



**MEKELLE UNIVERSITY
COLLEGE OF VETERINARY SCIENCES**

Department of Veterinary Clinical Medicine and Epidemiology

**SERO-PREVALENCE, MOLECULAR DETECTION AND ASSOCIATED
RISK FACTORS OF PESTE DES PETITS RUMINANTS (PPR) IN SELECTED
DISTRICTS OF EASTERN AND SOUTH EASTERN ZONES OF TIGRAY
REGION:**

By:

Tadele Gebereslassie

**A Thesis Submitted to the College of Veterinary Sciences, Mekelle University, in
Partial Fulfilment of the Requirements for the Degree of Master of Science in
Tropical Veterinary Medicine (TVM)**

January, 2025

Mekelle, Ethiopia

DECLARATION

I hereby declare that this thesis titled “**Sero- Prevalence, Molecular Detection and Associated Risk Factors of Peste Des Petits Ruminants (PPR)**” represents my independent work. It does not include, without acknowledgment, any material previously submitted for a degree or diploma at any university. To the best of my knowledge, it does not contain any material previously published or authored by another individual, except where proper references are provided in the text. All significant contributions made by others to this work, including jointly authored publications, have been clearly acknowledged.

Name: _____ Signature: _____

**SERO- PREVALENCE, MOLECULAR DETECTION AND ASSOCIATED
RISK FACTORS OF PESTE DES PETITS RUMINANTS (PPR) IN SELECTED
DISTRICTS OF EASTERN AND SOUTH EASTERN ZONES OF TIGRAY**

REGION:

By:

Tadele Gebereslassie (CVM/PR007/13)

BOARD OF EXTERNAL EXAMINERS

1.

2.

3.

Advisors:

1. **Mebrahtu Weldegebriel (DVM, MSc, Assist. Prof)** **Signature:** _____

2. **Biruk Mekonnen (DVM, MSc, Assist. Prof)** **Signature:** _____

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	III
LIST OF TABLES	IV
LIST OF FIGURES	V
LIST OF ABBREVIATIONS	VI
ABSTRACT.....	VII
1. INTRODUCTION	1
1.1. Objective	2
1.1.1. General Objective	2
1.1.2. Specific Objectives	3
2. LITERATURE REVIEW.....	4
2.1. Etiology	4
2.1.1. Structure and Genome Organization of the Virus	4
2.1.2. Physiochemical property of PPR virus.....	5
2.1.3. Lineage of the virus	6
2.2. Epidemiology of the Disease.....	7
2.2.1. Geographic distribution	7
2.2.2. Host Range and Reservoirs	9
2.2.3. Host determinants and seasonal occurrences	9
2.2.4. Transmission	11
2.2.5. Morbidity and Mortality	11
2.2.6. Pathogenesis	12
2.3. Clinical Sign.....	12
2.4. Gross Pathology of PPR Disease	14
2.5. Differential Diagnosis	14
2.6. Treatment	15
2.7. Diagnostic Techniques	16
2.8. Socioeconomic Impact of PPR.....	20
2.9. Prevention and Control.....	21
2.9.1. PPR Global Control and Eradication Strategy	22

3. MATERIALS AND METHODS	24
3.1. Description of Study Area.....	24
3.2. Study Animals	25
3.3. Study Design	26
3.4. Sample Size Determination	26
3.5. Sampling Techniques	26
3.5.1. Serum sample collection	27
3.5.2. Nasal and ocular swabs for molecular characterization	27
3.6. Laboratory Analysis	27
3.6.1. Serological analysis	27
3.6.2. Molecular detection of PPRV nucleic acid using RT-PCR.....	28
3.7. Data Management and Analysis.....	29
3.8. Ethical Considerations.....	29
4. RESULTS	31
4.1. Sero prevalence of PPR infection.....	31
4.2. Molecular Detection of PPRV	34
4. DISCUSSION	37
5. CONCLUSION AND RECOMMENDATIONS	41
7. REFERENCES.....	42
8. ANNEXES.....	54

ACKNOWLEDGEMENTS

Above all, I want to express my gratefulness and admiration for God for giving me the strength to finish this thesis project. Next, I would like to sincerely thank and express my gratitude to my advisors, Dr. Mebrahtu Weldegebriel and Dr. Biruk Mekonnen, for their enthusiastic support, perseverance, and encouragement throughout this research project, from the proposal development to the writing and defense of the thesis.

My sincere gratitude and admiration are extended to the Tigray Region Animal Health Laboratory, which provides me with a vehicle for fieldwork and all the facilities and equipment I need. In particular, I would like to thank Dr. Girmay Weldeselassie, the director of the laboratory and Mr. Goitom Eyasu, the focal person of the PPR control and eradication program, With the gracious assistance of Mr., Sisay Fesha, Geberemedhen Gebreslassie, Teklehayimanot, Dr.Berhe, Alemat Hagose, and Kahsu, the blood collection from sheep and goats and the data gathering from various kebelles were completed.

The Animal Health Institute (AHI) has provided study supplies and authorization for the laboratory work, for which I am incredibly appreciative. I would like to sincerely thank Dr. Dereje Shegu, Dr. Fasil Aklilu, Mr. Abde Aliy, and Mr. Bayeta for their support and hospitality. They gave me during the laboratory work. I am delighted that the NORAD Project provided me with the study funds.

Lastly, I want to express my gratitude to Mekelle University College of Veterinary Sciences and to all of my friends, family members who inspired me by offering their persistently helpful advice, moral support, prayers, and encouragement throughout my study sessions and thesis work.

LIST OF TABLES

Table 1: Test methods available for the diagnosis of peste des petits ruminants and their purpose.....	17
Table 2 : Sero prevalence studies of PPR in different regions of Ethiopia.....	23
Table 3: Analysis of Associated factors of PPR using Chi-square.....	32
Table 4: Univariate logistic regression analysis of risk factors for PPR sero prevalence	33
Table 5: Multivariate logistic regression analysis of risk factors for PPR sero prevalence	34
Table 6: Species, district, sex and age wise detection rate of PPRV nucleic acid using real time PCR.....	35

LIST OF FIGURES

Figure 1: Schematic representation of the PPR morbillivirus	5
Figure 2: Worldwide cumulative distribution of PPR virus lineages	7
Figure 3 : Location Map of the study Area.....	25
Figure 4: Real time PCR standard curve indicating PPRV detection result	36

LIST OF ABBREVIATIONS

AGID	Agar gel Immuno Diffusion
AHI	Animal Health Institute
BCS	Body Condition Score
CFSPH	Center for Food security and Public Health
CPE	CytoPathic Effect
CSA	Central Statistical Agency
Ct	Cycle threshold
DRC	Democratic Republic of Congo
ELISA	Enzyme Linked Immuno sorbent Assay
ESGPIP	Ethiopia Sheep and Goat Productivity Improvement Program
FAO	Food and Agricultural Organization
FMD	Foot and Mouth Disease
Ic-ELISA	Immuno capture Enzyme Linked Immuno sorbent Assay
M.a.s.l	Meter Above Sea level
OIE	World Organization for Animal Health
PPR	Peste Des Petits Ruminants
PPRV	Peste Des Petits Ruminants Virus
PPR GCES	PPR Global Control and Eradication Strategy
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RNA	Ribonucleic acid
RRLS	Regional Reference Laboratories
SDS	Sequence Detection Software
SLAM	Signaling Lymphocytic Activation Molecules
TADs	Transboundary Animal Diseases
TCID50	Fifty Percent Tissue Culture Infective Dose
VDS	Vero Dog SLAM cells
VNT	Virus Neutralization Test
WAHID	World Animal Health Information Data base
WOAH	World Organization for Animal Health

ABSTRACT

Peste des Petits Ruminants (PPR), also known as "goat plague, is a contagious, trans-boundary viral disease of both domestic and wild small ruminants associated with high mortality and morbidity rates. Cross-Sectional Study was carried out From January 2024 to December 2024 in Enderta and Kiltawulaelo districts in the eastern and southeastern zones of the Tigray region to determine the seroprevalence, related risk factors, and molecular detection of PPR virus in sheep and goats using a combination of purposive and simple random sampling. 384 serum samples 43 swab samples and were gathered in total, PPR virus-specific antibodies and viral nucleic acid were found using the virus neutralization test and real-time RT PCR, respectively. The relationship between PPR seroprevalence and the possible risk factors was examined using Pearson's chi-square and logistic regression analysis. Of the total 384 sera samples tested, 41.9% (n = 161) were found positive for PPRV antibody, the seroprevalence in the districts of Enderta and Kiltawulaelo was found to be 47.6% (n = 98) and 35.% (n = 63), respectively, while the species level was found to be 36.8% (n = 49) in sheep and 44.6% (n = 112) in goats. Out of all the sera analyzed, 39 samples (43.8%) were positive from males and 122 samples (41.4%) were positive from females. and from 43 samples tested with the real-time PCR, 23.26% (n = 10) were detected positive. The results of the research showed that the PPR virus was active in the investigated areas. To minimize the threat of PPR, strategic vaccination of sheep and goats should be implemented.

Keywords: *molecular detection, Peste des Petits Ruminants, Sero-prevalence, and VNT*

1. INTRODUCTION

Ethiopia is home to one of the biggest populations of small ruminants in Africa, with an estimated 42.91 million sheep and 52.46 million goats (CSA, 2021/22). FAOSTAT's livestock population figures indicate that this represents around 10% of Africa's total livestock population and 4% of the global small ruminant population (FAO, 2019). Small ruminants (sheep and goats) are an important part of the Ethiopian livestock system, contributing significantly to the food security of smallholder farmers, particularly women in Ethiopia (Beyene *et al.*, 2018; Weldemariam *et al.*, 2023). They play a great role in maximizing income generation, thereby securing economic feasibility, meeting food requirements, and ensuring the sustainability of livelihoods, particularly for smallholder farmers (Tarekegn, 2021).

Small ruminant production and productivity are far below the expectations due to the prevalent diseases and other factors. One of the major bottlenecks restricting maximum efficient small ruminant production is the presence of the significant negative impact of infectious animal diseases such as Peste des Petits Ruminants (PPR) (Abraham *et al.*, 2005; Biruk, 2014). The impact of livestock disease can vary from reduced productivity to the elimination of entire herds. It also restricts access to domestic and international markets through export bans, thereby causing the country to lose income from exports (FAO/OIE, 2012).

Peste des Petits Ruminants (PPR), also known as "goat plague, is a contagious trans-boundary viral disease of both domestic and wild small ruminants that was first reported from Cote- d'Ivoire in 1942 (Baaziz *et al.*, 2017). It is caused by Peste des petits ruminants virus (PPRV). 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). The disease is characterized by severe pyrexia, oculo-nasal discharge, necrotizing and erosive stomatitis, enteritis, and pneumonia (Balamurugan *et al.*, 2011; OIE, 2016). The PPR epidemics can cause

mortality of 50–80% in unvaccinated sheep and goat populations (Khalafalla *et al.*, 2010; Balamurugan *et al.*, 2013).

The disease is associated with high mortality and morbidity rates in naïve populations (Kaukarbayevich, 2009), significant economic losses, reduced production and productivity of small ruminants, and a constant threat to subsistence farmer's livelihoods, and ultimately is a threat to national food security (Banyard *et al.*, 2010; FAO, 2011). In addition, small ruminants and their products are internationally traded commodities, mainly in Africa and the Middle East; PPR has a significant impact on export revenues and causes supply shortages (WAHID, 2020). Given its social and economic impact, The United Nations Food and Agriculture Organization (FAO) and the World Organization for Animal Health (WOAH) started a campaign in March 2015 to eradicate PPR by 2030 after eliminating rinderpest. Globally, the expected pooled prevalence of PPR between 2000 and 2021 was 39.16%. (Mondal *et al.*, 2024)

Ethiopia has currently worked with FAO and OIE to build a plan for the gradual control and eradication of PPR. It is critical to recognize the disease's prevalence and identify the virus that is circulating in order to effectively control and eradicate the illness. The epidemiology of PPR disease, however, is poorly understood in Ethiopia, leading to a lack of information. In Tigray region where there is only one seroprevalence report (Afera *et al.*, 2014) and no molecular-level report, there is insufficient data on the seroprevalence and related risk factors of PPR infection in the study areas specifically and Tigray region generally. Therefore, to help Ethiopia's present initiative to control and eradicate the disease, more epidemiological data is needed.

1.1. Objective

1.1.1. General Objective

The overall objective of this research is to determine the seroprevalence and molecular detection of Peste des petits ruminants (PPR) in sheep and goats in selected districts of the southeastern and eastern zones of the Tigray Region, Ethiopia.

1.1.2. Specific Objectives

- To estimate the sero-prevalence of peste des petits ruminants virus infection in sheep and goats in the study areas.
- To identify associated risk factors of peste des petits ruminants virus infection in sheep and goats in the study area
- To isolate the PPR virus from specimens collected during the active cases of PPR in sheep and goats in the study areas.

2. LITERATURE REVIEW

2.1. Etiology

Pest 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 a close antigenic relation to the rinderpest 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).

2.1.1. Structure and Genome Organization of the Virus

PPR virus is an 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 of about 15,948 nucleotides that encode two non-structural and six structural proteins. The two non-structural proteins of the virus are V and C proteins, while the structural proteins are nucleoprotein (N), phosphor protein (P), matrix protein (M), fusion protein (F), hemagglutinin 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 the 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. Phospho protein 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 an infectivity and virulence factor. It is also indicated to be an interferon antagonist. The non-structural V protein highly inhibits interferon actions. Accordingly, it contributes to immune suppression induced by Morbilli virus infections (Rudra, 2019). However, N is the most abundant and the most immunogenic among PPRV proteins, which does not induce protective immunity against the virus. It has been used in the development of diagnostic tests (Diallo *et al.*, 2007).

