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MVSC THESIS

SEROLOGICAL INVESTIGATION AND MOLECULAR DETECTION OF PESTE DES PETITS RUMINANTS VIRUS IN BOSET AND FANTALE DISTRICTS OF EAST SHEWA ZONE OF OROMIA REGION

BY

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DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND VETERINARY PUBLIC HEALTH MVSC PROGRAM IN VETERINARY MICROBIOLOGY

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BISHOFTU, ETHIOPIA

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

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College of Veterinary Medicine and Agriculture

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DEDICATION

This thesis manuscript is dedicated to my late father Haji Alo Kadi whose departure happened recently.

STATEMENT OF AUTHOR

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

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LIST OF ABBREVATIONS

AHI	Animal Health Institute
cDN	Complementary Deoxyribonucleic Acid
cELISA	Competitive Enzyme Linked Immunosorbent Assay
CPE	Cytopathic Effect
CSA	Central Statistical Agency
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
IcELISA	Immunocapture Enzyme Linked Immunosorbent Assay
MAb	Monoclonal antibodies
NP	Nucleoprotein
OD	Optical density
OIE	Office International des Epizootics
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PPR	Peste des Petits Ruminants
PPRV	Peste des Petits Ruminants Virus
RNA	Ribonuclic acid
RPV	Rinderpest Virus
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SLAM	Signaling Lymphocyte Activation Molecule
VTM	Viral transport medium
VN	Virus Neutralization
mRNA	Messenger Ribonucleic acid

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ABSTRACT

A cross-sectional study was conducted for serological investigation and molecular detection of Peste des Petitis Ruminants virus from January 2023 to May 2023 in Boset and Fantale districts of East Shewa Zone of Oromia Region with the objective of revealing the sero-prevalence and associated risk factors of PPR in non-vaccinated small ruminants, estimate sero-conversion after PPR vaccination using c-ELISA and detection of PPRV circulating in the area using PCR. Of the total 331 sera samples collected, 165 samples were collected before administering vaccine in the two districts and tested for PPRV antibodies by using c-ELISA and 102 (61.82 %) were positive. Seroprevalence of 47.44 % in Boset and 74.71% in Fantale was recorded with a statistically significant difference in the prevalence between the two districts (χ^2=12.96, P value<0.001). Prevalence in sheep (46.88%) and (65.41%) in goat were recorded. PPRV antibody seroprevalence was higher in adult animals 65.16 % (95%CI=57.3-72.3) and lower in young animals 10 % (P< 0.001, χ2=36.12) and it was statistically significant. The majority of seropositive sheep and goats were those with poor body condition (70%). With flock size, statistically significant seropositivity was observed (P< 0.001). Out of 166 vaccinated sheep and goats tested for post vaccination sero-conversion assessment starting from the second-week post-vaccination, 137 (82.53%) animals were seropositive to PPR according to c-ELISA. So, in this study, rise in herd immunity from (61.82%) prior to vaccination to (82.53%) was observed after the vaccination campaign. From 9 goat samples taken at an outbreak and tested for viral RNA detection 4 (44.4%) were positive for viral nucleic acid. Serological investigation and molecular detection results from this study show that, PPRV was circulating in Boset and Fantalle districts. Therefore, measures to control and eradicate the disease have to incorporate early reporting of the disease outbreaks, effective targeted vaccination and strict animal movement control. More studies characterizing the circulating virus are also recommended.

Keywords*: Epidemiology, Pest des petits ruminants, molecular detection, small ruminant, Boset,Fantale,seroprevalenc,sero-conversion*

1. INTRODUCTION

Ethiopia possesses a huge number of livestock populations in African continent estimated to be 57.5 million cattle, 31.2 million sheep and 29.8 million goats found in the country (CSA, 2019). Owing to their high fertility, short generation interval and adaptation even in harsh environments, sheep and goats are considered as an important asset for poor farmers. Small ruminants are exploited in the country for diverse purposes. However, small ruminant production and productivity and producers' benefits are far below expectations due to diseases and other factors (Abraham *et al*., 2005). Peste des petits ruminant is one of the major limiting factors in increasing the productivity of small ruminants in developing nations, and it has a disproportionately negative impact on the income of impoverished farmers (Balamurugan *et al*., 2014).

Peste des petits ruminants (PPR) is a widespread, acute, highly contagious, virulent and devastating animal disease of domestic and wild ruminants caused by a virus belonging to the genus Morbillivirus of the family Paramyxoviridae (Albina *et al* 2013). Its name is derived from French for "disastrous disease of small ruminants" as it is a fatal disease of sheep and particularly goats it is also called 'goat plague'. The virus is antigenically very similar to the rinderpest virus and other members of the genus Morbillivirus including the measles virus, phocine distemper virus, canine distemper virus and dolphin morbillivirus (Bailey *et al*., 2005). It has an enveloped, single-stranded negative-sense RNA genome that is 15,948 nucleotides long. PPRV genome encodes six structural proteins, which are listed as follows: the nucleocapsid (N) protein, the phosphoprotein (P), the large polymerase (L) protein, the matrix (M) protein, the fusion (F) protein, and the hemagglutinin (H) protein. Two more nonstructural proteins (C and V) are also found (Bailey *et al*., 2005, Alemu *et al*., 2019). It has a single serotype with four distinct genetic lineages (I-IV) and is closely related to the rinderpest virus, which actually was assumed for a long time to be a variant of rinderpest adopted in small ruminants in causing PPR (Agnès *et al.,* 2008).

Peste des petits ruminants (PPR) is widespread in Africa and the Middle East and in some geographical areas of Asia, including much of the Indian subcontinent. Furthermore, because of outbreaks in Morocco and the existing commercial trade between Morocco, Algeria and Spain, the situation raised huge concern owing to the increased risk of introduction of the disease into free zones in northern Africa and into Europe (FAO, 2009; Khalafalla *et al.,* 2010). It is considered an emerging disease in new geographical regions that have not been identified triggering substantial socioeconomic deficits (Banyard *et al*., 2014). The Food and Agriculture Organization (FAO) and the World Organization for Animal Health (WOAH) set the goal of eradicating PPR by 2030, following the successful eradication of rinderpest (FAO, 2016).

Peste des Petitis Ruminants disease is transmitted by direct contact with newly 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 the introduction of new animals are very important to decrease the risk of infection with the disease (CFSPH, 2008). It is an economically important disease of sheep and goats (Ishag *et al*., 2015).

The disease is characterized by high fever, ocular and nasal discharge, pneumonia, necrosis and gastrointestinal tract leading to severe diarrhea (Afera *et al*., 2014). Currently, the disease is recognized as responsible for mortality and morbidity across many countries of the world. Heavy loss can be seen, especially in goats, with morbidity and mortality rates sometimes approaching 80-100% (Diallo, 2006).

Peste des Petitis Ruminants disease is routinely diagnosed on the basis of case history, geographic location, clinical examination, gross pathology and histological findings but clinical signs and lesions can be misleading for PPR diagnosis since a number of diseases have similar outcomes (Kwiatek *et al.,* 2011). However, conventional reverse transcription-polymerase chain reaction (RT-PCR) is routinely used for virus detection (Kwiatek *et al.,* 2007).

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). Vaccines have been available to control the disease for decades with two attenuated vaccine strains, Nigeria 75/1 and Sungri 96, being regularly employed in endemic areas with great success (Sen *et al*., 2010).

In Ethiopia, PPR affects small ruminant production and contributes to food insecurity, particularly in pastoral regions due to its potential for rapid spread and associated restrictions on the international trade of animals and animal products (Ebissa, 2020, Fourni *et al*., 2018). Many Sero-prevalence studies and few molecular detection studies have been conducted on PPR in various regions of the country. Even though these studies contribute to a great extent to the ongoing eradication plan that Ethiopia undertaking to eradicate PPR in collaboration with FAO and OIE. Owing to the depth and magnitude of the problem, these studies are just the tip of the iceberg. Therefore, parallel to the intensification of the efforts to eradicate this debilitating disease, more research is imperative to further understand the epidemiology of the disease and confirming the circulating virus. Therefore; the general objective of this study were to assess epidemiology of the disease and detect PPRV circulating in Boset and Fantale districts.

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Hence, the specific objectives of the study were:

- ✓ To assess the sero-prevalence and risk factors of PPR in non-vaccinated small ruminants
- ✓ To estimate sero-conversion following PPR vaccination in small ruminants
- ✓ To detect PPRV in small ruminants in Boset and Fantale districts. using molecular technique

2. LITERATURE REVIEW

2.1. General Description of the Disease

Peste des petits ruminants is a trans boundary viral disease of sheep and goats that is endemic in many countries of Africa and Asia, and is a major threat for pastoralist and small-holder farmers, making a significant impact on food security, livelihoods and trade (Banyard *et al*., 2010). It is one of the major limiting factors in increasing the productivity of small ruminants in developing nations, and it has a disproportionately negative impact on the income of impoverished farmers (Balamurugan *et al*., 2022). 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 are also called pseudo-rinderpest, goat plague, kata, stomatitis pneumo-enteritis syndrome, and pneumo-enteritis complex (SOP, 2013).

Peste des petits ruminants is most prevalent in third world countries, and more specifically in regions where the farming of small ruminants is a significant contributor to both global trade and food production. Moreover, although a highly effective vaccine is available, it has continued to spread worldwide. It is possible that diseases could spread to PPR-free nations via unlawful transport of contaminated animal products (Mohammed *et al*., 2020). When the infection results in overt and acute disease, the most common outcome is death with case fatality rates that may exceed 90% in naïve populations (Albina *et al*., 2013).

2.2. Etiology

Peste des petits ruminants caused by an RNA coated virus belongs to genus morbillivirus, family Paramyxoviridae and order Mononegavirales (Zakian *et al*., 2016). 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). The morbilli virus genus has different species such *Measles morbillivirus* (Canine), *Cetacean morbillivirus, Feline morbillivirus, Phocinemorbillivirus, Rinderpestmorbillivirus, Small ruminant Morbili virus* (OIE, 2013).

2.2.1. PPRV genome structure

Morbilliviruses are linear, non-segmented, single stranded, negative sense RNA viruses with genomes approximately 15–16 kb in size and 200 nm diameters (Rima *et al*., 2005). PPRV genome encodes six structural proteins, which are listed as follows: the nucleocapsid (N) protein, the phosphoprotein (P), the large polymerase (L) protein, the matrix (M) protein, the fusion (F) protein, and the hemagglutinin (H) protein. Two more nonstructural proteins (C and V) are also found (Bailey *et al*., 2005, Alemu *et al*., 2019). 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).

