ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

MSC THESIS

STUDY OF PESTE DES PETITS RUMINANTS (PPR) OUTBREAKS: ISOLATION, MOLECULAR DETECTION, AND SEROLOGICAL IDENTIFICATION IN SMALL RUMINANTS OF BORANA PASTORAL AREA, ETHIOPIA

DEPARTMENT OF VETERINARY MICROBIOLOGY, IMMUNOLOGY AND VETERINARY PUBLIC HEALTH

BY

TOLERA FUFA FEYISSA

JUNE, 2024

BISHOFTU, ETHIOPIA

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STUDY OF PESTE DES PETITS RUMINANTS (PPR) OUTBREAKS: ISOLATION, MOLECULAR DETECTION, AND SEROLOGICAL IDENTIFICATION IN SMALL RUMINANTS OF BORANA PASTORAL AREA, ETHIOPIA

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

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As MSc research advisor, I certify that I have read and evaluated the thesis prepared under my guidance by: **Tolera Fufa Feyissa**: Entitled "**Study of Peste des Petits Ruminants (RRP) Outbreaks: Isolation, Molecular detection, and Serological identification in Small ruminants of Borana pastoral area, Ethiopia"** and recommended that it be accepted as fulfilling the thesis requirement for the degree of: **Masters of Science in Veterinary Microbiology**.

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: **Tolera Fufa Feyissa** Entitled "**Study of Peste des Petits Ruminants (RRP) Outbreaks: Isolation, Molecular detection, and Serological identification in Small ruminants of Borana pastoral area, Ethiopia"** and recommend that it is accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Veterinary Microbiology.

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First, I certify that this thesis is my original work and that all resources utilized in its preparation have been properly referenced. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture, and is placed at the university/college Library to be made accessible to borrowers under the rule of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgment of the sources made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the materials in the interest of scholarship. In all other instances, however, the author must obtain permission.

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TABLE OF CONTENTS **PAGES**

TABLE OF CONTENTS (Continued)

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LIST OF ABBREVATIONS

AGID	Agar gel immunodiffusion
APCs	Antigen presentation cells
B-ELISA	Blocking-ELISA
C-ELISA	Competition ELISA
CIEP	Counter immune electrophoresis
CPE	Cytopathic effect
CSA	Central statistical agency
DIVA	Differentiation of infected from vaccinated animals
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra-acetic acid
FAT	Fluorescence antibody test
HN	Hemaglutination-neuraminidase
ICE	Immunecapture ELISA
IFAT	Immunofluorescent antibody test
MAbs	Monoclonal antibody
PCR	Polymerase chain reaction
PPR	Peste des petits ruminants
PPRV	Peste des petits ruminants virus
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
RPV	Rinderpest virus
RT-PCR	Reverse transcription polymerase chain reaction
$TCID_{50}$	Tissue culture infectious dose
SLAM	Signaling lymphocytic activation molecule
SRMV	Small ruminant morbillivirus
VNT	Virus neutralization test
VTM	Virus transport medium

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIXES

ABSTRACT

PPR is a severe, highly transmissible transboundary virus disease that primarily affects pastoral areas by seriously compromising the health of both domestic animals and wild herbivores. An outbreak investigation was carried out from October 2023 to January 2024 in Borana and East Borana zones to figure out the PPR status in sheep and goats by isolation, molecular detection and assessing the antibodies level amongst small ruminants by purposively collecting serum(n=102) and swabs (n=48) samples from the flocks exhibiting active clinical symptoms resembling PPR.. From 48 swabs and 102 serum, 26(54.2%) and 70(68.6%) was positive for RT-PCR and b-ELISA respectively. All positive samples identified by RT-PCR underwent further processing for virus isolation by infecting Vero Dog SLAM (VDS) cells. Among the 26 samples cultured, 17 (65.4%) displayed typical cytopathic effects. To measure the agreement of the two diagnostic techniques that performed on the common animals was analyzed by Kappa statistics (κ) which revealed an agreement between the two tests was 40.7% with kappa value -0.16, it indicating that no agreement between the two tests (RT-PCR and b-ELISA). Goats were the only species suffering with clinical symptoms but no pathological evidence of PPR reported in sheep. Serologically, female animals (72.7%) were highly tested positive than male (61.1%) and the highest percentage was found in old animals followed by adults, young was the least. The sex-wise percentage of RT-PCR value for PPRV was higher in male than females. Unauthorized animal movement, flock size, rearing practices and management system, communal grazing, mixing of unknown origin, lack of quarantine practices are the main factors identified for the continuous emerging of PPRV in study area. The persistent clinical manifestations, high anti-PRV antibody levels, successful virus isolation, and precise nucleic acid detection by RT-qPCR all suggest PPRV as the primary cause of the continuous outbreak in the study area. We recommended controlling animal movement, quarantine newly purchased animals, isolating the symptomatic animals from a flock and effective mass vaccination against the disease among small ruminants.

Key words: b-ELISA; Borana; Cell culture; Outbreak; RT-PCR; PPR; sheep and goat.

1. INTRODUCTION

Ethiopia has the greatest population of livestock in Africa with 51 million goats, 65 million cattle, 40 million sheep, 8 million camels, and 49 million chickens in 2020 (CSA, 2020). Ethiopian farmers prefer breeding sheep and goat due to their affordable costs of production, prolificacy, and ability to adapt to the environment by adaptive feeding habits, quick reproduction cycle, and rapid growth rate. One of the most important measures of the effectiveness of sheep and goat production is the extent to which the animals reach marketable age (Hailegebreal, 2019).

Because they play a vital role in ensuring household food security, small ruminants are preferred by pastoralists. Next to camels, small ruminants are the most significant animal able to endure the harsh climate conditions of arid and semi-arid regions. About 23-39% of farm cash income is generated by small ruminants, which are an essential component of the livelihood of the vast majority of Ethiopia's rural population (Tolera and Abebe, 2007).

Borana is Ethiopian lowland area that mostly vulnerable to climate change. Raising livestock is the main source of income for Borana pastoralists and it is adversely affected by climate change and unpredictability. These climate-related factors, together with other stresses like resource issues, marginalization, inadequate infrastructure, and land degradation, have reduced the resilience and coping skills of pastoralist communities (Gatew and Guyo, 2024).

Owing to their high rate of reproduction, they frequently serve as quick cash income generators as well as providing supplies of meat and milk for the household. However, a number of challenges, among them, animal diseases are frequently impair the productivity of sheep and goats. PPR is extensively found throughout several agro-ecological zones and production systems, contributing significantly to rural households' means of subsistence and the country's earnings from exports (Tolera and Abebe, 2007; Legesse *et al*., 2010).

The *peste des petits ruminants* is an acute viral disease, a highly transmissible that seriously impairs the health of domestic animals and wild herbivores. It is highly contagious and primarily affects small ruminants, however, in camels, pigs, and cattle, it can also result in subclinical signs (Amarasinghe *et al*., 2018). PPR was initially discovered in Côte d'Ivoire (West Africa) in 1942. Then it has expanded throughout much of Africa since the late of 1990s, from North Africa to Tanzania, and the Middle East. It is also prevalent across central Asia to South and East Asia. It is a threat to more than 80% of the world's small ruminant population, and has spread to over 65 countries in Africa, Asia, the Middle East, and Europe during the last 20 years (Banyard *et al*., 2010).

It is a known transboundary viral disease that mostly affects sheep, goats and dromedaries. Despite having a very efficient vaccine, PPR is still spreading geographically. An additional risk to the transmission of disease to countries free of the PPR is the unauthorized importation and export of live animals. Ethiopian rural communities that rely heavily on sheep and goats for livelihood perceive significant economic effects from the PPR disease (Parida *et al*., 2016). In a naive community, PPR morbidity and mortality rates can approach 90-100%. The introduction of PPRV in previously uninfected regions and the mixing of lineages in endemically suffering nations highlight the constantly changing and transboundary nature of this disease (Banyard *et al*., 2010; Adombi *et al*., 2017).

PPR had been believed to be restricted to West Africa, but later research from the Middle East and Asia, as well as from south of the Sahara to north of the Equator, throughout Africa (apart from a few southern African countries), was discovered. Currently, the World Organization and the Food and Agriculture Organization of the United Nations (FAO) for Animal Health (OIE) have set a target global eradication timeframe of 2030 for PPR due to its financial impact in many developing nations. This time line symbolizes the successful elimination of the rinderpest virus (Banyard *et al*., 2010; Parida *et al*., 2015; Agga *et al*., 2019).

1.2. Statement of problems

Millions of people throughout the Horn and East African countries rely on pastoralism as their primary source of income. According to estimates, pastoralists make about 10-12% of the population in Ethiopia (Galgalo, 2015). Small ruminants like sheep and goats are commonly raised by pastoral and agro-pastoral communities. However, livestock face numerous health challenges that peste des petits ruminants is the primarily significant constraint on production. PPR has an effect on Ethiopia's small ruminant production as well as health, particularly in pastoral areas.

The southern Ethiopian region, especially Borena and East Borana zone was a pastoral area where an enormous population of sheep and goats were raised. PPR outbreaks have been documented frequently in southern Ethiopia's pastoral regions, primarily in both Borena and East Borana zone. Since the first reported cases of PPR in Ethiopia, the disease has worsened food insecurity by affecting small ruminant productivity, particularly in the most vulnerable regions of the nation. Because few research have been done in pastoral and agro-pastoral area, little has been discovered regarding the current status of PPR disease. This study hypothesizes that the peste des petits ruminants is a highly circulating viral disease of sheep and goat in the pastoral community. Therefore; the general objective of this study were to isolate, molecularly detect and serologically identify Peste des petits ruminants in small ruminants during an outbreak in Borana and East Borana zone, pastoral area of Ethiopia.

Specific objectives:

- To isolate and detect PPR in small ruminants during outbreaks in Borana and East Borana zone.
- To assess the immunity of sheep and goat against PPR virus during outbreaks in Borana and East Borana zone.
- To assess the factors that contributing to recurrent occurrences of PPR in in study area.

2. LITERATURE REVIEW

2.1. Ethology and Structure of PPR

PPR is caused by Peste des petits ruminants virus (PPRV), it is a member of the genus M*orbilivirus,* family *Paramyxoviridae*, together with *canine distemper virus (CDV), measles virus (MV), rinderpest virus (RPV)* and *Phocine distemper virus (PDV).* PPR is enveloped and it contains close antigens with the rinderpest virus, which affects large ruminants and that makes it distinct. The two main glycoproteins on its surface are the fusion proteins and haemagglutinin-neuraminidase PPRV contains a genome made up of 15,948 nucleotides of arranged in a special negative single-strand RNA molecule that is enveloped. Eight transcriptional units, designated 3′-N-P/C/V-M-F-H-L-5′, make up the viral genome. Intergenic regions divide each gene (Abd El-Hakim, 2006; Alemu *et al*., 2019).

Six structural proteins are present in the genome (Fig. 1). The six structural proteins that it encodes and that provide protection against the disease in infected are the major nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the large protein (L), and the two external fusion (F) and hemagglutinin (H) glycoproteins. The ribonucleoprotein (RNP), which is the minimally necessary structure for viral replication in cells, is generated by the viral genomic RNA and the N, P, and L proteins. There are additionally two more nonstructural proteins, C and V (Alemu *et al*., 2019).

It was recently renamed Small Ruminant Morbillivirus (SRMV). SRMV present as a single serotype. The fusion protein (F) gene and the nucleoprotein (N) gene's C-terminus partial sequences can be used to distinguish the four genetically separate lineages I, II, III, and IV (Banyard *et al*., 2010; Amarasinghe *et al*., 2018). Lineages I-II have historically been found primarily in West Africa, while lineage III has used to be found in the Middle East and East Africa, Following its most likely West African origin, lineage IV moved eastward and became the main lineage throughout Asia until resurfacing in Africa, where it appears to be the dominant lineage once again. In accordance with phylo-geographical study, the

most recent common ancestor of PPRV lineages I, II, and III is believed to have emerged in Africa, while lineage IV may have originated in India (Banyard *et al*., 2010; Muniraju *et al*., 2014; Mahapatra *et al*., 2015).

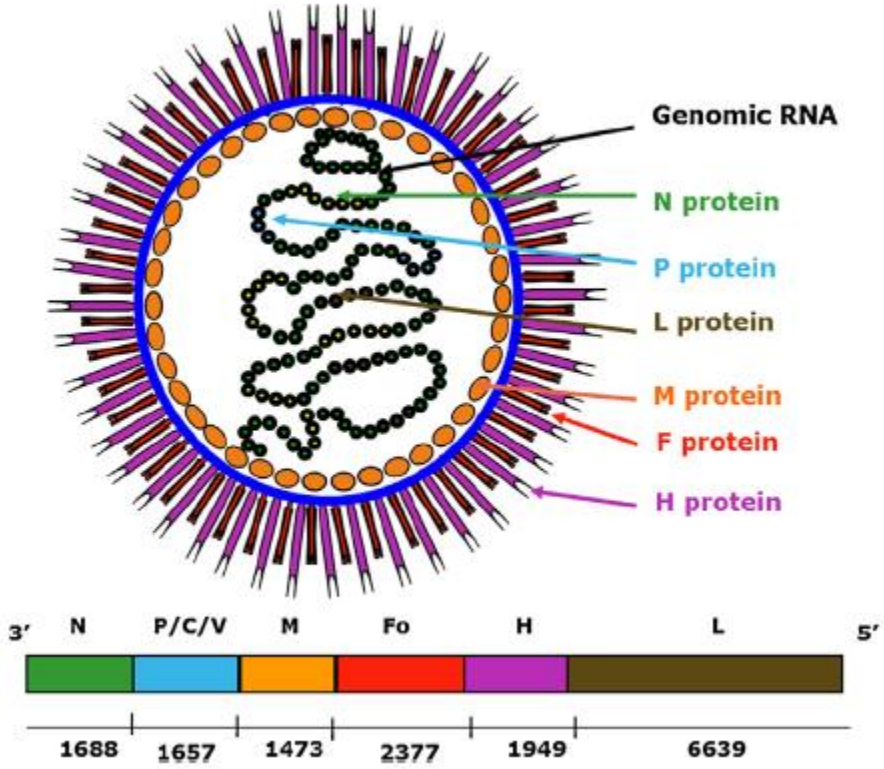


Figure 1: Schematic representation of the PPR morbillivirus

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

2.2. Host range of PPR virus

2.2.1. Types of domestic animals affected by PPR virus

Although both sheep and goats are primarily impacted by PPR, compared to sheep, goats are typically more seriously and frequently affected sheep (Ozkul *et al*., 2002; Singh *et al*., 2004a). However, following an outbreak, varying seroprevalence has been reported in sheep and goats. A variety of factors, particularly host species and breed, strain virulence, host density, and management techniques, could all contribute to these variations (Couacy-

Hymann *et al*., 2007). In natural conditions, cattle generate antibodies and to experimental inoculations mainly undergo subclinical reactions. Despite establishing an immunological response that indicating cattle are likely dead-end hosts, presently, research studies have not demonstrated any evidence of viral excretion of in-contact susceptible cattle (Schulz *et al*., 2019).

It is well known that PPRV infection typically occurs in sheep and goats at a younger age, and that they maintain seropositivity for 1-2 years after exposure. According to some reports, the virus is more specific to goat species than sheep species, and it may contribute to more fatalities in the goat population, especially among young goats, as compared to sensitive sheep. Geographical locations and ecological systems exhibit considerable differences in the disease's epidemiological pattern (Singh *et al*., 2004a).

Large ruminants in Asia and Africa have been reported to have a confirmed PPRV infection with sero-prevalence.The vast majority of these research investigations have been pertaining to cattle and camels. PPRV infections in cattle and camels are caused by sheep and goats cohabiting or living together. Asia and Africa are prominent regions for reports of clinical PPRV infection and seroconversion in camels (Zakian *et al*., 2016; Rahman *et al*., 2020).

There was also evidence of infection in other ruminants, such as cattle and buffaloes, which become subclinical infected but seroconvert (Schulz *et al*., 2018; Dundon *et al*., 2020). Cattle and camels have been shown to possess PPRV antigen, and there have been documented cases of PPRV transmit from infected goats to cattle (Lembo *et al*., 2013). PPRV has been identified in infected camels manifesting active clinical sign to PPR and it has been experimentally shown to spread to sheep and goats; nevertheless, these animals might be the virus's final host (Schulz *et al*., 2019; Saeed *et al*., 2022).

Woma *et al*. (2015) states that, different countries have reported PPRV-positive serology in camels, along with a high frequency of PPRV antibodies. And multiple investigations conducted in Ethiopia and Nigeria have also provided evidence of PPRV antigen in camels

and support the hypothesis that PPRV infection is a possibility in camels. A 2001 serological survey conducted in Ethiopia on inexperienced camels and other ruminants revealed that 3% of camels possess PPRV antibody. In addition to the finding of antibodies in camels, which suggests that camels may be susceptible to PPRV in natural environments, a PPR epidemic in camels and death was reported in Sudan in 2004 (Khalafalla *et al*., 2010). Although camelids in recent PPRV experimental infection did not show signs of clinical disease or PPRV shedding, a number of reports indicate that dromedaries are vulnerable to PPRV infection and can express disease clinically, as seen in Iran, Ethiopia, and Sudan (Zakian *et al*., 2016; Schulz *et al*., 2018).

The first PPRV outbreak in camels to be recorded was in 1996. But recently, a deadly respiratory disease associated with PPRV has claimed in camels from Sudan. It was established consisting of highly transmissible respiratory syndromes that had low fatality rates but large rates of sickness. It has been established that a lineage III PPRV is the causal agent. From the previous reports, camels and cattle was seroconverted for PPR. Cattle also susceptible for PPR with or without showing clinical signs (Abubakar *et al*. 2011a; Balamurugan *et al*. (2012).

2.2.2. Wild animal status in relation to the PPR virus

Since pigs are experimentally infected with PPRV lineage II and exhibit typical PPR clinical signs, they are regarded as the virus's dead-end host.
A new study regarding a virulent PPRV lineage type IV infection in domestic and wild boar revealed that PPRV might spread from goats to pigs and vice versa, implying that pigs could represent a potential source of PPRV infection (Schulz *et al*., 2018). While pigs experimentally develop viremia and can spread the virus to other individuals, natural illness in pigs remains undocumented (Schulz *et al*., 2019).