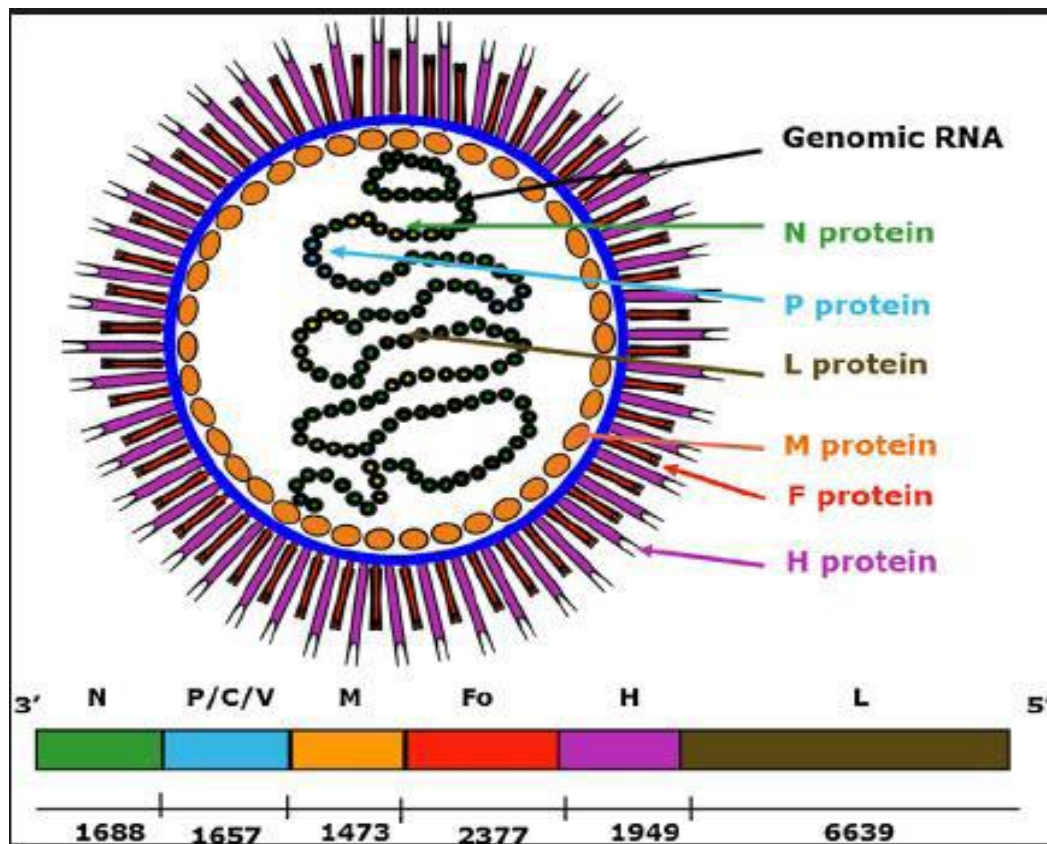


Figure 1: Schematic representation of the PPR morbillivirus

Source: (Albina *et al.*, 2013)

2.1.2. Physiochemical property of PPR virus

Molecular weight of the PPR virus genome is 5.8×10^6 . Intact virion has a diameter of about 130-390 nm, with the thickness of the ribonucleoprotein measuring approximately 14-23 nm. It is wrapped by a nuclear protein that is associated with the

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°C for 60 minutes or 37°C for 2 hours. However, it survives for long periods in chilled and frozen tissues (Dejene, 2016).

2.1.3. Lineage of the virus

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

All PPRV lineages are circulating in the African continent: (Baazizi *et al.*, 2017). Lineage I is commonly found in West Africa and has recently been reported in central Africa, whereas lineage II isolates are found in western African 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).

Virulence of PPR virus is found to vary between the lineages. A 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).

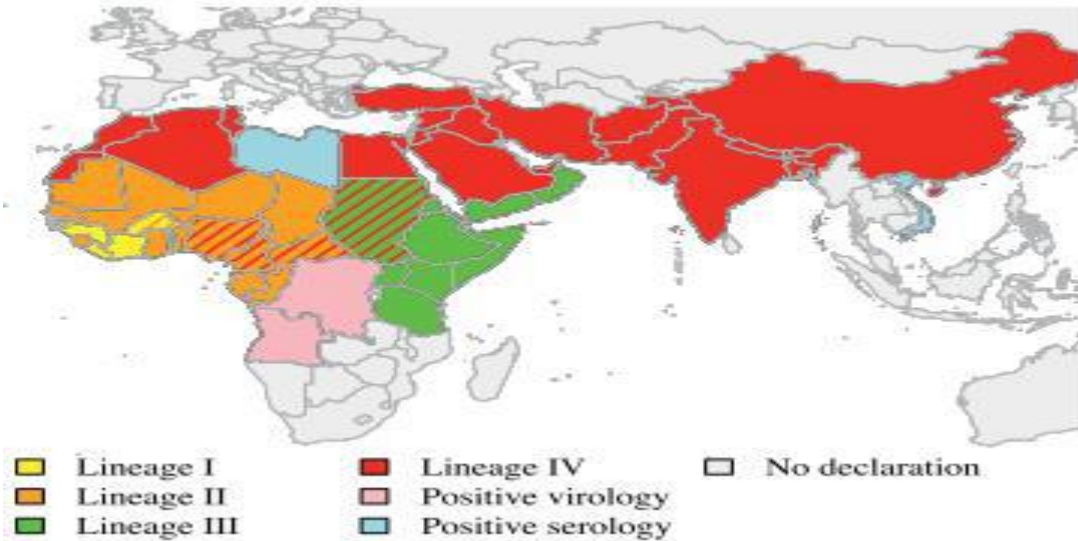


Figure 2: Worldwide cumulative distribution of PPR virus lineages

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).

2.2. Epidemiology of the Disease

2.2.1. Geographic distribution

Currently, PPR is the fastest-expanding and potentially the most economically important disease of sheep and goats in many regions of the developing countries where these domestic animals play an integral and important role in sustainable agriculture and development (Baron *et al.*, 2016). Peste des petits ruminants has spread so alarmingly during the last two decades that it has become a matter of concern for the Food and Agriculture Organization of the United Nations (FAO) and the International Organization Epizootic (OIE), which have now initiated efforts for its control and eradication (OIE 2015).

West Africa includes 16 countries distributed over an area of approximately 5 million square kilometers. Almost all of these countries have experienced significant outbreaks of PPRV. In recent years, material submitted to Regional Reference Laboratories (RRLs) has confirmed the presence of either antibodies to the virus or the detection of viral nucleic acid in samples from Burkina Faso (2008), Ghana (2010), Nigeria (2007, 2013, 2014), and Senegal (2010) (Banyard *et al.*, 2010).

Peste des petits ruminants virus strains from both lineages I and II are currently circulating across West Africa, although undoubtedly many outbreaks are not characterized at the molecular level. Characterization of PPRV in Nigeria from a recent outbreak indicated that PPRV lineage IV is also circulating alongside PPRV lineage II in Nigeria (Mantip *et al.*, 2016).

While Somalia, Djibouti, Ethiopia, and Eritrea are frequently included, the term "East Africa" is typically used to refer only to the region that currently encompasses the countries of Kenya, Tanzania, and Uganda. The majority of these nations are home to the endemic Peste des petits ruminants virus. In 1996, genetic typing of the virus identified a circulating strain in Ethiopia as being of lineage III. (Banyard *et al.*, 2010). Two isolates from animals in Oman (1983) and the United Arab Emirates (1986), an isolate from Sudan (1972), and an unexpected virus isolate from sheep in southern India (1992) are among the previously isolated viruses belonging to lineage III (Banyard *et al.*, 2010).

More recently, the discovery of PPRV antibodies in Kenya (1999 and 2009) and Uganda (2005 and 2007) has demonstrated the endemicity of PPRV throughout East Africa. Some viruses have been identified by molecular techniques as being of lineage III; isolates from Tanzania (2008 and 2010), Uganda (2007), and Sudan (2000) have all had their viruses characterized in this way (Banyard *et al.*, 2010). In 2006, PPRV also impacted Somalia, with the central regions suffering the most severe effects (Nyamweya *et al.*, 2009). lineages II and IV were also detected in Ethiopia (Aliy *et al.*, 2022).

and spleen specimens taken from an epidemic in a holding close to Addis Abeba (Roeder Although molecular typing for the latest outbreaks in Kenya and Somalia is lacking, lineage III virus is most likely the one spreading in these regions. Burundi, the Central African Republic (CAR), Angola, Cameroon, Chad, Gabon, the Democratic Republic of the Congo (DRC), and Rwanda comprise Central Africa, which is a core region of Africa. Lineage IV viruses are widespread throughout Central Africa, according to a phylogenetic study (Banyard *et al.*, 2010). PPR was first clinically suspected in 1977 in a goat herd in the eastern Afar area of Ethiopia. It was subsequently verified in 1991 using a cDNA probe in lymph nodes *et al.*, 1994). Eventually, it was discovered that the virus belonged to lineage III genetically (Banyard *et al.*, 2010).

2.2.2. Host Range and Reservoirs

While the primary natural host of PPRV is thought to be small ruminants, both domestic and wild, additional animals that can contract the virus include cattle, pigs, buffalo, camels, and, according to a recent investigation, even Asiatic lions (Balamurugan *et al.*, 2012). The literature currently in publication suggests that wild small ruminants may play a significant role in the epidemiology of PPR, despite the paucity of evidence regarding the disease's susceptibility, prevalence, and severity in ungulate species. (Munir, 2013).

In Sudan, camels may be susceptible to PPR because they can exhibit clinical signs of the disease, serological testing such as reverse transcription polymerase chain reaction (RT-PCR) has shown positive results, and PPRV has been isolated in cell culture (Khalafalla *et al.*, 2010; Kwiatek *et al.*, 2011). A possible source of PPR infection for domestic animals, wild ruminants such as antelope, buffalo, hippo traginae, tragelaphinae, nigale, laristan sheep, dorcas gazelles, Nubian ibex, and gemsbok are also susceptible to the disease. (Kinne *et al.*, 2010).

2.2.3. Host determinants and seasonal occurrences

Numerous studies have reported on the host-determining aspects of PPR spread, including age, sex, breed, and animal species. (Munir and others, 2013) Young animals are more vulnerable to PPRV because they are less likely to have generated protective antibody titers (Luka *et al.*, 2011). Ethiopia, Kenya, Pakistan, India, and Turkey have all documented high susceptibility in the young; as a result, the age of small ruminants is a significant risk factor for susceptibility or resistance to the disease (Abubakar *et al.*, 2009).

Sex has also been reported as a risk factor for susceptibility/resistance to the disease (Abdalla *et al.*, 2012). The off-take of male small stock for social and economic activities is higher and at an early age compared to females, who end up staying in the herds for longer periods for productive purposes; therefore, females are more likely to demonstrate antibody titers than the males. The recruited young males, having been in the herds for a shorter period, are less likely to have been in contact with the virus. In fact, research conducted in Bangladesh has revealed that male goats are noticeably more likely than female goats to contract PPR (Sarker S. and Islam H, 2011).

In endemic locations, PPR also demonstrates a seasonal pattern with an increased frequency of outbreaks at the beginning of the cooler wet season (Ruguet *et al.*, 2019). The seasonal epidemiologic patterns of the disease vary in different agro-ecological systems based on the culture and livelihood patterns of the smallholder farmers (Bedore *et al.*, 2019).

The seasonality of the disease is not evident in all geographic zones, though. PPR disease is said to be more widespread in subtropical regions during the winter and rainy seasons. In tropical nations experiencing rainy seasons, the confinement and limited mobility of animals can potentially impact their nutritional well-being and increase their vulnerability to PPRV infection. PPR is said to be more prevalent in the cool, dry season in the majority of endemic locations (Abubakar *et al.*, 2009; Munir 2013).

Climatic factors favorable for the survival and spread of the virus may also contribute to the seasonal distribution of PPR outbreaks. With the start of the rainy season between June/July and August/September, the migratory activity of animals is reduced due to the increased availability of local fodder. The nutritional status of the animals is also improved, resulting in an increased resistance to infection. These factors may play a key role in limiting the transmission of disease. PPR prevails throughout the year in the country (Ahmed, 2020).

Generally, it is believed that goats show more severe clinical signs than sheep in the same environmental conditions. This is supported by the fact that the level of PPRV antibodies is higher in sheep than goats, which may render sheep resistant to the disease (Munir *et al.*, 2009).

2.2.4. Transmission

PPRV 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; licking of bedding, feed, and water troughs is 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, the congregation of susceptible animals at grazing land and watering points, and intensive-type farming systems facilitate the transmission of this highly contagious disease (Biruk, 2014).

The affected animals are an important source of transmission during incubation periods, subclinical cases, or before the onset of clinical signs (Madboli and Ali, 2012). PPRV is secreted in tears, nasal discharges, and secretion from coughing and in feces of infected animals. 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).

2.2.5. Morbidity and Mortality

The morbidity and mortality rates of PPR can be up to 100% in severe outbreaks, but in milder outbreaks, the mortality rate may be reduced to 50% while the morbidity rate still remains high in both cases (Fentahun and Woldie, 2012). Mortality rate is high in the susceptible young animals (4-8 months) and animals with poor nutritional status. Stress and concurrent parasitic and bacterial infections also enhance the severity of the disease (Alemayehu *et al.*; 2015).

2.2.6. 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 the respiratory system, it replicates itself in lymph nodes (pharyngeal and mandibular lymph nodes) and tonsils (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 the spread of the virus to other lymphoid tissues and organs, such as the spleen, bone marrow, and mucosae of the gastrointestinal and respiratory tracts, where it causes severe damage by replicating in endothelial, epithelial, and monocyte cells (Abubakar *et al.*, 2011; Rudra, 2019).

2.3. Clinical Sign

The incubation period can range from two to 10 days; in most cases, clinical signs appear in 2-6 days (CFSPH, 2011). Most cases of PPR are acute, with a sudden fever that may last for 5-8 days before the animal either dies or begins to recover. The characteristic signs begin with a clear discharge from the nose that becomes grey and sticky. The discharge from the nose may remain mild or may progress to severe inflammation of the mucous membrane of the nose characterized by the presence of exudates that crust over, blocking the nostrils causing respiratory distress. The nasal mucous membranes may develop small areas of erosion. The conjunctiva may be congested with matted eyelids. The mucous membranes in the mouth may also be eroded. Concurrently, animals will most likely have profuse, non-hemorrhagic diarrhea resulting in severe dehydration, which may progress to emaciation, difficult

breathing and die within 5-10 days. Bronchopneumonia with coughing is common late in the disease. Infection of pregnant animals with the virus has also, albeit rarely, been linked to abortion (Abubakar *et al.*, 2008).

The prognosis of acute PPR is usually poor. The severity of the disease and outcome in the individual is correlated with the extent of the mouth lesions. Prognosis is good in cases where the lesions resolve within 2 to 3 days. It is poor when extensive necrosis and secondary bacterial infections result in a fetid odor from the animal's mouth. Respiratory involvement is also a poor prognostic sign. The severity of the disease varies with the species, as well as the animal's immunity to PPRV and its breed. Goats and sheep are not always affected to the same extent during an outbreak (CFSPH, 2011).

Per acute form: Per acute cases can be seen when PPR first occurs in native populations of sheep or goats. In this form, the clinical signs are generally limited to high fever, severe depression and death. More often, peste des petites ruminants' occurrence is as sub-acute or acute disease. Domesticated animals other than sheep and goats do not usually become ill. (CFSPH, 2011).