Peste des petits ruminants virus genome made up a single-stranded non-segmented negative sense RNA molecule encapsidated by nucleoprotein (N) constituting a helical nucleocapsid, combined with the phosphoprotein (P; polymerase complex) as co-factor and the RNA-dependent RNA polymerase (L; large polymerase) to make up the ribonucleoprotein (RNP) complex. These RNP complexes are found inside the viral envelope and look as a helical structure having a herringbone appearance. The matrix protein (M protein) forms an envelope inner surface serving as a bridge between the RNP and cytoplasmic tails of the F and H membrane glycoproteins. This virus is polyploidy and as such incorporates more than one functional and independent encapsidated genome in the appearance of RNPs (Rager *et al*., 2002). The polyploidy results in virions general pleomorphic shape.

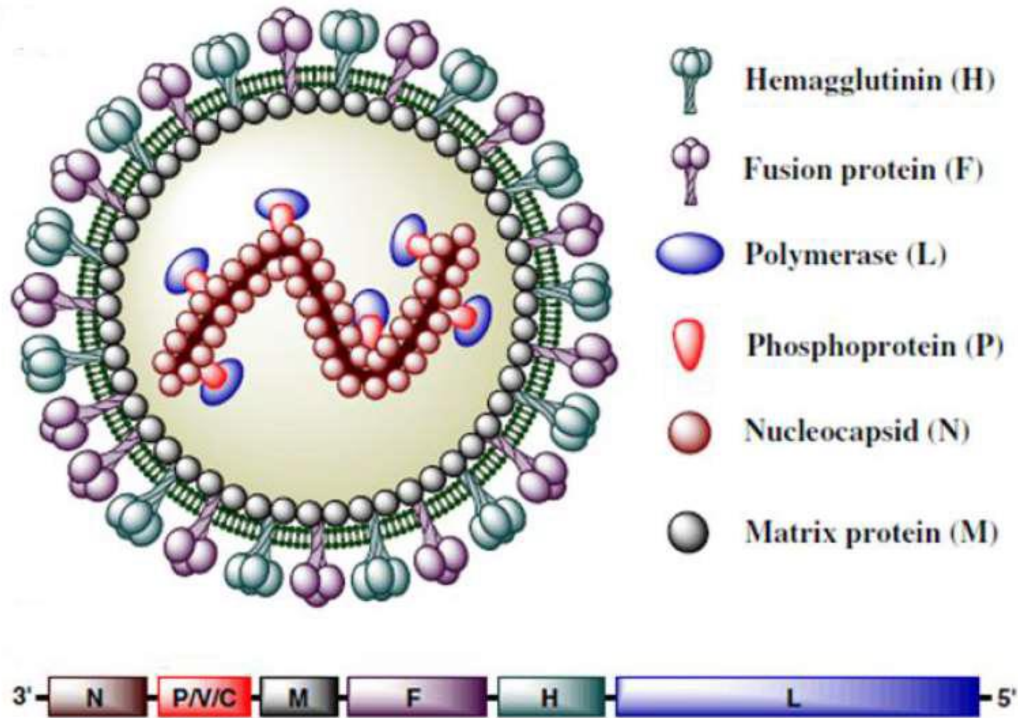

Figure 1: Schematic illustration of PPRV structure (A) and PPRV genome organization (B).

Source: Liu *et al.* (2016)

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).

2.2.2. Viral Replication

By definition, RNA viruses use RNA as genetic material and, thus, must use some relatively subtle strategies to replicate in a cell since the cell uses DNA. Ultimately, to express its genetic information, any virus must be able to present genetic information to the cell as translatable mRNA, but the way this happens with RNA viruses will depend on the type of virus and the nature of the encapsidated RNA (Edward *et al.*, 2008).

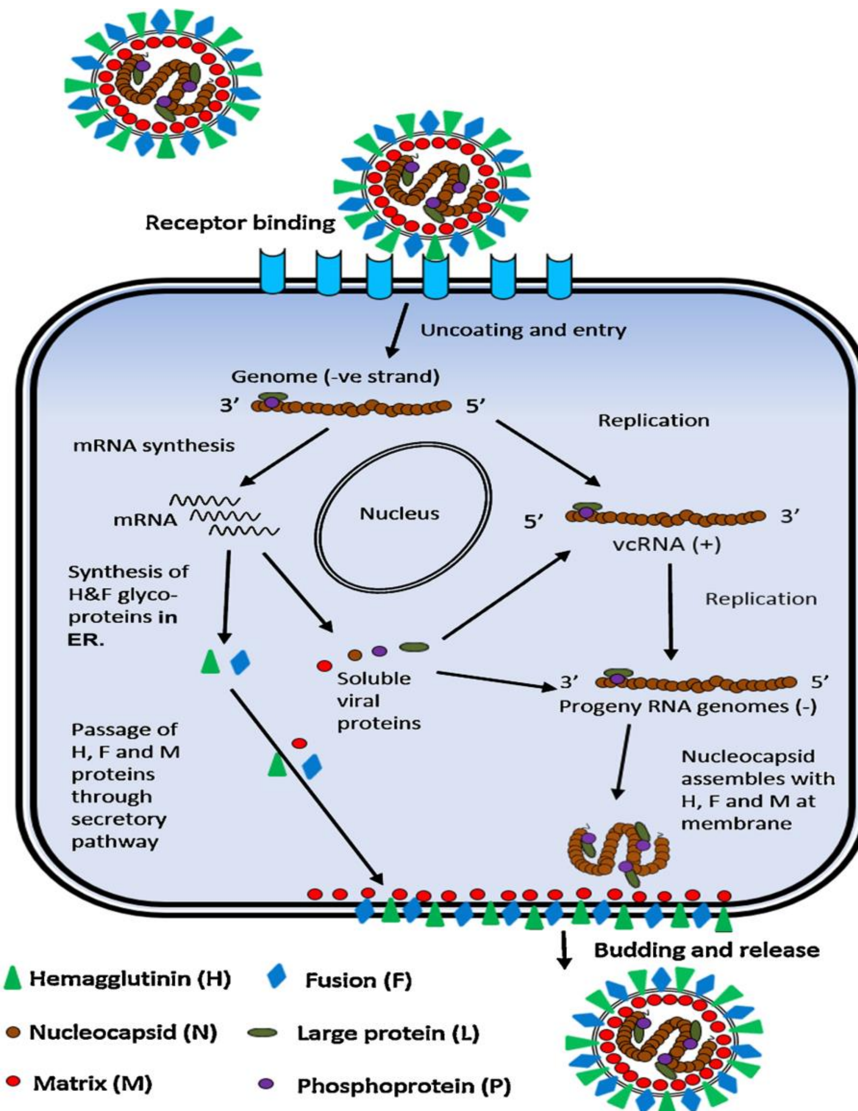

Figure 2: A schematic replication of life cycle of a morbillivirus (adapted from Moss and Griffin, 2006).

The first step in virus infection is the attachment of a virion to a host cell surface receptor which leads to the fusion of viral and cellular membrane. The negative sense RNA genome is released into the cell cytoplasm and transcription initiates to produce viral gene transcripts (mRNAs), which are translated using the host cell transcriptional machinery. Later, following the production of the necessary viral proteins, a switch to a replicative mode occurs that results in the production of a positive sense (+) viral complementary RNA (vcRNA +ve), a replicative intermediate which acts as a template for the generation of progeny negative sense genome RNA. The encapsidated genomes interact with the M protein and the viral glycoproteins,

leading to budding of new virions at the host cell plasma membrane. ER: endoplasmic reticulum (Parida *et al*., 2015).

2.2.3. Physiochemical property

Molecular weight of the PPR virus genome is 5.8 x 10^6. 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). All morbilliviruses are extremely labile in the environment and are inactivated by heat, ultraviolet (UV) light, and chemicals that alter pH or destroy their lipid envelopes. Therefore, although highly contagious, they require close contact between infected and susceptible animals for their transmission which normally occurs via infected air droplets (Mahy and Van Regenmortel, 2010).

2.2.4. Virus Propagation

All known morbilliviruses can be propagated on Vero cells, which lack the ability to produce interferon, but it generally requires several blind passages to adapt the virus to these cells. This adaptation can alter the receptor-binding characteristics of the virus and often attenuates it for the natural host. PPRV is normally grown on lamb kidney cells and typical cytopathic effects such as cell elongation, cell rounding, the formation of stellate cells and syncytia can be observed 3–12 days post infection of the cell cultures although several blind passages may sometimes be necessary before cytopathic changes are observed (Mahy and Van Regenmortel, 2010).

2.2.5. Lineage of the virus

PPRV strains that have been identified by different laboratories so far are divided into four phylogenetic lineages designated I to IV according to the sequence data derived from the nucleoprotein or from the fusion protein genes (OIE, 2013). Each lineage was found to have

specific geographic distribution pattern that has been changed in recent years (Dundon *et al*., 2020). Regardless of lineage, all strains belong to a single serogroup. Lineages I and II occur in West Africa; lineage III in East Africa, the Middle East, and southern India; lineage IV extends from the Middle East to Tibet (Maclachlan and Dubovi, 2011). 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).

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).

2.3. Epidemiology of the Disease

2.3.1. Origin and geographic distribution

PPR was first described in Ivory Coast, West Africa in 1942 and subsequently spread to other regions where it used to be named as Kata, psuedo-rinderpest, pneumoenteritis complex and stomatitis-pneumoenteritis syndrome. In the late 1970s sub-Saharan Africa, then the Middle East and Asia faced severe epidemics respectively (Libeau*et al*., 2014) .It is enzootic in West Africa and on the Indian subcontinent and is now spreading from Afghanistan into Central Asia. It first appeared as a recognized disease in India in 1988 and has subsequently been found as far east as Bangladesh and north as far as Nepal. Epizootics regularly occur in the Middle East and Turkey through import of infected animals. Turkey's proximity to Southern Europe poses a threat to small ruminants in that region. The widespread distribution of PPRV in southwest Asia suggests that the virus had been present on the continent for some considerable time before it was identified in India (Mahy and Van Regenmortel, 2010). 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 *et al*., 2018).