It remains unclear whether or not naturally occurring PPRV disease occurs in animals due to a lack of evidence regarding the clinical, pathological, epidemiological, and laboratory features that match with PPR investigations. Considering the vast numbers of PPRV-

susceptible wild species in Africa, this is unusual and requires more research (Asil *et al*., 2019).In the recent studies on PPRV's atypical hosts, warthogs, wild boar, and domestic pigs have been identified as capable of serving as the virus's hosts, capable of sustaining enough viral replication and shedding to allow PPRV dissemination (Schulz *et al*., 2018). Lions and elephants in wildlife from Asia and Africa, respectively, are a few instances of PPRV infection (Fernandez *et al*., 2020). There was seroprevalence finding in water buffalo and yaks (Rahman *et al*., 2020).

Reports from Sudan, Pakistan, Turkey, Ethiopia, Kazakhstan, Algeria, and West African nations on the prevalence of PPRV antibodies in buffalo and wild ruminants. Buffalos are susceptible to PPRV infection without exhibiting any symptoms and sero-convert, which results in the development of antibodies which combat against PPRV (Abubakar *et al*. 2011a). According to Halecker *et al*. (2020), the clinically less affected domestic herd of small ruminants may be the result of the lack of direct interaction between gazelles and domestic animals, since gazelles are believed to be the carrier of PPR.

2.3. Characteristic and means of PPR transmission

The likelihood of the role of domestic large ruminants in the virus's spread and as indicators of circulating virus within populations has been suggested by reports describing the isolation of live virus from sub clinically infected cattle three weeks after the disease was transmitted from experimentally infected goats (Lembo *et al*., 2013). Recent experimental transmission research has shown that European wild boar and domestic pigs are susceptible to PPRV infection, which can lead to clinical manifestations and the spread of PPRV to pigs and goats that come into contact with them (Schulz *et al*., 2018).

According to Lembo *et al*. (2013), PPRV has recently spread from sick goats to cattle.
The type of farming techniques and the strain of PPRV circulation in specific geographic locations may have an impact on the transmission of PPR from small ruminants to cattle. Since domestic animals and wildlife share grasslands and water sources, wildlife may play a role in the disease's transmission. But at the moment, it’s unidentified what their role is

(Banyard *et al*., 2010; Balamurugan *et al*., 2012). The detection of PPRV RNA in Culicoides imicola resulted in recent studies showing PPRV may be a vector-borne sickness (Sevik and Oz, 2019).

Kerdiles *et al*. (2006) state that both nasal and oral routes are the two main passageways for entry of PPR in animals. The PPR virus damages gastrointestinal and respiratory tract epithelial cells because it has a tropism for epithelial and lymphoid cells. The respiratory, gastrointestinal, and severe immunosuppressive symptoms that follow the infection are directly caused by the lesions that occur from the infection. Even though the virus has an immunosuppressive effect that makes opportunistic pathogens more likely to proliferate, recovering animals constantly develop a robust, lifelong immunity that eliminates the virus.

Important sources of the virus include the secretions from the mouth, nose, and eyes, as well as the feces of infected animals both during and after the start of clinical indications. When afflicted animals cough or sneeze, these discharges create tiny infectious droplets in the air that animals in close proximity inhale and are prone to contract from. Additionally, the infectious aerosols have the potential to contaminate bedding, water, and feed troughs, creating new opportunities for infection. However, indirect mode appears to be less significant because PPRV is also susceptible to lipid solvents and is not anticipated to survive for extended periods of time outside of the host (Tripathi *et al*., 2018).

The inoculation route does have an impact on the earliness and intensity of viral excretion and clinical signs (Enchery *et al*., 2019). PPRV is mostly spread through direct or indirect contact of infected and healthy animals. Still, it is impossible to entirely rule out indirect transmission across recently infected material, and epidemiological models must to take this into account. In both experimental and natural infections, PPRV's high multi-systemic virulence has been shown (Couacy-Hymann *et al*., 2007). It has a direct life cycle that is maintained by infection infected animals to vulnerable animals, without the use of vectors or carrier animals. The fundamental prerequisites are a large population that consistently supplies susceptible hosts and significant animal migration to for population mixing (Sevik and Oz., 2019).

Enormous amounts of contagious viruses are expelled by the oral, nasal, ocular, and faces in secretions. Sneezing and coughing create short-range aerosols, which are the main respiratory routes of infection. Since the virus is fragile, it is doubtful that fomites will spread it (Parida *et al.*, 2015). Şevik and Oz (2019) state that, there is no proof of the virus spreading through biting flies or mosquitos, however a recent study revealed that culicoides were infected with the virus during an outbreak, so the possibility of perform is still hypothetical.

The virus is very susceptible to being inactivated by outside influences like heat, sunlight, and chemicals since it is enveloped. The virus is extremely fragile and cannot withstand long periods of time without a host. At 56°C, its half-life is predicted to be 22 minutes, and at 37°C it is 3 hours. There doesn't appear to a known carrier state to exist (Woodford, 2005). Since PPRV is mostly extended by direct interaction among vulnerable and infected animals, live animal markets and a commonality grazing areas are crucial centers for the virus's dissemination (Banyard *et al*., 2010).

2.4. Pathogenesis of PPR virus

The initial connection between the host and pathogen is mediated by the sialic acid contained in the host cell membrane and the hemaglutination-neuraminidase (HN) protein present in the Peste des Petits Ruminants Virus (PPRV). The enzyme neuraminidase is responsible for viral budding, it removes sialic acid residues from glycoproteins' carbohydrate moiety. PPRV differs from other morbilliviruses in that its HN protein participates in both hem agglutination and neuraminidase activities; so, it is more appropriate to refer to PPRV as an HN protein rather than H protein (Munir *et al*, 2015).

Once the inexperienced animals get infected by the virus through their nasal and oral passageways, it begins to multiply in the oropharynx and nearby lymphoid tissues as soon as it enters the organism. Viruses can replicate in any type of immune cell, including reticular cells, macrophages, and lymphocytes. The newly generated virions spread throughout the host's organs and tissues, showing a predilection for the immune system,

lungs, and digestive tract. The diseases clinical signs, such as discharge, lacrimation, and diarrhea, are triggered by the following tissue destruction, which is apparent post-mortem (Gitao *et al.*, 2012; Munir *et al*., 2012).

Munir *et al*. (2012) states that, as confirmed by biochemical and enzyme tests, after the virus multiplies in cells, it causing abnormalities in kidney function (high urea and creatinine), and Hematocrit and erythrocyte levels that are low are linked to internal intestinal and renal hemorrhage. Furthermore, PPRV infection causes immune cells to undergo apoptosis, which results in cell death and severe immunosuppression. Leukopenia, a weakened immune response caused on by a low white blood cell count, weakens the animal's natural defenses and makes it more susceptible to secondary bacterial and viral infections and make diagnosis more difficult. The death rate linked to PPR is considerably raised by these opportunistic infections (Vinayagamurthy, 2017).

For the rest of their lives, animals that recover remain protected against PPRV. The pathogenicity of PPRV varies, and this variation in virulence is probably related to the host's susceptibility, which depends on the host's breed and species. There could be differences in the virus's affinity for the lymphocytes. Rapidly replicating viral strains might be the most virulent, whereas attenuated strains might be less infectious due to altered tissue affinities, which would lower their epitheliotropism (Munir *et al*., 2012; Vinayagamurthy, 2017).

PPRV is a type of epitheliotropic and lymphotropic virus that can cause pneumonia, stomatitis, gastroenteritis, rhinotracheitis, and conjunctivitis. Following PPRV infection, the virus is picked up by antigen presentation cells (APCs) in the respiratory nasopharynx and interepithelial space. It is then transferred to nearby lymphoid tissue to replicate. Viruses are spread throughout the body by infected lymphocytes found in the lymphoid and blood systems. Eventually in the course of illness and following a high dose of viral exposure, pneumonia develops (Gitao *et al.*, 2012).

Following entry into the body, the virus replicates in the respiratory and nasopharyngeal epithelium before moving on to infect regional lymphoid organs. There, it undergoes a second round of replication that extends the infection to different organs. Because of the morbilliviruses' strong affinity for lymphoid organs, there has been extensive lymphocyte destruction in the mesenteric lymph nodes (MLN) and Peyer's patches. And infections with them usually cause severe immunosuppression due to leucocyte loss (Abubakar *et al*., 2008a, 2012).

Rudra (2019) states that, primary viraemia is a consequence of the virus spreading from its original replication sites through lymph and blood, and the onset of clinical symptoms corresponds with PPRV replication in target tissues. The PPRV causes particular lesions in the respiratory and gastrointestinal tracts due to its unique affinity for lymphoid tissues and epithelial tissue. Both thymus-dependent and thymus-independent regions suffered during the pathogenesis.

Apart from aerosols, PPR infection can also be acquired through licking or eating from common water sources and grasslands. It penetrates the body by means of the oropharynx, multiplies in the lymph nodes that drain, and then circulates throughout the body. After that, it reaches the circulatory system and accumulates in lymphatic tissues. Following that, it travels to the tissue of the epithelium, where it replicates in cells that are vulnerable, leading to the appearance of local lesions and clinical symptoms related to damage to these areas (Pope *et al*., 2013; Şevik and Oz, 2019).

2.5. Clinical signs of PPR

In average an incubation period of PPR last within 4-6 days. The clinical presentation can differ significantly according on the virulence of virus, breed, age, and immunological status. The virus can also cause abortions in animals that are pregnant (Couacy-Hymann *et al*., 2007). According to Pope *et al.* (2013), PPRV-induced immunosuppression may make infected animals more vulnerable to subsequent infections, evidenced by the noticeably severe leukocytopenia that occurs in goats and pigs a few days after viral inoculation.

Variations in an individual's resistance to PPRV infection or concurrent infections with other pathogens may have contributed to different clinical sign manifestations following PPRV infection.

The clinical manifestations of PPRV infection might differ in accordance on the strain of the virus, the breed of the afflicted animal, and other factors like nutritional status, population resilience, co-infection with other illnesses, and stresses that contribute to the pathophysiology of PPRV infection. The immune condition of the animal determines how severe the disease is; for instance, once colostrum antibodies naturally wane, newborn animals become vulnerable to PPRV infection at three to four months of age (Banyard *et al*., 2010).

According to Alemu *et al*. (2019), the most common characteristics of PPR is the acute form, which is characterized by pneumonia, severe diarrhea, a high fever, conjunctivitis and nasal discharge, anorexia, and mouth erosive lesions. PPR can range in severity from highly suffer to subclinical and per acute to acute to subacute, contingent on the pathogenicity of the virus and a number of risk factors, including age, sex, breed, housing, and feeding techniques.

Three to five days after the incubation period, there is diarrhea, anorexia, muco-nasal discharge, hyperemia, and a protracted fever. There is a negative correlation between the viral dosage and the incubation period. After its exposure in susceptible species, an acute lung congestion and edema are common followed by mortality (Jagtap *et al*., 2012). In sheep and goats, the disease manifests as a high body temperature, catarrhal ocular discharges, mucopurulent nasal discharges, and erosive stomatitis in the early stages, which is followed by severe enteritis and bronchopneumonia (Baron *et al*., 2011).

The severity of the disease in livestock varies depending on the PPR virus strain, the host breed and species, age, immunity level, and production system (intense, semi-extensive, or free-roaming). PPR largely mimics the RP symptomatology, exhibiting clinical indications similar to those of other respiratory syndromes, such as coughing, discharges from the nose

and eyes, and more severe symptoms that can result in death in the acute form. It is reasonable to conclude that PPR affects all wild ungulates (Pruvot *et al*., 2020).

In both endemic and epidemic circumstances, mortality is higher in young animals than in olds, it is more severe in goat than sheep. Similar clinical symptoms have been seen in captive, managed free-range populations of ruminant animals as well as in PPR outbreaks impacting different wild species in Asia. PPRV seems to spread from domestic sheep and goats that are sick to nearby wild animals, resulting in infection and clinical illness (Baron *et al*., 2011; Mahapatra *et al*., 2015).

Sevik and Oz (2019) reported that, the infection develops quickly, taking 2-6 days for it to develop. Death typically happens 5 days of infection. The condition proceeds as a fever, coupled with discharges through the eyes and nose, erosions of the gums and/or mouth's epithelium, and diarrhea begins (Fig 2). Pneumonia and complicates of dehydration can leads to death. In goats and sheep, the sero-prevalence rate increases with age. According to Abubakar *et al.* (2008b), blood samples from the aborted dams tested positive for PPRV antibodies.

With the notable exception of PPRV's remarkable predilection for lung tissues that respiratory signs are a distinguishing feature typical of PPR, the clinical presentation of PPR is almost identical to that of RPV. Along with to these "3Ds," which are discharge, diarrhea, and death, the clinical picture of PPR may also include bronchopneumonia. Five distinct cardinal clinical indicators pyrexia, oculo-nasal discharge, oral lesions, respiratory indications, and diarrhea are included in the actual description of PPR (Balamurugan *et al*., 2019).

Figure 2: Severe form of PPR cases in sheep and goat.
(A): Mucopurulent nasal and oral discharge; (B): Sloughing of oral mucosa, (C): Formation of crust in and around nostril and eyes.

Source: (Mallinath *et al*., 2018).

2.6. Differential diagnosis

The clinical signs and symptoms of some other diseases, including as rinderpest, bluetongue, and contagious caprinepleuro pneumonia, can be confused with those of Peste des petits ruminants and it leading to misunderstanding. Laboratory diagnostic tests are used in addition to clinical observations to confirm the diagnosis because there is a possibility of secondary bacterial infections, which complicates the diagnosis (Younus *et al*., 2020). Pneumonic pasteurellosis is the respiratory disease which affects sheep and goats. When oral lesions and diarrhea are either absent or not readily apparent in PPR, the biggest challenge with distinguished status occurs. Pasteurella haemolytica bacteria can be readily isolated in pure culture from sheep pneumonic lungs by using pure culture (Gomes *et al*., 2007).

While Peste Des Petits Ruminants (PPR) induces necrotic and erosive stomatitis, Orf virus primarily causes erythematous and ulcerative papules in the oral and perilabial areas. This kind of resemblance in the location of lesions is what causes an incorrect recognition of

ORF as PPR in clinical settings. In consequence of the incorrect diagnosis of Orf, which has zoonotic potential, humans may acquire the disease from affected animals (Abubakar *et al*., 2015; Manzoor *et al*., 2018). Standard tests such as Penside, c-ELISA, Immuno-capture ELISA, and molecular test RT PCR are preferred for the confirmed diagnosis of PPR (Abubakar *et al*., 2011a).

Goats are vulnerable to a disease called contagious caprine pleuropneumonia (CCPP), but sheep are unaffected. Similar to PPR, CCPP is characterized by fever, dyspnea, and coughing, although it doesn't involve diarrhea or oral sores. Even if CCPP is suspected, it is advisable to rule out PPR in PPR high risk areas by laboratory tests by serological test from convalescent flocks (Gomes *et al*., 2007). Both peste des petits ruminants (PPR) and reinderpest (RP) are highly pathogenic infectious diseases that mostly attack small ruminants and cattle, respectively. And both are caused by Morbillivirus. The PPRV shows a notable affinity for the parenchymatous cells found in the lungs, setting it apart from the RPV (Vinayagamurthy *et al*., 2020).

The clinical signs of PPR closely resemble with those of rinderpest in large ruminants. Nonetheless, the postmortem abnormalities and clinical examination were strongly indicative of a PPR virus infection. PPR was also confirmed by the results of cELISA utilizing monoclonal antibodies specific to PPR (Ahmad *et al*., 2005). Compared to goats, sheep are more frequently affected by foot-and-mouth disease (FMD). Besides the appearance of the lesions, the most significant characteristics that set FMD apart from PPRV are an absence of diarrhea or breathing problems and the presence of lameness (Gomes *et al*., 2007).

2.7.Types of samples utilized for PPR test

Both peste des petits ruminants (PPR) and rinderpest (RP) are Studies have used a variety of samples to examine the prevalence of PPR in animals that have been recovered from PPR. Real-time PCR analysis of nasal, ocular, and oral swabs from infected animals with PPR virus revealed 40 days of PPRV persistence (Liu *et al*., 2018). Selection of sample

type and primers is critical for optimizing the accuracy of PPR diagnosis in sheep and goats, based on the findings of a PCR assay for PPRV detection in swab (ocular and nasal), buffy coat, and tissue (spleen, liver, lungs, lymph node, and intestine) samples using F and N gene-specific primers (Luka *et al*., 2012b).

For the purpose of detecting PPRV antigen, ante mortem samples such as nasal, anal, ocular, whole blood, and urine were taken from sheep and goats presenting PPR-like signs and lesions. Lymph node and lung samples had the greatest viral load when PPRV was relative detected across a variety of tissues from natural outbreaks. The samples from post-mortem sheep and goat cases exhibiting pathological changes and symptoms indicating of PPR such as the liver, kidney, trachea, bile, heart, tongue, brain, intestine, thymus, abomasum, rectum, lymph nodes and spleen were obtained (Chauhan *et al*., 2014).

For the purpose of detecting antigens, buffy coat samples were obtained by centrifuging blood contained in anticoagulant-containing tubes, separating the plasma, and storing the buffy coat at -70°C. But for swabs, after centrifuging and vortex ocular and nasal swabs, the supernatant was poured into sterile tubes and kept at -70°C until RNA extraction. One gram of tissue from each sample was weighed and homogenized using a tissue homogenizer. After homogenization, 9 ml of Phosphate buffered saline (PBS) was added to make a 10% tissue suspension and centrifuged. The supernatant was decanted into a sterile tube and stored at 4℃ until RNA extraction (Kerur *et al*., 2008; Luka *et al*., 2012a).

Blood, swabs (ocular, nasal, oral, and rectal) collected early in the course of the disease and from a dead animal's tonsil, mesenteric lymph nodes, spleen, part of the colon, and lung are the most appropriate samples to use for PPR virus isolation (Santhamani *et al*., 2016; Rudra, 2019). For PPR infected, animals that are alive may provide samples such as conjunctival sac swabs, nasal secretions, mouth and rectal lining, clotted and whole blood (with EDTA anticoagulant). Samples of the affected parts of the alimentary tract mucosa, the tonsil, the tongue, the spleen, and lymph nodes may be collected at post-mortem (Woodford, 2005).

