

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

STUDY ON MOLECULAR CHARACTERIZATION AND PATHOGENESIS OF PESTE DES PETITS RUMINANTS VIRUS (PPRV) IN SELECTED SITES OF ETHIOPIA

BY

FASIL AKLILU ASRES

A PhD DISSERTATION SUBMITTED IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD) IN TROPICAL AND INFECTIOUS DISEASES

AUGUST, 2025

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Addis Ababa University

Graduate Program

This is to certify that the dissertation prepared by Fasil Aklilu Asres, entitled "Study on Molecular Characterization and Pathogenesis of Peste des Petits Ruminants Virus (PPRV) in selected sites of Ethiopia" and submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Tropical and Infectious Diseases complies with the regulation of the Addis Ababa University and meets the accepted standards with respect to originality and quality.

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LIST OF ABBREVATIONS

AAU	Addis Ababa University
Abs	Antibodies
Ags	Antigens
AHI	Animal Health Institute
ALHRI	Aklilu Lemma Health Research Institute
ALIPB	Aklilu Lemma Institute of Pathobiology
BCs	Barcodes
BTV	Blue tongue virus
CCPP	Contagious Caprine Pleuropneumonia
CD150	Cluster of differentiation 150
cDNA	Complementary of deoxyribonucleic acid
CD	Canine Distemper
CDV	Canine Distemper Virus
cELISA	Competitive Enzyme Linked Immuno Sorbent Assay
CL	Clade
CPE	Cytopathic effect
CSA	Central Statistical Authority
DCs	Dendritic Cells
DIVA	Differentiating Infected from Vaccinated Animals
DMEM	Dulbecco's Modified Eagle's Medium
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide tri phosphate
EU	European Union
FAO	Food and Agricultural Organization
FP	Fusion Protein
GDP	Gross Domestic Product
GIT	Gastrointestinal tract
HA	Hemagglutinin
HP	Hemagglutinin Protein
HRP	Horse radish peroxidase
IcELISA	Immuno-Capture Enzyme Linked Immuno Sorbent Asssay
IFNs	Interferons
IHC	immuno histochemistry
IL	Interleukin
LFD	Lateral Flow Device
LP	Large Protein
LNs	Lymph Nodes
MAbs	Monoclonal antibodies
m.a.s.l	Meter above sea level
MeV	Measles Virus
MoA	Ministry of Agriculture

MP	Matrix Protein
MSA	Multiple Sequence Alignment
NAHDIC	National Animal Health Diagnostic and Investigation Center
NGS	Next Generation Sequencing
NP	Nucleoprotein
PAMPs	Pathogen Associated Molecular Patterns
PA/s	Peasant Association/s
PPBLs	Purified Peripheral Blood Lymphocytes
PBS	Phosphate buffered saline
PP	Polymerase Protein
PPR	Peste des Petits Ruminants
PPRV	Peste des Petits Ruminants Virus
PRRs	Pattern Recognition Receptors
qRT-PCR	Quantitative real time polymerase chain reaction
RNA	Ribonucleic acid
RNP	Ribonucleo-Protein
Rpm	Revolution per minute
RP	Rinderpest
RPV	Rinderpest Virus
RT-PCR	Reverse transcription Polymerase Chain Reaction
Rt-RT-PCR	Real time Reverse transcription Polymerase Chain Reaction
RRLs	Regional Reference Laboratories
SC	Sub- Clade
SLAM	Signaling Lymphocyte Activation Molecule
SNP	Single nucleotide polymorphism
SRMV	Small ruminant morbillivirus
TCID50	Tissue Culture Infective Dose 50
TCRV	Tissue culture rinderpest vaccine
Th1	Type 1 T helper
Th2	T helper 2
TLR	Tool Like Receptor
TMB	Tetramethyl benzidine
VDSC	Vero dog slam cells
VN	Virus neutralization
VNT	Virus neutralization test
VTM	Virus transport medium
WGS	Whole Genome Sequencing
WOAH	World organization for Animal Health

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ABSTRACT

Background: PPR has emerged as a significant and high-priority livestock disease in Ethiopia. A relatively recent study indicated that the disease leads to economic losses amounting to 14% of the yearly household income, posing a serious threat to the livelihoods of livestock owners. In connection to the worldwide significance of PPR, the FAO and WOAH developed a global strategy in 2015 to eradicate the disease by 2030. In alignment with this framework, countries such as Ethiopia have devised their own plans to achieve eradication by setting the same target year. However, over five years of control efforts, PPR outbreaks persist in Ethiopia and continue to expand into regions that were previously unaffected. This phenomenon coupled with others necessaitates to the need and urgency of investgating the factors that influence the continued presence of the virus in the field. One of the potential factors is the involvement of non-target host species, such as cattle, which may play a significant role in the epidemiology of PPR. Furthermore, much of the current understanding of PPR pathology and pathogenesis is derived from research on closely related viruses, such as the rinderpest (RP) virus signifying knowledge gap in the area. Moreover, updated knowledge of circulating PPRV lineages/strains and their virulence profiles is essential for targeted and effective PPR control and eradication efforts in Ethiopia.

Objectives: This study aimed to identify factors hindering the success of ongoing PPR control and eradication efforts in Ethiopia and beyond. The first study addressed whether infected cattle shed enough PPRV to transmit the virus to seronegative small ruminants or other seronegative cattle while also evaluating the role of cattle in PPR epidemiology. The second study aimed to investigate PPRV tissue tropism, shedding patterns, pathogenesis, clinical progression, and comparative pathology in goats and cattle to support the development of more effective surveillance and control strategies. The third study aimed to identify circulating strains and compare their virulence in an experimental setting. Such insights could aid in strategic planning for more effective disease control.

Methodology: The first experimental study aimed to investigate the role of cattle in PPR epidemiology by using both empirical and model-based approaches. The trial was conducted at the Animal Health Institute (AHI) experimental facility. Local virus strains, lineage IV isolate (Ethiopia/Habru/2014) and animal breeds were used in the study. The first experiment consisted

of five consecutive trials involving 15 sheep, 66 goats, and 37 cattle, with a total of 118 animals. To simulate natural transmission, sheep and goats were administered 1 ml of viral suspension, while cattle received 2 ml, all via the intranasal inoculation route. Similarly, the second experimental study was conducted using local breeds of goats and cattle, along with a locally circulating PPRV strain. A total of 32 animals, with 16 from each species, were included. Samples were collected from live animals, as well as postmortem samples from purposivelly sacrificed goats and cattle. In goats, sampling was performed daily, while in cattle, it was conducted at predetermined intervals. Finally, an experimental study was conducted on 21 goats, including both experimental and control groups, to assess the virulence of six PPRV strains circulating in specific regions of Ethiopia. Samples were collected from both live and sacrificed goats at predetermined intervals for further analysis. All animals used in the experiments were serologically negative for PPR, allowed to acclimatize for 10–15 days, randomly assigned to experimental barns, and analyzed using various diagnostic techniques.

Results: Studies on the role of cattle in the epidemiology of PPR demonstrated that the animals can naturally contract the virus through contact with infected goats, though they typically exhibited only mild and short-lived clinical symptoms. This indicates that cattle play a limited role in PPRV transmission due to the fact, cattle-to-goat transmission being rare, even when the animals share the same space for extended periods. While qRT-PCR detected PPRV RNA in a virusinfected sentinel calf, attempts to isolate the virus were unsuccessful. Additionally, cattle that were experimentally inoculated or housed with PPRV-infected goats did seroconvert. These findings underscore the need for further investigation into the mechanisms that may restrict disease development and viral shedding in this species. A comparative pathology study of PPRV in goats and cattle revealed moderate level of clinical signs in goats while, no obvious symptoms among cattle. Instead, cattle experienced a subclinical infection, suggesting they could act as silent carriers of the virus. The significant difference in clinical manifestations between the two species (P-value = 0.0070) underscores the variation in host responses to PPRV infection. Although the successful isolation of live PPRV from cattle samples was limited, it raises the need to reassess the epidemiological role of cattle in PPRV transmission. These findings indicate that under certain conditions, cattle could serve as potential reservoirs or silent spreaders of the virus. However, further research is needed before applying these results to other PPRV lineages or regions beyond the currently circulating lineage-IV PPRV in Ethiopia. Investigation of

comparative virulence of locally circulating PPRV strains in indigenous goats, demonstrated that five out of six strains displayed typical PPR clinical signs, with the exception of one lineage-IV strain (designated as 38920/19), which only caused a mild nasal discharge. Goats inoculated with viruses from two different lineages, lineage-IV and lineage-III, exhibited nearly identical clinical outcomes, indicating that lineage classification alone may not be a reliable predictor of disease severity. The observed reduction in virulence in one strain may be attributed to inherent biological differences or technical factors related to laboratory procedures.

Conclusions: The present study considered in-depth examination of various factors and insights that could potentially affect theongoing PPR control and eradication efforts, highlighting key areas for further research and consideration. The study on the role of cattle in PPRV epidemiology suggests that local breed (zebu) in mixed-species environments play a limited role in transmitting PPRV to sheep and goats. The detection of PPRV RNA in a naturally infected sentinel calf, despite failed virus isolation attempts, highlights the need for further comparative studies on both cattle and goats. A comparative pathology study of PPRV in goats and cattle provides evidence of subclinical infections and live virus shedding in cattle, suggesting that under certain conditions, cattle may play a more active role in PPRV epidemiology than previously recognized. However, the current study relied on a single local lineage (L-IV) limits the broader applicability of the findings to other strains and regions. To enhance understanding of PPRV, further research should investigate additional virus strains and their virulence characteristics. The comparative virulence study of Ethiopian PPRV strains reveals similar virulence profiles across lineages (L-III and L-IV) in local goats, indicating that lineage classification alone may not reliably predict clinical outcomes. This finding is essential for informing regional disease control strategies.

Key words: Cattle, comparative virulence, pathogenesis, experimental, goats, PPR, PPRV, strain characterization, virus transmission.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background

Peste des Petits Ruminants (PPR) is a highly contagious and often deadly viral disease primarily affecting small ruminants (sheep and goats). The disease is caused by Peste des Petits Ruminants Virus (PPRV), which has recently been renamed Small Ruminant Morbillivirus (SRMV) (Adams et al., 2017), it is characterized by causing high morbidity and mortality particularly in immunologically naive population. Since its initial identification in West Africa (Gargadennec and Lalanne, 1942), the disease has rapidly spread across vast regions, first affecting large parts of Africa before reaching Asia, the Middle East, and, more recently, Europe (Banyard et al., 2010; Niedbalski, 2019; Fayyad and Alzuheir, 2024). Currently, PPR has been reported in over 70 countries, with many others at risk of its introduction (Zhao et al., 2021). PPR severely impacts food security and livelihoods of smallholders, causing $1.2–1.7 billion in annual losses from animal deaths, reduced production, and disease control costs. Approximately a third of the financial impact occurs in Africa. PPR is considered as a priority pathogen set for global eradication by 2030, as targeted by the FAO and WOAH (FAO, 2015; Baron et al., 2017). PPR threatens 80% (1.7 billion sheep and goats) of the global small ruminant population and endangers the livelihoods of over 330 million farmers (WOAH, 2015).

Following the Global Strategy, infected countries like Ethiopia have developed national plans to eradicate PPR by 2030. However, despite five years of control efforts, outbreaks persist in endemic regions like Ethiopia and continue to spread; now reaching mainland Europe (Fayyad and Alzuheir, 2024).

The livestock industry makes a significant contribution to Ethiopia's economy and holds strong potential for further supporting its economic growth (CSA, 2021). The country is home to an estimated 70.29 million cattle, 42.9 million sheep, and 52.46 million goats (CSA, 2021). In Ethiopia, livestock contributes 45% to agricultural GDP, 19% to overall GDP, and 16–19% to foreign exchange earnings. Among the livestock, small ruminants play a crucial role in both animal production and the overall economy of Ethiopia. Livestock production in Ethiopia is hindered by various challenges, with animal diseases being a major concern. Peste des Petits

Ruminants (PPR) is particularly significant, as it leads to high mortality and morbidity in small ruminants. Based on reported morbidity and mortality of the infection and the size and structure of the small ruminant sector it is likely that PPR became one of the most economically important and priority livestock diseases in the country (Waret-Szkuta et al., 2008; Magona et al., 2016). Ethiopia's Ministry of Agriculture (MoA) has recently identified PPR as a key disease for targeted control and eradication within its strategic plan. A study in northwest Ethiopia found that PPR caused high morbidity and mortality in affected flocks, leading to economic losses equivalent to 14% of annual household income and severely impacting the livelihoods of flock owners (Jemberu et al., 2022). PPR outbreaks have caused a significant negative impact on the livelihoods, food security, and socio-economic activities of livestock keepers in affected regions, including Ethiopia. The direct economic losses from PPR are often worsened by the sanitary measures enforced by authorities, such as restrictions on animal movement and trade limitations on animal by-products (Abraham et al., 2005a).

A key challenge in controlling and eradicating infectious diseases is that many pathogens infect multiple host species, some serving as reservoirs (Portier et al., 2019). Pathogen control programs must identify each host species' role in transmission and determine which sustain pathogen persistence (Haydon et al., 2002; Buhnerkempe et al., 2015).

When eradication is the goal but longitudinal field studies are difficult, an interdisciplinary approach that integrates empirical data and modeling may be the most viable solution. This applies to the economically significant multi-host disease caused by the PPR virus (Herzog, C. et al., 2020).

Similar to other morbilliviruses, PPR affects a variety of livestock and wildlife species, exhibiting varying degrees of pathogenicity (Cosby, 2012), and triggers a robust immune memory in animals that recover from infection. Knowledge regarding its host range has been derived from documented outbreaks, serological findings, and a limited set of experimental studies (Hamdy and Dardiri, 1976; Nawathe and Taylor, 1979; Abd El-Hakim, 2006; Wernery, 2011; Schulz et al., 2018; Fakri et al., 2019; Saeed et al., 2022). There is concern that proposed eradication efforts, relying on mass vaccination of small ruminants, could be hindered if PPRV persists in currently unidentified livestock or wildlife reservoirs (Baron et al., 2017; Mancy et al., 2022). The high turnover of sheep and goats makes sustaining population-wide immunity

challenging. Mass vaccination alone may not eradicate PPR, as spillback into susceptible hosts remains a risk (Kock, R. et al., 2025).

Large ruminants like cattle may exhibit a subclinical form of PPRV, clearing the virus without symptoms while generating strong neutralizing antibody responses (Balamurugan et al., 2012a; Sen et al., 2014; Schulz et al., 2018). The role of atypical hosts, such as cattle, in PPRV infection remains debated, with conflicting findings. Sen et al. (2014) suggest that cattle may harbor the virus longer in blood and peripheral monocytes, potentially contributing to transmission. Moreover, Mornet et al. (1956) observed pyrexia and oral erosion in experimentally infected calves (Mornet et al., 1956). In contrast, some studies classify cattle as dead-end hosts, reporting seroconversion and short-term, low-level PPRV RNA detection in oronasal swabs (Couacy-Hymann et al., 2019; Schulz et al., 2019).

There is also a notable knowledge gap regarding the role of other host species, such as cattle, in the transmission dynamics of PPRV. Thus a study aimed to determine whether infected cattle shed sufficient amounts of PPRV to transmit the virus to seronegative small ruminants or other seronegative cattle is urgently needed. This study was designed to provide valuable insights that will enhance national PPRV control and eradication efforts in Ethiopia and other countries affected by PPRV.

Although much of the current knowledge on PPR pathology and pathogenesis is derived from studies on the closely related Rinderpest virus (RPV) and other morbillivirus infections, many aspects remain poorly understood (Domingo and Holland, 1997). Despite PPR's global impact, its pathology and clinical progression remain poorly understood, especially in atypical hosts like cattle. However, limited studies have focused specifically on PPRV pathology, and there remains a gap in understanding its impact, including the clinical progression in primary hosts (goats and sheep) and atypical hosts (such as cattle) using comparative experimental studies.

The role of species beyond sheep and goats in PPRV persistence is still unclear. A deeper investigation into both target and atypical hosts is crucial. Given these knowledge gaps and conflicting findings, a thorough investigation of PPRV tissue tropism, pathogenesis, shedding, clinical progression, and comparative pathology in goats and cattle is essential to inform the development of more effective surveillance and control strategies.

In Ethiopia, no published studies exist on the pathology of PPRV, and there is limited information on its pathogenesis and clinical manifestations in both susceptible and atypical hosts like cattle. Such studies are vital for understanding the infection's progression, virus circulation, shedding patterns, and for contributing to early disease detection.

Moreover, successful control and elimination of PPR depend on tracking the circulating virus strains in the field and analyzing their virulence. Also over the past few decades, there has been limited focus on the diversity of PPRV strains and their potential to cause varying clinical outcomes (Couacy-Hymann et al., 2007). Two field strains/isolates from Sudan show differences in virulence for goats (Taylor, 1984).

Field PPR outbreak investigations reveal a range of clinical outcomes, from mild to severe respiratory and gastrointestinal symptoms, sometimes leading to death (Roeder et al., 1994; Alemu et al., 2019; Mohammed et al., 2022). Some studies showed that PPR affects different species and breeds of small ruminants to varying degrees. Research on PPRV isolates in West African dwarf goats suggests that virulence characteristics could help classify PPRV isolates (Couacy-Hymann et al., 2007). Diop et al. (2005) observed variations in goat breed sensitivity to the same PPRV outbreak and concluded that susceptibility is more breed-related than virus-dependent (Diop et al., 2005). Although some isolation and molecular characterization of PPRV studies have been conducted in Ethiopia to study the circulating PPRV (Muniraju et al., 2016; Alemu et al., 2019; Rume et al., 2020), they have not fully characterized the circulating PPRV strains. A more detail analysis is needed to better understand the virus and improve control strategies. In Ethiopia, there is a lack of documented research on the clinical and pathological changes, as well as the assessment of the virulence of different PPR virus (PPRV) strains circulating in the country, especially in controlled experimental settings. Moreover, monitoring the virus in the field using whole genome sequencing (WGS) method allow detail monitoring of ongoing PPRV epidemic and may be beneficial to plan strategically. To gain a deeper understanding of the role played by strains with different levels of virulence and transmission potential, additional research is required in regions where PPRV is enzootic or endemic (Libeau et al., 2014). This current study aims to identify circulating PPRV strains and compare their virulence in an experimental setting.

1.2. Literature review

1.2.1 Peste des Petits Ruminants (PPR)

1.2.1.1 Etiology and classification

Peste des Petits Ruminants (PPR) is a disease that caused by the Peste des Petits Ruminants Virus (PPRV). PPRV is the fourth member of the genus Morbillivirus, within the family Paramyxoviridae and the order Mononegavirales, due to its similarities with rinderpest (RP), canine distemper (CD), and measles viruses (MeV) (Gibbs et al., 1979). PPRV has recently been renamed Small Ruminant Morbillivirus (SRMV) (Adams et al., 2017).

1.2.1.2 Host range

PPR is a highly contagious viral disease that mainly affects small ruminants, particularly sheep and goats, with goats experiencing more severe impacts than sheep (Nanda et al., 1996). Cattle and pigs can act as carriers of the PPR virus without exhibiting any clinical symptoms of infection (Taylor, 1979). Studies in Sudan suggest that camels may be susceptible to PPR, as evidenced by clinical symptoms and positive laboratory findings (Khalafalla et al., 2010; Kwiatek et al., 2010). An antibody prevalence of 3% was detected among 628 camels tested in Ethiopia (Abraham et al., 2005).

In addition to domestic small ruminants (sheep and goats) as natural hosts, camels (Zakian et al., 2016), large ruminants (cattle and buffalo), (Lembo et al., 2013; Sen et al., 2014) a wide range of wild animals (Wensman et al., 2018) and in unusual hosts like pigs, an experimental investigation revealed a subclinical infection of PPRV with no shedding and transmission of viruses to a native host (Nawathe and Taylor, 1979). Recent experimental studies have emphasized the epidemiological role of pigs and wild boars in both inter- and intra-species transmission of PPRV (Schulz et al., 2018). PPRV-positive Asiatic lion (Balamurugan et al., 2012b) and dogs (Ratta et al., 2016) were found negative for CDV.

1.2.2 Epidemiology

1.2.2.1 Global distribution and endemic areas of PPR

PPR is widely distributed across West and Central Africa, East Africa, the Arabian Peninsula, the Middle East, Southern Asia, and the Indian subcontinent (Nanda et al., 1996; Shaila et al., 1996; Banyard et al., 2010). PPR was first reported as a recognized clinical entity in 1942 in Côte d'Ivoire (Gargadennec and Lalanne, 1942) however the virus was isolated in Senegal in 1962 (Gilbert and Monnier, 1962). PPR was initially thought to be confined to West Africa until a disease affecting small ruminants was observed in Sudan in 1972, which was initially identified as Rinderpest (RP) (Ali, 1973). The first observation of PPR outside West Africa occurred in Sudan between 1970 and 1972 (El Hag Ali and Taylor, 1984). PPR was first reported in sheep, goats, as well as gazelles, ibex, and gemsbok in Al Ain, located in the Arabian Gulf (Furley et al., 1987).

In Central African countries, various laboratory techniques and phylogenetic analysis have revealed that lineage-IV viruses are circulating (Kardjadj and Luka, 2016). Historically, PPRV has been detected in Egypt at various time points, and recent molecular typing has identified the most recent PPRV discovery in the country as lineage-IV (Ahmed et al., 2021). In Morocco, genetic characterization of the PPR virus during the 2008 outbreak classified it as a lineage-IV virus (Khalafalla et al., 2010). Studies have also provided serological evidence of PPRV infection in Tunisia (Ayari-Fakhfakh et al., 2011). In 2002, an outbreak of PPR was reported in Saudi Arabia, Asia, the Arabian Peninsula, and the Middle East, with a 100% case fatality rate (Housawi et al., 2004). Seroprevalence results for both sheep and goats have also been reported in northern Jordan (Al-Majali et al., 2008) and in the Lebanon. In Qatar, both lineages-III and IV of PPRV have been circulating, with isolates from goats and other wild ruminants reported in 2010 (Kinne et al., 2010). PPR is endemic in most East African countries, including Ethiopia (Banyard et al., 2010). PPR is now spreading southward in Africa, particularly in areas with high densities of wild ruminants, as well as sheep and goats. .

Until June 2018, PPR had not been detected in Europe, except for the European part of Turkish Thrace (Parida et al., 2016). However, in June 2018, Bulgarian authorities reported cases of PPR

in sheep along the border with the Thrace region of Turkey (European part of Turkey). This marked the first occurrence of PPR in Bulgaria and the European Union (EU). The source of the PPR infection in Bulgaria remains unclear, but it may have been due to the illegal movement of animals, contaminated materials, or humans, such as refugees (Parida et al., 2016; Masalski and Najdenski, 2019).

1.2.2.2 History and current status of PPR in Ethiopia

PPR was first suspected in Ethiopia on clinical grounds in the Afar goat herds of eastern parts of the country in 1977 (Pegram and Tereke, 1981). Consequently, the suspected outbreaks due to PPRV have been confirmed using specific cDNA probes (Roeder et al., 1994). The serological results were also suggested that the source of infection were goats purchased from south-west Ethiopia (Roeder et al., 1994). Goats entering the marketing chain by this route can be derived from as far away as the Kenyan border. The disease studied was clinically and pathologically typical of PPR and not rinderpest.. Some authors speculated that PPR was introduced to Ethiopia in 1989 in the southern Omo River valley from where it moved east to Borana then northwards along the Rift Valley to Awash. The disease then spread northwards into the central Afar Region and eastwards into the Ogaden (Roeder et al., 1994).

Furthermore, both previous and recent serological study results have supported the circulation of the virus in various regions and areas of the country at different time points (Roeder et al., 1994; Abraham et al., 2005; Waret-Szkuta et al., 2008; Megersa et al., 2011; Agga et al., 2019; Rume et al., 2020; Dubie et al., 2022; Abesha et al., 2023; Ejigu et al., 2023; Kumbe et al., 2024; Mandefro et al., 2024; Senbeto et al., 2024).

Moreover, in the past decade several studies have been conducted on PPR, confirming the circulation of different PPRV lineages/strains in various parts of the country. These studies aimed to detect the presence of the disease and the virus using advanced laboratory techniques such as conventional RT-PCR, real-time RT-PCR, sequencing, and phylogenetic analysis (Muniraju et al., 2016; Alemu et al., 2019; Rume et al., 2019).

Lineage-III (L-III) PPR virus was confirmed and characterized first in 1994 (Roeder et al., 1994), while lineage-IV (L-IV) PPR viruses were identified and reported as circulating in the country in 2010 and 2017 (Muniraju et al., 2016; Alemu et al., 2019; Rume et al., 2019).

Muniraju et al. (2016) reported the first complete genome sequence of a field isolate of PPRV from East Africa. This isolate, collected during the 2010 outbreak in Ethiopia, was named PPRV Ethiopia/2010. The full genome sequences of this isolate clusters genetically with other lineage-IV PPRV isolates (Muniraju et al., 2016). Recently, Rume et al. (2019) and Alemu et al. (2019) reported the circulation of lineage-IV PPR viruses in different parts of the country through partial genome sequencing and phylogenetic analysis. Rume et al. (2019) further characterized the circulating lineage-IV virus into clade (Cl) and sub-clade (SCl) levels using sequencing and phylogenetic methods (Rume et al., 2019). Other studies, both past and recent, have explored the molecular aspects of PPRV on virulence and genes used for lineage determination (Abraham, 2005; Mohammed et al., 2022; Mengesha et al., 2024).

1.2.3 PPRV genetic structure and its functional roles

The genome of the PPR virus is a single-stranded, negative-sense RNA molecule that encodes eight proteins in the order of 3′-N-P/C/V-M-F-H-L-5′ (Bailey et al., 2005) comprising 15,948 nucleotides and encoding six structural proteins as the measles virus (MV) (Albina et al., 2013). These including, the major nucleocapsid protein (N), which encapsulates the viral genomic RNA; the phosphoprotein (P), which interacts with the polymerase protein (L, for large protein); the matrix protein (M); the fusion protein (F); and the haemagglutinin (H) glycoproteins that elicit protection against the disease in infected or vaccinated animals (Berhe et al., 2003). The matrix protein, which is closely linked to the inner surface of the viral envelope, plays a crucial role in connecting the nucleocapsid with the external glycoproteins H and F. These glycoproteins facilitate the virus's attachment to and entry into host cells. Moreover, the PPRV genome also encodes two nonstructural proteins, C and V (Bailey et al., 2005). The ribonucleoprotein (RNP), essential for minimal viral replication in cells, is formed by the viral genomic RNA along with the N, P, and L proteins (Bailey et al., 2007). It also has a length divisible by six, a feature shared with other members of the deoxyvirinae family (Calain and Roux, 1995).

PPRV exists as a single serotype. molecular epidemiology studies, based on the sequence analysis of partial segments of the fusion (F) gene (322 nt) or the nucleoprotein (N) gene (255 nt), have classified PPRV into four genetically distinct lineages: I, II, III, and IV (Banyard et al., 2010).

1.2.4 Phylogenetic relationships and molecular evolution of PPRV

The various lineages of PPRV are associated with specific geographic regions. Lineages-I and II are primarily found in West Africa, with historical records of lineage-II in Nigeria and Benin. Lineage-III was historically identified in both the Arabian Peninsula and East Africa, though more recent detections have been predominantly in East Africa. Initially emerging in Central Africa, lineage-IV has since spread extensively across Asia, particularly in India. This lineage appears to be dominant in endemic regions, and while its evolutionary patterns remain unclear, this trend plays a crucial role in understanding PPRV evolution (Mahapatra et al., 2021). The steady decrease in PPRV lineage-IV diversity since the late 1990s may be attributed to the effective implementation of preventive strategies, such as widespread mass vaccination campaigns undertaken in multiple countries (Padhi and Ma, 2014)

Evolutionary trends

Similar to other RNA viruses, PPRV exhibits genetic variability due to the inherently high mutation rate associated with RNA-dependent RNA polymerase activity (Drake and Holland, 1999). This variability can result in a population of viruses with differing levels of virulence and transmissibility (Libeau et al., 2014). The genetic variability of PPRV is demonstrated through comparative gene sequence analysis, which has led to the classification of PPRV strains into four distinct lineages (I-IV) (Barrett, 1999). A study examining the evolutionary and epidemiological dynamics of PPRV utilized Bayesian phylogenetic analysis of complete and partial genome sequences from all four PPRV lineages. The findings traced the time to the most recent common ancestor and the initial divergence of PPRV to a lineage III isolate from the early 20th century. Using a phylogeographic approach, the study estimated the probable root locations for ancestral

PPRV and its lineages, identifying Senegal for lineage-I, Nigeria/Ghana for lineage-II, Sudan for lineage-III, and India for lineage-IV (Muniraju et al., 2014).

Further subdivisions of lineage-IV have been proposed by (Kumar et al., 2014) who identified two clades (CI and CII) and four subclades (SCI to SCIV) based on the nucleoprotein (N) gene.

A recent comparison of genetic data from the F, N, and H genes showed that the N gene exhibits greater variability than the F and H genes. As a result, lineage classification based on the N gene is regarded as the most reliable method for typing novel isolates, since this region displays more divergence both between lineages and among isolates within the same lineage (Kumar et al., 2014).

All four PPRV lineages have been detected across Africa, with some countries reporting multiple lineages. For example, Tanzania has identified the presence of lineages-II, III, and IV (Mahapatra et al., 2015; Misinzo et al., 2015), while lineages-III and IV have been reported in Uganda (Luka et al., 2012). In Ethiopia, genetic characterization of PPRV from confirmed outbreaks in 1994 and 2010 identified lineage-III and lineage-IV, respectively (Roeder et al., 1994; Kwiatek et al., 2007; Banyard et al., 2010; Muniraju et al., 2014; Muniraju et al., 2016).

1.2.5 PPR virus host interaction and pathogenesis

In highly susceptible hosts, such as goats and sheep, the tissue tropism, pathogenesis, and disease manifestation following PPRV infection are linked to the virus's ability to infect immune cells through the Signaling Lymphocyte Activation Molecule (SLAM) receptor and epithelial cells via the nectin-4 receptor (Pawar et al., 2008; Birch et al., 2013; Schulz et al., 2019).

The initial step of PPRV infection involves the binding of the virus to the host cell and the delivery of the nucleocapsid into the host cell cytoplasm. This process is crucial for the virus's pathogenesis and plays a significant role in determining the host's susceptibility to infection (Kumar et al., 2014). The initial interaction between the host and the pathogen (attachment) is mediated by the binding of the virus to cell receptors via its H protein (Kumar et al., 2014).

Immune cells, such as macrophages and dendritic cells, located in the intraepithelial space and the lamina propria of the respiratory mucosa, absorb PPR virions from the respiratory tract lumen. These cells then migrate to T cell-rich areas of local lymphoid organs, where SLAM-mediated replication occurs within lymphoid cells before the virus enters circulation (Pope et al., 2013). Following general viraemia, PPRV utilizes nectin-4 receptors present in respiratory and gastrointestinal epithelial cells, where secondary viral replication takes place. This leads to the progression of the disease and the manifestation of further symptoms (Birch et al., 2013).

The initial site of PPRV multiplication is not within the epithelial cells of the respiratory mucosa, as previously reported for PPRV and RPV. Instead, the virus first multiplies within macrophages and dendritic cells, which then transport it to local lymph nodes for further replication. From there, the virus enters the circulation, causing viraemia, similar to the viruses of measles and canine distemper (Pope et al., 2013). Following viraemia, the virus specifically targets and damages epithelial cells in the alimentary and respiratory systems, as well as lymphocytes in the lymphoid system. Infected cells may proliferate and form syncytial giant cells before undergoing necrosis or apoptosis.

In goats, death may occur due to severe diarrhea and dehydration before respiratory lesions become severe, or it may be accelerated by concurrent infections such as pneumonic pasteurellosis, coccidiosis, or coliform enteritis (Pope et al., 2013). Most sheep and some adult goats recover from the disease. Lower respiratory involvement typically occurs late, only in the presence of a high viral load. It may be absent in animals that die early from dehydration or in those that recover from a mild form of the disease (Parida et al., 2015a). PPRV leads to harm in the epithelial cells of the intestinal and respiratory systems, along with damaging both circulating and resident lymphocytes. These lesions are the primary cause of the respiratory and digestive disorders, as well as the severe immunosuppression that follows infection. Despite the virus's immune-suppressive effects, which favor opportunistic pathogens (Kerdiles et al., 2006) animals that recover from the infection typically develop strong, lifelong immunity, effectively clearing the virus.

1.2.6 Clinical manifestations/signs

After incubation period (IP) of 2- 10 days, PPR presents with a range of clinical forms, including peracute, acute, subacute, and subclinical, depending on predisposing factors and the virulence of the infecting viral strain (Couacy-Hymann et al., 2007; Parida et al., 2015b). The peracute form of the disease causes severe symptoms, including severe depression, high fever, loss of appetite, and abortion in pregnant animals, often resulting in sudden death (Abubakar et al., 2009). Young animals are severely affected by this form of the disease. The most common i.e acute form is marked by a sudden onset of symptoms, including depression, fever, eye and nasal discharge, mucous membrane inflammation, eyelid matting, mouth ulcers, diarrhea, coughing, and foul breath, often leading to death (Taylor, 1984; Roeder et al., 1994; Banyard et al., 2010). Sub-acute cases exhibit milder clinical symptoms with lower mortality rates, and affected animals generally recover within 10–14 days. This form is more commonly observed in sheep (Lefèvre, 1989; Parida et al., 2015b). Large ruminants like cattle may display a subclinical form (Balamurugan et al., 2012b; Sen et al., 2014; Schulz et al., 2019), where the virus is eliminated without apparent clinical symptoms, accompanied by robust neutralizing antibody responses.

In addition, PPRV causes necrosis and ulceration of the mucuous membrane and inflammation of the gastro-intestinal tract leading to severe diarrhea (Gibbs et al., 1979). In most fatal infections, death is caused by primary viral bronchopneumonia or severe dehydration caused by acute diarrhea. Diarrhea become profuse may be mucoid and blood tinged. There is profound halitosis and the animal is unable to eat because of a sore mouth and swollen lips. PPR cause mortality rates exceeding 90% in immunologically naive populations. Due to its immunosuppressive effects, PPRV infections are often accompanied by secondary infections, which can complicate clinical diagnosis.

1.2.7 Diagnosis

A preliminary diagnosis of the disease relies on history, clinical symptoms, epidemiology, gross pathological observations, and histological examination. Laboratory confirmation is obtained

through various serological and molecular methods, including detecting specific anti-PPRV antibodies in serum or identifying viral antigens (Bao et al., 2008).

The post-mortem examination of gross lesions showed well-defined black-red zones, accompanied by congestion in the lungs, as well as in both the large and small intestines (Muhammad Abubakar et al., 2008). Severe lesions are characterized by congestion and distinctive "zebra stripes" in the large intestine. Additionally, bronchopneumonia, marked by lung consolidation and atelectasis, is frequently observed (Brüning-Richardson et al., 2011).

The laboratory diagnosis of PPR disease is mainly based on virus isolation, detecting viral antigens using lateral flow rapid tests or antigen capture ELISA or the viral RNA by RT-PCR, real-time (rt) RT-PCR (Kamel and El-Sayed, 2019). The diagnosis can also be performed by immunological/serological assays aiming to detect anti-PPRV specific antibodies by c-ELISA (Libeau et al., 1995). Detailed laboratory diagnostic methods are outlined in the molecular characterization section.