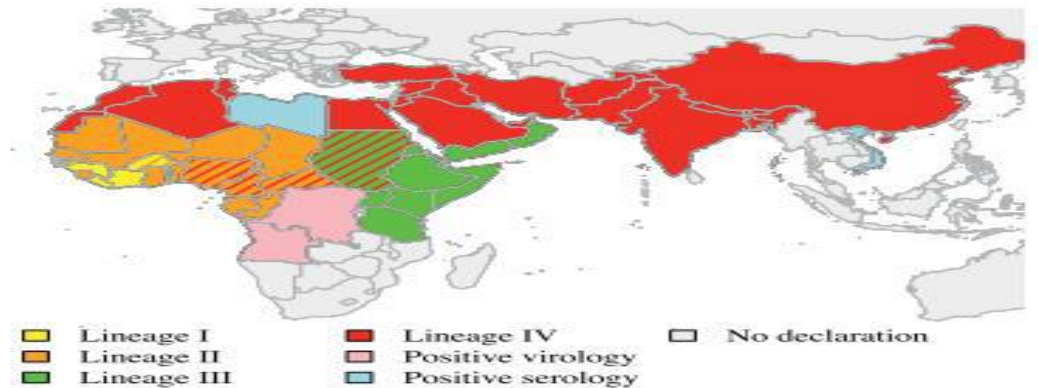

Figure 3: Worldwide cumulative distribution of the four PPR virus lineages. Source: Albina *et al.* (2013)

Different colors show different lineages and hached bars represent the last identified lineage in the corresponding country. The pink color indicates virological evidence of PPR infection. The blue color indicates serological evidence of PPR infection but no virus isolated. The grey color indicates missing information or disease never reported.

2.3.2. Susceptible hosts and reservoirs

Domestic animals such as sheep and goats, camel, cattle and pigs are susceptible to PPR in a variety of degrees. Clinically PPR is seen in both sheep and goats however, goats are more susceptible than sheep (Adel *et al.,* 2004). In addition to sheep and goats, several species of antelope have been fatally infected by contact with infected sheep. Outbreaks of PPRV have been reported in game reserves and zoos where the mortality was 100% in some species (Mahy and Van Regenmortel, 2010). Recent observations in Sudan suggest that camels could be affected by PPR, as they can show clinical expression of the disease and positive results were detected by serological tests, including reverse transcription polymerase chain reaction

(RT-PCR), and PPRV was isolated in cell culture (Khalafalla *et al*., 2010; Kwiatek *et al*., 2011). In one study, antibodies against PPR were detected in Ethiopia in 3 % of the 628 tested camels (Abraham *et al*., 2005). Indian buffaloes (Bulbalus bulbalis) have also been reported to have died from PPRV infections. Cattle have been found in West Africa which were seropositive for PPRV, with up to 80% prevalence in some herds, but there is no evidence that it can cause disease in cattle (Mahy and Van Regenmortel, 2010).

2.3.3. Transmission

PPRV is secreted in tears, nasal discharges, and secretion from coughing and in feces of infected animal. The virus is shed from the intestine and is found in feces at the end stage of the disease approximately 10 days after the onset of fever (Zakian*et al*., 2016). Therefore, it is mainly transmitted by the aerosol route during close contact between animals mainly through sneezing and coughing (Banyard *et al*., 2010). The virus spread through ingestion and conjunctival penetration; by licking of bedding, feed and water troughs are also common. Furthermore, infection may spread to offspring through the milk of an infected dam (Munir *et al*., 2013). Moreover, mixed populations of sheep and goats, the introduction of new animals into a herd/flock, congregation of susceptible animals at grazing land and watering points and intensive type farming system facilitate the transmission of this highly contagious disease (Biruk, 2014).

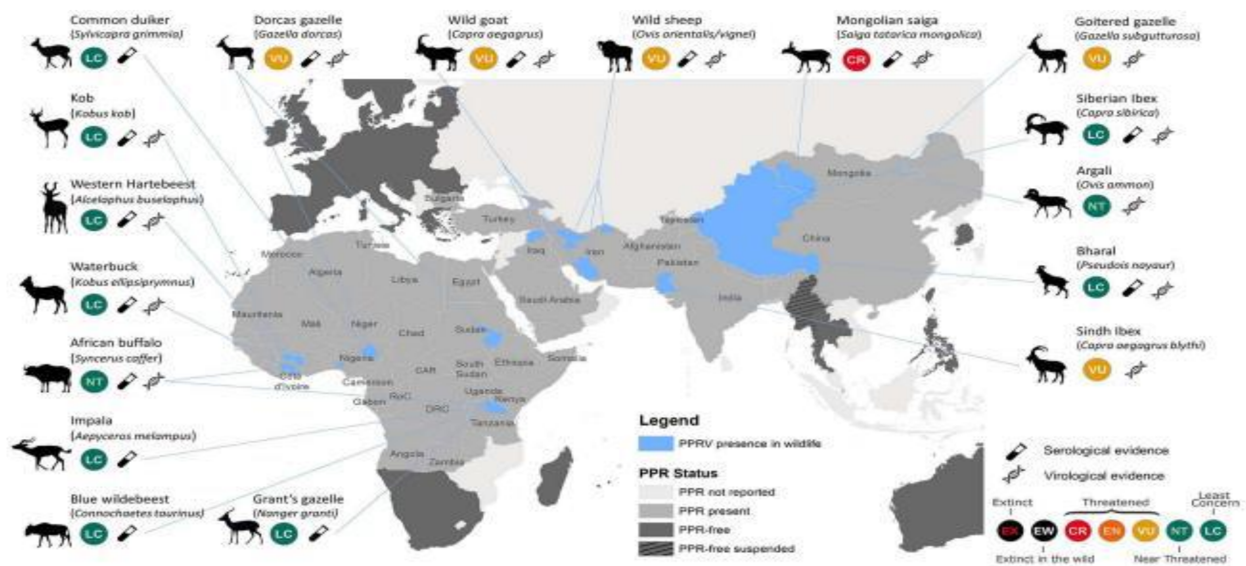

Figure 4: Map illustrating published reports of PPR virus detection in free-ranging wildlife species.

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

All morbilliviruses are extremely labile in the environment and are inactivated by heat, ultraviolet (UV) light, and chemicals that alter pH or destroy their lipid envelopes. Therefore, although highly contagious, they require close contact between infected and susceptible animals for their transmission which normally occurs via infected air droplets. All secretions and excretions potentially harbor virus and, along with fomites, can also be a source of infection. In the case of seals, contact at haulout sites allows the proximity required for aerosol transmission ((Mahy and Van Regenmortel, 2010).

2.3.4. Morbidity and mortality

The morbidity and mortality rates of PPR can be up to 100% in severe outbreaks but in milder outbreaks, mortality rate may be reduced to 50% while morbidity rate still remains high in both cases (Fentahun and Woldie, 2012). Mortality rate is high in the susceptible young

animals (4-8 months), animal with poor nutritional status, stress and concurrent parasitic and bacterial infections also enhance the severity of the disease (Alemayehu *et al*., 2015).

2.4. Pathogenesis

The pathogenesis of the infection is probably similar or identical to that of rinderpest virus, with infection of mononuclear cells with a resulting viremia, leukopenia, and systemic infection, principally involving lymphocytes, macrophages, and the epithelial cells lining the alimentary tract (Mahy and Van Regenmortel, 2010). After the entry of the virus through the respiratory tract system, it localizes first replicating in the pharyngeal and mandibular lymph nodes as well as tonsil. Viremia may develop 2-3 days after infection and 1-2 days before the first clinical sign appears. Subsequently, viremia results in dissemination of the virus to spleen, bone marrow and mucosa of the gastro intestinal tract and the respiratory system (Radostits *et al*., 2007). Acute disease is usually accompanied by lymphopenia and immuno-suppression, leading to secondary opportunistic infections. The virus can be isolated from nasal discharges from the day ninth of virus infection. PPRV then starts multiplying in the gastrointestinal tract, which leads to stomatitis and diarrhea. Apoptosis of infected cells also seems to play an important role in the pathogenesis of PPRV in goats and sheep (Jilo, 2016).

2.5. Clinical Signs

The course of the disease may be peracute, acute, or chronic, depending on strain of virus, age of animals and breed of host (Maclachlan and Dubovi, 2010). The most common form of PPR is the acute form which is characterized by sudden depression, high fever, anorexia, nasal and ocular discharge, mouth erosive lesions, pneumonia and severe diarrhea (Megersa *et al*., 2011). A clear nasal discharge that eventually becomes grey and sticky exudate with severe inflammation of the mucous membrane of the nose, causing respiratory distress is the characteristic sign of PPR. It also causes erosion of nasal and oral mucous membranes, severe oculonasal discharge and congestion of conjunctiva with matted eyelids profuse non-hemorrhagic diarrhea, severe dehydration, progressive emaciation, difficult of breathing and death within 5-10 days in affected animal. Bronchopneumonia with productive cough and

dyspnea is common late in the disease while abortion may be seen in pregnant animals (Abubakar *et al.,* 2008). At necropsy, erosions may be noted in the gastrointestinal and urogenital tracts. The lungs may show interstitial bronchopneumonia and often secondary bacterial pneumonia (Thomas *et al.*, 2006).

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). The sub-acute form, usually occurs in sheep, but also possible in goats. Necrotic ulcers are not obvious and most of the affected animals are recovered. Death is rare. The disease duration is usually more than two weeks (Abraham, 2005).

The peracute form of the disease starts often after a short incubation period of 2 days with a sudden high rise in body temperature up to 40–42°C accompanied by serous oculo-nasal discharges, depression, dyspnoea, anorexia and constipation become congested and occasionally eroded. Affected animals develop profuse watery diarrhoea and die within 4–6 days after the onset of fever. In s*ubacute* forms less severe illness is observed after an incubation period of 6 days and low-grade fever. Affected animals do not display all the clinical signs described above and the mortality rate is much lower. Many animals will recover after an illness of 10–14 days. Subclinical infections are characterized by seroconversion alone (Thomas *et al.,* 2006).

2.6. Diagnosis

Specimens for laboratory examination should be taken from animals in the acute phase of the disease. Suitable specimens include nasal and ocular swabs, unclotted blood and scrapings of buccal and rectal mucosae. Samples of lung, spleen and lymph node from animals slaughtered early in the course of the disease are also suitable (Quinn *et al.,* 2011*).*

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).

However, whatever the qualities of all the new techniques are, it is important to know though not necessarily used for all outbreaks that, virus isolation still remains the gold standard diagnostic technique (Ularamu *et al.,* 2012).