2.8. Major diagnostic techniques for PPR

Current laboratory diagnostic tests for PPR can be divided into three categories: tests based on the identification of viral antigens, tests based on the identification of genetic material derived from viruses, and tests based on the identification of antibodies directed against viruses (Younus *et al*., 2020). Libeau *et al*. (2014b) reported that, the data from more recent PPR-infected zones occasionally demonstrates complex polymicrobial disease, presenting the possibility of misidentifying PPR symptoms as those of other respiratory or gastrointestinal illnesses or misclassifying them as such. The identification of the antigen, viral isolation, nucleic acid amplification, indirect detection, and confirmation of virus-specific antibodies are among the methods for PPRV diagnosis that have been revealed.

2.8.1. PPR genome detection techniques

It is well known that morbilliviruses exhibit a significant level of genetic diversity. Primers employed in RT-PCR tests can identify alterations in the viral genome which affect target regions due to PPRV's genetic viability. The sensitivity and specificity of PPR virus primer sets used to identify PPRV vary, indicating that further primer sets may be needed to evaluate the genetic diversity of the virus (Rana *et al*., 2023).

The laboratory testing is required for an ultimate confirmation diagnostic of the disease. The presence of PPR virus in the outbreaks under investigation was clearly shown by the clinical symptoms, post mortem findings, and epidemiological observations. The PCR technique is limited to use with DNA strands. RT-PCR is a PCR variant in which RNA can be translated into complementary DNA (cDNA) with the aid of the reverse transcriptase enzyme, enabling PCR examination of RNA molecules (Batten *et al*., 2011; Gomes *et al*., 2016).

This RT-PCR assay was 1000 times more sensitive than the conventional titration technique (Couacy-Hymann *et al*., 2002). Protocols for PPRV antigen and nucleic acid detection can be applied when sampling is done for clinical surveillance or diagnosis

throughout active disease outbreaks. Many of these nucleic acid and PPRV antigen detection methods are only applicable between 4 and 17 days after infection. Furthermore, the sample quality must be carefully handled in order to detect antigens and genomes (Saravanan *et al*., 2010; Singh, 2011). The ability of RT-PCR to identify the PPR virus in both clinical and subclinical infections is one of its benefits. Because of this, it's a valuable tool for handling and detecting outbreaks in the future (Rana *et al*., 2023).

PPR infection is still possible to confirm with performing RT-PCR tests on sample in which the virus is no longer alive (Couacy-Hymann *et al*., 2002). For the particular diagnosis of PPR, a number of nucleic acid-based molecular diagnostics have been developed. These include reverse transcriptase PCR (RT-PCR), real-time RT-PCR, loop mediated isothermal amplification (LAMP), and complementary DNA hybridization (cDNA). Although they need a lot of time and effort for typical disease diagnosis from significant sample sizes, RT-PCR and cDNA hybridization methods are the most sensitive diagnostic approaches (Balamurugan *et al*., 2014).

2.8.2. Viral isolation techniques

Though virus recovery is not always successful, attempts at virus isolation in cell culture can also be made using a variety of cell lines. Previously, primary lamb kidney or African green monkey kidney (Vero) cell cultures have also been successfully employed, although the marmoset-derived cell line B95a was the most commonly utilized. SLAM cells from Vero dogs are currently being used to cultivate and recover morbilliviruses. In the days that follow the infection of a monolayer with doubtful material, cultures are often checked for cytopathic effect; virus neutralization or molecular techniques can be used to validate the identity of the virus (Sreenivasa *et al*., 2006).

Adombi *et al*. (2011) formulated that, the suspected materials such as 10% tissue suspensions, buffy coats, or swab material, are utilized to inoculate monolayer cultures. The resulting cultures are then monitored every day for signs of cytopathic effect (CPE). PPRV-produced CPE can take up to five days to develop, and in lamb kidney cells and cell

lines that express SLAM, it consists of cell rounding and aggregation that results in the development of syncytia. It might occasionally be challenging to see the syncytia in unaltered Vero cells. Couacy-Hymann *et al*. (2002) states that in a laboratory setting, seroneutralization or virus isolation is performed to confirm the PPR diagnosis. Isolating PPRV from infected materials in cell culture is a challenging task. An isolation procedure takes two to three weeks to be completed. A high-quality sample that has been preserved in cold condition until processing is required for screening.

2.8.3. Techniques for PPRV antigen detection

Penside (field) test is a commercially available PPRV antigen tests that can be used in the field. The "lateral flow technology". Swabs from doubtful animals' conjunctiva, nasal cavities, or oral swabs are required. The swabs are then cleaned with buffer and this buffer is placed on one end of a chromatographic strip. A specific MAb coated in colored beads that recognize the PPRV antigen is mixed in with the sample. It has been verified against isolates of PPRV from each of the four lineages (Baron *et al*., 2014).

According to Banyard *et al*. (2010), the Counter immune electrophoresis (CIEP) is the fastest test available and also used to find viral antigens. In order to generate the line of precipitation at the point of interaction, the antigen to be detected and the particular antibody employed to detect the antigen move in opposite directions in the electric field during this test. CIEP is faster and more sensitive than agar gel immunodiffusion (AGID). Both tests, however, are less sensitive in the early stages of infection and when the disease is moderate. Agar gel immunodiffusion (AGID), immunecapture ELISA (ICE), and counter immune electrophoresis (CIEP) can all be used to identify PPRV antigens. PPRV and RSV cannot be distinguished from one another using the AGID test, but they can be distinguished using ICE and CIEP. Because the viruses contain cross-reacting epitopes, they are unable to distinguish between PPRV and RPV (Santhamani *et al*., 2016).

The hem-agglutination test requires neither sterility nor complex equipment to perform, resulting in a straightforward, affordable, and effective test for PPRV diagnosis. Actually, it's additionally used to distinguish between RPV and PPR infections (Baron *et al*., 2014). Comparing cell ELISA to infectivity titration, the former has a relative sensitivity and specificity of above 97%. Results showed that cell-ELISA may be used to test the virus's identification and infectivity in vaccination samples (Santhamani *et al*., 2016). Immunochromotagraphic lateral flow devices using monoclonal antibody detection method are proving useful for field use (Baron *et al*., 2014).

A PPR-specific monoclonal antibody to a nucleocapsid protein epitope has been used to create a sandwich ELISA assay. Polyclonal sera are used in the test to extract the antigen from clinical samples, such as tissues and swabs. PPR-specific monoclonal antibodies are used to identify captured antigens from clinical samples. Since the rinderpest vaccine virus was not detected by the test, it is specific to PPR (Singh *et al*., 2004b). Using an immunofluorescent antibody test (IFAT) and a particular monoclonal antibody (mAb) to PPRV, the antigen of the peste des petits ruminants virus (PPRV) was found in conjunctival epithelial cells taken from goats in either the early or late stages of the disease (Santhamani *et al*., 2016).

Santhamani *et al*. (2016) states, by utilizing immunohistochemistry methods, PPRV antigen in tissues was identified. The fluorescence antibody test (FAT), which uses monoclonal antibodies (MAb) as its basis, was created to identify antigen in conjunctival smears. FAT is more sensitive. It's an approach to render syncytial cells observable. Even though FAT is quick and easy to do, skilled technicians and a fluorescence microscope are required.

2.8.4. Antibody detection techniques

The most significant diagnostic methods for virus antibody detection are the virus neutralization test (VNT) and competitive ELISA (c-ELISA), which are based on MAbs against the H or N proteins (Bedore *et al*., 2012). The most commonly used diagnostic

technique for PPRV antibody detection at the moment is c-ELISA. Compared to VNT, c-ELISA possesses a sensitivity of 92.2% and an overall specificity of 98.4% (Bello, 2013).

Virus neutralization test is agold-standard antibody detection test recommended for international trade. Before inoculating a 96-well microplate with cell culture, 100-1000 TCID50 of PPR is mixed with 100µL of serum dilutions and incubated at 37°C. The absence of virus neutralization can be detected by the development of CPE in the microplate wells containing a certain antibody dilution. The endpoint dilution assay known as the virus neutralization titer of a serum is used to quantify the infectious viral titer. It is defined as the highest dilution that leads to a 50% reduction in CPE. It is challenging to use VNT for routine serosurveillance and seromonitoring tasks because of the needs for sterile serum and cell culture facilities, especially when a significant number of samples need to be screened. Because of this, competing and blocking ELISA techniques have largely replaced it (Santhamani *et al*., 2016).

After strongly infected, antibodies are detectable as early as the diarrheal stage. Virus neutralization test (VNT) is the recommended test for international trade, whereby it serves as the basis for veterinary certification of animals confirming the presence or absence of antibodies (OIE, 2008). Competition ELISA, which uses a monoclonal antibody that is targeted to the virus nucleoprotein, is the most accurate and quick method for recognizing antibodies. This test is frequently used for PPR monitoring and sero-surveillance (Albina *et al*., 2013). Antibodies to the PPR and RPV can cross-neutralize, so it was necessary to compare a positive VNT result for the PPR virus to the titer that was found for the RPV. When a serum's neutralization titer for PPR is at least twice as high as that of rinderpest, it is considered positive for PPR (OIE, 2008).

Monoclonal antibodies to the epitope-blocking ELISA were developed to provide competition ELISA (C-ELISA) tests, which have increased access to serological testing and proven useful in field research on PPR epidemiology (Bodjo *et al*., 2018). Controls and the tested samples are added to the N protein coated micro wells in order to detect antibodies. If anti-N protein antibodies are present in the sample, an antibody-antigen

complex that masks the N protein epitopes is produced. Because of the high specificity and sensitivity of c-ELISA, it can be used even for samples not kept under ideal conditions (Bello, 2013; Abiyu, 2022).

A blocking-ELISA (b-ELISA) that is sensitive, reliable, inexpensive, specific, and reproducible for the detection of anti-PPRV antibodies. H protein-based MAb is utilized as a competitive antibody in the ELISA. Compared to the VNT, blocking ELISA has a 98.9% specificity and a 90.4% sensitivity. It has been shown to be a straightforward, more rapid and accurate for identifying PPR antibodies (Kamel and El-sayed, 2019). Blocking ELISA (b-ELISA) is a rapid detection of PPRV antibody in sera, in which two neutralizing monoclonal antibodies (MAbs) that are specific for the hemagglutinin protein of PPRV are used. It is a simple and fast test for distinguishing PPRV antibodies from those belonging to other morbilliviruses, particularly RPV (Libeau, 2014a).

2.9. Post mortem finding of PPR disease

According to (Gomes *et al*., 2016), in endemic locations, clinical indicators and post-mortem findings are adequate for an initial identification of PPR. Retrogressive and necrotic alterations in lymphoid tissues, respiratory and gastrointestinal epithelial cells characterize and predominate in PPR pathophysiology. Additionally, the lungs' regions primarily the anterior and cardiac lobes become rigid to the touch and turn dark red or purple. The presence of PPR virus in the outbreaks under investigation was clearly shown by the clinical indicators, post mortem findings, and epidemiological observations (Nafea *et al*., 2019).

Notable lesions in PPR-infected animals include consolidation, alterations to the appearance of the lungs, and occasionally, foamy fluid that appears in cut pieces of the lung on squeezing (Kumar *et al*., 2002). Balamurugan *et al*. (2014) state that pleuritis and hydrothorax are potential side effects of bronchopneumonia, which is a recurrent lesion. The most frequently afflicted lymph nodes are those linked to the gastrointestinal (mesenteric) and lung (mediastinal), which are typically swollen, edematous, and

congested. Spleen occasionally developed petechial on the capsule surface and became enlarged and obstructed.

Typically, congestion at the ileocecal valve, at the caecum-colic junction, and in the rectum is observed, along with necrotic or hemorrhagic enteritis. Irregular streaks of congestion, sometimes referred to as "Zebra" stripes or "Zebra markings," on the epithelial folds in the posterior part of the colon and rectum are symptomatic of PPR. The lymphoreticular system's tissue, especially the tonsils, head and lung-associated lymph nodes, mesenteric lymph nodes, and small intestinal Peyer's patches, was shown to be the most appropriate for postmortem diagnosis using PCR and immunohistochemistry for PPRV diagnosis (Balamurugan *et al*., 2014; Schulz *et al*., 2018).

2.10. Treatment and prevention of PPR

Since PPR has no approved treatment, medical treatment such as dextrose normal saline is required to replacement the body's ionic fluid lost while broad spectrum antibiotics are administered to avoid extra bacterial problems. Broad-spectrum therapies like penicillin, chloramphenicol and streptomycin for stomatitis, enteritis, and pneumonia in affected animals are required. Nonetheless, medications that manage the bacterial and parasite problems can lower the death rates. It also recommended to utilize oxytetracycline and chlortetracycline in particular to avoid developing secondary pulmonary infections (CIDRAP, 2003; Jilo, 2016).

Morbilli viruses are quickly rendered inactive at room temperature by desiccation and sun light. The PPRV can be rapidly inactivated in the environment by disinfectants because it can only survive outside of an animal for four days or less. Alkalis like sodium carbonate and sodium hydroxide, halogens like sodium hypochlorite, phenolic compounds, citric acid, alcohols, and iodophors may also assist in controlling and eradicate the virus (Abubakar *et al*., 2015). In sheep and goats, the rate of sero-prevalence increases with age. Sheep and goats have a widely recognized history of PPRV infection at a younger age,

with sero-positivity lasting 1-2 years after exposure. Sero-positivity to PPR was more likely in animals older than one year and younger than six months (Rudra, 2019).

After being infected with PPRV, animals begin to generate antibodies seven to ten days later. Because they remain resistant to PPR once after recovering, animals do not become infected repeatedly. In addition to receiving a lifetime immunization with live attenuated PPRV vaccine, animals that recover from illness also acquire a lifetime immunity which provides complete protection against reinfection. For up to three or four months after birth, immunological animal offspring have protective maternal antibodies (Parida *et al*., 2015; Enchery *et al*., 2019).

Jemberu *et al*. (2022) suggested that vaccination is essential for preventing a variety of animal diseases, such as PPR, for which a very efficient vaccine is frequently applied. PPRV antibodies are known to last a lifetime in sheep and goats following PPRV infection, and it's probable that this holds true for wild animals as well (Libeau, 2014b). The PPR virus is only has one serotype, and immunity is long-lasting possibly lifetime. Since colostrum antibodies protect young animals, most disease in endemic places emerges when colostrum immunity wanes, with protecting animals that have already been exposed (Fernandez *et al*., 2020).

In either vaccination or natural infection within ten days after the initial viral encounter, the PPRV-induced host antibody response may be noticed, and it can persist for a long duration of life. In areas where the virus is endemic and farmers cannot afford and implement the strict sanitary control measures such as the stamping out policy to control of PPR, widespread vaccination of small ruminants is the most effective means of controlling PPR in a given area after it has been confirmed (Saravanan *et al*., 2010; Singh, 2011; Albina *et al*., 2013).

For mass vaccination campaigns to put an end to the virus's epidemic cycle, strong livestock levels of immunity between 70% and 80% must be maintained (Hailegebreal, 2019). Nowadays an accessible vaccines are all attenuated PPRV strains. An increasing

number of vaccination programs are being supported by post-vaccination serum surveillance, enabling us an assessment of the manner in which the local immunization program is succeeding (Baron and Baron, 2015).

The beneficial influence of colostrum in providing passive immunity was confirmed by the significantly increased concentration of colostrum Ig's produced by vaccinated ewes and nanny during pregnancy against PPR in comparison with ordinary colostrum secreted from unvaccinated ewes/nanny during pregnancy. As vaccinations assisted to maintain nanny's/ewes' health by preventing any abnormalities in the pregnant nanny/ewes, the data clearly showed no cases of mortality or abortion (Burezq and Khalil, 2022).

Since there are currently no DIVA tests available in the research for PPRV detection and no differentiate of the source of diseases, Nafea *et al*. (2019) highlighted the necessity for DIVA diagnostic techniques and indicators to differentiate between field wild-type and vaccine PPRV strains. The maternal antibodies to the PPR virus were detected in young animals as early as a few months of age and these antibodies will be dropped below the protective threshold level at 3.5 and 4.5 months in lambs and kids respectively (Awa is *et al*., 2002; Diallo *et al*., 2002).

Controlling the movement of susceptible and infected animals, prohibiting the importation of sheep and goats from afflicted areas, and keeping newly introduced animals in quarantine for a minimum of three weeks are all crucial measures in the prevention and management of PPR disease. Additionally, barns, tools, and other items that have come into touch with sick animals have to be disinfected, and carcasses of dead animals should be buried or destroyed. In endemic regions, vaccination should be provided yearly before the beginning of the rainy season (Bedore *et al*., 2012).

According to Younus *et al*. (2020), a vaccination that can differentiate infected from vaccinated animals (DIVA) is thought to be helpful in controlling and eradication of PPRV. In order to develop a DIVA vaccine that can distinguish between vaccinated and diseased animals. Even though PPR has long been a problem, relatively little study has

been done to figure out the disease's incidence. In certain regions of Ethiopia, PPR is considered as one of the most economically significant livestock diseases due to the documented morbidity and mortality as well as the affected flock size of small ruminant production (Abraham *et al*., 2005). A National PPR Progressive Control and Eradication Strategy, sponsored by the European Union (EC-SHARE), has been created by the cooperation of Ethiopian government with the Food and Agricultural Organization of the United Nations (FAO) (Veronica *et al*., 2019).

2.11. Major factors for PPR distribution

Host genetics and non-genetic variables may also have a substantial impact on the immune system's response to viruses, innate resistance, and disease susceptibility. The immune level of the livestock, parasite infection in the host, nutritional status of the host, breed, age, and sex of the animal, besides other factors, all influence the capacity of the virus to multiply and cause disease (Munir *et al*., 2012). Some countries like Ethiopia, Kenya, Pakistan, India, and Turkey have reported age of small ruminants is a significant risk factor for susceptibility or resistance to PPR disease in which high susceptibility noticed in young animals (Abubakar *et al*., 2009).

There are more potential for PPRV transmission when small ruminants are traded at marketplaces where animals from different sources are brought into close contact with one another. Animals that are nomadic or migratory frequently interact with local populations of sheep and goats, from which they can pick up the virus. Once infected, migratory animals have the potential to spread the virus to further vulnerable local sheep and goat populations. As a result, animal mobility is crucial to the PPR virus's preservation and spread in the wild (Singh *et al*., 2004a).

According to Sevik and Oz (2019), the primary weaknesses in herd health management include keeping a large herd animal densities, by mixing animals that have various viral susceptibilities with different species of small ruminants; unrestricted animal movement, introduction of animals of unknown origin into a herd without health guarantees;

free pastured animal husbandry, reintroduced into a herd without adhering to any quarantine measures of unsold animals from market and the newly purchased animals causing animals of different ages to live closely together.