Acute form: The disease usually appears in the acute form, followed by a sudden rise in body temperature to 104-106°F (40-41°C). The temperature usually remains high for about 5 to 8 days before slowly returning to normal proceeding recovery or dropping below normal before death. Affected animals appear ill and restless and have a dull coat, dry muzzle and depressed appetite. Accompanying these nonspecific signs is a series of changes that make up a highly characteristic syndrome. From the onset of fever, most animals have a serious nasal discharge, which progressively becomes muco purulent. The discharge may remain slight or may progress, resulting in a profuse catarrhal exudate, which crusts over and occludes the nostrils. At this stage, animals have respiratory distress and there is much sneezing in an attempt to clear the nose. Small areas of necrosis may be seen on the visible nasal mucous membranes. The conjunctiva usually becomes congested and the medial canthus may have some

crusting. As with the nose, there may be profuse catarrhal conjunctivitis resulting in matting of the eyelids (Ahaduzzaman, 2020).

2.4. Gross Pathology of PPR Disease

Many pathological characteristics are common at post-mortem examination following PPRV infection. Ulcerative to necrotic lesions are clearly evident throughout the buccal cavity in PPRV infected sheep and goats at post-mortem. The buccal papillae, dental pad, gum, dorsal surface of tongue, palatine tonsil and hard palate are mainly affected. Congestion of the digestive tract, particularly the duodenum, abomasum, ileum, caecum and colon, is often seen. Extensive congestion along the longitudinal folds of the caecum, colon and rectum may be evident as zebra striping. The ileo-cecal valve can also demonstrate extensive mucosal hemorrhage. In severe cases, hyperaemic, edematous and ulcerative mucosa are also seen throughout the intestines (Munir, 2013).

The enlargement of lymph nodes accompanied with necrosis and hemorrhage, particularly the mesenteric lymph nodes and atrophied congested spleen are also sometimes seen in PPRV- infected goats in the field (khan, 2008) . Similar to the buccal mucosa, the nasal mucosa becomes hyperaemic. The caudal part of the trachea and bronchus may contain froth. Pulmonary congestion and oedema with varying degree of red and grey consolidations can be seen with severe infections. Congestion of lungs and bronchopneumonia was also seen in PPR-infected West African dwarf goats and was associated with bacterial infection (Emikpe *et al.* 2013).

2.5. Differential Diagnosis

Confirmatory diagnosis of PPR under field conditions is not always possible due to the prevalence of PPR-like diseases and the frequent occurrence of PPR as a mixed infection in sheep and goats. The common clinical manifestations of PPR in sheep and goats are pyrexia, oculo-nasal discharges, erosive stomatitis (involving the lips, tongue and gums) anorexia, diarrhea, and dehydration, followed by either recovery or death (Albina *et al.*, 2013). Further, secondary infections result in a change in the clinical picture of the original disease. This again complicates differential diagnosis based on

clinical signs typical of PPR. Therefore, other diseases of small ruminants causing PPR-like signs can cause problems in clinical diagnosis (Malik *et al.*, 2011).

Infected animals have noticeable lameness and do not exhibit respiratory issues or diarrhea, which sets foot and mouth disease apart from PPR. Furthermore, FMD cases typically do not exhibit the foul-smelling exudates that PPR-infected animals emit from their mouths. Similar to PPR, bluetongue illness can produce fever, nasal discharge, and mouth lesions. On the other hand, bluish coloring of the mouth cavity and edema of the head region are its characteristic. Clinically, diarrhea from PPR and the lack of a mouth lesion can also distinguish CCPP from PPR. Moreover, postmortem examinations of CCPP-infected animals reveal fibrin deposits on the lung and attachment of the lungs to the chest cavity. Because of the similarity between the thick scabs that are seen on the lips of animals in PPR infection and the nodules that Orf generates, the two conditions are often misunderstood. That being said, simple Orf does not typically present with pneumonia, diarrhea, or even oral lesions. Pneumonic pasteurellosis is only a respiratory illness in animals; there is no diarrhea or oral lesion in these animals. Still, PPRV detection is the only way to establish the diagnosis in cases where PPR manifests without oral lesions (Bello, 2013).

2.6. Treatment

Since PPR is a viral disease, there is no specific treatment for this disease. Post-exposure therapeutic approaches for PPR infections are not mentioned much in the literature. However, treatment of affected animals by administration of antibiotics (long acting oxy tetracycline, chlortetracycline) to prevent secondary bacterial infections and anti-diarrheal medicines has been practiced with supportive therapy (B-complex and Dextrose saline) for 5–7 days, which may be useful to reduce the severity of the disease. Treatment and management of clinical cases of PPR or in the event of outbreaks in sheep and goats is necessary in order to minimize the economic losses to farmers. (Balamurugan *et al.*, 2014).

2.7. Diagnostic Techniques

Four key concepts—virus isolation, antigen detection, nucleic acid detection, and antibody identification from serum with sequencing—are the foundation for the diagnosis of PPR infection. Field investigations frequently employ the ELISA test (Kihu *et al.*, 2015). Tests for virus neutralization and isolation in cell cultures are expensive, time-consuming. Antigen-ELISA, RT-PCR, and subsequent nucleotide sequencing are the diagnostic techniques that are now employed to diagnose PPR infection (Saritha *et al.*, 2015). Assays applied to individuals or populations may have different purposes, such as confirming diagnosis of clinical cases, determining infection status for trade and/or movement, estimates of infection or exposure prevalence (surveillance) or checking post-vaccination immune status (monitoring).

Table 1: Test methods available for the diagnosis of peste des petits ruminants and their purpose

Method	Purpose				
	Population freedom from infection	Individual animal freedom from infection	Confirmation of clinical cases	Prevalence of infection surveillance	Immune status in individual animals or populations post-vaccination
Competitive ELISA	++	++	-	+++	+++
Virus neutralization	+++	+++	-	+++	+++
RT-PCR	-	-	+++	-	-
Real-time RT-PCR (QRT-PCR)	-	-	+++	-	-
Virus isolation in cell culture	-	-	+++	-	-
Immuno-capture ELISA	-	-	+++	-	-
Agar gel immune diffusion	-	-	+	-	+
Counter Immune electrophoresis	-	-	+	-	-

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; - = not appropriate for this purpose.

Although not all of the tests listed as category +++ or ++ have undergone formal standardization and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable. Source (OIE, 2013).

Nucleic Acid Sequencing Molecular detection of PPRV is performed by using loop-mediated isothermal amplification techniques and a Real-Time Polymerase Chain Reaction (RT-PCR) assay based on sequence of the N protein gene. From those methods RT-PCR assay based on sequence of the N protein gene is the most and rapid methods for viral Nucleic acid sequencing (Batten *et al.*, 2011).

Pen side tests are field diagnostic procedures that use conjunctival, nasal, and oral swabs to diagnose diseases. These are quick tests that don't require extra equipment and can provide a result in 20 minutes. They cannot, however, be used on tissue and blood samples. Pen side assays have demonstrated sensitivity comparable to that of IC-ELISA and 100% specificity in laboratory testing, having been tested against PPRV isolates from all four lineages. In order to combine the sample with colored beads coated with a particular MAb that recognizes PPRV antigen, the swab samples are rinsed with buffer and the buffer is applied to one end of a chromatographic strip. As a result, the beads are moved along the chromatographic strip by the buffer flow. A line of anti-PPRV MAb is caught halfway down the strip by the antigen-beads complex, which is formed if the sample contains PPRV antigen. This colorful line indicates a positive result. The test line will not bind any beads if the sample contains no PPRV antigen (OIE, 2019).

Isolation of the virus :- In cell culture, several cell lines have been employed for PPRV isolation. These include African green monkey kidney (Vero) cells, transformed monkey cells, primary lamb kidney cells (ovine kidney cells), caprine kidney cells, bovine kidney cells, and marmoset-B- lymphoblastoid (B95a) cell lines. Even if primary lamb kidney and Vero cell cultures have been effective, B95a was previously primarily used for culturing (Alemu, 2014). For a very long period, Vero cells have been the preferred cells for PPRV isolation and propagation. Certain isolates, though, might not develop well in these cells. It has recently been noted that the sensitivity of modified monkey cells expressing sheep/goat signaling lymphocytic activation molecules (SLAM or CD150) to isolate the PPRV is enhanced (Alemu, 2014).

Agar gel immune diffusion :- Agar gel immune diffusion (AGID) is a very simple and inexpensive test that can be performed in any laboratory and even in the field.

Standard PPRV antigen is prepared from infected mesenteric or bronchial lymph nodes, spleen or lung material and ground up as 1/3 suspensions (w/v) in buffered saline). These are centrifuged at 500 g for 10–20 minutes, and the supernatant fluids are stored in aliquots at –20°C. The cotton material from the cotton bud used to collect conjunctival or nasal swabs is removed using a scalpel and inserted into a 1 ml syringe. With 0.2 ml of phosphate buffered saline (PBS), the sample is extracted by repeatedly expelling and filling the 0.2 ml of PBS into an Eppendorf tube using the syringe plunger. The resulting conjunctival/nasal swab extracted sample, like the tissue ground material prepared above, may be stored at –20°C until used. They may be retained for 1–3 years. Negative control antigen is prepared similarly from normal tissues. Standard antiserum is made by hyperimmunising sheep with 1 ml of PPRV with a titre of 104 TCID₅₀ (50% tissue culture infective dose) per ml given at weekly intervals for 4 weeks. The animals are bled 5–7 days after the last injection (OIE, 2022).

Virus neutralization test: - This test is sensitive and specific, but it is time-consuming. The standard neutralization test is now usually carried out in 96-well micro titer plates although roller-tube cultures may also be used. Vero cells are preferred, but primary lamb kidney cells may also be used. This test requires the following materials:

cell suspensions at 600,000/ml; 96-well cell culture plates; sera to be titrated (inactivated by heating to 56°C for 30 minutes); complete cell culture medium; PPRV diluted to give 1000, 100, 10 and 1 TCID₅₀ /ml. i) Dilute the sera 1/5, and then make twofold serial dilutions in cell culture medium. then Mix 100 µl of virus at 1000 TCID₅₀ /ml (to give 100 TCID₅₀ in each well) and 100 µl of a given dilution of serum (using six wells per dilution) in the wells of the cell culture plate ,then Arrange a series of control wells for virus and uninfected cells as follows: six wells with 100 TCID₅₀(100 µl) per well; six wells with 10 TCID₅₀ (100 µl) per well; six wells with 1 TCID₅₀ (100 µl) per well; six wells with 0.1 TCID₅₀ (100 µl) per well; and six wells with 200 µl of virus-free culture medium per well. Make the wells containing the virus dilutions up to 200 µl with complete culture medium, and incubate the plates for 1

hour at 37°C. v) Add 50 µl of cell suspension to each well, pat the sides of the plate lightly to distribute the cells in the well and cover. Incubate the plates at 37°C in the presence of CO₂. vi) Read the plates after 1 and 2 weeks of incubation. The results should be as follows: (OIE, 2022).

If the virus dilution has been done correctly, all the virus control wells with 100 and 10 TCID₅₀ /well will show CPE, 50% of the wells will show CPE for the 1 TCID₅₀ /well dilution, and none should show CPE for the 0.1 TCID₅₀ /well dilution. The test is only valid if the virus has been suitably diluted. For the serum titration, there will be no CPE in wells where the virus had been neutralized by serum during the test; any level of CPE means that the virus had not been neutralized by serum. The neutralizing titre is the dilution of serum that neutralizes virus in half the wells. A neutralizing titre of greater than 10 is positive. (OIE, 2022).

2.8. Socioeconomic Impact of PPR

The Peste des Petits Ruminants virus is widely distributed throughout Asia and Africa. These regions include a large portion of the developing globe, which is largely dependent on subsistence farming to offer commodities for trade or food, with tiny ruminants being an excellent source of both. Miserably, PPR and other transboundary animal diseases (TADs) pose a danger to small ruminant production and, consequently, the livelihoods of poor farmers in many parts of Asia and Africa. PPRV is one of the main barriers to subsistence farming because of its high morbidity and mortality rates (Banyard *et al.*, 2010).

The high death rate that is typical with PPR is the primary cause of the socioeconomic losses connected with the illness. This has an adverse effect on small ruminant marketing chains' revenue from production and value addition. International trade is hampered by Peste des Petits small ruminant disease, while this effect is lessened in local and regional marketplaces because of the illness's current widespread geographic dispersion. However, the trade limitations on animal by-products and the sanitary precautions enforced by authorities to control animal mobility exacerbate the direct economic losses caused by the disease (Elsawalhy *et al.*, 2010).

There can be significant financial losses linked to PPR. Globally, farmers' and pastoralists' livelihoods are severely impacted by peste des petits ruminants. Small ruminant herds, numbering 2.1 billion worldwide, are vital resources for poor rural households in developing nations. They offer high-quality milk, protein, nutrients, wool, and fiber in addition to income opportunities and financial freedom. Approximately 80% of the animals can be found in areas with PPR (FAO, 2013).

The virus is currently present or predicted to be present in over 70 countries, and it continues to spread, showing up virtually yearly in previously unaffected areas or nations and causing an estimated \$1.5 to \$2 billion in financial losses annually. Controlling, preventing, and eliminating PPR is connected to other global issues like reducing poverty, ensuring food security and nutrition, and fostering resilience, especially among women and smallholder farmers. Afar Regional State, North Eastern Ethiopia, conducted a study that revealed 63.3% of the total sheep and goat population lost annually as a result of PPR. The average financial loss resulting from animal farm mortality in the affected areas was 2,146,875.00 birr/92,140.56\$ for both goat and sheep farms. (Gizaw *et al.*, 2018)

2.9. Prevention and Control

Strong support for diagnostic techniques and appropriate, timely immunization of the susceptible population are essential for the effective control of PPR. Therefore, the strong recommendation made for the control program is supported by the availability of the attenuated cell culture vaccine and different diagnostic techniques/kits for the diagnosis of PPR. One of the most serious animal diseases, PPR control is thought to be crucial for reducing poverty in Southern Asia and Africa. (Balamurugan *et al.*, 2013).

Vaccination, appropriate carcass disposal, and movement control are the usual strategies used to contain PPR epidemics. Sheep and goat imports from impacted areas are prohibited, and newly introduced animals must spend three weeks in quarantine. Furthermore, barns, tools, and other items that have come into touch with sick animals should be disinfected using conventional disinfectants such as phenol, sodium

hydroxide 2%, virkon, as well as alcohol, ether, and detergents. Additionally, carcasses and contact fomites should be buried or burned. (OIE, 2013).