2.6.1. Laboratory diagnosis

The laboratory tests currently available for diagnosis of the disease can be grouped into three categories: (i) those detecting virus or viral antigen (e.g., virus isolation, antigen capture ELISA, lateral flow devices), (ii) those detecting genetic material from the virus (e.g., RT-PCR, real-time PCR, LAMP PCR) and (iii) those detecting antibodies against the virus (e.g., virus neutralization test, competitive ELISA, indirect ELISAs). However, the efficiency of laboratory diagnosis can be greatly influenced by the integrity of the sample received, often affected by the conditions of its collection and transportation (Parida *et al*., 2015).

I. Virus isolation:

For successful isolation of the virus, 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). Techniques for virus isolation normally not used as routine diagnostic tests as they are time-consuming and cumbersome (OIE, 2013). Virus isolation does, however, play an important role from a research perspective (Alemu, 2014).

PPRV may be isolated in primary lamb kidney/lung cells and some cell lines (Vero, B95a). Unfortunately, PPRV isolation using such cells is not always successful on first passage and may require multiple blind passages. Recently derivatives of cell lines (Vero, CV1) expressing the morbillivirus receptor, the signaling lymphocyte activation molecule (SLAM or CD150), have been developed that can enable isolation of field viruses from pathological specimens in less than 1 week, without requirement for blind passages. These include a derivative of the

monkey cell line, CV1, expressing goat SLAM and derivatives of Vero cells expressing dog SLAM. Monolayer cultures are inoculated with suspect material (swab material, buffy coat or 10% tissue suspensions) and examined daily for evidence of cytopathic effect (CPE). The CPE produced by PPRV can develop within 5 days and consists of cell rounding and aggregation culminating in syncytia formation in lamb kidney cells and cell lines expressing SLAM. In unmodified Vero cells, it is sometimes difficult to see the syncytia. If they exist, they are very small. However, small syncytia are always seen in infected Vero cells stained with haematoxylin and eosin. Syncytia are recognized by a circular arrangement of nuclei giving a "clock face" appearance. Cover-slip cultures may show CPE earlier than day 5. Some cells may contain intra cytoplasmic and intra nuclear inclusions, others may be vacuolated. Similar cellular changes may 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 (OIE, 2013).

II. Immunocapture enzyme-linked immunosorbent assay (icELISA)

The icELISA using two monoclonal antibodies (MAb) rose to the N-protein allows a rapid identification of PPRV. The instructions provided by kit supplier should be followed (OIE, 2013).

III. Lateral flow devices

Lateral flow test for detection of PPRV antigen and antibody was also developed, but not up to the mark in regular usage. However, it is a rapid screening test/pen-side test. The LFD-based PPRV test is based on the specificity and affinity of monoclonal antibody. This antibody recognizes the PPRV H-protein. The instructions provided by kit supplier should be followed (Purbright Ltd.)

IV. Competitive enzyme –linked immunosorbnt assay

Several competitive ELISAs (C-ELISA) have been described, based on the use of MAbs that recognize virus proteins. They are of two types: those where the Mab recognizes the N-protein and use recombinant N-protein produced in baculo-virus as the antigen and those with a viral

attachment protein (H) specific MAb and antigen consisting of purified or part-purified PPRV (vaccine strain). All the assays work on the principle that antibodies to PPRV in test sera can block the binding of the MAb to the antigen (OIE, 2013). 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).

V. Virus neutralization

Virus neutralization test is a gold-standard antibody detection test prescribed for international trade. In the VNT, 100-1000 TCID50 of PPRV is mixed with 100µL of twofold dilutions of serum and incubated at 37°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).

VI. Indirect ELISAs

An indirect ELISA, as a valuable alternative to competitive ELISA, was developed for PPR antibody detection (Singh *et al*.,2004b) with a relative diagnostic specificity of 95.09 % and diagnostic sensitivity of 90.81 % when compared to competitive ELISA (Singh *et al.,*2004a). Although a species-specific conjugated secondary antibody is required, this indirect ELISA could be used if the MAb clone used in competitive ELISA is lost due to some unavoidable situations or in laboratories where competitive ELISA is not available.

VII. Nucleic acid recognition methods

Diagnosis of the disease, aside from clinical signs, has shifted from virus isolation to quantitative RT-PCR tests. These tests can distinguish between peste des petits ruminants and rinderpest viruses, which has been critical in the rinderpest eradication program. Virus isolation in primary lamb kidney cells is still used to obtain isolates for molecular characterization and comparison (Maclachlan and Dubovi, 2010).

Polymerase Chain Reaction techniques (PCR)

Reverse transcription PCR (RT-PCR) techniques based on the amplification of parts of the N and F protein genes have been developed for the specific diagnosis of PPR. This technique is 1000 times more sensitive than classical virus titration on Vero cells with the advantage that results are obtained in 5 hours, including the RNA extraction, instead of 10–12 days for virus isolation. The two most commonly used protocols are given in some detail below. A multiplex RT-PCR, based on the amplification of fragments of N and M protein genes, has been reported. Another format of the N gene-based RT-PCR has also been described. Instead of analyzing the amplified product, the amplicon, by agarose gel electrophoresis, it is detected on a plate by ELISA through the use of a labeled probe. This RT-PCR-ELISA is ten times more sensitive than the classical RT-PCR. In recent years, nucleic acid amplification methods for PPR diagnosis have been significantly improved with quantitative real-time RT-PCR. This method is also ten times more sensitive than the conventional RT-PCR, as well as minimizing the risk of contamination. The application of nucleic acid isothermal amplification to PPR diagnosis has also been described. The sensitivity of this assay seems to be similar to that of the real-time RT-PCR. This assay is simple to implement, rapid and the result can be read by naked eye (OIE, 2013).

1.RT-PCR for the diagnosis of PPRV based on the amplification of part of the N-gene .The RT-PCR gives an amplification product of 351 bp. 10µl of these products are analyzed by electrophoresis on 1.5 % agarose-gel. For all positive results, 40µl of the final product may be directly used for sequencing. 2.RT-PCR for the diagnosis of PPRV based on the amplification

of part of the F-gene .Analyze 10µl of the reaction product by agarose gel electrophoresis using a 2% agarose gel in either TBE or TAE buffers. If present, PPRV RNA will be amplified to give a DNA fragment of 371 bp.

2.7. Treatment

There is no treatment for PPR but it helps to give broad spectrum antibiotics to stop secondary bacterial complications and supportive treatment like dextrose normal saline for restoration of body ionic fluid balance (Jilo, 2016). Affected goat with stomatitis, enteritis and pneumonia were treated with penicillin and streptomycin reinforced with broad-spectrum chloramphenicol. However, mortality rates can be reduced by the use of drugs that control the bacterial and parasitic complications. Specifically oxytetracycline and chlortetracycline is recommended to prevent secondary pulmonary infections (CIDRAP, 2003).

2.8. Control and Prevention

Effective implementation of control measures for PPR require rapid, specific and sensitive methods for diagnosis (Munir *et al.,* 2012a). The existence of only one serotype for each of the morbilliviruses, the absence of persistence of infectious virus, and lifelong immunity after recovery from the initial acute infection suggest that outbreaks of these viruses should be easy to control. In addition, the morbilliviruses need close contact for infection to occur, are labile and do not survive long in the environment, and disinfection of infected premises is fairly straightforward (Mahy and Van Regenmortel, 2010). Therefore, Control of PPR outbreaks routinely based on movement control combined with proper disposal of carcass and the use of vaccine. Restriction on importation of sheep and goats from affected areas or newly introduced animal should be quarantined for three weeks. Additionally, carcass and contact fomites should be buried or burned, Barns, tools trans and other items that have been in contact with the sick animals must be disinfected with common disinfectants such as phenol, sodium hydroxide 2%, virkon as well as alcohol, ether and detergents (0IE, 2013). 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). Vaccination should be carried before the start of the rainy season and annually in endemic areas (0IE, 2013).

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°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°C in the lyophilized form and for 21hr at 37°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).

Perspectives on PPR eradication

The goal of the overarching global strategy is the eradication of *peste des petits ruminants* by 2030. For infected countries, this requires a progressive reduction of the virus' incidence and spread, leading to its final eradication. In non-infected countries, it means confirming and maintaining the officially recognized PPR-free status (FAO/OIE, 2017-2021).

The specific characteristics of PPRV such as antigenic stability, a single serotype, and the requirement for close contact for virus transmission, combined with the availability of potent vaccines, long-lasting immunity, and sensitive diagnostics support the prediction that eradication can be achieved (Santhamani *et al.,*2016*).* Investing in PPR eradication contributes to food security and reduces poverty in the world's most vulnerable pastoral and rural

communities, directly benefiting the livelihoods and stability of millions of pastoralists and livestock smallholders in affected countries (FAO/OIE, 2017-2021).

2.9. Status in Ethiopia

PPR was introduced to Ethiopia in 1989 in the Southern Omo River Valley from where it moved East to Borana then north wards along the Rift Valley to Awash. The disease then spread northwards into the central Afar Region and eastwards into the Ogaden (Abraham *et al*., 2005). After the first confirmed cases of PPR in Ethiopia, the disease is continuously affecting small ruminant production and thus contributing to food insecurity, particularly, in vulnerable regions of the country (Waret-Szkuta *et al*., 2008).

Several agro-ecological conditions accompanied with seasonal occurrence of the disease, movement of infective small ruminants within the country and cross-border particularly, the pastoral areas of Afar, Somali and Oromia are well known for significant movement of small ruminants and other livestock species within these regions, to towards central high lands where important livestock markets and export abattoirs are located. Moreover, a cross-border seasonal movement in search of pasture and water in pastoral areas of Kenyan border is also a great challenge to control this widely spreading disease (Alemayehu *et al*., 2015). Therefore strict animal movement control within the country and cross-border should be effective and use of epidemiological intelligence to initially target endemic populations and high-risk areas will be essential (Jilo, 2016).

Clinical and serological evidence of its presence confirmed in 1991 in Addis Ababa (Abraham *et al.,* 1994, Gelagay, 1996) has reported that 14.6% of sheep sampled along four roads from Debre Berhan to Addis Ababa were seropositive for PPR. Waret-Szkuta *et al*., (2008) has also reported an overall seroprevalence of 1.7% in Oromia, 21.3% in Somali, Amhara Region of Ethiopia (Megersa *et al*., 2011) has also reported an overall seroprevalence of 30.9% from sheep and goat in pastoral and agro-pastoral area of afar and Gambella region of Ethiopia. 2.1% from sheep and goats in Four Districts of Bench Maji and Kafa Zones of South West Ethiopia has been reported by (Mussie *et al.,* 2007); Alemu (2014) also reported (28.1%) in Eastern Amhara region.