Ahaduzzaman (2020) reported that the transboundary movement of infected animals without proper quarantine, the existence of hot and humid climates that support disease epidemiology, the absence of vaccination or vaccine administration monitoring, the lack of knowledge about PPR among backyard farmers, and the lack of funding for disease eradication in developing nations are some of the factors that could contribute to variations in the prevalence of PPR and may facilitate disease spread. PPR outbreaks have also been correlated to transport stress, which has significant consequences since it causes a sudden herding of the grazing animals without feed or water for longer than 12 hours.

When animals are suddenly shifting from an adapted grazing system, especially from free grazing to an intense method of management and changing the feed and forage. In addition, an environmental stress, particularly hot and humid climate also favors precipitation of disease (Kumar *et al.*, 2001). Some common factors that impair health surveillance and contribute to the ongoing spread of the disease are lack of veterinarians, the limited access to veterinary care for vaccination and health monitoring systems for some animal owners, and limited knowledge of the disease in disease-free areas (Perry *et al*., 2018). Singh *et al*. (2004b) found that a possible reason for the increased incidence of PPR outbreaks between March and June has been suggested. In contrast, due to animal migrate during the hot, dry months in search of nourishment when fodder is limited, it could expose animal to affected area.

Understanding the role of wildlife in PPRV ecology and PPR dynamics is one of the possible obstacles to the effective eradication of PPR globally. In the socioecological context of rural Africa and Central-South Asia, where domestic stock coexists with a wide variety of wild ungulate species, many of which are vulnerable to PPR. There are many chances for virus transmission between domestic and wild hosts in these settings of wildlife-livestock interfaces (Fine *et al*., 2020). Wildlife has recently been shown to have

a possible epidemic function, and these populations may disperse the virus both temporally and spatially (Pruvot *et al*., 2020).

2.12. Socio-economic importance of PPR viral disease

PPR is one of the diseases that affect sheep and goats in developing countries which has most economically impact due to its high rates of morbidity and mortality, especially in small flocks (Aitken, 2007). Up to 100% of the flock's animals may be affected by the PPR virus and between 20% and 90% of animals exposed to it may dead during an outbreak. In affected populations, the disease has a major impact on livelihood, food security, and economic performance. PPR may interfere with commerce and the expansion of intensive small-ruminant production, in addition to causing significant losses in production associated with death, morbidity, and control expenditures (Jemberu *et al*., 2022).

It is not well known why diseases spread, especially in domestic ruminants. The Food and Agriculture Organization (Libeau *et al*., 2014a) estimates that 63% of small-ruminant populations are currently at risk of PPR, with a particular emphasis on those from southern Africa, central Asia, Southeast Asia, China, Turkey, and southern Europe. Presently, almost one billion sheep and goats are at risk of infection. Due to its significant effect on small-ruminant output, PPRV is becoming a major concern for animal health worldwide. Therefore, it has been suggested that PPR be eliminated following rinderpest (Libeau *et al*., 2014b).

PPR was included in the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs). PPR vaccine costs were predicted to have cost between US$270 million and US$380 million in 2015. Direct yearly losses from PPR were estimated to be between 1.2-17US$ billion in 2015. The potential yearly impact of PPR alone is estimated to be between US$1.45 billion and US$2.1 billion. Africa has about one-third of the world's financial burden associated with PPR, whereas South Asia bears the remaining quarter (FAO, 2015).

Diagnoses, surveillance, prevention and control, creating a legislative framework that includes veterinary services, and stakeholder involvement are the technical pillars of the worldwide PPR eradication strategy. Within five years of a successful eradication, an estimated US$ 7.1 billion investment in global PPR eradication might be returned (Santhamani *et al*., 2016). In PPRV-infected pregnant females, abortions may happen at any stage of gestation. The highly contagious characteristics of PPRV in combination with their migration and dissemination presents a serious transboundary importance that hinders commercial activity and increases economic losses that causes financial in the farming community (Banyard *et al*., 2010).

2.13. Geographical distribution of PPR

The climate change and genetic variation affect the PPRVs' capacity to adapt to new hosts can alter the set of outbreak patterns. As a result, new viral strains have emerged that may be undetected by the current primer sets. Additionally, improper handling of clinical materials during their transfer from the field to the testing laboratory can seriously affect the quality of the test results by causing viral antigens and RNA to degrade and complicating diagnosis (Rana *et al*., 2023)

2.13.1. World distribution of PPR

Since 1993, the virus has been reported to cause major outbreaks in the Middle East, Arabian, and a large portion of the Indian subcontinent; as consequently. Those region is currently considered to be endemic for PPR (Dhar *et al*., 2002). Formerly predominant in West Africa, the peste des petits ruminants virus has migrated throughout Bangladesh and Turkey, southern Asia, the Middle East, and East Africa. A wide area of sub-Saharan Africa, Arabia, the Middle East, and Southern Asia are known to be inhabited to PPR. Recently widespread outbreaks in Turkey and India have pointed to a significant rise in PPR prevalence worldwide (Ozkul *et al*., 2002). The World Organization of Animal Health (OIE) and the Food and Agriculture Organization (FAO) intend to completely eradicate the PPR disease worldwide by 2030,

Geographically, lineages I and II were primarily limited to western and central Africa, while lineage III was primarily associated with eastern Africa and the Arabian Peninsula. Lineage IV was associated with South Asia, the Middle East, and more recently, the northern, western, central, and eastern parts of Africa. Lineage IV PPRVs exhibit an extensive geographic range; findings have been made from Turkey, the Middle East, China and Tibet, South Asia, and, more recently, northern, western, central, and eastern Africa (Dhar *et al*., 2002; Dundon *et al*., 2020).

2.13.2. Distribution of PPR in Africa

In accordance with historical theories, PPRV originated in West Africa and traveled along trade routes through Sudan, Egypt, and the Middle East to reach North and East Africa (Abd El-Hakim, 2006). In North Africa PPR mainly reported in Algeria, Egypt, Libya, Morocco, Sudan, Tunisia (Khalafalla *et al*., 2010). Sudan is the first country in Africa where antigenic or genetic evidence of PPR disease in wildlife species has been published, according to Wensman *et al*. (2018). It's possible that PPR disease has affected other free-ranging wild species in other parts of Africa but has not been discovered or reported because there is limited capacity for monitoring diseases in wild animals, and dead or sick animals may be consumed by predators and scavengers before they are identified.

According to Banyard *et al*. (2010), PPR prevalence has been documented in a number of central African countries, particularly Burundi, the Democratic Republic of the Congo (DRC), Rwanda, Angola, Cameroon, Chad, and Gabon. PPRV is endemic across the majority East African countries belonging to lineage III with isolates being characterized in Ethiopia, Sudan, Uganda, Kenya, Somalia and Tanzania. Both lineages III and IV are circulating in the Sudan (Khalafalla *et al*., 2010). In Africa, all four lineages have been identified. Subsequently has been revealed that different countries have numerous lineages. For instance, lineages II, III, and IV have been confirmed in Tanzania (Mahapatra *et al*., 2015); lineages III and IV in Uganda (Luka *et al*., 2012b).

2.13.3. Status of PPRV in Ethiopia

Historically, the first clinical suspicion of PPR emerged in 1977 in a goat flock in the eastern Afar region of Ethiopia (Waret-Szkuta *et al*., 2008). After PPR first came to Ethiopia since 1989 in the Southern Omo River Valley. Then it spread eastward to the Borena region and then northward toward the Rift valley to Awash. It is endemic in goat (Abraham and Berhan, 2001). PPR existence was verified in 1991 using a cDNA probe in lymph node and spleen tissues taken from an epidemic around Addis Ababa. Ocular and nasal discharges, oral lesions, pneumonia, gastroenteritis, and diarrhea were among the symptoms of PPR at that time. Over 60% of the fatalities reported in this outbreak were due to the disease (Gopilo, 2005).

The first description of PPR in Ethiopia was published in 1994 and detailed the outbreak in goat within the country's capital city, Addis Ababa. The virus's entire genome was subsequently sequenced in 2014. Recent research on Ethiopian samples conducted since 2017 has only found lineage IV PPRVs, it indicating that lineage IV viruses may have replaced the earlier lineage (lineage III) (Dundon *et al*., 2020).

Muniraju *et al*. (2016) report that SRMV in Ethiopia was identified to be of lineages III and IV based on genomic analysis of documented outbreaks in 1994 and 2010 respectively. PPR outbreaks have been reported widely throughout Ethiopia, and these reports have often been verified through serological testing and looking for certain clinical signs. The outcome of the investigation showed that there had been about 45% mortality and 80% morbidity. Furthermore, since 1994, many PPR outbreaks with varying rates of illness as well as death have been documented in different regions of Ethiopia (Alemu *et al*., 2019).

During the 1990s, the PPR virus was widely spread among Ethiopia's small ruminant population. Considering the scale and makeup of the small ruminant industry, the documented morbidity and mortality of this disease, and the possibility that PPR is going to be among the most economically significant livestock diseases in the country (Gopilo, 2005). The majority of outbreaks in developing countries remain unreported or

uninvestigated (Couacy- Hymann *et al*., 2002). Around 1.7% and 85.12% is the estimated apparent sero-prevalence of PPR across countries. This variation can be determined by the prevalence of PPRV in a given region, variations in disease identification techniques, sampling method, stage of infection, laboratory equipment utilized, animal species, and sample size (Delil *et al*, 2012).

Besides of the previously reported shift in the SRMV lineage in Ethiopia, it was additionally observed that since the first identification of lineage IV in 2010 there have been periodic PPR outbreaks in various parts of Ethiopia (Muniraju *et al*., 2014). Ethiopia's small ruminant production suffering financially from the continued spread of PPRV lineage IV. It is crucial to isolate and characterize the genetic components of PPR viruses in order to properly understand the molecular epidemiology of PPR outbreaks in Ethiopia and to create practical preventative and control measures for the geographical region (Mohammed *et al*., 2022).

There were important differences in the prevalence across regions, from the highest to lowest, Somali, Tigray, Afar, Benishangul Gumuz, Amhara, SNNPR and Oromia was 21.3%, 15.3 %, 15.3%, 8%, 4.6%, 1.8% and 1.7% respectively. Oromia region showing the lowest prevalence and the Somali region presenting the highest. The estimated pooled sero-prevalence differs greatly amongst the regions; in comparison, it is higher in Benishangulgumz (60.97%) and Tigray (39.9%) (Waret-Szkuta *et al*., 2008).

The ecological features of a particular location, including the environment, population pattern, sanitary practices and socioeconomic may be responsible for variations in sero-prevalence. Both the Benishangul-Gumz and the Tigray region are close to the border and might become susceptible to infection from other regions. Despite being widespread in Ethiopia, PPRV frequently causes outbreaks and less known about the virus's genetic makeup (Ahaduzzaman, 2020).

3. MATERIAL AND METHODS

3.1. Description of the Study Area.

This study was conducted in Borana zone (Moyale) and East Borana zone (Arero and Dhas) of Oromia regional state. Arero, Dhas and Moyale are 655 km, 716km and 785km distance from Addis Ababa city (Fig 3). Agro-ecologically, both zone are identical and consists of semi-arid and dry ecological zones with bimodal rainfall patterns that typically vary from 400 to 700 mm (Berhanu and Beyene, 2015). They rearing cattle primary for milking followed by camels and goat. The warra and fora herd units are the division of household's pastoral herds. According to Berhanu (2011), the warra unit is made up of weak and milking animals that housed in semi-settled encampments with women, elders and children, but the fora divisions are movable herd units that travel to remote regions where there are capable male herders.

In the Borana zone, animals contribute to over 90% of the local economy, and the marketing of livestock and livestock products provides over 60% of the income for households. The Borana people are pastoralists who live as nomadic people, traveling with their animals in search of water and grasslands. Movement is influenced by the season and availability of forage, as well as by relationships with others, family structure, and their demands. From lowest to highest, the vulnerability's rank of livestock to harsh environments and drought can be associated with camel, goat, sheep, and cattle (Bogale and Erena, 2022).

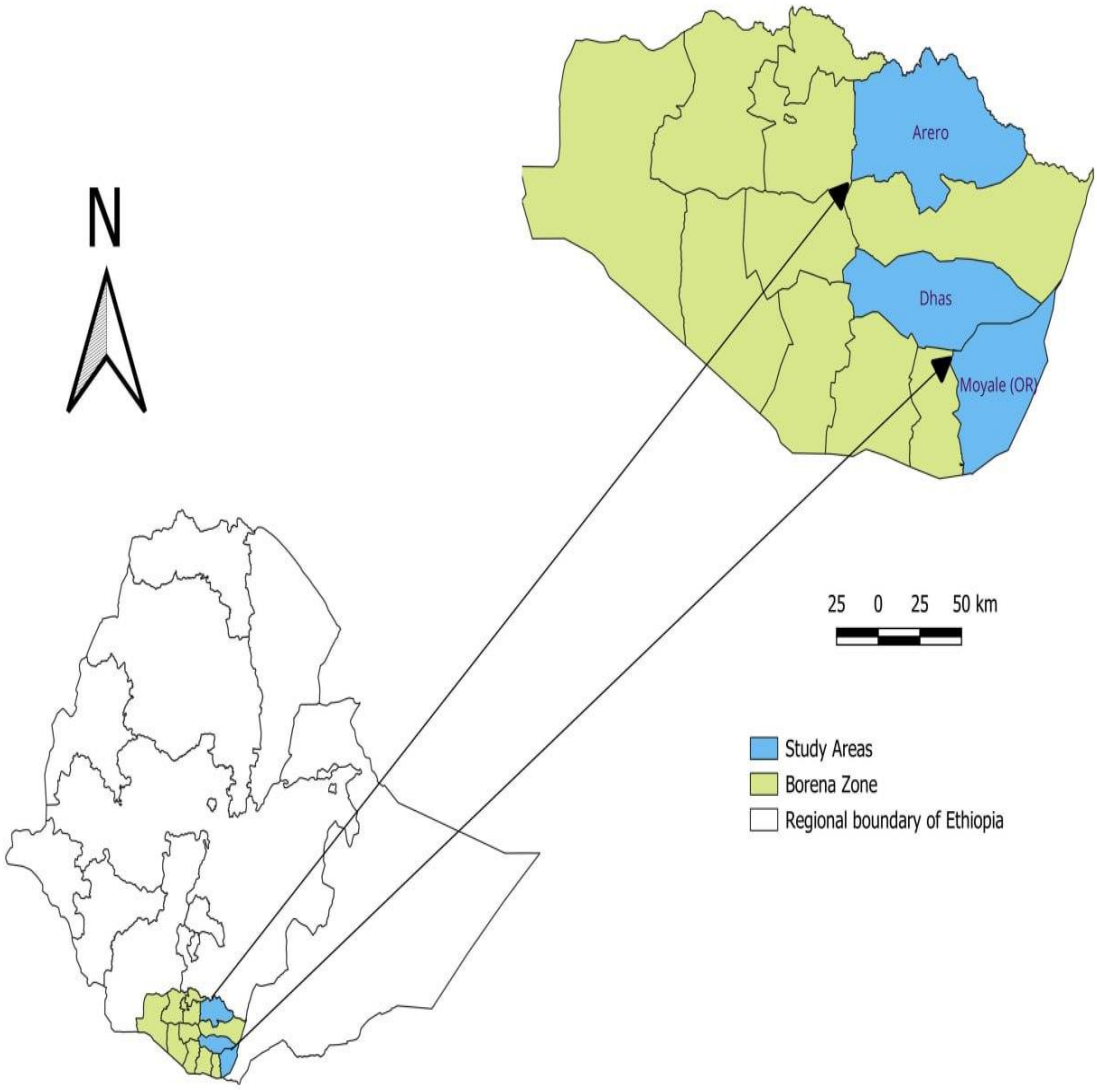


Figure 3: Map of the study area

3.2. Study design

An outbreak investigation was carried out from October 2023 to January 2024, in the Borana zone (Moyale) and East Borana zone (Arero and Dhas). The selection of pastoral study districts and kebeles was based on information regarding Peste des Petits Ruminants Virus (PPRV) outbreaks confirmed by the Yabello Regional Veterinary Laboratory (YRL) and validated through consultations with district and kebele animal health professionals. Rigorous clinical histories and vaccination records were reviewed to ensure accurate

identification of suspected cases. Owners of sheep and goats that take part in the study were selected in particular since their flocks may have had probable PPR cases. Sampling procedures focused on two groups of animals: those showing clinical signs of PPRV infection as reported by local animal health professionals and after interviewed with local authorities and animal owner those know in detail regarding health status of small ruminants, addition sheep and goats those with suspected infections were included in study for serological investigation (Reintjes and Zanuzdana, 2010).

3.3. Study Population

The sampling of the target study populations included all sheep and goats aged 6 months and above from flocks exhibiting active clinical symptoms resembling PPR, such as bi-nasal discharges with difficult breathing and binocular lacrimation, conjunctivitis, necrotizing and erosive stomatitis, with a complicated oral mucosal lesions with or without diarrhea and within which death reported with the same case included in the sampling during field study. Age was categorized into three groups young (<1 year), adult (1-3 year) and old (>3) (Husen *et al*., 2018). Based on their sizes, animals' herd sizes were classified as large (> 20 animals), medium (10-20 animals), and small (< 10 animals) Gebre *et al*. (2018).

3.4. Sampling techniques

Purposively an affected district and kebele was selected and all sheep and goat exhibited the complicated PPR's clinical signs and symptoms were sampled. Then blood samples were collected from suspected animals exhibiting a range of symptoms, from mild to severe, to detect antibodies produced against the PPR virus. Additionally, swab samples were obtained from active cases for nucleic acid detection and cell culture analysis. Three recently died animals were used to provide pathological and clinical samples, which included the lungs, spleen, intestine, liver and lymph nodes (OIE, 2008).

A semi-structured questionnaire was employed to gather flock-level information, and a standardized format was used to collect biodata on individual animals (OIE, 2008; Adombi *et al*. 2011).