1.2.8 Differential diagnosis

PPR should be differentially diagnosed from pasteurellosis, contagious ecthyma, contagious caprine pleuropneumonia (CCPP), bluetongue (BTV), heartwater, coccidiosis, mineral poisoning, and foot-and-mouth disease (Couacy-Hymann et al., 2005; Banyard et al., 2010).

1.2.9 Transmission and spread

1.2.9.1 Natural Transmission

Since PPR virus is rapidly inactivated in the environment, transmission typically occurs through direct contact between infected and healthy animals. During the early stages of infection, the virus is excreted in large amounts through exhaled air. PPRV is highly contagious and primarily spreads by aerosols among animals living in close proximity (Lefèvre and Diallo, 1990).

It can also spread easily through direct contact with the secretions (discharges from the eyes, nose, and mouth) and/or excretions (loose feces) of infected animals coming into contact with nearby healthy animals (Ezeibe et al., 2008). The virus is excreted in nasal, oral, and ocular

discharges at the onset of fever, as well as in feces when diarrhea begins. Animals in the incubation stage can also infect other animals (Diallo, 2003).

However, indirect transmission through recently contaminated materials (within hours) cannot be ruled out and should be considered in epidemiological models. The disease can spread through interactions between susceptible and infected animals, as well as through contact with contaminated equipment, water, feed troughs, bedding, oculonasal and oral discharges, and loose feces, which contain large quantities of the virus and can facilitate transmission (Abubakar et al., 2015).

In a specific flock, the risk of an outbreak significantly increases when new stock is introduced or when animals are returned unsold from livestock markets. Goats from markets can harbor and transmit the virus (Roeder et al., 1994).

1.2.9.2 Experimental transmission

The virus can be transmitted through various routes, including nasal, oral, subcutaneous, intraocular, intratracheal, and intravenous pathways (Durtnell, 1972). Infective lymphoid tissue suspension is used to infect goats and sheep after the virus has been propagated through serial passages in goats. Pigs can also be infected with PPRV either by inoculation or through interaction with infected goats (Nawathe and Taylor, 1979; Schulz et al., 2018).

1.2.10 Risk factors

Various factors may influence variations in infection rates and disease outcomes, including management strategies (communal grazing), host population density, and the virulence of the strain (Couacy-Hymann et al., 2007) as well as host species, age, sex and breed (Diop et al., 2005). Some of the risk factors are mentioned below.

1.2.10.1 Age

Kids are more vulnerable to the disease compared to adults (Couacy-Hymann et al., 2007). Young animals between 4 months and 1 year of age are more commonly affected. In contrast, older animals are more likely to be seropositive for PPR than younger ones (Zahur et al., 2011).

1.2.10.2 Sex

Ewes are more frequently affected and show higher seropositivity for PPR than rams, possibly due to poor management practices commonly observed in Pakistan (Jalees et al., 2013). Moreover, male animals are typically slaughtered at an early age, whereas female goats and sheep are kept for breeding and milk production (Khan et al., 2007).

1.2.10.3 Species

Research conducted in Pakistan, has confirmed that among the primary hosts goats are more susceptible to PPRV infection than sheep (Abubakar et al., 2009). Sheep tend to experience a milder form of the disease compared to goats (Lefèvre and Diallo, 1990).

1.2.10.4 Breed

West African goats are more susceptible to PPR than European goats, with kids in the former group being particularly vulnerable (Couacy-Hymann et al., 2007). Additionally, studies have shown that Sahelian goats are more resistant compared to Guinean dwarf goats, while Alpine goats exhibit high sensitivity following experimental infections (Hammouchi et al., 2012).

1.2.10.5 Season

PPR is often regarded as a seasonal disease, primarily occurring during the cool, dry season in many endemic regions of Africa (Lancelot et al., 2002; Abubakar et al., 2009). PPR outbreaks are more likely to increase between December and February due to the dry and dusty conditions,

along with reduced fodder availability in grazing areas, which facilitates disease transmission (Khan et al., 2008).

1.2.10.6 Production system

Seroprevalence was notably higher in pastoral animals compared to agropastoral animals across all age groups (Herzog et al., 2020). Outbreaks in sheep and goats typically arise when new livestock are introduced to a farm.

1.2.11 Immunity to PPRV

1.2.11.1 Innate Immune Response

The innate immune system can sense viruses, bacteria, parasites and fungi through the expression of pattern recognition receptors (PRRs), which recognize conserved structure in the pathogens called pathogen associated molecular patterns (PAMPs). The engagement of the viral PAMPs with the PRRs leads to the activation of a complex network of intracellular signaling pathways, which ultimately results in transcription of several cytokine genes to produce an antiviral state in the host (Platanias, 2005; Randall and Goodbourn, 2008). Interferons (IFNs) are the main group of cytokines that induce a virus-resistant state in host cells and also play an important role in modulating the adaptive immune response.

PPRV infection in goats leads to a classic inflammatory response characterized by enhanced expression of cytokines (Atmaca and Kul, 2012; Baron et al., 2014). However, Morbilliviruses are well known for their ability to inhibit IFN signaling. Currently, there is limited information on the pattern recognition receptors (PRRs) activated during PPRV infection. Attenuated Morbillivirus vaccines stimulate cell-mediated immunity (Lund et al., 2000), which may play a crucial role in protection. In general, cell-mediated immunity may contribute to protective immune responses. PPRV induces IL-8 (pro-inflammatory cytokines) by interacting with Tool

Like Receptor (TLR) 2 in a dose-dependent manner. Recent work identified PPRV H protein as a new agonist of TLR2 and a potential trigger of innate immunity to PPRV infection.

1.2.11.2 Adaptive Immune Response

Morbillivirus infection is characterized by severe immunosuppression alongside a strong virus-specific immune response. Protective humoral and cell-mediated immunity against Morbilliviruses is primarily directed toward the H, F, and N proteins (Naik et al., 1997; Naik and Shaila, 1997; Sinnathamby et al., 2001a). The surface glycoproteins H and F of RPV and PPRV elicit a strong and protective neutralizing antibody response (Romero et al., 1994; Sinnathamby et al., 2001b; Munir, 2013). Although N is the most prevalent viral protein, it does not trigger a neutralizing antibody response in the host (Diallo et al., 2007). Nevertheless, it has been shown to elicit a robust cell-mediated immune response, which is thought to play a role in protection. The specific immune effectors associated with protection after PPRV vaccinations remain uncertain, whether they are systemic neutralizing antibodies, cytotoxic T cells, or mucosal immunity. In the course of PPRV infection, an efficient immune response is generated that clears the virus and confers long-time protection (Rojas et al., 2021).

Activated Dendritic Cells (DCs) release cytokines that can direct the adaptive T cell response toward a Th1 or Th2 pattern (Luther and Cyster, 2001). Inactivated PPRV or PPRV H protein treated DCs stimulate IL-12 involved in directing adaptive immunity to a Th1 response. Animals infected with PPRV develop a strong cellular and humoral response, both components required to control virus clearance and dissemination.

Dendritic Cells (DCs) activated by TLR2 will secrete IL-12, resulting in differentiation of naïve T cells into Th1 cells. At the same time, maturation of Th2 cells will start by the release of IL-6 by DCs, and these Th2 cells will produce IL-4 to generate more Th2 in an autocrine loop. These interconnections between Th1 and Th2 response will allow for a strong humoral as well as cellular response during PPRV infection (Rojas et al., 2021).

Surface glycoproteins of morbilliviruses (H and F) are highly immunogenic and confer protective immunity. Immunized goats develop both humoral and cell-mediated immune

responses. Antibodies generated in the immunized animals could neutralize PPRV (Sinnathamby et al., 2001b).

In addition, the F and H proteins are the most immunologically relevant determinants of protection against morbilliviruses, containing several T cell epitopes and the majority of neutralizing antibody epitopes.

1.2.12 Characterization of PPRV

The most widely used virus detection methods rely on molecular biology. Recently, one-step real-time RT-PCR assays have been developed and identified as the most sensitive techniques for detecting the PPR genome (Bao et al., 2008; Kwiatek et al., 2010). In the absence of PCR machines, immuno-capture ELISA (ICE) can be used as an alternative. It is a rapid, specific, and relatively sensitive method for detecting PPRV antigens in infected animals (Libeau et al., 1994). When additional characterization of the disease cause is needed, the virus must be isolated and cultured in cells. The most reliable and rapid test for antibody detection is a competition ELISA, which uses a monoclonal antibody targeting the virus nucleoprotein (Libeau et al., 1995).

1.2.12.1 Description of important diagnostic techniques

Detection of agent

A) Immunocapture enzyme-linked immunosorbent assay (IC-ELISA)

The Ic-ELISA allows for the rapid detection of PPRV by utilizing two monoclonal antibodies (MAbs) that specifically target the N protein (Libeau et al., 1994). The Ic-ELISA procedure is depicted in **Appendix Text 1.1**.

B) Molecular diagnostic techniques (Nucleic acid Recognition Methods)

Molecular techniques for detecting the PPRV genome have become the preferred methods for definitive diagnosis, lineage determination, outbreak typing, and epidemiological studies. These techniques rely on the phylogenetic analysis of the N and/or F genes of PPRV through Polymerase Chain Reaction (PCR). RT-PCR assays targeting the N gene have proven effective in detecting all four genetic lineages of PPRV in tissues, ocular and nasal swabs, as well as in blood and tissue samples collected from the field. Additionally, a highly sensitive and rapid real-time RT-PCR method has been developed, capable of identifying PPR nucleic acid as early as three days post-infection and up to 20 days in swab samples from experimentally infected animals (Couacy-Hymann et al., 2002).

Reverse transcription PCR (RT-PCR) methods have been designed to specifically diagnose PPR by amplifying segments of the N and F protein genes (Couacy-Hymann et al., 2002). According to Couacy-Hymann et al. (2002), this method is 1000 times more sensitive than traditional virus titration on Vero cells. Additionally, it provides results within five hours, including RNA extraction, whereas virus isolation requires 10–12 days. In recent years, advancements in nucleic acid amplification techniques have greatly enhanced PPR diagnosis, particularly through the development of quantitative real-time (rt)- RT-PCR (Bao et al., 2008; Khalafalla et al., 2010; Batten et al., 2011) This technique is ten times more sensitive than conventional RT-PCR and reduces the risk of contamination. Additionally, a real-time RT-PCR assay has been developed specifically for detecting lineage-IV strains (Li et al., 2016).

Real time qRT-PCR

The real-time qRT-PCR assay allows the rapid, specific and sensitive laboratory detection of PPRV in tissue samples from field cases. While one-step real-time RT-PCR using a TaqMan probe is a sensitive and rapid method compared to traditional RT-PCR, offering lower contamination risk for detection and quantification, it requires advanced and costly equipment, limiting its accessibility for routine diagnostics.

In all cases, RNA must first be extracted from blood, swabs, or tissue samples. Viral RNA can be obtained from lymph nodes, tonsils, lung tissue, whole blood, or swabs collected from the eyes, rectum, or nose. The purification process follows the manufacturer's guidelines, and both magnetic bead-based and spin column-based extraction methods are suitable for tissues, blood, or swabs. In our study spin column-based extraction method was used. The extracted RNA is stored at -70^{o}C, or at –20°C if –70°C is unavailable, until needed. While real-time RT-PCR is the preferred diagnostic method for well-equipped laboratories, no existing assay has demonstrated complete reliability across all tested PPRV isolates. PPRV RNA extraction procedure is detailed in **Appendix Text 1.2.**

Real-time reverse transcriptase polymerase chain reaction (rt-RT-qPCR)

A real-time RT-qPCR assay was conducted on all extracted viral RNA samples using specific primers and a probe targeting the N gene. The one-step real-time RT-qPCR for PPRV was carried out in a 20µl reaction mixture, which included 10µl of Express Universal SuperScript, 2.0µl of Express SuperScript Enzyme Mix, and 0.4µl of passive reference ROX (Invitrogen, Thermo Fisher Scientific, EXPRESS SuperScriptTM MIX for one-step qPCR, reference no. A10316). Additionally, 0.8µl of each primer—PPRV forward (5'AGA GTT CAA TAT GTT RTT AGC CTC CAT 3') and PPRV reverse (5'TTC CCC ART CAC TCT YCT TTGT 3')—along with 0.4µl of PPR probe (FAM-CAC CGG AYA CKG CAG CTG ACT CAG AA-QSY), 2.6µl of RNase-free water, and 3µl of RNA template were included. The amplification process involved an initial step at 50°C for 15 minutes, followed by denaturation at 95°C for 20 seconds. This was succeeded by 40 cycles of denaturation at 95°C for 3 seconds, annealing, and extension at 60°C for 30 seconds. The reaction was performed using the Applied Biosystems 7500 FAST thermal cycler, following a previously established protocol (Batten et al., 2011). In this study, samples were classified as positive if the RNA extracted from swab and tissue samples yielded a cycle threshold (Ct) value of **≤35**. Samples with a Ct value exceeding **35** were considered undetermined or negative. The Ct value indicates the number of amplification cycles needed for the fluorescence signal to surpass the threshold, signifying the presence of detectable viral RNA.

c) Culture and Isolation methods

Virus isolation is both costly and time-intensive, making it impractical for routine diagnostics. Instead, it serves as the gold standard for disease confirmation and is primarily utilized in research. Therefore, developing cell lines with high susceptibility to PPRV, such as VDS (Vero-Dog SLAM) cells, would be valuable for detecting viable PPRV in the final stages of global eradication efforts (Kinimi et al., 2020). At the start of the study, VDS cells were kindly provided by the Pirbright Institute through Dr. Michael Baron. Viral isolation exclusively detects live viruses, necessitates specialized laboratories equipped with tissue culture facilities, and has relatively low sensitivity. However, it typically provides greater diagnostic accuracy compared to sandwich ELISA. Even when rapid diagnostic techniques are used, isolating the virus from field samples in tissue culture remains essential for further research. Recently, derivatives of cell lines such as Vero and CV1 cells, which express the morbillivirus receptor—signaling lymphocyte activation molecule (SLAM or CD150)—have been developed. These advancements allow for the isolation of field viruses from pathological specimens within a week, eliminating the need for blind passages. This includes a derivative of the monkey cell line CV1 expressing goat SLAM (Adombi et al., 2011) and Vero cell derivatives expressing dog SLAM. Monolayer cultures are inoculated with suspect samples, such as swab material or 10% tissue suspensions, and monitored daily for signs of cytopathic effects (CPE). The cytopathic effects (CPE) caused by PPRV can appear within five days in SLAM-expressing cell lines, characterized by cell rounding and aggregation, ultimately leading to syncytia formation. In cover-slip cultures, CPE may become evident even before day five.

To briefly describe virus isolation, Vero Dog SLAM (VDS) cells were prepared by washing with Phosphate Buffer Saline (PBS) and then inoculated with 100-200µl of supernatants from ocular, nasal, and rectal swabs or processed tissue, previously homogenized and centrifuged at 1650g for 20 minutes at 4°C. These cells, with over 70% confluence in 24-well plates, underwent a pre-adsorption process, followed by an hour of incubation at 37°C with 5% CO2 and 96% humidity, with intermittent agitation every 15 minutes to promote virus adsorption. After removing the inoculum and washing with serum-free Dulbecco's Modified Eagle Medium (DMEM), 500µl of DMEM with 2% serum was added to each well. The cultures were then observed under an

inverted microscope for non-specific reactions before a final incubation of five to seven days under the same conditions to identify CPE indicative of viral replication, characterized by cell rounding, cell fusion (syncytia formation), in line with the protocol described by (Gomes et al., 2016).

Virus titrations

Virus titers are determined by performing precise serial dilutions of virus suspensions, with the diluent typically being a tissue culture maintenance or growth medium. The resulting data are then used to calculate the Tissue Culture Infectious Dose fifty ($TCID_{50)}$ of the initial virus suspension using the Spearman-Karber method. This calculation determines the dilution at which the virus causes cytopathic effects (CPE) in 50% of the inoculated cells. The $TCID_{50}$ is defined as the dilution of a virus that is required to infect 50% of the cell cultures in a given batch (Von Messling et al., 2003). Animal viruses, such as PPRV, can be quantified through infectivity assays like $TCID_{50}$. This method helps in determining the concentration of total or infectious virus particles in a suspension, which is important for monitoring the stages of virus purification, assessing the pathogenicity of the virus and vaccine dosage.

1.2.12.2 Next Generation Sequencing (NGS)

NGS is a high-throughput technique capable of generating millions of short sequencing reads in a single run. To enable this, nucleic acids (such as total DNA, genomic DNA, RNA, etc.) must first be extracted, purified, and then fragmented into sequences that are compatible with the sequencing instrument through a process known as library preparation. The methods used for library preparation, along with the choice of sequencing platform and its associated technology, significantly influence the subsequent analyses that can be performed. The initial step in the sequencing process involves converting nucleic acids into libraries that can be read by the instrument. Furthermore, multiple samples can be sequenced simultaneously (known as multiplexing) to maximize the efficiency of the machine run and reduce reagent costs. This is done by incorporating unique identifiers (MIDs) or barcodes (BC) during the library preparation

process. Since 2006, the sequencing-by-synthesis technology used by Solexa/Illumina sequencers has dominated a large portion of the NGS market (Vincent et al., 2017).

In brief, total nucleic acids were extracted from collected samples/isolates using the MagMAX Core Nucleic Acid Purification Kit (ThermoFisher Scientific) on a KingFisher Flex automated extraction platform (ThermoFisher Scientific), following the manufacturer's guidelines. The nucleic acids were then eluted in 90 µl of elution buffer and stored at -20°C until further processing. For library preparation, total RNA was quantified with the Qubit RNA HS Quantification Kit, and a maximum of 50 ng of total RNA was used for library preparation with the Trio RNA-Seq Kit (Tecan), according to the manufacturer's protocol. The resulting libraries were subjected to paired-end sequencing (2x150 cycles) on an Illumina MiSeq using v2 reagents (Illumina).

PCR and Sanger sequencing

Sanger sequencing was used to fill in sections of the GC-rich part of the genome not determined by the NGS. First strand cDNA was synthesised using SuperScript III First-Strand Synthesis System (ThermoFisher Scientific), according to manufacturer's recommendations, with random hexamers as the primer. First strand cDNA was converted into double stranded cDNA using NEBNext Second Strand Synthesis reaction buffer and enzyme mix (New England Biolabs) in a final volume of 80 µl and incubated at 16˚C for 2.5 hours. Double stranded cDNA was purified using the Illustra GFX PCR DNA and Gel Band purification kit.

The GC-rich region was amplified in two fragments using primers designed from the next generation sequencing data, specifically for the isolate. The reaction mix comprised 1X KAPA HiFi reaction mix (Roche), 10 pmol of forward and reverse primer and 2 µl of first strand cDNA template (50 µl final volume). The PCR cycling conditions were as follows; initial denaturation at 95^{o}C for 5 min, followed by 40 cycles of 98˚C for 20 s, 65˚C for 15 s, 72˚C for 1 min, and a final elongation step of 72˚C for 1 min. PCR products were visualised on a 1% agarose gel stained with GelRed prior to purification using the Illustra GFX PCR DNA and Gel Band purification kit. Purified PCR products were sequenced bi-directionally using BigDye Terminator v3.1 reagents.

Genome assembly and phylogenetic analyses

NGS datasets were processed following the approach outlined (King et al., 2020), utilizing bowtie2 (Langmead and Salzberg, 2012) (v2.3.4.2) and bwa mem (Li, 2013) (v0.7.15) for sequence alignment. Consensus calling was performed with bcftools (Danecek et al., 2021) (v1.6), using PPRV/Ethiopia/2010 as the reference genome. Gaps in the M-F intergenic region, which were not covered by the NGS data, were filled using Sanger sequencing data. The final genome assembly was completed with SeqMan Pro software (v17.0.2). Phylogenetic trees based on maximum likelihood were generated using either RAxML (Stamatakis, 2014) (v8.2.12) or IQ-TREE (Minh et al., 2020) (v2.1.1), depending on the analysis.

1.2.12.3 Serological tests (eg. competition ELISA)

The detection of antibodies in sheep and goats infected with PPRV can support disease diagnosis alongside clinical symptoms. However, these antibodies may also be induced by vaccination with any of the available PPRV vaccines. The relative sensitivity and specificity of competition (c) - ELISA in reference laboratories are 94.5 and 99.4% respectively (Libeau et al., 1995).

Competitive enzyme- linked immunosorbent assay (c-ELISA)

Various competitive ELISA (C-ELISA) assays have been developed utilizing monoclonal antibodies (MAbs) that target viral proteins. These assays fall into two categories: one type employs MAbs that specifically bind to the N protein, using recombinant N protein expressed in baculovirus as the antigen (Libeau et al., 1995); the other type utilizes MAbs targeting the viral attachment protein (H), with the antigen consisting of either purified or partially purified PPRV (vaccine strain) (Saliki et al., 1993; Anderson and McKay, 1994). All these assays operate on the principle that PPRV-specific antibodies present in test sera can inhibit the binding of the MAb to the antigen. A competitive ELISA was developed based on the interaction between a

monoclonal antibody (MAb) and a recombinant nucleoprotein of the PPRV. The assay has been employed to detect PPRV-specific antibodies in sheep, goats, and other animals. In this competitive ELISA, the MAb and antibodies from a positive serum sample are simultaneously introduced, leading to competition for a specific epitope on the N-B. It is as sensitive and specific as the virus neutralization test (VNT) while offering the additional benefit of using a safe antigen that can be produced in large quantities. In principle, the wells are coated with purified recombinant PPR nucleoprotein (NP). The samples to be tested and the controls are added to the micro wells. Anti-NP antibodies, if present, form an antibody-antigen complex which masks the NP epitopes. An anti-NP-peroxidase (HRP) conjugate is added to the micro-wells. It fixes to the remaining free NP epitopes, forming antigen-conjugate-HRP complex. After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added. The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested: in the absence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution;

In the presence of antibodies, no coloration appears. The micro-plate is read at 450 nm. Brief testing procedure is shown in **Appendix Text 1.3**.

1.2.12.4 Gross Pathology

Following PPRV infection, various pathological changes are commonly observed during post-mortem examination across different tissues (Parida et al., 2015a). In sheep and goats infected with PPRV, post-mortem examination reveals ulcerative to necrotic lesions throughout the buccal cavity. The most affected areas include the buccal papillae, dental pad, gums, dorsal surface of the tongue, palatine tonsils, and hard palate. Additional necropsy findings often include congestion of the gastrointestinal tract (GIT) and nasal sinuses, consolidation of the anteroventral lobes of the lungs, an engorged spleen, and, in some cases, oedematous lymph nodes (Kumar et al., 2004). Congestion of the digestive tract, especially in the duodenum, abomasum, ileum, caecum, and colon, is commonly observed. A distinctive feature is extensive congestion along the longitudinal folds of the caecum, colon, and rectum, often appearing as zebra striping. In severe cases, the intestinal mucosa can become hyperaemic, oedematous, and ulcerated (Munir et al., 2013). A notable finding in a recent study (Pope et al., 2013) was the

challenge in detecting Peyer's patches in the ileum of virus-infected animals, even when necrosis or hemorrhage was not visibly present. Previous research has highlighted the association of PPRV with these lymphoid structures, with extensive necrosis and collapse of Peyer's patches reported in both natural and experimental infections (Taylor, 1984; Kumar et al., 2004; Kul et al., 2007). Like the buccal mucosa, the nasal mucosa also becomes hyperaemic. Additionally, the caudal portion of the trachea and bronchi may contain frothy exudate (Emikpe et al., 2013).

Enlarged lymph nodes with necrosis and hemorrhage, especially in the mesenteric region, along with an atrophied and congested spleen, are occasionally observed in PPRV-infected goats in the field (Khan et al., 2008). In one study, the prominent post mortem lesions seen were congested lungs, oedematous and congested retropharyngeal and mesenteric lymph nodes, and linear haemorrhages in the intestinal mucosa and splenomegaly (Ugochukwu et al., 2019).

1.2.12.5 Histopathology

PPR infection can also be identified through histopathological techniques. Examination of PPRV-infected tissues reveals key features typical of morbillivirus infections, such as syncytial formation and widespread necrosis (Parida et al., 2015a). Histopathological analysis of major gastrointestinal organs showed degeneration and necrosis of the labial mucosa, severe congestion in the mucosal and submucosal layers, degeneration and necrosis of the intestinal epithelium, and lymphoid cell depletion in Peyer's patches, occasionally accompanied by syncytial formation (Kumar et al., 2004). In the late stages of the disease, inclusion bodies were absent in tissue sections from animals with a mild form of the infection (Pope et al., 2013). A high number of syncytial cells, varying in size and viability, can be observed in the paracortex of lymph nodes from day 5 post-infection. By day 7, these cells are also present in the cortex and follicles of lymph nodes, splenic white pulp, and gastrointestinal submucosal lymphoid tissue (Pope et al., 2013). Lymph nodes may also become edematous with mild lymphoid depletion. Moderate to severe lymphoid depletion is observed in the germinal centers of the spleen. However, cellular necrosis, indicated by nuclear debris, was less evident in the tonsillar and splenic tissues compared to the lymph nodes (Pope et al., 2013). The lungs exhibited broncho-interstitial changes, along with the presence of intracytoplasmic and intranuclear eosinophilic inclusions in

alveolar macrophages and syncytial cells. These pulmonary alterations were often complicated by serofibrinous pneumonia (Kumar et al., 2004).

In severe PPRV infections, large alveolar macrophages containing both intranuclear and intracytoplasmic inclusion bodies, numerous neutrophils, fibrin exudates, and multinucleated giant cells have been reported (Emikpe et al., 2013). However, it is important to note that some of these histopathological changes could also be attributed to secondary bacterial or parasitic infections. The alveolar lining cells transform into a cuboidal shape, and the interstitial space becomes infiltrated with lymphocytes and neutrophils. In addition, throughout the intestines and abomasum, there is significant diffuse lymphoid infiltration and edema, leading to varying degrees of crypt disruption. Lymphoid syncytial formation is frequently observed within the lamina propria, accompanied by cellular necrosis (Pope et al., 2013).

1.2.13 Control and prevention of PPR

Different measures can be implemented for prevention and control of PPR. In countries free from infection, prevention can be achieved through conventional methods such as restricting the importation of sheep and goats from affected regions, quarantining newly introduced animals for a minimum of three weeks, conducting sanitary slaughter, and ensuring the proper disposal of carcasses and contaminated materials. Additionally, decontaminating affected premises in the event of an outbreak is crucial (Abera, 2023). Managing PPR outbreaks can involve movement restrictions (quarantine) along with targeted ("ring") vaccination and preventive immunization in high-risk populations (Abraham, 2005a; Jilo, 2016). Additionally, vaccinating animals is a highly effective strategy for reducing the risk of infection in healthy populations (Diallo et al., 1989).

Once PPR is confirmed, mass vaccination of small ruminants is the most effective control measure in affected areas. The currently available vaccine provides lifelong immunity with a single dose. This homologous vaccine, developed in the 1980s, was derived from the Nigeria 75/1 strain through repeated attenuation in Vero cells (Diallo, 2003).

1.2.13.1 Vaccines

Commercially available live attenuated homologous PPR vaccines, which are not thermostable, are derived from various PPRV isolates, such as Nigeria 75/1 and Shun-gri/96. These vaccines have been attenuated through multiple passages in Vero cells (Saravanan et al., 2010). These vaccines have proven to be highly effective and are believed to provide lifelong immunity in both sheep and goats. They are widely produced and used in various countries, including Ethiopia, where PPRV remains endemic (Munir et al., 2012).

Until recently, there was no commercially available vaccine capable of serologically distinguishing between vaccinated and infected animals, a characteristic known as the Differentiating Infected from Vaccinated Animals (DIVA) vaccine (Munir, 2015).

Novel recombinant PPR DIVA vaccines have been tested in goats for safety and efficacy, with all vaccinated animals showing clinical protection against an intranasal PPRV challenge. Additionally, newly developed ELISAs successfully differentiate between infected and vaccinated animals. These DIVA vaccines, along with their associated diagnostic tools, can improve serological monitoring and speed up global PPR eradication efforts, particularly in the later stages of the control program (Niedbalski, 2023).

A newly developed thermostable vaccine provides adequate stability for up to 30 days without requiring a cold chain, making it better suited for use in challenging field conditions (Mariner et al., 2017).

1.2.13.2 Other control measures

In non-endemic regions, PPRV outbreaks are best managed through measures such as culling infected herds, maintaining strict sanitation, enforcing import controls, implementing movement restrictions, and establishing quarantine protocols. However, the effectiveness of these strategies is often hindered by inadequate facilities and limited veterinary services. Regardless of the virus's prevalence in a given area, sero-monitoring through surveillance programs proven effective in past RPV eradication efforts remains essential for controlling PPRV infections and

preventing further spread (Banyard et al., 2010). Carcasses and contaminated materials should be either buried or incinerated. Additionally, barns, tools, and other items exposed to infected animals must be disinfected using common disinfectants such as phenol, 2% sodium hydroxide, Virkon, as well as alcohol, ether, and detergents. Furthermore, isolating infected animals from healthy ones helps reduce the risk of PPR virus transmission. Removing visibly infected, suspected, or seropositive animals also plays a crucial role in controlling the spread of the disease (Braide, 1981).

1.3 Statement of the problem

Despite the ongoing PPR control and eradication program, outbreaks of PPR continue to occur in various parts of Ethiopia (Rume et al., 2019; Dubie et al., 2022; Mohammed et al., 2022; Abesha et al., 2023; Ejigu et al., 2023; Kumbe et al., 2024; Mandefro et al., 2024; Mengesha et al., 2024; Senbeto et al., 2024). Outbreak findings from the above referred studies suggest that the PPR virus is continuously circulating within the country, possibly due to the involvement of other host species, in addition to the primary hosts, sheep and goats, in the maintenance and transmission of the virus. There is also a lack of solid data regarding the actual role of large ruminants, such as cattle, in the epidemiology of PPR disease. In addition, in developing countries like Ethiopia, sheep and goats are often raised alongside other host species such as large ruminants (cattle) and camels. However, the current national control and eradication program only considers sheep and goats. There are also conflicting findings from previous studies (Mornet et al., 1956; Balamurugan et al., 2012b; Sen et al., 2014; Schulz et al., 2018; Couacy-Hymann et al., 2019) regarding the role of cattle in the maintenance and transmission of PPRV.

Furthermore, the pathology, pathogenesis, and clinical progression of PPR are not well understood, particularly in atypical hosts like cattle. Much of the knowledge is derived from studies on closely related rinderpest (RP) and other Morbillivirus (MV) infections (Domingo and Holland, 1997). In Ethiopia, so far there is no available data on the comparative pathology of PPRV between goats and cattle performed in experimental settings.

Yet in Ethiopia, there is limited up-to-date knowledge regarding the PPRV lineages and strains circulating in the country, using whole-genome sequencing (WGS) technique. Furthermore, significant knowledge gaps remain in understanding the circulating virus strains and their associated virulence characteristics.

Therefore, it is crucial to conduct a study to investigate the role of cattle in PPRV epidemiology by determining whether infected cattle shed sufficient amounts of PPRV to transmit the virus to seronegative small ruminants or other seronegative cattle. The results of this research would provide valuable insights that could enhance national PPRV control and eradication strategies in Ethiopia and other countries affected by the virus.
Given the conflicting findings on the role of cattle in the maintenance and transmission of PPRV, a detailed investigation comparing goats and cattle through experimental studies is crucial. This research should focus on assessing virus tissue tropism, clinical progression, pathology, and shedding. The findings from this study will provide valuable insights to guide the development of more effective surveillance and control strategies.

Furthermore, tracking the circulating PPRV strains in the field and analyzing their virulence characteristics are crucial areas of research for the successful control and elimination of PPR. A more detailed analysis is needed to gain a deeper understanding of the circulating virus strains. The findings from this study could inform strategic planning and enhance the effectiveness of current control strategies.

1.4 Research questions

In light of the above background information and challenging issues of PPRV infection in Ethiopia and beyond, the following research questions were articulated to be addressed in this study:

a) Do cattle become infected through natural transmission from goats, and can they transmit PPRV in Ethiopia to goats?

b) Is there a difference in the pathogenesis and pathology of PPRV infection between goats and cattle?

C) What are the diversity and virulence characteristics of the PPRV lineages/strains circulating in Ethiopia?

1.5 Objectives

General objective

The study aimed to generate comprehensive knowledge and data on the factors responsible for persistence of PPRV particularly on the role of other host species like cattle in the epidemiology of PPR, as well as on the field-circulating PPR virus lineages/strains and their virulence. The findings were intended to enhance the effectiveness of the ongoing PPR control and eradication program in Ethiopia.

Specific objectives:

- To establish through experiments whether local cattle infected with locally circulating PPRV strains shed and transmit PPRV to seronegative (naïve) small ruminants;
- To investigate PPRV tissue tropism, pathogenesis, shedding, clinical course, and comparative pathology in both goats and cattle and
- To characterize the PPRV strains circulating in Ethiopia and assess their virulence in local goat breeds through experimental infection.

1.6 Significance of the study

PPR has a significant impact on food security and the livelihoods of smallholders, leading to annual losses of $1.2–1.7 billion globally due to animal deaths, reduced production, and disease control expenses. Around one-third of these financial losses occur in Africa. Despite ongoing disease control efforts, PPR outbreaks continue to occur and are expanding from lowland pastoral areas into highland mixed farming regions of Ethiopia that were previously free of the

disease. This highlights the need to investigate all contributing factors to the persistent occurrence of the disease. Among these factors, the role of other host species, such as cattle, in the maintenance and transmission of PPRV is a key consideration.

A Research investigating the role of cattle in PPRV maintenance and transmission through experimental animal trials that simulate mixed husbandry settings, along with the exploration of various transmission scenarios using computational models, suggests that cattle are unlikely to serve as a reservoir for PPRV.

A key insight from this study, in relation to the goal of PPR eradication by 2030, is that the most common cattle species in sub-Saharan Africa (Zebu) are for now unlikely to play a role in transmitting PPRV to small ruminants. The study demonstrates that, under co-housing conditions typical of mixed-species zero-grazing husbandry practices in East Africa, cattle can become infected but do not transmit PPRV to goats. Although PPRV could not be isolated from the samples, the detection of its RNA by qRT-PCR in an infected sentinel calf highlights the importance of ongoing surveillance in cattle. It also emphasizes the need for further investigation into the mechanisms that may prevent clinical disease and viral shedding in these animals.

A study investigating the comparative pathogenesis of the Ethiopia/Habru/2014 Lineage-IV PPRV in goats and cattle, which focused on disease progression and virus shedding from different sites over time, offers important insights into reassessing the role of cattle in PPR epidemiology. The results of this study could contribute to the creation of more effective strategies for PPR surveillance and control in the future.
Furthermore, the information on circulating field PPR viruses and their virulence characteristics in the country, as explored in one of the current study, makes a crucial contribution to optimizing the ongoing PPR control and eradication program.

1.7 Scope of the study

The scope of this PhD dissertation focuses on investigating the role of cattle in the epidemiology of PPR, and to compare the pathology of PPRV in goats and cattle by studying the virus's tissue

preferences, clinical development, and shedding patterns. Additionally, the research seeks to identify the PPRV strains present in Ethiopia and assess their virulence in experimentally infected local goat breeds.

Thes tudy aimed at understanding the role of cattle in PPR epidemiology involved five consecutive trials across sheep, goats, and cattle. Sheep and goats received an intranasal inoculation of 1 ml of viral suspension, while cattle were given 2 ml of the virus. The research was carried out in a randomized experimental design at the AHI animal experimental facility, adhering to all ethical, biosafety, and biosecurity protocols. In a similar fashion, the second experimental study was conducted using local breed of goats and cattle, along with a locally circulated virus. In this study, samples were collected from live animals, and postmortem samples from strategically sacrificed goats and cattle were also analyzed for a comparative pathology evaluation. Finally, a randomized experimental study was carried out on 21 goats, including both experimental and control groups, to assess the virulence of six PPR virus strains/isolates circulating in specific regions of Ethiopia. These strains were characterized using various methods.