2.9.1. PPR Global Control and Eradication Strategy

Despite the availability of an efficacious and cheap live-attenuated vaccine, the virus has continued to spread. The reasons include growing population of and dependencies on small ruminants, which are increasingly popular livestock, more resilient in the face of climate change and they provide a ready trade and cash economy for poorer communities. In April 2015, the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (WOAH) launched the PPR Global Control and Eradication Strategy (PPR GCES) with the vision for global eradication by 2030. There is a strong and lasting international consensus to eradicate the disease in order to protect the livelihoods of the world's poorest populations (Njeumi *et al.*, 2020).

Vaccination is being taken forward as the key strategy along with epidemiological surveillance to target vaccination efforts and eradicate the disease (Mahapatra *et al.*, 2020). Phase I of the global strategy has been completed and Phase II is in its inception aiming at eradication in 2030 (Zhao *et al.*, 2021).

The main shifts in the approach are firstly, to more comprehensively engage in episystems (geographically distinct, transnational, ecologically connected host populations infected with the virus) for coordinated elimination and secondly, attention to the whole spectrum of species associations and epidemiologies, learning what matters to the eradication, which remains focused on sheep and goat. This is possible in the knowledge that even where infection spreads beyond these domestic animals, it is ultimately dead end. These events can be catastrophic to wildlife and biodiversity also bringing stronger arguments for eradication beyond the economic benefits to the livestock industry alone. (Zhao *et al.*, 2021)

Table 2 : Sero prevalence studies of PPR in different regions of Ethiopia

Study regions/areas in Ethiopia	Number of animals tested and their prevalence	Reference
Tigray (southern zone)	240 Goats (47.5%)	Afera <i>et al.</i> , 2014
Afar (Adar, mille)	229 sheep and goats (41.5%)	Gizaw, 2018
Afar (mille, Asayita)	384 sheep and goats (60.15%)	Dubie, 2022
Eastern Amhara	612 Sheep and Goats (56.5%)	Alemu, 2014
Amhara	672 sheep and goats (18.3%)	Fentie <i>et al.</i> , 2018
Oromia (East Shewa, Arsi Zone)	700 sheep and goats (48.43%)	Gari <i>et al.</i> , 2017
Oromia (Horo Guduru)	806 Sheep and goats (27.42%)	Gelana <i>et al.</i> , 2020
Oromia (Buno-Bedele)	680 sheep and goats (4.7%)	Seyoum, 2020
Oromia	662 sheep and goats (10.3%)	Ejigu, 2023
Benishangul (Metekel)	452 Sheep and Goats (73.45%)	Woldemichael <i>et al.</i> , 2018
Benishangul (Asosa)	321 Sheep and Goats (75.7%)	Yalew <i>et al.</i> , 2019
Benishangul (Awi and Metekel)	714 Sheep and Goats (65.4%)	Abiyu, 2022
SNNPR (Kafa zone)	968 Sheep and Goats (2.1%)	Gebre <i>et al.</i> , 2018
SNNPR (Gurage zone)	221 Sheep and Goats (33%)	Hailegebreal, 2018
Somalia	472 Sheep and Goats (41%)	Dejene, 2016
	7734 Sheep and Goats (62%)	Nijue , 2018

3. MATERIALS AND METHODS

3.1. Description of Study Area

The study was conducted in the selected districts of eastern and south eastern zones of Tigray region, Ethiopia. Specifically, kilte Awulaelo district from Eastern Zone and Enderta district from south eastern zones .Eastern Zone is located at longitude and latitude of 14°16'N 39°27'E coordinates: with an elevation of 2457 m.a.s.l. The mean annual rainfall is 552 mm. The annual minimum and maximum temperatures are 7.77 °C and 24.21 °C respectively (CSA, 2014).The zone has livestock population of 377,033cattle, 483,160goat, 370,439sheep, 196,363equine, 419 camel, 775,837 poultry. (source bureau of agriculture and natural resource un published document 2020).South eastern zone is located in latitude of 13° 14' 60.00" N and longitude 39° 09' 60.00" E. The altitude of the area ranges from 1470-2370 meter above sea level (m.a.s.l.). The average annual temperature and rain fall ranges from 13 to 25 degree Celsius and 350mm to 700mm, respectively. The livestock population estimated to be 407,014 cattle, 408,432 goat, 154,408 sheep, 71390 equine, 5168 camel,502,823 poultry (CSA, 2014).

Enderta According to current administrative structure of Districts in Tigray Region, the District is bounded geographically with Afar Regional National State in the East, District DeguaTemben in the West, Hintalo in the South, Agulae in the North. The absolute location of the District is 13.44292⁰ East and 39.507154⁰ North. The district covers 141,051 hectare and 1410 km² total area coverage. The administrative center of the district is Kuiha which is found 13 KM far from the Mekelle City. The livestock population estimated to be125,933 cattle, 19,495 sheep, 77,242 goats, 48,639 donkeys, 2008 mules, 636 horses, and 96,940 poultry. (Tigray Bureau of Agriculture and Rural Development, 2020).The main economic activity of the people in the District depends on mixed agriculture.

Kilte Awulaelo district is located at altitude of in between 870 to 1920 m.a.s.l with The absolute location of 13.51217⁰ north and 39.68612⁰ east . It has 13 Kebeles covering an area of 51,297.6 hectare and 512.9 km². The area experiences a

temperature ranging from 19.40°C to 37.6°C and an annual rain fall ranging from 375 to 875 mm. The livelihood of the society largely depends on mixed livestock and crop production The district have livestock population of 68, 893 cattle, 42,822 goat, 37,776 sheep, 13,642 equines, 304,343 poultry (Tigray Bureau of agricultural unpublished report, 2020).

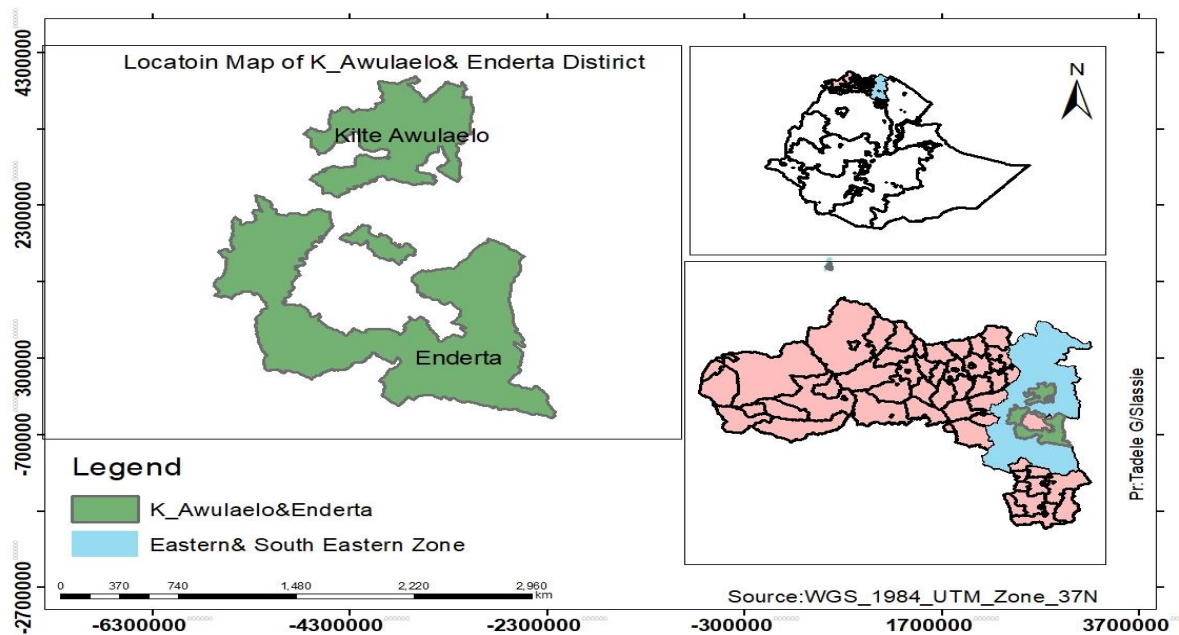


Figure 3 : Location Map of the study Area

Source: Tigray Bureau of agricultural and natural resource water shade management department

3.2. Study Animals

The native breeds of sheep and goats that have not received a vaccination against Peste des petits ruminants (PPR) was made up the research population of small ruminants kept under intensive production systems. Animals older than six months, regardless of gender, were included in the study. Age, species, sex category, body condition, and herd size were documented for each individual animal. Following that, the research animals' herd sizes was divided into three categories: large (> 20 animals), medium (10–20 animals), and small (< 10 animals) in accordance with (Gebre *et al.*, 2018). The age of the research animals was ascertained by interviewing the owner and using

the ESGPIP 2009 dentition to divide them into three age groups: young (less than a year), adult (between one and three years), and elderly (more than three years). (Husen *et al.*, 2018).

3.3. Study Design

From January 2024 to December 2024, a cross-sectional study was conducted to ascertain the sero-prevalence of Peste des petits ruminants (PPR) in small ruminants. Using a checklist, the project was gather epidemiological data on the age, species, sex, body condition and size of the sheep and goat herds that are linked to the associated risk factors for PPR. Goats and sheep of every age were sampled; however, in order to rule out maternal antibodies, only young animals older than six months were taken into consideration for sampling.

3.4. Sample Size Determination

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

$$n = \frac{(1.96)^2 P \exp (1 - P \exp)}{d^2} = \frac{3.8416 * 0.5 * 0.5}{(0.05 * 0.05)} = 384$$

Where n is the required sample size, $P \exp$ is the expected sero-prevalence, d^2 is the desired absolute precision. Accordingly, a sample size of 384 was estimated using the formula and 43 clinical Samples for molecular characterization was taken.

3.5. Sampling Techniques

The two zones were selected randomly using lottery method and then Enderta district from south eastern zone and Kilde awulaelo district from eastern zone were purposively selected based on accessibility of transport and small ruminant population potential. The sampling peasant associations were chosen based on the number of

sheep and goats in each area. In this way, two peasant associations from the Kilte awulaelo and two from the Enderta district were selected. Based on the population of sheep and goats, the number of sheep and goats sampled for the serological study was proportionally distributed at each district level to peasant associations. Next and finally, a simple random sampling method was employed.

3.5.1. Serum sample collection

Five milliliters of blood were extracted from the jugular vein using sterile vacutainer tubes. The samples were labeled with the animal's species, identification number, and collection date. They were then left to stand slantwise and incubated for twenty-four hours at room temperature to promote clotting. Then using a sterile micropipette, the serum was extracted into cryogenic vials. The samples from which the serum could not be clearly separated were centrifuged for 10 minutes at 1500 rpm to extract the clear serum and remove any leftover red blood cells. Before being shipped to the Animal Health Institute in Sebeta, Ethiopia, the serum was first kept at -20°C in the Mekelle livestock Regional Laboratory.

3.5.2. Nasal and ocular swabs for molecular characterization

A total of 43 nasal and ocular swabs were collected from active cases of sheep and goat flocks in Enderta and Kilte awulaelo districts suffering from fever, respiratory distress, coughing, nasal and ocular discharge, erosive stomatitis and diarrhea suggestive of PPRV infection. The swabs were stored in the college of veterinary science Laboratory and then transported to animal health institute (AHI), Sebeta, Ethiopia for molecular identification of the PPR virus circulating in the study area.

3.6. Laboratory Analysis

3.6.1. Serological analysis

A gold-standard antibody detection test recommended for international trade is the virus neutralization test. Before being inoculated into a 96-well micro plate, 100-1000 TCID₅₀ of PPRV is combined with 100µL of serum dilutions and incubated at 37°C in the VNT. The absence of virus neutralization is indicated by the development of

CPE in the micro plate wells containing a certain antibody dilution. A serum's virus neutralization titer is defined as the maximum dilution that causes a 50% reduction in CPE. When it comes to morbilli viruses, this test finds antibodies that neutralize the virus, which may be a sign of in vivo defense. (Santhamani *et al.*, 2016).

3.6.2. Molecular detection of PPRV nucleic acid using RT-PCR

Viral genomic RNA was extracted from collected samples using a commercial RNA extraction kit (Qiagen® RNeasy Universal Mini Kit) as per the manufacturer's instructions. RT-PCR was performed for the N-gene and F-gene of PPRV using the QIAGEN® One-step RT-PCR kit as per the manufacturer's instructions (Vandna *et al.*, 2020).

RNA extraction of The PPR virus the swab samples were equilibrated to room temperature and processed for RNA extraction. 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. (Vandna *et al.*, 2020).

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 micro centrifuge tube, 60µl elution 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, RNA was extracted. (Vandna *et al.*, 2020).

Real time RT-PCR was done for the extracted samples by using Applied Bio systems 7500 fast one step real time PCR thermo cycler machine. The extracted RNA was converted to cDNA using a reverse transcriptase enzyme and the cDNA was amplified using PPRV specific forward and reverse primers (Hodgsan, 2018). In brief, a master mix was prepared, vortexed and 17µl 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°C for 15 minutes, one cycle of reverse transcription inactivation/Tag activation at 95°C for 20 seconds and 45 cycles of denaturation at 95°C for 3 seconds followed by annealing and final extension at 60°C for 30 seconds (Hodgsan, 2018; Tolessa, 2020).

3.7. Data Management and Analysis

The data was processed in MS Excel and analyzed using STATA version 14. Logistic regression was used to identify the link between predictor variables and PPRV antibodies sero-prevalence. Variables include district, species, sex, age, body condition and herd size, those independent variables that were statistically significant was again subjected to multivariate logistic regression. A p-value of < 0.05 was considered significant. Descriptive analysis was used for molecular characterization.

3.8. Ethical Considerations

Institutional ethical clearance was obtained from the ethical and research review committee of the College of Veterinary Sciences, Mekelle University, with approved

certificate reference number of AEEC 27/2024. Permission was obtained from the Regional Agriculture bureau, Tigray Region. Study participants were received a full explanation about the study including the purpose and benefits of the study. Informed consent was obtained from each sheep and goat owner before collecting epidemiological data and blood samples for laboratory analysis. The owners had the right to withdraw from this study at any time and confidentiality was maintained. The sampled sheep and goats was dewormed using anthelmintic, and sick ones were treated using antibiotics.

4. RESULTS

4.1. Sero prevalence of PPR infection

Overall, 161 (41.9%) of the 384 serological samples that were examined using VNT for PPR virus antibodies were found to be positive. The sero prevalence in the districts of Kildeawlalo and Enderta was found to be 35.4% and 47.6%, respectively. In the studied areas of the Tigray region, the sero prevalence of the PPR virus among the species level was found to be 36.8% in sheep and 44.6% in goats. Out of all the sample analyzed, 39 samples (43.8%) were positive from males and 122 samples (41.4%) were positive from females.

As shown in (Table 4), associated factors including species, sex, age, district, flock size, and body condition score (BCS) were evaluated using a structured framework for each animal sampled in order to determine the sero prevalence of PPRV infection.