3.4.1. Samples collection for molecular detection and pathological finding

After restraining the animal's movement, swab samples (nasal or ocular) were aseptically collected from active clinically suspect cases for molecular detection using sterile cotton swabs from either the nasal or ocular area (Appendix 16). The swab sticks were cut at an appropriate site and placed in cryogenic vials containing 1-1.5ml of virus transport medium (VTM) with BPS, antibiotics, and antifungal agents (Appendix 9). In parallel, in sample collection format, an accurate data regarding the sampled animals was filled (Appendix 14). The vials were labeled and kept in pre-prepared ice packs in an icebox before being transported to the Yabello Regional Veterinary Laboratory. Upon arrival at the laboratory, the collected swab samples were stored at -20°C until further transportation to the Animal Health Institute (AHI), where they were maintained at -80°C for until culture. Animals clinically suspected of PPR were isolated from the flock and treated with procaine penicillin to manage secondary bacterial infections (OIE, 2008; Adombi *et al*. 2011).

3.4.2. Samples for serological investigation

Blood samples (n=102) were collected from clinically suspected sheep and goats in the designated area through jugular vein puncture using sterile needles and vacutainer tubes. Prior to sample collection, individual animals were restrained by their owners to prevent unexpected injuries and minimize unnecessary stress. The area was cleaned and disinfected with 70% alcohol before puncture (Appendix 15). After disinfecting the jugular vein site, 5ml of blood was collected by sterile disposable needle into a sterile plain vacutainer tube free of coagulants (without EDTA) under aseptic conditions (Appendix 9). Corresponding to each sample, the date of collection, specific identification number, age, species, origin, and sex of the animal were recorded on the vacutainer tube. In parallel, all relevant

information regarding the infected animal(s) was documented using a format of sample collection (Appendix 14) (OIE, 2008; Adombi *et al*. 2011).

The blood samples collected in plain vacutainer tubes were allowed to stand in a slanted position for 24 hours at room temperature to facilitate serum separation. After 24 hours, samples with unseparated serum were centrifuged until the clear serum was separated from the whole blood. The serum was then harvested using sterile micropipettes or by pouring into cryogenic vials and kept chilled in a cool icebox until reaching the Yabello Regional Veterinary Laboratory, where it was stored at -20°C. Finally, the samples were transported to the National Veterinary Institute (NVI) for serological analysis.

3.5. Molecular detection of PPR virus

By utilizing a one-step real-time RT-PCR kit that targets the virus's N-gene, swab (nasal and ocular) samples taken from clinically sick sheep and goats were examined for the presence of PPRV RNA.

3.5.1. Extracting RNA.

After bringing the swab samples at room temperature, they were prepared for RNA extraction. Then to determine the presence of PPRV RNA, the viral RNA was extracted from the processed nasal and ocular swab samples using a commercial RNA extraction kit (Qiagen ® RNeasy Universal Mini Kit, Germany) and following the manufacturer's instructions, viral genomic RNA was extracted from collected samples following the manufacturer's instructions (its procedures are listed in (Appendix 1). And an extracted viral RNAs were kept for later analysis at -80°C.

3.5.2. Real time RT-PCR

The reverse transcription polymerase chain reaction (RT-PCR) technique was employed to figure out whether genomic RNA was present or not in the samples that had been collected.

Standardized RT-PCR was used to detection of PPRV nucleic acid from the extracted sample. The extracted samples were subjected to real-time RT-PCR using an Applied Biosystems 7500 fast one step real time PCR thermocycler machine. PPRV-specific forward and reverse primers were also used to amplify the cDNA after reverse transcriptase was employed to convert the extracted RNA into cDNA (Batten *et al*., 2011).

The RT-qPCR was performed in a final reaction volume of 20 µl (Appendix 3) containing that contains 10 µl of Express Universal superscript (Invitrogen), 2 µl of superscript enzyme, 0.4 µl of passive reference Rox, 0.8 µl of each primer PPRV forward primer (5′AGA GTT CAA TAT GTTRTT AGC CTC CAT 3′), PPRV reverse primer (5′TTC CCCART CAC TCT YCT TTGT 3′) and 0.4 µl of PPR probe (FAM-CAC CGG AYA CKG CAG CTG ACT CAG AAQSY), 2.6 µl of RNAse-free water, and 3 µl of RNA template. By estimating the total amount for total samples, master mix was prepared in aseptic environment to minimize contamination (Appendix 3). Then 17 µl was added to each plate by adding 3 µl from each sample. Amplification was performed at 50°C for 15 min, 95°C for 20 seconds, followed by 45 cycles of denaturation and annealing at 95°C for 3 seconds and extension at 60°C for 30 seconds. The samples that had a Ct value < 35 were considered positive (Batten *et al*., 2011).

3.6. Culture and viral isolation

Isolation of PPRV through cell culture methods remains the gold standard for virus detection (Hemmatzadeh *et al*., 2016). The PPR virus was cultivated and propagated using Vero Dog SLAM (VDS) cells (Pirbright Laboratory, UK) cultured in 24 well culture flasks with a 2 cm2 growing area. Thus, positive samples identified via RT-PCR underwent further processing for virus isolation by infecting Vero Dog SLAM (VDS) cells using the technique outlined by (OIE, 2008). Following homogenization and centrifugation of the swab samples at 3000 rpm for 20 minutes at 4°C, the resulting supernatant was utilized to inoculate Vero Dog SLAM (VDS) cells grown on 24-well plates.

By adding 100 µl each sample to each test wells with VDS monolayer cell culture pre washed twice by 500µl PBS followed by inoculation of VTM containing antibiotics and antifungals on negative control flask wells. The inoculated cells were then monitored under an inverted microscope for any nonspecific reactions and incubated at 37°C, 5% CO2 and 96% humidity for 7 days. Over the course of consecutive days, the cells underwent daily examination under an inverted microscope to assess the presence of cytopathic effects (CPE) indicative of viral replication (OIE, 2008; Mallinath *et al*., 2018).

3.7. Serological detection of antibodies against PPR Virus

Serum samples from sheep and goats were tested using blocking ELISA (b-ELISA) to detect anti-PPRV antibodies. Sera collected from goat (n = 94) and sheep (n = 8) were tested for the presence of PPRV-specific antibodies using blocking ELISA (b-ELISA). All procedures were carried out according to the instructions in the manual included with the kits and as described by (Bodjo *et al*., 2018). The assays and its procedural steps were performed and analyzed following the manufacturer's instructions (Appendix 5). The optical density (OD) of each well was read using a spectrophotometer reader with the filter at 450 nm wavelength. The reader was connected to computer loaded with ELISA data interchange software that was used to automate reading. Serum samples with percentage inhibition (PI) value $\leq$30% (PI$\leq$30%) were considered negative. Samples with percentage inhibition (PI) value $\geq$35% (PI$\geq$35%) were considered positive. And it is doubtful when percentage inhibition (PI) value becomes between 30% and 35% (30 %< PI<35%) (Bodjo *et al*., 2018).

3.8. Questionnaire survey

To gain insight into potential factors affecting PPR occurrence in the study area, all household heads of the sampled small ruminant flocks were interviewed. Through in-person interviews with the owners and careful observation of the sheep and goats, relevant data were collected using a semi-structured questionnaire translated into the local language (Afan Oromo) (Appendix 17). The questionnaire gathered information on species, age, sex,

breed, physical body condition, flock size, livestock movement to neighboring countries, various management practices, shared water and feed sources, contact with other flocks and wildlife, source of animals for restocking, distance from livestock markets, and vaccination status. During the sample collection and face-to-face interview data collection phase, one expert veterinarian and a local trained enumerator were involved (Fig 4). In parallel, awareness was raised in the community about PPR disease, its transmission, and control and prevention practices. This comprehensive approach aimed to identify key risk factors associated with PPR occurrence and transmission in the study area, which is crucial for developing targeted prevention and control strategies.

Figure 4: During interviewing the owner to gather PPR related information.

3.9. Data Management and Analysis

Data obtained from serological tests, PCR result, questionnaires survey from the field and laboratory data were incorporated into a Microsoft Excel 2013 spreadsheet. Then the data was thoroughly checked to evaluate errors and any irregularities before sorting, coding, and testing for integrity. The collected data was analyzed by STATA/IC 14.2. To measure the agreement of the two diagnostic techniques that performed on the common animals,

and to know how well the two measurements of the same animals agree or not, we use the Cohens kappa. Subsequently, Along with positive and negative predictive values, Kappa statistics (κ) was analyzed to measure the agreement between the two dependent categorical samples measured by using online statistical program with 2-way Contingency Table Analysis (Hripcsak and Heitjan, 2002).

3.10. Ethical Consideration

The study was carried out in accordance with the Animal Research Ethics Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University and ethical approval for the study was ensured by the approval certificate with reference number VM/ERC/04/15/2022 indicated in (Appendix 18). The owners were informed about the purpose of the study and consent was sought. The samples for this investigation were carefully gathered without causing any harm to the animals and in accordance with all ethical standards for sample collection and sampling methods.

4. RESULTS

4.1. Factual evidence and clinical observation at study area

Based on information available in outbreak records, during field study and data collection the most frequent clinical findings in diseased animals was a complicated clinical signs such as a serous discharges from the eyes and conjunctivitis, serous nasal discharge, crusts around the nostrils, necrotic lesions around the lip, erosion of gums and buccal mucosa and anorexia, a sudden onset of depression, fever, off feed, difficulty in breathing and coughing, mild to severe diarrhea, emaciation and death were observed in the affected sheep and goats (Fig 5). The hemorrhage of gastro intestinal tract, congested lung with splenomegaly was identified in dead goat by PPR cases. Sheep did not exhibit any clinical symptoms and signs.

From 48 animals, showed notable clinical indicators, the common observed exhibited clinical signs were serous nasal discharge and difficulty in breathing as a result of occluded air passage by a thick mucopurulent deposit in nostrils (85.4%), followed by crusts around the nares (81.3%) and sudden depression and emaciation (77.1%). Lacrimation of an eye and diarrhea cases were the least clinical manifested in study area.

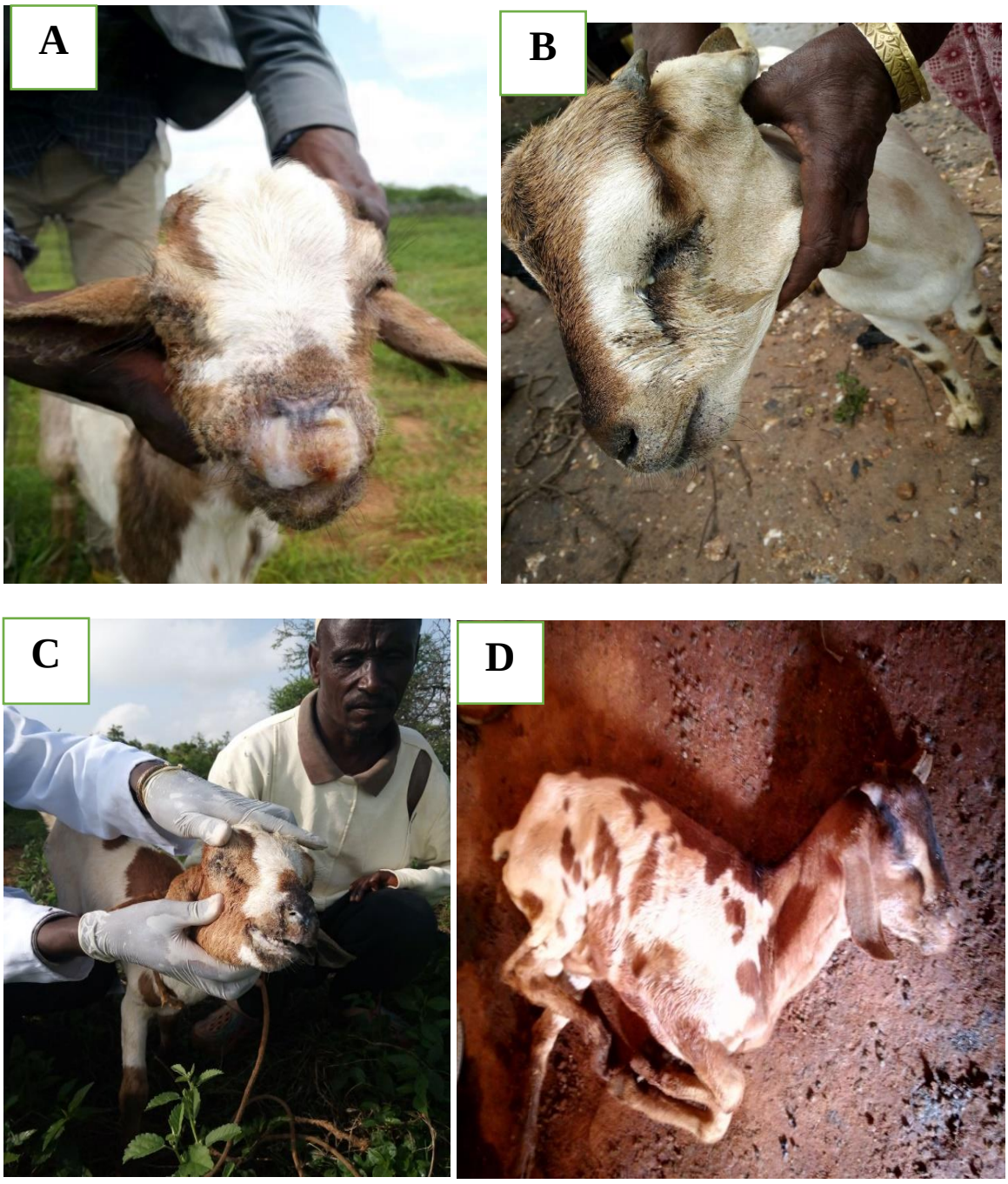


Figure 5: Typical clinical signs of PPR observed in study area.

(A): Sever nasal and ocular discharges with necrotizing and erosive stomatitis; (B): Acute case of PPR with eye discharge; (C): Oculo-nasal discharges with necrotizing, erosive stomatitis and unable to visualize, (D): Goat unable to stand due to severe and chronic PPR case.

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

To detect the PPRV nucleic acid, a total of 48 samples (ocular and nasal swabs) were taken from sick animals exhibiting clinical signs related to PPR. Among them 54.2% (26/48) were positive by RT-qPCR with variable coefficient thresholds *Ct* values ranging from 16 to 31 for the sheep and goats swab samples (Fig 6). The higher viral load was reported from nasal swab with Ct value of 16.04, while the highest viral load in ocular had a Ct value of 16.28. The overall range of coefficient thresholds was 16.04 to 30.9 from nasal and ocular swabs respectively.

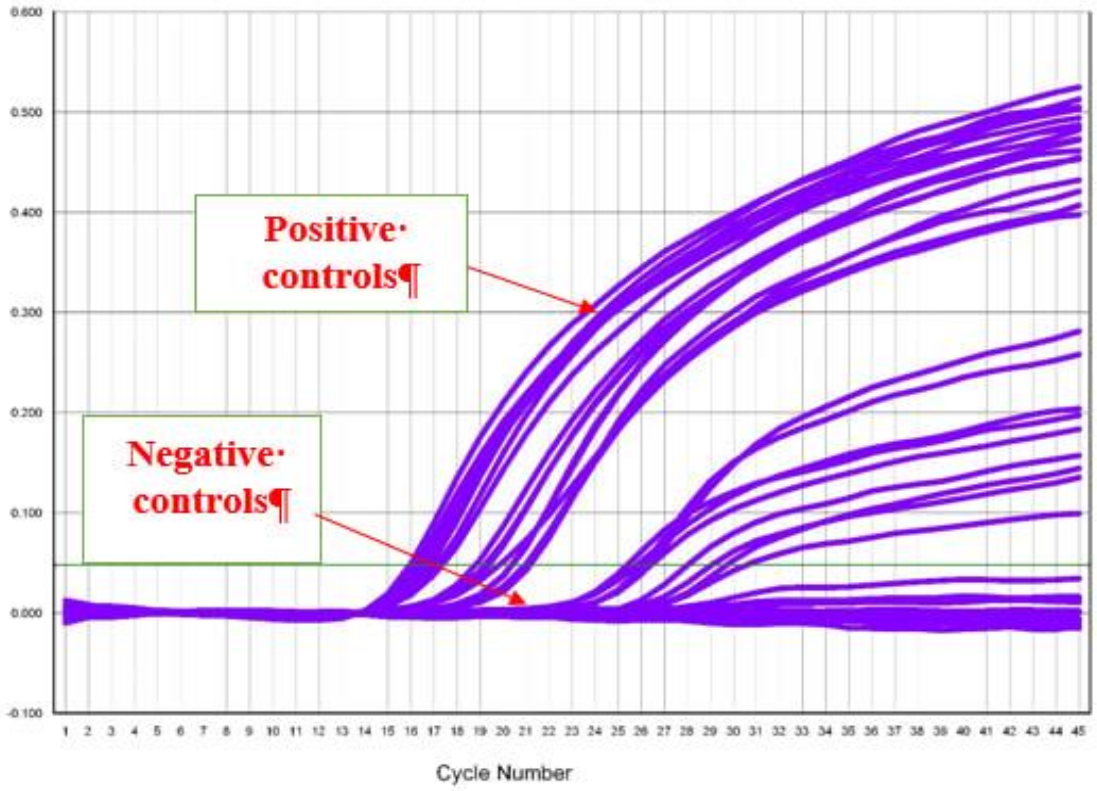


Figure 6: Real time PCR standard curve indicating PPRV detection result.

With an individual cycle threshold (Ct) value of the present study outcome, the majority of infected animals (53.8%) had a high viral load (16≤21), nearly one-third (30.8%) had the lowest viral load with Ct value (26≤31) and 15.4% had a moderate viral load (21≤26) Ct value (Fig 6).

Table 1: Zone, district and kebele wise detection rate of PPRV nucleic acid

Factors		**Levels**	**No. tested**	**No. positive (no)**
Zones		Borana	42	23(54.8%)
		East Borana	6	3 (50%)
		Total	**48**	**26(54.2%)**
Districts		Moyale	42	23(54.8%)
		Arero	3	1(33.3%)
		Dhas	3	2(66.7%)
		Total	**48**	**26(54.2%)**
Kebeles	Moyale	Lag sure	29	19(65.5%)
		Madomigo	13	4(30.8%)
	Arero	Mata gafarsa	3	1(33.3%)
	Dhas	Mata Arba	3	2(66.7%)
		Total	**48**	**26(54.2%)**

Zone level indicated that more samples were gathered from the Borana zone (Moayale) than from East Borana. District-wise investigation revealed that Dhas district (East Borana zone) showed the highest percentage of PPRV's nucleic acid detection, reaching 66.7%, while Moyale district (Borana zone) demonstrated a substantial percentage of 54.8%. Conversely, Arero district (East Borana zone) demonstrated the lowest percentage (33.3%) (Table 1). The clinical exhibition of PPR was varied in species (Table 2), in which it was higher in goat (95.8%) than sheep 2(4.2%).