More recently, an overall seroprevalence record of 60.15% from sheep and goat in the pastoral community from Afar region of Ethiopia has been reported by Dubie *et al.* (2022), Abiyu, (2022) reported (65.4 %) in Awi and Metekel zone and Wondimagegn (2016) reported 41% from sheep and goats in some selected pastoral areas of Somali regional state of Ethiopia. Furthermore, Yalew *et al*. (2019) and Woldemichael *et al*. (2018) reported 75.7% and 73.45% prevalence in Asosa and Metekel zones respectively, Gari *et al*. (2017) and Fentie *et al*.(2018) reported (48.43%) in Eastern Shewa and Arsi Zones of Oromia Regional state and in Tigray (47.5%), respectively.

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Boset and Fantale districts of East Shewa Zone of Oromia Regional state.

Fentale District

Fentale district extends between 8°42’-8°09 and latitudes and39°39’-40°04’E longitudes. It is located in the northeast part of East Shewa Zone. It is bordered with Amhara Regional State in the west and northwest; with Afar Regional State in the north and northeast; with West Hararghe and Arsi zones in east; and with Boset district and Arsi zone (Merti district) in south and southeast. Because of geographical location i.e. crossed by road that leading east part of the country and coming to Addis Ababa, do pass through this district it has a great advantage for accessing the local products to the market and creates ideal conditions for the provision of the demanded commodities to the local communities (Shimelis, 2020).

Annual Minimum and Maximum Temperature: 18°C, 39°C respectively and, annual Rainfall: 350mm-450mm. Total area of land in the district 133,967.00, Cultivated land 19677.25, Forest land 457.00, Grazing land 79329.37, Land used for construction 6,302.9, Others 28,200.00, Total livestock 781,099, Goat 129,424, Sheep 106,932 (Shimelis, 2020).

Boset District

Boset District extends between 8°24′–8°51′ north latitude and 39°16′–39°50′ East longitude. It is located in the northeast part of East Shewa Zone, Oromia Regional State. It is bordered by the Adama District in the west; by Amhara Regional State in the north; by Fantale district in northeast; and by Arsi Zone in southeast. Boset District is located in the midst of the Rift

Valley, which extends from the north to south. Most parts of the district (about 89%) belong to tropical agro-climatic zone and the remaining small section (about 11%) is subtropical (BDFED, 2012). Similarly, the document showed that the district is characterized by hot and dry weather with an average annual temperature that varies between 25–30 °C for the tropical and 15–20 °C for the subtropical. The rainfall is weakly bimodal with spring (a small rainy season) during the months of April and May, while summer (a long rainy season) during the months of July–September. The average annual rainfall ranges between 700 and 800 mm with the intensity and variability being high in the district. In terms of drainage system the district falls in the Awash River Basin, with no other major streams and lakes. Total livestock 581,585, Goat 193,306, and Sheep 67146 (BDFED, 2012).

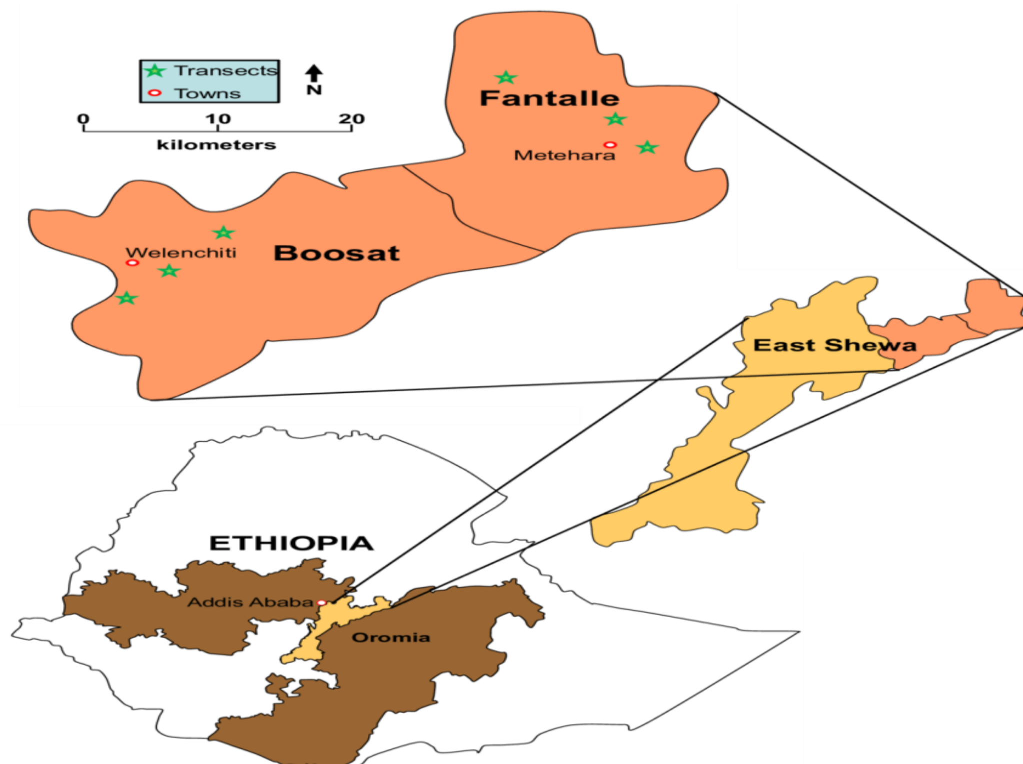

Figure 5: Map showing study area **Source:** Debela *et al.,* 2012

3.2. Study Population

The study population was small ruminants in the selected kebeles of Fantale District and Boset District. Both non-vaccinated and vaccinated sheep and goats were included for serological investigation to study the sero prevalence before and sero conversion after vaccination. Sheep and goats of both sex and all body condition were also included in the study. All age groups of the animals with clinical disease or active lesion were considered for the molecular detection.

3.3. Study Design and Sampling Technique

A Cross-sectional study design was conducted from January 2023 to May 2023. In such a way two kebels from Fantalle and three kebeles from Boset districts were selected. Villages and households having sheep and goats were selected from each PAs using a systematic random sampling method and, simple random sampling method was used to select the small ruminant animals to be sampled. Proper information collection channel was established at the beginning of the study with Assela Regional Laboratory, and used to collect information about the occurrences of the disease outbreaks in the region. A simple random sampling method was employed to collect blood sample for seroprevalence determination, and purposively sample from lesions of animal of all age groups and sex that exhibit clinical sign of the disease was collected for molecular detection of PPRV. Samples included for molecular detection purpose was nasal, conjunctival and rectal swab samples following reported outbreak and field visit while blood collection.

3.4. Sample Size Determination

The sample size for this study was determined at 5% desired absolute precision based on the formula described by (Thrusfield, 2005) using 95% confidence interval and 25.1% expected prevalence which is the average previous sero prevalence study findings of 48.43% (Gari *et al*., 2017) and 1.7% (Faris *et al*., 2011) in Eastern Showa and Arsi Zones of Oromia Regional State and from Awash Fentale District, Afar respectively using the formula;

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

Where N= required sample size, Pexp = expected prevalence and d=desired absolute precision. Thus, substituting the respective values in the formula, 289 sheep and goats were required. However, 331 sera samples were collected for this study.

3.5. Sample Collection and Transportation

Following outbreak reports from the district veterinarians to the Assela Regional Laboratory, the body that supervise PPRV eradication campaign in Oromia Region State, investigation by pen-side test kit (Peste-test rapid test for PPRV infection) was done both in the two districts at different times.

After careful restraining of the study animals, jugular vein area was disinfected using 70% alcohol and 5ml blood was collected from the vein using venoject needle and vacutainer tubes. The collected blood standing in slant position for 24 hours at room temperature to collect serum. Then, the serum was harvested into cryogenic vials and stored at -20°C in aliquots. Nasal, ocular and rectal swabs from active clinically suspected cases were collected using sterile Rayon cotton swab and put in cryogenic vials containing virus transport medium (VTM) containing BPS, antibiotic and antifungal. Both serum and swab samples were transported to Addis Ababa University College of Veterinary Medicine and Agriculture, Molecular laboratory in cool ice box for temporary storage at -20°C. Then, the samples were transported to the Animal Health Institute (AHI) for serological analysis and for molecular detection. Moreover, species, body condition, place of sample collection, age, sex, and vaccination status of the animals were recorded during sample collection to determine association between these risk factors and seroprevalence of PPR.

3.6. Laboratory Investigation

3.6.1. Serological Study

Laboratory analysis of the collected sera samples was tested at Animal Health Institute (AHI) for the detection of specific PPRV antibody using N protein-based c-ELISA kit according to the manufacturer's instruction (ID Screen® PPR Competition, IDvet innovative diagnostics, France). In brief, all reagents were allowed to come to room temperature ($21^oC \pm 5^oC$) and were homogenized by vortex before use. Test plate layout was prepared for the samples and 100µl of each sample was dispensed into microplate wells using a single channel pipette based on the plate layout, sealed with adhesive plastic sealants and temporarily incubated at $+4^oC$. Then, the procedure commences on other new plates as follows; 25µl of dilution buffer 13 was added to each microplate well. A 25µl positive control (to A1 and B1 wells) and 25µl of the negative control (to C1 and D1 wells) was added to the respective plate wells. Subsequently, 25µl of each 100µl dispensed samples were added to the remaining wells using a 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^oC for 45 minutes and each plate wells was 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^oC 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^oC 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 a computer loaded with Gen 5TM 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.

3.6.2. Molecular detection of PPRV

Conventional RT-PCR

Nine(9) samples were examined for the presence of PPRV RNA by one step reverse transcription- polymerase chain reaction (RT-PCR) assays (OIE, 2013). The RNA was extracted from the processed samples by using NucleoSpin® RNA kit (QIAGEN, Cat. no.52906, Hilden, Germany) according to the manufacturer's instructions.

Reverse Transcription- Polymerase Chain Reaction (RT-PCR) was performed for the N-gene of PPRV using QIAGEN® one step RT-PCR kit as per the manufacturer's instructions. The reverse transcription and PCR were carried out sequentially in the same tube. The RNA obtained was converted to cDNA using a reverse transcriptase enzyme. The cDNA was amplified using PPRv specific NP3 and NP4 primers as previously described by Couacy-Hymann *et al.* (2002).

