PPRV in test sera can block the binding of the MAb to the antigen. Because of the high specificity and sensitivity of c-ELISA, it can be used even for samples not kept under ideal conditions (Bello, 2013; Kihu, 2014; OIE, 2019). To detect antibodies using N protein coated micro wells, the samples to be tested and the control are added to the wells and antibody-antigen complex which masks the N protein epitopes is formed if anti-N protein antibodies are present in the sample.

Then, anti-N protein-peroxidase conjugate is added to the micro wells. Thus, theanti-N protein-peroxidase monoclonal antibody conjugate competes with the serum antibodies in order to fix to the N protein epitopes on the remaining coated antigen. Accordingly, where no serum anti-N protein antibodies are present, the anti-N protein-peroxidase monoclonal antibody conjugate fixes the free N protein epitopes forming an antigen-conjugate-peroxidase complex. After washing, substrate solution Tetra methyl benzidine (TMB) is added to the complex in order to eliminate the excess conjugate (Kihu, 2014). The absorbance in PPR ELISA is converted to percentage of inhibition (PI) using the formula: PI=100-(absorbance of the test wells/ absorbance of the MAb control wells) x 100. Thus, sera with PI greater than 50% are scored positive (Bello, 2013).

African Union Pan African Veterinary Vaccine Center (AU-PANVAC) has developed an affordable, highly sensitive, specific, repeatable and reproducible blocking-ELISA (b-ELISA) for detection of anti-PPRV antibodies. The ELISA use H protein based MAb as a competitor antibody. It also exhibits good diagnostic performance for detection of anti-PPRV antibodies after vaccination. Blocking ELISA has 98.9% specificity and 90.4% sensitivity in comparison to the VNT (Kamel and El-sayed, 2019). It is proved to be simple, more rapid, sensitive and specific method for detection of PPR antibodies. Unlike the VNT, b-ELISA may be less affected by the quality of sera, cytotoxicity and contamination (Kihu, 2014; Bodjo *et al*., 2018).

Precipitinogen Inhibition test (PIT) is used to detect antibody based on ability of the antibody in serum to inhibit diffusible virus antigen (precipitinogen) from developing a precipitin line against hyper immune serum. This test is more sensitive than VNT. Hemagglutination inhibition (HI) test is also another technique used to detect antibodies produced against PPRV by adsorbing out the cross-reacting antibodies to rinderpest antigen from PPR serum and leaving the specific antibody to PPR antigen. Indeed, the CIEP PPRV antibody detection method that is highly adaptable for use in titration of serum antibody. It can be conducted for sero-epidemiological and experimental studies on PPR. Furthermore, AGID test was also used to detect antibodies produced against PPRV in the sera of infected animals by using specific PPR antigen. This test is considered useful for field diagnosis of PPR (Kihu, 2014).

2.6.4. Genome detection methods

Polymerase chain reaction (PCR) techniques have been developed targeting F, N, M and H genes and used for detection of specific PPRV genetic material from clinical samples. In addition, PCR for PPRV or RPV using PPR virus-specific external F gene primers, specifically F1 and F2 primers has also gained great importance in differential diagnosis and epidemiological studies. However, this method may not always be suitable for diagnosis of every virus strain, variant or isolate because of the changes occurring at 3′ end of the primer binding sites may yield a false-negative result as a result of variation between strains in the immunogenic protein coding region (Balamurugan *et al*., 2014).

Several nucleic acid based molecular tests have been developed for the specific diagnosis of PPR including complementary DNA (cDNA) hybridization, reverse transcriptase PCR (RT-PCR), real time RT-PCR and Loop mediated isothermal amplification (LAMP). The RT-PCR and cDNA hybridization techniques are the most sensitive diagnostic methods though they are time consuming and difficult for routine diagnosis of the disease from large sample size (Balamurugan *et al*., 2014).

Reverse transcriptase PCR is an accurate, rapid and reliable diagnostic method used for detection and quantization of specific DNA molecules based on the amplification of parts of the N and F protein genes. This diagnostic method is about 1000 times more sensitive than classical virus titration on Vero cells. It is also more advantageous in that the results are obtained within 5 hours, including the RNA extraction (Kihu, 2014).

Currently, there is also highly sensitive Real-time RT-PCR to diagnose PPR. This test is ten times more sensitive than conventional RT-PCR. It also minimizes the risk of contamination (OIE, 2019). Furthermore, N gene based radio isotope ^{32}P labelled cDNA probes were also developed for the detection and differentiation of PPRV from the RPV. However, their use in routine diagnosis is not recommended due to the short half-life of the ^{32}P and the need for special equipment to protect the users due to hazardous nature of the isotope. It could differentiate between the two viruses without need for culture and virus isolation (Santhamani *et al*., 2016).

The cDNA directed against M, F and P protein genes were found to cross hybridize to a much greater extent and were not suitable for use as discriminating probes. Therefore, probes using nonradioactive labels such as biotin or digoxygenin which are safe were developed. The biotin labeled cDNA was found to be as specific as the one using the radioactive label. It is more rapid in differentiation between PPRV and RPV (Rudra, 2019).

Table 1: Diagnostic tests available for diagnosis of PPR and their purpose

Method	**Purpose**					
	For population freedom from infection	Individual animal freedom from infection	For eradication policies	Confirmation of clinical cases	For Prevalence and surveillance	To check Immune status post vaccination
Agent Identification						
RT-PCR	-	++	++	+++	+	-
Real time RT-PCR	-	++	+++	+++	+	-
Isolation of the virus from culture	-	-	-	++	-	-
Ic- ELISA		+	++	+++	+	-
LFD	-	-	++	++	-	-
AGID	-	-	+	+	-	-
CIEP	-	-	-	+	-	-
Detection of immune response						
VNT	+++	+++	-	++	++	++
C- ELISA	+++	+++	+++	+	+++	+++
AGID	-	-	+	+	-	+
CIEP	-	-	-	+	-	-

Key: +++= recommended method, validated for the purpose shown; ++= suitable method but may need further validation; += may be used in some situations, but cost, reliability or other factors severely limits its application; – = not appropriate for that purpose. **Source**: (OIE, 2019)

2.7. Socio-Economic Importance of the Disease

Peste des petits ruminants is found in about 70 countries in Africa, the Middle East and Asia, affecting more than 1.7 sheep and goats. It also affect livelihood, food security and nutrition of more than 330 million people in poor farming communities in these countries that depends solely on small ruminant production for their survival. The disease does not only affect the society who raise small ruminants, but also the complex and well defined value chains that the animals production system supply (OIE and FAO, 2015a).

Peste des petits ruminants is considered a major constraint for sheep and goat production causing serious economic losses due to its extremely contagious nature. Economic losses caused by PPR strike at the heart of vulnerable livelihoods as well as national and regional livestock production. Countries have experienced a yearly economic losses ranging from tens to hundreds of millions of dollars (OIE and FAO, 2015a; Robi, 2019). It is reported that PPR causes annual loss of 1.5 million US$ in Nigeria, 39 million US$ in India, 15 US$ million in Kenya and at least 1.5US$ million in Iran (Robi, 2019).

At global level, PPR is found to cause an estimated range of 1.45 to 2.1 billion US$ economic losses each year due to reduced production, animal deaths and cost of caring for sick animals, including vaccination. Almost half of these losses are in Africa while about quarter of the loss is in South Asia (OIE and FAO, 2015a; Fentie *et al*., 2018; Fine *et al*., 2020).

In Ethiopia, the financial losses due to PPR was found to be varied based on season. Estimated economic losses of 652, 595 birr in drought time and 1,683,120 birr in non-drought period of time with a total loss of 43,478.3$ is reported in the country only from death of seep and goat population of 3905 heads in the study farms (Gizaw *et al*., 2018).

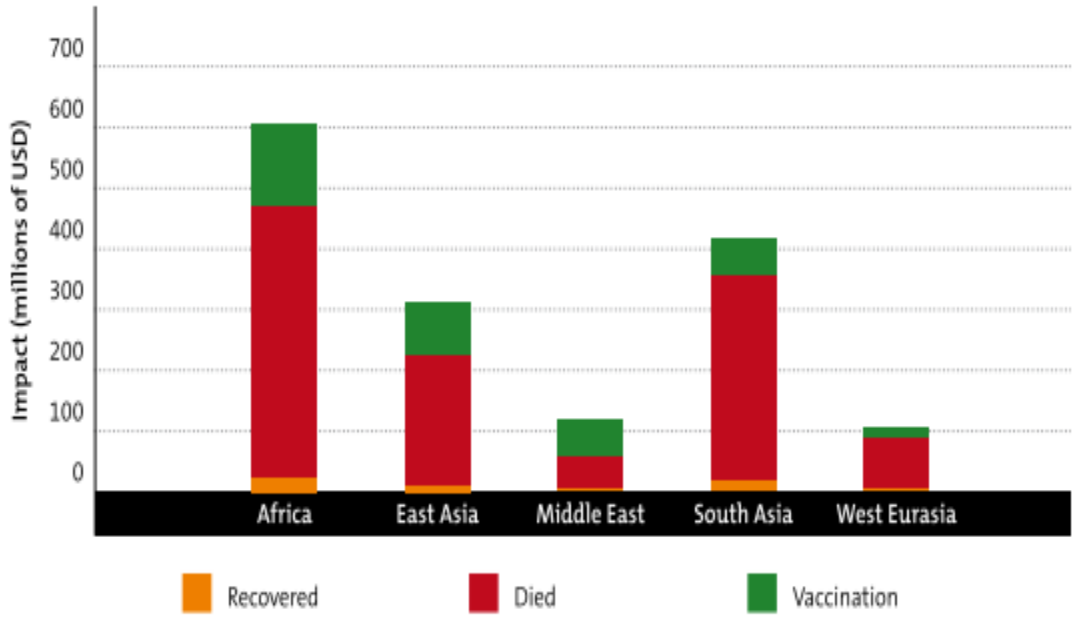


Figure 6: Yearly economic impact of PPR by region

Source: (OIE and FAO, 2015a).

2.8. Prevention and Control

Peste des Petits ruminants can be prevented, controlled or eradicated with a combination of measures such as quarantine, movement control, euthanizing infected/exposed animals, and cleaning and disinfection of infected premises. Rapid inactivation of the PPRV in the environment also aids for control and eradication in that this virus is thought to remain viable only for less than four days outside the animal. In addition to that, PPRV can also be inactivated by disinfectants; alkalis such as sodium carbonate and sodium hydroxide, halogens like sodium hypochlorite, phenolic compounds, citric acid, alcohols and iodophors. Moreover, PPR is controlled in endemic areas by vaccination. Animals that recover from infection develop good immunity, which persists for at least four years and possibly lifelong. To prevent infections in susceptible wildlife and captive wild animals such as gazelles, they should be prevented from having contact with sheep and goats. Vaccination might also be possible in these species (CFSPH, 2008; Abubakar *et al*., 2015).

Susceptible and infected animal movement control, restriction on importation of sheep and goats from affected areas and quarantining newly introduced animal for at least three weeks is also very important in prevention and control of PPR disease. Moreover, carcass of dead animals and contact fomites should be buried or burned and the barns, tools and other items that have been in contact with the sick animals must be disinfected. Vaccination should be carried before the start of the rainy season and annually in endemic areas (Bedore *et al*., 2019).

The most effective way to control PPR in a given area is mass immunization of small ruminants. Very effective commercial vaccines conferring a life-long immunity after a single administration are available. Homologous vaccine has been developed in the 1980s by attenuation of the Nigeria 75/1 strain through multiple passages on Vero cells. This vaccine provides a life-long immunity against PPR after a single shot and is used worldwide. However, it has a low thermal stability with half-life of 2-6 h at 37^{o}C after reconstitution. To prolong preservation time of sufficient virus titer, the vaccine strain has been mixed with cryo-protectant mixture containing trehalose. Thus, the vaccine can be stored for 5-14 days at 45^{o}C in the lyophilized form and for 21hr at 37^{o}C after reconstitution. These thermo stabilizing additives are compatible with the shipment of the vaccine to remote areas without the need for a cold chain. Alternative thermo-tolerant PPR-recombinant pox virus vaccines have been also engineered in the past (Albina *et al*., 2013).

Current PPRV attenuated vaccines are thermo labile and to avoid their thermal inactivation they require uninterrupted maintenance of the cold chain until their application to the animal. The currently commercially available vaccines are in freeze-dried form and they are stable for at least two years at 2°C to 8°C and for several years at -20°C. Once the vaccine is reconstituted, it needs to be utilized as soon as possible, but not later than 30 minutes after dilution. For PPR control, cell culture attenuated vaccine strains such as PPRV-Nigeria75/1, PPRV-Sungri/96 and also other strains are available for small ruminants. PPRV-Nigeria75/1 is commonly used in Africa, the Middle East, and parts of Asia, while PPRV-Sungri/ 96 strain vaccine is used in India and some of the countries in the Middle East and South Asia. However, currently there is no vaccine present with ability to identify infection from vaccination (DIVA) even though different trials have been done (Santhamani *et al*., 2016).

2.9. Status of the Disease in Ethiopia

Peste des petits ruminants was clinically suspected for the first time in Ethiopia in 1977 in a goat herd in the Afar region, east of the country. Later on clinical and serological evidence of its presence has been reported and confirmed in 1991 with cDNA probe in lymph node and spleen samples collected from an outbreak that happened in a goat holding area near Addis Ababa (Gebre *et al*., 2018; Woldemichael *et al*., 2018). This outbreak of the disease had caused more than 60% mortality and it was clinically characterized by ocular discharge, nasal discharge, mouth lesions, pneumonia, gastroenteritis and diarrhea (Delil, 2007).

The virus was also detected in 1994. Subsequently, the virus was isolated from this outbreak at the Institute of Livestock and Veterinary Medicine of Tropical Countries, Maisons-Alfort, France and the isolate was reported in 1996 being genetically determined to cluster in lineage III. The full genome of this virus was then sequenced in 2014 (Alemu *et al*., 2019; Dundon *et al*., 2020).

Full genome of another PPRV isolate from the intestine of a goats suffering from severe PPR clinical disease during an outbreak in 2010 was also characterized in 2016 and the isolate was classified to lineage IV, indicating that two PPRV lineages have been present in the country. However, two more recent studies on samples from 2011, 2014 and 2017 outbreaks in Ethiopia detected only lineage IV PPRVs, suggesting that the lineage III viruses may have been replaced by lineage IV viruses. Currently, there are five full genome sequences of PPRVs available in Gen Bank with KJ867540, KJ867541, MK991798, MK991799 and MK991800 accession numbers from Ethiopia (Dundon *et al*., 2020).

In 1999, national sero-surveillance of PPR was conducted in Ethiopia and the overall sero-prevalence of 6.4% in goats and sheep was estimated (Gari *et al*., 2017).Currently, PPR is endemic in Ethiopia and National Veterinary Institute (NVI) is producing live attenuated vaccine using PPR75/1 (LK6 Vero74) strain to combat the disease (Bedore *et al*., 2019). Different serological studies conducted across the country in different areas reported a sero-prevalence ranging from 0.7 to 75.7 in small ruminants (**Table 2**).

Table 2: Seroprevalence studies of PPR in different hosts and regions of Ethiopia

Study regions/areas in Ethiopia	Number tested by c-ELISA, species of animal (prevalence)	Reference
Afar, Borena, East Shewa, Gambella, Jijga	835 Sheep (13%), 442 Goat (9%) and 910 Cattle (9%)	Abraham, 2005
Afar (Awash Fentale)	238 Sheep and Goats (36.6%)	Delil *et al*., 2012
	1239 Sheep and Goats (1.7%)	Delil, 2007
Gambella	779 Sheep and Goats (27.2%)	Megersa *et al*., 2011
Afar	384 Sheep and Goats (38.3%)	
	1653 Sheep and Goats (15.3%)	Waret-szkuta *et al*.,2008
Amhara	5992 Sheep and Goats (4.6%)	Waret-szkuta *et al*., 2008
	672 Sheep and Goats (18.3%)	Fentie *et al*., 2018
Amhara (North Shewa)	613 Cattle (10.6%)	
	1065 Sheep (11%)	Agga *et al*., 2019
	1325 Goats (9.6%)	
Eastern Amhara	612 Sheep and Goats (56.5%)	Alemu, 2014
Benishangul-Gumuz	729 Sheep and Goats (8%)	Waret-szkuta *et al*., 2008
Benishangul (Asosa)	321 Sheep and Goats (75.7%)	Yalew *et al*., 2019
Benishangul (Metekel)	452 Sheep and Goats (73.45%)	Woldemichael *et al*., 2018
Oromia	700 Sheep and Goats (48.43%)	Gari *et al*., 2017
Oromia (Horo Guduru)	806 Sheep and goats (27.42%)	Gelana *et al*., 2020
Oromia	2290 Sheep and Goats (1.7%)	Waret-szkuta *et al*., 2008
SNNPR(Siltie Zone)	160 Sheep and Goat (24.2%)	
SNNPRGurage zone)	221 Sheep and Goats (33%)	Hailegebreal, 2018
SNNPR(Bench Maji)	429 Sheep and Goats (3.7%)	
SNNPR (Kafa zone)	539 Sheep and Goats (0.7%)	Gebre *et al*., 2018
Somali	465 Sheep and Goats (21.3%)	Waret-szkuta *et al*., 2008
	472 Sheep and Goats (41%)	Dejene, 2016
Tigray	900 Sheep and Goats (15.3%)	Waret-szkuta *et al*.,2008
	240 Goats (47.5%)	Afera *et al*., 2014

3. MATERIALS AND METHODS

3.1. Study Area Description

The study was conducted in selected districts of Metekel and Awi zones, North West Ethiopia. Specifically, Pawi district from Metekel Zone and Jawi and Guangua districts from Awi zones were selected for the study. Metekel zone is found at 567 km Northwest from Addis Ababa in Benishangul Gumuz Regional State and lies between 9.9 and 12.5° N and 34.9 and 36.6° E. The zone covers a total area of 25,705 km^2 and has road density of 28.4 km per 1000 km^2. The minimum and maximum altitudes of the zone are 300 and 2700m respectively. The zone has also 23°C mean minimum and 31.1°C mean maximum annual temperature (Metekel zone department of Agriculture).

Awi zone is one of the eleven administrative zones of Amhara National Regional State. Enjibara is the administrative town of the zone that is located about 430 km far away from Addis Ababa in the Northwest direction. The zone is bordered with Benishangul Gumuz region in the West, North Gondar in the North, Oromia region in the South and West Gojam in the East direction. It has a mean annual rainfall of 1,750 mm and a mean monthly temperature that ranges from 17°C to 27°C (Mazengia, 2016).

Pawe district is located at altitude of 1064m.a.s.l with latitude of $11^0 15´24.7´´N$ and longitude of $36^0 23´10´´E$. It has 20 Kebeles covering an area of 64,300 hectare with estimated human population of 59,127 (50.76% male) inhabitants. The area experiences a temperature ranging from 19.40°C to 37.6°C temperature and an annual rain fall ranging from 1186 to 1977 mm. The livelihood of the society largely depends on mixed livestock and crop production (Asmamaw and Getachew, 2016; Berhanu *et al*., 2018). The district have livestock population of 75, 873 cattle, 7868 goat, 6862 sheep, 1348 equines, 29378 poultry and 3076 beehives (Pawe district agricultural office unpublished report, 2020).

Jawi is found within the geographical location of 10^0 38` to $11^0$ 30` N latitude and 36^0 to 37^0 E longitude (Wondim, 2019). It receives alternating rain fall with long summer rain fall (June to September) and a winter dry season (October to May) with mean annual rain fall of 1569.4mm. The altitude of the district ranges from 648-1300 m.a.s.1 with a mean temperature varying from 16.68^{o}C to 37.6^{o}C. The area is covered by different vegetation types such as savanna grass land, forest, riverine and bush lands. Jawi district is characterized both by crop and livestock production with the major agricultural products like sorghum, maize, sesame and cotton. The livestock population of the district is estimated to be about 362297 cattle, 27521 sheep, 87683 goats, 23718 equines, 122121 poultry and 23396 bee hives (Jawi district agricultural office unpublished report, 2020).