The study population showed varying levels of sero prevalence according to age groups: 34/80 (42.5%) in the young age group, 55/138 (39.9%) in the adult age group, and 72/166 (43.4%) in the old aged group. The prevalence of older age groups was somewhat higher than that of younger age groups. According to the data, the sero prevalence of PPR infection varied by sex group, with 39/89 (43.8%) and 122/295 (41.4%) in males and females, respectively.

The majority of seropositive sheep and goats to PPR antibodies were found to be in good body condition (7/16; 43.8%), followed by those in medium body condition (86/204; 42.2%) and poor body condition (68/164; 41.5%). District level sero prevalence was higher in enderta 98/206 (47.6%) than in k/awoulealo 63/178 (35.4%) and 76/151(50.3%), 49/130 (37.7%) and 36/103 (35%) were sero positive in small, medium and large flock size respectively.

The results of the chi-square (χ^2) analysis in this study showed that, of the risk factors linked to the incidence of PPRV seroprevalence, species, sex, age, and body condition were not determined to be statistically significant, as shown (Table 3), where it was determined that flock size at p-value (0.025*) and seroprevalence incidence of

Districts at P-value (0.016*) were statistically significant.* P<0.05 indicates significance.

Table 3: Analysis of Associated factors of PPR using Chi-square

Factors	Sample size	Seropositive	χ^2 Value	<i>P-value</i>
Species				
Sheep	133	49	2.17	0.142
Goats	251	112		
Sex				
Male	89	39	0.17	0.680
Female	295	122		
Age				
Young	80	34	0.39	0.820
Adult	138	55		
Old	166	72		
District				
Enderta	206	98	5.82	0.016*
Kilte Awlalo	178	63		
Flock size				
Small	151	76	7.39	0.025*
Medium	130	49		
Large	103	36		
Body condition				
Poor	164	68	0.04	0.980
Medium	204	86		
Good	16	7		

* Significance at P<0.05

Through the use of univariate logistic regression analysis, the non-important predicted associated risk variables were decreased. According to the findings of a univariate logistic regression analysis, the risk factors flock size (P = 0.016) and district (P = 0.016) had a significant correlation with VNT sero-positivity. On the other hand, Table 4 shows that there was no significant correlation between VNT seropositivity of the disease and sex (p = 0.68), age (p = 0.702), or body condition score (P = 0.893).

Table 4: Univariate logistic regression analysis of risk factors for PPR sero prevalence

Factors		Positive samples	Prevalence, %	<i>P-value</i>	<i>COR</i> (95% <i>CI</i>)
Species	Sheep	49	36.8	-	Ref
	Goats	112	44.6	0.142	1.38 (0.897- 2.12)
Sex	Male	39	43.8	-	Ref
	Female	122	41.4	0.68	0.90 (0.56 - 1.46)
Age	Young	34	42.5	-	Ref
	Adult	55		0.702	0.89 (0.51- 1.57)
	Old	72	39.9	0.897	1.04 (0.60- 1.78)
District	Enderta	98	47.6	-	Ref
	KilteAwulealo	63		0.016	0.6 (0.40- 0.91)
Flock size	Small	76	50.3	-	Ref
	Medium	49		0.034	0.60 (0.37- 0.96)
	Large	36	37.7	0.016	0.53 (0.32- 0.89)
Body condition	Poor	68	41.5	-	Ref
	Medium	86		0.893	1.03 (0.68- 1.56)
	Good	7	42.2	0.859	1.10 (0.39- 3.09)
			43.8		

For the multivariate logistic regression analysis, variables from the univariate analysis with P values less than 0.05 are taken into account. as a result, for multivariate logistic analysis, we have two variables: flock size and district. But as (Table.5) demonstrates, there is a strong correlation between the two variables. Thus, the problem of multicollinearity will arise variables with a p-value of less than 0.25 would be chosen.

In order to determine the true significant contribution of these related risk factors without compounding their effects on one another, species and herd size were found to be statistically significant variables in univariable logistic regression. These variables were then fitted to the final multivariable logistic regression model, as shown in (Table 5).

Table 5: Multivariate logistic regression analysis of risk factors for PPR sero prevalence

Factors		No. of animals tested	Positive samples	<i>P-value</i>	AOR (95% CI)
Species					
	Sheep	133	49	-	Ref
	Goats	251	112	0.574	1.15 (0.70-1.90)
Flock size					
	Small	151	76	-	Ref
	Medium	130	49	0.039**	0.60 (0.37-0.97)
	Large	103	36	0.065*	0.57 (0.32-1.03)

* Significant at 1% and ** is significant at 5 %.

Flock size was found to be a possible risk factor for PPRV sero positivity, which was substantially correlated with the prevalence of viral antibodies ($P < 0.05$) based on multivariable logistic regression analysis.

4.2. Molecular Detection of PPRV

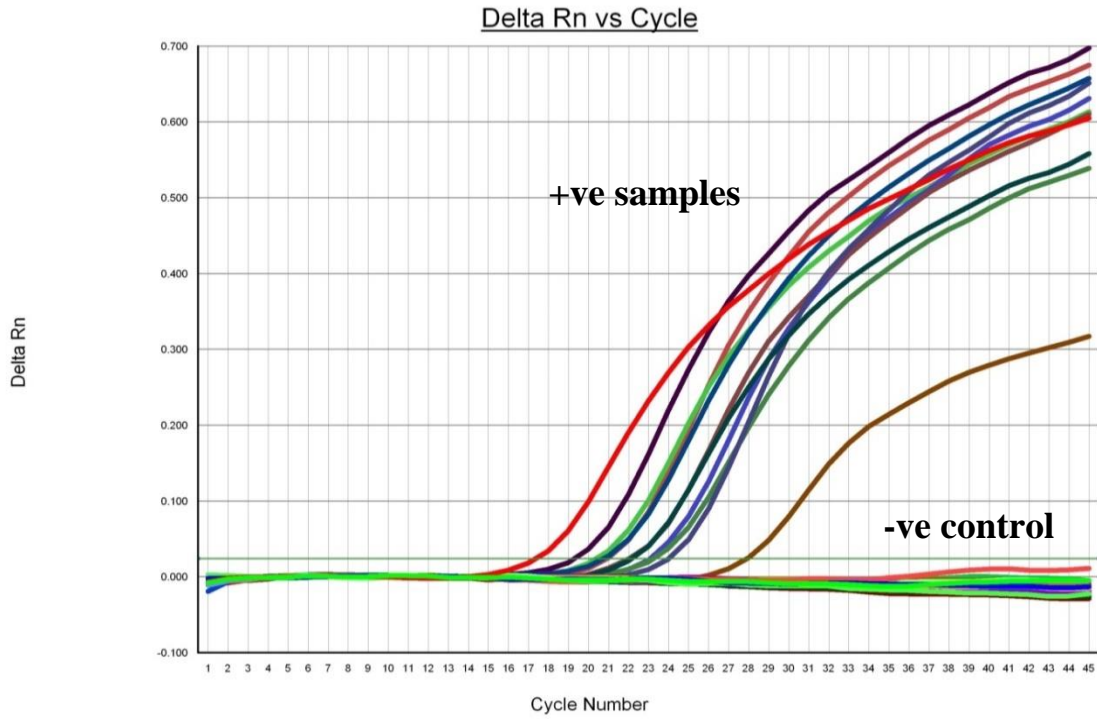
The nucleic acid of PPRV was found using the RT-PCR test using swab samples taken from sheep and goats in the research locations that were clinically suspected of having PPR. 10/43 (23.26%) of the 43 probable clinical samples that were subjected to real-time PCR testing tested positive for PPRV nucleic acid. The positive control was discovered less than 35 cycle threshold (Ct) value, whereas the negative control remained unnoticed. The positive samples were detected at Ct values ranging from 19.69 to 28.25. 23.3% (n= 10/43) of the positive samples overall came from goats; no

positive samples from sheep were found. As a result, samples taken from goats had a higher rate of viral detection than ones from sheep. It was also discovered that the nucleic acid of the virus was more prevalent in males.3/12 (25%) compared to females 7/31 (22.5%). Kiltawulaelo has a larger percentage of PPRV nucleic acid (47.4%) than Enderta (4.2%), according to districts.

Table 6: Species, district, sex and age wise detection rate of PPRV nucleic acid using real time PCR

Factor	category	No. tested	No. positive	percent %
Species	Caprine	34	10	29.4 %
	Ovine	9	0	0
	total	43	10	23.26 %
District	Enderta	24	1	4.2 %
	Kiltawulaelo	19	9	47.4 %
	total	43	10	23.6 %
Age	Young (< 1 year)	27	9	33%
	Adult (1- 3 years)	11	1	9.1%
	Old (> 3 years)	5	0	0
Sex	male	12	3	25 %
	female	31	7	22.5 %

The current investigation clarified why PPRV was found in clinical samples and verified that PPRV was circulating in various areas of the Kiltawlaelo and Enderta districts. Frequent outbreaks may be caused by animal trading, animal migration, or animal mixing for the market from various locations.



Selected Detector: All
 Well(s): A1-B5,B7-C9,C11-D3,D5-H12
 Document: PPR Tadele Research from Mekele 230424.sds (Standard Curve)

Figure 4: Real time PCR standard curve indicating PPRV detection result

5. DISCUSSION

PPR continues to be a significant threat to small ruminant productivity, spreading across the country. The disease leads to the death of animals and production losses, causing substantial economic impact and potentially worsening food insecurity, especially in the most vulnerable regions of the country (Alemu *et al.*, 2019).

The sero-prevalence study revealed that the PPR virus was circulating among the sheep and goat populations in the Enderta and KildeAwlalo districts of the Tigray region. The overall sero-prevalence rate in the study was 41.9%, which aligns with findings from previous research: 40.2% in Afar (Gizaw, 2018), 46.53% in goats from Tigray (Afera *et al.*, 2014), and 48.43% in Oromia (Gari, 2017). However that the current study produced lower results than earlier research conducted in Ethiopia, which reported 60.15 percent in the Afar area (Dubie *et al.*, 2022), 65.4% in the Awi and Metekel zone (Abiyu, 2022), 75.7% in Asosa (Yalew *et al.*, 2019), and 62% in Somalia. (Njue *et al.*, 2018). Studies from other Asian and African countries also showed higher rates, such as 73.8% in Turkey (Bello *et al.*, 2016), 58.2% in Sudan (Fatima, 2018), and 53.86% in India (Mahesh, 2022).

On the other hand, the current results were comparatively higher than those in some previous reports, including 18.3% in the Amhara region (Fentie *et al.*, 2018), 29.2% in the South Region (Hailegebreal *et al.*, 2018), 6.98% and 10.3% in Oromia (Gelana, 2023; Ejigu, 2023), 14.8% in Rwanda (Shakaka *et al.*, 2021), 34.2% in Pakistan (Aziz-ul, 2016), and 33% in Libya (Dayhum, 2017). The discrepancies between the current sero-prevalence studies and previous reports may be attributed to variations in sample size, the season of specimen collection, production systems, host population density, agro-climatic conditions, and the movement of shoat, all of which can affect contact rates. Higher livestock density can lead to more frequent contact between animals, increasing the likelihood of PPR transmission.

In the current study, sero-prevalence of the PPR disease among the study districts was observed. Antibodies against the PPR virus were detected in all the study districts of the Tigray region. Enderta district exhibited the highest PPR sero-prevalence at 47.6%,

while Kilde Awulalo district had the lowest at 35.4%. Significant differences ($p < 0.016$) in PPR sero-prevalence were observed between the study districts. This may be due to free animal movement from nearby afar region.

The current study reported a species-specific prevalence of Peste des Petits Ruminants (PPR), with 44.6% in goats and 36.8% in sheep. The sero-prevalence results showed that PPR is more common in goats than in sheep, though the difference between the species was not statistically significant ($p > 0.05$). This finding aligns with previous researches indicating higher PPR prevalence in goats (Fentie *et al.*, 2018; Abesha *et al.*, 2023), but contrasts with studies by Yalew *et al.* (2019), Gizaw *et al.* (2018), and Gelana *et al.* (2020).

In the study area, goats were more populous than sheep, and sheep were confined to grazing around the village, while goats migrated longer distances. The lack of significant difference between species may be attributed to the communal grazing system and free movement of animals in the region, which likely equalized their exposure to disease risk factors. Despite biological differences between sheep and goats, factors such as management practices, viral strains, animal distribution, and sampling methods can influence sero-prevalence. Additionally, PPRV may preferentially infect one species over the other, with varying severity depending on the environment (Yizengaw *et al.*, 2021; Truong *et al.*, 2014).

In the sex-wise subgroups, the prevalence rate was slightly higher in males (43.8%) compared to females (41.4%), but this difference was not statistically significant. This may be due to the higher demand for male animals' meat, leading to their increased presence in markets, while females are typically kept at home for reproductive purposes. Genetic variability among animals could also contribute to the observed differences in infection rates (Rony *et al.*, 2017). These findings are consistent with studies by Mahesh (2022), Thakor *et al.* (2016), and Sakhare (2019), but differ from studies by Dubie *et al.* (2022), Fatima *et al.* (2018), and Joseph *et al.* (2023), which reported higher sero-prevalence in females.

The current study found a slightly higher prevalence of PPR in older animals (43.4%) compared to younger ones (42.5%), though this difference was not statistically significant. This contrasts with the findings of Yizengaw *et al.* (2021), who reported a significantly higher prevalence in young animals (36.53%) than in older ones (8.08%). However, our results are consistent with studies by Ahaduzzaman (2020), Acharya *et al.* (2018), and Gari *et al.* (2017). The marginally higher prevalence in adults could be attributed to several factors, including increased virus circulation, aging, and the decline of maternally derived antibodies in older animals. PPRV is highly immunogenic and can persist in tissues, particularly in endemic areas, making older animals more likely to be exposed (Acharya *et al.*, 2018; Gebre *et al.*, 2018). Furthermore, animals infected at a younger age may test positive for the virus longer, leading to higher antibody detection rates in older animals (Yalew *et al.*, 2019).

The present study found a seroprevalence of 35.0% in large flock sizes and 50.3% in small flock sizes, with this difference being statistically significant. These rates are higher than the flock prevalence rates of 18.8% and 25.86% reported by Gebre (2018) and Gelena (2023), respectively. However, they are lower than the seroprevalence at the flock level reported by Fatima (2018) and Wondimagegne (2016), which were 100% and 98%, respectively. The lower prevalence in larger flock sizes could be attributed to better husbandry practices among owners of larger flocks compared to those with smaller ones. It may also be related to the endemic nature of the disease in the study area.