1.8 Limitations of the study

The study on the role of cattle in the epidemiology of PPR was carried out using a single PPRV lineage-IV isolate/strain (Ethiopia/Habru/2014) as the challenge virus. However, since different PPRV lineages may vary in their ability to shed infectious virus from atypical hosts like cattle and initiate transmission chains, the findings of this study may not be directly applicable to other lineages or strains. Furthermore, the controlled delivery of the same virus dose to each species during the experiment may not accurately reflect field conditions, where the virus dose received could vary.

While the comparative pathology study of PPRV in goats and cattle offers important insights for the development of control strategies and diagnostic approaches for PPR in endemic regions, the use of a single PPRV lineage (lineage IV) restricts the generalizability of the findings to other strains or geographic regions. Additionally, the small number of animals included in the study limits the statistical strength of the results. Furthermore, this study did not consider potential

confounding factors, such as pre-existing infections or exposures, which could impact the observed disease progression and immune responses.

The study aimed at characterizing locally circulating PPRV strains and evaluating their virulence in experimentally infected local Ethiopian goat breeds had several limitations. These included small group sizes (n=3 per strain), which reduced the statistical power and may have obscured subtle differences between strains. The relatively short observation period of 12 days also likely limited the detection of late-emerging clinical signs or mortality that might have occurred after this timeframe. Furthermore, the focus on only the Central Highland goat breed restricts the generalizability of the findings to other indigenous Ethiopian breeds, especially those from lowland areas that may exhibit different susceptibility patterns.

1.9 Structure of the dissertation

This dissertation is organized into five main chapters, following an article compilation approach. The first chapter provides the introduction, while chapters two through four present articles focusing on the role of cattle in PPR epidemiology, as well as the PPR virus strains circulating in Ethiopia and their virulence. More specifically, chapter two presents a study on investigating role of cattle in the epidemiology of PPR using experimental data and modeling approach. The study, titled "Empirical and model-based evidence for a negligible role of cattle in peste des petits ruminants virus transmission and eradication" was published in "C*ommunications Biology*" (https://doi.org/10.1038/s42003-024-06619-2).

With regard to chapter three findings of in-depth comparative analysis of PPRV infection dynamics in goats and cattle, focusing on PPRV tissue tropism, clinical progression, virus shedding, and pathology are presented. The study, titled "Comparative pathogenesis of Ethiopia/Habru/2014 Lineage-IV Peste des Petits Ruminants virus in goats and cattle" was published in "*BMC Veterinary Research*" (https://doi.org/10.1186/s12917-024-04313-3).

Chapter four details the circulating PPR virus strains in Ethiopia and examines their virulence characteristics at the experimental level. The study, titled "Comparative virulence study of

locally circulating Peste des Petits Ruminants Virus (PPRV) strains in Indigenous Ethiopian Goats" was submitted to "*BMC Veterinary Research*".

The final chapter five contains the general discussion, conclusion, and recommendations.

CHAPTER TWO

CHAPTER TWO: EMPIRICAL AND MODEL-BASED EVIDENCE FOR A NEGLIGIBLE ROLE OF CATTLE IN PESTE DES PETITS RUMINANTS VIRUS TRANSMISSION AND ERADICATION

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2.1. ABSTRACT

Peste des petits ruminants virus (PPRV) is a multi-host pathogen with sheep and goats as main hosts. To investigate the role of cattle in the epidemiology of PPR, we simulated conditions similar to East African zero-grazing husbandry practices in a series of trials with local Zebu cattle (Bos taurus indicus) co-housed with goats (Capra aegagrus hircus). Furthermore, we developed a mathematical model to assess the impact of PPRV-transmission from cattle to goats. Of the 32 cattle intranasally infected with the locally endemic lineage IV strain PPRV/Ethiopia/Habru/2014 none transmitted PPRV to 32 co-housed goats. However, these cattle or cattle co-housed with PPRV-infected goats were seroconverted. The results confirm previous studies that cattle currently play a negligible role in PPRV-transmission and small ruminant vaccination is sufficient for eradication. However, the possible emergence of PPRV strains more virulent for cattle may impact eradication. Therefore, continued monitoring of PPRV circulation and evolution is recommended.

Keywords: peste des petits ruminants, cattle, goat, transmission, multi-host, reservoir host

2.2. INTRODUCTION

A challenge in the control and eradication of many infectious diseases is the ability of several pathogens to infect multiple host species, some of which can act as reservoirs of infection (1). Pathogen control programs need to identify the role of each host species in transmission dynamics and to determine which host species permit pathogen persistence within the host community (2, 3). Tackling this challenge requires leveraging multiple tools simultaneously: experiments that shed light on host species susceptibility and transmission competency, models that capture our understanding of the dynamics of transmission and explore outcomes of potential intervention scenarios, and the collection of epidemiological and ecological field data that inform and improve the models. Such combined approaches have been used effectively for rabies to disentangle the role of multiple hosts in viral persistence (4), to identify specific mechanisms that alter transmission at varying spatial scales (5), and to inform progress toward control and elimination (6,7). If eradication is the goal but longitudinal field studies are challenging, an interdisciplinary approach that combines both empirical and modelling scenarios may be the only feasible approach for progress. This is the case for the economically important, multi-host disease caused by the livestock pathogen peste des petits ruminants virus.

Peste des petits ruminants (PPR) virus (PPRV; family: Paramyxoviridae; genus: Morbillivirus; species: Small ruminants morbillivirus) is a priority pathogen that is targeted for global eradication by 2030 by the Food and Agricultural Organization (FAO) and the World Organization for Animal Health (WOAH) (8, 9). PPRV threatens nearly 80% of the global small ruminant population and the livelihoods of over 330 million farmers (10). The virus can cause high morbidity and moderate to high mortality rates in the main hosts, domesticated goats and sheep. PPRV is typical among morbilliviruses in that it infects a range of livestock and wildlife host species, with varying levels of host pathogenicity (11), and results in robust immune memory in recovered hosts. Information on host range has come from observed outbreaks, serological data, and a small number of experiments (12–18) in white-tailed deer, pigs, and camels which directly address the ability of each species to transmit PPRV. Importantly, there is concern that the proposed eradication efforts, which based on mass vaccination of small ruminants, may be thwarted if PPRV has the potential to persist within currently unidentified livestock or wildlife reservoirs (5,9). The turnover of sheep and goat populations is high and

maintaining high immunity at a population scale may be challenging. Mass vaccination of sheep and goats alone could be insufficient to eradicate PPR and the potential for spillback events into susceptible host populations would remain. Therefore, there is an urgent need to better understand the role of a larger number of livestock and wildlife species in PPR epidemiology and to identify what additional control measures may be needed to account for these atypical host species.

Cattle are an important focus for investigation as they are abundant, live in close proximity to sheep and goats, and serological studies indicates that they are frequently exposed to PPRV infection. Previous cross-sectional studies found widespread PPRV seroconversion in cattle in Africa and Asia (19–33). Experimentally infected cattle seroconvert (34, 35) and shed PPRV RNA detectable from 1-10 days post infection (dpi) 35, but not infectious virus (34, 35). However, of these studies, one did not use local cattle breeds from the same region where the PPRV strain was isolated (35), which limits the conclusions that can be drawn around transmission and susceptibility of locally-circulating PPR viruses and hosts. The second study did not determine if infectious virus was present in cattle and used a host sample size that would only be able to detect infection if a high rate of cattle-to-goat transmission was present (i.e. n = 12, with 24 possible cattle-goat contacts) (34). Hence, it remains unclear if PPRV-infected cattle are capable of shedding PPRV at a level sufficient for transmission to sheep and goats or other cattle. Whether cattle can transmit PPRV to small ruminants in experimental conditions and understanding the scenarios of inter-species transmission would enable PPRV to persist in the field are key questions that need to be answered to resolve the role of this atypical host.

The objectives of the current study were twofold: first, to establish through experiments whether local cattle infected with locally circulating PPRV strains shed and transmit PPRV to seronegative (naïve) small ruminants and second, to use a mathematical modeling approach to quantify the extent to which varying scenarios of potential cattle transmission rates could impact PPR eradication efforts. This was achieved through a series of PPRV transmission trials in Ethiopia using local PPRV isolate and local breeds of cattle, sheep, and goats. The trials were designed to emulate mixed species zero-grazing husbandry practices commonly observed in East Africa, where PPR is endemic. We hypothesize that cattle are capable of transmitting PPRV to small ruminants and thus contribute to PPRV persistence in the field. However, since cattle-to-small ruminant transmission was expected to be rare, this study was designed to be able to detect a low rate of cattle-to-small ruminant transmission and define an expected upper limit on the probability of such transmission. We developed a cross-species susceptible-infected-recovered (SIR) model with small ruminant vaccination to estimate the impact of cattle transmission on the effective community reproductive number, Re, and to determine the proportion of vaccinated small ruminants needed to control community transmission under varying cattle-to-goat transmission scenarios. Together, the experimental trials and modeling framework suggest that cattle are currently not playing a significant role in the transmission of PPRV to sheep and goats and that there is no need to modify the PPR eradication campaign to include control measures for cattle.

Significance Statement

The presence of reservoir hosts can impede attempts to control important pathogens. Peste des petits ruminants virus (PPRV) can infect multiple animal species and represents a major risk to food security and poverty alleviation and is therefore targeted for global eradication by mass vaccination of sheep and goats. Through animal trials conducted in mixed species zero-grazing husbandry settings and examination of transmission scenarios with computational models, these results showed that cattle are unlikely to serve as a reservoir of PPRV and potential transmission from cattle is unlikely to interfere with the current eradication program in sheep and goats. The models also highlight the need for active monitoring of PPRV transmission and strain evolution across multiple domestic and wild species in order to continually assess risk.

2.3. MATERIALS AND METHODS

2.3.1. Virus

The PPRV lineage IV (LIV) isolate was collected in 2014 from pooled samples of a single female goat in the Habru district in the Oromia Zone of the Amhara region (36). This isolate caused 21.9% morbidity and 8.4% mortality with a case fatality rate of 38.4% in a population of 511 (121 sheep, 390 goats) (36). After being passaged twice on CHS-20 cells, which are (Flp-In-CV1) CV1 African Green Monkey kidney cells stably expressing the goat SLAM receptor (37), the isolate PPRV/Ethiopia/Habru/2014 was stored at the Animal Health Institute (AHI, formerly National Animal Health Diagnostic Investigation Center - NAHDIC) at -80° C (36). For the trials, the virus was further grown on Vero-dogSLAM (VDS) cells (38) and harvested starting at the third passage (Table S1). The full genome sequence was determined at VDS passages 3 and 4 (Genbank accession ON110960) and no difference was found between these two preparations. Tissue culture infectious dose (TCID50) was calculated using the Spearman-Kärber Method (39).

2.3.2. Animals and Study Design

Local breeds of sheep (Ovis aries), goats (Capra aegagrus hircus), and Zebu calves (Bos taurus indicus) were purchased from markets within a 200 km radius of AHI (Sebeta, Ethiopia). There was no reported history of PPRV vaccination in any animal purchased. Animals were of both sexes and ranged in age from 6 months to 1.5 years. Once seronegative status was confirmed (see Serological Analysis), animals were moved into the six-barn experimental facility for acclimatization, followed by randomization of experimental animals and controls to specific experimental barns (also randomized). More details on animal recruitment, sampling, barn conditions, biosecurity, and positive and negative control animals are provided in the supplement (**Text S 2.1- S 2.3**). The cattle-to-goat transmission trial was designed with the necessary sample size to have an 80% chance of detecting a cattle-to-goat transmission event, if it occurred, for an event probability as low as 0.05 (**Text S 2.4**, **Figure S 2.1**). We have complied with all relevant ethical regulations for animal use (see Inclusion & Ethics Statement for details).

A series of 5 trials were conducted (**Figure S 2.2**) in which sheep or goats and cattle were infected intranasally with 1 ml of viral isolate in Dulbecco's Modified Eagle's Medium (DMEM), 0.5 ml per nostril. Cattle were given 2 ml total virus, 1 ml per nostril. Specifically, passage 3 or 4 was used, and TCID50 ranged between $10^{5.3}$ and $10^{5.6}$. Virus titre and passage for each trial are in Table S 2.1. For transmission trials, sentinel (seronegative) animals were added to the barn 1 day post-infection (dpi). Within the barn, animals were allowed to move freely and shared feeding and watering troughs (**Figure S 2.3**). Each day animals were monitored for clinical signs using an established clinical scoring scheme which included rectal temperature (35, 40). Each of the 6 component scores ranged from 0-4 and were summed to the final score. Samples (whole blood, cloted blood, and ocular, nasal, and rectal swabs) were collected from every animal on 0, 4, 7, 10, 14, 17, 21, 28 dpi. For trials 3-5, sampling days were extended to include 32 and 35 dpi to better understand pathogen shedding in animal feces. Total animals used in each barn and each trial are listed in **Figure S 2.2**, and in the conceptual figured inset in the upper left of each results figure.

2.3.3. Clinical Validation of Isolate

Trials 1 and 2 established that a single 1 ml intranasal dose of the local isolate PPRV/Ethiopia/Habru/2014 produced typical clinical signs in local breeds of sheep and goats and transmitted readily from sheep to sheep and goat to goat (**Figures S 2.4** – **S 2.9**, **Text S 2.1**). Sheep were not used after Trial 2. A supplemental PPRV challenge trial confirmed that seroconversion protected goats from clinical disease (**Figure S 2.9**, **Text S 2.1**). Results from trials 1-2 are reported in the supplement and we focus here only on the investigation of cattle-to-goat transmission (trials 3-5). For each trial, new positive (inoculated) and negative (PPRV seronegative) control animals were run at the same time, in the same facility, in a randomly assigned barn (**Figures S 2.10** - **S 2.12**).

2.3.4. Serological Analyses

Goat, sheep, and cattle sera were tested in duplicate for antibodies against the PPRV N protein using competitive ELISA (41) (sensitivity: 94.5%, specificity: 99.4%) according to the manufacturer's instructions (**Text S 2.2**).

2.3.5. Molecular Analyses

PPRV RNA from swabs and whole blood taken on each sampling day was extracted and PPRV RNA assayed using quantitative reverse transcription real-time PCR (qRT-PCR) based on Batten et al (42) with custom Taqman QSY Probe and Express One Step kit (**Text S 2.2**). Samples were tested in duplicate and those with a cycle threshold (Ct) value of 35 or less were considered positive.

Swabs were also tested by commercial antigen sandwich ELISA kit (**Text S 2.2**) and included nasal swabs from 4 dpi + and rectal swabs from 14 dpi + (trials 1-3) or 4 dpi+ (trials 4-5). Ocular swabs were not tested. This test detected PPRV nucleoprotein using anti-PPRV N protein antibodies. For trials 2-5, the qRT-PCR data were compared with the antigen ELISA results (**Figures S 2.8 - S 2.13**). The commercial kit had not been validated in cattle.

Swab samples were not taken from negative control animals after trial 1 and they were monitored solely for clinical signs at a daily interval and seroconversion on sampling days. Whole blood was not tested for PPRV RNA after trial 2.

2.3.6. Virus Isolation

Ocular, nasal, or rectal swab samples that tested positive by qRT-PCR or antigen sandwich ELISA were tested for infectious virus by inoculating into Vero-dogSLAM (VDS) cells (38) and recorded as PPRV-positive if typical CPE was observed within 5 days. Details in **Text S 2.2**.

2.3.7. Statistics and Reproducibility

For each trial, plots of rectal temperature, clinical score (35, 40), serology, viral RNA and or viral protein/antigen from nasal swabs over time (dpi) were created using the ggplot package in R statistical software 4.3.3. Local regression (LOESS) smoothed curves and accompanying 95% confidence bands (from t-based approximation) were added in bold to the plots using the stat-smooth function. These smoothed lines represent all animals in the specific group (control, inoculated, sentinel). The dpi at which the peak value for each metric in each animal group was achieved was extracted and plotted as a vertical line corresponding to the color of the group and an inter-peak interval was calculated by taking the difference in dpi of the peak values (sentinel peak dpi – inoculated peak dpi).

Using the trial results in a beta-binomial Bayesian model from the bayes rules package in R (43, 44), posterior distributions were estimated for the following transmission probabilities: cattle-to-small ruminants (βCS), small ruminants-to-cattle (βSC), and small ruminant-to-small ruminant (βSS). These reported probabilities represent a net transmission from one species to another in the trials. The individual animal transmission probability is calculated by dividing the posterior mean by the number of inoculated animals. Transmission probabilities were converted into rates using the following relationship:

$$p(t) = 1 - e^{-rt}$$

where p(t) is the transmission probability, r is the transmission rate, and t represents the infectious period (10 days (35,45) used in main text, results with 8 and 14 available in supplement). Credible intervals- (CrI) were calculated for the posterior mean and transmission rates using the appropriate quantile functions in R (qbeta or rbeta for sampling and then quantile). Calculated transmission rates were used as transmission rate parameters between animal species (βCS, βSC, βSS) in the mathematical model. Code is available in Text S 2.5 and on GitHub (see Data Accessibility) and visualization of the priors and posterior distributions in **Figure S 2.14**.

2.3.8. Mathematical Modeling

To explore the impact of potential cattle transmission to small ruminants under the current eradication plan in which only small ruminants are vaccinated, a Susceptible-Infected-Recovered (SIR) dynamical model was developed and applied to multiple species.

$$\frac{d\vec{\mathbf{S}}}{dt} = \underbrace{\mu\vec{\mathbf{N}}(1-\vec{\mathbf{p}})}_{\text{recruitment}} - \underbrace{\phi\vec{\mathbf{S}}}_{\text{infection}} - \underbrace{\mu\vec{\mathbf{S}}}_{\text{death}}$$

Force of infection: $\phi = \beta \times \left(\vec{\mathbf{I}}/\vec{\mathbf{N}}\right)$

$$\frac{d\vec{\mathbf{I}}}{dt} = \underbrace{\phi\vec{\mathbf{S}}}_{\text{infection}} - \underbrace{\gamma\vec{\mathbf{I}}}_{\text{recovery}} - \underbrace{\mu\vec{\mathbf{I}}}_{\text{death}}$$

$$\frac{d\vec{\mathbf{R}}}{dt} = \underbrace{\gamma\vec{\mathbf{I}}}_{\text{recovery}} - \underbrace{\mu\vec{\mathbf{R}}}_{\text{death}}$$

In the above system of equations, which represents species-specific populations (N), vaccination of the susceptible sheep or goats is included (1 - p), as well as birth (μN), death (μS, μI, μR), and recovery (γI). Cattle were not vaccinated (pcattle = 0). The parameter p represents small ruminant vaccination coverage and effectiveness combined. This model assumes homogeneous mixing of hosts.

Using the next-generation framework (46), the community reproductive number (R0) – the average number of secondary infections produced by one infected animal – was calculated over a range of small ruminant vaccination (0-100%) and cattle-to-small ruminant transmission rates (βCS = 0-slightly above the rate of goat-to-goat transmission). Parameter values are found in **Table S 2.2**. Mortality rates were drawn from the literature (47) and the inverse of the rate yielded reasonable life expectancy for small ruminants (2.14 years) and cattle (10 years). Recovery from PPRV infection takes approximately 14 days; the recovery rates (γ) for small ruminants and cattle were assumed the same (1/14). Transmission rates between each species (βCS, βSC, βSS) were calculated from the posterior distributions of the transmission

probabilities from the trials. As control was applied and small ruminant population immunity changed, the values of the effective community reproductive number RE were estimated. Four plausible transmission patterns (two symmetric, two asymmetric) were explored as the cattle transmission rate (βCS; where C = cattle and S = small ruminants) was varied, but other values in the 2x2 transmission parameter β matrix remained fixed. Specifically, scenario 1 explored a symmetric transmission rate for all species (βSS = βSC = βCC; βCS varies 0-0.26), scenario 2 explored a symmetric transmission rate within each species (βSS = βSC; βCC; βCS varies 0-0.26), scenario 3 explored an asymmetric transmission rate between species (βSS; βSC = βCC; βCS varies 0-0.26), and scenario 4 explored an asymmetric transmission rate within and between species (βSS; βSC; βCC = 0; βCS varies 0-0.26). R software code for the model is provided on GitHub (see Data Accessibility).

2.4. RESULTS AND DISCUSSION

Cattle can be infected naturally by contact with PPRV infected goats and can show mild, transient clinical signs

The PPRV/Ethiopia/Habru/2014 isolate infected local Zebu cattle under natural conditions. Two PPRV-negative cattle co-housed with 4 inoculated goats seroconverted, though these cattle seroconverted later than 2 PPRV-negative goats co-housed in the same barn (17-21 dpi vs 14 dpi, respectively; **Figure 2.1**). In this trial, days were counted starting with goat inoculation, so cattle were exposed to the excreted virus only from 4-5 dpi. Calf 1 had no clinical signs. Calf 2 had only mild, transient clinical signs between 13-18 dpi, including slightly elevated rectal temperature (≥ 39.5° C but ≤ 40° C) and displayed mildly inactive, tired behavior. PPRV RNA was detected by qRT-PCR in both replicates of nasal (7 dpi) and rectal (17 dpi) swabs, and one replicate of ocular (17 dpi) swabs from Calf 2 (Figure 2.1). These positive qRT-PCR samples (all Ct > 27) were negative when virus isolation in cell culture was attempted. When testing for viral antigens by AgELISA, Calf 2 had positive nasal swabs on 7 and 10 dpi and positive rectal swabs on 21, 28, and 32 dpi. Calf 1 had positive nasal swabs on 7, 10, 17, and 21 dpi but did not have positive rectal swabs. Antigen results from these sentinel calves are not visualized.

This experiment demonstrated that calves can be infected naturally with PPRV by contact with infected goats and show mild, transient clinical signs. Based on this, we suggest that cattle in the field are most likely exposed to PPRV as calves (< 1 year) through direct contact when co-housed with goats or when a PPR epidemic spreads through a co-housed or communally grazed herd after new animals are introduced. Previously published studies reported low seroconversion rates among cattle in the field across several African and Asian countries (1.8-18%, n=24-1,158) (19–33) which may be due to few encounters with PPRV-infected sheep or goats by management practice or chance, pre-existing cross-immunity from infections with other circulating morbilliviruses (48), or infection with PPRV strains that are either able to evade (49) or are unable

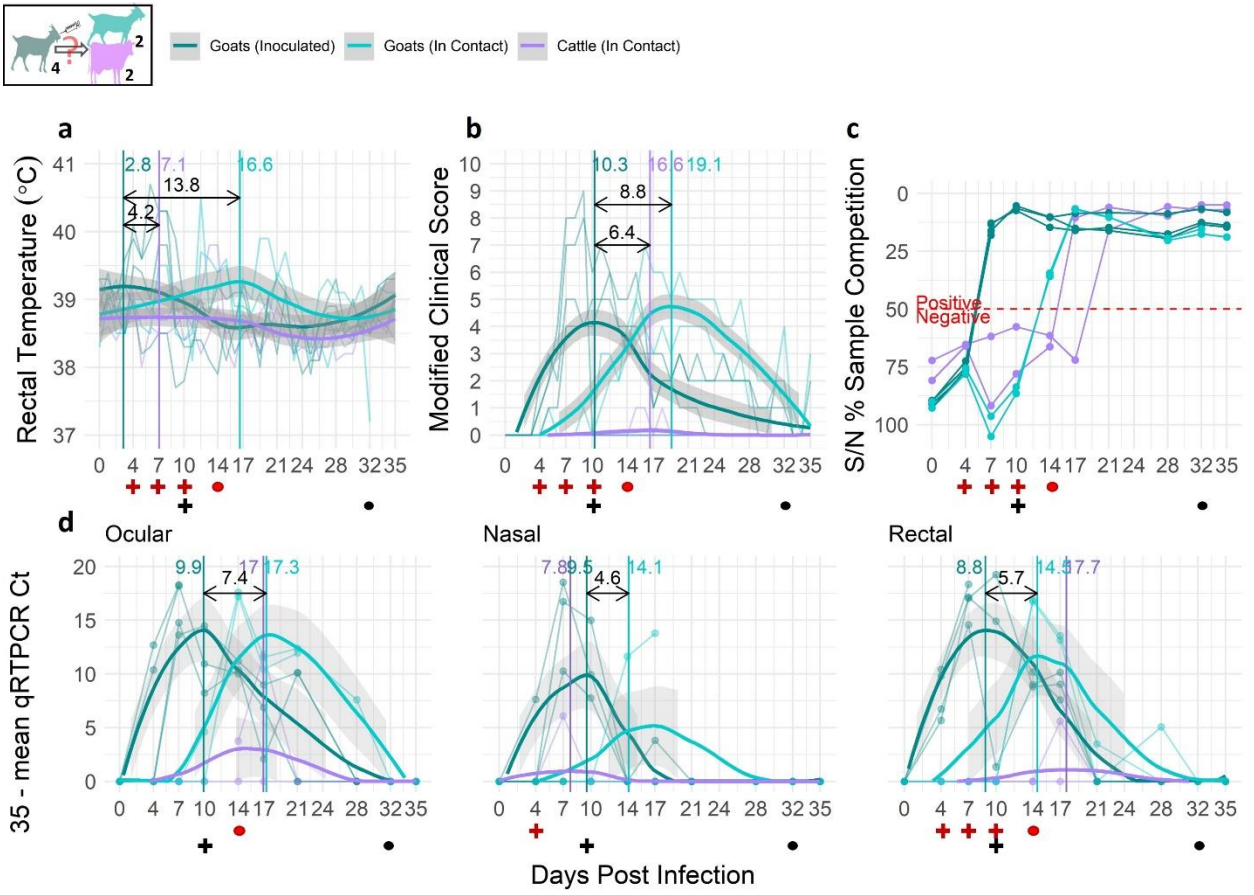


Figure 2.1: Cattle are naturally infected and seroconvert after contact with co-housed PPRV infected goats. **A.** daily rectal temperature, **B.** daily clinical score, **C.** serology (competitive ELISA), and **D.** viral RNA from ocular, nasal, and rectal swabs (qRT-PCR). Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (control, inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). Vertical lines indicate day post infection (dpi) of peak value and inter-peak interval (difference in dpi of peak

value for each animal group) is indicated in black. Sampling days on which PPRV could be isolated (cross if isolated from inoculated animal, circle for sentinel animal) are indicated with red and dpi with deaths are indicated with black (cross for inoculated animal deaths, circle for sentinel deaths, x for euthanized) along the x-axis. No PPRV was isolated from cattle samples. Two deaths occurred, one inoculated and one sentinel goat. Image Icon Credits: GetDrawing.com (goat (73), cattle (74)), Clker.com (needle (75)).

to overcome the cattle innate immune response – both of which might reduce seroconversion. In contrast, two field studies from Pakistan and Sudan report high PPRV seroprevalence among cattle (41.9%-42%, n=43-1000) (20, 23). Animals sampled either experienced (20) or were hypothesized to have experienced (23), shared grazing and watering. Additional explanations for high seroprevalence could include: cattle have longer lifespans, relative to sheep and goats, and so have a greater chance of PPRV exposure and seroconversion; an ongoing source of PPRV exposure is present such as frequent contact with newly introduced PPRV-infected animals, extended shedding of PPRV from infected sheep and goats, or extended PPRV survival in some environments. More data is needed to support any of these explanations. Future work could vary the number of co-housed infectious goats as a proxy for varying PPRV dose to better understand the minimum PPRV dose that consistently would produce clinical signs in cattle.

Cattle experimentally infected with PPRV do not develop clinical disease and do not transmit PPRV to goats

Cattle-to-goat transmission trials were conducted to determine if inoculated cattle could transmit PPRV to co-housed goats. Intranasally inoculated cattle seroconverted between 7 to 14 dpi (21.9% by dpi 7 and 93.7% by dpi 10, **Figure 2.2**), developed no pyrexia and no, or only mild, transient clinical signs. None of the inoculated cattle shed PPRV RNA as detected by qRT-PCR. Nasal and rectal swabs from the inoculated cattle were positive for PPRV antigens as detected by AgELISA (**Figure S 2.13**), but antigen presence was transient and at lower levels than infected goats (Figure S 2.8). Low levels of antigen were detected in cattle nasal swabs up to 21 dpi (11 of 12 detected up to 10 dpi) and rectal swabs up to 32 dpi (**Figure S 2.13**), which contrasted with the clear peak of antigen detection in infected goats, which resolved by 17 dpi (**Figure S 2.8**).

The PPRV/Ethiopia/Habru/2014 isolate transmitted efficiently among goats (**Figure S 2.5**) and from inoculated goats to cattle (**Figure 2.1**): all 12 sentinel experimental animals became infected and all 4 sentinel positive control goats. The small ruminant-to-small ruminant transmission probability (9.29 x 10-1, 95% CrI: 7.53 x 10-1, 9.98 x 10-1) was 1.24 times the small ruminant-to-cattle transmission probability (7.50 x 10-1, 95% CrI: 2.92 x 10-1, 9.92 x 10-1), and the calculated small ruminant-to-small ruminant transmission rate was nearly double the small

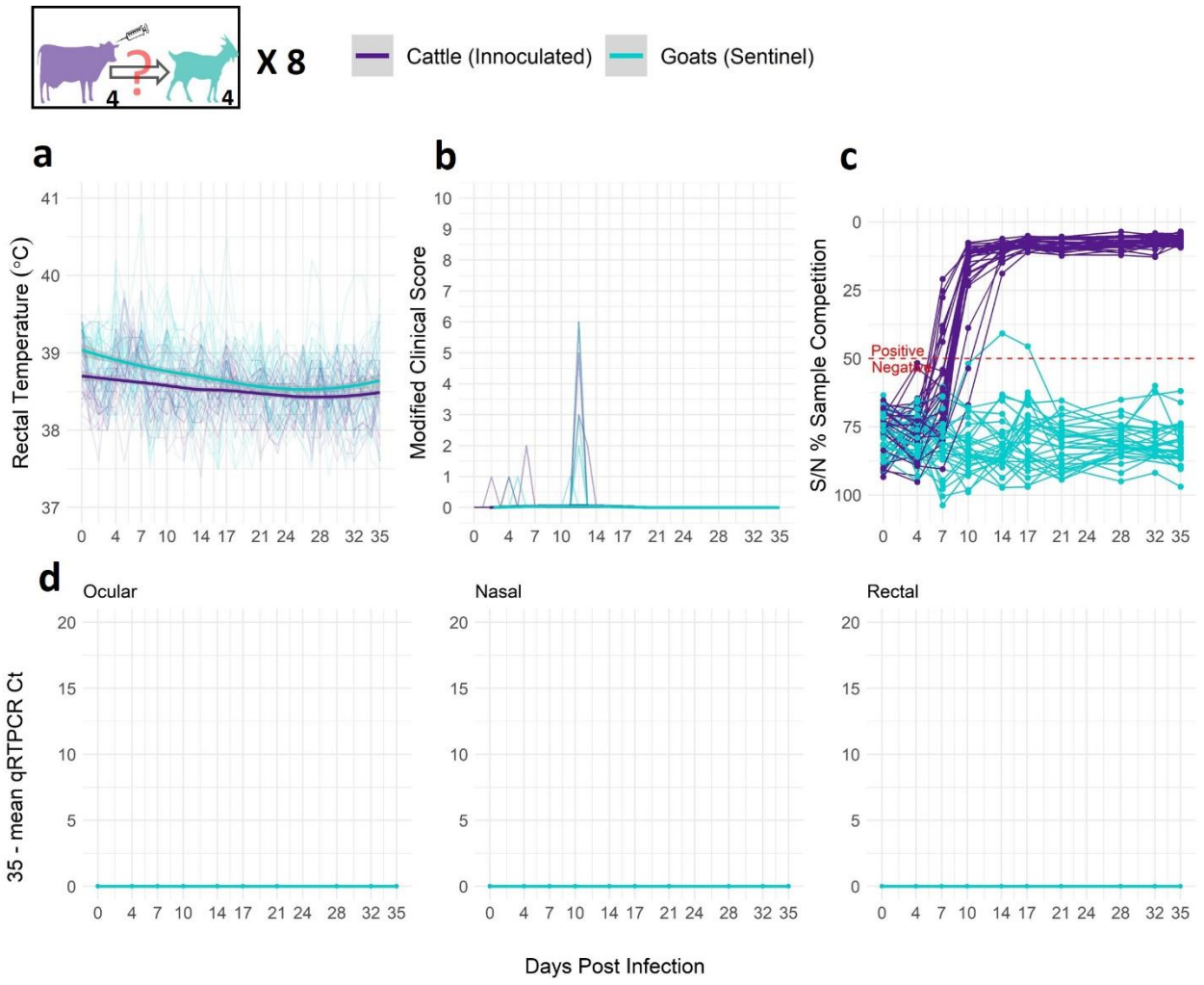


Figure 2.2: Inoculated cattle do not transmit PPRV to co-housed sentinel goats. **A**. daily rectal temperature, **B**. daily clinical score, **C**. serology (competitive ELISA), and **D**. viral RNA from ocular, nasal, and rectal swabs (qRT-PCR). Each trial used 4th passage isolate TCID50 105.5-5.6. Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (control, inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). No deaths occurred (with the exception of the death of one goat due to injury unrelated to infection) and no isolates were recovered from cattle. Image Icon Credits: GetDrawing.com (goat (73), cattle (74)), Clker.com (needle (75)).

ruminant-to-cattle transmission rate (βSS = 2.64 x 10-1 (95% CrI: 1.44 x 10-1, 6.33 x 10-1); βSC = 1.39 x 10-1 (95% CrI: 3.45 x 10-2, 4.87 x 10-1)). In contrast, no evidence was found for PPRV transmission from inoculated cattle to co-housed goats (**Figure 2.2**). Co-housed goats developed no lasting PPRV antibodies as detected by cELISA, shed no virus detectable by qRT-PCR, and showed minimal clinical signs not consistent with a typical course of disease (e.g. transient days of more inactive behavior up to score 2, and/or changes of score 1 in feces, discharges, or respiration (40)). Each barn contained 4 inoculated cattle and 4 sentinel co-housed goats, giving 16 potential transmission contacts per barn; across all cattle-to-goat trials, this was replicated 8 times giving a total of 128 potential transmission contacts. With no cattle-to-goat transmission detected among the 32 inoculated cattle and 32 co-housed goats, this study provides strong evidence that the probability of cattle-to-goat transmission is rare, including when animals are co-housed for an extended period (**Figure S 2.1**). This is supported by the nearly 32 fold difference in the estimated posterior mean of the small ruminant-to-small ruminant transmission probability (9.29 x 10-1, 95% CrI: 7.53 x 10-1, 9.98 x 10-1) compared to the cattle-to-goat transmission probability (2.94 x 10-2; 95% CrI: 7.67 x 10-4, 1.06 x 10-1), which translates to an 88 fold difference in transmission rates (βSS = 2.64 x 10-1 (95% CrI: 1.44 x 10-1, 6.33 x 10-1), βCS = 2.99 x 10-3 (95% CrI: 8.60 x 10-5, 1.13 x 10-2 (**Text S 2.5**, **Figure S 2.14**).