Conventional RT-PCR was conducted following a standard method as described in the OIE manual (OIE, 2013) using primer pairs: NP3 (5′GTC TCG GAA ATC GCC TCA CAG ACT 3′) and NP4 (5′ CCT CCTCCT GGT CCT CCA GAA TCT 3′) (Couacy-Hymann *et al.,* 2002). The amplification was carried out in a final reaction volume of 25 μl containing 7.5 μl of RNase-free water, 5 μl of 5XRT-PCR buffer (Qiagen), 1 μl of deoxyribonucleotide triphosphate (dNTP), 5 μl of *Q* solution, 1.5 μl of each primer NP3 forward and primer NP4 reverse, 1 μl of one-step enzyme mix and 2.5 μl of RNA template at 50°C for 30 min, 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 5 min in an Applied Biosystems 2720 thermal cycler. The PCR products were analyzed by gel electrophoresis on a 1.5% (w/v) agarose gel (Couacy-Hymann *et al.,* 2002).

3.7. Data Analysis

The data was entered into Microsoft Excel spreadsheet 2016 and data were summarized using descriptive statistics (mean, median etc.) and prevalence was estimated for all related risk factors by dividing number of PPR infected individuals to number of individuals sampled multiplied by 100 using STATA version 14.0. Additionally, the Pearson's chi- square ($\chi 2$) was utilized to identify risk factors of prevalence of PPR between Districts, Kebeles, Age and Flock size.

The association between the risk factors and the disease was evaluated using both univariate and multivariate logistic regression. Moreover, the strength of the association between the risk factors and PPR sero-positivity was estimated using the odds ratios (OR). 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.

3.8. Ethical Clearance

The present study underwent thorough review by the Animal Welfare and Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture at Addis Ababa University, with consideration of all pertinent ethical and animal welfare considerations. The approval was duly accorded and endorsed, accompanied by a reference number VM/ERC/23/04/15/2023 indicated in **Annex 6.** In order to ensure the ethical integrity of the study, due diligence was exercised in obtaining the necessary ethical clearances and consents from all relevant participants, as well as adherence to relevant principles in public health and animal welfare ethics.

4. RESULTS

4.1. Outbreak investigation

4.1.1. Clinical Findings

Following outbreak reports from the districts, the predominant clinical signs observed were fever, ocular and nasal discharge, respiratory distress, lacrimation, matting of eyelids and profuse diarrhea in both sexes and all age group (**Figure 6**). A prominent clinical sign was observed in goat than sheep.

Figure 6: clinical signs of PPR a) Conjunctivitis b) Nasal discharge c) profuse diarrhea (picture taken from Boset District).

4.1.2. Virus Detection and Confirmation Using RT-PCR

From 9 samples examined with RT-PCR for viral nucleic acid, 4 (44.4%) samples were positive on gel electrophoresis of the PCR products that were analyzed. The fragment size of the amplified products was 913 bp but the positive control used was that of 351 as reported by Couacy- Hymann *et al.* (2002).

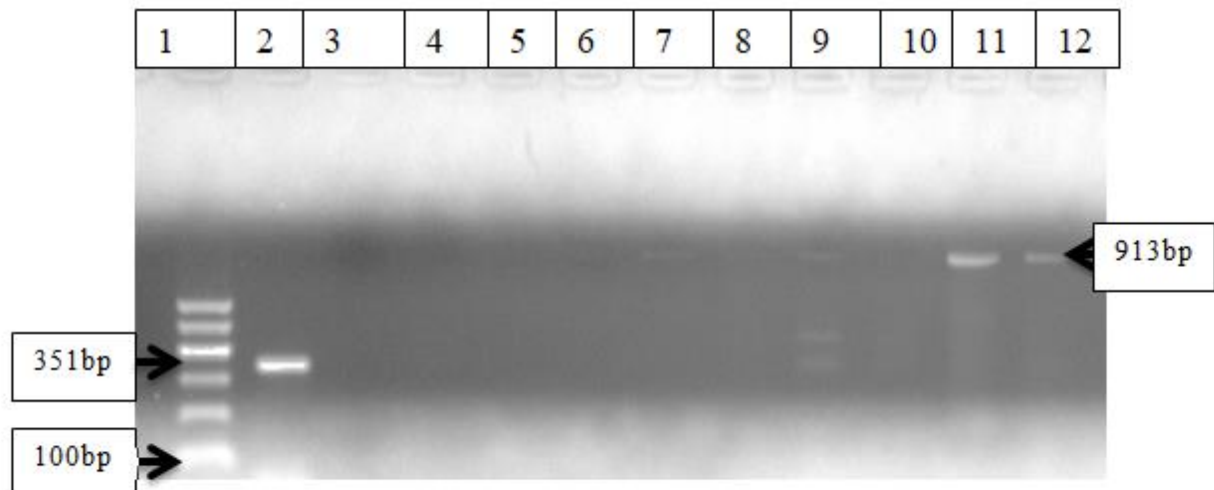

Figure. 7: Agarose gel electrophoresis of PCR products (913 bp) amplified with NP3 and NP4, PPR specific primers. Lane 1 is the DNA ladder of 100 bp, and 2 and 3 lanes are the positive and negative control, respectively. Lane represented by No. 4, 5, 6, 7, 8, 9, 10, 11, and 12 are study samples.

4.2. Serological Study

From a total of 165 sera samples collected from two districts and tested for PPRV antibodies by using c-ELISA kit for unvaccinated small ruminants in the area, a seroprevalence of 102 (61.82%,95% CI) was documented. Sero prevalence of 37 (47.44%, 95% CI) in Boset and 65 (74.71%, 95% CI) in Fantale was recorded with a statistically significant difference in the prevalence between the two districts (χ^2=12.96, P value<0.001). Prevalence was the highest in Tututi kebele of Fantale 15 (100%, 95% CI) and the lowest prevalence in Tri kebele of Boset

district 3 (30%, 95% CI). Differences in disease sero-prevalence between kebeles were statistically significant (X^2= 19.6, P<0.001) (**Table 2**). Additionally, age and herd size had a statistical significant difference (P<0.05) with the prevalence of the disease. Out of total 32 sheep and 133 goat samples tested, 15 (46.88%, 95% CI) and 87 (65.41%, 95% CI) were found to be positive for PPR antibody respectively. It was shown that no statistically significant difference in the prevalence between species of the animals (P>0.05).

Table 1: Seroprevalence of PPRV antibody based on risk factors

Factor	Categories	No. tested	No. positive	% prevalence (95% CI)	χ2	P value
Districts	Boset	78	37	47.44(36.5-58.6)	12.9641	0.000
	Fantale	87	65	74.71 (64.4-83)		
Kebeles	Buta	22	12	54.55(26-66.4)	19.6	0.001
	Butab	46	22	47,83(37.7-66.3)		
	Ilaala	72	50	69.44(21-42.2)		
	Tri	10	3	30(35.7-90.7)		
	Tututi	15	15	100(no observation)		
Species	Ovine	32	15	6.88 (30.2-64.2)	3.7557	0.053
	Caprine	133	87	65.41 (56.9-73.1)		
Age	Young	10	1	10 (1.2-50)	12.11	0.001
	Adult	155	101	65.16(57.3-72)		
Sex	Male	21	10	47.62 (27.3-68.8)	2.0554	0.152
	Female	144	92	63.89 (55.6-71.4)		
Body condition	Poor	10	7	70 (35.7-90.8)	1.4507	0.484
	Good	9	4	44.44 (16.4-76.5)		
	Medium	146	91	62.33 (54.1-70.8)		
Flock size	Large	113	76	67.26 (58.8-75.3)	4.4930	0.034
	Small	52	26	50 (36.5-63.5)		

Taking into consideration the age of animals, PPRV antibody seroprevalence was higher in adult animals 65.16 % (95%CI=57.3-72.3) and lower in young animals 10 % ($P<0.001$, $\chi^2=36.12$) and it was statistically significant. The prevalence in female animals is higher (63.89 %) than male animals (47.62 %, 95% CI) but no statistically significant difference between the sex groups ($P>0.05$, $\chi^2=2.0554$). The majority of seropositive sheep and goats were those with poor body condition (70 %, 95% CI) and then those with medium body condition (62.33%, 95% CI). Nevertheless, the difference in the seropositivity was statistically not significant ($\chi^2=1.4507$, $P>0.05$). With flock size, high prevalence was seen in animals from medium sized flocks and it was statistically significant (62.33 %, $P<0.05$, 95%CI=54.1-70).

To determine the potential individual risk factor for seropositivity Uni-variable logistic regression analysis was conducted for PPRV antibody. Therefore, from the putative risk factors evaluated for the seropositivity, districts, kebeles, and age and flock size, were found to be statistically significant risk factors. (**Table 3).**

Table 2: Factors in univariable logistic regression analysis for PPRV seropositivity

Variable	Category	No. tested	No. positive	Odd ratio (95% CI)	P value
District	Boset(ref)	78	37		
	Fantale	87	65	3.3 (1.7-6.3)	0.000
Species	Caprine(ref)	133	87		
	Ovine	32	15	0.5 (0.2-1)	0.056
Age	Young (ref)	10	1		
	Adult	155	101	0.06 (0.007-0.48)	0.008
Sex	Female (ref)	144	92		
	Male	21	10	0.5 (0.2-1.3)	0.157
B,con	Good (ref)	9	4		
	Medium	146	91	2 (0.5-8)	0.294
	Poor	10	7	2.9 (0.44-19)	0.266
Flock size	Large (ref)	113	76		
	Small	52	26	0.5 (0.25-0.95)	0.035

District and age, of the animals were found to be possible risk factors for PPR seropositivity being strongly linked with viral antibody prevalence (P< 0.05) using multivariable logistic regression analysis. The likelihood of being seropositive to PPRV infection in fantale district was 2.96 higher compared to Boset district .Young small ruminants found to be at lower risk for PPR infection (OR=0.06, P <0.001).