Field observations and clinical samples from this study indicated that PPRV is currently circulating in the Enderta and Kilde Awlalo districts of the Tigray region. The clinical signs of the disease observed align with those reported by Almu *et al.* (2019), Ahmed *et al.* (2021), and Adeoye *et al.* (2022), including fever, depression, dullness, diarrhea, ocular and nasal discharge, coughing, anorexia, erosive lesions on the gums, and animal deaths. In regions without vaccination programs against PPR, serological detection of PPRV antibodies, alongside clinical symptoms, can confirm the presence of the disease. A definitive diagnosis can be made by detecting the virus's nucleic acid in clinical samples.

In this study, real-time PCR was used to confirm suspected PPR cases by detecting PPRV nucleic acid in clinical samples. Out of 43 samples tested, 10 (23.26%) were positive, confirming that the virus is circulating in the study areas. These findings are consistent with previous studies, such as Khalefe *et al.* (2024) with 33/133 (25.2%) positives, and Abiyu (2022) with 16/42 (38%). However, higher rates of PPRV nucleic acid detection were reported in studies by Abdi *et al.* (2022) at 54%, Maheshm *et al.* (2021) at 56.3%, and Bhoyar *et al.* (2019) at 52.2%. In contrast, lower rates were reported by Adeoye *et al.* (2022) at 7.4%, Altan *et al.* (2019) at 10.4%, Candela *et al.* (2017) at 11.8%, and Rudra (2019) at 15.3%. The differences in detection rates could be attributed to variations in targeted gene types, the stage of virus infection, sample size, sample types, and detection methods, as suggested by Alemu *et al.* (2019). This molecular study also found that goats had a significantly higher PPR infection rate (29.4%) compared to sheep (0%). This result is in line with previous research by Abiyu (2022) and Alemu *et al.* (2019). The disparity could be due to sheep's relative resilience to the virus or a potential decrease in the virulence of the Ethiopian PPRV strain in sheep, as noted by Alemu *et al.* (2019).

6. CONCLUSION AND RECOMMENDATIONS

The study provides evidence that PPRV is widely circulating among small ruminant populations in the Enderta and Kilde Awlalo districts of the Tigray region. It confirms that PPR is endemic in the area, with high sero-prevalence rates of 47.6% in Enderta and 35.4% in Kilde Awlalo. The sero-prevalence was significantly associated with risk factors such as district and flock size. Common clinical signs of PPR were observed, and the disease was confirmed by real-time PCR detection of the PPR virus, marking the first documented occurrence in these districts. These findings underscore that PPR remains endemic in the region and could contribute to further viral transmission within the area and to neighboring disease-free regions.

Therefore, based on the above conclusion the following recommendations were proposed:-

- Regular vaccination of sheep and goats should be implemented with a strategic plan that includes both the studied and neighboring districts to prevent further virus spread.
- Farmers should be made aware of disease preventive and control systems in order to increase their contribution and the way the disease investigation and control program is implemented.
- Further research should be conducted to determine the gene sequences and lineage of the PPR virus isolated in this study area.

7. REFERENCES

- Abdalla A.S., Majok A.A., El Malik O.K. and Ali ALSO. (2012) :Sero-prevalence of Peste des petitsruminants virus (PPRV) in small ruminants in Blue Nile, Gadaref and North Kordofan States of Sudan. *J. Pub. Hlth. Epidemiol*, 4(3):59-64.
- Abde Aliy Mohammed, Tesfaye Rufael Chibssa , Waktole Terfa, Fasil Aklilu, Delesa Damena, Redeat Belayneh, and Menbere Kidan (2022). Isolation and Molecular Characterization of Peste des Petits Ruminants Virus from Outbreaks in Southern Ethiopia, 2020. National Animal Health Diagnostic and Investigation Center (NAHDIC), P.O. Box 04, Sebeta, Ethiopia. *Advances in Virology Volume 2022*, Article ID 5329898, PP.2-7.
- Abesha, H., Teshome, Y., Alemu, Y.F., Dejene, H., Tarekegn, Z.S. and Assefa, A., 2023. Seroepidemiology of peste des petits ruminants virus in small ruminants in selected districts in Northwest Ethiopia. *Veterinary Medicine and Science*, 9(2), pp.884-890.
- Abraham G., Sintayehu A., Libeau G., Albina E., Roger F., Laekemariam Y., Abayneh D, Awoke KM (2005): Antibody seroprevalences against peste des petits ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia. *Prev Vet Med*, 70(1–2):51.
- Abubakar M., Jamal M.S., Arshed M.J., Hussain M. and Ali Q. (2009) : Peste des petitsruminants virus (PPRV) infection: Its association with species, seasonal variations and geography. *Trop. Anim. Health. Prod*, 41: 1197-1202.
- Abubakar, M., Jamal, S.M., Hussain, M. and Ali, Q.(2008): Incidence of peste des petits ruminants (PPR) virus in sheep and goat as detected by immuno-capture ELISA (Ic ELISA). *Small ruminant research*, 75(2-3): 256-259.
- Afera B.,Hussen D., Amsalu K. (2014): Seroprevalence of Peste des Petits Ruminants in Goats of Southern Parts of Tigray Region. *Global Veterinaria*, 12; 512–516.
- Ahaduzzaman, M., (2020) : Peste des petits ruminants (PPR) in Africa and Asia: A systematic review and meta-analysis of the prevalence in sheep and goats between 1969 and 2018. *Vet. Med. Sci.*, 6(4): 813–833. <https://doi.org/10.1002/vms3.30>.

- Ahmed, S., Hosny, W.A.E.W., Mahmoud, M. and Mahmoud, M.A.E.F., (2021). Isolation and identification of peste des petits ruminants virus from goats in Egyptian governorates. *Veterinary World*, 14(4), p.926.
- Albina E, Kwiatek O, Minet C, Lancelot R, de Almeida RS, G. L.(2013): Peste des petits ruminants, the next eradicated animal disease? *Veterinary microbiology* 165(2): pp.38–44.
- Alemayehu, G., B. Hailu and N. Seid, (2015) : Participatory Assessment of Major Animal Health Constraints to Sheep Export from Afar Pastoral Production System. *Global Veterinaria*, 15(1): 48-56, ISSN 1992-6197 © IDOSI Publications, DOI: 10.5829/idosi.gv.2015.15.01.9572.
- Alemu, B. (2014) : Epidemiology and identification of peste des petits ruminants (PPR) virus circulating in small ruminants of Eastern Amhara region bordering Afar, Ethiopia. MSc Thesis, Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of Veterinary Clinical Studies, Bishoftu, Ethiopia.
- Alemu, B., Gari, G., Libeau, G., Kwiatek, O., Kidane, M., Belayneh, R., Siraw, B., Wieland, B., Asfaw, W. and Abdi, R.D. (2019). Molecular detection and phylogenetic analysis of Peste des petits ruminants virus circulating in small ruminants in eastern Amhara region , Ethiopia. *BMC Veterinary Research*, 15: 84.
- Altan, E., Parida, S., Mahapatra, M., Turan, N. and Yilmaz, H. (2019). Molecular characterization of Peste des petits ruminants viruses in the Marmara Region of Turkey. *Transboundary and Emerging Diseases*, 66: 865-872.
- Anselme Shyaka , Marie Aurore Ugirabe and Jonas Johansson Wensman .,(2021) : serological Evidence of exposure to pestdespetits ruminants in small ruminants in Rwanda. *School of Veterinary Medicine, College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda* pp .4-6.
- Apurbo Kumar Mondal, Md. Rabiul Auwul, Md. Momotaj Hossen, Md. Sodrul Islam1, Narayan Paudyal, Md. Shahidul Islam, and Kazi Khalid Ibne Khali (2024) Global Prevalence and Associated Risk Factors of Peste des Petits Ruminants (PPR) Virus in Sheep and Goats: A Meta-Analysis at: <https://www.researchgate.net/publication/380162663> pp.2-14.

- Baazizi R, Mahapatra M, Clarke BD, Ait-Oudhia K, Khelef D, Parida S. (2017) : Peste des petits ruminants (PPR): a neglected tropical disease in Maghreb region of North Africa and its threat to Europe. PLoS ONE. 2017;12:e0175461.
- Balamurugan V., Hemadri D., Gajendragad MR., Singh RK., Rahman H.(2013): Diagnosis and control of peste Des petits ruminants: A comprehensive review. *Virology*; 25: 39-56.
- Balamurugan, V., Sen, A., Venkatesan, G., Bhanot, V., Yadav, V., Bhanuprakash, V. and Singh, R.K. (2012a) : Peste des petits ruminants virus detected in tissues from an Asiatic lion (*Panthera leo persica*) belongs to Asian lineage IV. *Journal of Veterinary Science* 13, 203–206.
- Banyard, A.C., Parida, S, Batten, C., Oura, C., Kwiatek, O. & Libeau, G., (2010) : ‘Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control’, *Journal of General Virology* 91, 2885–2897. <https://doi.org/10.1099/vir.0.025841-0>.
- Baron, M.D., Diallo, A., Lancelot, R. & Libeau, G., (2016): ‘Peste des Petits Ruminants virus’, *Advances in Virus Research* 95, 1–42. <https://doi.org/10.1016/bs.aivir.2016.02.001>.
- Batten, C.A., Banyard, A.C., King, D.P., Henstock, M.R., Edwards, L., Sanders, A., Buczkowski, H., Oura, C.C. and Barrett, T. (2011): A real time RT-PCR assay for the specific detection of Peste des petits ruminants virus. *Journal of virological methods*, 171(2): 401-404.
- Bedore, B., Mustefa, M., Tamire, M. and Geinoro, T. (2019) : Current Status of Occurrence and Socio-Economic Impacts of Peste Des Petits Ruminants Virus (PPRV) on Small Ruminant Population in Ethiopia. *American Journal of Epidemiology and Public Health*, 3 (1): 012-016.
- Bello, A.M., Lawal, J.R., Dauda, J., Wakil, Y., Lekko, Y.M., Mshellia, E.S., Ezema, K.U., Balami, S.Y., Wazari, I., Mani, A.U. (2016): *Direct Research Journal of Agriculture and Food Science*, 4 (8): 193-198.
- Bello, M. B. (2013): Serological studies on peste des petits ruminants (ppr) in sheep, goats and camels in Sokoto state, Nigeria. MSc Thesis, Ahmadu Bello University, Faculty of Veterinary Medicine, Zaria, Nigeria.

- Beyene, A., Alilo, A. A., and Mola, M. (2018): Assessment of sheep and goat (small ruminants) production system in Esera district, of Dawro Zone, Southern Ethiopia. *J. Adv. Dairy Res*, 6(4), 215.
- Biruk, A. (2014): Epidemiology and identification of peste des petits ruminants (PPR) virus circulating in small ruminants of Eastern Amhara region bordering Afar, Ethiopia. MSc Thesis, Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of Veterinary Clinical Studies, Debre Zeit, Ethiopia.
- Candlan, E.P., Khoran, F.P. and Hana, L., (2017). Molecular identification of peste des petits ruminants virus in wild goat and domestic small ruminants by real-time-PCR technique in Erbil-Iraq. *Iraqi Journal of Veterinary Sciences*, 31(1), pp.51-54.
- Centere for Food Security and Public Health (CFSPH), (2011): Peste des Petits Ruminants. Iowa State University: CFSPH; Peste des Petits Ruminants.
- Central Statistical Agency (CSA) (2021/2022) : Agricultural Sample Survey. Volume II. Report on livestock and livestock characteristics (private peasant holdings), Statistical Bulletin, 589. Addis Ababa: Central Statistical Agency (CSA) . pp. 12-17
- Central Statistical Agency (CSA) (2014): Federal Democratic Republic of Ethiopia, Central Statistical Agency, Agricultural sample survey 2013/14 (2006 E.C).Vol II. Report on livestock and livestock characteristics (Private Peasant Holdings). Statistical Bull. PP. 573.
- CFSPH. (2011). Peste des Petits Ruminants. The Center for Food security and Public Health, Institute for International Cooperation in Animal Biologics, Ames, Iowa
- Dayhum. A., M. Sharif., Eldaghayes., A. Kammon ., P. Calistri., M. L. Danzetta., D. Di Sabatino., A. Petrini., G. Ferrari., S. Grazioli., G. Pezzoni., E. Brocchi., (2017) : Sero-prevalence and epidemiology of peste des petits ruminants in Libya Department of Preventive Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya. pp. 3-5.
- Dejene, W. (2016): Sero-epidemiology and spatial distribution of peste des petits ruminants virus antibodies in some selected pastoral areas of Somali regional

- state, Ethiopia. MSc Thesis, Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia.
- Diallo A., Minet C., Le Goff, C. Berhe, G. Albina, E. Libeau, G. and Barrett T. (2007) :the threat of peste des petits ruminants: progress in vaccine development for disease control. *25: 5591–5597.*
- Dubie T., Betelhem D., Esrael G., Wossene N., Fentaw H., and Mulatu W. (2022): Seroprevalence and associated risk factors of peste des petits ruminants among ovine and caprine in selected districts of Afar region, Ethiopia. *BMC Veterinary Research (2022) 18:429 <https://doi.org/10.1186/s12917-022-03528-6>.*
- Dundon, W.G., Diallo, A. and Cattoli, G. (2020): Peste des petits ruminants in Africa : a review of currently available molecular epidemiological data, 2020. *Archives of Virology, 165: 2147-2163. <https://doi.org/10.1007/s00705-020-04732-1>.*
- Emikpe BO, Ezeasor CK, Sabri MY, Anosa VO (2013) Clinico pathological evaluation of intranasal, subcutaneous and intramuscular routes of vaccination against intratracheal challenge of peste des petits ruminants virus in goats. *Small Ruminant Res 113:290–296.*
- ESGPIP (2009) :Estimation of weight and age of sheep and goats. *Technical Bulletin 23, Ethiopia.*
- Eyoel Ejigu , Tadele Tolosa , Feyissa Begna , Hailehizeb Tegegne , (2023): Sero-Prevalence and Associated Risk Factors of Peste Des Petits Ruminants in Dera and Gerar Jarso Districts of Oromia Region, Ethiopia. *Veterinary Medicine: Research and Reports*.pp.115-116.
- FAO (2011): Deadly animal virus peste des petits ruminants threaten to spread to Southern Africa. *Goal Geology*, viewed 16 January 2011, from [-animalviruspeste-des-petits-ruminants-threatens-to-spread-to-southernafrica;/8302/](#). 2010.
- FAO and OIE. (Food and Agricultural Organization of the United Nations/World Organization for Animal Health), (2012):2nd Global conference on Foot and mouth disease control. Bangkok, Thailand 27-29 June 2012.
- FAO.(2019) : FAOSTAT Statistical Database. Rome: Food and Agriculture Organization of the United Nations.