Serological findings in this study can be compared to two smaller trials (34, 35). Specifically, this study found cattle seroconverted between 7-14 dpi, whereas Schulz et al (35) and Couacy-Hyman et al (34) documented seroconversion at 10 dpi and 9 or 15 dpi (depending on the isolate), respectively. Unlike Schulz et al (35), this study found no PPRV RNA by qRT-PCR from any swab of an inoculated cattle. However, PPRV RNA was detected by qRT-PCR in one naturally infected sentinel calf, but PPRV was not isolated from these samples. This finding suggests it may be important to continue to monitor PPRV in cattle and to further investigate the mechanisms that prevent PPRV-induced pathogenesis and viral excretion in cattle, such as differences in viral localization (35), receptor usage (50), intercellular transmission pathways, or replication dynamics in lymph nodes and immune compartments vs the rest of the cattle host (51) as well as if these mechanisms vary with PPRV strain or cattle breed. Lastly, this study detected PPRV antigens in nasal and rectal swabs by AgELISA among cattle in contact with

inoculated goats; however it is highly likely that the early positive nasal swabs (7 and 10 dpi) represents goat-excreted virus that the calf inhaled and that positive rectal swabs represents dead tissue shedding as opposed to live virus. Inoculated cattle positive nasal swabs (4, 7, 10, 21 dpi; **Figure S 2.13**) were likely due to remaining PPRV from nasal inoculation, but could potentially also be from local replication in nasal epithelial cells as has been observed with measles and canine distemper viruses (52). We note as well that the AgELISA commercial assay used in this study has been validated for use in sheep and goats but to our knowledge has not yet been validated for use in cattle.

Implications of cattle transmission to small ruminants for eradication

Transmission from host species that are not targeted for control will increase the multi-species reproductive number R0 and impede eradication efforts. Therefore, we developed a general cross- species SIR modeling framework to explore the impact of not vaccinating cattle on the rate of small ruminant vaccination needed to bring the effective community reproductive number RE below 1 across varying cattle-to-small ruminant transmission scenarios. For PPRV, simulations from this framework suggest that an increasing level of small ruminant vaccination would be needed to reduce RE < 1 as transmission from the non-target host cattle increased in two of the four transmission scenarios explored (**Figures 2.3B**, **2.3D**), rising from low levels to 40-60% coverage needed. In one scenario (**Figure 2.3C**), a small level of small ruminant vaccination may be sufficient across a wide range of increasing cattle transmission rates.

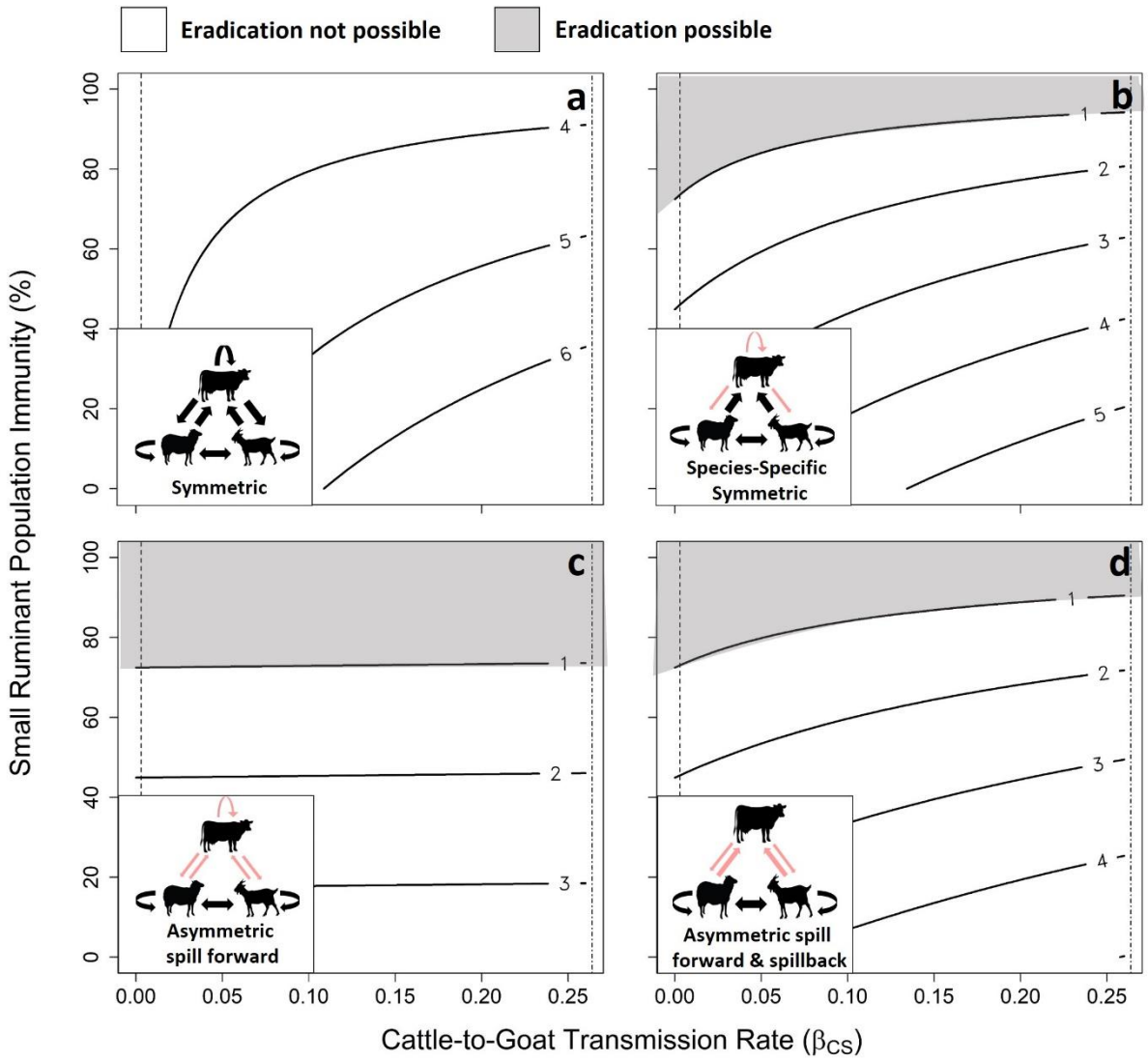


Figure 2.3: Increasing cattle-to-small ruminant transmission increases the amount of small ruminant vaccination needed in some but not all transmission scenarios. Eradication is possible in scenarios **B**, **C**, and **D** when RE <1 (i.e. area above the contour labelled "1" in **B**, **C** & **D** and shaded in gray), but is not possible in scenario **A**. Community RE values are indicated in the numeric values on labelled contours. For an infectious period of 10 days, the estimated transmission rates for cattle-to-small ruminant and small ruminant-to-small ruminant are shown as dashed (farther left) and dot dashed (farther right) vertical lines, respectively. In scenarios **B** and **D**, increasing levels of small ruminant vaccination is indicated, as the rate of cattle-to-small ruminant transmission increases from the observed cattle-to-goat transmission rate to slightly above the goat-to-goat transmission rate observed in the trials. For each transmission scenario, cattle (C) transmission rate to small ruminants (S) varies from 0 to 0.1 (x-axis, gray arrow). In panels **B**, **C**, **D** changes in transmission scenarios relative to panel A are indicated with red arrows or removal of an arrow in the inset figure. Scenario 1 (A) explores a symmetric transmission rate for all species (βSS = βSC = βCC = 2.6 x 10-1; βCS varies 0-0.26). Scenario 2 (B) explores a symmetric transmission rate within each species (βSS = βSC = 2.6 x 10-1; βCC = 3.0 x 10-3; βCS varies 0-0.26).). Scenario 3 (C) explores asymmetric transmission rate between species (βSS = 2.6 x 10-1; βSC = βCC =3.0 x 10-3; βCS varies 0-0.26).). Lastly, scenario 4 (D) explores asymmetric transmission rate within and between species (βSS = 2.6 x 10-1; βSC = 1.4 x 10-1; βCC = 0; βCS varies 0-0.26).). See Methods,

Text S 2.5, **Text S 2. 6**, and **Table S 2. 2** for more information on parameter values selected and model code. See Figures **S 2.15 - 2.16** for results considering infectious periods of 8 and 14 days. Image Icon Credits: Cliparts Zone (sheep76), GetDrawing.com (goat (73), cattle (74)).

In most realistic transmission scenarios (**Figure 2.3B-D**), eradication is possible. However, if PPRV were to gain transmission competency in cattle and then transmit to small ruminants and other cattle at the same rates that small ruminants transmit PPRV (**Figure 2.3A**), no amount of small ruminant vaccination is predicted to bring community RE <.1.Although these trials were designed with a large sample size to be able to detect low cattle-to-goat transmission, a limitation of this study is the use of a single PPRV lineage IV isolate. While PPRV/Ethiopia/Habru/2014 is from a lineage commonly circulating in Ethiopia (53), where PPR is endemic, and more broadly in Africa (54), PPRV lineages may differ in their ability to shed infectious virus from atypical hosts and start transmission chains. Additionally, we delivered a single dose and the size of dose (2ml for cattle and 1ml for goats) was not varied; in the field, both may vary. However, PPRV/Ethiopia/Habru/2014 demonstrated key characteristics observed in the field including development of conventional clinical signs of appropriate severity in small ruminants, the ability to transmit between goats and cattle, and cattle seroconversion. As no cattle-to-goat transmission was observed, cattle-to-cattle transmission was not explored on the basis that sentinel goats would have provided the greatest chance of detecting PPRV transmission from cattle. Additionally, host species origin of the strain, host breed, and additional stressors (e.g. environmental stress, coinfection) may impact infection and transmission, and could be explored in future long term field trials. Finally, as measurements of inter- and intra-species transmission rates and host mixing patterns improve, the SIR modeling framework presented will need to be updated.

Concluding Remarks

The critical insight to arise from this study toward the goal of 2030 PPR eradication is that the most common cattle species found in sub-Saharan Africa (Zebu) are unlikely to be involved in transmission of PPRV to small ruminants. This study shows that, under co-housing conditions representing mixed species zero-grazing husbandry practices found in East Africa, cattle can become infected but do not transmit PPRV to goats. Given the experimental sample size, this

study had an 80% chance of detecting a cattle-to-goat transmission event with an event probability as low as 0.05. No transmission was observed. Additionally, statistical analysis estimated a cattle-to-goat transmission probability of 2.94×10^{-2} (95% CrI: 7.67×10^{-4}, 1.06×10^{-1}), which was nearly 32 times smaller than the estimated small ruminant-to-small ruminant transmission probability. This demonstrates that cattle-to-goat transmission, if it exists, is rare, including when animals are co-housed for an extended period. This finding supports the alternative hypothesis that cattle act as dead-end hosts in the epidemiology of PPR and currently have a negligible role as reservoirs of PPRV transmission to sheep and goats. Additionally, by using a SIR modeling framework with biologically realistic parameters, this study found that bringing the community RE <1 should be achievable with only sheep and goat vaccination in most realistic scenarios. However, if PPRV were to gain the ability to transmit from cattle hosts, intervention strategies would need to be revisited. For now, continued monitoring of circulation among potential non-target host species is warranted.

Going forward, a key test of the hypothesis that small ruminants are the only maintenance hosts for PPRV will be large-scale monitoring of PPRV circulation in the community of host species carried out alongside mass vaccination campaigns. During the early phase of the rinderpest (RP) eradication campaign, similar questions around wildlife reservoirs were posed and definitive confirmation that cattle were the sole maintenance host population was obtained only through observation of RP virus (RPV) elimination in wildlife following mass vaccination of cattle (55). For PPR, a further important consideration for monitoring morbillivirus circulation at a population scale is that successful PPR eradication and the loss of host population immunity to PPRV may result in the vacant ecological niche (56) being occupied with another morbillivirus (57–60) through immunological competitive release. A similar explanation has been identified for monkeypox in filling the immunological niche created by the cessation of smallpox vaccination (61). This process may have already contributed to successful PPR spread after RP eradication, and may already be underway with primary canine distemper virus (CDV) infection among cattle in northern Tanzania (60). As PPR eradication efforts continue (10), selective pressure could result in novel PPRV mutations that enable more efficient replication and transmission from currently unrecognized livestock or wildlife hosts to be favored, as was predicted (62) and seen in suspected spillover of SARS-CoV-2 into wildlife hosts such as white-tailed deer (63) and mink (64) or highly pathogenic avian influenza into mink (65). Broadly,

morbilliviruses are adaptable with only a few amino acid changes enabling host range expansion in nature and in-vitro for CDV (66–68), and only one amino acid change enabling PPRV to enter human SLAM receptors and evade anti-Measles antibodies in-vitro (50). Additionally, selective pressure could result in either increased virulence or decreased virulence in main hosts, the latter of which was sen in the reduced virulence of RPV lineage 2 in cattle in the final stages of RP eradication (69–71). Therefore, PPR molecular epidemiology studies should include whole genome sequencing and clinical sign documentation of circulating PPRV strains to detect and monitor mutations that could potentially increase viral fitness (e.g. increased replication and shedding from cattle) or adaption to other atypical hosts. Such work should be combined with animal movement data (72). Importantly, continued vigilance is required and monitoring PPRV circulation and evolution across hosts (9) is a critical area for investment during PPR eradication.

Inclusion and Ethics Statement

PPR is endemic in Ethiopia and has a major impact on small ruminant production and human livelihoods and investigating other potential hosts for PPRV that need to be considered for control was considered a relevant topic for study by AHI leadership. AHI researchers and other relevant local Ethiopian and international PPR researchers were included, consulted, and cited in all stages of the research process including study design, implementation, sample collection, processing, testing, analysis, manuscript editing, and as co-authors on this publication. Those who made valuable contributions to the project but did not meet the criteria for authorship are named in the Acknowledgements. All researchers were informed of personal risks and appropriate personal protective equipment and training for use and biosafety and biosecurity practices were provided and adhered to. Local researchers are in possession of all samples collected and a copy of all data produced have ownership of both. As part of this study, infrastructural, laboratory, and analytical capacities were supported and expanded through the following: construction improvements to the biosecure animal facility, introduction and training on protocols for managing and use of cell lines (VDS), molecular equipment maintenance, established collaborations with researchers in US and UK, and funding for local researchers to attend bioinformatics and epidemiological modeling trainings. Additionally, prior to the study, funded training was provided by the Association for Assessment and Accreditation of Laboratory

Animal Care (AAALAC) in preparation for future certification. This research study was held to international animal welfare and biosafety standards and approved by the Animal Health Institute's Animal Research Scientific and Ethics Review Committee (ARSERC) under reference ARSERC/EC/001/17/04/2019.

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Competing Interest Statement

"The authors declare no competing interests".

Data Availability:

Whole genome sequence information for PPRV/Ethiopia/Habru/2014 passage 4 can be found at Genbank accession number: ON110960. Clinical, serological, molecular, and tissue culture data from main and supplementary figures are available in the following repository: https://github.com/cherz4/pprv-cattle-transmission, doi: 10.5281/zenodo.12791123

Code Availability: Code is available on GitHub: https://github.com/cherz4/pprv-cattle-transmission with doi for release v1.0.0: 10.5281/zenodo.12791123.

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CHAPTER THREE

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CHAPTER THREE: COMPARATIVE PATHOGENESIS OF ETHIOPIA/HABRU/2014 LINEAGE-IV PESTE DES PETITS RUMINANTS VIRUS IN GOATS AND CATTLE

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3.1 ABSTRACT

Background: - Peste des Petits Ruminants (PPR) is a highly contagious viral disease primarily affecting goats and sheep, with clinical manifestations ranging from peracute disease to subclinical infection, particularly in atypical hosts such as cattle. The role of atypical hosts such as cattle to the spread of PPR remains controversial, with conflicting reports in the literature. Despite its worldwide significance, considerable knowledge gaps exist regarding the pathogenesis and clinical progression in both primary (goats) and atypical (cattle) hosts. This study aimed to elucidate the tissue tropism, pathogenesis, virus shedding, clinical progression, and pathology associated with experimental PPR virus infection in indigenous goats and cattle. To this end, 32 animals - 16 goats and 16 cattle - were intranasally inoculated with the Ethiopia/Habru/2014 Lineage-IV strain of the PPR virus followed by detailed clinical evaluations and systematic sampling at pre-established intervals to assess serological conversion, viral shedding, and the pathogenesis of the infection across both species.

Results:-The results show that goats exhibited typical clinical signs 4 days post-inoculation, with seroconversion by day 6 and early detection of viral RNA in swabs and tissues by day 3 and virus isolation starting day 4. In contrast, cattle exhibited minimal clinical signs, with seroconversion occurring at day 8 with viral RNA detected in tissue samples at day 4 and virus isolation starting day 6 in tissues and in a single nasal swab at day 8. Clinical scores and tissue positivity rates significantly differed between goats and cattle ($P=0.007$ and $P< 0.001$, respectively). While goats exhibited expected gross and histopathological lesions, cattle showed only nonspecific lesions.

Conclusions:-Together, our findings highlight the importance of comparative pathology studies for better understanding virus dynamics and transmission pathways that may help inform more effective PPR control programs. Future research should explore the pathogenesis of different PPRV lineages in cattle, assessing variations in disease progression and potential for epidemiological impact.

Key words: **cattle, comparative pathology, goats, disease pathogenesis, experimental, PPR, PPRV Lineage-IV,**

3.2 BACKGROUND

Peste des petits ruminants (PPR), a highly contagious viral disease primarily impacting goats and sheep, was initially identified in, Côte d'Ivoire, West Africa in 1940's [1]. It has since become widespread, affecting regions across Africa, the Middle East, and Southeast Asia [2], with a global presence in over 70 countries and territories [3]. The disease poses a significant threat to subsistence farmers, causing substantial economic losses due to its elevated morbidity and mortality rates [4]. Outbreaks of PPR not only disrupt the livelihoods of livestock owners but also have far-reaching consequences on food security and socio-economic activities, leading to a considerable disruption in affected regions [5].

PPR exhibits various clinical manifestations, including peracute, acute, subacute, and subclinical forms contingent upon predisposing factors and the virulence of the infecting virus types [6, 7]. The peracute manifestation results in severe symptoms such as depression, anorexia, abortion in pregnant animals, and often leads to death [8]. The acute form is characterized by a sudden onset of symptoms like depression, pyrexia, discharge from the eyes and nose, inflammation of mucous membranes, eyelid matting, mouth inflammation, diarrhea, coughing, and foul breath, ultimately culminating in death [2, 9, 10]. Subacute cases present milder clinical signs, resulting in lower mortality rates, with animals typically recovering within 10-14 days, a pattern more prevalent in sheep [7]. Large ruminants like cattle may display a subclinical form [11-13], where the virus is eliminated without apparent clinical symptoms, accompanied by robust neutralizing antibody responses [7].

In highly susceptible hosts (goats and sheep) the tissue tropism, pathogenesis and disease expression after PPRV infection is associated with infection of immune and epithelial cells via Signaling Lymphocyte Activation Molecule (SLAM) and nectin-4 receptors, respectively [13-15]. However, the role of atypical hosts, such as cattle, in PPRV infection remains a subject of debate with conflicting findings in the literature. Sen et al. (2014) report that cattle may have prolonged viral presence in blood and peripheral blood monocyte cells and are a potential source of PPRV transmission [12]. In contrast, other groups classify cattle as dead-end hosts and report seroconversion and short-term low levels of PPRV RNA in oronasal swab samples [13, 16]. The inconsistency in results from field and experimental studies can be attributed to various

factors, including differences in methods, strains, breeds, virus doses, and environmental conditions.

Despite the global impact of PPR, there is a paucity of knowledge regarding its pathology and clinical progression, particularly in atypical host species like cattle. Questions regarding the potential role of species other than sheep and goats, particularly cattle, in PPRV persistence remain unanswered. Recognizing the importance of understanding PPRV in both target and atypical hosts, a more comprehensive examination becomes imperative to establish effective surveillance and control measures.

In light of these knowledge gaps and the conflicting results reported in previous studies, the present research investigated PPRV tissue tropism, pathogenesis, shedding, clinical course, and comparative pathology in both goats and cattle. The findings provide important insights on disease progression and virus shedding patterns from various sites over intervals in both primary and atypical (cattle) hosts. These insights may be used to better guide decisions about the future development of more effective PPR surveillance and control strategies.

3.3 MATERIAL AND METHODS

Experimental design and procedure

Indigenous goats (Central and Western Highland breeds) and cattle (Arsi breed), aged between one to two and a half years, were acquired from North Shoa, Ejere, and Degem animal markets, located within a 200-km radius of Addis Ababa, Ethiopia. These areas are recognized for being relatively free of PPR. Selection criteria included age, apparent health, breed authenticity, and no history of vaccination against PPR. Age verification was based on dentition [17] and corroborated by information from the sellers. Beyond visual inspection and temperature measurement, no further health assessments were conducted at purchase. The animals were then transported to the Animal Health Institute's (AHI's) experimental facility. Upon arrival, competitive enzyme-linked immunosorbent assay (c-ELISA) testing (ID.Vet, Grabels, France) was performed to confirm their seronegative status for PPR. Following a 10 to 15-day acclimatization period, during which their health was continuously monitored, the animals were ear-tagged and divided into two groups: 16 goats (Group A) and 16 cattle (Group B). Comprehensive daily evaluations of rectal temperature and clinical signs were conducted, alongside veterinary care as detailed in Supplementary file (S) **Text S 3.1**. The overall study design is elaborated in **Text S 3.2**.

For the virus infection, we utilized the PPRV/Ethiopia/Habru/2014 isolate, Lineage-IV (GenBank Accession Number ON110960), cultured in Vero Dog Slam (VDS) cells at AHI. The virus strain was taken from a female goat displaying typical clinical signs of PPR in Habru area, Ethiopia. The virus was isolated and propagated up to the fourth passage using Vero Dog Slam (VDS) cells. Detail information on the strain can be found in published papers [18, 19]. The viral titer was determined to be $10^{5.3}$ $TCID_{50}$, (50% Tissue Culture Infective Dose) per ml at the fourth passage (P4), with its presence confirmed through real-time reverse transcription-PCR (RT-qPCR) and observation of cytopathic effects (CPE) in virus cell culture as described in virological analysis section. Inoculation was performed using a 3ml syringe attached to a MAD-300 intranasal mucosal atomization device (Teleflex Incorporated, Wayne, PA, USA), delivering 2 mL of the virus suspension per cattle (1 mL per nostril) and 1 mL per goat (0.5 mL per nostril),

to mimic natural transmission. Care was taken to secure the animals and elevate their heads to ensure the effective administration of the inoculum and facilitate deep inhalation.

Clinical investigation and scoring

Following inoculation, daily examinations were conducted on all animals to identify the onset and progression of clinical signs indicative of PPR as outlined by other groups [9, 20, 21]. Prior to each sampling event, a thorough clinical assessment was performed on every animal, encompassing rectal temperature measurements and the observation of ocular and nasal discharges, mucous membrane congestion, oral lesions, diarrhea, coughing, respiratory distress, among other signs. These observations were systematically scored according to previously described criteria [20] and goats and cattle exhibiting a rectal temperature of 39.5°C or higher were classified as febrile. In this study, we adhered to humane endpoints for virus-infected animals as outlined by Pope et al. (2013), which recommend euthanasia on ethical grounds when animals reach a clinical score of 20. However, none of the infected animals in our research reached this maximum score within the set timeframe, so early euthanasia was not necessary.

Sampling and sample preparation

For both the goat and cattle cohorts, antemortem blood for serological analyses, swabs from the eyes, nose, and rectum, and tissue samples from sacrificed animals were systematically collected at predetermined days post-infection (dpi) to monitor for viral shedding, tissue tropism and seroconversion. Upon collection, swab samples with medium from goats and cattle were transported on ice for immediate analysis. Goats were euthanized daily, with two randomly selected starting from 1 dpi until 8 dpi, whereas two cattle were euthanized on 2, 4, 6, 8, 10, 17, 24, and 28 dpi. Swabs were stored in 1 ml Dulbecco's Modified Eagle Medium (DMEM), and postmortem tissue samples were placed in sterile falcon tubes with 5-7 ml PBS for transport, while tissues intended for histopathological examination were fixed in 10% buffered formalin.

Collected tissue samples including, retropharyngeal lymph node (LN), mediastinal LN, bronchial LN, mesenteric LN, tracheal LN, lung cranial, lung caudal, colon, jejunal and ileal Peyers patch, spleen, tongue (apex), palatine tonsil, nasal mucosa, and 3rd (third) eyelid. These samples

allocated for RT-qPCR and viral culture analysis were preserved in 5-7ml phosphate-buffered saline (PBS) and stored at -80°C. These samples were homogenized upon thawing followed by centrifugation and the resultant supernatant was used for both RT-qPCR testing and culture. RT-qPCR was performed on swab and tissue samples collected during the study and specimens that were positive underwent virus isolation in VDS cells to further confirm the presence of viable virus. Concurrently, for serological assessment, 5-7 ml of blood was collected from the experimental animals into plain vacutainer tubes at predetermined intervals post-infection.

Virological analysis (PCR, Ag-ELISA, virus isolation)

The detection of PPRV antigen was conducted using real-time RT-qPCR and immunocapture-ELISA (Ic-ELISA) as described below. RT-qPCR positive samples were further subjected to virus isolation. Despite not being fully validated with cattle samples, the PPR Ic-ELISA was utilized to examine nasal and rectal swabs from both species.

For RT-qPCR, in brief, viral RNA extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) per manufacturer's protocol, and used in an RT-qPCR assay targeting the N gene of PPRV using the specific primers and probe described by (Batten et al., 2011). The reaction mixture comprised 10µl of Express Universal SuperScript mix, 2.0µl of Express SuperScript enzyme mix, 0.4µl of ROX reference dye (Invitrogen, Thermo Fisher Scientific), 0.8µl of each primer (forward and reverse), 0.4µl of the PPR probe, 2.6µl of RNase-free water, and 3µl of the extracted RNA, totaling a 20µl reaction volume. Amplification was performed using an Applied Biosystems 7500 FAST thermal cycler with reverse transcription at 50°C for 15 minutes, followed by DNA polymerase activation at 95°C for 20 seconds, and 45 cycles of denaturation at 95°C for 3 seconds, and annealing/extension at 60°C for 30 seconds, based on the protocol by Batten et al., [21].

For virus isolation, VDS cells were prepared by washing with PBS and then inoculated with 100-200µl of supernatants from ocular, nasal, and rectal swabs or processed tissue, previously homogenized and centrifuged at 1650g for 20 minutes at 4°C. These cells, with over 70% confluence in 24-well plates, underwent a pre-adsorption process, followed by an hour of incubation at 37°C with 5% CO2 and 96% humidity, with intermittent agitation every 15 minutes

to promote virus adsorption. After removing the inoculum and washing with serum-free DMEM, 500µl of DMEM with 2% calf serum was added to each well. The cultures were then observed under an inverted microscope for non-specific reactions before a final incubation of five to seven days under the same conditions to identify CPE indicative of viral replication, in line with the protocol described by Gomes et al., [22].

For antigen detection, we used the ID Screen® PPR Antigen Capture kit from ID.Vet (Grabels, France) following the Ic-ELISA method Libeau et al., [23]. Results interpretation was straightforward, with a sample-to-positive (S/P%) ratio below 20% indicating negative outcomes, and ratios of 20% or higher signaling positive detections according to the manufacturer's instruction.

Serology, postmortem examination and histological analysis

In parallel, antibody responses and baseline exposure to PPRV in serum samples from goats and cattle were assessed using the ID Screen® PPR Competition kit (ID.Vet, Grabels, France). This c-ELISA kit was designed to detect anti-PPRV-N protein antibodies Libeau et al., [24] indicative of virus exposure. The criteria for result interpretation classified samples as positive with a competition percentage (S/N%) of 50% or less, doubtful for percentages greater than 50% but at or below 60%, and negative when exceeding 60% according to the manufacturer's instruction.

In this experiment, we employed a captive bolt stunning to render the animals unconscious before euthanasia. This approach enhances animal welfare by preventing unnecessary pain and cruelty during the process and also improves safety standards. The stunning was promptly followed by effective bleeding, resulting in euthanasia. The captive bolt pistol used was the Bolt Stunner Model KS from Karl Schermer GmbH, Germany, selected for its suitability for both goats and cattle. Gross pathology and photographic documentation of any gross tissue lesions was undertaken at postmortem examination. Subsequently, a comprehensive postmortem examination was conducted on each animal to evaluate for gross pathological changes. Tissue and lymph node (LN) inspections were meticulous, focusing on areas affected by the PPR virus. This included the buccal and nasal cavities, conjunctiva, head lymph nodes (including palatine tonsils), mesenteric regions (encompassing both LNs and bowel tract), the respiratory system

(lungs and associated LNs), and visceral organs such as the liver, spleen, and kidneys. Documented changes, including any deviations in structure, morphology, and color, were systematically scored to quantify the extent of the lesions, with scoring criteria detailed in **Table S 3.1**.

Histopathological examination was performed on a total of 480 tissue samples to assess cellular changes induced by PPRV infection. Standard operating procedures (SOPs) of the AHI pathology laboratory were followed. In brief, formalin-fixed tissue samples were dehydrated in graded ethanol and clarified with xylene with the Shandon Citadel 1000 tissue processor (Thermo Fisher Scientific, Waltham, MA, USA) prior to embedding in paraffin using the TEC2900 modular Tissue Embedding Centre (Histo-Line Laboratories, Milan, Italy). Tissue sections were prepared at a thickness of 4-5µm and stained with Hematoxylin and Eosin, were examined under light microscopy at 4x, 10x, and 40x magnifications, and histopathological lesions, were scored using a grading system from 0 (no lesions) to 4 (severe lesions) as outlined in **Table S 3.1**.

Data analysis and visualization

Analysis and visualization of the dataset were conducted in R statistical software (version 4.1.3), employing the ggplot2 package for generating graphical representations. To illustrate trends within the groups of inoculated animals, local regression (LOESS) smoothed curves with corresponding 95% confidence intervals, calculated through t-based approximation, were incorporated into the plots using the stat-smooth function to provide a visual summary of the collective data trends across the study duration. Comparative analyses, focusing on tissue positivity rates and clinical scoring between goats and cattle, were performed using a non-parametric, Mann-Whitney test within GraphPad Prism (version 10.1.1). To assess the concordance between different methods of sample analysis, Cohen's kappa coefficient was calculated using the psych package in R, offering a statistical measure of inter-rater agreement for categorical items. It was interpreted as per [22]. If value of kappa is "< 0", No agreement; "0-0.20", Slight agreement; "0.21-0.40", Fair agreement; "0.41-0.60", Moderate agreement; "0.61-

0.80", Substantial agreement; "0.81-1.00", Almost perfect agreement. All data and R-code are provided (https://github.com/cherz4/pprv-cattle-goat-comparative-pathology).

3.4 RESULTS

Experimental infection with Lineage-IV PPRV/Ethiopia/Habru/2014 strain results in moderate clinical signs in goats but no obvious clinical signs in cattle

Goats inoculated with Lineage-IV PPRV/Ethiopia/Habru/2014 presented no obvious clinical signs until 3 dpi. From 4 dpi, fever was noted in 3 of the 10 goats, and a peak rectal temperature of 40.5°C was noted at 6 dpi **(Figure 3.1A and 3.1B).** Serous nasal discharge appeared in 5 of the 10 goats at 4 dpi, increasing to 6 of the 8 remaining goats at 5 dpi **(Figure S3.1A)**. Additionally, at 5 dpi, congested mucous membranes were observed in the conjunctiva of 5 of the 8 goats (62.5%), while diarrhea was observed in a single animal (12.5%) **(Figure S3.1B).**

By 6 dpi, 5 of the 6 remaining goats (83.3%) continued to display serous nasal discharge, while 1 of 6 (16.7%) exhibited muco-purulent nasal discharge and diarrhea. Congested conjunctiva and coughing were noted in 2 of 6 goats. At 7 dpi, 2 of the 4 remaining goats had serous nasal discharge, and the other 2 displayed muco-purulent nasal discharge; congested conjunctiva was noted in 3 of the 4 goats.

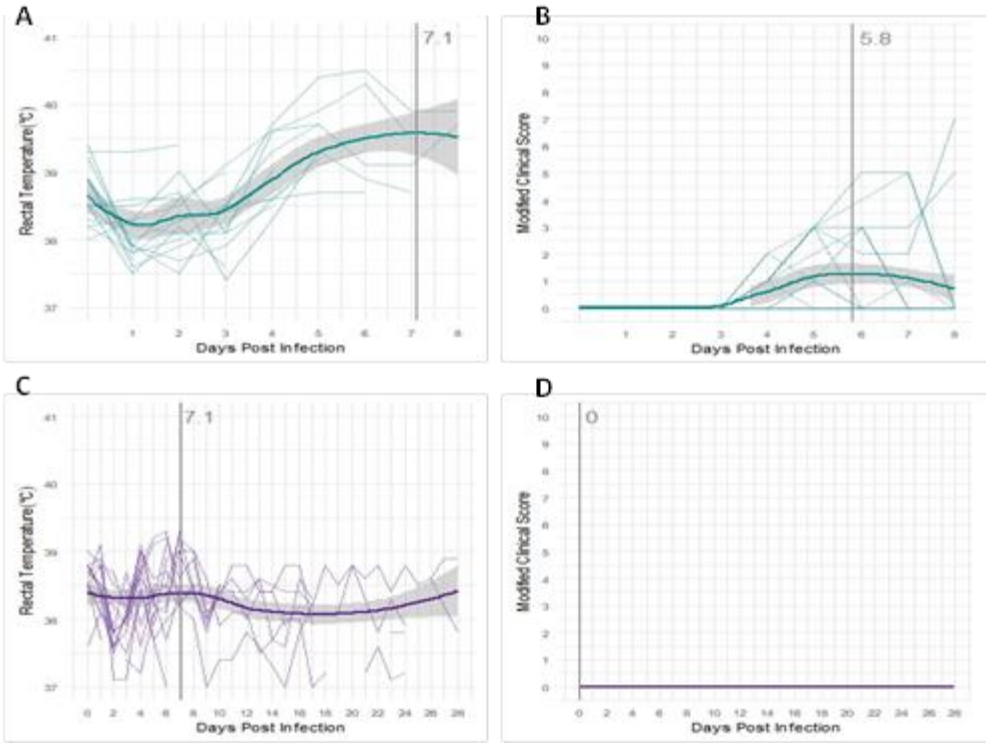


Figure 3.1: Clinical Signs and Rectal Temperature in Goats and Cattle infected with PPRV (**A**) Trends in daily rectal temperature in goats post-infection (**B**) Modified composite clinical scores in goats without rectal temperature readings. (**C**) Daily rectal temperatures in cattle post-inoculation, with all values remaining below the febrile threshold of 39.5°C. (**D**) Composite clinical scores in cattle, indicating the absence of significant clinical signs or elevated temperatures. In all subfigures, thin lines depict individual animal data, while bold LOESS curves represent the collective pattern. The bold vertical lines in **Figures 3.1A**, **3.1B** and **3.1C** denote the dpi of the peak value, while the zero value in **Figure 3.1D** indicates no recorded clinical signs or fever.

At the conclusion of the trial (8 dpi), both remaining goats showed signs of conjunctival congestion and diarrhea. One of the goats exhibited serous nasal discharge, and the other muco-purulent nasal discharge and cough. Moreover, one animal presented with distinct oral lesions, including lesions and sloughing of the oral mucosa and gums **(Figure S 3.1C)**. A detailed summary of the clinical signs observed post-inoculation in the goat cohort is provided in **Table S 3.2.**

In contrast, no clinical signs were observed following experimental infection of cattle with lineage-IV PPRV/Ethiopia/Habru/2014 strain. Rectal temperature ranged from 36.1^{o}C to 39.3^{o} C, and clinical scores remained 0 through the trial **(Figures 3.1C and 3.1D)**. Significant differences in clinical observations were noted between the goat and cattle groups (P-value = 0.0070, Mann-Whitney test), underscoring the variability in host response to PPR virus inoculation.