Guangua district is located at about 52 and 513 km away from Enjibara and Addis Ababa respectively. It has latitude of 10.950°N and longitude of 36.500°E with an elevation ranging from 1583 to 1710 m.a.s.l. Its temperature ranges from 22 to 31^{0}C with annual rainfall of 1300-1800mm. The total area of the district is about 106,914 hectares with an estimated total human population of 223, 066 and the house hold size of 11,936. Guangua is bordered on the South and West by the Benishangul-Gumuz Region, on the North by Dangila, on the Northwest by Faggeta Lekoma and Banja Shekudad, and on the East by Ankasha Guagusa (Mazengia, 2016; Dawud and Aki, 2018). The district has an estimated livestock population of 125683 cattle, 29828 sheep, 24,714 goats, 11039 equines, 83, 446 poultry and 1989 bee hives according to 2020 Awi zone agricultural office unpublished report.

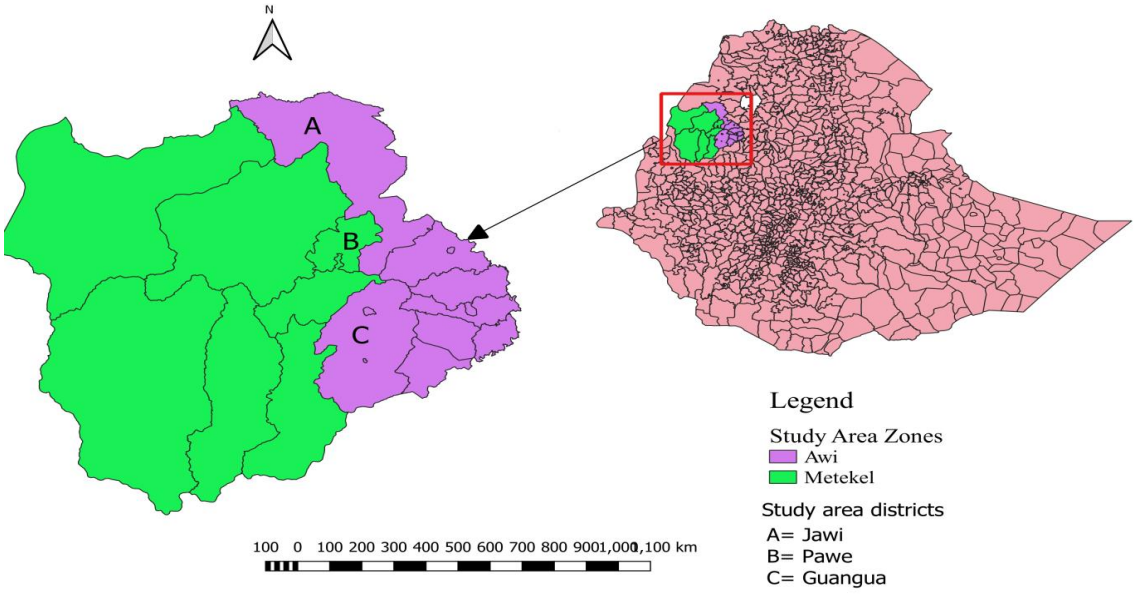


Figure 7: Map of the study area

3.2. Study Population

This study was conducted on small ruminant animals found in Metekel and Awi zones. Animals with ages greater than 6 month were considered for serological investigation to exclude possibility of sero-positivity results due to maternal antibody. Only non-vaccinated sheep and goats were included for serum collection. However, all age groups of the animals with clinical disease or active lesion were considered for the molecular detection. Sheep and goats of both sex and all body condition were also included in the study.

Herd size of the study animals was categorized as large (> 20 animals), medium (10-20 animals) and small (< 10 animals) according to Gebre *et al*., 2018. The age of the study animals was determined by asking the owner and by dentition according to ESGPIP 2009 and categorized in to three age group as young (<1 year), adult (1-3 year) and old (>3) (Husen *et al*., 2018). Body condition of the animals was recorded and categorized as poor, medium and good (ESGPIP, 2009; Yasin *et al*., 2017).

3.3. Study Design

This study was cross-sectional study conducted from January 2020 to May 2021 to reveal epidemiology of PPR, to isolate and molecularly detect PPRV circulating in selected districts of Metekel and Awi zones, North West Ethiopia. For the isolation and molecular detection of the virus, blood and swab samples were collected from active cases of the disease and outbreaks.

3.4. Retrospective Epidemiological study

A six year (2016-2021) retrospective epidemiological data describing place, number, season, year and month of PPR outbreak, number of sick and dead animals, number and species of animals at risk was collected from Ministry of Agriculture and analyzed to appreciate current epidemiological status of the disease.

3.5. Questionnaire Survey

Semi-structured questionnaire was developed and 8-10 flock owners from each study area Kebeles were interviewed to reveal possible risk factors that can have associations with PPR occurrence. The objectives of the survey were explained and informed consent was obtained from the respondents before the start of the interview. Accordingly, information on risk factors such as flock size, age, sex, vaccination history, health status, grazing management, raising type, introduction of new animals, inter-herd contact, animal movement, live animal market visiting frequency, clinical signs of disease encountered, number of diseased and dead animals and awareness of the respondents on PPR was collected.

Figure 8: Interviewing the farmers to gather risk factor and other PPR related information

3.6. Sample Size Determination

The sample size for this study was determined at 5% desired absolute precision based on the formula described by Thrusfield (Thrusfield, 2005) using 95% confidence interval and 64.4% expected prevalence which is the average previous sero prevalence study findings of 73.45% (Woldemichael *et al*., 2018) and 55.34% (Fentie *et al*., 2018) in Metekel and Awi zones respectively using the formula;

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

Where N= required sample size, P_{exp} = expected prevalence and d=desired absolute precision. Thus, substituting the respective values in the formula, 352 sheep and goats were found to be required for the study. However, to increase precision of the sample estimate a total of 714 sera samples were collected for seroprevalence part of this study.

3.7. Sampling Techniques

Pawe district from Metekel Zone, and Jawi and Guangua districts from Awi Zone were purposively selected based on history of PPR occurrence, accessibility to transport and small ruminant population potential. Then, lists of Kebeles found within the districts were obtained from respective agricultural offices from which the sampling Kebeles were selected based on sheep and goat population. In such away, four Kebeles from Pawi district and three Kebeles from each Jawi and Guangua districts were purposively selected based the study animal population potential. From each Kebeles, three to four peasant associations (PAs) were also selected for the study. Accordingly, the number of sheep and goats sampled for serological study were proportionally allocated at each level throughout districts to Kebeles depending on small ruminant density. Subsequently, limited numbers of households having sheep and goats were selected from each PAs using systematic random sampling method and finally, simple random sampling method was used to select the small ruminant animals to be sampled.

3.8. Sample Collection and Transportation

After careful restraining of the study animals, blood samples using clot activator plain vacutainer tubes for serological analysis and swabs (nasal, rectal and ocular) from active clinically suspect cases for molecular detection were aseptically collected. Blood sample was also collected using K3 EDTA vacutainer tubes from suspect cases for molecular diagnosis. Briefly, jugular vein area was disinfected using 70% alcohol and 5ml blood was collected from the vein using venoject needle and vacutainer tubes. The nasal swabs were collected using sterile Rayon cotton swab and put in cryogenic vials with virus transport medium (VTM) containing BPS, antibiotic and antifungal. The animals from which the sample was collected were treated with ivermectin 1% injection. Animals which were clinically suspected with PPR were also treated with tylosin.

In such away, the collected blood and swab samples were labeled with date of collection, specific identification number and species of the animals and transported to Pawe Agricultural Research Center laboratory in cool ice box for temporary storage. In the laboratory, the blood sample collected with plain vacutainer tubes were allowed to stand in slant position for 24 hours at room temperature to collect serum. After 24 hours, the samples from which serum was not clearly separated were centrifuged at 5000 rpm for three minutes to remove the remaining red blood cells and collect clear serum. Then, the serum was harvested using sterile micropipette into cryogenic vials and stored at -20°C in aliquots. The blood collected in EDTA tubes and the swab samples were also stored at $+4^{o}$Cand -20°C respectively. Finally, the samples were transported to the Animal Health Institute (AHI) being chilled in a cool ice box for serological and molecular analysis.

Place of sample collection, age, sex, species, body condition and vaccination status of the animals was recorded during sample collection. Other risk factors such as herd size, housing, origin of animals, physiological status, grazing management, presence of inter herd contact, recent introduction of new animal and isolation practice of infected small ruminant animals were also collected during sampling.

3.9. Serological Study

Laboratory analysis of the collected sera samples were tested at AHI for the detection of specific PPRV antibody using N protein based c-ELISA kit according to the manufacturer instruction (ID Screen® PPR Competition, IDvet innovative diagnostics, France).

In brief, all reagents were allowed to come to room temperature ($21^{o}C \pm 5^{o}C$) and were homogenized by vortex before use. Test plate lay out was prepared for the samples and 100µl of each samples was dispensed in to micro plate wells using single channel pipette based on the plate lay out, sealed with adhesive plastic sealants and temporarily incubated at $+4^{o}C$. Then, the procedure commences on other new plates as follows; 25µl of dilution buffer 13 was added to each micro plate wells. A 25µl positive control (to A1 and B1 wells) and 25µl of the negative control (to C1 and D1 wells) were added to the respective plate wells. Subsequently, 25µl of each 100µl dispensed samples were added to the remaining wells using multichannel pipette, sealed with adhesive plastic plate sealants to prevent cross contamination and evaporation of the samples during incubation.

Then, the plates were incubated at $37^{o}C$ for 45 minutes and each plate wells were washed three times with 300µl working Wash Solution prepared from Wash Solution 20X avoiding drying of the wells in between the washings. Conjugate 1X was prepared by diluting conjugate 10X to 1/10 in dilution buffer 4 and 100µl of the conjugate was added to each well. The plates were incubated at $21^{o}C$ for 30 minutes and each well was washed three times with approximately 300µl of the wash solution. 100µl of the substrate solution was added to each well and incubated at $21^{o}C$ for 15 minutes in the dark. Finally, 100µl stop solution was added to each plate well in order to stop the reaction and the optical density (OD) for each sample was read and recorded at an inference filter of 450nm using ELx800 BioTek ELISA reader (BioTek, USA). The reader was connected to computer loaded with Gen 5^{TM} 3.04 software for automated reading and calculation of competition percentage (S/N %) values. Thus, samples with S/N% $\leq$ 50 % and S /N % > 60 % were considered positive and negative respectively.

3.10. Culture and Isolation of the Virus

Vero Dog SLAM (VDS) cells (Pirbright laboratory, UK) grown in CORNING® 24 well culture flask of 2cm^2 growth area was used for culture and propagation of the PPR virus. The samples taken from the same village were pooled together and processed for inoculation to the VDS cells. Thus, a total of 10 pooled samples were inoculated to confluent monolayer VDS cells for isolation of the virus. In brief, the swab samples were thoroughly macerated in VTM used for collection, centrifuged at 3000rpm for 20 minutes and filtered using sterile 0.22µm filter syringe (Millex™ Syringe Driven Filter unit, Millipore Corporation, USA). The whole blood samples were also prepared by washing 3ml of the samples three times with 3x PBS solution and centrifuging at 2500rpm for five minutes in each wash steps. Then, 1ml of RBC sediment was lysed with 9ml sterile distilled water, centrifuged at 600g for 10min and the supernatant was collected for inoculation (Mallinath *et al*., 2018).

Accordingly, 0.1 to 0.2 ml of the swab and whole blood supernatants were inoculated to test wells with VDS monolayer cell culture (about 80% confluence) pre washed twice by 500µl PBS followed by inoculation of VTM containing antibiotics and antifungals on negative control flask wells. Then, the flask was incubated at 37^{o}C, in 5% CO_2 and 96% humidity adjusted incubator for 60 minutes with intermittent shaking to allow adsorption of the virus on to the cell culture. After incubation, the virus inoculum was decanted, the infected cells washed with serum free Dulbeccos`s Minimum Essential Medium (DMEM) (Gibco campany, USA) and incubated at 37^{o}C with maintenance medium (DMEM with 2% serum). The cells were then inspected daily for evidence of CPE under inverted light microscope for 7days. Cultures not showing CPE were processed up to 3 blind passages. Finally, the flask showing CPE was frozen as soon as the CPE involved about 70% of the cell layer and the presence of the virus in the medium was confirmed by testing the cell culture supernatant with RT-PCR (Alemu *et al*., 2019).

3.11. Molecular detection

The blood and swab (nasal, ocular and rectal) samples collected from clinically sick sheep and goats were tested for the presence of PPRV RNA by one step real time RT-PCR kit targeting N-gene of the virus.

3.11.1. Sample processing and RNA extraction

The swab and whole blood samples were equilibrated to room temperature and processed for RNA extraction. The swab samples were thoroughly macerated in the VTM used for collection using vortex mixer and the whole blood samples were mixed carefully by inverting the tubes to prevent hemolysis. The RNA was extracted from the processed samples and culture isolates by using QIAmp® Viral RNA Mini kit (QIAGEN, Cat. no.52906, Hilden, Germany) according to the manufacturer's instructions.

Briefly, buffer AVL containing carrier RNA was prepared and 560µl of the buffer was pipetted into 1.5 ml micro centrifuge tubes prepared based on the number of the samples to be processed. 140µl of the samples was added to the Buffer AVL-carrier RNA in the tubes, thoroughly mixed with the buffer and incubated at room temperature for 10 minutes.

Then, 560µl absolute ethanol (96-100%) was added to the samples and thoroughly mixed by vortexing for 15 seconds. Subsequently, 630 µl of the solution was carefully transferred to QIAamp Mini column in 2ml collection tube and centrifuged at 8000 rpm for 1minute closing the cap of the Mini column. After centrifugation, the Mini column was placed into a clean collection tube and the remaining 630 µl solution was added to the column followed by centrifugation at 8000 rpm for 1 minute. Next, 500µl Buffer AW1 was added to the Mini column in clean collection tube and centrifuged at 8000 rpm for 1 minute. Then, 500µl Buffer AW2 was added to the Mini column by changing the collection tube and centrifuged at 14000 rpm for 3 minutes.

Subsequently, the QIAamp Mini column was placed in a new collection tube and re-centrifuged at 14000 rpm for 1 minute to eliminate any chance of Buffer AW2 carryover. Finally, the Mini column was placed in a clean 1.5 ml microcentrifuge tube, 60µl elusion buffer (Buffer AVE) equilibrated to room temperature was added to the mini column and centrifuged at 8000 rpm for 1 minute after following incubation at room temperature for 1 minute. Accordingly, the extracted RNA product was labeled and stored at -80^{o}C for further processing.

3.11.2. Real time RT-PCR

Real time RT-PCR was done for the extracted samples by using Applied Biosystems 7500 fast one step real time PCR thermocycler machine. The extracted RNA was converted to cDNA using a reverse transcriptase enzyme and the cDNA was amplified using PPRV specific forward and revers primers (Hodgsan, 2018). In brief, a master mix was prepared (Table 3), vortexed and 17µ l of the mix was added to plate wells followed by addition of 3µl extracted RNA product to each wells. Subsequently, Rnase free water as negative control and standard Nigerian 75/1 strain vaccine as positive controls were added to two other wells each. Then, the plates were sealed with adhesive sealants and loaded into thermal cycler machine for processing. The amplification reaction was set by creating a plate sheet for the PCR machine to run using 7500 Fast System SDS Software. Accordingly, the amplification was carried out with the final reaction volume of 20µl containing 17µl master mix and 3µl RNA template submitted to a thermal profile of one cycle of reverse transcription at 50^{o}C for 15 minutes, one cycle of reverse transcription inactivation/Taq activation at 95^{o}C for 20 seconds and 45 cycles of denaturation at 95^{o}C for 3 seconds followed by annealing and final extension at 60^{o}C for 30 seconds (Hodgsan, 2018; Tolessa, 2020). In such away, the clinical samples detected at cycle threshold (Ct) values < 35 were declared positive.

Table 3: Master Mix recipe used for PPRV detection using one step real time RT-PCR

No.	Component	Volume per well (µl)
1	EXPRESS Super Script® qPCR Super Mix Universal	10
2	Forward primer (10µM) (5′-AGAGTTCAATATGTTRTTAGCCTCCAT-3′)	0.8
3	Reverse primer ((10µM) (5′-TTCCCCARTCACTCTYCT TTGT-3′)	0.8
4	RNase free water	2.6
5	Rox (diluted 1 to 10 factor)	0.4
6	EXPRESS Super Script® Mix	2
7	Probe (5µM) (5′-FAM-CACCGGAYACKGCAGCTGACTCAGAA-TAMRA-3′)	0.4
	Total volume	17

3.12. Data Analysis

The collected raw data was entered into Microsoft Excel spread sheet and analyzed by using R software version 4.0.2. Descriptive statistics was used to summarize the data and the prevalence was calculated for all associated risk factors as the number of PPR infected individuals divided by the number of individuals sampled multiplied by 100. The Pearson's chi- square ($\chi 2$) was also used to test the existence of differences in prevalence between species, age groups, sex, body condition scores and place of sample collection.

Both univariate and multivariate logistic regression were used to assess association the risk factors have with the disease. For multivariable logistic regression analysis, all putative risk factors considered in the study were run together and simplified by backward step wise model simplification method to get the final regression model. Moreover, the strength of the association between the risk factors and PPR sero-positivity was estimated using the odds ratios (OR). The risk factors with OR equals to 1 are considered to be unlikely associated with PPR sero-positivity and those with OR greater or less than 1 are considered to be more likely associated with PPR sero-positivity and the stronger the association. Statistically significant association between variables was considered to exist if the computed P-value at 95% confidence interval and 5% degree of precision is less than 0.05 (Dejene, 2016). The study area map and maps indicating retrospective data based spatial distribution of PPR in this paper were created using QGIS 3.20.1 version.

3.13. Ethical clearance

Ethical clearance for the study was approved by and obtained from Addis Ababa University College of Veterinary Medicine and Agriculture animal research ethical review committee with approved certificate reference number of VM/ERC/17/03/13/2021 indicated in **Annex 14**. The samples for this study were carefully collected without causing any harm to the animals following all ethical procedures for sampling, enrollment and sample collection. Prior to sample collection from the animals, objectives of the study were discussed and informed consent was sought from the owners.

4. RESULTS

4.1. Serological study

Out of the total 714 sera samples tested for PPRV antibodies, 467 (65.4%) were detected positive. Sero prevalence of 60.8% (231/380) in Awi and 70.7% (236/334) in Metekel zone was recorded with statistically significant difference in the prevalence between the zones (P=0.007, χ^2=7.2). Significantly different PPR antibody prevalence was also observed in between the study area districts (χ2=47.7, P value=0.0001). The highest prevalence (76.2%) was detected in Jawi district (CI=69.4-81.9) followed by Pawe (70.7%) indicated in **Table 4**.

Table 4: Seroprevalence of PPRV antibody in the study area zones, districts and Kebeles

Factors		**Levels**	**No. tested**	**No. positive**	**%Prevalence (95% CI)**	**χ2**	**P value**
Zones		Awi	380	231	60.8 (55.7-65.7)	7.2	0.007
		Metekel	334	236	70.7 (65.4- 75.4)		
		Total	**714**	**467**	**65.4 (61.8-68.9)**		
Districts		Guangua	191	87	45.5 (38.4-52.9)	47.07	0.0001
		Jawi	189	144	76.2 (69.4-81.9)		
		Pawe	334	236	70.7 (65.4-75.4)		
		Total	**714**	**467**	**65.4 (61.8-68.9)**		
Kebeles	Guangua	Bizrakani	70	27	38.6 (27.4-51)	64.3	0.0000
		Luisdegera	66	28	42.4 (30.6-55.2)		
		Tirubirhan	55	32	58.2 (44.1-71)		
	Jawi	Adisweyni	72	48	66.7 (54.5-77.1)		
		Ayma	65	53	81.5 (69.6-89.7)		
		Jayimela	52	43	82.7 (69.2-91.3)		
	Pawe	Almu	80	66	82.5 (72-89.8)		
		Mender 14	83	57	68.7 (57.4-78.2)		
		Mender 23/45	96	62	58.2 (54.1-73.9)		
		Mender 50	75	51	68 (56.1-78)		
		Total	**714**	**467**	**65.4 (61.8-68.9)**		

Highest Kebele wise prevalence of PPR was recorded in Jayimela (82.7%) followed by Almu (82.5%) and Ayma (81.5%) while the lowest seroprevalence was recorded from Bizrakani Kebele (38.65). There was highly significant difference in prevalence and association of the disease between the Kebeles (X^2=64.3, P=0.0000) (**Table 4**). Out of total 364 sheep and 350 goat samples tested, 243 (66.8%) and 224 (64%) were found to be positive to PPR antibody respectively. It is revealed that there is no statistically significant difference in the prevalence between species of the animals (P=0.49) (**Table 5**).