- Fatima A. Saeed, Sana A. Abdel-Aziz, Mohammed M. Gumaa. (2018) : Seroprevalence and Associated Risk Factors of Peste des Petits Ruminants among Sheep and Goats in Kassala State, Sudan. *Open Journal of Animal Sciences*, 8, 387-392.
- Fentahun, T. and M. Woldie, (2012) : Review on Peste Des Petits Ruminants (PPR) *European Journal of Applied Sciences* 4 (4): 160-167, ISSN 2079-2077 © IDOSI Publications, DOI: 10.5829/idosi.ejas. 2012.4.4.66151.
- Fentie, T., Teshome, Y., Ayele, B., Molla, W., Fenta, N., Nigatu, S., Assefa, A. and Leta, S. (2018): Sero-epidemiological study of peste des petits ruminants in small ruminants in Amahara region, Ethiopia. *Comparative Clinical Pathology*, 1-9. <https://doi.org/10.1007/s00580-018-2697-2>.
- Fiyinfoluwa Adeoye , Adedamilola Kolapo , Oluyemi Ogunmolawa , Adegboyega Aluko , Clement Meseko , Oluwayelu. (2022): Molecular Detection of Peste des Petits Ruminants Virus (PPRV) in Goats and Sheep in Ibadan, Oyo State, Nigeria, Pan African University Life and Earth Sciences Institute. PP 405-413.
- Gari, G., Biressaw, S., Dejene, N., Fethu, L., and Hagos, A. (2017): Serological Investigation of Peste Des Petits Ruminants in East Shewa and Arsi Zones, Oromia Region, Ethiopia. *Veterinary Medicine International*. <https://doi.org/10.1155/2017/9769071>.
- Gebre, T., Deneke, Y. and Begna, F. (2018) : Seroprevalence and Associated Risk Factors of Peste Des Petits Ruminants (PPR) in Sheep and Goats in Four Districts of Bench Maji and Kafa Zones, South West Ethiopia. *Global Veterinaria*.2018, **20** (6): 260-270. <https://doi.org/10.5829/idosi.gv.2018.260.270>.
- Gelana, M., Gebremedhin, E.Z. and Gizaw, D., (2020): Seroepidemiology of Peste des Petits ruminants in sheep and goats in the selected district of Horu Guduru Zone, Western Ethiopia. *Res. Vet. Sci.*, 132: 527–534. <https://doi.org/10.1016/j.rvsc.2020.07.019>.
- Gizaw, F., Merera, O., Zeru, F., Bedada, H., Gebru, M. and Abdi, R.D. (2018): SeroPrevalence and Socioeconomic Impacts of Peste Des Petits Ruminants in Small Ruminants of Selected Districts of Afar, Ethiopia. *Journal of Veterinary*

ScienceanTechnology,9(1):513.<https://doi.org/10.4172/2157-7579.1000513>.pp .3-8.

- Hailegebreal, G. (2018): Seroprevalence of Peste Des Petits Ruminants in Selected Districts of Siltie and Gurage Zones, South Region, Ethiopia. *Journal of Veterinary Science and Technology*, 9 (2): 529. doi:10.4172/2157-7579.1000529.
- Hodgson, S. (2018). The immune response to live , attenuated peste des petits ruminants virus vaccines. PhD Thesis, University of Surrey, Pirbright Institute, Surrey, Uk
- Husen, M., Aliyi, F., Damtew, S., Negassa, T. and Abebe, H. (2018): Prevalence of Small Ruminant Helminthiasis in and Around Tullo District in Western Harerghe Zone, Eastern Ethiopia. *Austin Journal of Veterinary Science and Animal Husbandry*.5 (1): 1-5.
- Kaukarbayevich KZ(2009): Epizootological analysis of PPR spread on African continent and in Asian countries Available online at [https:// www. researchgate. net/ publication/ African Journal of Agricultural Research](https://www.researchgate.net/publication/African%20Journal%20of%20Agricultural%20Research); 4(9):787–790.
- Khalafalla, IA., Saeed, K., Yahia, H., Ali, M., Abdurrahman BK, et al.(2010): An outbreak of peste des petits ruminants (PPR) in camels in Sudan. *Acta Trop*; 116: 161-5.
- Khalefa A. Mansour, Muthanna H. Hussain, Saad H. Al-Husseiny, Asaad J. Abid, and Qassim H. Kshash (2024).Clinical Manifestation and Phylogenetic Analysis of Peste des Petits Ruminants in Local Iraqi Breed Sheep in Al-Diwaniyah Province, *Veterinary Medicine International* Volume 2024, Article ID 5579913, 11 pages.
- Khan, H.A., M. Siddique, R. Sajjad, M. Abubakar and M. Ashraf, M. (2008):The detection of antibody againstpeste des petits ruminants virus in sheep, goats, cattle and buffaloes. *Tropical Animal Health Production*, 40: 521-527.
- Khan, H.A., Siddique, M., Abubakar, M., Arshad, M.J. and Hussain, M. (2008) : Prevalence and distribution of PPRV infection in small ruminants. *Small Ruminants Research*, **79**: 152-157.
- Kihu SM, Gachohi JM, Ndungu EK, Gitao GC, Bebora LC, John NM, Wairire GG, Maingi N, Wahome RG, Ileri R (2015) : Sero-epidemiology of Peste des Petits

- Ruminants virus infection in Turkana County, Kenya. *BMC. Vet. Res.* 11: 87-90.
<http://dx.doi.org/10.1186/s12917-015-0401-1>
- Kinne, J., R. Kreutzer, M. Kreutzer, U. Wernery and P. Wohlsein, (2010) : Peste des petits ruminants in Arabian wildlife. *Epidemiology and Infection*, 138(08): 1211-1214.
- Kwiatk O, Ali YH, Saeed IK, Khalafalla AI, Mohamed OI, Obeida AA, Abdelrahman MB, Osman HM, Taha KM, Abbas Z, El Harrak M, Lhor Y, Diallo A, Lancelot R, Albina E, Libeau G (2011) : Asian lineage of peste des petits ruminants virus, Africa. *Emerg Infect Dis* 17:1223–1231.
- Luka, P.D., Erume, J., Mwiine, F.N. and Ayebazibwe, C. (2011): Seroprevalence of Peste des petits ruminants Antibodies in Sheep and Goats after Vaccination in Karamoja, Uganda: Implication on Control. *International Journal of Animal and Veterinary Advances*, 3 (1): 18-22
- Mahapatra, M., Selvaraj, M., Parida, S., (2020) : Comparison of immunogenicity and protective efficacy of PPR live attenuated vaccines (Nigeria 75/1 and Sungri 96) administered by intranasal and subcutaneous routes. *Vaccines*, 8(2). DOI : 10.3390/vaccines8020168.
- Mahesh M. Tajpara, Nalinkant M. Shah, Bhaveshkumar B. Javia, Dilipsinh B. Barad, Umed V. Ramani, Dharmesh R. Patel, Vinay Q, and A. Kalaria.(2021). Molecular Detection and Sequencing of Peste Des Petits Ruminants Virus of Saurashtra Region of Gujarat, India pp. 18-22.
- Malik YS, Singh D, Chandrashekar KM et al (2011) : Occurrence of dual infection of peste-des-petits-ruminants and goatpox in indigenous goats of central India. *Transbound Emerg Dis* 58:268–273. doi:10.1111/j.1865-1682.2011.01201.x.
- Mantip, S., Quan, M., Shamaki, D. & Van Vuuren, M., (2016) : Comparison of nucleotide sequences of recent and previous lineages of peste-des-petits-ruminant viruses of sheep and goats in Nigeria’, *Onderstepoort Journal of Veterinary Research* 83(1)1163<https://doi.org/10.4102/ojvr.v83i1.1163>.
- Milkessa Gelana (2023) : Seroprevalence, Risk Factors, Detection and Isolation of Peste Des Petits Ruminants Virus from Sheep Oromia Agricultural Research

- Institute, Bako Agricultural Research Center, Animal Health Researcher, Bako, Ethiopia .pp.4-6.
- Munir, M., S. Zohari and M. Berg, (2013) : Epidemiology and distribution of peste des petits ruminants. In *Molecular Biology and Pathogenesis of Peste des Petits Ruminants Virus*, 69-104. Springer Berlin Heidelberg.
- Munir, M., Siddique, M. and Ali, Q. (2009) : Comparative efficacy of standard AGID and precipitinogen inhibition test with monoclonal antibodies based competitive ELISA for the serology of Peste des Petits Ruminants in sheep and goats. *Tropical Animal Health and Production* 41, 413–420.
- Narayan Acharya., Shankar Prasad Poudel, Krishna Prasad Acharya (2018) : Cross-sectional sero-prevalence study of Peste des Petits Ruminants (PPR) in goats of Syangja and Kaski districts of Nepal. Pp. 176-179.
- Njeumi, F., Bailey, D., Soula, J.J., Diop, B., Tekola, B.G., (2020) : Eradicating the scourge of peste des petits ruminants from the world. *Viruses*, 12(3). DOI: 10.3390/v12030313.
- Njue S, Saeed K, Maloo S, Muchai J, Biao C, Tetu K.(2018): Sero-prevalence study to determine the effectiveness of Peste de Petits Ruminants vaccination in Somalia. *Pastoralism: Research, Policy and Practice*. 8:1.
- Nkamwesiga, J., Lumu, P., Nalumenya, D.P., Korennoy, F., Roesel, K., Wieland, B., Perez, A., Kiara, H. and Muhanguzi, D., (2023). Seroprevalence and risk factors of Peste des petits ruminants in different production systems in Uganda. *Preventive veterinary medicine*, 221, p.106051.
- Nyamweya, M., Otunga, T., Regassa, G. & Maloo, S., (2009) : *Technical brief of Peste Des Petits Ruminants virus*, ELMT Livestock Services Technical Working Group, Food and Agricultural Organisation (FAO), Addis Ababa.
- OIE (2020): OIE technical disease cards. Updated January 2020. Accessed on December 20,2021.https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/PESTE_DES_PETITS_RUMINANTS.pdf.

- OIE (Office international des Epizooties), (2013) :Version adopted by the World Assembly of Delegates of the OIE Terrestrial Manual 2013, Chapter 2.7.11, Peste des petits ruminants.
- OIE Terrestrial Manual (2022): Peste des petits ruminants (infection with small ruminant morbillivirus) Chapter 3.8.9.pp.6-9.
- OIE. (2016): Infection with peste des petits ruminants virus. Terrestrial Animal Health Code.Of Int Epizooty. 2016:1;1–12.
- OIE. (2019): Peste des petits ruminants (infection with peste des petits ruminants virus). OIE Terrestrial manual,World Organization for Animal Health, pp 1-16.
https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.07.09_PPR.pdf
 .
- Pragati Bhojar, Prabhakar Tembhurne*, Ruchi Bhate, Prashant Tarale, Anuradha Nemade Anand Kadam, Jivan Kesharkar, and V.C. Ingle. (2019) Molecular Detection of Peste des Petits Ruminants Virus (PPRV) by RT-PCR. International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 8 Number 11 PP 2594-2601.
- Rony, M.S., Rahman, A., Alam, M.S., Dhand, N.K. and Ward, M.D., (2017): Peste des Petits Ruminants risk factors and space-time clusters in Mymensingh, Bangladesh. Transb. Emerg. Dis., 64: 2042–2048. <https://doi.org/10.1111/tbed.12615>.
- Rudra, P.G. (2019): Prevalence and molecular characterization of Peste Des Petits Ruminants (PPR) in Goat Pran Gopal Rudra. MSc Thesis, Chattogram Veterinary and Animal Sciences University, Department of Medicine and Surgery, Chittagong, Bangladesh.
- Ruget, A.S., Tran, A., Waret-Szkuta, A., Moutroifi, Y.O., Charafouddine, O., Cardinale, E. and Cetre-Sossah, Catherine Chevalier, V. (2019): Spatial Multicriteria Evaluation for Mapping the Risk of Occurrence of Peste des Petits Ruminants in Eastern Africa and the Union of the. Frontiers in Veterinary Science, 6: 455. doi: 10.3389/fvets.2019.00455.

- Sakhare, P. S. (2019): Detection, isolation and molecular characterization of peste des petites ruminants virus in small ruminants of south Gujarat. Ph.D. Thesis, Navsari Agricultural University, Navsari. India. pp. 1554-1556.
- Santhamani, R., Singh, R.P. and Njeumi, F. (2016). Peste des petits ruminants diagnosis and diagnostic tools at a glance: perspectives on global control and eradication. *Archives of Virology*, 161(11): 2953-2967. <https://doi.org/10.1007/s00705-016-3009-2>.
- Saritha G, Shobhamani B, Rajak KK, Sreedevi B (2015). Sophycate Njue1 , Khalid Saeed1 , Seiffuddin Maloo , Joan Muchai , Cyprien Biauou and Kennedy Tetu., (2018): Sero-prevalence study to determine the effectiveness of Peste de Petits Ruminants vaccination in Somalia pp 5-11.
- Saritha G, Shobhamani B, Rajak KK, Sreedevi B (2015): Detection and confirmation of PPR virus antigen in sheep and goats by sandwich-ELISA and RT-PCR in Andhra Pradesh, India. *J. Adv. Vet. Anim. Res.* 2(2): 210-212.
- Sarker S. and Islam H. Md. (2011): Prevalence and Risk Factor Assessment of Peste des petits ruminants in Goats in Rajshahi, Bangladesh. *Vet. World*, 4(12):546-549. 67.
- Seifudin Kassim Ahmed. (2020): Review on Peste Des Petits Ruminants (Ppr) and its Economic Importance. *International Journal of Research Studies in Biosciences (IJRSB)* Volume 8, Issue 8, 2020, PP 17-31.
- Shahriari, R., Khodakaram-tafti, A. and Mohammadi, A. (2019): Molecular characterization of Peste des Petits ruminants virus isolated from four outbreaks occurred in southern Iran. *BMC Veterinary Research*, **15**: 177. <https://doi.org/10.1186/s12917-019-1920-y>.
- Tarekegn D. (2021): Contribution of small ruminant production in food and economic security: A Review. *Journal by Innovative Scientific Information & Services Network*. **12**(1):28-33.
- Thakor RB, Patel MD, Patel RM, Kalyani IH. (2016): Seroprevalence of peste des petits ruminants in goats of south Gujarat. *Indian J small ruminants*. 22(2): 252-254.