Serological Responses of goats and cattle after experimental infection with PPRV/Ethiopia/Habru/2014 strain

Seroconversion in the experimental goat cohort, as detected by c-ELISA, was observed at 6 dpi in 1 of the 6 goats (16.7%), but by 7dpi all remaining goats in the cohort had seroconverted (**Figure 3.2A, Table 3.1**). In cattle, seroconversion was detected in 6 of 10 animals at 8 dpi, and in all cattle from 10 dpi **(Figure 3.2B, Table 3.1)**.

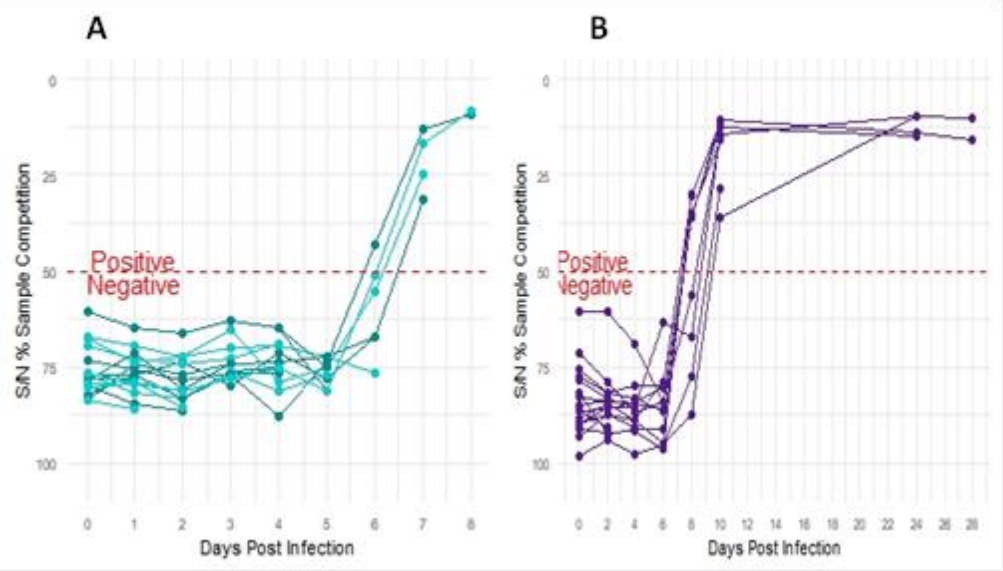


Figure 3.2: Time course of seroconversion in goats and cattle inoculated with PPR Virus highlight goats seroconverting earlier than cattle. **(A)** In the goat cohort, seroconversion was detected from 6 dpi onwards. Each line represents the serological response of an individual goat, with seropositivity defined by a competition percentage below 50%. The dashed red line denotes the cutoff threshold for a positive test result. **(B)** Among cattle, seroconversion commenced from 8 dpi. Similar to goats, individual cattle responses are shown by lines, with the cutoff for seropositivity marked by a dashed red line at the competition percentage of less than 50%.

Temporal dynamics of PPR virus antigen and RNA detection in antemortem and post-mortem samples in goats and cattle experimentally infected with PPRV/Ethiopia/Habru/2014.

Within the goat cohort subjected to experimental inoculation, viral antigen in nasal swabs was first detected at 2 dpi in 1 of 16 goats. Fecal swab analyses revealed the presence of PPRV-antigen starting at 4 dpi in 1 of 10 goats. The frequency of detection in nasal swabs rose to 2 out of 12 goats by 3 dpi, escalating to 7 out of 10 goats by 4 dpi. From 5 dpi onward, nasal swabs from all goats tested positive for PPR virus antigens. The trend of antigen positivity in fecal swabs saw gradual increase from 4 dpi (1/10 goats), escalating systematically to 100% positivity by the 8th dpi **(Table 3.1, Figure 3.3)**.

In the cattle cohort, the emergence of PPR virus antigens in nasal swabs was noted as early as 2 dpi in 1 out of 14 cattle, with a progressive increase in detection rates, albeit with some fluctuations observed at various dpi benchmarks. Specifically, at 4 dpi, 3 out of 14 cattle tested

positive; this positivity rate evolved to 6 out of 12 by 6 dpi, and reached 100% by 10 dpi and was, maintained through 17 dpi (6/6), 24 dpi (4/4), and 28 dpi(2/2). Fecal swabs in cattle revealed antigen detection beginning at 4dpi (1/14), at 6 dpi (1/ 12); at 8 dpi (1/10); at 10 dpi (3/8); at 17 dpi (4/ 6); at 28 dpi (1/2) **(Table 3.1, Figure 3.3)**.

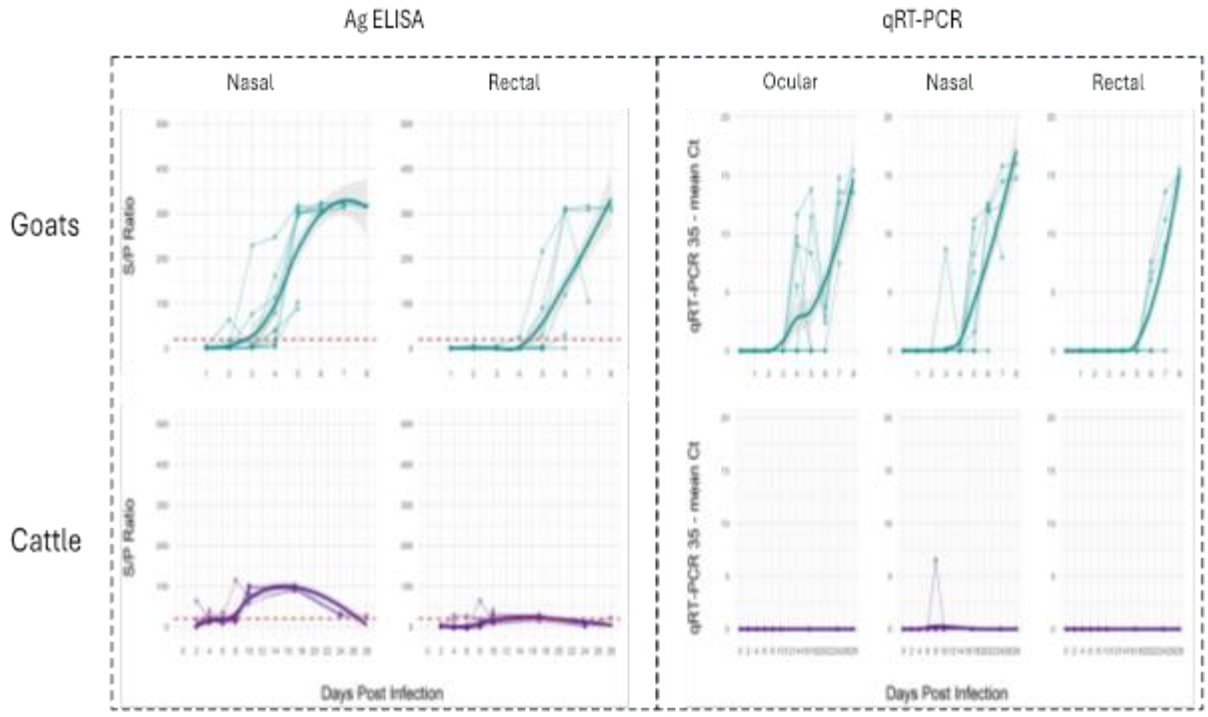


Figure 3.3: Kinetics of PPRV detection in experimentally inoculated goats and cattle. The left panels illustrate the sample-to-positive (S/P) ratio values from Ic-ELISA of the nasal, and rectal swabs, while the right panels show the cycle threshold (35-mean Ct) values from RT-qPCR of the ocular, nasal and rectal samples. The top row represents data from goats (teal color plots), and the bottom row from cattle (purple color plots). Each line depicts the virus antigen shedding pattern of individual goat or cattle, with antigen positivity defined by a percent positivity ratio of ≥ 20%. The dashed red line indicates the cutoff threshold for a positive test result. Bold lines represent smooth local regression (LOESS) curves encompassing all animals in both experimental groups.

The presence of PPRV-RNA was initially detected in goats at 3 dpi, with subsequent increases observed over time. Specifically, at 4 dpi, the virus was detected in 4 of 10 goats, primarily in ocular swabs. By 5 dpi, the virus was present in 6 of 8 goats, found in both ocular and nasal swabs. This trend continued, with the virus being detected in increasing numbers of goats and in various swab types up to 8 dpi (**Figure 3.3, Table 3.1**).

RT-qPCR testing revealed an escalating presence of PPR virus RNA in post-mortem tissue and LN samples from inoculated goats, with no detection at 1 and 2 dpi, but a progressive increase from 3 dpi onward. Cumulative data indicated an increase in positive samples: starting with 25% at 3 dpi, it peaked at 89% by 8 dpi. Cattle, conversely, showed a singular instance of viral RNA positivity in a nasal swab at 8 dpi (eartag ID 211), with a subsequent uptick in tissue samples over time, highlighting species-specific resistance to the virus.

Table 3.1: Temporal dynamics of clinical scores, PPRV antigen and RNA detection and pathological scores in experimentally infected goats and cattle

Species	ID	DPI	Clin Score	cELISA	AgELISA	PCR Tissue Positivity (Ct values)														Total PCR Positive	Path Scores	
						NasMuc	Tons	3rdEyelid	RPLN	Tongue	TrachLN	BronchLN	LungCran	LungCaud	MediLN	Spln	PP	Colon	MesLN		Gross	Histo
Goats	235	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-
	246	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	1
	239	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	1
	208	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1	1
	234	3	0	-	-	-	23.3	-	19.3	-	20.4	-	-	-	30.3	-	-	-	-	4	1	2
	200	3	0	-	-	-	-	-	20	-	20.5	-	-	-	-	25.6	-	-	-	3	1	2
	198	4	1	-	38.4	27.8	19.6	22.3	17.5	-	20.5	21.6	-	-	-	22.5	21.4	-	22.8	9	2	2
	195	4	0	-	20.7	-	21.1	20.6	17.7	-	19.3	18.4	-	-	20	20.6	26.6	-	21.4	9	2	2
	241	5	4	-	88.4	24.7	17.5	19.7	15.8	26.5	16.2	16.5	-	-	16.6	19.8	-	26.6	16.7	11	2	2
	209	5	1	-	103.1	-	18	-	18.2	-	20.2	20.2	-	-	20.3	22	-	-	24.2	7	2	2
	150	6	3	-	310.8	24.2	15.9	18.9	16.8	-	18.4	17.8	-	-	19.3	20.3	24.7	-	17.5	10	2	2
	178	6	3	-	302.7	26.5	20	27.8	21.4	-	28.2	20.4	-	-	20.5	21.3	23.9	-	20.8	10	2	2
	206	7	6	24.7	313.1	17.9	-	31.3	22.3	18.5	16.5	-	-	-	-	16	16.8	-	-	7	2	2
	196	7	5	31.1	314.2	24.7	17.7	19.9	23.4	20.1	18.3	15.6	20	19	16.7	15.9	15.7	18.8	17.6	14	2	2
	254	8	6	8.4	319	20.4	19.1	18	15.4	20.5	15.6	15.5	-	22.5	15.5	16.4	19	15.4	14.7	13	2	2
	253	8	8	9.2	316.7	19.4	17.8	17.2	16.1	18.1	17.2	16.7	-	-	16.5	16.6	24.7	15	15.9	12	2	2
Cattle	222	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	1
	227	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	1
	213	4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	1
	220	4	0	-	-	-	-	-	30.2	-	29.3	-	-	-	-	-	-	-	-	2	1	1
	221	6	0	-	22.8	-	32.5	-	24.5	-	-	26.6	-	-	28.6	-	-	-	-	4	1	1
	229	6	0	-	-	-	24.7	-	22	-	-	24	-	-	24.5	-	-	-	-	4	1	1
	212	8	0	29.8	22	-	22.5	24.1	24	-	29.6	25.4	-	-	26.6	-	-	-	-	6	1	1
	225	8	0	29.7	-	-	22.5	26.2	-	-	-	31.3	-	-	32.6	-	-	-	-	4	1	1
	217	10	0	28.6	42	-	29.7	27.7	-	-	-	-	-	-	-	-	-	-	-	2	1	1
	219	10	0	15.9	-	-	-	-	-	24.4	-	-	-	-	-	-	-	-	-	1	1	1
	216	17	0	9.5	88.3	-	24.8	-	28.8	-	-	-	-	-	-	-	-	-	-	2	1	1
	226	17	0	9.8	103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1	1
	211	24	0	9.9	29.9	-	24.9	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1
	214	24	0	14.9	29.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1	1
	223	28	0	10.3	28.8	-	24.7	-	27.6	-	-	26.8	-	-	26.5	-	-	-	-	4	1	1
	230	28	0	15.6	20.7	-	-	-	-	28.6	-	-	-	-	-	-	-	-	-	1	1	1

"DPI", days post-infection ; NasMuc - Nasal Mucosa; Tons - Tonsil; 3rdEyelid - Third Eyelid; RPLN - Retropharyngeal Lymph Node; Tongue - Tongue (Apex); TrachLN - Tracheal Lymph Node; BronchLN - Bronchial Lymph Node; LungCran - Lung Cranial; LungCaud - Lung Caudal; MediLN - Mediastinal Lymph Node; Spln - Spleen; PP - Ileal & Jejunal Peyer's Patches; Colon - Colon; MesLN - Mesenteric Lymph Node.; "-" Undetermined with RT-qPCR CT > 35 or apparently normal Gross and Histology results in tissues, or >50% threshold (negative) results in cELISA, or <20% threshold (negative) results in AgELISA tests. "Total PCR Positive" implies the number of tissue samples out of 14 that yielded positive results for PPRV RNA at each respective time point.

Viral culture results from samples primarily positive for RNA in goats confirmed active viral replication based on typical characteristic CPE, with a high culture positivity rate from 5 dpi onwards in swab (**Table S 3.5**) and tissue samples (**Table S 3.6)**. In contrast, for cattle, culture positivity was considerably lower, with only one nasal swab (from animal ID 211) and a limited number of tissues yielding live virus, chiefly from the head and thoracic regions at 6 dpi (from animal ID 229) (**Table 3.2**). Figures of virus-induced CPE in a culture-positive nasal swab sample (Animal ID 211), along with a comparison figure without CPE in a negative control sample are shown in Figure 4. For the outcomes of entire cultured cattle samples, refer to (**Table S 3.7**). The significant difference in tissue positivity rates between goats and cattle ($P < 0.0001$, CI=33.75-70.11) further illustrates the striking differences in patterns of disease and infection observed between the species.

Table 3.2: Observed Cytopathic Effect in Positive Cattle Tissue Samples*

ID	DPI	Sample Type	Culture Day						
			1	2	3	4	5	6	8
229	6	Retropharyngeal LN	-	++	+++	+++	+++	+++	+++
229	6	Mediastinal LN	-	-	-	++	++	++	nd
229	6	Bronchial LN	-	-	-	-	-	+	++
229	6	Palatine Tonsil	-	-	+	+++	+++	+++	nd
211	8	Nasal Swab	-	-	++	+++	+++	nd	nd

*Cytopathic effect (CPE) observed in positive tissue samples from animals 229 and 211 over various culture days. Symbols indicate CPE intensity: "-" (none), "+" (mild), "++" (moderate), "+++" (strong), and "nd" (not determined). Samples from animal 229 were collected at 6 days post-infection (DPI), and from animal 211 at 8 DPI. Only positive samples are shown; tissues and swabs collected from all other animals were negative based on culture and observed CPE.

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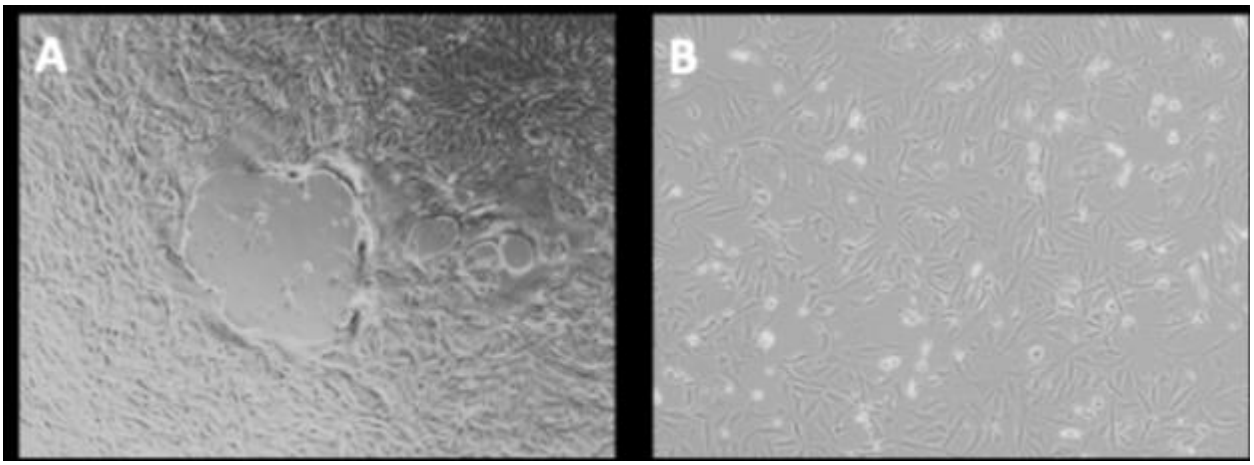


Figure 3.4. Cytopathic effects of PPRV in Vero Dog SLAM (VDS) cells. **(A)** VDS cells infected with PPRV isolated from a bovine nasal swab (Animal ID 211) at 8 days post-infection. The image shows pronounced cytopathic effects (CPE) characteristic of PPRV infection, including a large central syncytium formed by cell fusion. Surrounding the syncytium are elongated fibroblast-like cells and some rounded cells, indicating viral-induced cellular changes. **(B)** Uninfected control VDS cells showing normal monolayer morphology with typical elongated, fibroblast-like appearance and regular distribution. Images were converted from color to monochrome and minimally adjusted for brightness and contrast uniformly across the entire image using Microsoft PowerPoint. No other adjustments were made (Magnification 10x, regular tissue culture inverted microscope).

The agreement between different virological analysis methods was assessed using Cohen's kappa method and was restricted to experimental goats given the low positivity rates observed in cattle. The analysis included real-time RT-qPCR, Ic-ELISA, virus culture, and c-ELISA. The findings indicated substantial agreement between Ic-ELISA and RT-qPCR tests for PPRV, with a Cohen's kappa score of 0.75.. However, the majority of the tests exhibited fair agreement, with one test suggesting slight agreement between Ic-ELISA and c-ELISA tests. Detailed test agreement results interpreted as per [22] are provided in (**Table S 3.4)**.

Gross and histopathological lesions associated with PPR virus infection in goats and cattle

Gross and histopathological lesions associated with PPR virus infection were semi-quantitatively evaluated using a prepared lesion scoring system (**Table S 3.1**), which provided a comparative perspective on the clinical and pathological impacts of PPR virus in goats and cattle.

In brief, gross pathology findings in goats revealed a spectrum of lesions that evolved over the course of the study. Starting from 2 dpi, there was notable swelling and edema in lymph nodes, especially retropharyngeal and bronchial, along with vascular congestion in the mesenteric region and hemorrhages in the jejunal and ileal Peyer's patches. This pathology intensified by 4 dpi, with almost all lymph nodes exhibiting enlargement and hemorrhaging, and the lung lobes showing calcification in some cases. The spleen was often enlarged, and hemorrhagic enteritis became apparent. Lesions diversified further by 8 dpi, with more severe respiratory and GIT involvement.

Cattle exhibited milder, less specific gross lesions. Early signs included lymph node swelling and palatine tonsil changes, with the addition of enteritis and lung hemorrhage by 8 dpi. More prolonged disease courses in cattle revealed spleen atrophy and lung hepatization by the later stages of the study.

Histopathologically, goats displayed early lymphoid depletion and pulmonary changes, which progressed to bronchopneumonia and parenchymal necrosis in lung tissues **(Figs S 3.2C and S 3.2D)**. By 8 dpi, severe characteristic PPRV lesions were identified, including syncytial cells and marked lymphoid depletion. Necrosis was prominent in the palatine tonsil, along with infiltrating neutrophils and syncytial cell formations, as depicted in supplemental figures **(Figs S 3.2A and S 3.2B)**. The spleen showed hemosiderin deposits and heightened cellularity **(Figs S 3.2E and S 3.2F)**, while broncho-interstitial pneumonia and severe hemorrhage and congestion marked the lung pathology **(Figure S 3.2G and S 3.2H)**. Cattle, in contrast, showed more subtle histopathological changes with less frequency and severity. Lymphoid depletion was observed, but the intestinal eosinophil infiltration and mucosal necrosis noted by 10 and 24 dpi highlighted the species' distinct response to PPR virus challenge **(Figs S 3.3A and S 3.3B).**

Together, the gross and histopathological findings underscore the stark contrast in the pathogenesis of PPRV between goats and cattle, similar to the difference observed clinical signs between the two species.

3.5 DISCUSSION

Our study was designed to provide comparative insights into the pathology of PPRV lineage-IV in goats and cattle, with a specific focus on viral shedding patterns and the potential epidemiological role of cattle. Our findings of live PPR virus recovered from bovine samples add a new perspective to our understanding of PPRV dynamics. While these findings challenge the established notion that cattle are mere dead-end hosts, and improve our understanding of PPRV pathophysiology and epidemiology, they require further study before generalization to other lineages or geographic regions beyond the lineage-IV PPRV circulating in Ethiopia.

In this study, the onset of pyrexia in goats starting from 4 days post-inoculation (dpi) is consistent with previous experimental research by [25, 27, 28], which reported similar early fever responses in caprine subjects infected with different strains of PPRV. This early fever manifestation also corresponds closely with clinical patterns documented during PPR field outbreaks as described by [9], suggesting that the experimental model may reflect natural disease progression. In contrast, the lack of noticeable clinical signs post-inoculation in cattle are consistent with findings from studies such as those by [13, 16], which also reported minimal to no clinical manifestations in bovine hosts, supporting the hypothesis that cattle may exhibit a subclinical course of PPRV infection, potentially acting as silent carriers of the virus. This has raised a concern that such asymptomatic carriers may play a critical but understudied role in the epidemiology of PPR, particularly in mixed farming systems where goats and cattle coexist, and where silent infection in cattle could go unnoticed while contributing to disease dynamics.

The timelines for seroconversion observed in this study suggest an earlier immune response post-infection i.e. 6 and 8 dpi in goats and cattle respectively, when compared to prior studies [13, 27, 29] that reported sero-conversion at 7 and 10 dpi in goats and cattle, respectively. This somewhat accelerated seroconversion could be attributed to several factors including the specific PPRV strain used, which was Lineage-IV, the species of the host animals involved, virus titre, route of inoculation and the methodical timing of the sampling schedule. Such differences highlight the complex interplay between the viral genetics and the host's immune system, and

they underline the necessity to consider these variables when designing control strategies and diagnostic approaches for PPRV.

The RT-qPCR analyses performed on clinical and tissue samples from caprine and bovine hosts suggest a pronounced viral RNA shedding pattern in goats, with peak periods (5-8 dpi's) of shedding aligning with those previously reported by [25, 30], indicating the high potential for the transmission of PPRV through excreted bodily fluids during these peak periods. More importantly, the presence of viral RNA in samples collected before the appearance of any clinical symptoms as highlighted in earlier work [31] emphasizes the critical need for early and proactive sampling in disease monitoring and outbreak management. Detecting PPRV before clinical signs manifest offers significant advantages for controlling spread, particularly in densely populated livestock areas where rapid transmission can lead to large-scale outbreaks.

In cattle, the sporadic RT-qPCR positivity noted throughout the study period indicates a more contained viral presence compared to goats, with the virus tending to localize within specific tissues. This distinct pattern of viral distribution may be explained by species-specific differences in the expression of viral receptors. Previous studies [32-34] have identified that variations in the expression of PPRV receptors, namely SLAM and nectin-4, across different species and tissues, play a crucial role in determining the susceptibility and resistance to PPRV infection. In cattle, these receptors are expressed differently compared to goats, potentially contributing to the lower and more localized presence of viral RNA found in bovine tissues [34].

The gross and histopathological examinations experimentally infected goats and cattle highlighted the wide range of lesions that result from infection with PPRV, with goats exhibiting a more severe and extensive range of pathology, typical of morbillivirus infections. These findings are consistent with reports from previous studies [20, 27, 35], highlighting the consistent pathological impact of PPRV in caprine hosts. In contrast, cattle inoculated with PPRV/Ethiopia/Habru/2014 presented, nonspecific mild lesions, unlike from recent studies [13], which noted an absence of observable PPR-related pathology in cattle. These differences highlight variable pathogenic impact of the virus on different host species or might reflect variations in individual or breed-specific immunity.

The use of VDS cells in our study merits further discussion. These cells, derived from the original Vero cell line, are particularly suited for PPRV propagation due to their lack of beta

interferon production, which increases their susceptibility to morbilliviruses [36, 37]. While VDS cells offer exceptional virus production capabilities, it's important to note that repeated passages in cell culture can lead to genetic drift and potential attenuation of the virus. Importantly, studies on morbilliviruses, including PPRV, have shown that mutations can accumulate during serial passages in cell culture. For instance, the Rinderpest virus (RPV) isolated after 49 passages resulted in an attenuated strain that did not revert to its pathogenic form [38]. Similarly, the Nigerian strain PPRV Nig 75/1 was attenuated through serial passage in Vero cells, leading to the development of an avirulent vaccine strain [39]. Eloiflin et al. [40] further demonstrated that the PPRV vaccine strain Nigeria 75/1 undergoes genetic changes during cell culture passage, resulting in attenuation [40]. These mutations, while individually may not be fully attenuating, can cumulatively lead to complete attenuation of the virus [41]. In our study, we used the virus at the fourth passage to minimize potential genetic drift. However, we recognize that even this limited number of passages may have introduced some mutations. This consideration is important when interpreting our results, particularly in the context of virus isolation from cattle samples. Future studies could benefit from deep sequencing analysis to track any genetic changes that may occur during cell culture passage.

Importantly, the successful isolation of live PPR virus from bovine samples, though limited, necessitates a reconsideration of the epidemiological role of cattle in the spread of PPRV. The isolation of the virus from a nasal swab at 8 dpi and from select tissues at 6 dpi is particularly revealing. These findings suggest that cattle may potentially act as reservoirs or silent spreaders of the infection under certain conditions, with implications for PPR control and eradication strategies.

Our results both align with and contrast previous studies. Sen et al. [12] reported PPRV detection in cattle blood and PBMCs for extended periods, though not in natural secretions [12]. Conversely, Couacy-Hymann et al. [16] reported that cattle do not exhibit signs of viral replication in epithelial cells and do not excrete the virus in quantities sufficient to infect animals in close proximity [16]. While our study also showed no clinical signs of infection in cattle following PPRV exposure, we did confirm viral excretion through both RT-qPCR and virus culture.

The discrepancy in results could be attributed to several factors, including differences in virus strain, route of infection, virus titer used for inoculation, sampling schedule, and cattle breed. These variables may have contributed to the recovery of live virus from natural secretions (nasal swab at 8 dpi) in our study, unlike in some previous studies.

Study strengths and limitations. This study offers several strengths, including the use of a local PPRV strain and indigenous breeds, providing regionally relevant data. Our daily postmortem examinations from 1 to 8 dpi offer a detailed temporal analysis of PPRV tissue tropism, which is not commonly reported in such granularity. Additionally, our comparative approach, studying both goats and cattle simultaneously under the same experimental conditions, provides direct insights into species-specific differences in PPRV pathogenesis.

However, some limitations should be noted. Only a single lineage of PPRV (Lineage-IV) was used, limiting generalizability to other strains or regions. The use of a small number of animals, while larger than many previous studies, limits the statistical robustness of the findings. Immunohistochemistry studies were not performed due to lack of access to necessary reagents in Ethiopia, which could have provided additional insights into viral localization and tissue-specific viral dynamics.

Our study did not account for potential confounding factors such as pre-existing infections or exposures, which could influence the observed disease progression and immune responses. Importantly, our findings did not confirm that the virus isolated from cattle was transmitted to susceptible animals, such as goats, through contact. However, we addressed this aspect in another study using the same local virus [19].

Addressing these limitations in future research is essential for a deeper understanding of PPRV pathogenesis and for refining disease control strategies.

Concluding comments: Taken together, this study presents a comprehensive comparative analysis of PPRV infection dynamics in goats and cattle. The evidence of subclinical infection and live virus shedding in cattle, compared with the moderate clinical manifestations in goats, highlights the complex interaction between PPRV and its hosts. Importantly, the results suggest

that, under certain conditions, cattle might play a more active role in PPRV epidemiology than previously understood. The implications of these findings are important for the development of control strategies and diagnostic approaches for PPR in endemic regions. Overall, the findings highlight an urgent need for further experimental studies on cattle PPRV with diverse PPRV lineages in future studies to enhance our understanding of cattle's role in PPRV epidemiology, particularly in regions where the disease is endemic. Additionally, future studies should explore the effects of co-infections and induced stress in goats to better understand how these factors influence the clinical and molecular dynamics of PPRV infection.

LIST OF ABBREVIATIONS

AHI –Animal Health Institute, ALIPB- Aklilu Lemma Institute of Pathobiology, ARSERC- Animal Research Scientific and Ethics Review Committee, CT- Cycle Threshold, dpi- days post inoculation, Ic-ELISA- Immunocapture Enzyme Linked Immuno Sorbent Assay, LOESS- Local Regression, PPR- Peste des petits Ruminants, PPRV- Peste des petits ruminants virus, PSU- Pennsylvania State University, SLAM- Signaling Lymphocyte Activation Molecule, VDS- Vero Dog Slam.

ETHICS STATEMENT

The study protocol was ethically and scientifically evaluated by Animal Research Scientific and Ethics Review Committee (ARSERC) of AHI formerly NAHDIC and awarded Ethical Clearance Certificate with certificate Ref. No. ARSERC/EC/001/17/04/2019 on date May 03/2019. In addition, Institutional Review Board (IRB) of Addis Ababa University, Aklilu Lemma Institute of Pathobiology (ALIPB) has awarded Ethical Clearance Certificate with Ref. No. ALIPB IRB/83/2014/22 on date July 21, 2022. This study was reported according to ARRIVE guide lines.

CONSENT FOR PUBLICATION

“Not Applicable**”**

DATA SHARING STATEMENT

All data and R-code are provided (https://github.com/cherz4/pprv-cattle-goat-comparative-pathology). Whole genome sequence information for PPRV/Ethiopia/Habru/2014 passage 4 can be found at Genbank accession number: ON110960.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was carried out without any commercial or financial affiliations that could pose a potential conflict of interest.

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AUTHOR CONTRIBUTIONS

FA, DSi, and DSh conducted the experiments. FA, AA, RB, MK, DSi, DSh, and AS performed laboratory analyses. FA, CMH, VK and HASh designed the study. CMH created analysis code and plotted figures. FA, CMH, VK, AS and GM analyzed the data. FA, DSi, DSh, and HASg collected samples and data. FA, CMH, and HASg were responsible for data management. FA, DSi, TC, GM, CMH and VK for overall project management. TC and GM project administration. FA wrote the original draft that was extensively revised by CMH and VK. FA, HASh, TK, HC, GM, AS, CMH, CS and VK critically edited and reviewed the manuscript. All authors read and approved the final manuscript.

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CHAPTER FOUR

CHAPTER FOUR: COMPARATIVE VIRULENCE STUDY OF LOCALLY CIRCULATING PESTE DES PETITS RUMINANTS VIRUS (PPRV) STRAINS IN INDIGENOUS ETHIOPIAN GOATS

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4.1 ABSTRACT

Background: Peste des Petits Ruminants (PPR) is a highly contagious viral disease affecting small ruminants with considerable socioeconomic impacts in Ethiopia. Despite its importance, comparative virulence of locally circulating PPR virus strains in indigenous goats remains poorly characterized. This study evaluated six distinct PPRV strains (four lineage-IV, two lineage-III) in experimentally infected Ethiopian goats. Twenty-one Central highland breed goats (12-30 months) were randomly allocated into six experimental groups and one control group, with three goats in each group and experimental animals inoculated intranasally ($10^{4.5}$ $TCID_{50}$ per ml). Clinical, serological, molecular, and pathogenesis data were collected.

Results: Five of six strains produced consistent clinical manifestations beginning at 4 days post-infection (dpi), with seroconversion by 7 dpi and viral antigens detectable in nasal swabs from 3 dpi. No substantial lineage-specific differences in virulence were observed between lineage-III and lineage-IV strains. One lineage-IV isolate (38920/19) produced significantly milder clinical manifestations ($P<0.05$) and no seroconversion, suggesting potential strain-level variation. Gross and histopathological findings were consistent across virulent strains, with nasal swabs proving most reliable for early diagnosis.

Conclusions: This first comparative study of Ethiopian PPRV strains establishes similar virulence profiles across lineages in local goats, suggesting that lineage classification alone may not predict clinical outcomes - an important finding for regional disease control strategies. The reduced virulence in one strain suggests factors beyond genomic lineage may influence experimental outcomes, though additional work with standardized protocols is needed to confirm these findings.

Key words: **-Comparative virulence, Experimental infection, Indigenous Goats, Pathogenesis, PPRV, Strain characterization,**

4.2 INTRODUCTION

Peste des Petits Ruminants (PPR), also known as 'goat plague' or 'Kata' [1], is a highly contagious and frequently fatal viral disease that affects both domestic and wild small ruminants [2]. The disease has a severe socio-economic impact on rural poor farmers [3], who heavily rely on small ruminant rearing, due to its high morbidity and mortality rates. In most PPR infected regions, it poses a significant threat to small ruminant production, greatly impacting livelihoods of poor farmers who predominantly own sheep and goats [4]. Since its emergence in Côte d'Ivoire [5], the disease has progressively expanded its geographical range from Africa to the Middle East, Asia, and more recently, Europe [6, 7]. The etiological agent of the disease (PPR virus) belongs to family Paramixoviridae, genus Morbillivirus and species *Morbillivirus caprinae*. The disease primarily manifests in an acute form, clinically characterized by symptoms such as depression, fever, oculo-nasal discharges, oral lesions, pneumonia, and diarrhea, often resulting in death. However, the sub-acute form is observed, particularly in sheep in endemic areas [8] and a subclinical form in atypical hosts like cattle [9-11].

During field outbreak investigations, a spectrum of clinical outcomes is typically observed, ranging from mild to severe respiratory and gastrointestinal symptoms, and in some cases, resulting in death [12-14]. Previous studies have shown that different species and breeds of small ruminants are affected by PPR to varying degrees. For instance, a research on different PPRV strains investigated their virulence in West African dwarf goats and indicated that virulence characteristics might serve as a valuable marker for classifying PPRV strains [15]. Diop et al. (2005) also noted variations in the sensitivity of different goat breeds to the same PPRV outbreak during field investigations and concluded that this variation in susceptibility is more related to the breed than to differences in the virus itself [16].

Furthermore, current understanding of PPR pathology and pathogenesis has been largely inferred from studies of the closely related rinderpest virus (RPV) and other morbillivirus infections [17]. Only a few studies [18-22] have examined the pathology of PPRV, leading to a limited understanding of the disease's pathology, clinical progression, and outcomes in susceptible hosts like goats. Furthermore, in previous years, there has been limited focus on the diversity of viral strains and their potential to produce varying clinical outcomes [15]. To assess the impact of

these differences, including their potential for transmission, further studies are required in regions where PPRV is enzootic or endemic.