Table 5: Seroprevalence of PPRV antibody based different risk factors

Factors	**Categories**	**No. tested**	**No. positive**	**% prevalence (95% CI)**	**χ2**	**P value**
Species	Ovine	364	243	66.8 (61.6-71.5)	0.48	0.49
	Caprine	350	224	64 (58.7-69)		
Age	Young	280	146	52.1 (46.1-58.1)	36.12	0.0000
	Adult	216	157	72.7 (66.1-78.4)		
	Old	218	164	75.2 (68.9-80.7)		
Sex	Female	523	363	69.4 (65.2-73.3)	13.18	0.0003
	Male	191	104	54.4 (47.1-61.6)		
Body condition	Good	265	164	61.9 (55.7-67.7)	3.2	0.2
	Medium	400	267	66.8 (61.9-71.3)		
	Poor	49	36	73.5 (58.7-84.6)		
Origin	Born in herd	560	356	63.6 (59.4-67.5)	3.5	0.06
	Brought in	154	111	72.2 (64.2-78.8)		
Flock size	Small	527	345	65.5 (61.2-69.5)	7	0.03
	Medium	137	97	70.8 (62.3-78)		
	Large	50	25	50 (36.6-63.3)		
Inter herd contact	Absent	84	57	67.9(56.7-77.4)	0.14	0.7
	Present	630	410	65.1 (61.2-68.8)		
Introduction of new animal	Absent	448	288	64.3 (59.6-68.7)	0.54	0.46
	Present	266	179	67.3 (61.3-72.8)		

Table 5 (continued).

Factors	Categories	No. tested	No. positive	% prevalence (95% CI)	χ2	P value
Housing	Alone and bed	223	132	59.2 (52.4-65.6)	6	0.049
	Alone and floor	326	219	67.2 (61.7-72.2)		
	Floor and mixed	165	116	70.3 (62.6-77)		
Grazing management	Zero grazing	25	14	56 (32.3-75)	1.01	0.6
	Private	61	40	65.6 (52.2-77)		
	Communal	628	413	65.8 (61.9-69.4)		
Isolate sick	No	685	455	66.4 (62.7-70)	6.65	0.001
	Yes	29	12	41.4 (24.1-60.9)		

The age wise PPRV antibody seroprevalence was significantly higher in old animals (75.2%, CI=68.9-80.7) and lower in young animals (52.1%) (P=0.000, $\chi2$=36.12). The prevalence was also higher in female animals (69.4%) than male animals with statistically significant difference between the sex groups (P=0.000, CI=65.2-73.3). Sheep and goats with poor body condition were found to be most seropositive to PPR antibody (73.5%) followed by those with medium body condition (66.8%) even though the difference in the seropositivity was statistically not significant ($\chi2$=3.2, P=0.2). Statistically significant prevalence was observed with categories of flock size, housing system and isolation of sick animals (P< 0.05). The prevalence was significantly high in animals from medium flock size (70.8%, P=0.03, CI=62.3-78) and living in floor housing system being mixed with other animals (70.3%). It was also higher in condition where there is no isolation practice of sick animals from herd (66.4%, P=0.001, CI=62.7-70). Prevalence based on absence/presence of inter-herd contact, grazing management and absence/presence of introduction of new animals to the herd from which the animals sampled were recorded to be non-significant in between respective categories (P> 0.05) (**Table 5**).

Uni-variable logistic regression was run to identify the possible individual risk factors for seropositivity to PPRV antibody. Accordingly, from the putative risk factors evaluated for the seropositivity district, age, sex, flock size, physiological status and isolation status of sick animals were found to be statistically significant risk factors. Origin and housing systems of the animals were also identified as significant factors to PPR seropositivity (**Table 6**).

Table 6: Univariable logistic regression analysis of factors for PPRV seropositivity

Variable	Category	No. tested	No. positive	Odd ratio (95% CI)	Coeffi cients	P value
District	Guangua (ref)	105	56			
	Jawi	71	53	3.8 (2.5-6)	1.34	0.000
	Pawe	188	134	2.9 (2-4.2)	1.1	0.000
Species	Ovine (ref)	364	243			
	Caprine	350	224	0.9 (0.6-1.2)	-0.12	0.44
Age	Young (ref)	280	146			
	Adult	157	216	2.4 (1.7-3.6)	0.89	0.000
	Old	218	164	2.8 (1.9-1.8)	1.03	0.000
Sex	Female (ref)	523	363			
	Male	191	104	0.5 (0.4-0.7)	-0.64	0.000
Body	Good (ref)	265	164			
condition	Medium	400	267	1.2 (0.9-1.7)	0.21	0.2
	Poor	49	36	1.7 (0.9-3.5)	0.53	0.12
Flock size	Small (ref)	527	345			
	Medium	137	97	1.3 (0.9-1.9)	0.25	0.24
	Large	50	25	0.5 (0.3-0.9)	-0.64	0.03
Origin	Born in herd(ref)	560	356			
	Brought in	154	111	1.5 (1-2.2)	0.4	0.05
Inter-herd	Present (ref)	630	410			
contact	Absent	84	57	1.1 (0.7-1.9)	0.12	0.62
Grazing	Communal (ref)	628	413			
management	Zero grazing	25	14	0.7(0.3-1.5)	-0.41	0.3
	Private	61	40	1 (0.6-1.8)	-0.008	0.9
Housing	Alone and floor (ref)	326	219			
	Alone and bed	223	132	0.7 (0.5-1)	-0.34	0.05
	Floor and mixed	165	116	1.2 (0.8-1.7)	0.15	0.48
Introduction	Absent (ref)	448	288			
of new animal	Present	266	179	1.1 (0.8-1.6)	0.13	0.4
Isolate sick	No (ref)	685	455			
	Yes	29	12	0.3 (0.2-0.8)	-1.03	0.007

From multivariable logistic regression analysis, district, age, body condition and housing system of the animals were identified as potential risk factors for PPRV seropositivity being significantly associated with the virus antibody prevalence (P< 0.05). The likelihood of being seropositive to PPRV infection was 3.5 higher in old animals and 2.8 times higher in adult animals compared to young ones. Sheep and goats housed separately in a bed house are also found to be at lower risk for PPR infection (OR=0.6, P Value=0.038) in reference to those housed separately on floor. The odd of being positive to PPR seropositivity is also 1.5 times higher when the animals are housed together on floor. Sheep and goats with poor body condition are more likely to be seropositive to PPRV antibody (OR=1.5, P value=0.25). Isolating PPR infected sheep and goats from the flock are also found to decrease a chance of transmission of the disease by 0.5 times (**Table 7**).

Table 7: Multivariable logistic regression analysis of the risk factors

Variable	**Category**	**No. tested**	**No. positive**	**Odd ratio (95% CI)**	**Coeffi cients.**	**SE**	**P-value**
District	Guangua (ref)	105	56				
	Jawi	71	53	4.5 (2.8-7.4)	1.5	0.25	0.000
	Pawe	188	134	4 (2.6-6.2)	1.4	0.22	0.000
Age	Young (ref)	280	146				
	Adult	157	216	2.8 (1.9-4.2)	1.02	0.21	0.000
	Old	218	164	3.5 (2.3-6.2)	1.3	0.22	0.000
Body	Good (ref)	265	164				
condition	Medium	400	267	1.4 (1-2.0)	0.33	0.18	0.057
	Poor	49	36	2.2(1- 4.8)	0.78	0.39	0.04
Flock	Small (ref)	527	345				
size	Medium	137	97	1.4 (0.9-2.3)	0.36	0.24	0.12
	Large	50	25	0.7 (0.4-1.4)	-0.33	0.34	0.33
Housing	Alone and floor (ref)	326	219				
	Alone and bed	223	132	0.7 (0.4-1.0)	-0.44	0.21	0.038
	Floor and mixed	165	116	1.5 (0.9-2.4)	0.4	0.24	0.09
Isolate	No (ref)	685	455				
sick	Yes	29	12	0.5 (0.2-1.1)	-0.74	0.42	0.08

4.2. Retrospective Outbreak Epidemiological Data Analysis

4.2.1. Temporal distribution of PPR

A retrospective data (September 2016 to August 20210 showed a total of 632 PPR outbreaks with 255794 cases, 116341 deaths and 20,344,804 populations at risk from different agro-ecological regions of Ethiopia. The highest number of outbreak was reported in 2019 year (243 outbreaks) followed by 2020 (132 outbreak while the minimum outbreak report of the disease was reported in 2018 year (26 outbreaks) (**Figure 9**).

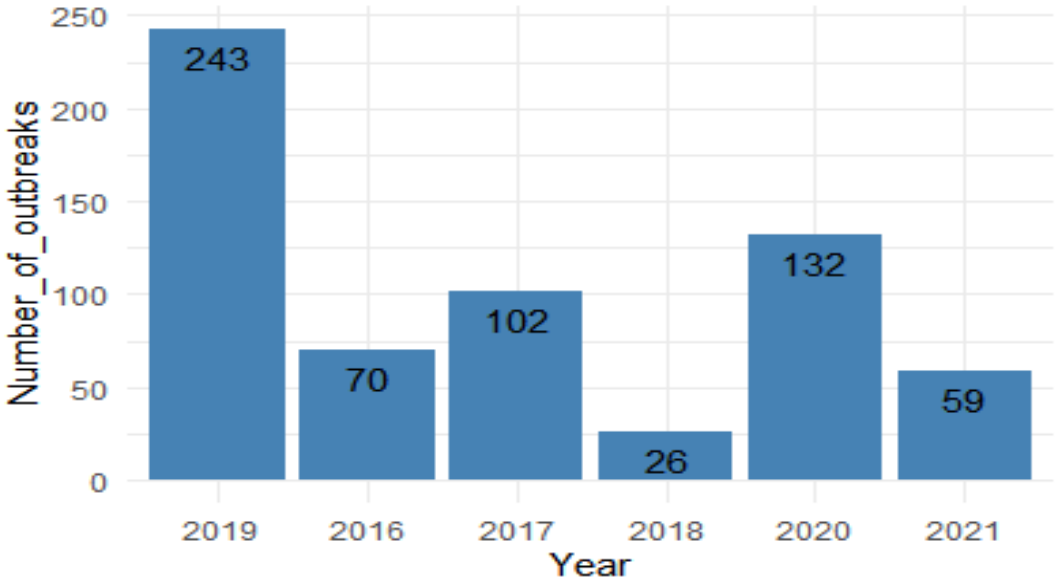


Figure 9: Number of PPR outbreaks reported from September 2016 to August 2021

The monthly and overall patterns of the disease for each year are respectively indicated below in **Figure 10**. The highest year based seasonal PPR incidence was reported in January 2019 (41 outbreaks) followed by April (35 outbreaks) and February 2019 (34 outbreaks). The next highest report was from November 2017 (32 outbreaks). The overall seasonal outbreak report was found to be high in April (84 outbreaks) followed by February (81 outbreaks) and January (75 outbreaks) while the lowest incidence of the disease was reported in May (25 outbreaks) based on the analyzed retrospective data.

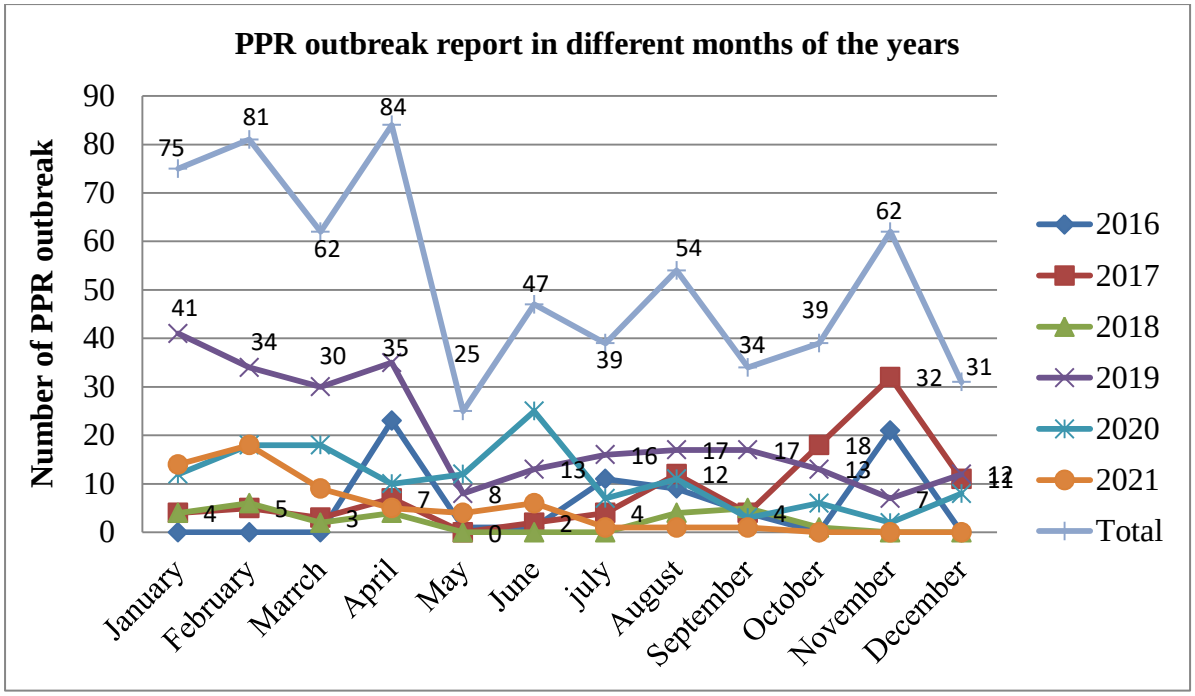


Figure 10: PPR year based and overall seasonal outbreak pattern from 2016 to 2021

4.2.2. Spatial distribution of PPR disease

According to the retrospective outbreak data obtained from MoA, only 7 regions of Ethiopia had reported incidence of PPR disease throughout the study years (2016 to 2021) despite the presence of the disease in the remaining regions as well. The highest number of PPR outbreak was recorded in Amhara region (258 outbreaks) followed by Oromia, Somali, Afar, SNNP, Tigrai and Benishangul Gumuz regions, respectively (**Figure 11**).

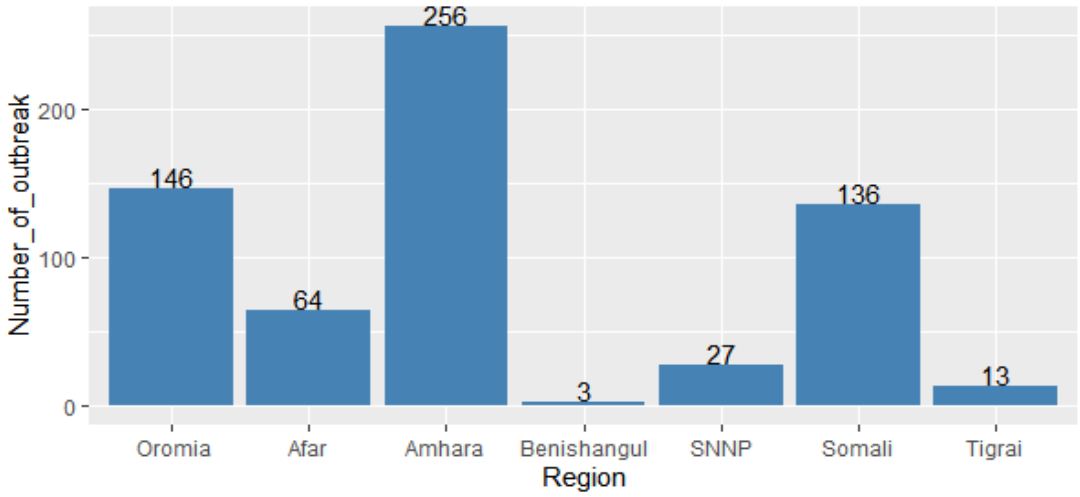


Figure 11: Region wise PPR outbreak reports from September 2016 to August 2021

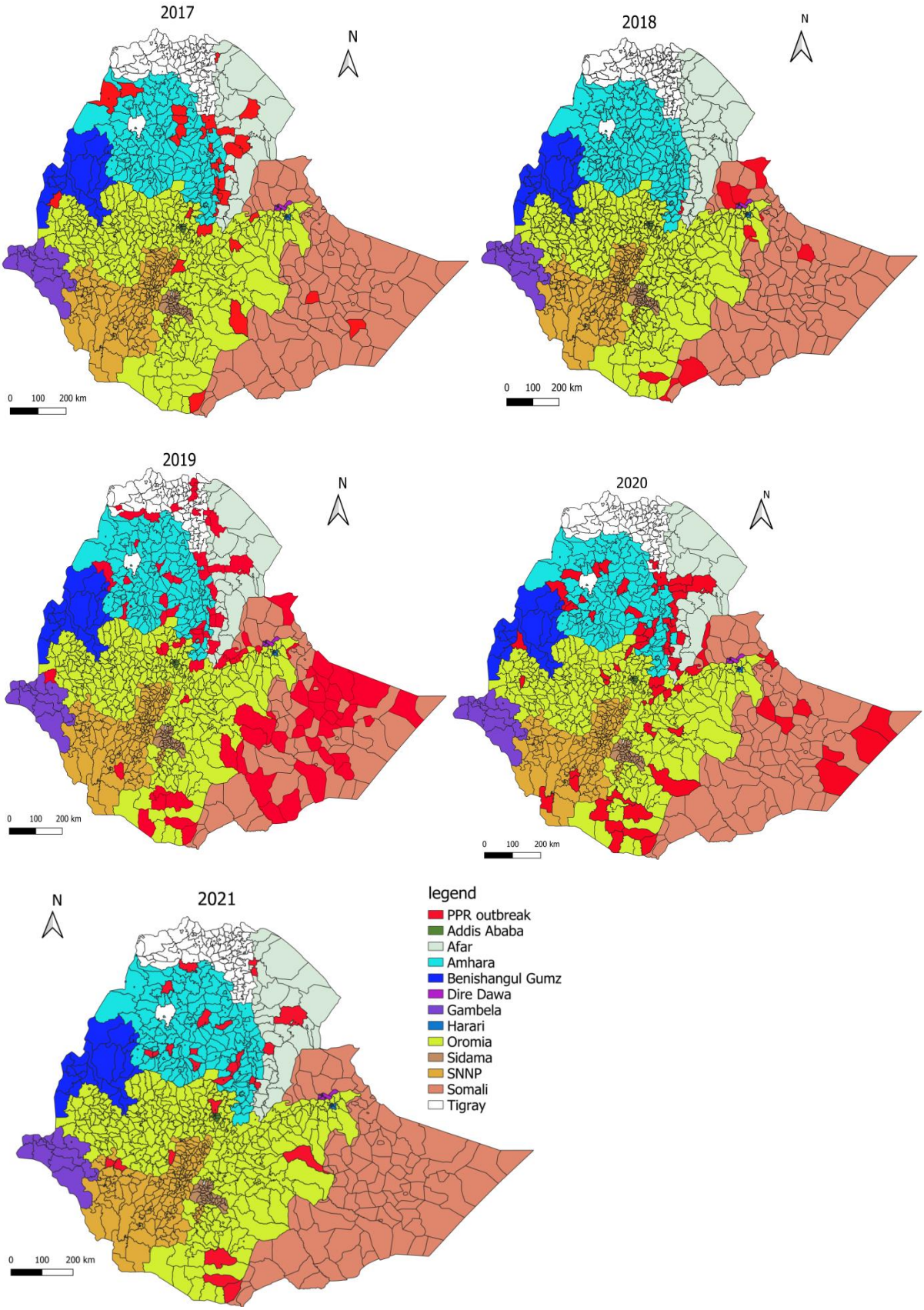


Figure 12: Maps showing status of PPR outbreak report in Ethiopia from 2017 to 2021consecutive years