- Thrusfield, M (2007): Sampling in Veterinary Epidemiology.3rd ed., Black well Science Ltd, London, pp. 214-256.
- Vandna Bhanot, Aman Kumar and Naresh Jindal (2020): Disease Investigation Laboratory, *Journal of Current Microbiology and Applied Sciences*, 9(9).
- WAHID.(2020): World animal health information data base. Disease information PPR disease.http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail (accessed on October, 2020).
- Weldemariam. L.F., Patrick S., Ayansina A. (2023): Household food-security strategies and migration in Tigray, Northern Ethiopia. *Scientific African***21**. <<https://doi.org/10.1016/j.sciaf.2023.e01801>>.
- Wondimagegne D. (2016): Seroepidemiology and Spatial Distribution of Peste des Petits Ruminants Virus antibodies in Some Selected pastoral Areas of Somali Regional State, Ethiopia [MSc thesis], Addis Ababa University, College of Veterinary Medicine and Agriculture. Ethiopia: Bishoftu.
- Yalew Abiyu Senbeto. (2022): Epidemiology of Peste Des Petits Ruminants, Isolation and Molecular Detection of the Virus in Selected Districts of Awi and Metekel Zones, North West Ethiopia.
- Yalew, S., Woldemichal, G. and Mamo, M. (2019): Seroprevalence of Peste Des Petits Ruminants Virus Antibody in Assosa Zone, Benishangul Gumuz Region, Ethiopia. *ARC Journal of Animal and Veterinary Sciences*, 5 (3): 29-33.
- Zhao, H., Njeumi, F., Parida, S., Benfield, C.T.O., (2021): Progress towards eradication of peste des petits ruminants through vaccination. *Viruses*, 13(1). DOI:10.3390/v13010059.

8. ANNEXES

Annex 54:: Sample collection format

Zone: _____ District: _____ Kebele: _____ village _____

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

No	Date	Flock size		Species	Sex	Age	Body condition	Vaccination states	Sample type
		Sheep	Goat						

* flock size (1= >20 animals, 2= 10-20 animals, 3= <10 animals) * Species (1=sheep, 2= goats) * sex male= 1, Female= 2 * Age (young <1 year, adult 1-3 year, old > 3 year). * Body condition (1=poor, 2= Medium, 3=Good) *Vaccination status (1= vaccinated, 2= Non-vaccinated)

Annex 54: 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 55: Body condition scoring method of sheep and goats

Condition	Score	Description
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.

Annex 55 55: Test method for neutralization (vn) of Pest des Petits ruminants (PPR) virus.

PRINCIPLE

To detect the presence of serum specific antibodies against PPR virus by *in vitro* neutralization of the viral cytopathic effect on cell culture. This method is based on a reaction between the virus and specific antibody in the test serum. Virus and products containing a neutralizing antibody are mixed under appropriate conditions and then inoculated into cell culture. The presence of un-neutralized virus is detected by plaque formation/cytopathic effect/. A loss of infectivity is caused by interference by the

bound antibody with any of the steps leading to the release of the viral genome from the host cells including attachment, infection, or viral release.

1. Materials

graduated pipettes of 1ml, 2ml,5ml and 10ml, Pipetter , tips of 100µl,200µl and1000µl, multichannel 50-300 µl and single channel 5-50,Sterile tubes,96 flat bottom wells sterile plate (micro plates tissue culture grade),Troughs for reagent distribution, Water bath set at 56°C, Incubator 37°C with 5% CO₂ atmosphere, Bio Safety cabinets , Inverted microscope , Refrigerator and freezer, Vortex mixer, Centrifuge and Pipette cylinder containing water and disinfectant.

2. Reagents

VERO Cells, Titrated PPR virus suspension in media without FCS, Cell culture medium (MEM) ,L Glutamine, Foetal Calf Serum, Antibiotic-antimycotics, Test sera ,Ethanol 70% and Other disinfectant (for example Bleach or virkon's).

Procedure

a) Working sheet

Take the record sheet and fill in the layout of the 96-plate according to the samples to be tested/

Each sample must be recorded individually, PPR virus stock used, the sera dilution (inverse log) and all information required.

B) Preparation of the medium used in the plate

Prepare a sufficient volume of medium: MEM + 2% antibiotic/antimycotic + 1% L-Glutamine of 200 mm with and without serum.





C) Preparation of the serum to be tested

Thaw and de complement the test sera, stored at -20, by heating at 56 °C for 30 minutes in a water bath.

d) Dilution of the virus

- Remove the viral suspension from -80°C just before use.

The virus stock, with a known titer, should be diluted to give a 10^3 TCID₅₀ /ml. Use MEM without serum but with 2% antibiotics & antimycotics and 1% glutamine. 100 µl (10^2 TCID₅₀) of this viral dilution will be added to each well, therefore prepare enough volume according to the number of plate you want to do (you need 10 ml for one plate).

- For the control plate, take four sterile tubes labeled (1,2,3 and 4) and put 1.5 ml of the 1000TCID₅₀/ml virus dilution in the tube (1) and add 1.350 ml of MEM without serum in the other three tubes (2,3 & 4) and prepare serial dilutions (1:10) of the virus at 10^3 TCID₅₀/ml (doing a serial dilution of the 100 TCID₅₀ virus suspension (150 µl in 1350 µl of medium, thus giving tubes containing:
 - 1.5 ml of virus 1000 TCID₅₀/ml  = 100 TCID₅₀/100µl
 - 1.350 ml of virus 100 TCID₅₀/ml  = 10 TCID₅₀/100µl
 - 1.350 ml of virus 10 TCID₅₀/ml  = 1 TCID₅₀/100µl
 - 1.350 ml of virus 1 TCID₅₀/ml  = 0.1 TCID₅₀/100µl

e) Preparation of the Vero cells suspension

- Vero cells are trypsinized. After counting the viable cells, they are diluted to make a suspension of 4×10^5 cell/ml in MEM + 10% CSF + 2% antibiotic/antimycotic + 1% L Glutamine (You need 5 ml for one plate).

f) Addition in the test plate

Test plate:

Take a 96 well tissue culture plate. One plate allows testing **8 sera** in duplicate in serial dilutions (1:5)

Addition of medium: 100 µl of medium without serum is distributed in all wells.

Addition of samples: Add in wells A1and B1 25 µl of a serum 1 to be tested.

Add in wells C1and D1 25 µl of a serum 1 to be tested.

Add in wells E1and F1 25 µl of a serum 1 to be tested.

Add in wells G1and H1 25 µl of a serum 1 to be tested.

Repeat the same operation with sera in wells of column 7.

With the multichannel pipette set at 25 µl, perform 5 fold serial dilutions (from column1-6, and Column7-12) with initial dilution 1/5 and discard 25 µl of suspension from the end point dilution 1/15625 i.e. from column 6 and 12.

- Addition of virus: Add 100µl of 1000 TCID₅₀/ml viral suspension to each wells.

Control plate: The plate should contain positive and negative controls and prepare separately (Layout 2).

-Negative control: 6 wells in the last row are without virus.

-Positive control: 6 wells in each 4 rows are filled with different dilutions of

PPR

Viruses (100TCID₅₀, 10TCID₅₀, 1TCID₅₀ and 0.1TCID₅₀ in 100 µl suspension) then incubate in 37°C for 1 hour.

- Addition of cells: After 1 hour add in each well of the plate 50 µl of Vero cells suspension of (4x10⁵ cell/ml). Then the plates are incubated at 37°C with 5% CO₂.
- Microscope reading: Plate reading to monitor for CPE formation at 8,10 and 14 days
- Interpretation of the result and Calculation of the serum sample titer: CPE is observed when the serum is negative (the virus is not neutralized), in the contrary, no CPE will be observed if there is an antibody in the serum against PPRV.

· The test result is valid only if CPE does not occur in the Negative control wells and

· For the positive controls the CPE formation should be according to the virus titer in each four rows and expected standard protocol would be (++++, +++, +- +-, --- -).

100TCID₅₀/100µl: + + + + +

10TCID₅₀/100µl: + + + + +

1TCID₅₀/100µl: + - - - +

0.1TCID₅₀/100µl: - - - - -

Calculation is done automatically in the work sheet. On the two dilutions series made for a serum sample, the titer can only be calculated if the difference between the two negative results (no CPE) and the two positive results(CPE) is not more than two dilutions. The different acceptable possibilities are shown in the table:

Dilutions	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1/10	-	-	-	-	-	-	-	-	-	-	-	-
1/20	-	-	-	-	-	-	+	-	-	+	-	-
1/40	+	+	-	+	+	-	-	-	+	-	+	+
1/80	+	+	+	+	+	-	+	+	+	+	-	-
1/160	+	+	+	+	+	+	+	+	+	+	+	+

The titer of the sample is equal to the inverse of the first dilution where the two wells are positives plus inverse of the last dilution where the two wells are negative, all being divided by two.

$$T = \frac{\left(\frac{1}{\text{first dilution with two wells positives}} + \frac{1}{\text{last dilution with two wells negatives}} \right)}{2}$$

Therefore, in the above example, the titer of sample 1 and 2 are respectively 30 and 50.

Example of moving positive wells:

Dilutions	Sample 3		Sample 4		Sample 5		Sample 6	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1/10	-	-	-	-	-	-	-	-
1/20	-	-	+	-	-	+	-	-
1/40	+	-	-	+	+	-	+	+
1/80	+	-	+	+	+	+	-	-
1/160	+	+	+	+	+	+	+	+

If the progression of CPE is not regular in the two series of dilution, (jump of well as in the case of samples 3 to 6 where CPE's do not match with dilutions), the calculation of the titer is done as before, after having moved the positivity of the well to the dilution just above (higher dilution) Therefore, the titer of sera 3, 4, 5 and 6 are respectively 60, 50, 30 and 60.

Plat layout 1: Test plate layout in duplicate for 8 test sera.

1/5 1/5 1/25 1/125 1/625 1/3125 1/5 1/5 1/25 1/125
 1/625 1/3125

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Layout 2: Control plate layout for the positive and negative controls.

	1	2	3	4	5	6	7	8	9	10	11	12	
A													
B													100 TCID50 / 100 µl
C													10 TCID50 / 100 µl
D													1 TCID50 / 100 µl
E													0.1 TCID50 / 100 µl
F													
G													No virus, only cells
H													

Annex 61: sample collection and processing



A. Briefing the owners about PPR



B. Age determination by dentation

C. Blood collection at field



D. Nasal swab collection

E. Rectal swab collection



F. Ocular swab collection households

G. participation of female households



H. Collected Blood by vacutainer tube collection



I. Centrifuged the sample for serum collection



J. collected serum



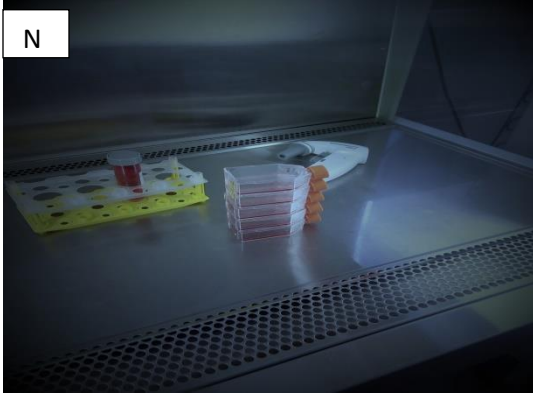
L. stored the serum at -80°C



L. media preparation



M. Subculture at biosafety level II



N. Vero cells used for culture

Several clinical indications of PPR were observed in sheep and goats in the study districts during the field observation. Nonetheless, the clinical condition was more prevalent in goats than in sheep. Clinical indicators that have been noted include fever, depression, matting of the eyelids, profuse serous nasal and ocular discharges, emaciation, coughing, difficult breathing, and ulcers on the gums. erosive stomatitis, diarrhea and conjunctival reddening.

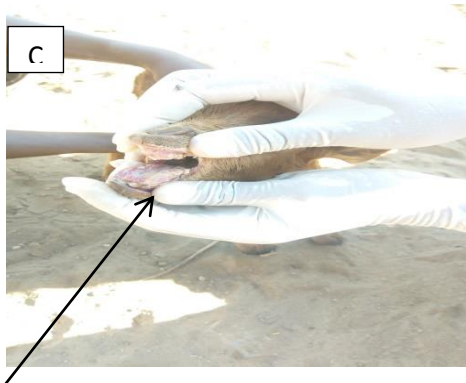
Annex 64 : Clinical signs shown by PPRV infected animals.



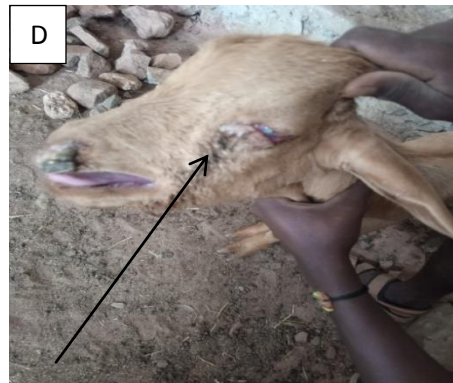
A. Nasal discharge
faeces)



B. Diarrhea (The hindquarters are soiled with liquid
faeces)



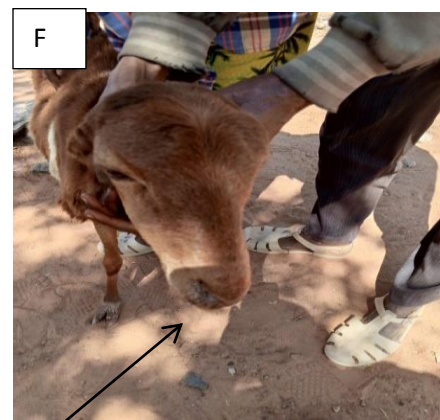
C. Ulcers inside the Mouth
the conjunctiva.



D. Ocular discharge and reddening of
the conjunctiva.



E. Bilateral ocular and nasal discharge
sheep



F. necrosis around the mouth in
sheep

Annex 65: Ethical clearance letter



To: Tadele Gebreslassie, Principal Investigator (PI)
Mekelle

Subject: Notification of AEEC decision on your research proposal

AEEC No: AEEC 27/2024

Protocol (Title of the study): Sero prevalence, molecular detection and associated risk factors of PPR in selected districts of Southeastern and Eastern zones of Tigray Region

Dear PI,

The aforementioned research proposal which was submitted by you for animal ethics and use clearance letter has been reviewed by the Animal Ethics and Experimentation Committee (AEEC).

The AEEC has discussed and examined the research proposal in detail from animal ethics and use principles and values perspective.

Finally, the AEEC has (**approved**, approved with modification or withhold approval) your research proposal.

This animal ethics clearance letter is valid for only one year (23/09/2024 – 23/09/2025).

Furthermore, any other correspondence and inquiries concerning your research proposal with committee must include the AEEC No., the name of the PI and the proposal title.

Best Regards,

AEEC Chairperson

Name: Nigus Abebe (PhD)

Signature: _____

Date: 23/09/2024

AEEC Secretary

Name: Enquebahe Kassaye (PhD)

Signature: _____

Date: 23/09/2024



Tel No. +251-3-44-40-16-10

P. O. Box 2084, Mekelle, Tigray, Ethiopia