Table 2: Species, age and sex wise detection rate of PPRV nucleic acid

Factors	Categories	No. tested	No. positive
Species	Goat	46	25(54.3%)
	Sheep	2	1(50%)
Age	Young	14	8(57.1%)
	Adult	11	6(54.5%)
	Old	23	12(52.8%)
Sex	Male	21	15(71.4%)
	Female	27	11(40.7%)

Regarding age factors, a considerable sample collected from the old, accounting for 47.9%, with the young constituting 29.2% samples. The adult was the least (22.9%). Interestingly, despite this variance in sample size, the detection of PPRV exhibits a little variation, from highest to lowest young, adult and old by 57.1%, 54.5% and 52.8% respectively (Table 3). Although there were more female (56.2%) in the sampled, male (71.4%) showed more RT-PCR positive outcomes than female (40.7%) (Table 2).

4.3. Isolation of PPR virus

Samples that tested positive for PPRV using RT-qPCR were cultured on Vero Dog SLAM (VDS) cells. Among the 26 samples cultured, 17 (65.4%) displayed typical cytopathic effects starting from three days post-inoculation. These isolates exhibited characteristic cytopathic effects (CPE) in VDS cells, showcasing aggregation, clustering, and syncytia formation, detachment of the cell (Figure 7 A, B and C). Conversely, the negative control (Phosphate buffered saline -inoculated) cell culture did not show any cytopathic effects (Figure 7 D).

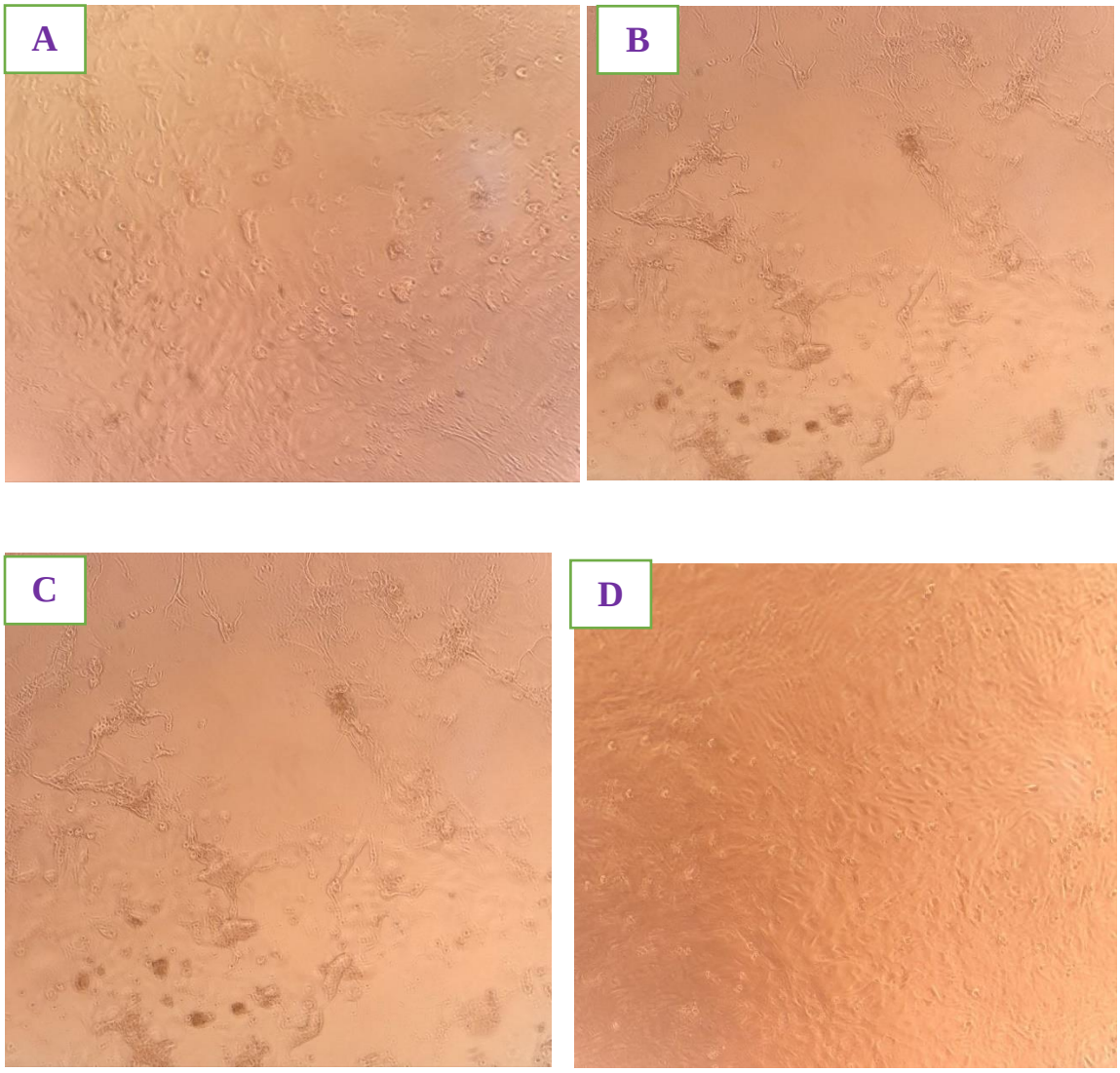


Figure 7: Virus isolation in VDS cell Culture.

(A) And (B): Forming rounding, aggregation and syncytia formation, (C): detachment of the cell, (D): Negative control.

4.4. Screening of PPR antibodies by b-ELISA

Out of the total 102 sera samples tested for PPRV antibodies, 70 (68.6%) were detected positive by blocking ELISA (b-ELISA). Among the species the higher percentage of goat (92.2%) was included in sampling compared to sheep (7.8%). From the total, seropositive was highly verified in goat (71.3%) than sheep (37.5%) (Table 3).

Table 3: The total seropositive during an outbreak in study area

Sampled species	Reason of sampled	Screened (n)	Seropositive (%)	Seronegative (%)
Goats	Outbreak	94	67(71.3%)	27(28.7%)
Sheep	Outbreak	8	3(37.5%)	5(62.5%)
	Total	**102**	**70(68.6%)**	**32(31.4%)**

Notably, since the majority of small ruminants in the study had not been previously vaccinated against PPR, the detection of PPRV antibodies suggests the likelihood of PPRV infection rather than vaccination-induced immunity. This finding highlights the widespread of the virus in the Borana and East Borana zones. Unlike sheep, goat revealed a higher sero-conversion rate (68.6%) (Table 4).

Table 4: Zone, districts and kebele wise antibody detected against PPR

Factors		Levels	No. tested	No. positive (no)
Zones		Borana	74	48(64.9%)
		East Borana	28	22 (78.6%)
		Total	**102**	**70(68.6%)**
Districts		Moyale	74	48(64.9%)
		Arero	14	10(71.4%)
		Dhas	14	12(85.7%)
		Total	**102**	**70(68.6%)**
Kebeles	Moyale	Lag sure	54	36(66.7%)
		Madomigo	20	12(60%)
	Arero	Mata gafarsa	14	10(71.4%)
	Dhas	Mata Arba	14	12(85.7%)
		Total	**102**	**70(68.6%)**

The outbreak was firstly identified in Moyale districts, where there had been more affected animals reported compared to Arero and Dhas districts. The seropositivity rates varied across each districts that 64.9% in Moyale district (Borana zone), 71.4% and 85.7% in Arero and Dhas district, respectively (Table 4). Based on age of animals, the collected serum samples was 59.8 %, 15.7% and 24.5% from old, adult and young respectively. The positivity rate varied across age groups, with the highest percentage found in old animals (78.7%), followed by adults (62.5%), and the least was found in the young (55%) (Table 5).

Table 5: Species, age and sex wise detection rate of antibody against PPR

Factors	Categories	No. tested	No. positive
Species	Goat	94	67(71.3%)
	Sheep	8	3(37.5%)
Age	Young	25	12(48%)
	Adult	16	10(62.5%)
	Old	61	48(78.7%)
Sex	Male	36	22(61.1%)
	Female	66	48(72.7%)

Out of the total samples collected, 64.7% were from female animals and 35.3% were from males. For serological investigation, the samples tested positive from female animals was 72.7%, while 61.1% in males (Table 5).

4.5.Quantify agreement with Cohen's kappa results

Out of 27 samples collected from a common animals for detection of PPR's nucleic acid and immunogenic level of animals, in RT-PCR tested reported, 13 (48.1%) samples was positive as compared to b-ELISA which reported 21(77.8%) samples was positive for PPR virus. Further analysis of samples revealed that 4 (14.8%) samples which were negative by b-ELISA reported positive with RT-PCR. By contrast 12 (44.4%) samples shown negative by RT-PCR techniques were positive in b-ELISA test (Table 3).

Table 6: The frequency of the data with respective test results

		B-ELISA		
		Positive	Negative	**Total**
RT-PCR	Positive	9	4	**13**
	Negative	12	2	**14**
	Total	**21**	**6**	**27**

The results of both tests in identifying samples that are positive or negative, as shown in the contingency table (Table 6), it suggest there is minimal correlation between b-ELISA and RT-PCR. Both RT-PCR and b-ELISA tests were in agreement with each other showing 9(33.3%) samples as positive and 2(7.4%) samples as negative. Number of observed agreements (Po) = 11/27=40.7% that the overall agreement between the two tests was of 40.7% with kappa value -0.16. Since Kappa <0, it suggesting that no agreement between the two tests (RT-PCR and b-ELISA).

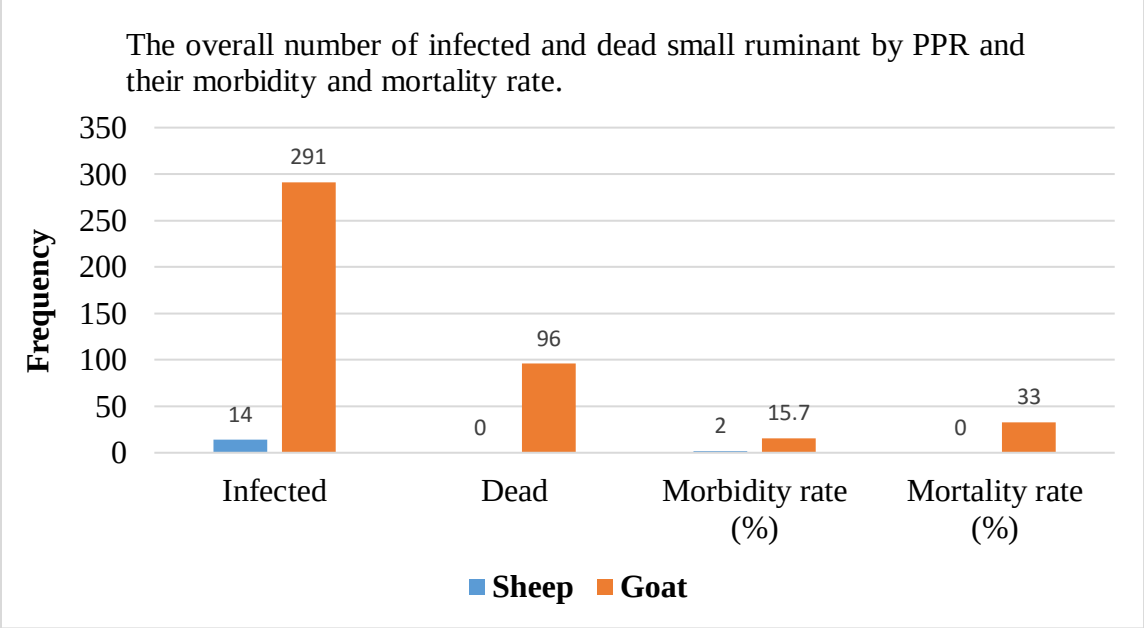
4.6. Questionnaire Survey analysis

To better understand the risk factors associated with PPR and the perceptions of small ruminant owners regarding its emergence, a total of 61 interviews were conducted. Among the participants, 47 (77%) were male and 14 (23%) were females. Education levels varied, with 49 (80.3%) having no formal education, 8 (13.1%) completing primary education, and 4 (6.6%) achieving a secondary education. The owned flock sizes of the community ranged from 10 to 150 animals, with an average of 20-30 sheep and goats per household.

According to the survey, the primary suspected source of the disease was restocking, reported by 43 (70.5%) respondents, often facilitated by non-governmental organizations (NGOs) bringing animals from unknown locations to replenish livestock lost during droughts. The survey also revealed that nearly all respondents (98%) practiced extensive management systems, allowing communal free-range grazing without separating sheep and goats from non-native animals and wild ruminants leading to frequent contact at watering points. Housing systems predominantly involved fencing (54.1%) or housing (45.9%), with kids separated from does at night for milking, while lambs and ewes remained together as ewes were not utilized for milk production.

Clinical signs of PPR reported by owners included serous discharges from the eyes and nose, oral sores, necrotizing stomatitis, crusts around nostrils, labored breathing, fever, depression, coughing, diarrhea, and eventual death. Morbidity and mortality rates varied

across districts, reaching up to 11.9% and 31.5% respectively as indicated in (Figure 8). Moyale district emerging as a central livestock market for Ethiopian and Kenyan traders, lacking quarantine practices at the Kenya-Ethiopia border. Majority of respondents (93.4%) do not isolate sick animals from the flock during both in day and at night. Goat infections were reported to be more severe (96.7%) than sheep, with all reported deaths occurring in goats alone. Mortality rates were highest in Moyale district (82.2%), followed by Arero district (19.8%), with no reported deaths in Dhas district. In estimation the total economic loss from death of small ruminant, especially goat of the interviewed farmers was 384,000 ETB.



Total no. (Goat =1857, Sheep = 711)

Figure 8: Bar chart showing the infected and dead animals by PPR in study area.

5. DISCUSSION

The current study, the manifested typical clinical sign of PPR was eye discharge, muco-purulent nasal discharge, complicated with breathing difficulty and diarrhea. The observed clinical signs was comparable with (Abd El-Hakim, 2006; Abubakar *et al*., 2008b; Mishra *et al*., 2020; Ba *et al*., 2024). It is noticed that, clinical signs might be associated with immunohistochemically and pathological symptoms stated by Kumar *et al*. (2004) and Toplu *et al*. (2004). Immune suppression associated with morbillivirus infection was caused by reducing CD46, inhibiting leukocyte proliferation, and leading peripheral blood mononuclear cells to die (Heaney *et al*., 2002). These all mechanisms could influence the disease's clinical appearance and animal morbidity by PPR case.

On the basis of clinical signs from an outbreak, goats was the only species suffering with clinical symptoms but amazingly no clinical evidence of PPR reported in sheep flock. It is in line with (Ahmad *et al*., 2005; El-Yuguda *et al*., 2009) stated that while sheep grazing among PPR-infected goats, none of them died or developed any clinical symptoms. Once a goat showed the typical clinical signs and symptoms of PPRV, there was certainly an elevated probability of death. It supported by (El-Yuguda *et al*., 2009; Ba *et al*., 2024).

But intestinal hemorrhage and pneumonia, splenomegaly, and enlargement of lymph node are the most common post-mortem pathological finding in PPR cases. This finding is matched with (Abd El-Hakim, 2006; El-Yuguda *et al*., 2009; Kardjadj *et al*., 2015; Abiyu, 2022), in which the causes of death in animals infected with PPRV are pneumonia-related respiratory failure and dehydration driven from enterocolitis.

The current study revealed an overall 54.2% samples were positive by RT-qPCR which is very similar with Mohammed *et al*. (2022) (54.2%) which recorded prior from the same study area (Arero district) since 2020. Compared to the previous finding, the current PPRV confirmed by RT-PCR was higher (Saritha *et al*., 2015) (25%), (Alemu *et al*., 2019) (46.4%), (Mishra *et al*., 2020) (35.6%) and (Rahman *et al*., 2023a) (35.6%). Still the percentages of the current PPRV nucleic acid detected was less than the earlier reported

by Kardjadj *et al*. (2015), Liu *et al*. (2018), and Kabir *et al*. (2020) with 58.06%, 63.3%, and 78.95%, respectively. These all variation in positive result could be the consequence of different targeted gene types in detection, initial concentration of virus in the clinical material, viral-host infection phases, sample type, sampling approaches and delivery time of the sample to laboratory all these could contribute to different results (Ahmad *et al*., 2005; Alemu *et al*., 2019).

The percentage of young animals that showed clinical PPR symptoms was higher than adult and old. It is consistent with (Gomes *et al*., 2016; Nabi *et al*., 2018; Ba *et al*., 2024) who found young goat were more commonly affected by PPR than adults. It disagree with (Mallinath *et al*., 2018) who found young was more severe than old. Further (Reta, 2024) found no variation related to animal's age. The probability to high percentage in young animal could be due to the decreasing the protective threshold of inherent immunity level of the young animal at 4 to 5 months (Diallo *et al*., 2002). Furthermore, compared to kids, lambs are protected by maternal antibodies for a longer duration of time (Awa *et al*., 2002; Diallo *et al*., 2002).

In species-wise finding, morbidity and mortality rate were highly reported in goat than sheep. It's in congruent with (Kumar *et al*. 2002; Abubakar *et al*., 2008a) who stated PPR virus mortality was higher in goat than sheep. The same results and observation were reported by (Singh *et al*., 2004b; Abd El-Hakim, 2006; Wernike *et al*., 2014), in which clinically goats was demonstrated the typical moderate to severe clinical symptoms than sheep. It also supported with earlier outbreak investigation (Singh *et al*., 2004b; Abubakar *et al*., 2008a; Delil *et al*., 2012) in which, goats are more severe than sheep. This variation in severity and clinical symptoms was clarified by Rajak *et al*. (2005) that, the PPR virus has a severe immunosuppressive effects in infected goats while fails to exhibit similar effects in infected sheep.