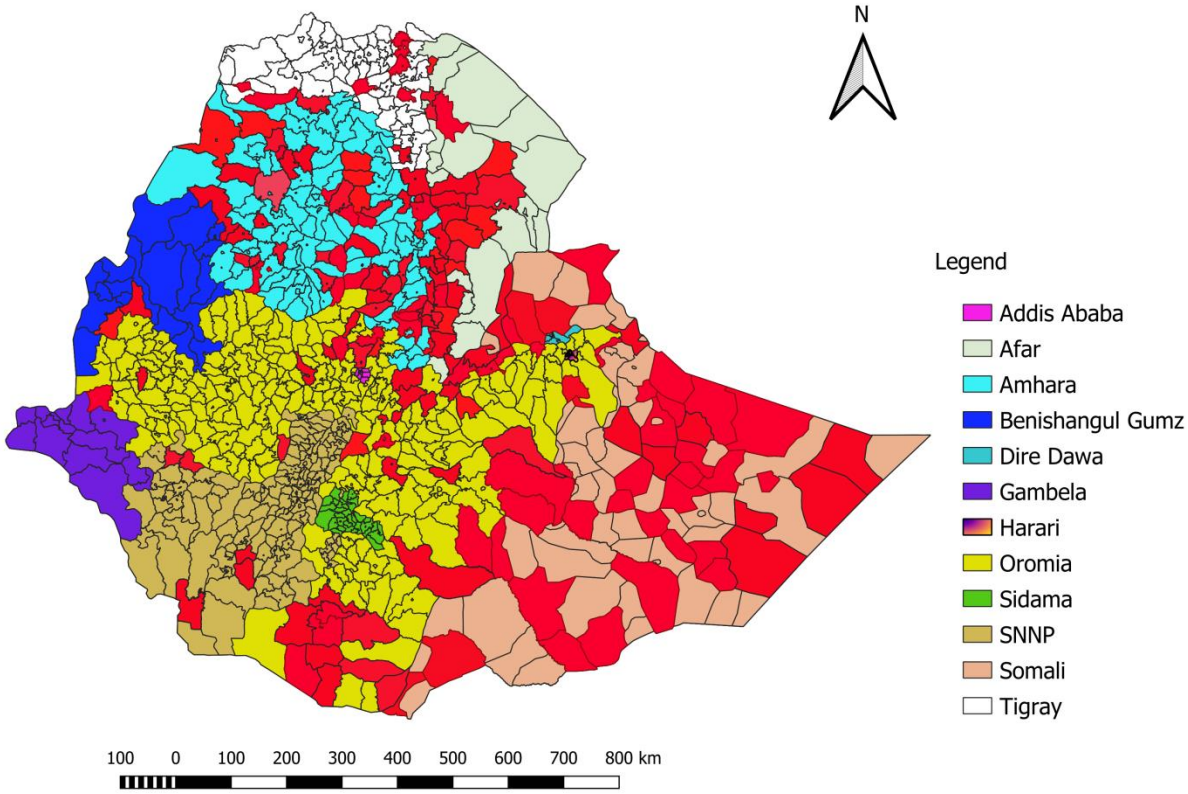
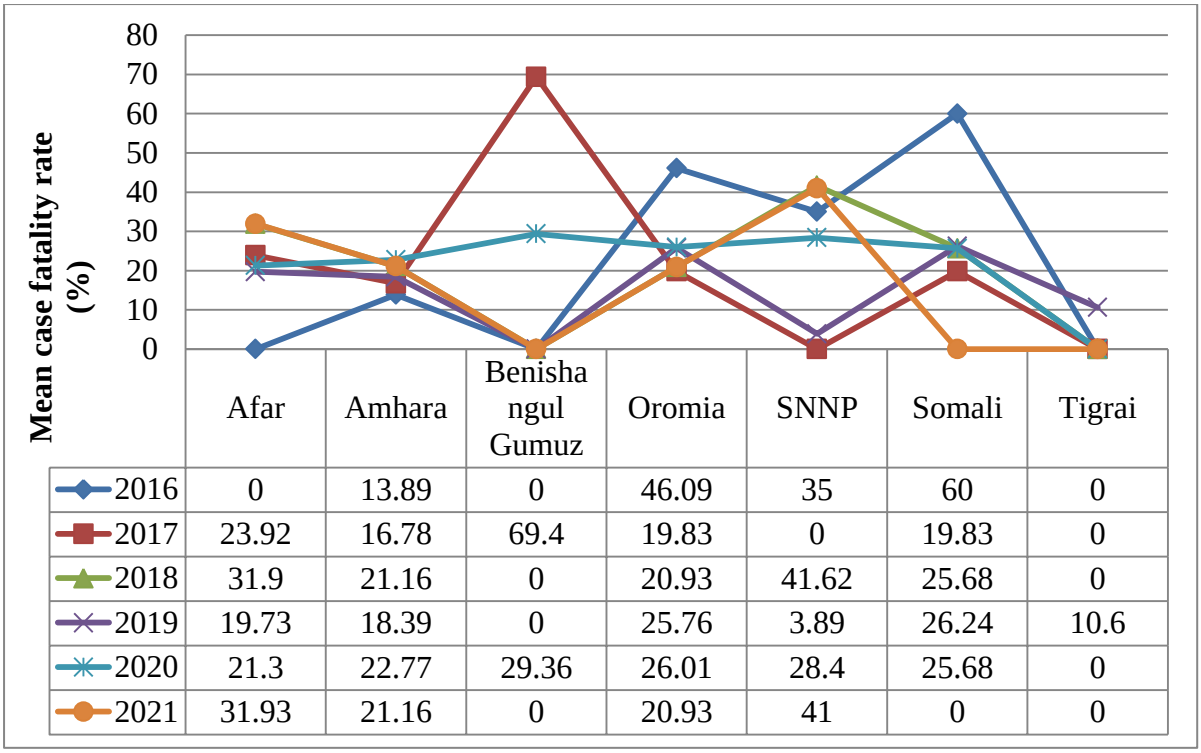


Figure 13: Cumulative spatial distribution of PPR outbreak report in Ethiopia (January 2017 to August 2021)

4.2.3. Epidemiological parameters estimates of PPR

The highest year based mean case fatality rate of PPR was reported in 2017 from Benishangul Gumuz regional state (69.4%) followed by 2016 report from Somali (60%) and Oromia region (46.09%), 2018 report from SNNP (41.6%), 2021 report from SNNP (41%), 2020 report from Benishangul Gumuz regional state (29.36%) and 2019 report from Somali region (26.24%). The lowest mean case fatality rate of the disease was also reported in 2016 from SNNP (3.89%) and Amhara regional states (13.89%). Similarly, the highest overall PPR mean morbidity rate (43.62%), mean case fatality rate (42.71%) and mean mortality rate (9.11%) were also reported from Benishangul Gumuz regional state. However, the lowest mean mortality rate was reported from Oromia region (0.22%) while the lowest morbidity (0.7%) and case fatality rates (10.61%) were reported from Tigrai region (**Figure 14**).



	Afar	Amhara	Benishangul Gumuz	Oromia	SNNP	Somali	Tigrai
2016	0	13.89	0	46.09	35	60	0
2017	23.92	16.78	69.4	19.83	0	19.83	0
2018	31.9	21.16	0	20.93	41.62	25.68	0
2019	19.73	18.39	0	25.76	3.89	26.24	10.6
2020	21.3	22.77	29.36	26.01	28.4	25.68	0
2021	31.93	21.16	0	20.93	41	0	0

Figure 14: PPR outbreak case fatality rate reported from 2016 to 2021 in Ethiopia. Zero value indicates unreported outbreak of PPR.

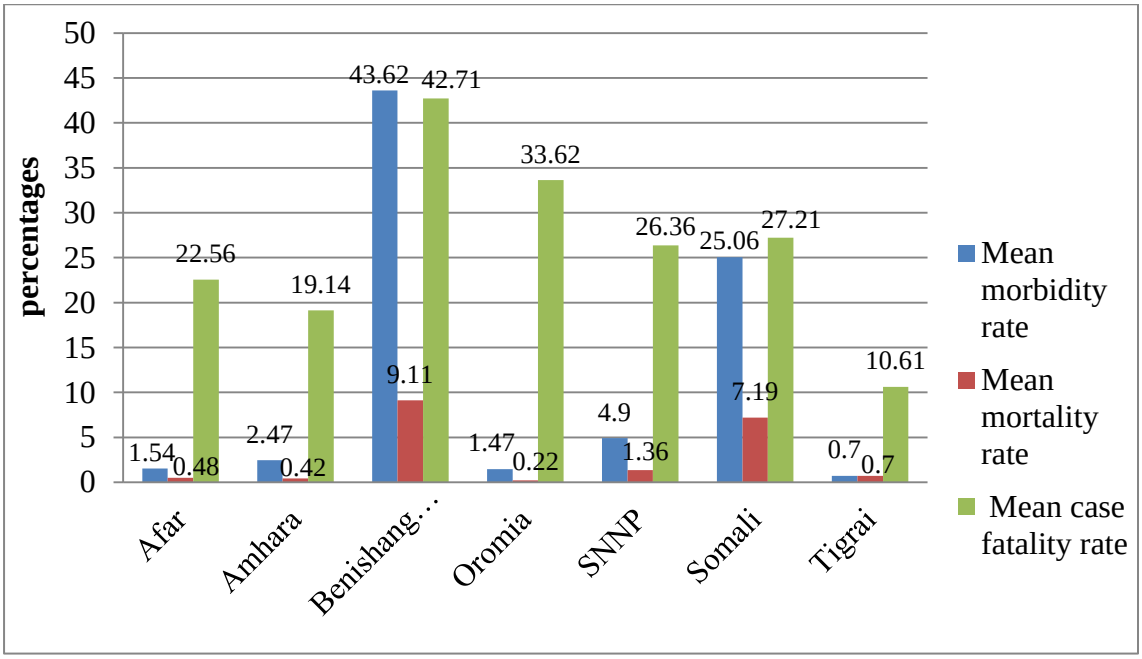


Figure 15: Region wise mean morbidity, mortality and case fatality rates of PPR outbreaks (2016-2021).

4.3. Questionnaire Survey Analysis

A total of 78 small ruminant owners were interviewed to reveal information on risk factors of PPR and awareness the owners have on PPR disease and associated risk factors. Out of the total individuals interviewed, 89.7 % of them responded that their shoats were infected with PPR disease. The clinical signs such as depression, erected hair with dark hair coat, anorexia, thirst, watery foul smelling diarrhea, tenesmus, rough hair coat, cough, nasal discharge, ocular discharge, dyspnea, sneezing, granting, abortion, erosion of oral mucosa, lesion on muzzle, emaciation, lethargy and death are reported by the farmers. They said that the disease can happens as a result of contact at market, introduction of infected animals by trades, contact at grazing areas, poor sanitation and poor management, stresses from cold, drought and hunger. More than half of the farmers (64.1%) interviewed responded that PPR outbreak occurs in the area every year. About 82.1% of the participants were treat their animals by themselves buying drugs from veterinary pharmacy (76.6%), veterinary clinics (18.8%) and both veterinary pharmacy and clinics (4.7%).

In response to a question for absence or presence of isolation of sick sheep and goats from clinically healthy flock practice, majority of the participants (84.6%) responded that they did not isolate sick animals. From the survey result, age (83.3%), sex (29.5%) of the animals, species of the animal (76.9%), season (91%), herd size (60.3%), housing system (32.1%) and body condition (28.2%) were also reported to be potential risk factors affecting severity of PPR disease in small ruminant animals. Most of the interviewees (84%) are also reported that the disease has a potential to infect both sheep and goats with higher severity in goats (66.7%).

The survey result also indicated that kids and lambs are the most severely affected age groups of the animals followed by young mature animals. Few participants (30.7%) responded that PPR vary with sex of which 69.6% them reported that female animals are the more affected sex group as a result of stress from pregnancy, giving birth, lactation and increased contact during mating. The surveyed individuals also indicated that PPR happens both in dry and wet season (52%) the highest monthly seasonal occurrence being from June to November.

Nearly all (90.1%) of the small ruminant owner's participated in the survey also responded that the disease causes abortion in pregnant sheep and goats. ninety sev79.5% of them have lost their animals due to mortality from PPR disease with average death of 8 animals ranging from 1 to 22 animal deaths in the past 2020/2021 year. According to the survey result, higher mortality (66.9%) and morbidity (45.8%) rate was reported in goats than sheep. The overall morbidity and mortality rate of the small ruminants were also found to be 53.7% and 64.2% respectively. The domestic economic loss from death of the shoats of the interviewed farmers and from treatment cost due to PPR was also estimated to be 565,600 ETB and 30,893 ETB respectively with total loss of 596, 493 ETB from 1024 flock size small ruminants as indicated in **Table 4** below.

Table 8: Morbidity, mortality and economic importance of PPR based on questionnaire survey

Species	Number owned	Number infected	No. died	Mortality rate	Morbidity rate	Loss due to death (ETB)	Loss from treatment cost(ETB)	Total loss (ETB)
Sheep	443	203	121	59.6%	45.8%	199600		
Goats	586	350	234	66.9%	59.7%	354100		596, 493
Overall	1029	553	355	64.2%	53.7%	56, 5600	30893	

Almost all (96.2%) of the respondents are rearing their small ruminant animals by communal grazing systems and 78.2% them use communal watering points for drinking. Three production systems namely sedentary mixed farming (85.9%), pastoral (7.7%) and agro-pastoral (6.4%) were recorded. About eighty percent of the participants raise sheep and goats grazing separately and most of them use separate housing system (84.6%) with free movement of the animals (89.7%) in the area. There is also free contact of the animals at market which a common practice in the country as well. Only few number of the farmers participated in this survey were reported isolation of sick animals, report to animal health expert, vaccination, treatment, good hygiene practice and good management as important measures to be taken for control of the disease.

4.4. Field Observation and Clinical Signs

During the field observation, different clinical signs of PPR were encountered in both sex of sheep and goats found in the study area districts. However, the clinical disease was rare in sheep and more common in goats with more obvious signs in goats. The clinical signs observed include, fever, foul smelling diarrhea, depression, cough, labored breathing, erosion on gum, nasal discharge, lacrimation, matting of eyelids. The field observation result also revealed higher clinical morbidity and mortality of the disease was in goats than sheep (**Figure 15**).

Figure 16: Clinical signs of PPR in sheep and goats from field observation

Nasal discharge, lacrimation and matting of eyelids in goat (A), diarrhea in goat (B and C), nasal discharge and crust and nodules on muzzle of goat (D), ocular discharge in goat (E), Diarrhea in sheep (F), nasal discharge in sheep (G), erosion of gum mucosae and nasal discharge in sheep (H).

4.5. Molecular Detection

Out of the total 42 suspected clinical samples tested with the real time PCR, 16 (38.1%) were found positive for PPRV nucleic acid. The samples were detected at cycle threshold (Ct) values ranging from 18 to 42.96 and the positive control was detected at 21.3 Ct value while the negative control remained undetected. The highest viral load was reported from rectal swab followed by nasal swab with Ct value of 18 and 18.2 respectively while the lowest viral load was detected from whole blood sample (**Figure 16**).

Of the total positive samples, 15/42 (35.7%) were from goats and 1/42 (2.4%) were from sheep. Thus, detection of the virus was higher in samples collected from goats compared to that of sheep. The presence of the virus nucleic acid was also found to be higher in females than males (**Table 9**).

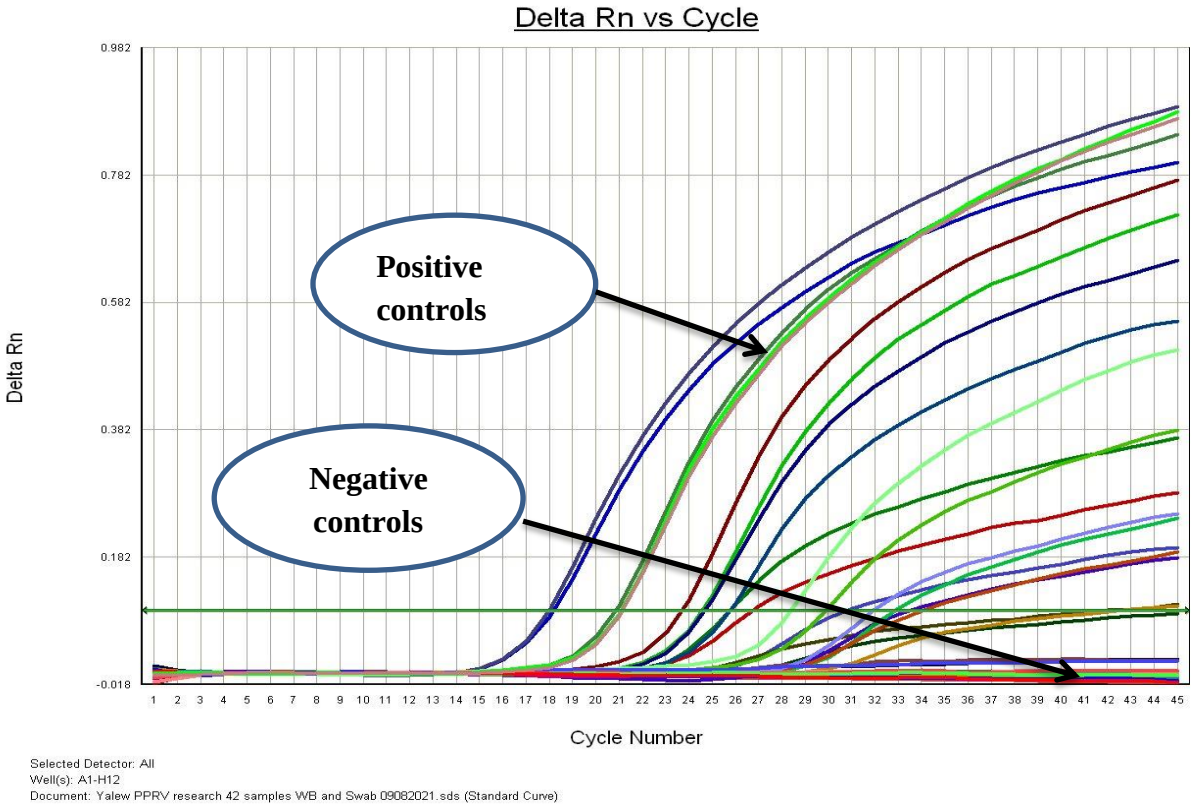


Figure 17: Real time PCR standard curve indicating PPRV detection result
Positive controls (green and red coloured lines indicated by the arrow) and negative controls (blue and red line indicated by arrow).

Table 9: Species and sex wise detection rate of PPRV nucleic acid

Factor	Category	No. tested	No. positive (%)	95% CI	P value
Species	Caprine	30	15 (50)	33.2 -66	0.01
	Ovine	12	1 (8.3)	0.4 – 40	
	Total	42	16 (38.1)	24-54	
Sex	Male	21	6 (28.6)	12-52	0.34
	Female	21	10 (47.6)	26-69	
	Total	42	16 (38.1)		

Based on district, higher percentage of PPRV nucleic acid was detected in Pawe (45.8%) with highest record of detection of the virus from rectal swab samples (66.7%) followed by that of nasal swab samples (50%) within the district (**Table 10**). The overall sample wise nucleic acid detection rate of the virus was higher in rectal swab samples (57.1%) followed by whole blood samples (35.3%).

Table 10: Detection rate of PPRV nucleic acid based on species and sample type in the study area districts

District	Sample type	Sheep		Goats		Over all	
		N. tested	**N.positive (%)**	**N. tested**	**N. positive (%)**	**N. tested**	**N.positive (%)**
Pawe	Nasal swab	1	0(0)	5	3 (60)	6	**3 (50)**
	Rectal swab	1	0(0)	2	2(100)	3	**2 (66.7)**
	Ocular swab	1	0 (0)	2	1 (50)	3	**1 (33.3)**
	Whole blood	5	1 (20)	7	4 (57.1)	12	**5 (41.7)**
	Total	**8**	**1(12.5)**	**16**	**10 (62.5)**	**24**	**11 (45.8)**
Jawi	Nasal swab	3	0 (0)	6	2 (33.3)	9	**2 (22.2)**
	Rectal swab	-	-	4	2 (50)	4	**2 (50)**
	Whole blood	1	0 (0)	4	1 (25)	5	**1 (20)**
	Total	**4**	**0 (0)**	**14**	**5 (35.7)**	**18**	**5 (27.8)**

4.6. Culture and Isolation

From the total pool of samples inoculated to confluent monolayer VDS for isolation, 6/10 (60%) have showed typical cytopathic effect from second to third day of inoculation with characteristic CPE of rounding of the cells, vacuolation, aggregation and syncytia formation and detachment of the cells. The cultures failed to show CPE on subsequent seven days follow up and the cultures with sign of contamination were blind passaged up to three times for declaration of the result as negative for isolation of the virus. Accordingly, the remaining four pooled sample cultures were declared negative after the subsequent passages (**Table 11**).