Table 3: Multivariable logistic regression analysis of the risk factors

Variable	Category	No. tested	No. positive	Odd ratio (95% CI)	SE	P-value
District	Boset(ref)	78	37			
	Fantale	87	65	2.96 (1.31-6.69)	1.23	0.009
Age	Adult (ref)	155	101			
	Young	10	1	0.06(0.006-0.47)	0.06	0.008
Flock size	Large (ref)	113	76			
	Small	52	26	0.80 (0.35-1.87)	0.35	0.613

4.3. Sero-conversion and efficacy of vaccines

Out of 166 vaccinated sheep and goats bled for post vaccination sero-conversion assessment starting from the second week post vaccination, 137 (82.53%, 95%CI) animals were seropositive to PPR according to c-ELISA. The percentage of goats sero-converted was greater than sheep and the difference was not statistically significant (P >0.05) using Chi-squared test. In this study (61.82%, 95%CI) sero-prevalence before vaccination and (82.53%, 95%CI) sero-conversion after vaccination was recorded. A rise in herd immunity from prior to vaccination was observed after the vaccination campaign.

Table 4: Association of fixed variables with sero-conversion of PPR antibody in vaccinated population

Factor	Categories	No. tested	No. positive	% prevalence (95% CI)	$\chi2$	P value
Districts	Boset	72	61	84.72 (74.3-91.4)	0.4238	0.515
	Fantale	94	76	80.85 (71.5-87.7)		
	Total	166	137	82.53 (72.9-89.55).		
Kebeles	Buta	32	26	81.25 (63.5-91.4)	4.5028	0.480
	Butab					
	Ilaala	70	54	77.14 (66-85.5)		
	Tri	6	6	100 no obs		
	Tututi	24	22	91.67 (71.2-98)		
	tiyo	10	9	90 (50-99)		
Species	Ovine	89	69	77,53 (67.5-85)	3.3296	0.068
	Caprine	77	68	88.31 (79-94)		
Age	Young	33	41	80.49 (65.2-90)	1575	0.691
	Adult	104	125	83.20 (75.5-88.8)		
Sex	Male	37	27	72.97 (56.3-85)	3.0163	0.082
	Female	129	110	85.27 (80-90)		
Body condition	Poor	4	4	100	0.9193	0.632
	Good	42	34	80.95 (70-90)		
	Medium	120	99	82,50 (74.5-88.3)		
Flock size	Large	38	33	86.84 (71.6-94.5)	6355	0.425
	Small	128	104	81.25 (73.4-87)		

5. DISCUSSION

Results of the current serological study showed an overall seroprevalence of 61.82 % which agrees with studies conducted by Dubie *et al.* (2022) (60.15%) Afar region, Saritha *et al*. (2014) (67.9%) in India, Abiyu (2022) (65.4 %) in Awi and Metekel zone, Abdalla *et al*. (2012) (61.8%) in Sudan, Saeed *et al*. (2010) (62.8%) again in Sudan. However, this finding was lower than that reported by, Yalew *et al*. (2019) and Woldemichael *et al*. (2018) who reported 75.7% and 73.45% prevalence in Asosa and Metekel Zone respectively. The current serological finding was slightly higher than prevalence reported by Gari *et al*. (2017) (48.43%) in Eastern Shewa and Arsi Zones of Oromia Regional State. Additionally, a lower seroprevalence reported by Alemu (2014) (28.1%) in Eastern Amhara Regional State and by Megersa *et al.* (2011) (30.5%) in postural and agropastural region in Ethiopia and Fentie *et al*. (2018) in Tigray (47.5%). However; the current prevalence is higher than Al–Dubaib (2009) (50.27%) in Bangladesh, Rahman *et al.* (2011) (55.1%) in Saudi Arabia, Sarker and Islam (2011) (49.5%) in Tanzania, Bonny *et al*. (2011) (55.2%) in Uganda and (Salih *et al*., 2014) (45.6%) in Sudan. Variations might be due to geographical location, agroecology and animal population density and various management systems.

The present result indicated a prevalence rate of 65.41% and 46.88% in goat and sheep respectively. This finding showed a higher prevalence in goat than sheep in the study area. The current finding was in harmony with Gari *et al*. (2017) sheep (46.68%) and goats (50.85%), Fentie *et al*. (2018) Sheep (14.89%) and Goat (21.57%), Hailegebreal *et al*. (2018) Sheep (24.2%) and Goat (34%), Dubie *et al.* (2022) sheep (38.18%) and Goats (60.15%), Saudi Arabia (Abdellatif *et al*., 2016) sheep (33.2%) and Goat (62.9%), (Zahur *et al*., 2011) Sheep (49.29 %) and Goats (65.94 %) in adult animals in Pakistan.

Contrary to the current study, others reported higher seroprevalence in sheep than goats. Works by (Abiyu, 2022) sheep (66.8%) and Goat (64%), (Abraham *et al.,* 2005) sheep (13%) and goats (9%), Saeed *et al. (*2010) sheep (67.2%) and goats (55.6%). The higher prevalence of PPR in sheep than in goats has been previously documented which is mainly due to the high mortality observed in goats so lower percentages of survivors exists (Khan *et al*. 2008). In

other words, Goats showed greater susceptibility to infection with PPRV while recovery rate of goats to infection is considerably less than that of sheep (Dhar *et al*., 2002, Couacy-Hymann *et al*., 2015). The variability of prevalence in the present study might be due to the fact that, in Ethiopia goats are affected more severely to PPR virus exposure compared to sheep and they exhibit striking clinical sign while sheep undergo mild form of the disease (Taylor, 1984).

Sex wise, in this study no significant difference in seroprevalence between male (47.62%) and female (63.89 %) even though, females were more affected than males. This observation was consistent with the findings by Kihu *et al*. (2015) who reported a significantly higher seroprevalence rate of anti-PPRV antibodies in females than in male sheep and goats. It is generally known that male animals are not usually kept in a flock for a long period of time (Al-Majali *et al*, 2008); because; households sell male animals due to demands of male animals for meat purpose and keep the female ones to breed. As a result of that; there was no enough representation of male small ruminants in the study samples.

In the current study, seroprevalence of the disease among the study districts was observed and there was statisticaly significant variation in seroprevalence between the two districts. It is more likely that animal from Fantale district to be seropositive than that in Boset (OR=3.3, p<0.001). This finding agrees with Gari *et al.* (2017), Abd-El Rahim *et al*. (2010) and Shuaib *et al*. (2014). This could be due to the fact that; while the two districts have similarity in agro-climatic conditions to some extents, Fantale is purely postural with larger herd size and Boset is agro postural were relatively animals of smaller herd size kept in mixed farming system. Additionally; the significant difference observed in seroprevalence of PPRV among study districts could be due to the variation in small ruminant population, animal health services, and the movement of sheep and goat flocks for market as well as seasonal grazing and management system. Prior study by Waret-Szkuta *et al.* (2008) pointed out that there is large variation between regions and woredas of the country.

Two flock size categories were used, thus medium flock size was also considered as a large flock and therefore, in this study seroprevalence of (67.26%) in large flock size, (50%) in

small flock size was recorded. It was statistically significant difference ($p < 0.05$). This finding was in agreement with Munir *et al*. (2008) and Selvaraju (2014), Gelana *et al*. (2020) Al-Majali *et al*. (2008), Alemu (2014) and Dejene (2016).This direct association might be an indication of the contagious nature of the disease and mode of transmission, which is attributed to crowding of animals that can facilitate the frequency of direct contact and hence escalating chances of transmission. In Jordan, large herd size was identified as a risk factor for PPR seropositivity in sheep and goat flocks and mixed (sheep and goats) farming was identified as a risk factor only in sheep (Al-Majali *et al*. 2008).

The association of age groups with PPR seroprevalence occurrence showed that age factor was found to be significantly associated risk factor ($p<0.05$). Higher PPR seropositivity in adult (65, 16%) and young (10%). This agrees with Dubie *et al. (*2022) and Abubakar *et al*. (2009). This finding was consistent with earlier studies (Abubakar *et al*., 2011, Abdalla *et al*., 2012 and Mahajan *et al*., 2012) that reported a decreasing seroprevalence rate as age decreases, and it was due to the higher likelihood of adult animals being exposed to PPRV than younger animals. In addition, the higher seroprevalence among adults may be because long life time allowing more exposure to PPRV. On the other hand, passive immunity from dam to the young animals might have effect on the result to some extent. Thus, older animals could have greater probability of exposure to the PPRV (Bello, 2013; Gebre *et al*., 2018). But it contradicts with Sarker and Islam (2011).

Seroprevalence from animals having poor body condition was higher (70%) than medium body condition (62.33%) and good body condition (44.44%). The current finding was also supported by Rath *et al*. (2020) study who revealed higher PPR seroprevalence in animals with poor body condition compared to the others.

The objectives of vaccination campaigns for diseases, such as PPR Global Control and Eradication Strategy (GCES), are that all small ruminants over 3 months of age should be vaccinated to reach a post- vccination level of 70% immunity at flock, geographical area, or farming system level to break the epidemiological virus maintenance and spread cycle (FAO/OIE, 2015). Kivaria *et al.* (2013) determined that 63.8% of small ruminants in a

population in agropastoral and 84.6% in pastoral farming system would have to be vaccinated with a 100 % efficient vaccine to reduce an effective reproductive number *(R*t) value of 4 to less than 1, with the overall threshold level of vaccination necessary to eradicate the disease in small ruminants was 74.9%. The results of this study's herd immunity assessment using c-ELISA for antibody seroconversion showed that the level of herd immunity against PPR in the area that received vaccination was high (82.53%) and this fall within the range of threshold level of vaccination necessary to eradicate the disease.

The chances of PPR eradication success are directly linked to the ability to vaccinate the vast majority of small ruminants, and this can be a significant challenge in smallholder village production systems because of the low density of small ruminants or in very remote areas where vaccine supply can be problematic. The quality and adaptability of vaccine delivery systems are also a key element that impacts on strategic vaccine implementation (FAO, 2015). Failure to follow stringent requirements for effective vaccination leads to less than expected herd immunity development post vaccination. Studies by Alemu, (2014) and Faris *et al*. (2011) reported (64.5%) and (61.1%) seroprevalence of antibody post vaccination respectively. These were less than the required range of threshold level of vaccination necessary to eradicate the disease. The possible explanation for this could be inability to keep cold chain at some stage of vaccine delivery. Contrary to this, Yirga *et al* (2020) reported (93.9%) above threshold level. This implies that when and where appropriate measures put in place the vaccine is effective in combating the disease.

Possible reasons for high antibody post vaccination monitoring in the current study were timely and accurate outbreak report and prompt response from experts from Assela Regional Laboratory to take samples and rapid and accurate diagnosis at Anima Health Institute that after confirmation of the disease followed by vaccination campaign. Strictly monitored and delivered vaccination campaign while keeping the cold chain produces protective herd immunity.