To date, PPRV strain virulence studies have not been undertaken on locally circulating strains in Ethiopia and there is a lack of knowledge on how viral strain relates to clinical outcomes observed in local Ethiopian goat breeds. Hence, this study focused on characterizing locally circulating PPRV strains and assessing their virulence in experimentally infected local Ethiopian goat breed.

4.3 MATERIAL AND METHODS

4.3.1. Experimental animals

Goats of indigenous breed (Central highland breed), sex all males, aged between 12 to 30 months, were obtained from animal markets in North Shoa, Ejere, and Degem, all within a 200-km radius of Addis Ababa, Ethiopia. Animal selection criteria involved factors such as age, overall health, breed validity, and absence of PPR vaccination history. Age determination relied on dental examination [23] and information provided by the sellers. Animals were subsequently transported to the Animal Health Institute (AHI). Seronegative status was confirmed by competitive enzyme-linked immunosorbent assay (c-ELISA, ID.Vet, Grabels, France) after 3 to 4 days of arrival. Seronegative goats were acclimatized for about 10 to 15-days, ear-tagged, and randomly divided into experimental and control groups.

In total, 21 goats each were randomly allocated across 7 barns: 6 for experimental groups and one for the negative control group, with each barn housing 3 goats. Study design details can be found in Supplementary (S) **Text S 4.1**.

4.3.2. Virus

Viral suspensions were prepared using Vero Dog Slam (VDS) cells at AHI laboratory. Viral strains were chosen from archived outbreak investigation samples. Selection criteria included:

geographic location, collection year, and test result. Specifically, candidates had to pass two consecutive tests—RT-qPCR and virus isolation—demonstrating positivity in both, as well as meeting quality control standards for sequencing.

In this study, a total of six strains were utilized, four belonging to lineage-IV, and two from lineage-III. Virus strain characterization work was conducted at AHI (RT-qPCR and viral culture) and at Penn State University (whole-genome sequencing (WGS)). Viral suspension titer, passage number, codes, geographic location, lineage type and animal species, age, sex and sample type collected and processed for the purpose are presented in **Table 4.1**. Each experimental goat received a total virus dose of 1 ml (concentration $10^{4.5}$ $TCID_{50}$ per ml), with 0.5 mL administered per nostril during inoculation as per [11, 24].

Table 4.1: Information on PPRV strains used for inoculation

Gen-Bank Accession number	Isolates/ strains code	Identity of isolates/strains by Lab code	Geographic location	Passage number	$TCID_{50}$ per ml	Species	Age	Sex	Sample type	Remark
NA	2	24475/21	Benishangul Gumuz, Assosa, Bambasi	3rd	10^4.5	Caprine	1.5yrs	Female	Swab (Nasal)	Lineage-IV
ON110960	6	Ethiopia/Habru/ 2014	Amhara, North wollo, Habru	4th	10^4.5	Caprine	3yrs	Female	Swab Pooled	Lineage-IV
PV110162	1	38920/19	Benishangul Gumuz, Assosa, Bambasi	2nd	10^4.5	Caprine	2yrs	Female	Swab (Nasal)	Lineage-IV
PV110160	5	26173/19	Oromia, Borena, Arero	2nd	10^4.5	Caprine	1yr	Female	Tissue (Intestine)	Lineage-III
PV110161	4	35368/19	Oromia, East Harerge	2nd	10^4.5	Caprine	2yrs	Female	Swab (Nasal)	Lineage-III
PV110159	3	25870/21	SNNPR, Gurage, Agena	3rd	10^4.5	Caprine	1yr	Male	Swab (Ocular)	Lineage-IV

NA: not available

4.3.3. Clinical investigation and scoring

After infection daily comprehensive clinical examinations were conducted on all goats to detect the emergence and progression of clinical signs indicative of PPR [11, 12, 24-26]. Veterinary attention was provided as described in **Text S 4.2**. As per the criteria set by Pope et al. (2013), goats with a rectal temperature of 39.5°C or above were categorized as febrile.

4.3.4. Sampling and sample processing

Sera and ocular, nasal, and rectal swabs from experimentally infected goats were collected at 0, 1, 3, 5, 7, 10, and 12 days post-infection (dpi). These were tested for viral antigen and RNA. Only serum samples were collected and tested from control animals on the same dpi and only c-ELISA was performed to confirm seronegativity, and that swab sample testing was not done because of biosecurity and economic considerations.

Detection of PPRV antigen and PPRV/RNA was performed using immunocapture-ELISA (Ic-ELISA, ID Screen® PPR Antigen Capture kit, ID.Vet, Grabels, France) and real-time RT-qPCR, respectively. The PPR Ic-ELISA was used only on nasal and rectal swabs from experimental goats, utilizing the method as described in [24, 27].

RT-qPCR was conducted on both swab and tissue samples (see Gross and Histological Sample section below) collected throughout the study and positive specimens underwent virus isolation to confirm the presence of viable virus. Viral RNA extraction was conducted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The extracted RNA was utilized in an RT-qPCR assay targeting the nucleoprotein (N) gene of PPRV using specific primers and probe as described by [28]. Detailed procedure of RT-qPCR is presented in **Text S 4.3.** Viral isolation occurred in Vero Dog Slam (VDS) cells, following the protocol outlined by [29]. A detail virus isolation procedure is outlined in **Text S 4.4.**

Baseline seronegativity and experimental antibody responses for anti-PPRV-N protein antibodies were evaluated using the ID Screen® PPR Competition kit (ID.Vet, Grabels, France) as described in [24, 27]. Moreover, sequencing and phylogenetic analysis for lineage-level classification were conducted as per the methods outlined in Simon King et al. (2023).

4.3.5. Gross and histopathological examination

Postmortem tissue samples were obtained from experimental goats after euthanasia by captive bolt stunning. Penetrating type captive bolt (**Text S 4.5**) was used, followed immediately by exsanguination to ensure death. These protocols were in full compliance with relevant AHI, Animal Research Scientific and Ethics Review Committee (ARSERC) and consistent with AVMA guidelines (2020)." The timeline was one goat per barn/strain (a total of six goats per day) sacrificed, starting at 7 dpi, followed by 10 dpi, and concluding at 12 dpi to aid in the collection of tissue samples. We set 12 dpi as end date for this experiment based on primary objective of the study (pathogenesis study during early disease course) and previous pathogenesis studies recommendations [11, 24, 25]. Subsequently, a comprehensive postmortem examination was conducted on each animal to evaluate for gross pathological changes. In total, 14 tissue specimens were collected, including the retropharyngeal Lymph Node (LN), palatine tonsil, third (3^{rd}) eyelid, nasal mucosa, tongue apex, tracheal LN, bronchial LN, mediastinal LN, cranial lung, caudal lung, mesenteric LN, spleen, jejunal and ileal Peyer's patches, and colon.
Tissue and LN inspections were meticulous, focusing on localized areas affected by the PPR virus. Documented changes, including any deviations in structure, morphology, and color, and histopathological changes induced by PPRV infection were also systematically scored by measuring the extent of the lesions, with scoring criteria detailed in [24]. For detail procedure on histological analysis and grading refer to **Text S 4.6**.

4.3.6. Data analysis and visualization

Analysis and visualization were conducted in R statistical software (version 4.1.3), employing the ggplot2 package for generating figures. Local regression (LOESS) smoothed curves with corresponding 95% confidence intervals, calculated through t-based approximation, were incorporated into the plots using the stat-smooth function to provide a visual summary of the collective data trends across the study duration. For comparative clinical score and tissue positivity percentage analysis we applied a non-parametric test (Kruskal-Wallis test) using GraphPad Prism version 10.1.1, as the data did not follow a normal distribution and the sample size was small.

4.4. RESULTS

Clinical signs following inoculation with different PPRV strains

Goats experimentally infected with six distinct strains of PPRV (four Lineage IV and two Lineage III) exhibited the typical clinical signs of PPRV, except for one strain (strain 38920/19), which only caused a mild nasal discharge between 6 to 9 days post-inoculation (dpi). The majority of strains showed clinical signs starting from 4 dpi including general signs, fever ($\geq$39.5^{o}C), oculo-nasal discharge, congested mucous membrane, and at later stage respiratory signs, diarrhea and some goats manifested erosive mouth lesions. Examples of some of the observed clinical signs are presented in (**Figs. S 4.1A- 4.1D**). The detailed results of the clinical signs, including rectal temperature (**Figure 4.1**), summarized clinical scores (**Table S 4.1**), and observed clinical signs for each individual strain **(Text S 4.7),** are presented in their respective sections.

Comparison of the average clinical score data across the experimental groups using the non-parametric Kruskal-Wallis test, revealed a statistically significant difference between strain 38920/19 and other groups ($P < 0.05$, 0.0039). Statistically, significant differences were observed between strain 38920/19 with the following strains from the group: 38920/19 vs 24475/21 (P=0.037); 38920/19 vs 2014/Habru isolate (P=0.011); 38920/19 vs 35368/19 (P=0.015); 38920/19 vs 25870/21 (P=0.045). Strain 26173/19 was not significantly different from 38920/19.

Key clinical observations in all the inoculated goats were recorded between 4 and 12 dpi and are summarized in **Table S 4.1**. In each group, at least one goat exhibited a characteristic clinical sign of PPR. Goats inoculated with the PPRV strain (38920/19 lineage-IV) displayed milder clinical signs compared to those inoculated with other strains.

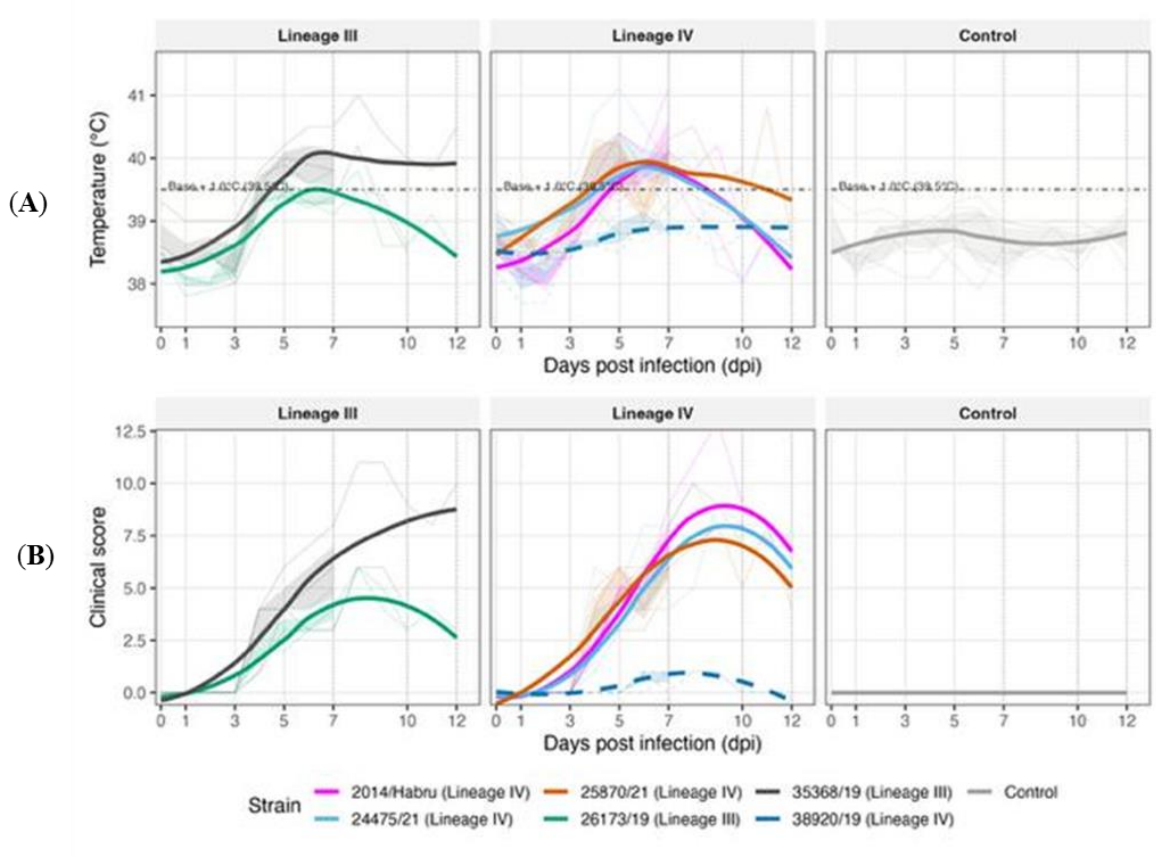


Figure 4.1: Observed Clinical signs and Rectal Temperature (^{o}C, febrile threshold ≥39.5) in experimental goats in both infected with PPRV **strains and uninfected controls. (A)** Trends in daily rectal temperature (**B**) Modified composite clinical scores without rectal temperature readings. In all subfigures, thin lines depict individual animal data, while bold LOESS curves represent the collective pattern for the group for each strain.

Viral dynamics and immune response

Viral dynamics as detected by Ag-ELISA

In general, viral antigen detection in nasal swabs began at 3 dpi with cumulative result of 5/18 (27.7%), increased at 5 dpi with 8/18 (44.4%) positivity, peaked at 7 dpi with 16/18 (88.8%) positivity, decreased to 8/12 (66.6%) at 10 dpi, and dropped to 1/6 (16.6%) at 12 dpi as detected by Ic-ELISA. In rectal swabs antigen positivity started at 3 dpi with cumulative result of 2/18

(11.1%), at 5 dpi 5/18 (27.7%), peaked at 7 dpi 13/18 (72.2%), decreased to 6/12 (50%) at 10 dpi, and were completely negative by 12 dpi. Detail viral dynamics results as detected by Ic-ELISA for all strains categorized at lineage level are presented in **Figure 4.2**. While detail results of antigen detection of individual strains are presented in **Text S 4.8.**

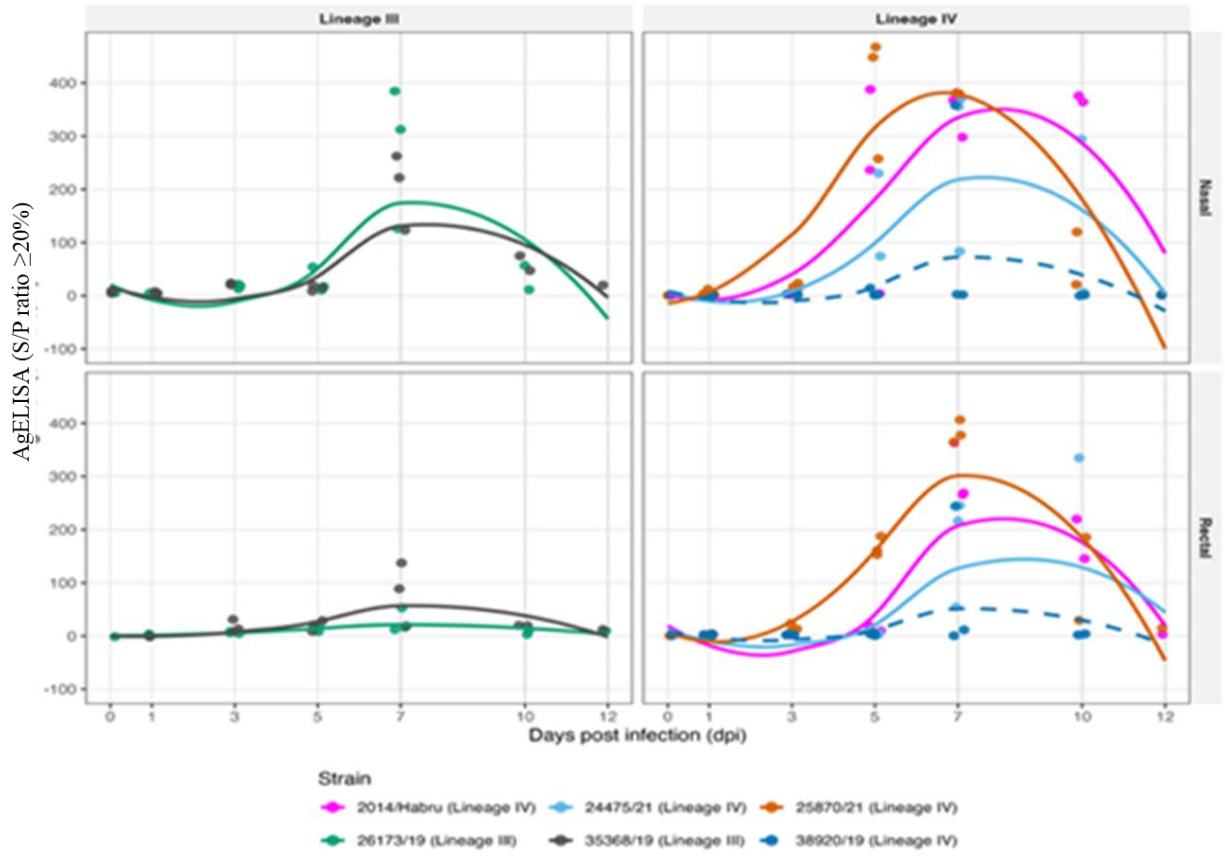


Figure 4.2: Kinetics of PPRV **(L-III and L-IV) antigen shedding (with S/P ≥20 positivity threshold) in experimentally virus infected goats.** Panel of figures illustrate the sample-to-positive (S/P) ratio values from Ic-ELISA of the nasal and rectal swabs. In the top row (Nasal swab) all sub figures (LIII and LIV groups) represented different strains within the group; each dot depicts the virus antigen shedding pattern of individual animal data while bold lines represent smooth local regression (LOESS) curves represented average result for each group. Among the results a strain, that represented lab code 38920/19 and among the lineage IV strains showed lowest Ic-ELISA positive results in both nasal and rectal swab samples. Antigen positivity defined by a percent positivity ratio (S/P) of ≥ 20%.

Viral dynamics as detected by RT-qPCR

Experimental goats inoculated with lineage-IV viruses of different strains produced RT-qPCR positive results starting from 5 dpi with average value of 50%, (18/36), increased at 7 dpi 29/36 (80.55%), slight decrease in 10 and 12 dpi's with 18/24 (75%) and 9/12 (75%), respectively. Similarly experimental goats inoculated with lineage-III viruses of different strains produced positive RNA results started from 5 dpi with 15/18 (83.33%) positivity, increased at 7 dpi 17/18 (94.44 %), slight decrease at 10 dpi with 11/12 (91.66%) and further deceased at 12 dpi 3/6 (50%) in average. For detailed viral dynamics results as a result of inoculated virus strains see **Figure 4.3**. Detailed RT-qPCR result of swab samples of individual strains is presented in **Text S 4.9.**

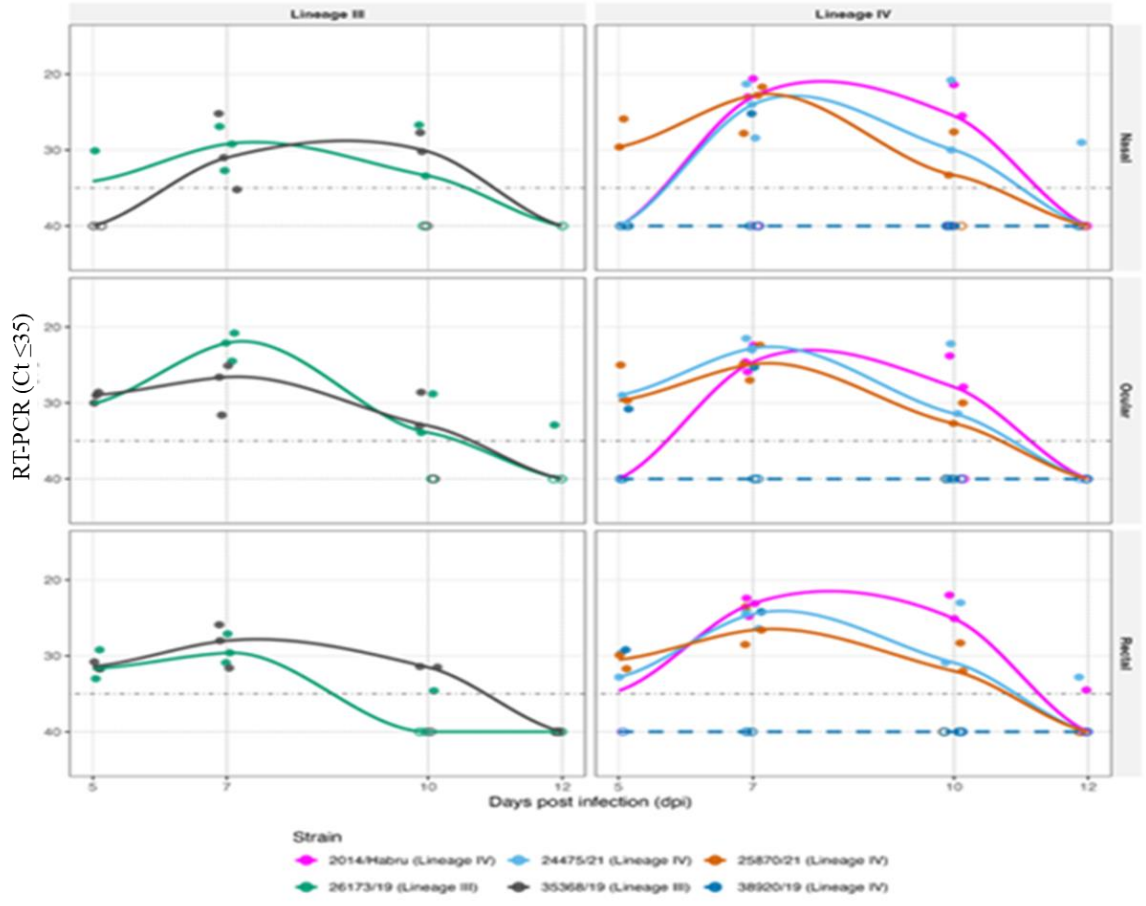


Figure 4.3: Kinetics of PPRV **(L-III and L-IV) RNA detection in experimentally virus infected goats.** The top row (**Nasal swab**) in both sub figures (**L-III and L-IV**), dots depict individual animal data while bold loess line represented average result for each strain in each lineage L-III and LIV group. Among the results a strain represented by blue broken line in all sample type (Nasal, Ocular and Rectal), that represented lab code 38920/19 and among the lineage IV strains showed very low RT-qPCR positive results (Ct>35). Each dot depicts the virus RNA shedding pattern of individual PPRV strain in infected goats. The dashed black line in the middle of the figure indicates the cutoff threshold for a positive test result (Ct ≤35).

Immune response to infection with different PPRV **strains**

The immune response to the virus strains was evaluated using a c-ELISA test. The 2 out of 3 experimental goats infected with PPRV lineage-IV (24475/19), had detectable anti-PPRV antibody starting from 7 dpi. By 10 dpi, all goats in the group were found seropositive. All goats inoculated with other lineage-IV virus strains (2014/Habru and 25870/21), were found to have detectable anti-PPRV antibody from 7 dpi until the end of the experiment (12 dpi). Conversely, goats inoculated with another lineage-IV virus, (38920/19), remained seronegative throughout the experiment. Goats inoculated with lineage-III viruses (26173/19, and 35368/19), tested seropositive with c-ELISA from 7 dpi until the end of the experiment (12 dpi). Negative controls remained negative until the end of the experiment (see **Figure 4.4**).

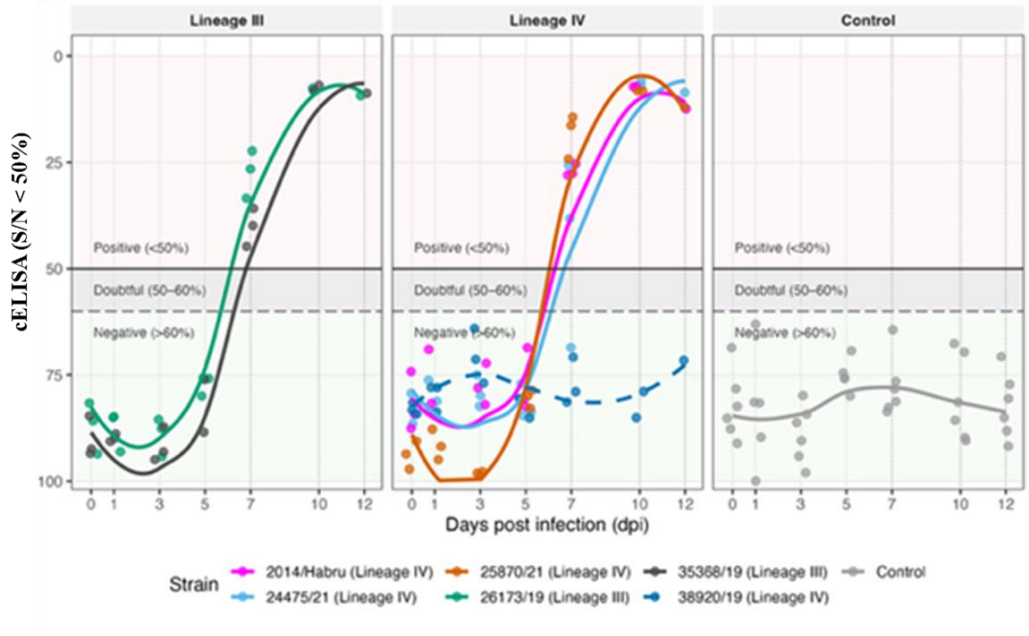


Figure 4.4: Time course of seroconversion in experimentally infected goats with six different PPRV **strains from L-III and L-IV and uninfected control groups.**

In the figure, seroconversion in the infected cohort (L-III and L-IV), was detected from 7 dpi onwards in most strains except strain 38920/19 from L-IV group which showed no seroconversion until end of the experiment day (12 dpi), indicated in blue broken curved line in subfigure Lineage IV. Each dot represents the serological response of an individual goat, while bold loess line in each group (L-III, L-IV and Control) represented average result for each sub groups, with seropositivity defined by a competition percentage below 50%. The black bold line in the middle of each figure denotes the cutoff threshold for a positive test result (S/N <50%).

Assessment of PPRV **tissue tropism in various post-mortem samples using RT-qPCR at various dpi's (7, 10 and 12 dpi's)**

PPRV tissue tropism on various postmortem tissues and LNs samples was evaluated by performing RT-qPCR (**Table S 4.2**). Overall, at 7 dpi, an average of 88.09% (74/84) of tissue samples tested positive. By 10 dpi, positivity rate decreased to 54.76% (46/84), and at 12 dpi, it further decreased to 51.19% (43/84). Even though differences in the tissue positivity percentage results among six experimental groups, it was not statistically significant (P>0.05, i.e 0.61).

The RT-qPCR analysis of 14 individual tissue and lymph node samples collected at post-mortem on different days post-infection from 18 experimentally infected goats revealed variable viral RNA detection level. Detailed results are presented in **Table S 4.3**.

PPRV isolation in cell culture

Virus culture results from swab and tissue samples that were initially positive by RT-qPCR are presented in **Table S 4.2**. Samples collected at 7 dpi both during antimortem and postmortem times produced higher proportion of culture-positive results, 58.69 % (27/46) and 57.8% (37/64), respectively whereas, samples collected at 10 and 12 dpi both during anti-mortem and postmortem times produced negative results.

Gross and histopathology findings

Gross pathology

Goats infected with different strains from both lineages-IV and III manifested PPRV characteristics grade-2 lesions. Grade-2 signs and lesions included swelling with edema, congestion and hemorrhage in most of the inspected tissues and lymph nodes (LNs), distinct signs on lung tissue like hepatization and meaty appearance (**Figure S 4.2**) and emphysema, necrotized thickened mucosa of jejunum, congested Peyer's patch and catarrhal enteritis. However, a goat infected with lineage-IV, 38920/19 strain and sacrificed at 7 dpi, showed grade-1 gross lesions, like slight enlargement in some of inspected LNs and slightly hepatized cranial lung. While goats sacrificed at 10 and 12 dpi's and infected with the same virus strain (38920/19) showed no typical gross lesions observed on inspected tissues and LNs. Detailed individual strain gross pathology results are presented in **Text S 4.10**.

Histopathology

Histopathological lesions characterized as grade-2 lesions were consistently observed beginning at 7 dpi in sacrificed goats. These grade-2 lesions:- included lymphocyte cell depletion (characterized by pyknosis and karyolysis), congestion, and hemorrhage of the head, lung-associated, and mesenteric lymph nodes, as well as in other inspected non-lymphoid organs. In contrast, goats inoculated with 38920/19 lineage-IV strain showed only mild histological lesions from one goat sacrificed at 7 dpi. The remaining two goats infected with 38920/19 strain and sacrificed at 10 and 12 dpi's showed no histological signs. Detailed histopathological findings for specific tissues can be found in **Text S 4.11** and **Figures S 4.3A – 4.3F**.

4.5 DISCUSSION

The current study characterized and compared PPRV strain virulence in indigenous Ethiopian goats for 6 locally circulating strains. In this study, viruses from two identified lineages, Lineage-IV and Lineage-III, produced nearly similar clinical outcomes in the inoculated goats except the very mild signs observed from 38920/19, a lineage-IV strain marked by significant ($P<0.05$, 0.0039) difference in clinical scores with other experimental groups. This study built on previous PPR virus pathogenesis studies, which showed that various strains produced a range of clinical outcomes. Historically, mild infection was seen with the lineage-II strain, acute to mild disease with the lineage-III strain, acute disease with the lineage-IV strain, and per-acute fatal disease with the lineage-I strain [15]. To date, one other study has compared PPRV pathogenesis and tissue tropism in goats [21]. Moreover, a study conducted in the UK reported no major lineage-specific differences in virulence among the six virus strains tested on British male goats [30]. The current study both aligns with and diverges from the previous works, and establishes similar virulence profiles across two lineages in local goats, suggesting that lineage classification alone may not predict clinical outcomes - an important finding for regional disease control strategies.

To our knowledge, this is the first report describing PPR virulence in a local breed of goats in Ethiopia. Although the strains used belonged to different lineages (L-III and L-IV) and had varying passage numbers (second to fourth), most of them caused similar clinical signs of disease. However, there were slight differences in the severity of symptoms observed both during anti-mortem and postmortem inspection times among the groups. The study, although conducted with a limited number of goats (three per group/strain), demonstrated that disease severity (including clinical signs, virus shedding pattern (viremia), tissue positivity and gross and histopathological alterations) varied at the strain level within the same lineage. Goats inoculated with virus strains, 24475/21(IV), 2014/Habru(IV) and 35368/19(III) showed peak temperature (rectal temperature $\geq 41^0$C) while other strains, 26173/19(III) and 25870/21(IV) manifested lower temperature and strain 38920/19 showed lowest temperature record (**Figure 1 and Table S 4.1**). Virus antigen shedding (viremia) pattern also showed variation. Shedding was prominent in strains 24475/21(IV), 2014/Habru(IV) and 25870/21(IV) while strains 38920/19(IV), 26173/19(III) and 35368/19(III) marked lower shedding pattern (**Figure 2**). Variation in disease

outcomes at the strain level was also been observed in prior research. Studies by Eloiflin et al. (2022) and Couacy-Hymann et al. (2007) identified differences in virulence between two strains from PPRV lineage-I. According to Eloiflin et al. (2022) the strain they used resulted in mild disease symptoms, whereas Couacy-Hymann et al. (2007) documented a severe disease outcome from the same lineage-I with a different strain.

While we observed some variation in disease manifestation, statistical analyses revealed no significant differences in clinical signs, viremia, viral tissue tropism, or gross and histopathological findings between lineage-III and lineage-IV groups ($P = 0.61$ for tissue positivity). Five of the six virus strains produced characteristic PPR clinical signs including fever ($\geq 39.5^{o}C$), oculo-nasal discharge, congested mucous membrane, and at later stage respiratory signs, diarrhea and some goats manifested erosive mouth lesions starting from 4 dpi, consistent with previous field and experimental studies [14, 15, 18, 31-33].

The notable exception was strain 38920/19 (lineage-IV), which produced significantly milder symptoms ($P < 0.05$) compared to other strains and performed poorly across all evaluation criteria, exhibiting only limited serous nasal discharge. This finding contrasts with earlier reports that lineage-IV PPRV, particularly those originating from Asia, typically display high virulence and cause acute disease [15]. The reduced virulence could reflect genuine biological differences or technical factors related to laboratory handling. Virus culture passages can produce defective interfering particles, which might reduce virulence by hindering the replication of standard virus [30, 34]), and serial passages are well-documented to attenuate PPRV [35, 36] and that confirmation will require neutralization assays and longer-term studies. Interestingly, our preliminary sequencing efforts (to be reported in a future study) have not yet revealed genomic variations that would explain this phenotypic difference, suggesting complex interactions between viral genetics, handling history, and host responses may be involved. These observations highlight the importance of considering both biological properties and technical factors when interpreting experimental outcomes in PPRV research.

In this study, no mortality occurred up to 12 dpi across all groups. This differs from some previous studies which reported mortality within 7-12 dpi, with lineages-I, III and IV PPRV [15], and from 9-21 dpi, with lineage-IV strain (PPR/Izatnagar/94), [37]. However, one study did not find mortality during the observation period up to 28 dpi [38], similar to our study. Absence

of mortality in our study might be due to various factors including virus infectious dose used, breed, use of relatively shorter duration of observation prior to sacrifice (12 days), and potentially altered virulence due to cell culture passaging [30, 34].

Most of the experimental goats seroconverted starting from 7 dpi in agreement with previous studies [11, 15, 18, 36], while goats infected with the 38920/19 strain failed to seroconvert and were serologically negative up to the end of our experiment on 12 dpi. This could be explained by decreased strain virulence or failure to infect with appropriate dose and shorter duration of the experiment (12 dpi).

Viral kinetics from swabs samples and viral predilection sites in postmortem tissues and LNs (Table 3 and Figure 2) were in agreement patterns described in previous studies [18, 31, 39, 40].

PPRV antigens were detected in both nasal and rectal swabs from 3 to 12 dpi. Detection in rectal swabs was intermittent and occurred at a later stage of the disease. These findings align with previous studies [11, 25], which also reported PPRV antigens detected later in the disease course in rectal swab samples and that the detection was intermittent. The results from this study suggest that nasal swabs are the most suitable samples for molecular diagnosis of PPRV (**Figure 2**) as has been in previous studies [25].

Limitations of the study

This study had several methodological constraints that should be considered when interpreting the results. The small group sizes (n=3 per strain) limited statistical power, potentially masking subtle differences between strains. The relatively short observation period of 12 days may have precluded detection of late-developing clinical manifestations or mortality that might have occurred beyond this timeframe. Additionally, our use of only Central highland goat breed restricts the generalizability of findings to other Ethiopian indigenous breeds, particularly those from lowland areas that may have different susceptibility profiles. While we characterized the strains by lineage, more detailed genomic analysis would be valuable for identifying specific genetic determinants of virulence variations observed, especially regarding strain 38920/19.

Conclusion and recommendations

This comparative assessment of locally circulating PPRV strains in Ethiopia provides important baseline data for understanding strain virulence in indigenous goats. Our findings demonstrate that both lineage-III and lineage-IV PPRV strains produce similar clinical outcomes. The reduced virulence of one lineage-IV strain highlights the complex interplay between viral genetics, laboratory handling, and host factors in determining disease outcomes.

Although this study has isolated and characterized different virus strains from the commonly circulating lineages (LIII and LIV) in the country, they remain controllable with the currently available Nigeria 75/1 (L-II strain) vaccine. However, to strengthen these findings and address current limitations, we recommend future studies incorporate: (1) larger sample sizes to increase statistical power; (2) extended observation periods beyond 12 dpi to capture the complete disease progression; (3) inclusion of diverse goat breeds from both highland and lowland regions to assess breed-specific susceptibility; and (4) comprehensive genomic and phenotypic characterization of viral strains to better understand the molecular basis of virulence differences. Such expanded research would provide more robust evidence to inform targeted control measures for this economically significant disease affecting Ethiopia's small ruminant population.