Table 11: Isolation result of pooled sample culture:

District	Kebeles	Pool by sample type	No. of pooled samples	Culture result
Pawe	Village 50	Nasal swab	3	+
		Rectal swab	2	+
		Whole blood	4	+
		Ocular swab	1	+
	Village 14	Whole blood	2	-
Jawi	Ayima	Nasal swab	1	-
		Rectal swab	1	+
		Whole blood	1	-
	Adis weyini	Nasal swab	2	+
		Rectal swab	1	-

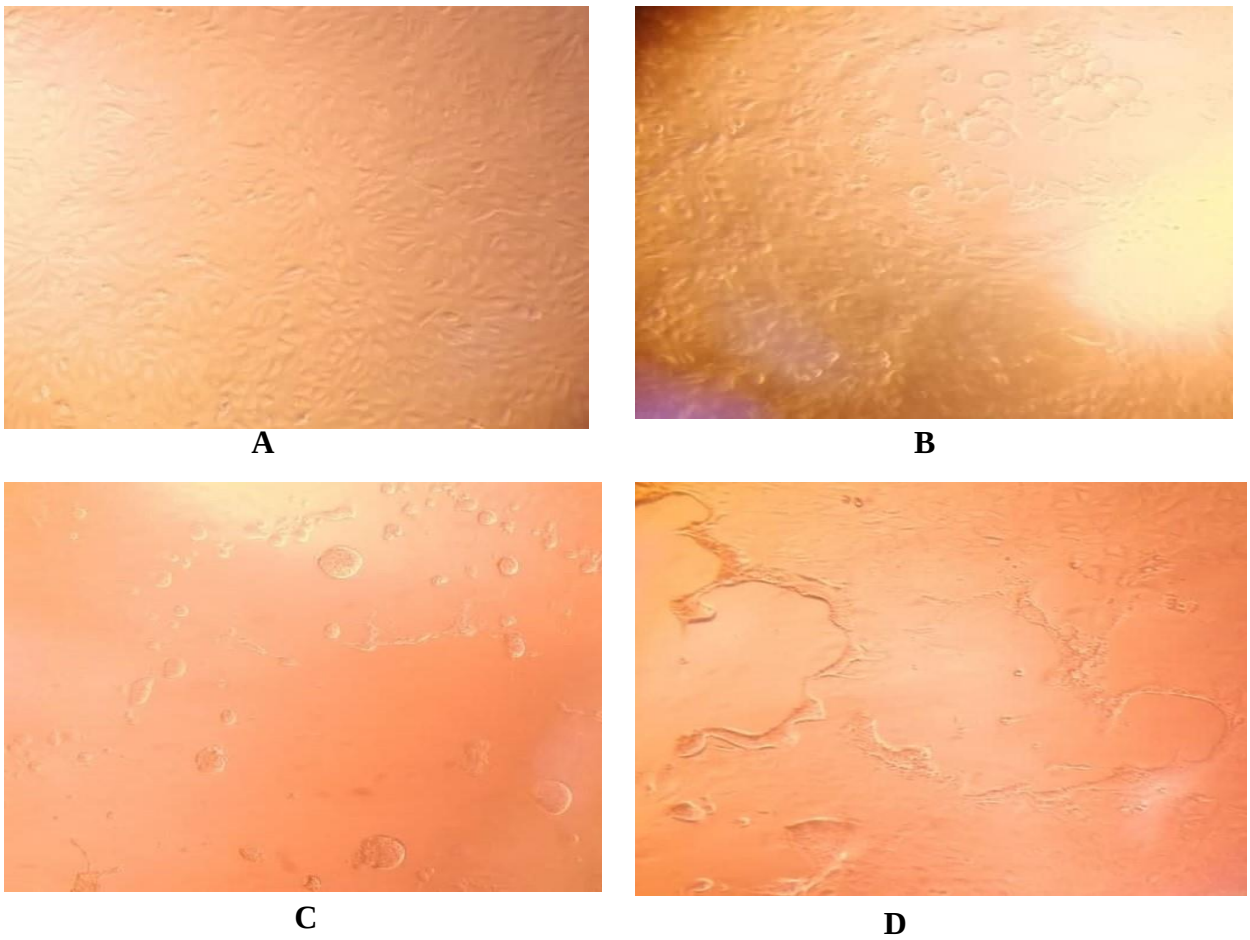


Figure 18: Cytophatic effect of PPRV on Vero Dog Slam (VDS) cell

Un-infected confluent normal VDS cell (A), rounding, aggregation and syncytia formation (B), rounding and ballooning of the cells(C) and detachment of the cell (D)

5. DISCUSSION

5.1. Antibody Seroprevalence of PPRV

The current study revealed an overall PPR seroprevalence of 65.4 % which is in agreement with findings of Abd El-Rahim *et al*. (2010) (63.4%), Saeed *et al*. (2010) (62.8%), Abdalla *et al*. (2012) (61.8%) and Saritha *et al*. (2014) (67.9%). However, it is lower than the findings of Yalew *et al*. (2019) and Woldemichael *et al*. (2018) who reported 75.7% and 73.45% prevalence in Asosa and Metekel zone respectively. Similarly, higher prevalence was also reported in Pakistan (74.9%) by Zahur *et al*. (2008) and in North and Central Sudan (80.9%) by Ibrahim (2018). Slightly lower prevalence was reported by Afera *et al*. (2014), Gari *et al*. (2017) and Fentie *et al*. (2018) in Tigray (47.5%), Oromia (48.43%) and Amhara (55.34%) regions respectively. The current prevalence is also a bit higher than results of 55.2% in Uganda (Bonny *et al*., 2011) and 45.6% in Sudan (Salih *et al*., 2014). Moreover, it is much higher than the reports of Nigusu and Fantie (2012) (26.3%), Hailegebreal *et al*. (2018) (29.2%) and Gebre *et al*. (2018) (2.1%) in Ethiopia. Variation in the prevalence can be due to difference in sample size, sampling procedure used, geographical location, season, small ruminant population density, management system and husbandry practices.

The present study reported slightly higher seroprevalence in sheep (66.8%) than in goats (64%) with no significant difference. The non-significant difference could be due to equal chance of exposure to a disease risk factors resulting from the usual free animal movement and communal grazing system in the area. This finding agrees with the findings of Gari *et al*. (2017), Gizaw *et al*. (2018), Agga *et al*. (2019), Yalew *et al*. (2019) and Gelana *et al*. (2020) who reported higher PPRV antibody prevalence in sheep. Studies from other African and Asian countries such as Enan *et al*., 2011 and Abdalla *et al*., 2012 from Sudan; El-Yuguda *et al*., 2013 and Woma *et al*., 2016 from Nigeria; Akwongo *et al*., 2022 from Uganda; Abubakar *et al*., 2009 and Jalees *et al*., 2013 from Pakistan and Balamurugan *et al*., 2020 from India also reported higher seroprevalence in sheep. This might be attributed to the low case fatality rate of the disease in sheep and higher susceptibility of goats to the virus with considerable lower recovery rate. The presence of large proportion of sheep previously recovered from infection and increase in mortality rate of goats prior to sampling can lead to the higher prevalence in sheep (Couacy-Hymann *et al*., 2015; Abdellatif *et al*., 2016).

In contrast to the current study, previous works in Tanzania (Swai *et al*., 2009), Kenya (Kihu *et al*., 2015, Saudi Arabia (Abdellatif *et al*., 2016), Nigeria (Bello *et al*., 2016) and Ethiopia (Fentie *et al*., 2018; Hailegebreal *et al*., 2018) reported significantly higher serum level PPRV antibody prevalence in goats than sheep. Luka *et al*. (2011), Delil *et al*. (2012) and Salih *et al*. (2014 were also revealed slightly higher prevalence goats in Uganda, Ethiopia and Sudan respectively. Similarly, reports by Waret-Szkuta *et al*. (2008) and Saritha *et al*. (2014) were documented non-significantly higher PPR antibody distribution in goats. This higher finding could be due to the fact that there is higher fecundity in goats than sheep which leads to increase in susceptible population of goats each year (Khan *et al*., 2008; Gelana *et al*., 2020).

The age wise antibody distribution of PPR in this study was significantly higher in old, adult and young age category respectively (P=0000) indicating the increasing PPRV antibody prevalence with increasing age of the animals similar to the reports of Waret-Szkuta *et al*. (2008), Dejene (2016), Gari *et al*. (2017), Yalew *et al*. (2019) and Lysholm *et al*. (2022). Earlier studies in Ethiopia (Fentie *et al*., 2018; Hailegabreal *et al*., 2018; Gelana *et al*., 2020), Nigeria (El-Yuguda *et al*., 2013), Pakistan (Khan *et al*., 2008; Nizamani *et al*., 2015) and India (De *et al*., 2016) also detected higher PPRV antibody in adult and above small ruminants. The increase in the prevalence with age might be due to increase in susceptibility of the animals after five month age as a result of declining maternal antibody. Thus, older animals could have greater probability of exposure to the PPRV (Bello, 2013; Gebre *et al*., 2018). It is also reported that sheep and goats exposed to natural infection of the virus at earlier age remains positive for long period of time leading to higher antibody detection in old animals (Yalew *et al*., 2019).

The present study showed significantly higher antibody seroprevalence in female sheep and goats (69.4%) than male shoats (54.4%). This might be attributed to the higher exposure of female animals as a result of stress from pregnancy and lambing/ kidding. This finding is in agreement with previous findings of Waret-Szkuta *et al*. (2008), Megersa *et al*. (2011), Salih *et al*. (2014), Bello *et al*. (2016), and Akwongo *et al*. (2022) who showed significantly higher prevalence in females. Additionally, a research done by Khan *et al*. (2008), Swai *et al*. (2009) and Fentie *et al*. (2018) concurs well with the current finding. Despite the fact that the difference is non-significant, the serum level PPRV antibody distribution was also reported to be higher in female small ruminats by other earlier studies (Farougou *et al*., 2013; Afera *et al*., 2014; Gari *et al*., 2017; Gebre *et al*., 2018; Gelana *et al*., 2020).

The higher prevalence in female animals might be due the owner's usual habit of keeping female sheep and goats in a flock for long period of time for breeding purpose and selling out or slaughtering of the males at earlier age (Khan *et al*., 2008; Swai *et al*., 2008; Gebre *et al*., 2018).

Seroprevalence rate of PPRV antibody obtained in this work is higher in animals having poor body condition (73.5%) than those having medium (66.8%) and good body condition (61.9%) with no statistical significant difference (P >0.05). In concordance to this finding, Yalew *et al*. (2019) also reported higher prevalence in animals with poor body condition (95.95%) than with medium body condition (8.16%). The higher antibody seroprevalence in poor body conditioned animals manifest the importance of the disease in causing body weight loss. The current finding is also supported by Rath *et al*. (2020) study who revealed higher PPR seroprevalence in animals with poor body condition compared to the others.

Similar to the reports of Gelana *et al*. (2020), the present study reported significantly higher seroprevalence in medium flock size (70.8%) compared to small (65.5%) and large flock size (50%). This finding is also supported by the earlier study done in Turkey (Ozkul et al., 2002). Indeed, the previous reports by Al-Majali *et al*. (2008), Alemu (2014) and Dejene (2016) also found higher antibody seroprevalence in medium flock size though the difference was not significant. However, a work done by Nkangaga (2014) in Nigeria and by Gebre *et al*. (2018) in Ethiopia reported higher PPRV antibody seroprevalence in small and large flock size respectively contrary to the present result. The smaller prevalence in large flock size in this finding might be due to the small sample size of the group considered in the study. It might also be due to endemic occurrence of the disease in the study area.

Origin of the study animals whether they are born in flock or brought in to the flock from outside was considered as the putative risk factor to PPR antibody seropositivity in this study. The result of the study revealed higher seroprevalence in those that were brought in compared to the ones born in herd (P = 0.06). This finding is in accordance with the findings of work done by Alemu (2014) and Gelana *et al*. (2020) who reported significantly higher prevalence in animals brought in from outside of the flock. Earlier studies by Mbyuzi *et al*. (2014) and Saeed *et al*. (2018) also indicated the finding that supports the current study report.

The serological profile reported in the present work have showed slightly higher PPR antibody seroprevalence in animals from flock to which new animal was recently introduced (67.3%) than those sampled from flock with no introduction of new animal (64.3%). This could be happened as a result of introduction of the animals to the flocks from the areas where the disease is endemic.This finding is supported by Gelana *et al*. (2020) who reported higher prevalence in flocks with recent new animal introduction the difference being statistically non-significant. In addition to this, the report of the current finding is in agreement with the findings of other previous studies (Alemu, 2014; Gebre *et al*., 2018) who reported significantly higher prevalence in animals sampled from flock having recently introduced new animals. However, the work of Shauib *et al*. (2014) and Saeed *et al*. (2018) showed non-significantly higher prevalence in those animals from flock with no introduction of new animal contrary to the current finding. This could be due to non-proportional number of animals sampled from the flock categories or it might also be resulted from introduction of the new animals from disease free area.

With respect to housing system, significantly higher PPR antibody distribution was observed in sheep and goats housed together on floor (P=0.049) with a prevalence of 70.3% (CI= 62.6-77, χ^2=6). Thus, the finding of this work indirectly signify rearing sheep and goats together as a risk factor for seropositivity to PPRV antibody which is in agreement to the finding of Akwongo *et al*. (2022). Previous works in Ethiopia (Alemu, 2014; Gelana *et al*., 2020) and Sudan (Shuaib *et al*., 2014 also reported similar finding with the present report. The serum level PPR antibody prevalence was also found to be higher in sheep and goats separately housed on floor compared to those housed on bed house (OR= 0.7, P=0.05). The lower risk could be attributed to the suitability of bed house for management leading to low level of risk of contamination.

As to grazing management, the finding of the present work showed non-significantly higher serum level PPRV antibody distribution in animals from communal grazing system. This reflection agrees with the finding of Gelana *et al*. (2020) who reported non-significant association of PPR seropositivity to grazing management. The reason for absence of significant difference in the association might be due to endemicity of the disease in the area. In contrast, Alemu (2014), Mbyuzi *et al*. (2014) and Dejene (2016) revealed significantly higher PPRV antibody prevalence in animals from communal grazing management system.

This can be due to the fact that communal grazing increases contact rate of the animals that can subsequently increase the chance of infection with the virus.

In the present study, significantly higher PPR antibody seroprevalence was observed in animals from flock owners not practicing isolation of sick animals (66.4%) compared to those isolate sick from herd (41.4%) (P=0.01). From univariable logistic analysis, the odd of the animals to be seropositive to PPRV antibody is found to be higher when there is no isolation of infected animals from herd (OD = 0.3, P = 0.007). Thus, isolation status of sick animals from herd is found to be a risk factor for PPR seropositivity. Isolating PPRV infected animals can reduce the risk of infection by reducing the chance of transmission of the disease by contact with infected animals and fomites as well as the chance of transmission by aerosol (OIE, 2020)

5.2. Risk Factors for Seropositivity to PPRV Antibody

In this study, multivariable logistic regression showed district, age, body condition, flock size, housing and isolation status of sick sheep and goats. Sex and physiological status of the animals were also found to be associated risk factors by univariable logistic regression but not by multivariable logistic regression. This finding agree with findings of Saeed *et al*. (2018) and Akwongo *et al*. (2022) who reported location and age as potential risk factors. Sex of the animals was also previously reported to be a risk factor for PPR seropositivity (Waret-Szkuta *et al*., 2008; Torsson *et al*., 2017)

In the present study, study area districts are found to be strongly associated with seropositivity of sheep and goats to PPRV antibody as indicated by the multivariable regression analysis. The probability of the animals to be seropositive for PPR serum antibody was found to be significantly higher in Jawi district in reference to that of Guangua district (OR=4.5, P=0.000). Sheep and goats from Pawe district are also about 4 times at higher risk of being seropositive to PPR antibody (P=0.000). In agreement to this finding, Abd-El Rahim *et al*. (2010) and Shuaib *et al*. (2014) also reported locality of the animals to be associated with risk of infection with PPR virus. Study by Saker and Islam (2011) also revealed similar finding.

The odd of the old animals to be seropositive for PPR antibody was found to be 3.5 times higher than young animals (P=0.000) indicating that age is potential risk factor for PPR seroprevalence in line to Ozkul *et al*. (2002), Waret-Szkuta *et al*. (2008), Abd El-Rahim *et al*. (2010) and Yalew *et al*. (2019) findings. In addition to this, previous works of Mahajan *et al*. (2012) and Rume *et al*. (2020) also indicated age as a risk factor to the disease.

In contrast, research work by Shuaib *et al*. (2014) showed non-significant association between age and positivity to serum level PPRV antibody. The reason for this variation could be because of highly immunogenic nature of PPRV, seropositivity of naturally infected sheep and goats for long time after recovery and high mortality rate of highly susceptible animals when infected.

Similar to the previous findings of Yalew *et al*., (2019), the current finding reported higher prevalence of serum PPR antibody in animals with poor and medium body condition compared to good body condition. The likelihood of poor body condition animals in being seropositive to PPR antibody was 2.2 times higher compared to good body condition animals while those with medium body condition were 1.4 times at higher risk in relation to those with good body condition.

Similar to the reports of Zahur *et al*. (2011) and Kardjadj *et al*. (2015), the finding of this research showed housing system of the animals as one of the potential risk factors to PPR seropositivity. Sheep and goats housed together on floor were found to be 1.5 times at higher risk of being seropositive to PPR antibody than those housed separately on flour (P=0.09, SE=0.42). Indeed, it was revealed that sheep and goats reared in separate bed house were less likely to be seropositive than those reared on floor separate house (OD=0.7, P=0.038). This result is also supported by Al-Majali *et al*. (2008) who reported rearing sheep and goats together to be potential risk factor to the seropositivity.

5.3. Retrospective Data Analysis

Despite inadequacy of disease reporting and surveillance system in Ethiopia, the retrospective outbreak data used to identify temporal and spatial distribution of PPR along with some epidemiological measures in this study provides information on status of the disease in the country. Understanding temporal and spatial distribution of a disease is important to design appropriate prevention and control measures of the disease. For instance, identifying temporal distribution is very important to design vaccination calendar and Knowledge of spatial distribution of a disease help to identify risk areas and the disease hot spots which is important to apply appropriate control measures.

The current retrospective study indicated a total number of 632 PPR outbreak which is lower reports of 832 (2009 to 2013 year) and 1282 number of outbreaks (2006 to 2015 year) by Alemu (2014) and Dejene (2014) respectively. The decrease in number of outbreaks observed in the present study could be resulted from variation in study period/number of years considered. It might also be due to the gradual improvement in surveillance system and implementation of the disease prevention and control measures. A minimum number of the outbreak reports were from 2018 year followed by 2021 year. The lower number of outbreak reports in these years might be due to effectiveness of the strategic vaccination that is being given to control the disease or the hindrance to the reporting system as a consequence of the security problems that has been prevailed in different parts of the country. Moreover, the lower number of outbreak report for 2021 can be due to the fact that only the 6 month report (January to August) is considered for the year.

The overall seasonal distribution of the disease throughout the study years also indicated that the disease occurred in all seasons of a year. It is revealed that PPR outbreak was most common in dry season (March to April) followed than rainy season (June to December). This finding agrees with the study done in India (Balamurugan *et al*., 2021) who observed higher number of outbreaks in dry season (January to march) .Alemu (2014) and Dejene (2016) have also reported higher incidence of the disease in wet dry season in Ethiopia from previous outbreak retrospective data.