Contradict to the current study, Wernike *et al*. (2014) reported that although the disease appears to be less severe in sheep, following a large PPR outbreaks, a thousands of sheep have been killed in Europe. Furthermore, in Benin (Adombi *et al*., 2017) informed that

only sheep were affected by PPRV. This difference in susceptibility might be due to the simultaneously exposure of host by multiple pathogen and distinct host genetics and non-genetic variation (Munir *et al*., 2012). Abraham *et al*. (2005) suggested that the seemingly non-pathogenic nature of the Ethiopian PPRV strains in sheep might be due to a specific species resistance or a decrease in the virulence of the strains.

In the present finding, the influence of sex on PPR outbreaks was found to be higher by RT-PCR in male than female. It is also comparable to that of (Nabi *et al*., 2018; Rahman *et al*., 2023a). But in contrast to the current finding, Ahaduzzaman, 2020) reported that PPR was higher in female animals than in male animals.

The agreement between RT- PCR and b-ELISA was 7.4% and 33.3% tested negative and positive respectively with overall agreement was 40.7%. The confirmed Cohen's kappa value was -0.16. Since kappa value < 0, it suggesting that no agreement between the outcome of the two tests. It indicated that an affected animals were at different stage of infection that, while they still excreting the virus, starting to generate antibodies within a few days post infection. It is in line with Baron *et al*. (2014) in which PPRV is highly concentrated in secretions and tissue samples; however, after a few days, antibody responses develop and making PPRV difficult to detect in secretions.

It also consistent with the earlier experimental study (Balamurugan *et al*., 2006; Wernike *et al*., 2014) starting from 7 days confirmed positive by using both the one-step RT-PCR and ELISA tests. It is further corroborated with Halecker *et al*. (2020), who found that animals infected with the highly virulent seroconverted virus 4-7 days after the onset of the first clinical indications and the maximum RNA loads were detected between 4 and 10 days post infection. This variance may be caused by the type of virus strain and the host animal's immunological response to the disease.

In contrast to the experimental testing conducted by Pope *et al*. (2013), in which all of the animals that survived the disease were seropositive by 12 days after viral inoculation. The animals reached their peak clinical symptoms and started to recover by 9 days following

infection. An elevated antibody levels against PPRV during an outbreak was 68.6%. It revealed that the likelihood of antibody generated in active case was also high. It is very similar with (Devi *et al*., 2016), who reported higher generated antibody percentage was observed in clinically suspected animals (68.6%) than samples collected from apparently healthy (5.3%).

From all clinical samples (26 samples) positive for viral nucleic acid detection were inoculated on VDS cells for isolation and propagation of PPRV. Out of them 17/26 (65.4%) were grown on the VDS cells. This finding is higher than the previous finding 21% (Ahmed *et al*., 2021), 60% (Abiyu, 2022), 25% (Mohammed *et al*., 2022) who found positivity of the virus on the Vero cell. This variation in outcomes might be because the virus is an RNA virus, that heat-labile, and because there could be leakage in the cold chain during sample shipment and cell cultivation done (Latif *et al*., 2018).

The probability of cultured samples failed to show CPE might be due to viral load in specific sample and the probability of dead virus detected in RT-PCR, but not growth in cell culture. Furthermore, an exact time of sample collection could have impact on an outcome for nucleic acid detection and cell cultures for a common sample(s). It supported by (Couacy-Hymann *et al*., 2007; Pope *et al*., 2013) who concluded that even before any clinical symptoms manifest, as early as five days post-infection (dpi), PPRV (nucleic acids) can be found in oral, nasal, and lachrymal secretions. However, it cannot be demonstrated within a first days and it takes seven days after infection (dpi) for the infectious virus to virus isolation from conjunctival swabs.

The characteristics cytopathic effect (CPE) of isolated virus in VDS cells was observed within 3 days post-inoculation rounding and aggregation culminating in syncytia formation, detachment of the cells. It is comparable with that is similar with the previous outcome by (Adombi *et al*., 2011; Mallinath *et al*., 2018; Abiyu, 2022; Mohammed *et al*., 2022), who found that the cytopathic effect (CPE) in VDS cells developed aggregation or clustering together, and syncytia formation of the cells. It also agreed with (Ahmed *et al*., 2021; Rana *et al*., 2023). Compared to the previous finding in Ethiopia (Veronica *et al*.,

2019) within 5 days, Egypt (Ahmed *et al*., 2021) within 5 days, Pakistan (Rana *et al*., 2023) 14 days' post-infection, the cytopathic effect of the present study was observed early within 3 days.

Serologically 70 (68.6%) out of 102 screened sheep and goat from flock were seropositive for PPR. The finding showed that the antibodies found were associated with an active illness because there was no history of vaccination. The present outcome almost similar with those of Devi *et al*. (2016) and Kabir *et al*. (2020) who reported samples tested from infected animals showed presence of PPR viral antibody was 68.6% and 68.4% respectively. The current finding was lower than (Abd El-Hakim, 2006) 83% and (Ahmad *et al*., 2005) 100%. But it was higher than that reported from other region of Ethiopia, Awash Fentale district of Afar with 36.6% (Delil *et al*., 2012).

Based on their sex, percentage of PPR was higher in female 72.7% compared to male (61.1%). It agree with the previous finding (Megersa *et al*., 2011). It disagree with (Rahman *et al*., 2023a) who confirm that it was higher in male than female. But (El-Yuguda *et al*., 2009; Reta, 2024) confirm no variation between male and female animals. Arguably, variations in positivity rates could potentially be attributed to the type of serological kit utilized, size of sample, and the collection technique, physiological variations between both participant species.

Specie-wise percentage was higher in goat (71.3%) than sheep (37.5%) it matched with other finding in Ethiopia (Megersa *et al*., 2011; Delil *et al*., 2012). Similar finding in China (Liu *et al*., 2018), India (Balamurugan *et al*., 2021) and Pakistan (Ahmad *et al*., 2005). But it contradict with (Abubakar *et al*., 2009; Younus *et al*., 2020) who revealed that the PPR antibodies were found to be more prevalent in sheep than in goats.

According age category, the finding was age related, in which old was the highest seroconverted (78.7%) followed by adult 62.5%. Young was the least (55%) seropositive for b-ELISA. The findings in line with (Ahmad *et al*., 2005; Abubakar *et al*., 2009; El-Yuguda *et al*., 2009), who observed that percentage was increased gradually with age and

it related to the accumulation of recovered sick animals over time which maintained in a flock for production. But contradict with the previous finding (Ba *et al*., 2024; Reta, 2024) in which there is not a correlation between the age of animals and the PPR outbreak occurrence. An exposure rate, the host's immunological status, and the wide range of virus strains could all contribute to a notable variation in disease severity.

According to the interviewees the source of disease in study area were associated with three reason; mixing to original flock of the newly purchased animal from a local market, re-stocking funded by non-governmental organization (NGO) by purchasing small ruminants from unknown source and contact of native species with migratory small ruminant at open free grazing area. It is in line with (Gebre *et al*., 2018; Liu *et al*., 2018; Abiyu, 2022) who reported the animal mixed in to the flock from outside was considered as the putative risk factor to PPR. It also agreed with (Abubakar *et al*., 2009; Bwihangane *et al*., 2016; Rahman *et al*., 2023b) who states an introduction of recently purchased animals from a common marketplace without proper vaccination details and failure to implement strict quarantine measures was the main source of PPR disease.

Further (Luka *et al*., 2012a; Bwihangane *et al*., 2016) reported that, the main factors contributing to the spread of disease was the uncontrolled and unauthorized movement of livestock for trade or grazing purpose. In current study keeping together of a large stocking density on a common feeding or watering source was identified as the means of disease transmission. This is in corroborated with (Selvaraju, 2014; Gebre *et al*., 2018; Rahman *et al*., 2023b) who revealed that, due to increased stresses in overcrowded animal predisposes the outbreak of PPR, high concentration of animals in close proximity to each other predisposes for the occurrence of PPR. In opposite, Hasan (2012) revealed that non-grazing small ruminants have been greater seropositive that free grazing.

From current questioner survey, mortality rate was higher in goat (33%) than sheep (0%), it is very similar with the previous reported by (Mishra *et al*., 2020; Balamurugan *et al*., 2021). In the current study, the morbidity and mortality rate was 11.9% and 31.5% respectively that was lower than the previous finding in Ethiopia (Abiyu, 2022) morbidity

and mortality rate was 43.6% and 9.1%, respectively, India (Mishra *et al*., 2020) the morbidity and mortality rate was 90% and 42%, respectively, Nigeria (El-Yuguda *et al*., 2009), morbidity and mortality rate was 63% and 17% respectively. This variation could be related to a mutation in the viral genome or to specific characteristics of the host and surrounding environment and an immediate control measures taken to minimize its dissemination. .

From the responder, one of the risk factor for PPR was isolation status, in which no habitual of segregating sick animals from health flock. It supported by (OIE, 2008), that states an isolation of PPRV-infected animals from healthy can lower the risk of infection and the likelihood of disease transmission through contact with other infected animals and fomites. Small ruminant migrations that have not been under control and interactions with wild animals could be the main reason for the emergence of PPR in the study area. It agree with Parida *et al*. (2016) who reported, wild ruminant populations could act as a link between remote populations of healthy and infected small ruminant populations, accelerating the spread of PPRV. Mahapatra *et al*. (2015) also noticed the PPRV infection in wildlife primarily in semi-free range environments. And sero-survey conducted at the interface between domestic small ruminants and wildlife found the spread of a virus from infected domestic animals to wild animals.

6. CONCLUSION AND RECOMMENDATIONS

The evidence generated in this study, including repeated outbreaks accompanied by notable fatalities, consistent clinical presentations, elevated antibody levels against PPRV, successful viral isolation, and precise nucleic acid detection through RT-qPCR all converge to unambiguously designate Peste des Petits Ruminants Virus as the driving force behind the ongoing outbreaks in the study area. Thus, this study affirms the endemic presence of PPRV within goat and sheep populations across the three studied districts. This study confirms the presence of PPRV, however, the specific circulating strains remain unidentified. Therefore, it is crucial to conduct further investigations to identify the common strains circulating in the area and in the country at large. These findings underscore the critical role of unauthorized animal mobility between Ethiopia-Kenya border, lack of isolation and quarantine practices in PPR occurrence, emphasizing the need to integrate such data into surveillance and management frameworks, alongside other risk indicators.

Therefore based on this conclusion the following recommendations are forwarded.

- A regular and effective mass vaccination against the disease among small ruminants should be done and since stress influences on the production of antibodies, reducing stress during vaccination.
- Monitor animals closely and frequently for any developing illness or signs of disease.
- Differentiation of infected from vaccinated animals (DIVA) diagnostic tests should be available.
- Strengthening surveillance and post-vaccination sero-monitoring at the national level.
- Transportation of animals across the borders should be restricted.
- Movement of sheep and goats from affected region(s) should be limited and implement the quarantine practices on border and for newly purchased animals.
- Isolating the symptomatic animals from a flock and disinfect the contaminated properties and eliminate contact fomites to limited the spread and severity of PPR outbreaks as well as prevent the incidence of the disease.

- Aware the community by mass media (by Radio, TV, newspapers and etc.) on prevention and control of PPRV transmission.
- Since the role of wildlife in the maintenance and transmission of PPR remains unclear, restricting livestock-wildlife interactions is required and the natural reservoirs of PPRV in wild animals should be known.

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8. APPENDICES

Appendix 1: Procedure of RNA extraction.

Extraction has four steps

1. **Lysis**

- Prepare lysine buffer solution= n* 560 µl, when n= number of sample
- Prepare Carrier RNA= n* 5.6 µl
- Mix the lysine solution and carrier RNA
- Dispense 560 µl of mix solution in to sterilize centrifuge tube
- Vortex swab specimen fluid
- Transfer 140 µl of the sample into the micro centrifuge tube containing carrier RNA and lysine solution.
- Vortex for 3 seconds to mix and incubate at room temperature for 10 min.

2. **Binding**

- Add 560 µl of absolute alcohol (ethanol) to a centrifuge tube containing lysine solution, carrier RNA and sample
- Vortex for 3 seconds and bring Q Amp mini spin column with silica- membrane
- Add 630 µl from total solution to Q Amp mini spin column and keep it in a clean 2ml collection tube.
- Add 630 µl from total solution to Q Amp mini spin column
- Centrifuge at 8000 rpm for 1 min
- Discard the flood and transfer column filter to a new collection tube

3. **Washing**

- Add 500 µl of wash buffers AW-1 in Q Amp mini column
- Centrifuge at 8000 rpm for 1 min
- Discard the flood and transfer column filter to a new collection tube
- Add 500 µl of wash buffers AW-2 in Q Amp mini column
- Centrifuge at 14000 rpm for 3 min
- Discard the flood and transfer column filter to a new collection tube
- Centrifuge at 14000 rpm for 1 min to dry.

4. Elusion

- Add 60 µl of elusion buffer and incubate at room temperature for 3 min
- Centrifuge at 8000 rpm for 1min to elute RNA from the Q Amp mini spin column
- Remove Q Amp mini spin column and the elute RNA was stored at -80 ^{O}C until additional utilize.

Appendix 2: Master Mix preparation of RT-PCR

- Before starting keep the environment free arrange all needed materials
- Thaw all reagents, except reverse transcriptase and Taq polymerase and possibly keep them on ice
- The master Mix preparation must be carried out in an ice bath (rice flakes)
- Before preparing the reaction mix, it is necessary to calculate the correct volume of the reagents to be used as the protocol.
- Always prepare a mix that will be enough for the number of samples to be tested including the positive and negative controls plus one. The extra one will be compensate for the loss during pipetting.
- Prepare a reaction mixture according to the table below QIAGEN One-step RT-PCR kit.

Appendix 3: Table showing the recipe used in master mix to identify PPRV

Master mix Components	**µl X 1 reaction**	**Final Volume**
Express Universal Superscript	10 µl	10* n, n= No of sample
RNase free water	2.6 µl	2.6 *n, n= No of sample
Superscript enzyme	2 µl	2 *n, n= No of sample
Forward primer NP3	0.8 µl	0.8 *n, n= No of sample
Reverse primer NP4	0.8 µl	0.8 *n, n= No of sample
Passive reference Rox	0.4 µl	0.4 *n, n= No of sample

Probe	0.4 µl	0.4 *n, n= No of sample
Total Volume	17 µl	17 µl is dispensed to each wells
RNA	3 µl	3 µl is dispensed to each wells
Final reaction volume	**20 µl**	**20 µl total solution for amplification**

Appendix 4: General content and information of b-ELISA

1. **Buffers**

a. Blocking buffer (BB)

b. Working buffer (WB)

2. **Assay procedure**

a. Plate layout

b. Distribution of controls and test samples

c. Washing

d. Control buffer and distribution of conjugate

e. Washing

f. Distribution of TMB substrate

g. Stop the reaction (1M H_2SO_4) and read the plate

3. **Result interpretation**

a. Validation of optical density (OD) CB and NC

b. Calculation of the percentage of inhibition (PI)

c. Validation of PI of controls PC and NC

d. Interpretation of the result

Appendix 5: Procedure of b-ELISA

1. Prepare **Blocking buffer** BB (freshly prepared): Dilution buffer (DB + 3% of Skim milk)

i.e.: For 50 ml of BB: 50 ml of dilution buffer (DB) and 1.5g of Skin milk power

2. **Washing buffer** (WB) 1X (it can be stored for a week)

i.e.: For 1000 ml of WB: 960 ml of Fresh distiller or deionized water and 40 ml of WB 25X.

3. Plate layout

NB: Equilibrate the plate and reagents at room temperature for 15 minutes before starting the test.

Appendix 6: Table used in steps of plate layout.

	Control		**Samples**									
	1	2	3	4	5	6	7	8	9	10	11	12
A	**CB**	**CB**	S9									
B	**PC**	**PC**	S10									
C	**NC**	**NC**	S11									
D	NC	NC	S12									
E	S1	S5	S13									
F	S2	S6	S14									
G	S3	S7	S15									
H	S4	S8	S16									S88

4. Distribution of control and test samples

- Dispense 75 µl of b-ELISA BB to all wells
- Control samples
- Control buffer (CB): Dispense 25 µl of BB to well A1 and A2
- Positive control (PC): Dispense 25 µl to well B1 and B2
- Negative control (NC): Dispense 25 µl to well C1, C2, D1 and D2
- Test samples: Dispense 25 µl of each serum per well as (Table 13)
- Shake gently and cover the plate for incubation at room temperature (18-25^{O}C) for 1 hour.

5. Washing

- Empty the test plate and wash all the plate 3 times with 300 µl. At the last washing, blot the plate against clean paper towels to remove the remaining buffer.

6. Control Buffer and distribution of conjugate

- Dilute the C4F3-HRP Conjugate at 1:100 in BB (i.e.: for one plate take 9.9 ml of BB and add 100 µl of C4F3-HRP Conjugate stock).
- Dispense 100 µl of BB to well A1 and A2 (Control Buffer)
- Dispense 100 µl of diluted C4F3-HRP Conjugate to the remaining wells.
- Shake the plate gently and cover the plate for incubation at room temperature (18-25^{O}C) for 45 minutes.

7. Distribution of TMB Substrate

- Distribute 50 µl of TMB (equilibrated at room temperature) to all wells
- Cover and incubate the plate in dark room at 37 ^{O}C for 15 minutes.

8. Stop the reaction and read the plate

- Distribute 50 µl of 1M H_2SO_4 to all wells.
- Read optical density (OD) in wells using ELISA reader with a filter at 450 nm.

9. Assay validation and interpretation

- It justified by validation of optical density (OD) CB and NC as;
- When average OD of wells control buffers (CB) becomes less than or equal to 0.1 ($\leq$0.1).
- When median OD of wells negative control (NC) becomes greater than or equal to 0.7 ($\geq$0.7)

The percentage of Inhibition (PI) was calculated as

PI (%) = (ODnc-ODx/ ODnc-ODcb)*100

ODx=OD Control or sample.