LIST OF ABBREVIATIONS

AHI –Animal Health Institute, ALIHR- Aklilu Lemma Institute of Health Research , ARSERC- Animal Research Scientific and Ethics Review Committee, CCPP- Contagious Caprine Pleuropneumonia, CDV- Canine Distemper Virus, c-ELISA- Competitive Enzyme Linked Immuno Sorbent Assay, CPE-Cytophatic Effect, CSV- Comma separated Values, CT- Cycle Threshold, dpi- days post inoculation/infection, DMEM- Dulbecco’s Modified Eagle Medium, DNA- Deoxyribo nucleic acid, GIT- Gastro Intestinal Tract, Ic-ELISA- Immunocapture Enzyme Linked Immuno Sorbent Assay, LNs- Lymph Nodes, LOESS- Local Regression, MeV- Measles Virus, PBS- Phosphate Buffered Saline, PPR- Peste des petits Ruminants, PPRV- Peste des petits Ruminants Virus, PSU- Pennsylvania State University, RNA- Ribo-Nucleic Acid, RNP- Ribo-nucleo Protein, RPV-Rinder Pest Virus, RT-qPCR- real time quantitative polymerase

chain reaction, SLAM- Signaling Lymphocyte Activation Molecule, SOPs- Standard Operating Procedures, , $TCID_{50}$- Tissue Culture Infectious Dose Fifty, VDS- Vero Dog Slam, WGS-Whole Genome Sequencing.

ETHICS STATEMENT

The study protocol underwent thorough ethical and scientific evaluation by the Animal Research Scientific and Ethics Review Committee (ARSERC) of AHI, formerly NAHDIC, and received an Ethical Clearance Certificate (Ref. No. ARSERC/EC/001/17/04/2019) on May 3, 2019. Additionally, the Institutional Review Board (IRB) of ALIHR, formerly Addis Ababa University's Aklilu Lemma Institute of Pathobiology (ALIPB) granted an Ethical Clearance Certificate (Ref. No. ALIPB IRB/83/2014/22) on July 21, 2022. The study was conducted following the ARRIVE guidelines.

CONSENT FOR PUBLICATION

"Not Applicable**"**

DATA SHARING STATEMENT

All analyzed data results are included in the manuscript and supplementary material. Additionally, data and R code is available in GitHub weblink https://github.com/cherz4/pprv-comparative-virulence-goats. Whole genome sequence (WGS) information for PPRV/Ethiopia/Habru/2014 can be found at Genbank accession number: ON110960. WGS information for the other distinct strains (25870/21, 26173/19, 35368/19 and 38920/19) can be found at Genbank accession numbers: PV110159, PV110160, PV110161 and PV110162, respectively.

CONFLICT OF INTEREST STATEMENT

The authors state that the research was conducted with no commercial or financial ties that could be interpreted as a potential conflict of interest.

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AUTHOR CONTRIBUTIONS

FA, DSi, and DSh conducted main experiments. FA, AA, RB, MK, DSi, DSh,, AS and MB performed laboratory analyses. FA, CMH, VK and HASh designed the study. CMH and VK created analysis code and plotted figures. FA, CMH, VK, AS and MB analyzed the data. FA, DSi, DSh, and HASg collected samples and data. FA, CMH, and HASg were responsible for data management. FA, DSi, TC, GM, CMH and VK handled overall project management. TC and GM project administration. FA wrote the original draft. FA, HASh, TK, HC, GM, AS, CMH, BM and VK critically edited and reviewed the manuscript. All authors read and approved the final manuscript.

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CHAPTER FIVE

CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Given the reported morbidity, mortality, and the size of the small ruminant sector, PPR has likely become one of the most economically significant livestock diseases in Ethiopia (Waret-Szkuta et al., 2008; Magona et al., 2016) and is now prioritized for control and eradication in the strategic plan. It is also designated as a priority pathogen for global eradication by 2030, as outlined by the FAO and WOAH (FAO, 2015; Baron et al., 2017). In line with the Global Strategy, countries affected by PPR, including Ethiopia, have developed national plans to eradicate the disease by 2030. Ethiopia launched its PPR control and eradication program five years ago with the support of EUSHARE project. Despite ongoing control efforts, PPR outbreaks continue to spread from lowland pastoral areas into highland mixed farming regions of Ethiopia that were previously unaffected. This underscores the need to investigate all factors contributing to the disease's persistence. One key consideration is the role of other host species, such as cattle, in maintaining and transmitting PPRV. The involvement of species beyond sheep and goats in PPRV persistence remains unclear. Additionally, characterizing and assessing the virulence of circulating PPR viruses is crucial for developing more effective control and eradication strategies.

This dissertation tackles these challenges through a series of interconnected experimental studies aimed at optimizing the ongoing PPR control and eradication program for small ruminants (sheep and goats) in Ethiopia. The research began by evaluating the role of cattle through five sequential randomized controlled trials and a model-based approach. This was followed by a comparative pathology study using the Ethiopian lineage-IV strain in goats and cattle. The trial examined PPRV tissue tropism, clinical progression, viral shedding, and pathology in both species, using an experimental design to compare disease pathogenesis and outcomes.

Additionally, an experimental study was conducted to evaluate the virulence of field virus strains circulating in specific regions of Ethiopia, with characterization performed using various methods.

Exploring the Role of Species beyond Sheep and Goats in PPRV Epidemiology for Effective Disease Control

One of the studies explored the role of species beyond sheep and goats, particularly cattle, in PPRV epidemiology using an experimental design. The study replicated conditions similar to East African zero-grazing husbandry practices through a series of trials involving local Zebu cattle (Bos taurus indicus) co-housed with goats (Capra aegagrus hircus). The experiment was carried out at the AHI experimental facility using the locally circulating PPRV (lineage-IV) and local cattle and goat breeds, adhering to all ethical, biosafety, and biosecurity protocols. During the experiment, each cattle received 2ml, while each goat received 1ml of the viral suspension via the intranasal route to mimic natural transmission.

The results showed that, cattle can become infected through contact with PPRV-infected/inoculated goats and may exhibit mild, transient clinical signs. However, cattle that are experimentally infected/inoculated with PPRV do not exhibit clinical symptoms of the disease and do not spread PPRV to goats. Of the 32 cattle intranasally infected with lineage-IV PPRV, none transmitted the virus to the 32 co-housed goats. However, these cattle, or those co-housed with PPRV-infected goats, did seroconvert. The results confirm previous studies that cattle currently play a minimal role in PPRV transmission (Schulz et al., 2018; Couacy-Hymann et al., 2019), and that vaccinating small ruminants is sufficient for eradication.

The current study also offers compelling evidence that the likelihood of cattle-to-goat transmission is infrequent, even when the animals are housed together for long durations. This suggests that cattle-to-goat transmission, if it occurs, is infrequent, even when animals are housed together for extended periods. This supports the alternative hypothesis that cattle might serve as dead-end hosts (Schulz et al., 2018; Couacy-Hymann et al., 2019) in PPR epidemiology and currently play a minimal role as reservoirs for PPRV transmission to sheep and goats.

In this study, PPRV RNA was detected by qRT-PCR in a naturally infected sentinel calf, although PPRV could not be isolated from these samples. This result underscores the necessity for continued monitoring of PPRV in cattle and calls for further research into the factors that may inhibit PPRV-related disease and viral shedding in cattle. Potential factors to explore

include variations in viral localization, receptor interactions, or differences in replication processes within lymph nodes and immune tissues compared to other organs in the cattle host.

However, if PPRV were to gain the ability to transmit from cattle, intervention strategies would need to be reevaluated. In the meantime, it is crucial to maintain surveillance of the virus's circulation in potential non-target host species. The visible findings of the first study lead to the foundation of the second study that —focused on the comparative pathology of PPRV in goats and cattle. It was designed to offer comparative insights into the pathology of PPRV lineage-IV in goats and cattle, with a particular emphasis on viral shedding patterns and the potential epidemiological role of cattle. The study focused on investigating the virus's tissue tropism (localization), pathogenesis, clinical progression, shedding, and pathology in both species. This experimental study involved a total of 32 animals, with 16 from each species, all of which were seronegative for PPR disease. Samples for analysis were collected from both live and sacrificed animals at predetermined time points. The results indicated that experimental infection with the Lineage-IV PPRV/Ethiopia/Habru/2014 strain caused moderate clinical signs in goats, while the absence of noticeable clinical signs post-inoculation in cattle aligns with findings from studies (Sen et al., 2014; Schulz et al., 2018; Couacy-Hymann et al., 2019), which also observed minimal to no clinical manifestations in bovine hosts. These results support the hypothesis that cattle may experience a subclinical course of PPRV infection, potentially serving as silent carriers of the virus. Significant differences in clinical observations were noted between the goat and cattle groups (P-value = 0.0070), highlighting the variability in host responses to PPR virus inoculation.

The seroconversion timeline in goats and cattle inoculated with PPRV showed that goats seroconverted sooner (at 6 dpi) compared to cattle (at 8 dpi). In general, seroconversion occurred earlier than what was observed in previous studies, which reported it at 7 dpi for goats and 10 dpi for cattle (Schulz et al., 2018; Eloiflin et al., 2022; Schmitz et al., 2023).

Culture positivity in cattle was considerably lower compared to the goats, with only one nasal swab (from animal ID 211 at 8 dpi) and a limited number of tissues, mainly from the head and thoracic regions, testing positive for live virus at 6 dpi (from animal ID 229). The marked difference in tissue positivity rates between goats and cattle ($P < 0.0001$, CI = 33.75-70.11)

further underscores the stark contrast in disease and infection patterns observed between the two species.

In cattle, the sporadic RT-qPCR positivity observed throughout the study period indicates a more confined viral presence compared to goats, with the virus primarily localized to specific tissues. This may be attributed to differences in the expression of SLAM and nectin-4 receptors between cattle and goats, which could account for the more restricted and localized viral RNA presence in bovine tissues (Sarkar et al., 2009). Gross and histopathological examinations also revealed differences between goats and cattle, highlighting the varying pathogenic impact of the virus on different host species. These differences may also reflect variations in individual or breed-specific immunity. The successful isolation of live PPR virus from bovine samples, though limited, highlights the need to reassess the epidemiological role of cattle in the transmission of PPRV. Notably, the virus was isolated from a nasal swab at 8 dpi and from specific tissues at 6 dpi, which is particularly significant. These findings suggest that cattle may act as potential reservoirs or silent spreaders of the infection under certain conditions, carrying important implications for PPR control and eradication strategies. While these results challenge the established notion that cattle are simply dead-end hosts, they deepen our understanding of PPRV pathophysiology and epidemiology. However, further research is necessary before extrapolating these findings to other PPRV lineages or regions beyond the lineage-IV PPRV currently circulating in Ethiopia.

Implications of Characterizing PPR Viruses and Assessing Virulence for Control Strategies

To the best of our knowledge, no virulence studies have been conducted on locally circulating PPRV strains in Ethiopia, and there is a limited understanding of how viral strain correlates with the clinical outcomes observed in local Ethiopian goat breeds. Therefore, one of our experimental studies aimed to investigate the comparative virulence of locally circulating PPRV strains in indigenous goats, following virus characterization using various methods. The study assessed six distinct PPRV strains (four lineage-IV and two lineage-III) circulating in different regions of Ethiopia in experimentally infected local goat breed. A total of 21 goats were

randomly assigned to six experimental groups and one control group, with three goats in each group. Each experimental group received 1 ml of virus suspension through the intranasal route from the challenge virus assigned to that group. The results showed that five out of six strains displayed typical PPR clinical signs starting at 4 days post-infection (dpi), with the exception of one strain (38920/19), which only caused a mild nasal discharge between 6 to 9 dpi. The majority of strains exhibited clinical signs from 4 dpi. In this study, viruses from two distinct lineages, lineage-IV and lineage-III, produced almost identical clinical results in the inoculated goats, with the exception of the mild symptoms seen in goats infected with the 38920/19 lineage-IV strain. This strain exhibited a notable ($P<0.05$, 0.0039) difference in clinical scores when compared to the other experimental groups. The current study both supports and contrasts with prior research, highlighting similar virulence profiles across two lineages in local goats. This suggests that lineage classification alone may not reliably predict clinical outcomes, a key factor to consider for regional disease control strategies. Despite the study being conducted with a small sample size (three goats per group/strain), it demonstrated that disease severity varied at the strain level within the same lineage. This strain-specific variation in disease outcomes aligns with findings from previous studies. Research by Eloiflin et al. (2022) and Couacy-Hymann et al. (2007) also identified differences in virulence between two strains of PPRV from lineage-I. Although some variation in disease manifestation was noted, statistical analyses revealed no significant differences in clinical signs, viremia, viral tissue tropism, or gross and histopathological findings between the lineage-III and lineage-IV groups ($P = 0.61$ for tissue positivity). The notable exception was the 38920/19 strain (lineage-IV), which led to significantly milder symptoms ($P < 0.05$) compared to the other strains and showed poor performance across all evaluation criteria, with only limited serous nasal discharge observed. This reduced virulence may reflect genuine biological differences or could be influenced by technical factors related to laboratory handling. These findings highlight the importance of considering both biological characteristics and technical factors when interpreting experimental results in PPRV research.

In this study, most of the experimental goats seroconverted starting from 7 dpi, which is consistent with previous studies (Couacy-Hymann et al., 2007; Enchery et al., 2019; Eloiflin et al., 2022). However, goats infected with the 38920/19 strain failed to seroconvert and remained

serologically negative until the end of the experiment on 12 dpi. This could be due to decreased strain virulence and/or insufficient viral dose for successful infection. No significant lineage-specific differences in virulence were observed between lineage-III and lineage-IV strains. However, one lineage-IV isolate (38920/19) caused significantly milder clinical manifestations ($P<0.05$) and did not lead to seroconversion, suggesting potential strain-level variation. Gross and histopathological findings were consistent across the virulent strains, with nasal swabs proving to be the most reliable for early diagnosis as has been in previous studies (Parida et al., 2019).

Conclusions and recommendations

This research emphasizes the need to investigate the crucial factors that sustain the PPR virus, including the role of other host animals, particularly cattle, in the virus's survival and spread. Furthermore, tracking the circulation of PPR viruses in local populations and evaluating their virulence are essential activities for effectively controlling and eradicating PPR.

The results from both empirical and model-based approaches regarding the role of cattle in PPRV epidemiology indicate that local breed (zebu) cattle in mixed-species environments have a limited role in transmitting PPRV to sheep and goats, thus having a minimal impact on PPR control and eradication efforts. These findings may support the previous idea that cattle serve as dead-end hosts in the epidemiology of PPR, contributing little to the virus's transmission to small ruminants. The identification of PPRV RNA in a naturally infected sentinel calf, despite unsuccessful virus isolation attempts, indicates the need for further comparative studies on both atypical hosts, such as cattle, and typical hosts, like goats. Such research would provide valuable insights into virus tissue tropism, shedding patterns, and pathogenesis, ultimately enhancing our understanding of the role cattle may play in PPRV persistence.

The study on the comparative pathology of PPRV in primary (goats) and atypical (cattle) hosts offers valuable insights into disease progression, virus tissue tropism, and shedding patterns across different sites over time. Evidence of subclinical infections and live virus shedding in cattle, in contrast to the typical PPR symptoms seen in goats, underscores the complex interaction between PPRV and its hosts. Under certain conditions, cattle may play a more active

role in PPRV epidemiology than previously acknowledged. These findings have important implications for developing more targeted control strategies and diagnostic tools for PPR, particularly in endemic areas, and are critical for enhancing future PPR surveillance and control efforts. However, the use of a local single lineage (L-IV) in this study limits the ability to generalize the results to other strains and regions. Therefore, further research should explore additional virus strains and their virulence characteristics to expand the understanding of PPRV.

The comparative study of Ethiopian PPRV strains demonstrates similar virulence profiles across lineages (L-III and L-IV) in local goats, suggesting that lineage classification alone may not be a reliable predictor of clinical outcomes. This insight is crucial for shaping regional disease control strategies. The observed reduction in virulence in one lineage-IV strain emphasizes the intricate interplay between viral genetics, laboratory conditions, and host factors in determining disease outcomes. These findings have important implications for PPRV control efforts in Ethiopia, suggesting that vaccines derived from either lineage could offer cross-protection against locally circulating strains.

In conclusion, the study underscores the need to examine all factors contributing to PPR virus persistence and the recurring outbreaks of PPR to develop effective control and eradication programs. Additionally, the research provides valuable data on recently circulating PPRV strains and their virulence profiles, which will be essential for improving future PPR surveillance and control efforts.

Based on the above conclusions, the following recommendations are suggested:

- Continued monitoring of PPRV circulation among primary and potential non-target host species is crucial and should be conducted alongside mass vaccination campaigns for small ruminants.
- Research should prioritize investigating the pathogenesis of different PPRV lineages in cattle, exploring variations in disease progression and assessing their potential epidemiological impact, to improve our understanding of cattle's role in PPRV epidemiology, especially in regions where the disease is endemic.

- Future research should include a variety of goat breeds from both highland and lowland regions to assess breed-specific susceptibility, using larger sample sizes and extended observation periods beyond 12 dpi.
- Additionally, a comprehensive genomic and phenotypic characterization of viral strains is crucial for a deeper understanding of the molecular basis of virulence variations. This expanded research would offer stronger evidence to inform the development of targeted control measures for this economically significant disease affecting Ethiopia's small ruminant population.
- Moreover, the national priority and attention given to PPRV control should be sustained to adequately address key and important issues in the disease management/epidemiology

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7. ANNEXES

APPENDIX TEXTS

Text 1.1: The Ic-ELISA procedure for PPRV detection involves the following steps:

1. **Coating**: Wells are pre-coated with an anti-PPRV-N antibody.
2. **Sample Addition**: Test samples and controls are introduced into the wells. If PPRV is present, it binds to the antibody, forming an antibody–antigen complex.
3. **Conjugate Binding**: After washing to remove unbound material, an anti-PPRV-N monoclonal antibody (MAb) conjugated to horseradish peroxidase (HRP) is added, forming an antibody–antigen–MAb–HRP complex.
4. **Substrate Reaction**: Following another wash to eliminate excess conjugate, the tetramethyl benzidine (TMB) substrate is added. If PPRV is present, a blue color develops, which turns yellow upon addition of the stop solution. In the absence of PPRV, no color change occurs.
5. **Reading Results**: The color intensity is measured at 450 nm, providing results within two hours.

This method is highly specific and sensitive, making it an efficient tool for PPRV detection.

Text 1.2: PPRV RNA extraction procedures

1. Prepare lysis buffer using AVL buffer and carrier RNA reagents in the following amount/proportions AVL in 0.56 mL required for one sample and 5.6 micro liter of carrier RNA for one sample, mix thoroughly the two prepared solutions,
2. Add 560 micro liter of the prepared buffer AVL carrier RNA into 1.5 micro centrifuge tube.
3. Add 140 micro liter of sample to the buffer AVL carrier RNA in the micro centrifuge. Mix by pulse-vortexing.
4. Incubate at room temperature(15-25^{o}C) for 10 minutes,

5. Add 560 micro liter of ethanol (96-100%) to the sample, and mix by pulse- vortexing for 15 seconds. After mixing, briefly centrifuge the tube to remove drops from the inside lid.
6. Carefully apply 630 micro liter of the solution from step 5 to the QIAamp mini spin column (in a 2ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 xg (8,000 rpm) for 1 min. Place the QIAamp mini spin column in to a clean 2 ml collection tube, and discard the tube containing the filtrate.
7. Carefully open the QIAamp mini spin column and repeat step 5.
8. Carefully open the QIAamp mini spin column and add 500ul of buffer AW1 close the cap and centrifuge 6000 xg (8000 rpm) for 1 min. Place the QIAamp mini spin column in to a clean 2ml collection tube (provided) and discard the tube containing the filtrate.
9. Carefully open the QIAamp mini spin column and add 500ul of buffer AW2. Close the cap and centrifuge at full speed at 20000 xg (14000 rpm) for 3 min.
10. Recommended: Place the QIAamp mini spin column in to a new 2ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 minute.
11. Place the QIAamp mini spin column in to a clean 1.5 ml micro centrifuge tube (not provided). Discard the old collection tube containing the filtrate. Carefully open the QIAamp mini spin column, and add 60ul of buffer AVE equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 6000 xg (8000 rpm) for 1 min.

Viral RNA is stable for up to 1 year when stored at -20^{o}C or -70^{o}C.

Text 1.3: Test procedure for c-ELISA test for PPR

Allow all the reagents to come to room temperature before use;

Homogenize all reagents by inversion or Vortex.

1. Add 25 µl dilution buffer 13 to each well;
2. Add 25 µl positive and negative controls to the specified wells;
3. Add 25 µl sample to be tested to the remaining wells.
4. Incubate for 45 min +/- 4 min at 37^{o}C +/-3^{o}C

5. Wash each well 3 times with approximately 300 µl of the Wash Solution. Avoid drying of the wells between washings.

Prepare the conjugate using Dilution buffer 4;

6. Add 100 µl of the prepared Conjugate to each well;

7. Incubate 30 min +/- 3 min at room temperature (21^oC +/-5^oC)

8. Wash each well 3 times similar with step 5;

9. Add 100 µl of the Substrate Solution to each well;

10. Incubate 15 min +/- at room temperature in the dark;

11. Add 100µl of the Stop Solution to each well in order to stop the reaction.

Read and record the O.D at 450 nm.

Validation

The test is validated if: The mean value of the Negative control OD (OD_{NC}) is greater than 0.7

$OD_{NC} > 0.7$; The mean value of the Positive Control (OD_{PC}) is less than 30% of the OD_{NC}.

$OD_{PC}/OD_{NC}<0.3$

Interpretation

For each sample calculate the competition percentage (S/N %);

$S/N\ \% = OD_{sample}/OD_{NC} \times 100$

Samples presenting a S/N %: Less than or equal to 50% are considered positive. Greater than 50% and less than or equal to 60% are considered doubtful. Greater than 60% are considered negative.

- Result	- Status
- S/N % ≤ 50%	- POSITIVE
- 50% < S/N % ≤ 60%	- DOUBTFUL
- S/N > 60%	- NEGATIVE

Supplementary Text 2.1: Trial 1 and 2 Detailed Results

Local PPRV isolate produced expected clinical disease in sheep and goats and transmitted from sheep to sheep and goat to goat.

Trials 1 and 2 (**Figures S 2.4 - S 2.5**) established that a single 1 mL intranasal dose of the PPRV/Ethiopia/Habru/2014 isolate produced typical clinical signs in local breeds of sheep and goats including fever, conjunctivitis, severe ocular and nasal discharge, diarrhea, altered respiration and coughing, changes in behavior (increasingly inactive, inappetence), and death from infection (trial 2). In both trials, rectal temperature in sheep was higher than that of goats throughout the trial. A modified clinical score (no rectal temperature) removed the difference in the rise and peak of clinical score between sheep and goats (**Figure S 2.6**), but sheep still had a significantly different and slower return to baseline clinical score than goats (evidenced by non-overlapping confidence intervals), driven by Trial 2 sheep observations. qRT-PCR peak Ct values for swabs generally occurred after peak rectal temperature and before or coinciding with peak clinical score (**Figure S 2.4**), except for viremia in whole blood which peaked at approximately the same time as peak rectal temperature for inoculated and sentinel sheep but appeared delayed for inoculated goats in both trials (Figures S 2.4-S2.5). This delay is likely an artifact of the LOESS smooth, and a closer inspection of combined Trial 1 and 2 whole blood data and individual trajectories revealed a biphasic pattern in both sheep and goats (Figure S2.7). As the pattern of viremia in whole blood was different to that seen in swabs, and as the important measure for this study was the excretion of PPR virus (as shown in swabs), whole blood was not assayed for PPRV RNA after Trial 2. In trial 1, one goat was euthanized due to symptom severity and one control animal died at the start of the trial due to other causes.

The isolate transmitted readily from sheep to sheep and from goat to goat (Trial 2). The co-housed animals all developed pyrexia and clear clinical signs with peak values for rectal temperature and clinical score at 10-12 and 7-11 days, respectively, after the corresponding peak for the inoculated animals. Similarly, seroconversion was detected by 7 dpi for inoculated animals and by 17 dpi for sentinel animals (**Figure S 2.5**). The lag observed for measures of virus excretion (qRT-PCR) was shorter (5-9 days for sheep, 1-6 days for goats; NB: LOESS smooth did not capture first goat peak in whole blood well so modified range for all goat measures is likely 4-7 days). PPRV was isolated in cell culture from swabs collected on 4, 7, 10 dpi from inoculated animals in trials 1 or 2 (**Figure S 2.4** and **S 2.5**, red crosses) and on 10, 14, and 17 dpi from co-housed animals (trial 2, **Figure S 2.5**, red circles). All the co-housed animals were infected with PPRV and developed the same level of disease seen in the inoculated animals. When comparing antigen ELISA and qRT-PCR results from nasal swabs taken from both inoculated and co-housed sheep and goats, findings were comparable in trajectory, dpi of peak value, inter-peak interval in days, and uncertainty (**Figure S 2.8**).

Challenge experiment and controls confirm expected disease progression and facility biosecurity

Seropositive and seronegative goats remaining from trial 3 were challenged with PPRV 13 days after the end of trial 3. The 3 PPRV-seropositive goats were placed in the same barn and were all

protected from disease upon challenge. The 7 seronegative goats were not moved and developed clinical signs of PPR and seroconverted upon challenge (**Figure S 2.9**). No virus isolation data were collected during the challenge experiment and no animals died.

Positive (inoculated) and negative (PPRV seronegative) control animals (see Methods) from each trial were run at the same time in a randomly assigned barn. The results from these control animals in trials 1-2 (**Figures S 2.4 – S 2.5**) and trials 3-5 (**Figures S 2.10 - 2.12**) were as expected with the positive controls developing PPR (clinical signs, seroconversion, RNA shedding) and negative controls showing no detectable clinical signs of PPR or seroconversion.

Supplemental Text 2.2: Supplemental Methods

Virus

The PPRV lineage IV (LIV) isolate was stored as a pooled sample of nasal and ocular swabs and gum debris according to Alemu et al .(2019).

Animals and Study Design

Markets animals were purchased from were in Western and Northern Showa and included Muger (Inchiny), Ginchi-Dendi, Ejere, and Degem. Sellers reported bringing animals to market from within a 20 km radius. Animals were housed on the AHI campus and baseline serological testing for PPRV was conducted to confirm seronegative status. Water and hay were provided ad libitum and refreshed daily. On each day, animals were fed, the barn cleaned of old feed and waste, the rectal temperatures of all animals was determined, and animals were monitored for clinical signs.

All animals were infected intranasally using a 3mL syringe fitted with a nasal atomizer (MAD-300, Dixie EMS Supply). Nasal, ocular, and rectal swabs were collected in duplicate and sent directly to the molecular and cell culture laboratories for processing. Swabs were collected using dry cotton or synthetic swabs and directly soaked in 1 ml of DMEM with 2% fetal bovine serum (FBS), and 1x (ocular and nasal swab) or 5x (rectal swab) antibiotic and antimycotic (Corning: 10,000 units/ml penicillin, 10mg/ml streptomycin sulfate, 25 μg/ml amphotericin B combination, VWR 45000-616).

Negative controls were used in each trial to have comparative rectal temperature, clinical signs, and serology data and assess biosecurity practices to ensure PPRV was not spreading between barns. Positive controls were used to check for expected transmission between small ruminants or expected clinical signs from isolate passage used. To reduce the number of animals used, the number of controls was reduced over the course of the five trials. For Trials 1-2 there were 3 each of sheep and goat negative controls. For Trial 3 there were positive controls (4 inoculated, 4 sentinel goats), and 3 each of cattle and goat negative controls. For Trial 4 there were positive controls (2 inoculated, 2 sentinel goats), and 2 goat negative controls. For Trial 5 there were 2 inoculated goat positive controls and 2 goat negative controls.

Serological and Molecular Analysis

Serological analysis was conducted using the ID Screen PPR Competition kit (IDvet PPRC-4P and PPRC-10P, France).

For molecular analysis, RNA was extracted using the QIAamp Viral RNA Mini kit (Qiagen 52904 and 52906), and real time quantitative RT-PCR conducted with custom Taqman QSY probe (Thermofisher 4482777, FAM-5-CACCGGAYACKGCAGCTGACTCAGAA- 3-QSY) and Express One Step kit (Thermofisher 1178101K) on an Applied Biosystems 7500 FAST instrument. The forward and reverse primers of the PPRV N gene were 5-AGAGTTCAATATGTTRTTAGCCTCCAT-3 and 5-TTCCCCARTCACTCTYCTTTGT-3, respectively. To observe molecular dynamics during the trials, swabs were tested by sandwich ELISA (ID Screen PPR Antigen Capture, IDvet PPRAG-2P, France). Antigen testing with this kit could be run more rapidly after sampling than qRT-PCR testing, which was typically conducted after a trial was complete. Antigen results from Trial 2 (Fig S2.8), the post Trial 3 challenge (**Fig S 2.9**), all cattle-to-goat transmission trial barns (**Fig S 2.13**), and positive control barns in Trials 3-5 (**Figs S 2.10- 2.12**) were visualized.

Virus Isolation

VDS cells were grown under selection with Zeocin (at 100 µg/ml every third passage; Alfa Aesar Thermofisher Scientific: 100 mg/ml in HEPES, VWR AAJ67140-XF) in DMEM growth media (Corning: VWR 45000-316) containing 2% fetal bovine serum (Avantor Seradigm Select

Grade FBS, USDA approved origin, heat-inactivated, VWR 89510-188), supplemented with L-glutamine (Gibco: Glutamax 100x, Thermofisher 35050) and 1:100 antibiotic and antimycotic preparation (Corning: 10,000 units/ml penicillin, 10mg/ml streptomycin sulfate, 25 μg/ml amphotericin B combination, VWR 45000-616).

Supplemental Text 2.3: Experimental Settings

Facility

The experimental facility has six experimental barns inside the facility, three on each side of a central hallway (**Figure S 2.3**). Each barn had an enclosed, small courtyard (approximately 4 meters by 3 meters) with no roof and a closed, roofed barn (approximately 8 meters long, 3 meters wide) with skylight and wire mesh ventilation that allowed air to enter the front or back, but not between barns. During acclimatization the animals were allowed to roam in their assigned barn courtyard (no roof) and inside their assigned roofed barn; however, upon the start of the trial, animals were solely kept inside the roofed barns. The maximum number of animals in a barn was 8 (4 inoculated, 4 co-housed PPRV seronegative animals). Water and feed were provided ad libitum and refreshed daily and the barn was cleaned of debris and fecal material. Specifically, a half bail of hay (6kg) and 2kg concentrate per calf per day and 3kg hay and 1 kg concentrate per sheep/goat per day. From the second trial onward, the barn-length feeding trough had metal bars to discourage sheep and goats from jumping into feeding trough. In cold weather, grass was added for bedding and drafts around the door were blocked with additional boards.

Biosecurity

All personnel entering the facility, including animal attendants, were required to shower to enter and exit the facility (**Figure S2.3A**). All personnel wore boots, scrubs, a full body Flexothane suit (Sioen Montreal coverall 4964A2FC1), and N95 masks (masks starting in Trial 2) for use solely on this project while in the facility. A footbath was present to be used upon entry and exit just after the showers and at the entrance to the central hallway. For each barn (**Figure S2.3B**) there were two footbaths, one just inside the door of the courtyard from the central hallway and one just in front of the door to the roofed barn and all personnel stepped in both baths on the way

in or out of the barns. Each animal attendant was assigned to a specific barn and did not enter other barns when a trial was actively running, whereas the sampling team of veterinarians and researchers (typically 3 or less) moved between barns for sampling, which started in the morning and ended by early afternoon. In total, to enter or exit a particular barn, personnel stepped in 4 footbaths filled with a dilution of FAM-30 disinfectant (Evans Vanodine, R067 KEV). When exiting any barn, while stepping in the first footbath, the sampling team and barn-specific attendants assisted each other to spray and sponge down their Flexothane suits with FAM-30 dilutions available in spray bottles. They waited for the suits to dry before entering the courtyard of the next barn. Clinical signs data was recorded by photo of a whiteboard in each barn and of each animal (photos included: eartag, head profile, frontal profile, conjunctival tissue, oral view, rectal view). A Go-Pro Hero 7 camera in waterproof housing case, used to record photos and video of clinical data and signs in the barn, was also sprayed with FAM-30 disinfectant on the way out of each barn, as were any items leaving the barn with the sampling team. Each barn had its own supply of latex gloves, biohazard trash bags, trash container, whiteboard makers and erasers, sample labelling markers, and broom that stayed within the barns. New gloves were put on by the personnel once they arrived in each barn and these gloves were discarded before leaving a barn. Prepared racks with labeled blood and serum vials and cryovials for collecting molecular swabs where only brought into the assigned barns, sprayed with FAM-30 upon exit, and stored in a cooler with icepacks while sampling was completed. They were then transferred immediately to AHI sample reception and appropriate laboratories. At the end of a trial, barns were cleaned of feed and waste and were sprayed down with FAM-30 disinfectant and left to dry before moving on to the next trial. Each barn had its own drain in the floor to take liquid waste to a tank on the side.

Supplemental Text 2.4: Power analysis details to determine detectable probability of cattle-to-goat transmission

Transmission is a binary event that can be modeled by binomial distribution Bin (n, p) where the distribution is the number of successes (transmissions) in n number of independent trials with a probability p of success (transmission) for each trial. In this trial there were 32 cattle goat pairs (trials). We simulated 1000 trial outcome observations, summing the outcomes and divided by 1000 to obtain an average, simulated a range of probabilities of cattle-to-goat transmission (we

used 0.0001 to 0.1 or 0.01-10%), and plotted these values. We drew a horizontal line on the y axis at 0.8 – indicating the cutoff where there was an 80% chance of detecting cattle-to-goat transmission, if it occurred. The vertical line intersects the simulated data at a probably of cattle-to-goat transmission of 5%, which is the lowest probability of transmission this study is powered to detect.

Figure S 2.1 can be recreated by running the following R code. Comments provided for clarity:

creates an empty vector of length 100 filled with NA. this vector will hold the y axis values, the probability of detecting transmission.

q <- rep(NA,100);

creates a vector of length 100 filled with a sequence of numbers to represent low probability values which ranged between 0.0001 and 0.1. this vector will hold the x-axis values, the probability of C->G transmission.

pp <-seq(0.0001,0.1,length = 100);

loops over the 100 indexed spots in the q vector and generates random draws from a binomial distribution (1000 observations of 32 trials with probabilities greater than 0 given in the pp vector), which are summed and divided by 1000 to generate an average value, and the average value is placed into the respective spot in the empty q vector)

for(i in 1:100){q[i] <- sum(rbinom(1000,32,pp[i])>0)/1000}

plots pp and q vector and adds text for x and y axes

plot(pp,q, xlab = "Probability of C -> G Transmission", ylab= "Probability of Detecting")

adds vertical and horizontal lines to the plot

abline(v=0.05), abline(h=0.8)

Supplemental Table 2.1: Concentrations and passage information for isolations used in all experiments.