Likewise, Balamurugan *et al*., (2012) also reported higher outbreak numbers in April which is in complete agreement to the current finding. The higher outbreak report observed in dry season could be attributed to limited availability of feed resources and increased contact rate of the sheep and goats during the season. In dry season the animals usually travel a long distance in search for feed and water leading to susceptibility of the animals as a result of stress from starvation and long distance movement. In addition, the usual close contact at communal grazing and watering points in dry season leads to increased probability of transmission of the disease. Consequently large number of small ruminants becomes infected and circulation of the virus is maintained through ought the year by animal to animal transmission (Balamurugan *et al*., 2021).

The spatial analysis of the outbreak data in this study indicated highest number of PPR outbreak in Amhara region (256 outbreaks) followed by Oromia region (146 out breaks). The highest number of report in these two regions could be resulted from the large sheep and goat population potential found in the regions. In contrary, a study reports of Alemu (2014) and Dejene (2016) showed highest number of outbreak from Amhara region followed by that of Oromia region. The lowest number of the PPR outbreak report was from Benishangul Gumuz region (3 outbreaks). The variation in outbreak number might be due to difference in strength of disease reporting system, production system and management practices in the regions.
The region with low and high number of outbreaks does not necessarily mean that the disease is low or high in respective areas. The more a region reports the disease outbreak, the more will be a number reported and vice versa.

The epidemiological parameters estimation analysis in this work indicated highest mean morbidity (43.62%) and mortality rate (9.11%) of PPR in Benishangul gumuz regional state even though the number of outbreak report from the region was relatively low. Mean casa fatality rate (42.71%) was also observed from the region retrospective data. Mortality rate of 8.4% and case fatality rate of 38.4% reported by Alemu (2014) is comparable to this finding but the morbidity rate is found to be higher in the present study. The highest morbidity rate in the present work might be due to the collective animal rearing practice and the presence of large number of animals at risk in the region.

Indeed, the present finding is also consistent to a previous work done in Egypt by Abd El-Rahim *et al*. (2010) who reported almost similar mortality (10.5%) and case fatality rate (40.2%) of small ruminant animals. However, it is higher than the previous morbidity (13.7%), mortality (3.7%) and case fatality (26%) reports from Asosa zone in Ethiopia (Ebissa, 2020). Study done in Algeria by Kardjadj *et al*. (2015) was also reported lower morbidity, mortality and case fatality rate contrary to the present one. This variation could be due to difference in the strategic vaccination coverage, number of outbreaks considered, the report data quality, outbreak reporting system and pattern of the disease. It might also be attributed to the difference in management system, the length of the study period, approaches in estimating the parameters and number of animals at risk in the study areas.

5.4. Questionnaire Survey

From the total number of farmers interviewed, majority of them have experienced PPR disease outbreak with their small ruminant animals. Most of them have also explained a typical clinical signs of the disease including depression, erected hair with dark hair coat, foul smelling diarrhea, tenesmus, nasal discharge, ocular discharge, cough, dyspnea, abortion, erosion of oral mucosa, lesion on muzzle, anorexia, emaciation, lethargy and death. This is in agreement with the clinical signs described by Dejene (2016) and Rahman *et al*. (2016).

Most of the owners are also found to treat the infected animals by themselves buying drugs from Pharmacy rather than taking the animals to nearby clinic. This might be due to the fact that there is lack of easily accessible veterinary services, lack of animal health professionals and in sufficient drug supply in the area.

The survey result revealed some important risk factors to infection with PPR disease. These include species, age, sex, season, herd size and housing system of the animals. Regarding species of the animals, the participants have reported higher severity and mortality of the disease in goats than sheep. The survey result has also revealed a young small ruminant specifically kids/lambs and female animals to be the severely affected groups by the disease. They also explained that the disease morbidity is higher in large herd size and in animals closely confined in small house. This awareness on PPR disease might be developed due to frequent occurrence of the disease outbreak in the area.

Nearly all of the participants responded to the questionnaire were explained that they rear their small ruminants mixing together at communal grazing and watering points in the extensive production system. This can lead to further spread of the PPR disease in the area by increasing the risk of transmission of the disease. Regarding awareness on prevention and control of PPR disease, only a small number of respondents were found to be aware of some prevention and control measures. Indeed, only few of the interviewees (15.4%) practiced isolation of sick animals from health flock. These are also one of the factors to the higher impact of the disease in the study area.

5.5. Field Observation and Molecular Detection

Since the confirmation of presence of PPR disease as one of the most economically important sheep and goat disease in Ethiopia (Waret-Szkuta *et al*., 2008), the disease is circulating in different parts of the country, continuously posing significant treat to small ruminant production. Thus, PPR have significant economic impact due to production loss and mortality of the small ruminant animals. Subsequently, it can contribute to food insecurity especially in vulnerable areas of the country (Alemu *et al*., 2019).

In the present study, field observation and detection of the PPRV from clinical samples imply that PPRV has been recently circulating in Awi and Metekel zone. The observed clinical signs, serological findings, detection and isolation of the virus from clinically suspected animals indicated that the causative agent of the disease to be PPRV. The clinical signs of the disease observed in field were in agreement to those described by Khan *et al*. (2007), Abubakar *et al*. (2009), Kardjadj *et al*., (2015) Alemu *et al*., (2019), and Ebissa (2020) who reported fever, depression, dullness, diarrhea, ocular discharge, nasal discharge, coughing, anorexia, erosive lesions on gums and death of the animals. The clinical finding was also similar to that reported by Kgotlele *et al* (2014), Abubakar *et al*. (2016) and Mishra *et al*. (2020). Serological detection of PPRV antibody in combination with clinical signs can confirm status of PPR disease especially in areas where there is no vaccination practice against the disease. However, if there is specific vaccination practice in or around the area, serological tests can lead to false picture of PPRV antibody prevalence in which case detection of the virus nucleic acid from clinical samples can confirm the disease.

In this study, real time PCR technique was used to confirm suspected PCR cases by detecting PPRV nucleic acid from collected clinical samples. Out of a total 42 samples tested for the viral nucleic acid, 16 (38.1%) were detected positive confirming that the virus is circulating in the study areas. This is in line to previous findings of 33.3% positive sample in Nigeria (De Nardi *et al*., 2012) and 44.4% positive sample in Morocco (Kwiatek *et al*., 2011) by N and F protein gene amplification respectively. The present finding is also comparable to that of Alemu *et al*. (2019) and Ebissa (2020) who reported 46.4% and 45.4% positivity using N gene based PCR method. Indeed, positivity finding of 42.6% (Saeed *et al*., 2009 and 34.3% (Abubakar *et al*., 2011) obtained by Ic-ELISA technique also support the current finding.
In contrast to the current finding, a much higher PPRV nucleic acid detection rate of 51.2% by Luka *et al*. (2011), 58.06% by Kardjadj *et al*. (2015) and 78.95% by Kabir *et al*. (2020) was reported using RT-PCR method. Twenty five percent (25%), 10.4% and 15.3% positivity of clinical samples to the virus nucleic acid which is much lower than the present report was also indicated by Anees *et al*. (2013), Altan *et al*. (2019) and Rudra (2019) respectively. The difference in this positivity might be resulted from variation in detection method, sample size, sample type, the virus infection stage and targeted gene type in detection (Alemu *et al*., 2019).

The present study showed significantly higher PPR infection rate in goats (50%) compared to sheep (8.3%, p=0.01) which might be due to the virulence loss of the Ethiopian PPRV strain for sheep or relative resistance that sheep have against the virus (Alemu *et al*., 2019). This finding is in concordance to earlier works by El-Rahim *et al*. (2010), Elhaig *et al*. (2018) and Mantip *et al* (2021) who reported higher severity of PPR in goats. Similarly, Mahajan *et al*. (2013) also revealed higher incidence of PPRV infection in goats than sheep. Moreover, Abubakar *et al*. (2008) noted that outbreak reports in Pakistan were relatively more severe in goats. This could be due to a reason that PPRV can cause significant immune suppression in goats as a consequence of proliferation of leukocytes and mononuclear blood cell apoptosis (Ebissa, 2020).

5.6. Culture and Isolation of PPRV

In this work, all clinical samples (16 samples) positive for viral nucleic acid detection were pulled together to 10 sample pull based on sample type and inoculated on VDS cells for isolation and propagation of PPRV. Accordingly, 6 pulled samples (60%) were grown on the cell with characteristic CPE of the virus including rounding of the cells, vacuolation, aggregation and syncytia formation and detachment of the cells as described by OIE (2013). This isolation percentage is lower than that of the previous works by Zahur *et al*. (2014) and Ebissa (2020) who isolated PPRV from 9.3% and 9% of VSD cell cultured samples respectively. In contrast to this, finding of 83% positivity of the virus on the vero cell was reported by Elhaig *et al* (2018). This difference might be observed because of difference in quality of the sample, load of the virus in the sample, storage duration and storage temperature.

Moreover, the CPE finding observed in the present work is in agreement with that of Sannat *et al*. (2014), Zahur *et al*. (2014) and Mallinath *et al*. (2018) who reported similar characteristic CPE of rounding, ballooning, aggregation and fusion of the cells on VSD cell. Similarly, Ebissa (2020) isolated PPR from a culture that showed cell rounding, vacoulation and aggregation of the cells on vero cells. The signs observed on the VDS cell in the current study were also agree with the finding of Hemida *et al*. (2020) who revealed rounding and clustering together of the cells, detachment and death of the cells as a culture result on similar cells.

6. CONCLUSION AND RECOMMENDATIONS

The current study revealed PPR is endemic in the study area with high seroprevalence in Awi and Metekel zones. Seroprevalence of the disease showed significant association with risk factors such as districts, age, body condition, flock size, housing and presence or isolation of sick animals. Typical clinical signs of PPR were observed and the case was confirmed by cultural isolation and detection of the PPR virus with real time PCR which is the first report in the study areas. Higher distribution/morbidity and fatality rate of PPR in the areas with higher number of outbreaks in dry season that peaks at April was also observed indicating that it requires priority attention. Most of the farmers in the area were aware of the disease being able to characterize it by clinical sign though most of them were not familiar with prevention and control measures of the disease. It was also concluded that molecular detection of PPR virus circulation in the study area with the usual free movement of animals by traders and migrants in Ethiopia along with that of between Metekel zone and Sudan boarders, the practice of communal grazing in extensive farming system of the area may contribute to the endemicity of the disease and further spread of the virus within the area and to other disease free areas which can lead to significant production loss posing continuous challenge to small ruminant production.

Based on the above conclusive remarks, the following recommendations are forwarded:

- Further study/research works should be done on PPR virus characterization, sequencing of circulating virus to characterize the lineage
- Strengthening early warning systems and proper implementation of prevention and control measures including regular surveillance, targeted vaccination and monitoring is important
- Priority attention should have to be given to the area by the control and eradication campaign and awareness on control and prevention measures of the disease should be created to the farmers to enhance participation and better implementation of the disease surveillance and control program.

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8. ANNEXES

Annex 1: Small ruminant age estimation by dentition

No. of permanent incisors	Estimated age range	
	Sheep	Goat
0 pair	Less than one year	Under one year
1 pair	1-1½ years	1-2 years
2 pairs	1½-2years	2-3 years
3 pairs	2½-3years	3-4 years
4 pairs	More than three years.	More than four years
Broken mouth	Aged	Aged

Source: ESGPIP, 2009

Annex 2: Body condition scoring method of sheep and goats

Condition	Score	Description
Starving	0	Extremely emaciated and on the point of death. It is not possible to detect any muscle or fatty tissue between the skin and the bone.
Very thin	1	The spinous process is prominent and sharp. The transverse processes are also sharp, the fingers pass easily under the ends, and it is possible to feel between each process. The eye muscle areas are shallow with no fat cover.
Thin	2	The spinous process feels prominent but smooth, and individual processes can be felt only as fine corrugations. The transverse process is smooth and rounded, and it is possible to pass the fingers under the ends with a little pressure. The eye muscle area is of moderate depth, but has little fat cover
Moderate	3	The spinous process is detected only as a small elevation; it is smooth and rounded and individual bones can be felt only with pressure. The transverse process is smooth and well covered, and firm pressure is required to feel over the ends. The eye muscle area is full, and has a moderate degree of fat cover
Fat	4	The spinous processes can just be detected with pressure as a hard line between the fat covered eye muscle areas. The end of the transverse process cannot be felt. The eye muscle area is full, and has a thick covering of fat.
Very fat	5	The spinous process can't be detected even with firm pressure, and there is a depression between the layers of fat in the position where the spinous process would normally be felt. The transverse process cannot be detected. The eye muscle area is very full with thick fat cover. There may be large deposits of fat over the rump and tail.

Source: ESGPIP, 2009

Annex 3: Sample collection format

Sampling format

Zone:_____District:_____Kebele:_____village_____

GPS coordinate: Northing: _____ Easting: _____ Elevation: _____

No.	Date	Flock size		Species	origin	Sex	Age	Phys. Status	Body condition	Inter herd Contact	Grazing Management	Housing	Introduction of new animal	Vac. status	Isolate sick	Sample type
		Sh	G													

* Species (1=sheep, 2= goats)
* Origin (1= born in herd, 2= gift , 3= Market)
* Phy. Status (Dry, lactating, pregnant, Keb)
* Grazing management (1= Private, 2= zero grazing, 3= communal)
* Housing (1=alone and floor, 2= alone and bed, 3= floor and mixed)
*Vaccination status (1= vaccinated, 2= Non-vaccinated)
* Body condition (1=poor, 2= Medium, 3=Good)
* Inter herd contact (1=present, 2= absent)
* Isolate sick (1=yes, 2= no)
* Introduction of new animal (1= Present, 2= Absent)

Annex 4: Description, principle, validation and interpretation of c-ELISA test

Description and Principle of the test

The wells are coated with purified recombinant PPR N protein. The samples to be tested and control are added to the micro wells. Anti-N protein antibodies, if present form an antibody-antigen complex which masks the N protein epitopes.

An anti-N protein-Peroxidase (HRP) conjugate is added to the micro wells and fixes to the remaining free N protein epitopes, forming an antigen-conjugate-HRP complex. After washing in order to eliminate excess conjugate, the substrate solution (TMB) is added and observed for presence or absence of color development.

The resulting coloration depends on the quantities of specific antibodies present in the sample to be tested. In the absence of antibodies in the serum, a blue solution which becomes yellow after addition of the stop solution appears where as in the presence of antibodies, no coloration appears.

Validation of the test

According to the IDvet innovative diagnostic ID Screen® PPR competition manual, the test is validated if

- ✓ The mean OD value of the negative control (OD_{NC}) is greater than 0.7 and
- ✓ The mean OD value of positive control (OD_{PC}) is less than 30% of the OD_{NC}.

Interpretation of the test result

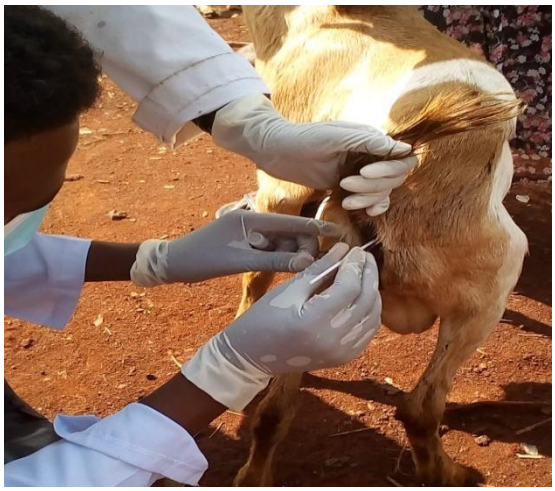
Interpretation of the test result depends on competition percentage (S/N %) of the samples that is obtained by dividing OD value of the samples for OD_{NC} multiplied by 100.
Accordingly;

- ✓ Samples with S/N % less than or equal to 50% are considered positive
- ✓ Samples with S/N% greater than 50% and less than or equal to 60% are considered doubtful
- ✓ Samples with S/N% > 60% are negative.

Annex 5: Sample collection and processing

Discussion and introduction of objective of the study to owner

Age estimation by dentition



Rectal swab sample collection

Ocular swab sample collection

Nasal swab sample collection

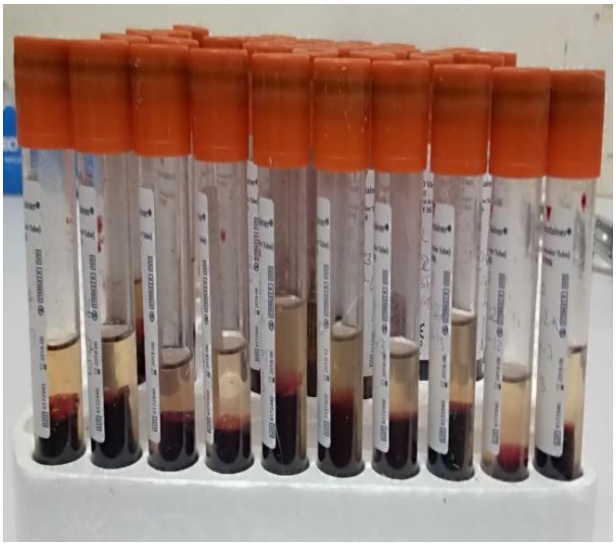
Putting the swab samples in VTM

Blood sample collection with EDTA

Blood collection with Plain vacutainer tube

Drugs for treatment during sample collection

Centrifugation for clearing serum



Samples with separated serum

Arranging the swab and serum samples

Setting storage temperature of refrigerator

Annex 6: Plate lay out for PPRV antibody detection using c-ELISA

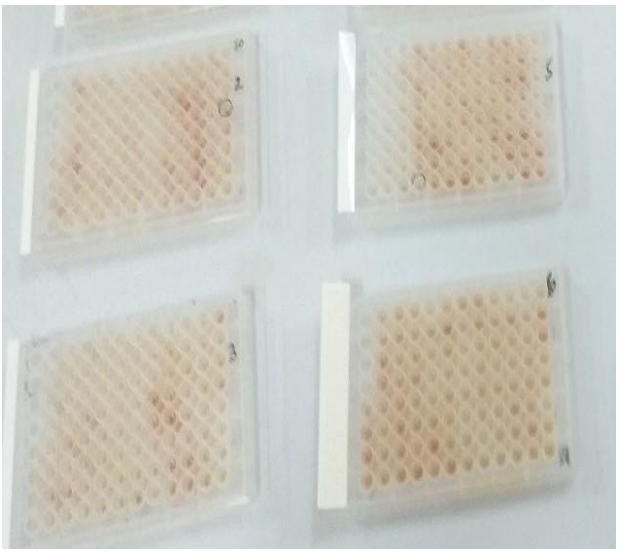
Type of test: PPR C -ELISA Date: 03-08-2021 Plate No: 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	P	21339	21347	21355	21363	21371	21379	21387	21395	21403	21411	21419
B	P	21340	21348	21356	21364	21372	21380	21388	21396	21404	21412	21420
C	N	21341	21349	21357	21365	21373	21381	21389	21397	21405	21413	21421
D	N	21342	21350	21358	21366	21374	21382	21390	21398	21406	21414	21422
E	21335	21343	21351	21359	21367	21375	21383	21391	21399	21407	21415	21423
F	21336	21344	21352	21360	21368	21376	21384	21392	21400	21408	21416	21424
G	21337	21345	21353	21361	21369	21377	21385	21393	21401	21409	21417	21425
H	21338	21346	21354	21362	21370	21378	21386	21394	21402	21410	21418	21426

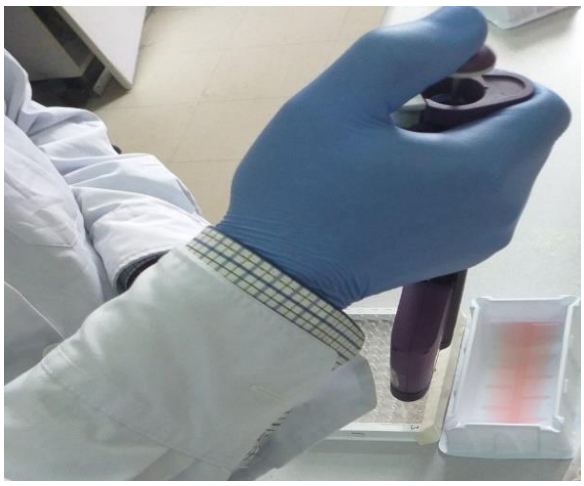
Hint: This is plate lay out is used as sample of the rest seven plate layouts that are prepared in the same way. P and N indicates positive and negative control plate wells layout respectively. The numbers indicate code of the respective samples.