In the current study, suspected PPRV cases were tested to detect PPRV nucleic acid from collected clinical samples by using RT- PCR. In conformation of PPRV circulation in the

study area, 4 (44.4%) out of 9 samples taken from outbreak site were positive. This agrees with findings by Alemu (2014) reported (46.4%) in Easten Amara region, Abiyu (2022) (38.1%) in Awi and Matakal Zones of Benishangul Region and from Morocco 44.4% positive (Kwiatek *et al*., 2011) by F protein gene amplification and Nigeria 33.3 % (De Nardi *et al*., 2012) by N protein gene amplification. Both Alemu *et al*. (2019) Eastern Amhara Region, Ethiopia. and Ebissa (2020) Asossa Zone, Benishangul-Gumuz Region reported 46.4% and 45.4% positivity using N gene based PCR method respectively. However, higher nucleic acid detection rate of 51.2% by Luka *et al*. (2011), 78.95% by Kabir *et al*. (2020) and 58.06% by Kardjadj *et al*. (2015) was reported using RT-PCR method. The difference in this positivity might be resulted from variation in detection method, sample type, the virus infection stage and targeted gene type in detection (Alemu *et al*., 2019).

6. CONCLUSION AND RECOMMENDATIONS

The current serological investigation and molecular findings showed and confirmed the circulation of PPR virus among populations of sheep and goats in the study areas. Significant association with risk factors like districts, flock size and age of the animals were observed. PPR clinical symptoms were confirmed by RT-PCR PPR viral detection. It was found that the practice of communal grazing in extensive farming systems and the geography of the study area, agro ecology as well as the free movement of animals by traders and migration to and from the area may contribute to the presenc of the disease and to further spread of the virus in the area. Early diagnosis of infection and subsequent control of the PPR disease in Ethiopia will be aided by the quick detection of PPRV by adequate and appropriate methods of antigen and nucleic acid detection in infected animals.

In light of the foregoing conclusions, the following recommendations are made:

- ✓ In the research area, PPR control efforts should be stepped up, including vaccination with PPR homologous vaccine.
- ✓ To identify the actively circulating lineages in the study area, a thorough molecular epidemiology analysis must be conducted.
- ✓ Strengthening early warning systems and regular surveillance and monitoring is important

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8. ANNEXES

Annex 1: Description, principle, validation and interpretation of c-ELIS

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 ODNC 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.

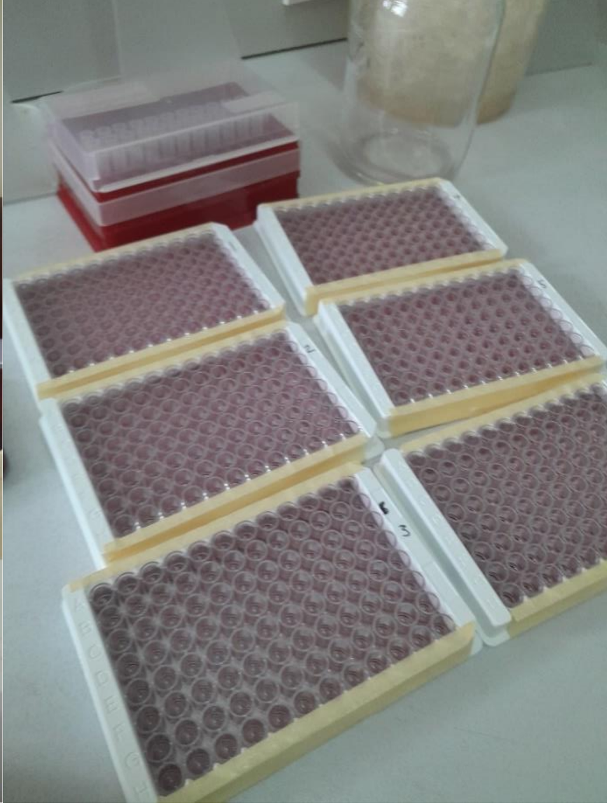
Annex 2: Sample collection and processing

Awareness Creation

Serum sample collection

Swab sample collection

Planning sample collection and trip to kebeles



Serum sample dispensing **Dispensed serum samples**

Annex 3: Sample processing and RNA extraction

Sample processing and RNA extraction: The swab 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. The RNA was extracted from the processed samples and culture isolates by using NucleoSpin® RNA kit (QIAGEN, Cat. no.52906, Hilden, Germany) according to the manufacturer's instructions

cDNA synthesis

Allow kit component to thaw, then mix and briefly centrifuge. Store the tubes on ice.

1. Add the following reagents in to sterile, RNase free tube on ice in the following order

10X ds DNase buffer	1µl
ds DNase	1µl
Template RNA	1µl
Total RNA or	1pg to 5µl
Poly (A) mRNA or	0.1pg to 500ng
Specific RNA	0, 01 to 500ng
Water nuclease free	to 7 µl
Total volume	10 µl

2. mix gently and centrifuge
3. Incubate at 370c in a preheated thermomixer or water bath for 2 minutes.
4. Chill on ice, briefly centrifuge and place on ice.
5. Add the following components to the tube on the ice

Maxima cDNA H Minus synthesis Master Mix (5X)	4 µl
Water, nuclease free	6 µl

6. Mix gently and centrifuge
7. Incubate at 250c for 10 minutes
8. Incubate at 50^{0}c for 15 minutes

Note: If using > 1µg RNA template, increase the reaction time to 30 minutes.For RNA template that are GC –rich or have a large amount of secondary structure, the reaction temperature can be increased to 65^0c

9. Terminate the reaction by heating at 85^0c for 5 minutes

The reaction product of the first strand cDNA synthesis can be used directly in qPCR store at -20^0c for up to one week, or -70^0c for long time storage. Avoid freeze- thaw cycles of the cDNA .

qPCR use the product of cDNA synthesis reaction directly qPCR .Normally , 2µl of the RT product is used as a template for subsequent qPCR in a 25 µl total volume .

CONTROL REACTIONS

Use the following negative control reactions to verify the results of first strand cDNA synthesis

.NO RT control to assess genomic DNA contamination of the RNA sample. The Maxima NoRT control reaction includes all reagents for reverse transcription reaction except the Maxima H Minus RT

No template control (NTC) to assess for reagent contamination. The NTC reaction includes all reagents necessary for the reverse transcription reaction except the RNA template.

ANNEX 4: Procedure of RT-PCR

- Thaw all reagents, except reverse transcriptase and taq polymerase and possibly keep them on ice.
- Master mix preparation, Before preparing the reaction mix, it is necessary to calculate the correct volume of reagents to be used as in attached protocols.. Prepare a reaction mix as follow

RNase-free water (7.5µl)
5XPCR buffer (**5**µl)
DNTPs Mix 10mM (**1**µl)
Q solution (**5**µl) Per sample
Primer forward NP3 (**1.5**µl)
Primer reverse NP4 (**1.5**µl)
Qiagen enzyme mix (**1**µl)
RNA (**2.5**µl)

- Aliquate 22.5 µl of the prepared master mix in to approximately labeled 0.2microcentrifuge tubes in PCR work station for master mix
- Add 2.5 µl of RNA template in sample dispensing PCR work station and transfer them to the thermocycler
- Place the reaction tubes in the thermal cycler and setup temperature according to the protocol in the table below
- **Amplification cycle**

Steps	**Temperature**	**Time**	**Cycle**
1st	50 oC	30 min	1 cycle
	95 oC	15 min	
2nd	94 oC	30 sec	40 cycles
	60 oC	30 sec	
	72 oC	1 min	
3rd	72 oC	5 min	1 cycle
Put at	4 oC		Till machine off

ANNEX 5: Procedure of Agarose Gel Electrophoresis

- Prepare an adequate volume of 1xTBE-buffer to prepare the gel and to fill the electrophoresis tank
- The amount of agarose that is used depends on the size of the tray and the concentration required. For general purpose a 2% gel is used. Gels are typically between 0.5 and 1 cm thick.
- Prepare a solution of molten agar as required by adding the 1xTBE-buffers to the appropriate amount of agarose powder in a suitable flask/bottle. Bring to boil in the microwave oven. Screw the cap loose in order to guarantee the pressure balance within the bottle/flask. After the agarose has been allowed to cool down to about 55oc, add the required amount of Ethidium bromide in chemical hood. This prevents warping of the gel apparatus
- Seal the ends of the edge of gel casting tray with masking tape and pour the gel in to it. Insert the comb and make sure that there are no bubbles trapped underneath the combs and that all bubbles on the surface of the agarose are removed before the gel sets. Stand for 45-60 min to allow the gel to solidify.
- After the gel has set, remove the tape from the casting tray, place the gel casting tray containing the set gel in the electrophoresis tank. Add sufficient 1xTBE-buffer to cover the gel to a depth of 1mm (or until the wells are just submerged) and withdraw the gel comb, taking care not to tear the sample wells. Make sure no air pockets are trapped within the wells.
- Apply 1ul of tracking dye to each 10ul of sample and add samples to the individual wells. Take care not to over load and be sure to include appropriate DNA molecular weight markers.
- Cover with the safety cover and run gel at 110mA (7x10cm tray) or 200mA (15x10cm tray)- typically 1 to 10v/cm of gel. When the bromophenol blue marker is about two thirds from the top, the gel can be stained with Ethidium bromide (EtBr).
- Turn off the power supply when the bromophenol blue has merged a distance judged sufficient for separation of the DNA fragments.
- The DNA can be visualized on a UV transilluminatorand photographed

Annex 6: Ethical clearance certificate

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
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Animal Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/23/04/15/2023

Name of Applicant: Dr Samson Leta (MSc, Associate Professor)

Address: Department of Biomedical Sciences, College of Veterinary Medicine and Agriculture (Addis Ababa University)

Title of the project: *Advancing animal health through development of field-deployable diagnostic assay, bivalent vaccine and promotion of indigenous knowledge for peste des petits ruminants (PPR) and Sheep and Goat Pox (SGP): to promote early detection and progressive control of major small ruminant diseases – DABV-project*

Date of application: **December, 2022**

Nature of the project: **Field investigation and experimental vaccine trial**
Target animal species: **Small ruminants**
Number of animals involved: **2000**
Study area: **Different parts of Ethiopia**

Minutes No. and date of review: **VM/ERC/04/15/022, 15/02/2023**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Dr Samson Leta.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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Please quote Our Ref. No. When replying

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