10. Validation PI of controls PC and NC

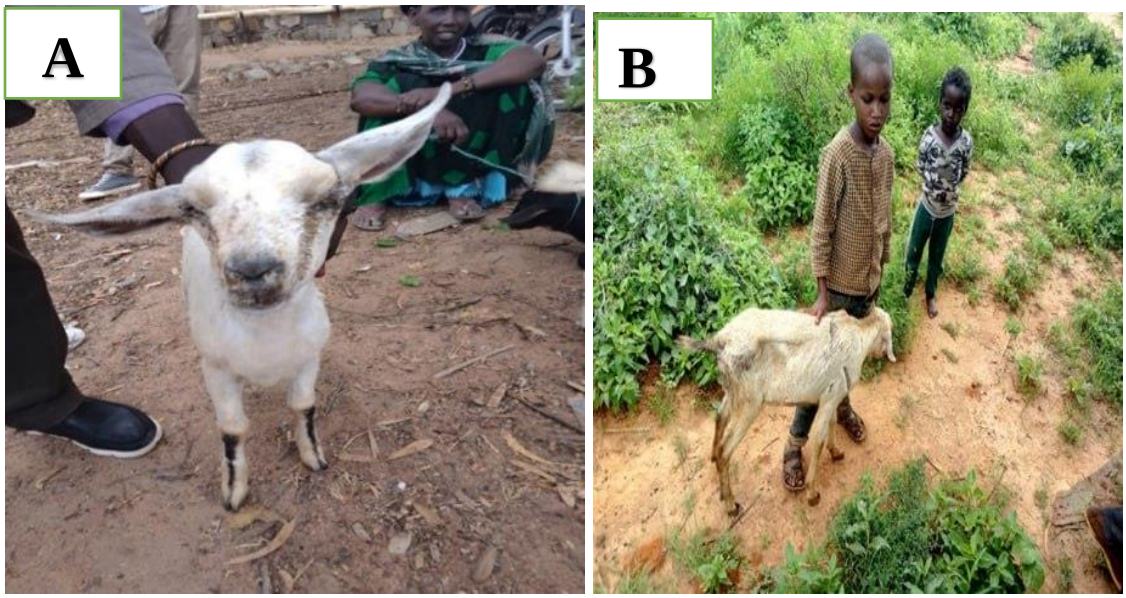
- Positive control (PC): All wells with PI > 70%
- Negative control (NC): At least 3 wells with PI < 15%

11. Interpretation of result

- Serum samples with percentage inhibition (PI) value $\leq$30% (PI$\leq$30%) will be considered negative.
- Samples with percentage inhibition (PI) value $\geq$35% (PI$\geq$35%) will be considered positive.

❖ It is doubtful when percentage inhibition (PI) value becomes between 30% and 35% (30 %< PI<35%).

Appendix 7: Photo during inspecting any anomalies within the flock



Appendix 8: Images illustrating the clinical indicators of PPR seen in outbreak cases.

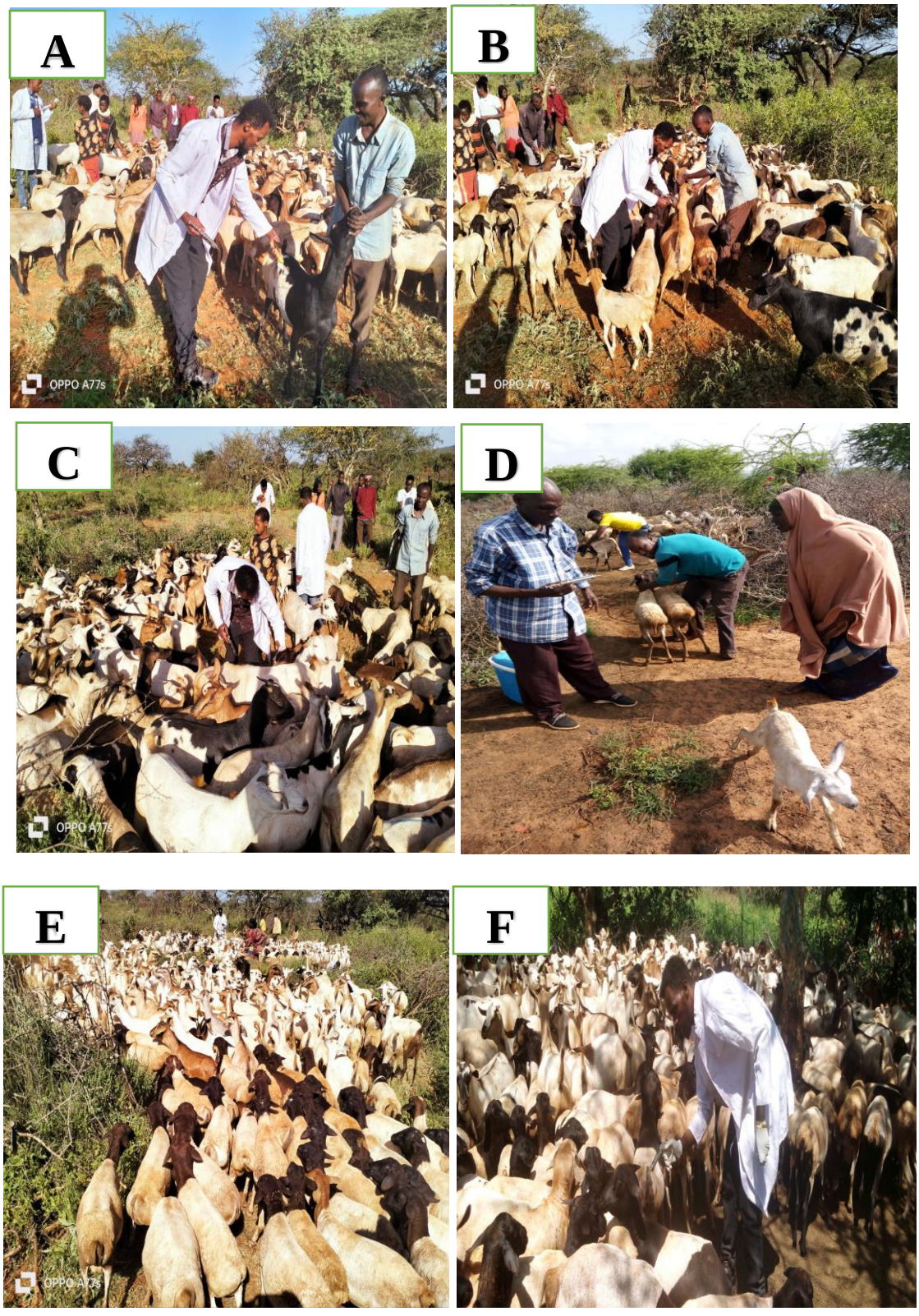
(A): Serous chronic ocular and nasal discharge; (B): Extremely emaciated goat with diarrhea.

Appendix 9: Photo during sample collection.

(A): Collecting blood sample from goat's jugular vein; (B): While obtaining ocular swab from goat.

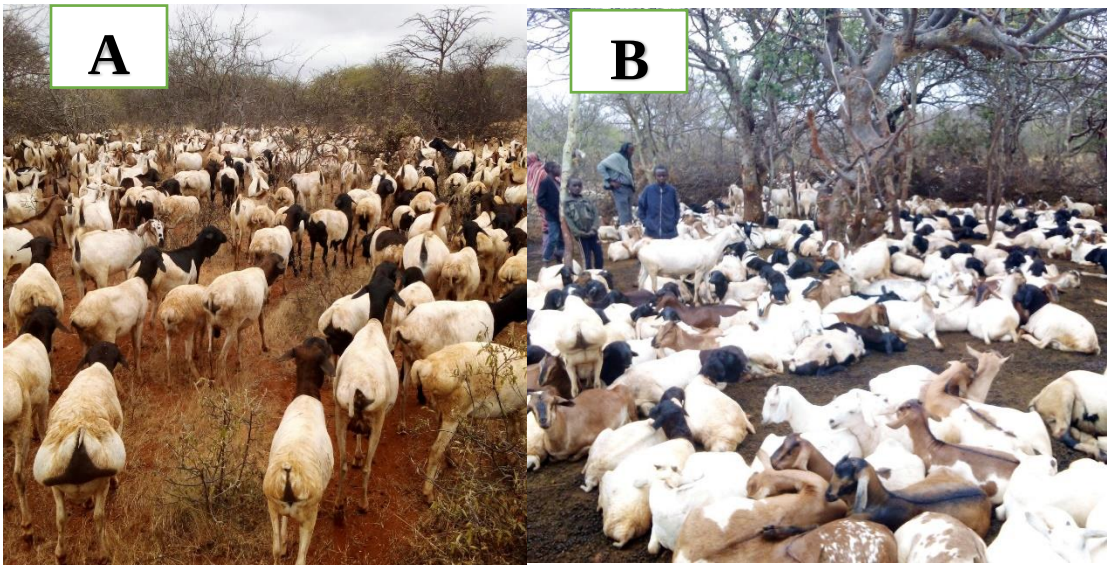
Appendix 10: Photo showing the ready vaccine for ring immunization against PPRV.

(A): Vaccine stored in icebox at YRVL to remain chilled until they are delivered to the district; (B): Vaccines remain chilled in icebox after it has been sent to the district.

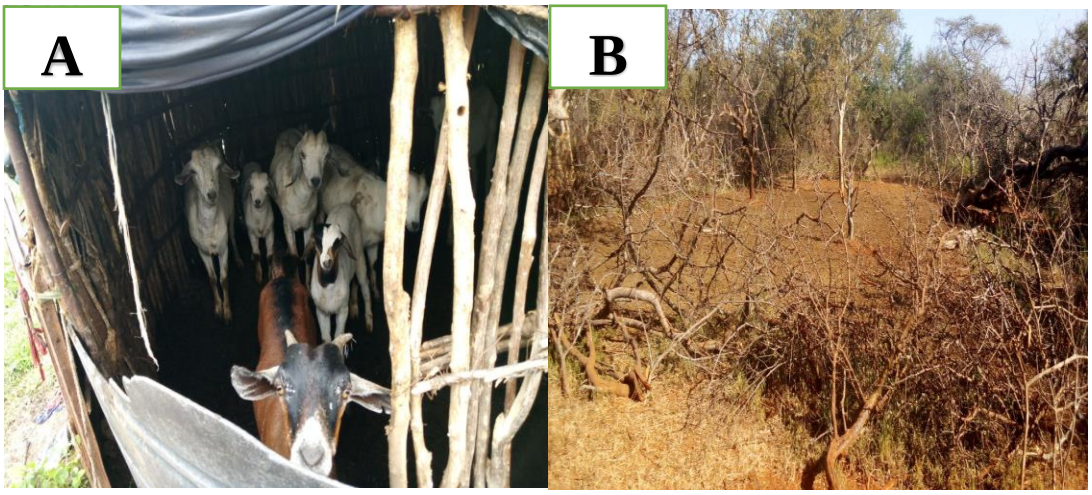


Appendix 11: Photo during mass vaccination of sheep and goat against PPR.

(A) and (B): During mass vaccination of sheep and goat against PPR in Arerodistrict.
(B) and (D): During mass vaccination of sheep and goat against PPR in Moyale district.
(C) and (D): During mass vaccination of sheep and goat against PPR in Dhas district.



Appendix 12: Photo showing mixed together of sheep and goat.
(A): Co-grazing of sheep and goats at free grassland; (B): Keeping sheep and goat in fence in common during the night at settlement area (foora).



Appendix 13: Photo showing the housing system of small ruminant in Borana.
(A): Keeping goats in house at night; (B): Fence (housing type) for sheep and goat

Appendix 14: Format for Sample Collection

Animal code	Date	Owner name	Species	Animal origin	Sex	Age	Inter herd contact	Introduction of new animals	Isolation/Quarantine	Previous access to other livestock	Vaccine status	Sample type	Animal body condition
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													

Appendix 15: Step followed during blood sample collection

1. Wearing gown and protective disposable glove
2. Prepare all materials of blood collection (needle, needle holder and vacutainer tube).
3. Restraining individual animal by owner
4. Ask an assistant to hold the animal smoothly.
5. An appropriate site of sample collection should be identified
6. Disinfecting the area around the bleeding site by swabbing with 70 percent alcohol.

7. The jugular vein should be seen enough to gain collect sufficient blood.
8. Direct the needle into the jugular vein in the direction of blood flow.
9. Once the tip of the needle is inserted in the vein, gently pull the plunger of the syringe.
10. Blood sample should be collected to vacutainer tube that free of EDTA.
11. After removing the needle, pressure will be applied to the vein for a few seconds to depress further bleeding.
12. Blood collecting site will be disinfected again if necessary.
13. Collected blood samples was labelled with a full information by permanent marker
14. It kept by placing at 45° for separating pure serum from whole blood
15. Within 24 hours separated serum was poured to cryogenic vials and kept in -20^{o}c.
16. If it remain unseparated, it centrifuged and separated serum was collected and stored in -20^{o}c.
17. At the end it transported to National Veterinary Institute (NVI), Bishoftu for investigations.

Appendix 16: Step followed during swab (ocular and nasal) sample collection.

1. Wearing gown and protective disposable glove
2. Prepare all materials of swab sample (cryogenic vials, rayon cotton swab scissor, and permanent marker).
3. Restraining individual animal by owner
4. Ask an assistant to hold the animal smoothly.
5. An appropriate site of sample collection should be identified and swab sample will be collected
6. After cutting swab stick at appropriate area, it will be putted in a cryogenic vials that contains viral transport media (VTM) which possess Phosphate Buffered Saline (PBS), antibiotics, and antifungals.
7. Then the sample should be appropriately labeled, kept chilled by putting in a cool box with ice packs it will be submitted and sent to Yabello Veterinary regional laboratory and it kept at deep freeze until it transported to Animal health institute (kept at -80 ^{O}C) until laboratory analysis done.

Appendix 17: Questionnaires on PPR for animal owner.

I. General information about livestock owner

1. Are you volunteered for a meantime to interview with you? Yes_____ No _____

1. If yes, Name of respondent.....Sex: (a) Male (b) Female, Age.....
2. Region.....Zone.....District.....kebele .....Village.....
3. Educational level: (a) Non-formal education (years) (b) Primary education (c) Secondary school (d) Preparatory school
4. Marital status: (a) married (b) unmarried (c) widowed (d) divorced
5. Role of the respondent with livestock (multiple answers possible)

(a) Household (b) Marketing (c) keeper (d) None

6. Household size (number of people who share a meal in a house).....
7. How many years since you have been involved in keeping livestock? .....
8. Number of Livestock owned. What type of livestock do you have and tell us the number of each livestock you have?
9. Cattle..... b. Sheep..... c. Goats..... d. Camels..... e. Equine (donkey....., Mule....., horse ....., f. Poultry.....
10. What are the main sources of food in the household? List the main sources of food in the household? …….....
11. What are the contribution of livestock to household food? .....
12. What are the age structures of sheep and goats? List of age sets of sheep and goats? .....
13. What period are major cultural ceremonies? .....

II. <u>PPR-related disease occurrence</u>

14. List the diseases of sheep and goats you have observed in last one years (local language).....
15. Do you have any dead sheep and goats? (a) Yes ……. (b) No ……
16. If yes, please, mention signs that sheep and goat manifested prior to die. .....……

17. Do you encounter in your sheep/goats that have ocular and nose discharge with stomatitis and diarrhea? (a) Yes …….. (b) No ……

18. Have you seen PPR (Marareeba) in your shoats? (a) Yes …… (b) No ……

19. If yes, as you think, where does it comes from?

    .....

20. Have you seen PPR related outbreak in your area in the last one year?

(a)Yes ….. (b) No …….

21. Would you tell me the following information about the morbidity and mortality of the outbreak in your small ruminant herd?

A. No. of affected species (a) Sheep..... (b) Goats.....

B. No. of died (a) Sheep..... (b) Goats.....

22. How do the diseases get into your flocks? .....

23. What signs are seen on sick sheep or goats?

A. Nasal, oral, and ocular discharges and contagious.
B. Diarrhea and nasal discharge and contagious
C. Mouth lesions and diarrhea
D. If other, specify …….

24. When did the disease start in the area (Kebele)? Season …… Month …….year ……

25. What ages are affected by the diseases? .....

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

Categories	Species	Breed	Sex	sick	Died
Old					
Adult					
Young					

27. How frequent PPR reoccurs in the area? Don't Know …….. Every 1yr …….. Every 2yrs ……. other, specify …….

III. PPR risk factor-related issues

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

5. June to September …. (b) October to January ……(c) February to May ……

29. Did you encounter any critical season of feed shortages? (a) Yes …. (b) No ….

30. If yes in which season? .....

31. What period of the year are livestock grazing in common? ……

32. Did you move your livestock to another place for grazing or for watering purpose?

(a) Yes … (b) No ….

33. If yes, in which season? ……..... Where are the alternative for feeding/ watering? .....? How long did you move them for searching feed/water? .....

34. What is your watering point for your animal? .....

35. What is your grazing system of your livestock? .....

36. How do you raise your sheep and goat?

a. Sheep and goat grazing separately (b) Sheep and goat grazing together

(c) Sheep and goat grazing with other livestock (d) If other, specify……...

37. Have you purchased new sheep/goats recently? (a) yes (b) no

38. If yes, do you know its origin? (a) yes (b) no

39. How many of them? …….species …… sex ….. age ……

40. What is your livestock market frequently used? ……..……distance (km).....

41. How often are goats/sheep/sheep taken to the market and returned home? (a) never (b) sometimes (c) always

42. Did you cross into neighboring countries in such of grassland and water? (a) yes (b) no

43. If yes, when did you cross into neighboring countries with livestock? .....

44. Do you cross into neighboring countries to restock/buy flocks? (a) yes (b) no

45. If yes, when did you cross into neighboring countries to restock/buy flocks?

Year ……..… month ……

46. Are there herds from other countries entering for grazing to your land? (a) yes (b) no

If yes, when did herds from other countries entered your land to share your water/feed sources? Year ……..… month ……

IV. Community perception of PPR control methods

47. What does the community do when the animals get sick with PPR? .....

48. What are the traditional treatments/control options for small ruminants clinically signed with PPR-related disease? .....

49. What are the adopted PPR treatment, control and prevention methods that your village uses? .....
50. What measures are taken to prevent PPR? (a)Traditional treatment (b) Modern treatment (c) Vaccination (d) No treatment (e) if other, specify.....
51. Did you vaccinate your shoats for PPR? (a) yes (b) no
52. If yes, when did you vaccinate before? …….
53. What are the potential risk factors for PPR in your community?

……

54. How you conclude the previous and current occurrence of PPR in small animals?

……

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethics Review Committee

Ethical clearance certificate

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

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

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

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

Date of application:	**December, 2022**
Nature of the project:	**Field investigation and experimental vaccine trial**
Target animal species:	**Small ruminants**
Number of animals involved:	**2000**
Study area:	**Different parts of Ethiopia**

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

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

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

Federal Democratic Republic of Ethiopia * Addis Ababa University College of Veterinary Medicine & Agriculture

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Appendix 18: Ethical clearance certificate photo.