Annex 7: Photographs indicating some serological test procedures

Dispensing the serum samples

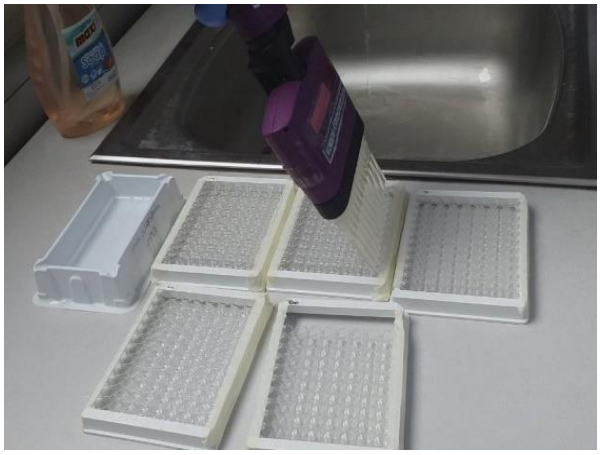


Dispensed serum samples



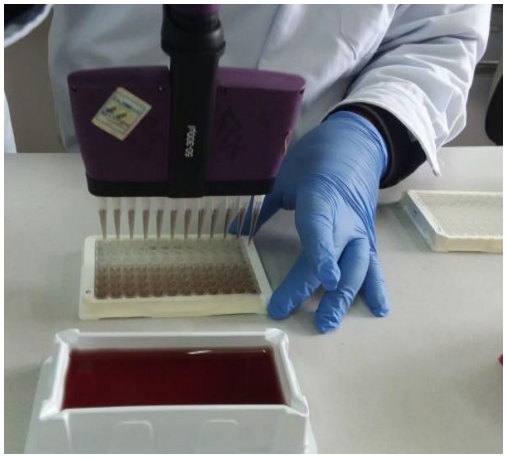
Adding dilution Buffer 13 to plate

Incubation of the diluted samples

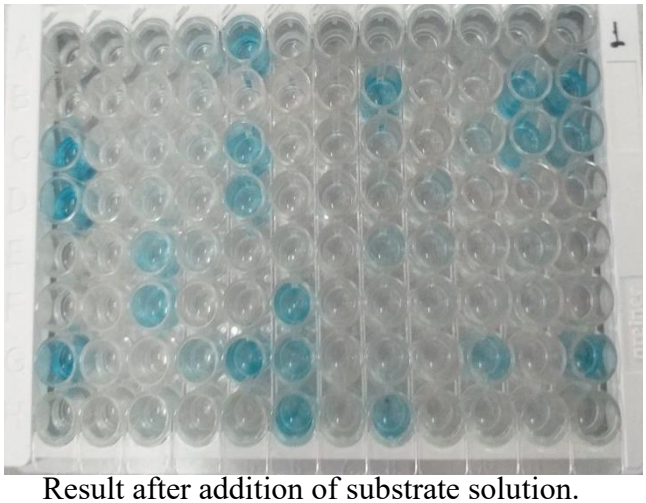


Washing the plates wells after incubation.

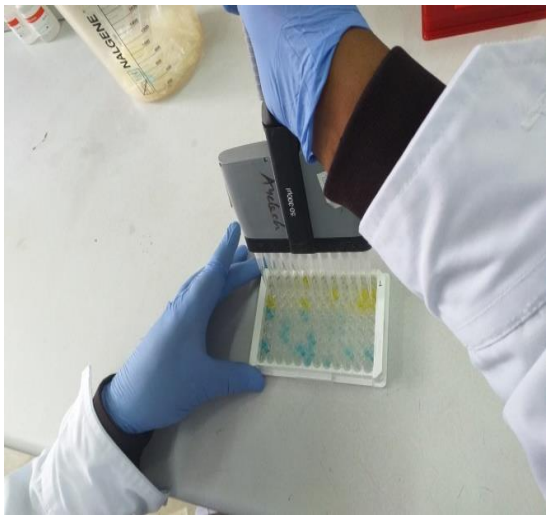
Drying the washed plates



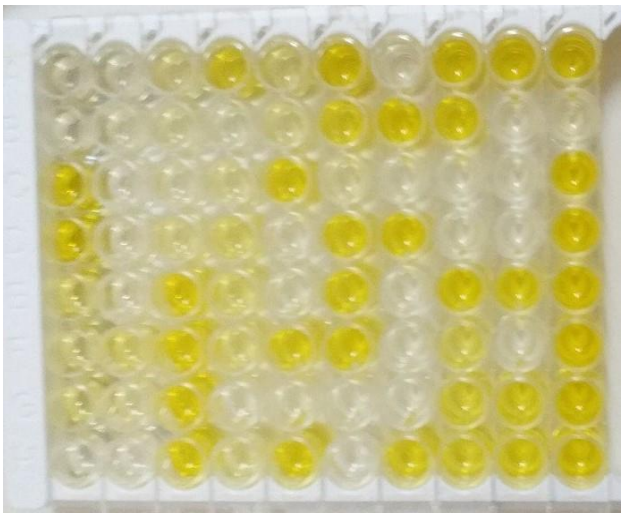
Adding conjugate solution to
Plate wells



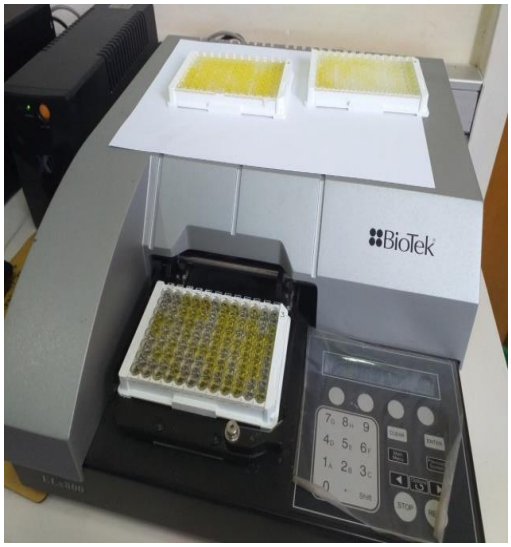
Result after addition of substrate solution.
The blue color indicates negative result



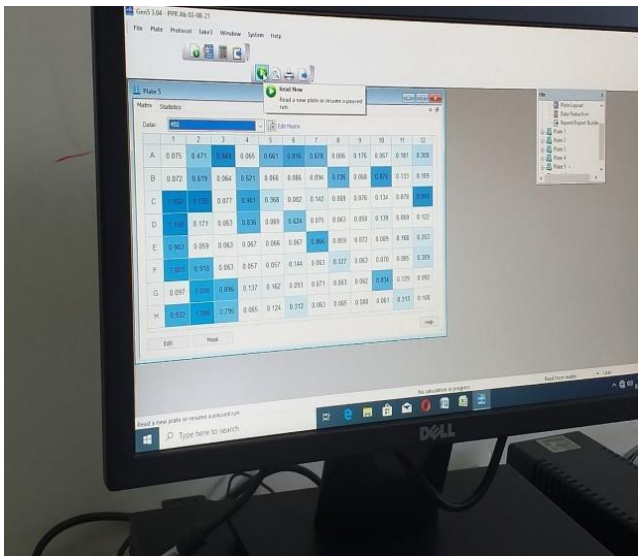
Adding stop solution to the plate wells
with substrate solution result.



Result after addition of stop solution.
Yellow color indicates negative result

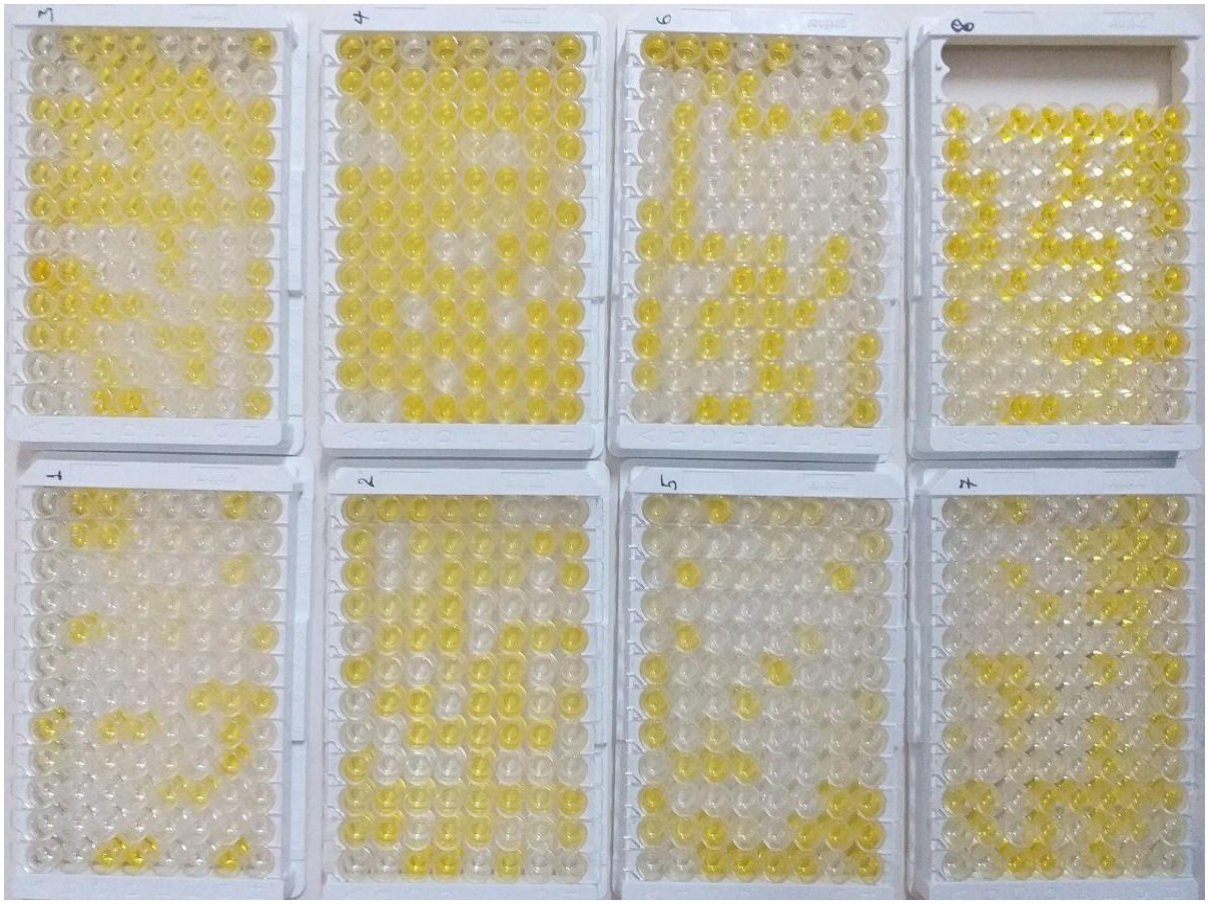


Loading the plates in to ELISA
Plate Reader



Reading result of ELISA test

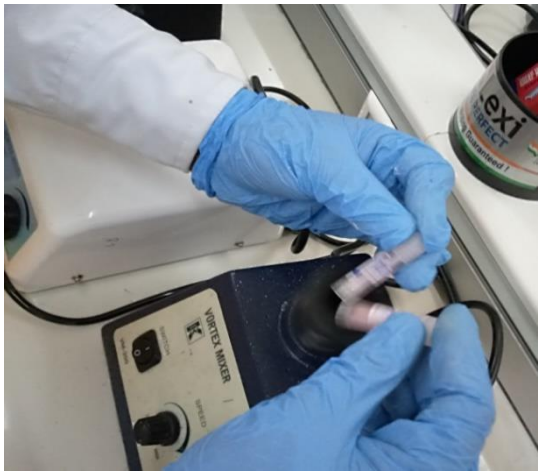
Annex 8: The c-ELISA microplates indicating the results of tested sera samples



Yellow colored plate wells indicate negative results and the non colored wells indicate positive result to PPR antibody

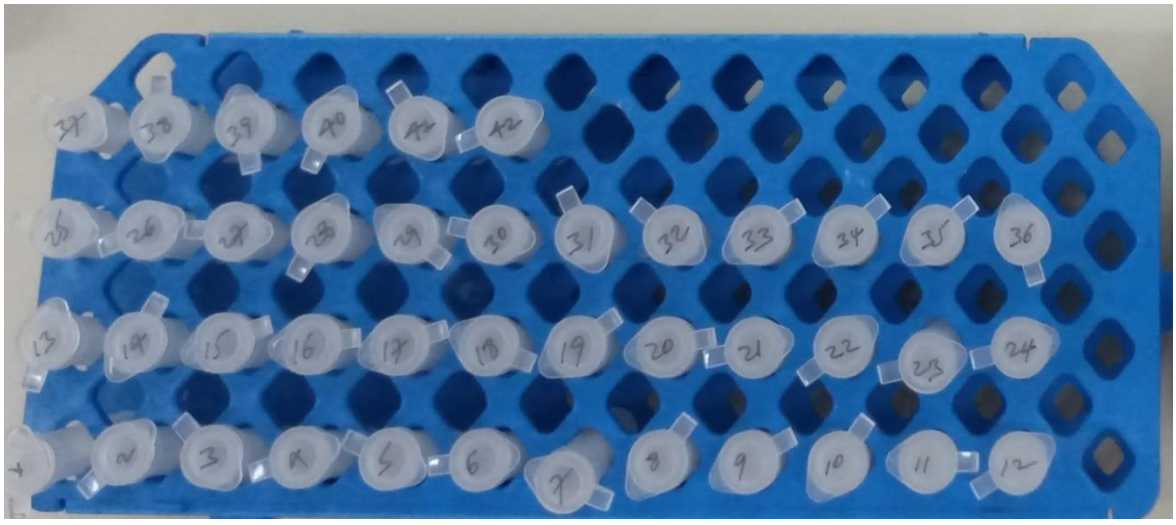
Annex 9: Pictures indicating some RNA extraction and real time PCR procedures

Whole blood and swab samples used for isolation of PPRV RNA

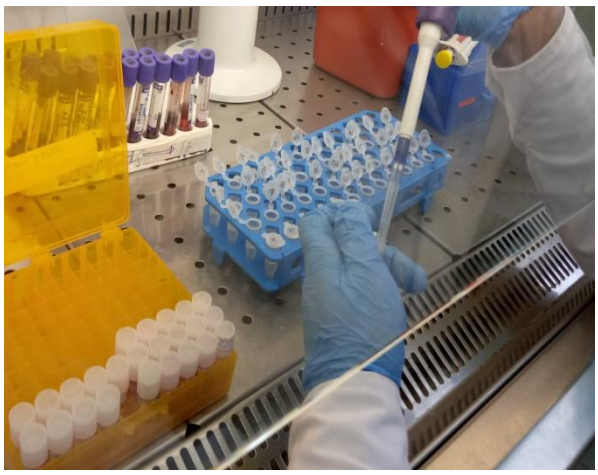


Vortexing swab samples

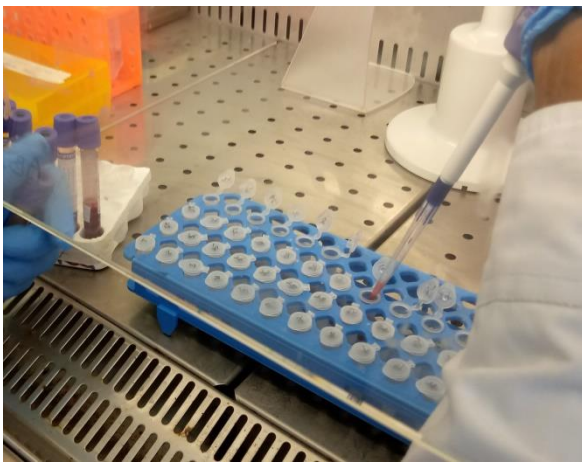
Viral lysis and wash buffers used



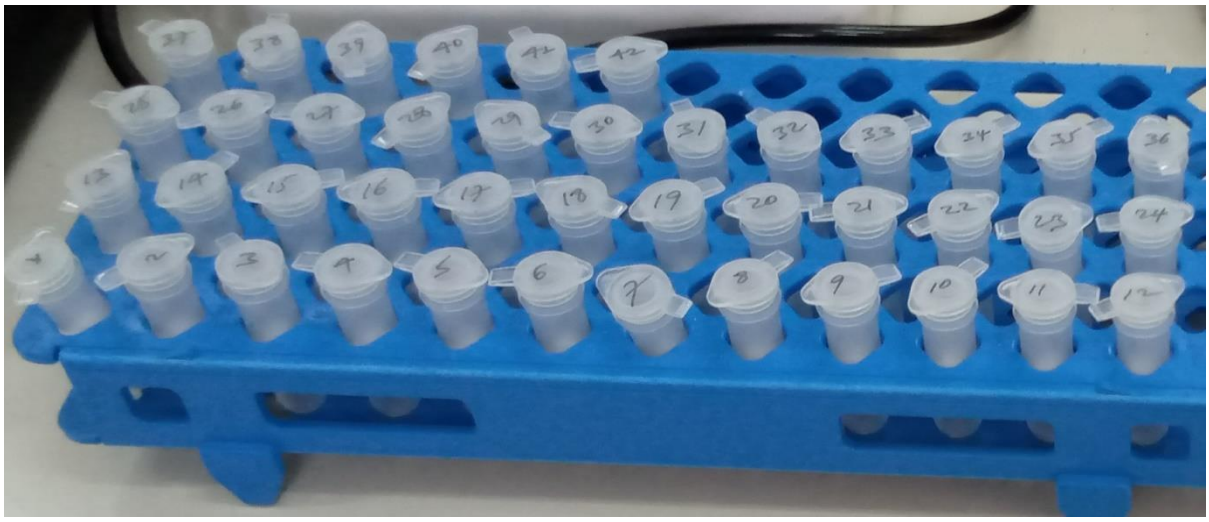
Arranged and coded sterile micro-centrifuge (Eppendorf) tubes for extraction



Adding swab samples to Eppendorf tube holding lysis buffer

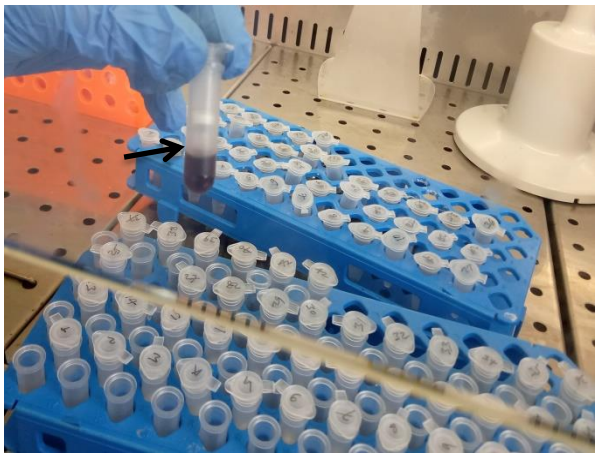


Adding blood samples to Eppendorf tube with lysis buffer

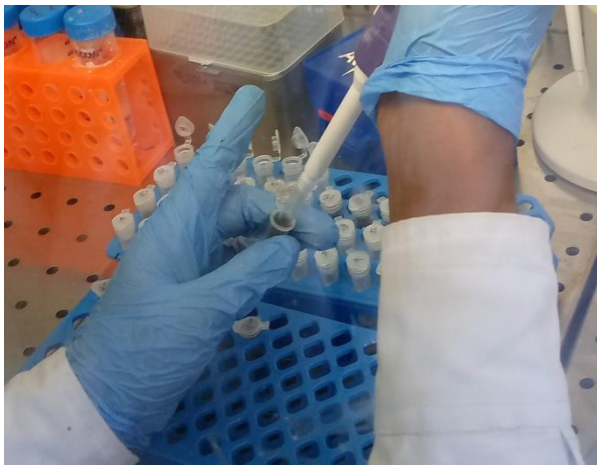


Transferring eppendorf tube containing solution of lysis buffer, samples and absolute ethanol into Mini spin column for centrifugation

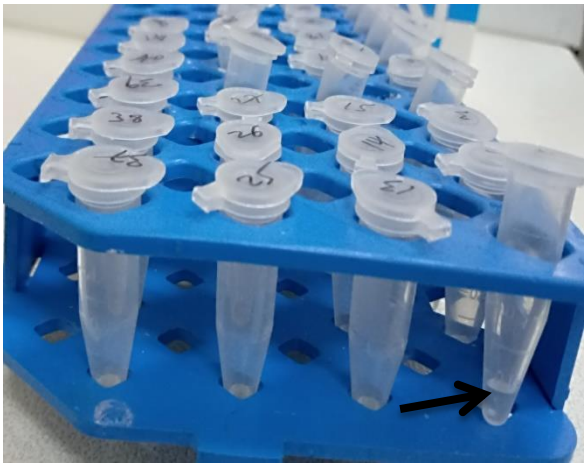
Centrifugations of the Mini spin column holding extraction solution.



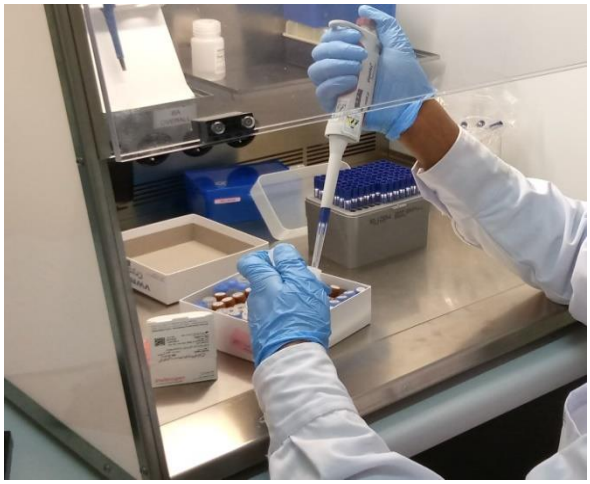
Changing collection tubes for Mini spin column. The arrow indicates the collection tube with filtrate after centrifugation.



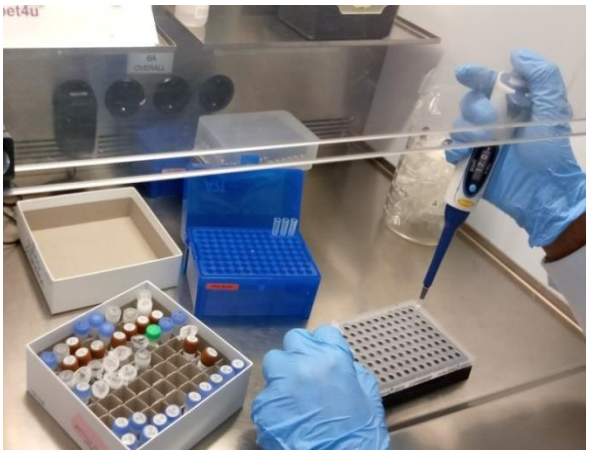
Adding Elusion buffer to the tubes



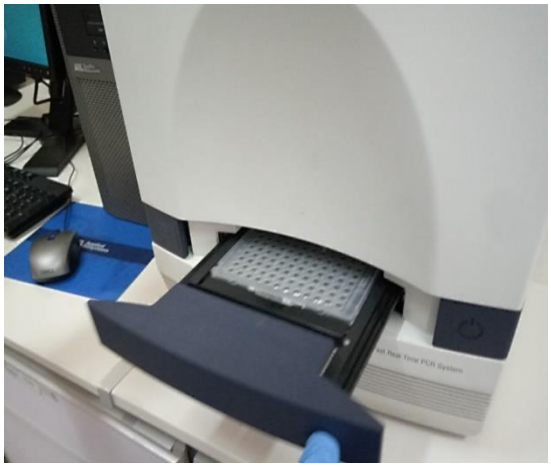
Extracted RNA product



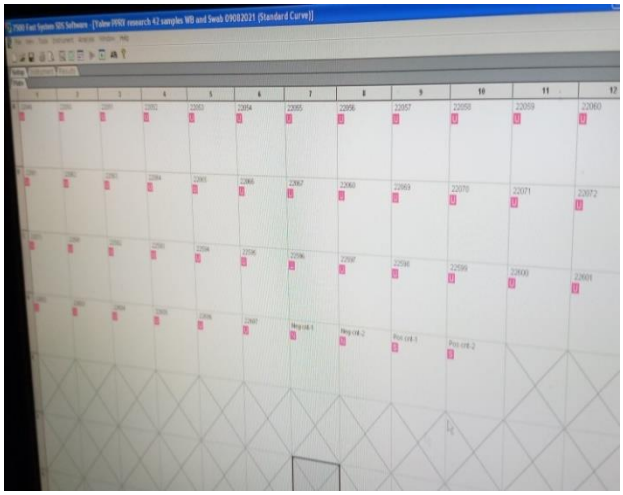
Master Mix preparation



Adding Master Mix, RNA extract and controls to PCR plate wells.



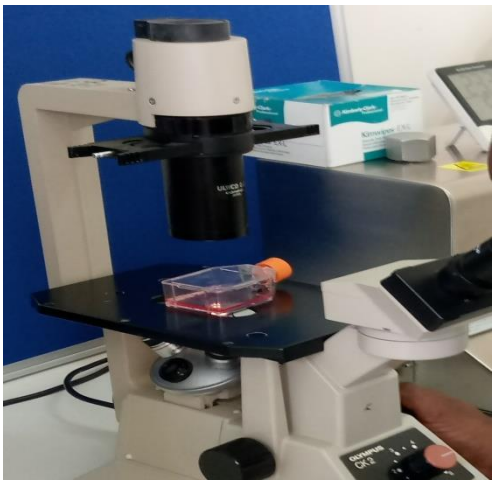
Loading sealed plates in to a PCR thermal cycler Machine.



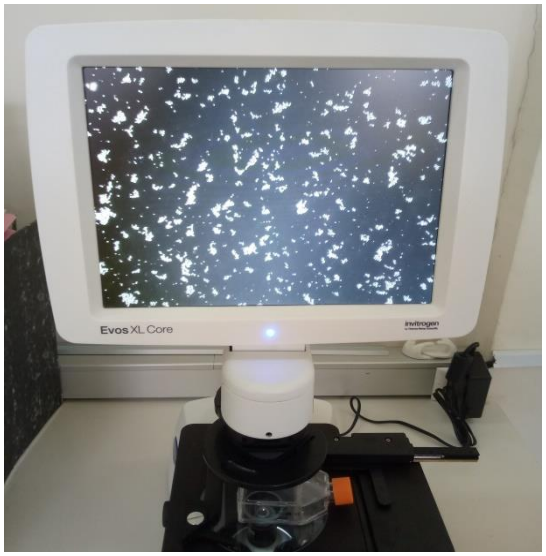
Amplification reaction plate sheet

Annex 10: Pictures indicating some PPRV culture procedures

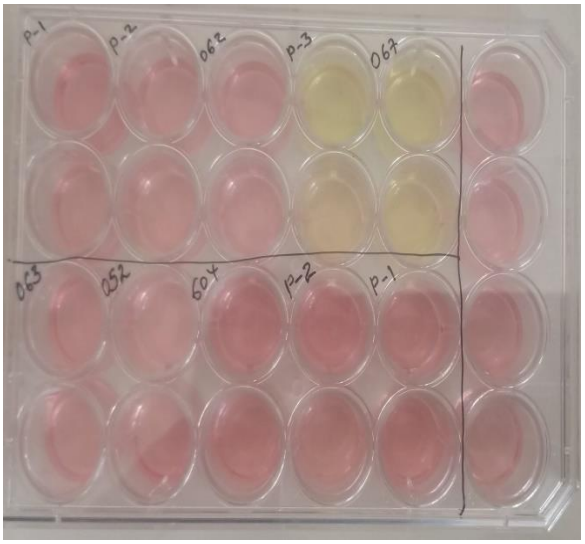
Vero dog SLAM cells used for culture



Examining the cells under light microscope



Observing VDS cell population density using Invitrogen



VDS culture plate after incubation for 24hr. The yellow coloration of P-3 and 067 wells indicate s contamination.

Annex 11: Preparation procedure of 1X conjugate from 10X conjugate

1. Calculate total volume (TV) of 1X conjugate required for the samples to be tested by multiplying amount required per sample (100µl/sample) with total number of plates and plate wells. To compensate for volume of the solution that can be lost during preparation and addition to wells, n+2 number of plates and 100 wells/plates were used for the calculation.

   TV= 100µl/sample X no. plates X no. wells

   = 100µl x (8+2) x (96+4) = 100, 000µl= 100ml
2. Calculate total amount of 10X conjugate required by multiplying TV of 1X conjugate to dilution factor

   Total 10X conjugate= 1/10x100ml = 10ml
3. Calculate amount of Dilution buffer 4 required by subtracting total 10X conjugate from TV of 1X conjugate

   Amount of Dilution buffer 4= 100ml-10ml = 90ml
4. Mix the Dilution buffer 4 and the total 10X conjugate to get the final 1X conjugate required.

   Total 1X conjugate required = 90ml Dilution buffer 4 + 10ml 10X conjugate.

Annex 12: Preparation of working wash solution 20X

1. Calculate total amount of Working solution (TotWS) required by multiplying volume required per sample (300µl) with number of plates, number of wells per plates, number of times the plates are washed
   TotWS= 300µlx10x100=300,000µl= 300ml
2. Calculate total amount of Wash Solution 20X (TWS20X) required by dividing the TotWS for Wash solution concentrate.
   TWS20X = 300ml/20 = 15ml
3. Calculate total amount of distilled water(TDW) required to dilute the wash concentrate
   TDW = TotWS-TWS20X = 300ml-15ml = 285 ml distilled water
4. Dilute the total Wash Solution 20X with the total distilled water required
   TotWS= 285ml distilled water +15 ml TWS20X= 300ml total Working Solution

Annex 13: Buffer AVL-Carrier RNA preparation

1. Calculate volume of Buffer AVL needed for the number of samples to be processed based on volume required per sample which is 0.56.
   Total Buffer AVL volume needed = 0.56ml/sample X total No. samples
   = 0.56X50= 28ml
2. Calculate total volume of carrier RNA-Buffer AVE needed to add to Buffer AVL based on volume required per each sample (5.6µl).
   Total carrier RNA-Buffer AVE needed = 5.6µl/sampleX50
   =280µl
3. Add the total Buffer AVL and the total carrier RNA- Buffer AVE calculated in 50ml Falcon tube and gently mix by inverting the tube 10 times. Do not vortex the mix to avoid foaming.
   Total buffer AVL-carrier RNA = 28ml Buffer AVL + 280µl carrier RNA-buffer AVE
   = 28.28ml

Annex 14: Questionnaire survey

SURVEY IDENTIFICATION

Survey record number: _____

Interviewers' code (Name):_____

Date: _____/_____/_____

I. GENERAL INFORMATION

1.Name of the respondent_____ Age_____ Sex? 1. Male 2. Female

2. Marital status? 1. Single 2. Married 3. Widowed 4. Divorced

3. Telephone_____

4. Educational level? 1. Illiterate. 2. Read and write 4. Primary (1-6) 5. Elementary (7-8)

6. Secondary (9-10) 7. Preparatory (11-12). 8. College. 9. University. 10. Other_____

5. Family members: Male: ____ Female: _____ Total: _____

6. Household head 1.Male. 2. Female

7. Address of the respondent: Zone_____ District_____Village_____
   GPS: Altitude_____latitude_____ longitude_____

8. What is the source of income for the family?

No.	Source of income	Rank
1	Crop	
2	Livestock	
3	Crop and live stock	
4	Trade	
5	Salary	
6	Traditional treatment	
7	Other	

9. What is the total land holding of the family in hectares? _____
   1. Grazingland_____2.crop land_____3.Fallow land_____ 4. Other_____

10. Do you have sheep and goats? 1. Yes 2. No

11. What is purpose of keeping sheep and goats? 1. Meat 2. Milk 3. Multipurpose

12. How many animals do you have at present?
    1. Cows_____ 2. Oxen _____ 3. Heifers_____4. Calves_____ 5. Goats_____

5. Sheep_____ 6. Equines_____7. Poultry_____8. Beehive_____ 9. bull_____

13. What are the main constraints of small ruminant animal production in this area?

No.	Possible lists of constraints	Constraints in your animals	Rank	Constraints in the area	Rank
1	Disease				
2	Feed				
3	Water				
4	Market				
5	Predator				
6	Other				

15. What are the major causes for death of your small ruminant animals? 1. Disease 3. Feed shortage 4. Toxicity 5. Predator 6. Other_____

16. Which type of your animals is mostly affected by disease? 1. Cattle 2. Sheep 3.Goat 4. Equine. 5. Poultry. 6. Honey bee 7. Others _____

17. Which type of animals is mostly affected by disease in the area? Rank them

No.	Species	Rank	Remark
1	Cattles		
2	Sheep		
3	Goats		
4	Poultry		
5	Equines		
6	Bees		

II.Questionnaire related to PPR

1. Do you know PPR (Indicate local name or sign)? 1. Yes 2. No
2. What is local name of the disease? _____meaning_____
3. How is the disease happen? _____
4. Are your sheep and goats has been affected by PPR? 1. Yes 2. No
5. What do you do when PPR infect your Sheep and goats? 1. Take to clinic for treatment 2. Take to market for sell 3. Buy drug and treat by myself 4. Isolate and manage 5, other_____

6. Which type of small ruminant affected? 1. Sheep 2. Goats 3. Both
7. In which animals the disease is more severe? 1. Sheep 2. Goats
8. If your answer is yes for Q4, how many of your animals were affected in last year (2021/2022)? _____
9. If answer for Q28 is yes, which age group is mostly affected?
   1. Kids/lambs 2. Young mature 3. Adult 4. Old 5. Other----------
10. Does the disease vary with sex of the animals? 1. Yes 2. No
11. If yes which sex type is most affected? 1. Male 2. Female what do you think the reason? _____
12. Does PPR infected pregnant sheep and goats abort? 1. Yes 2. No
13. Are sick sheep/ goats separated/isolated from the flock? 1. Yes 2. No
14. Have you ever lost an animal (dead) due to PPR? 1. Yes 2. No
15. If yes how many any animals you have lost in last year (2020/20 21)?

Sheep (በግ)				
	No affected	No died	Price at the time	Total loss in birr
Lamb (የበግ ግልገል)				
Ewe (እናት)				
Ram (አዉራ)				
Ram lamb (ወጠጤ)				
Ewe lamb (ቄብ)				
Wether(ሙክት)				
Goat (ፍየል)				
		No died	Price at the time	Total
Kid (ግልገል)				
Buck (አዉራ)				
Doe (እናት)				
Buckling (ወጠጤ)				
Goatling (ቄብ)				
Wether (ሙክት)				

16. How much ETB you have paid for PPR infected sheep and goats' treatment in 2021/2021? _____
17. What are the symptoms of the animals affected by PPR? _____
18. What are the factors affecting severity of the disease? 1. Age, 2. Sex 3. Body condition 4. Season 5. Housing, 6. Species of animals, 7. Herd size, 8. Other_____

19. If PPR occurrence vary with season why? _____

20. At what season does PPR is common? 1. September – November 2. December – February 3. March – May 4. June- August 5. Not related with season

21. If the disease morbidity is increasing from time to time? Why?

_____

22. How do PPR come to the areas that you are present right now?

_____

23. How do you think the disease could be controlled? _____

III. QUESTIONS RELATED TO HEALTH MANAGEMENT

1. Do your animals get treatment when they become sick? 1. Yes 2. No
2. If yes, how they are treated? 1. Modern treatment 2. Traditional treatment 3. Both
3. Do you treat the animals by yourself? 1. Yes 2. No
4. If yes what do you use to treat animals? 1. Modern drugs 2. Medicinal plants 3. Both
5. If your answer in Q4 above is both, which option you prefer more? 1. Modern drugs 2. Medicinal plants 3. Other_____

And why_____

6. If you use modern drugs for treatment where you get the drugs? 1. Market 2. Pharmacy 3. Government vet clinic 4, Private vet clinic, 5. Community animal health workers 6. Other_____
7. Do you treat PPR using traditional medicinal plants? 1. Yes 2. No
8. If yes, what are the plants used

No.	Plant used	Part used	Prep. method	Trt route

FEEDING AND WATERING SYSTEM

1. what the feeding system for your sheep and goats

1. Rotational grazing, 3. Zero grazing 4, communal grazing

2. Where do your sheep and goats drink water? 1. Separately at home 2. Communal watering points 3. Both
3. What is the source of water? 1. Private pond at home 2. Tape water 3. Communal River 4. Communal pond.
4. Feeding system? 1. Individual 2. Group feeding

PRODUCTION SYSTEM

1. What is production system of sheep and goats in your area?
   1. Extensive 2. Semi-intensive, 3.Intensive
2. What is the farming system
   1. Sedentary mixed farming system 2. Modern production system 3. Pastoral 4. Agro pastoral
3. How do you raise your sheep and goat? 1. Sheep and goat grazing separately
   2. Sheep and goat grazing together 3. Sheep and goat grazing with other livestock.
   4. Sheep and goat tethered feeding at home 5. Other_____

HOUSING

1. Does your sheep and goats housed together? 1. Yes 2. No
2. Do you clean small ruminant house? 1. Yes 2. No. if yes how often?
3. Does your sheep and goats housed by age group? 1. Yes 2. No
4. How your sheep and goats are housed? 1. Fenced barn 2. Separate house 3. Housed with human. 4. No house

VACCINATION

1. Is PPR vaccination practice present in your area? 1. Yes 2. No
2. If yes what is the source of vaccine? 1. Private 2. NGO, 3. Government
3. When did you last have vaccinations against PPR diseases? 1 only when outbreak occurs
   2. Annually 3. When outbreak occurs in neighbor districts/zones
4. Are you willing to vaccinate your animals? 1. Yes 2. No

ANIMAL MOVEMENT

1. Do you move your shoats to other place for grazing or watering seasonally? 1. Yes 2. No If yes, when_____ and for how long did you keep them there_____?
2. Is there free movement of animals at your area? 1. Present 2. Absent
3. Have you recently purchased small ruminant animals? 1. Yes 2. No
4. Animal marketing system? 1. free contact of animals at market 2. no contact at market
5. Have you recently visited market with your sheep and goats? 1. Yes 2. No
6. How frequent you visit market? 1. Once weekly 2. Twice/week 3. Rarely 4----------
7. Have you moved your animal recently for breeding purpose? 1. Yes 2. No.

OUTBREAK HISTORY

1. How often PPR outbreak re occur in your area? 1. Every year 2. Every two year 3. Every three years 4. Other (specify)_____
2. Season of occurrence: 1. dry season 2. rainy season 3. Both
3. When is the last outbreak of PPR in the village occurred? _____
4. What are the most likely sources of PPR outbreaks in your area? 1. Introduction of infected animal/animals to herd 2. Contact at communal watering points 3. Contact at market 4. Contact at communal grazing land 4. Other_____
5. What measures are taken to prevent and control PPR in your area? 1.Traditional treatment 2. Modern treatment 3. Vaccination 4. Report to expert 5. Other _____

Annex 15: The research ethical clearance certificate

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: Certificate Ref. No: VM/ERC/17/03/13/2021

Name of Applicant: **Yalew Abiyu Senebeto (DVM, MSc fellow)**

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

Title of the project *Epidemiology, isolation and molecular characterization of peste des petites ruminants in selected districts of Metekel and Awi zones, Northwest Ethiopia*

Date of application:	**December, 2020**
Nature of the project:	**Mildly invasive /little stress**
Target animal species:	**Small ruminants**
Number of animals involved:	**714**
Study area:	**Awi and Metekel zones, Ethiopia**

Minutes No. and date of review: **VM/ERC/03/13/021, 20/04/2021**

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

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when deemed necessary

Getachew Terefe (DVM, PhD)
Chairman

Signature

The Federal Democratic Republic of Ethiopia
Addis Ababa University
College of Veterinary Medicine and Agriculture

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Please quote Our Ref. No. When replying

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