Trial	Concentration ($TCID_{50}$/ml)	Passage
1	$10^{5.3}$	3rd
2	$10^{5.3}$	3rd
3	$10^{5.6}$	4th
Challenge	$10^{5.6}$	4th
4	$10^{5.5}$	4th
5	$10^{5.5}$	4th

Supplemental Table 2.2: Parameter table

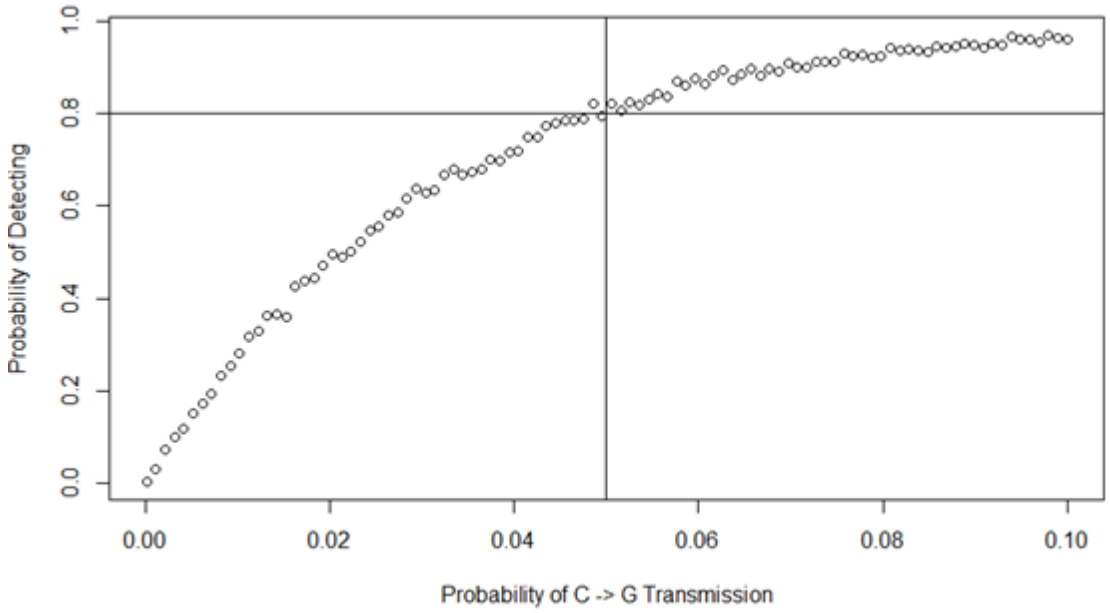
Parameter	Description	Scenario 1	Scenario 2	Scenario 3	Scenario 4
β matrix β_{SS} β_{SC} β_{CS} β_{CC}	Transmission rate matrix	β_{SS} β_{SC} 0-.26* β_{CC}	β_{SS} β_{SC} 0-.26* β_{CC}	β_{SS} β_{SC} 0-.26* β_{CC}	β_{SS} β_{SC} 0-.26* β_{CC}
β_{SS}	Transmission rate	0.2639057	0.2639057	0.2639057	0.2639057
β_{SC}	Transmission rate	0.2639057	0.2639057	0.002985296	0.1386294
β_{CS}	Transmission rate	0.2639057	0.002985296	0.002985296	0.002985296
β_{CC}	Transmission rate	0.2639057	0.002985296 (assumed)	0.002985296 (assumed)	0
μ_1	Small ruminant demographic rate (births and deaths)**	.4655/365	.4655/365	.4655/365	.4655/365
μ_2	Cattle demographic rate (births & deaths)***	0.1/365	0.1/365	0.1/365	0.1/365
γ_1	Small ruminant recovery rate	1/14	1/14	1/14	1/14
γ_2	Cattle recovery rate	1/14	1/14	1/14	1/14
p_1	Small ruminant vaccination proportion	Varies 0-100%	Varies 0-100%	Varies 0-100%	Varies 0-100%
p_2	Cattle vaccination proportion	0	0	0	0
N_1	Small ruminant population (as density)	1	1	1	1
N_2	Cattle population (as density)	1	1	1	1

(C) = Cattle; (S) Small ruminants

* β_{CS} varies between 0 and 0.26 (from 0 to rate of small ruminant-to-small ruminant transmission for an infectious period of 10 days) as seen in Figure 2.3.

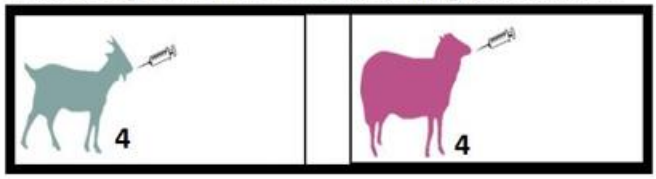
** Yitagesu et al 2022 reports mortality rate per animal year for kids and adults. We took the mean of these rates and converted to a daily rate. The inverse of this rate yields a lifespan of 2.14 years for small ruminants.

*** The inverse of this rate yields a lifespan of 10 years for cattle.

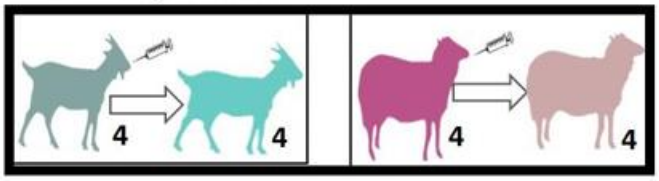


Supplemental Fig. 2.1: Power analysis to determine detectable probability of cattle-to-goat transmission. One thousand simulated draws for a range of cattle (C) to goat (G) transmission probabilities (x-axis) were simulated from a binomial distribution with trial size 32. The horizontal line on the y-axis represents a 80% chance of detecting transmission, if it exists. The vertical line intersects the simulated data and horizontal line at a probably of cattle-to-goat transmission of 5%, which is the lowest probability of cattle-to-goat ransmission this study was powered to detect.

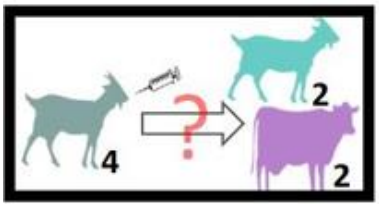
Trial 1: Does PPRV/Ethiopia/Habru/2014 produce clinical signs? Is live virus shedding detectable?



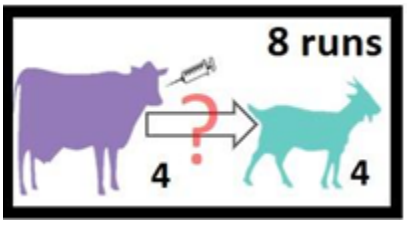
Trial 2: Does PPRV/Ethiopia/Habru/2014 transmit? What are dynamics?

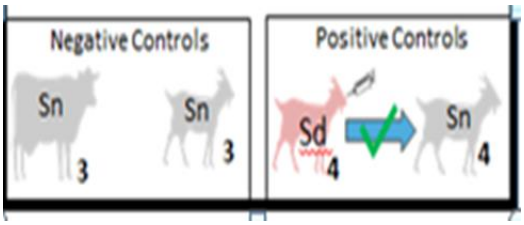


Trial 3: Can cattle can be infected & seroconvert naturally?



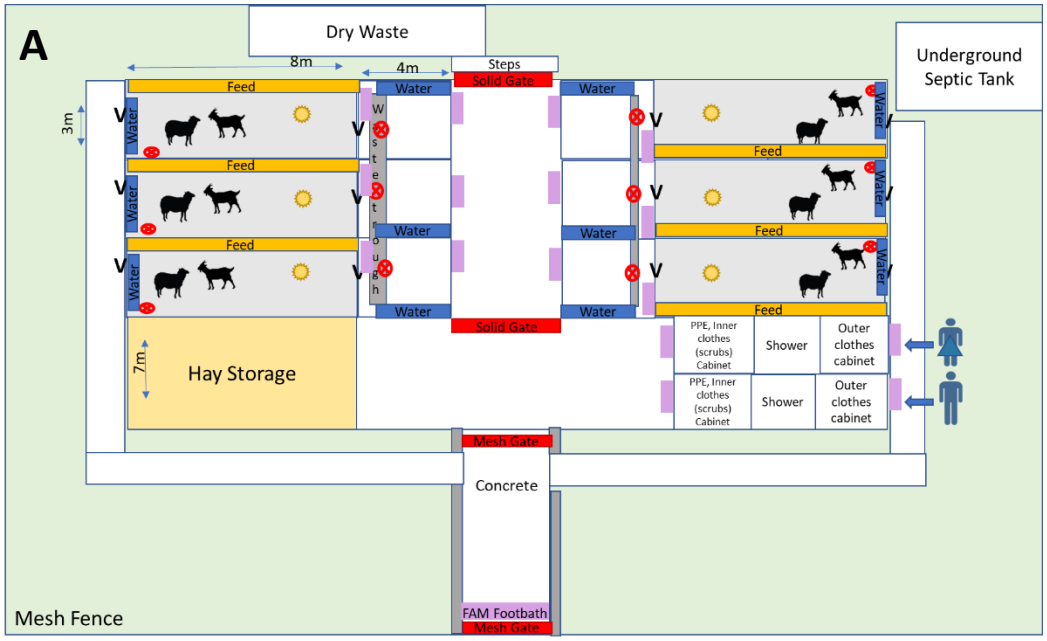
Trials 4 & 5: Do infected cattle transmit PPRV onward?

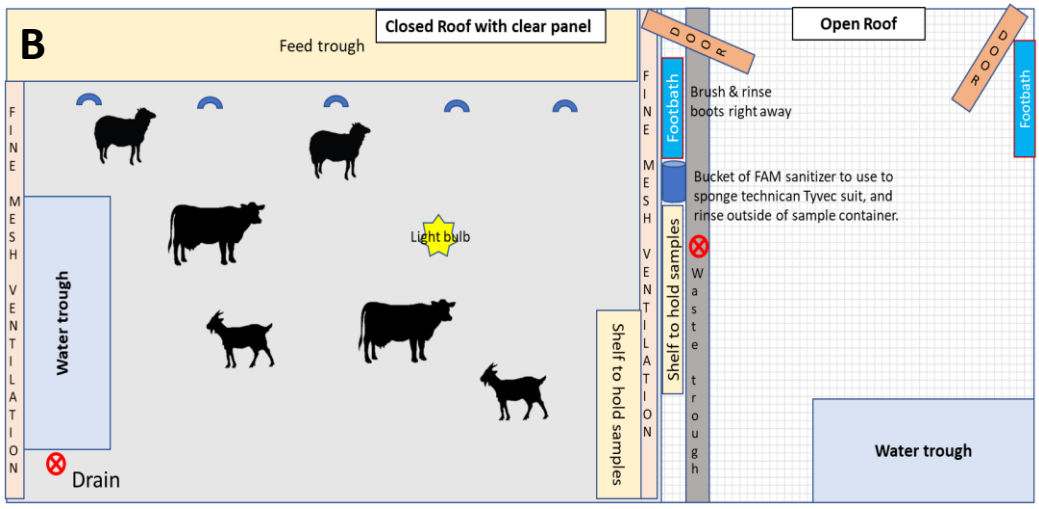




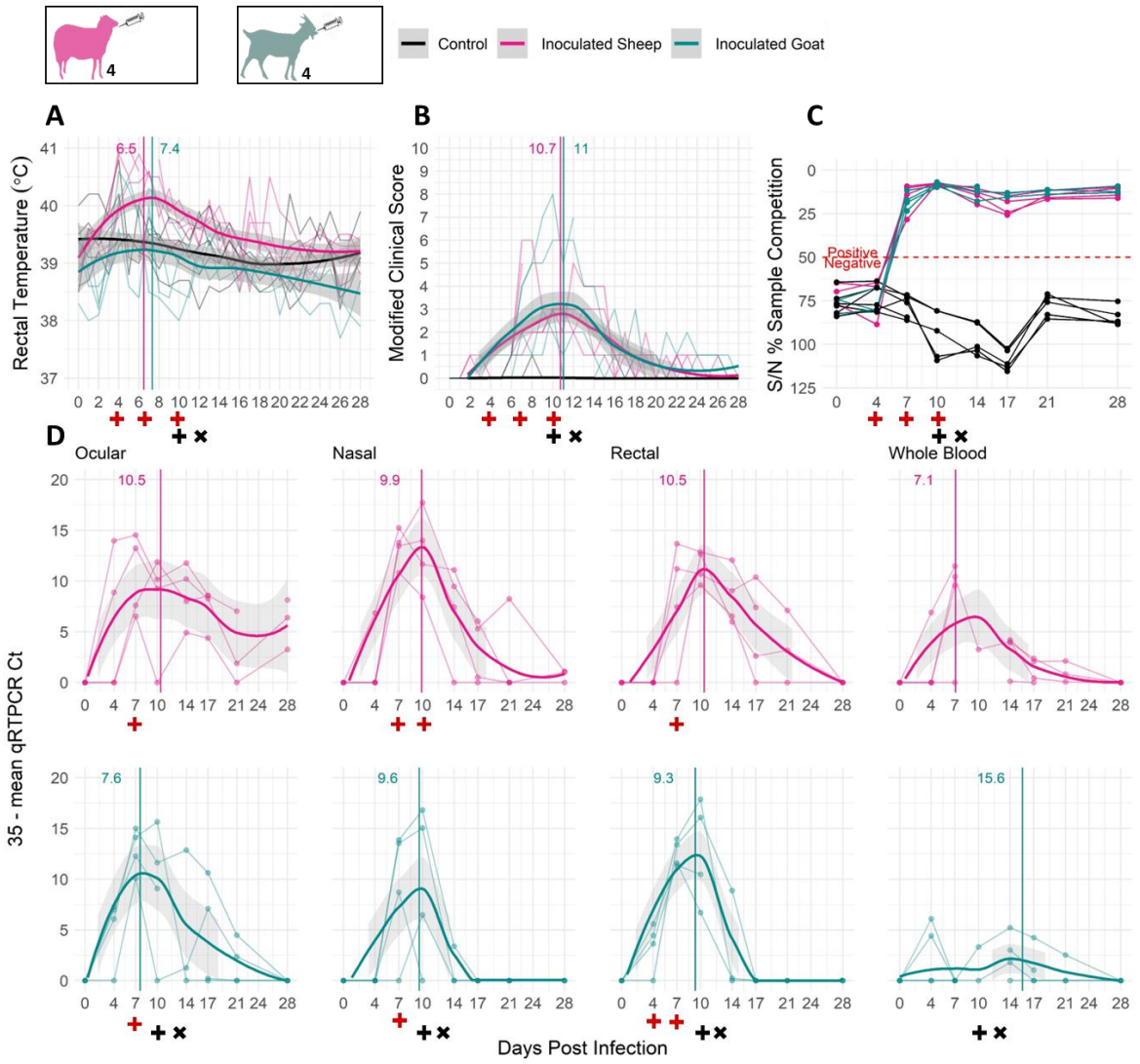
Supplemental Fig. 2.2: Study design of Trials 1-5.

The series of trials tested that PPRV/Ethiopia/Habru/2014 could infect, cause clinical signs and mortality, and transmit among small ruminants before addressing the open question of cattle spillback transmission to goats directly in Trials 3-5. Controls are not shown but were randomly assigned to one of the six barns in the facility and monitored concurrently with experimental barns during each trial. Darker colored animals with syringes are inoculated, lighter colored animals without syringes are co-housed, PPRV-seronegative animals.



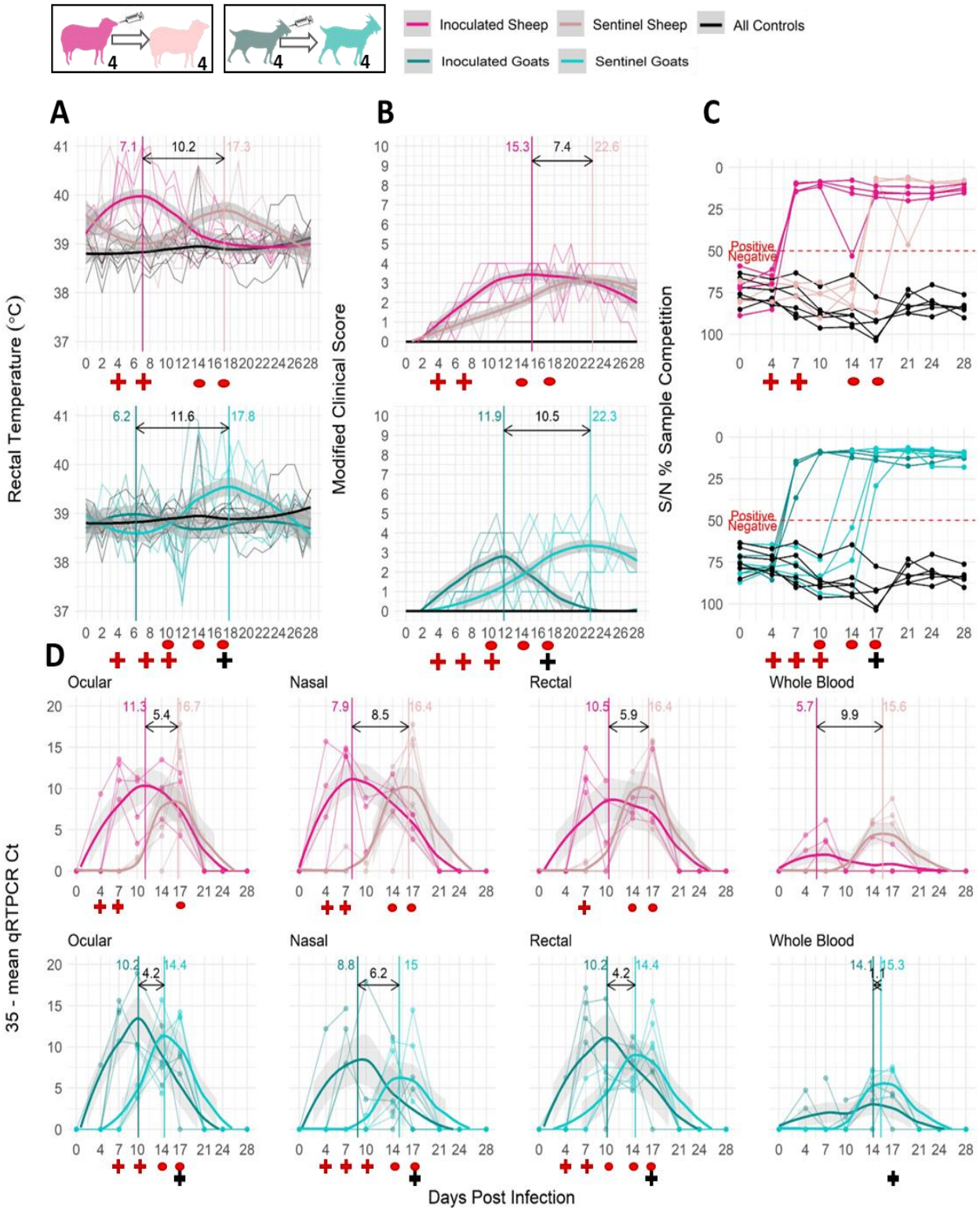


Supplemental Fig. 2.3: Animal facility (A) and within barn (B) biosafety and biosecurity workflow.



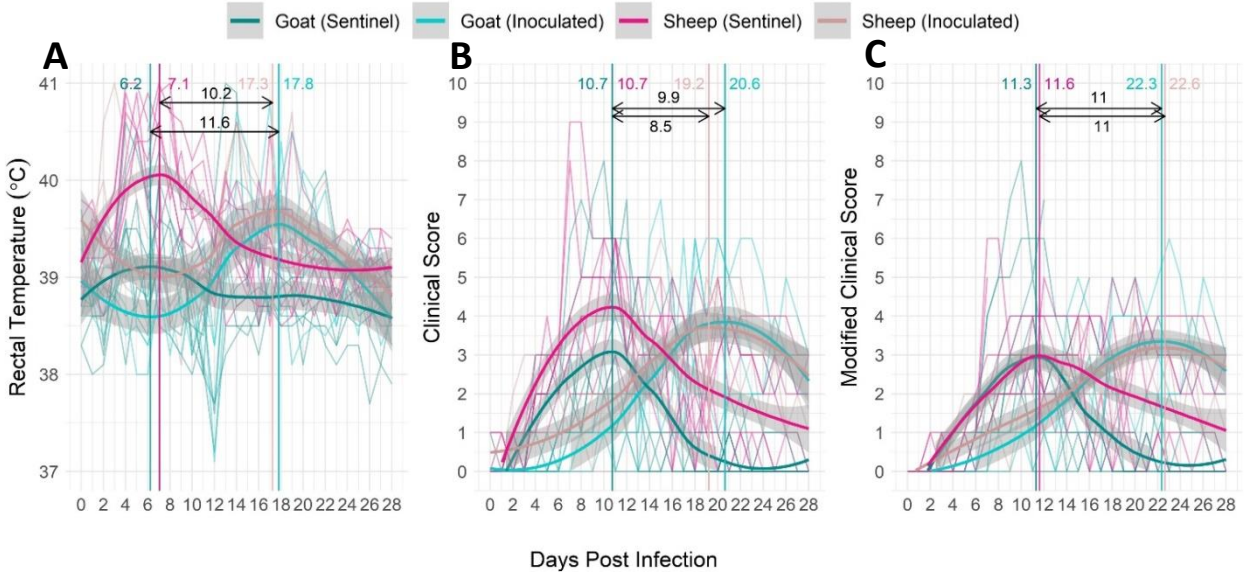
Supplemental Fig. 2.4: Infection dynamics of PPRV among local breeds of Ethiopian sheep and goat breeds.

Trial 1 **A**. daily rectal temperature, **B**. daily clinical score, **C**. serology (competitive ELISA), and **D**. viral RNA from ocular, nasal, and rectal swabs and whole blood (RT-PCR). Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (control, inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). Vertical lines indicate day post infection (dpi) of peak. Sampling days on which PPRV could be isolated (cross if isolated from inoculated animal, circle for sentinel animal) are indicated with red and dpi with deaths are indicated with black (cross for inoculated animal deaths, circle for sentinel deaths, x for euthanized) along the x-axis. Isolation was not conducted on whole blood samples.One inoculated goat was euthanized.

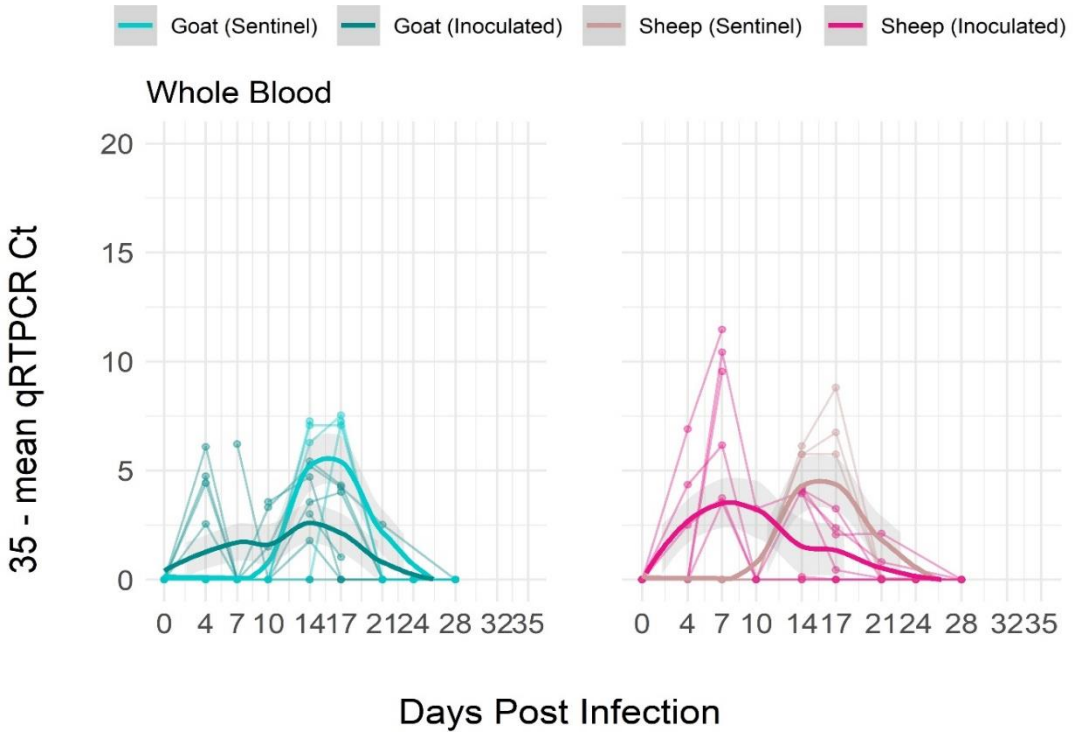

Inoculated Sheep
Sentinel Sheep
All Controls
Inoculated Goats
Sentinel Goats
A
B
C
D
Rectal Temperature (°C)
Modified Clinical Score
S/N % Sample Competition
Positive
Negative
Ocular
Nasal
Rectal
Whole Blood
35 - mean qRTPCR Ct
Days Post Infection

Supplemental Fig. 2.5: Within host and transmission dynamics of PPRV among co-housed local breeds of Ethiopian sheep and goats.

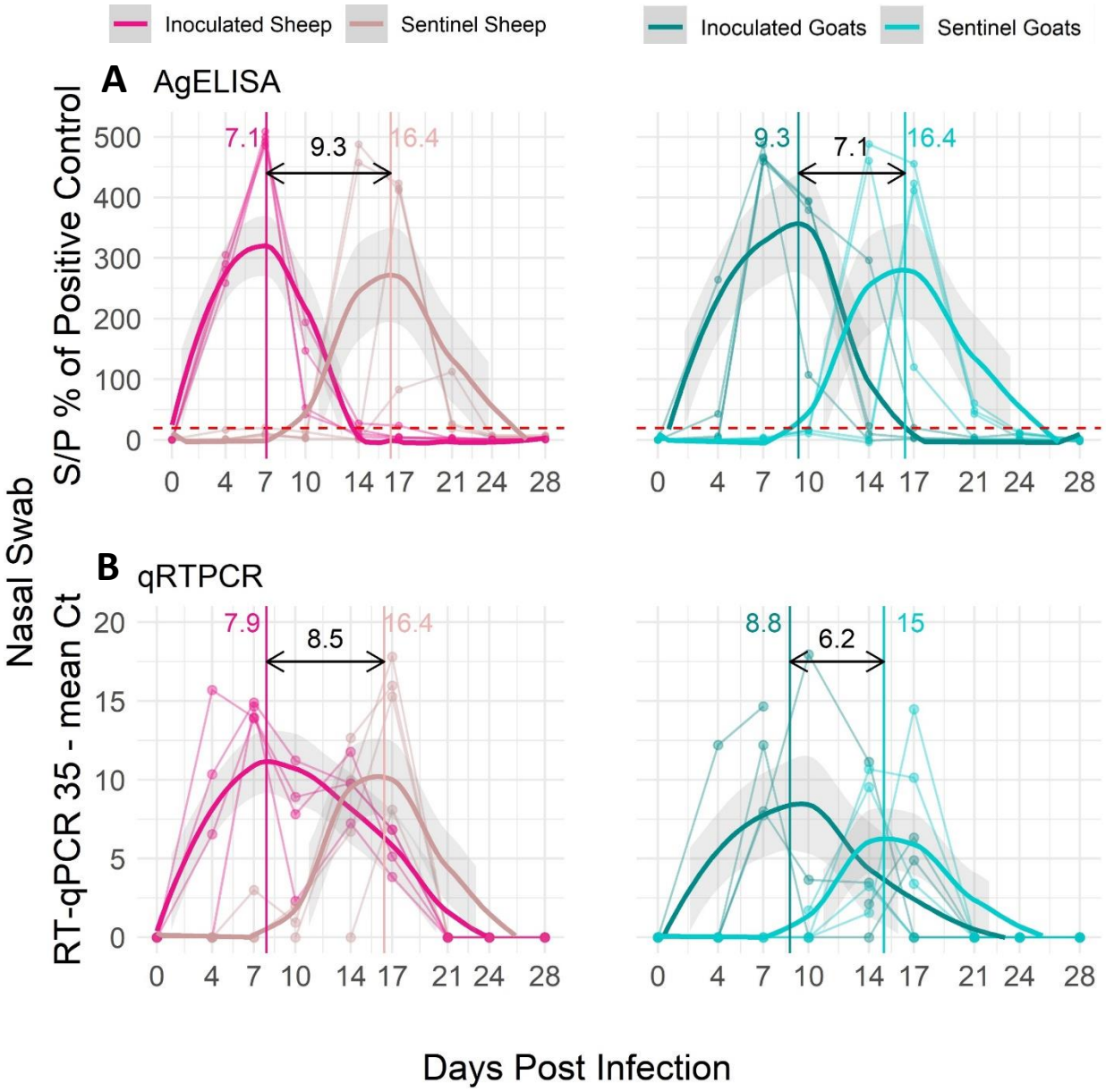
Trial 2 **A**. daily rectal temperature, **B**. daily clinical score, **C**. serology (competitive ELISA), and **D**. viral RNA from ocular, nasal, and rectal swabs and whole blood (RT-PCR). Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (control, inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). Vertical lines indicate day post infection (dpi) of peak value and inter-peak interval (difference in dpi of peak value for each animal group) is indicated in black. Sampling days on which PPRV could be isolated (cross if isolated from inoculated animal, circle for sentinel animal) are indicated with red and dpi with deaths are indicated with black (cross for inoculated animal deaths, circle for sentinel deaths, x for euthanized) along the x-axis. Isolation was not conducted on whole blood samples. One death occurred in an inoculated goat.



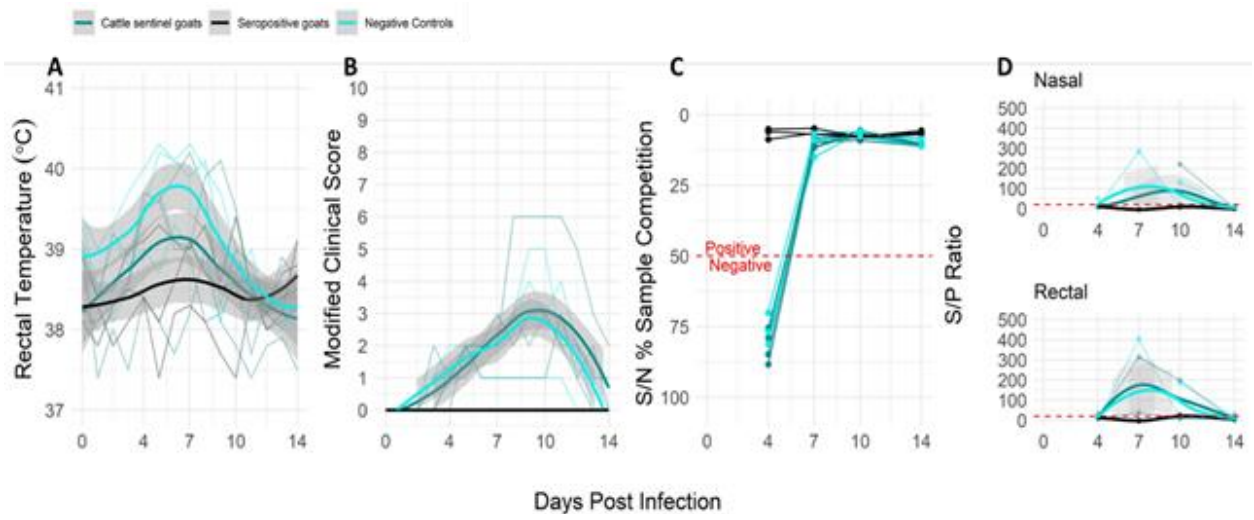
Supplemental Fig. 2.6: Impact of rectal temperature (**A**) on clinical score calculated with (**B**) and without (**C**) rectal temperature shows that the rise and peak of clinical score does not differ for sheep and goats, though the return to baseline clinical score may take longer for sheep (**C**) Combined sheep and goat data from Trials 1 and 2. Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). Vertical lines indicate day post infection (dpi) of peak value and inter-peak interval (difference in dpi of peak value for each animal group) is indicated in black.



Supplemental Figure 2.7: Biphasic pattern in PPRV RNA detected in whole blood samples of sheep and goats in Trials 1 and 2.
Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation).

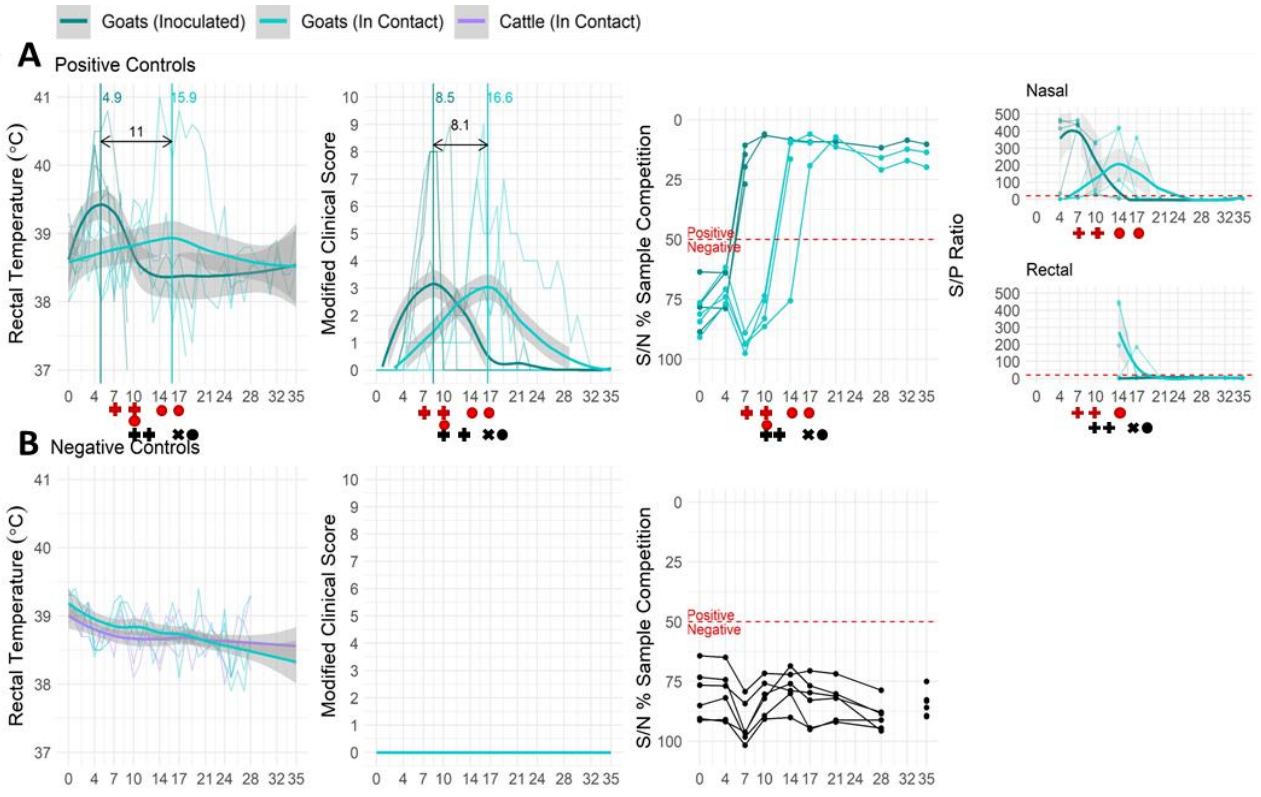


Supplemental Fig. 2.8: Comparison of RNA detection between antigen ELISA (**A**) and RT-PCR (**B**) among nasal swabs from inoculated and sentinel sheep and goats from Trial 2.
Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). Vertical lines indicate day post infection (dpi) of peak value and inter-peak interval (difference in dpi of peak value for each animal group) is indicated in black.

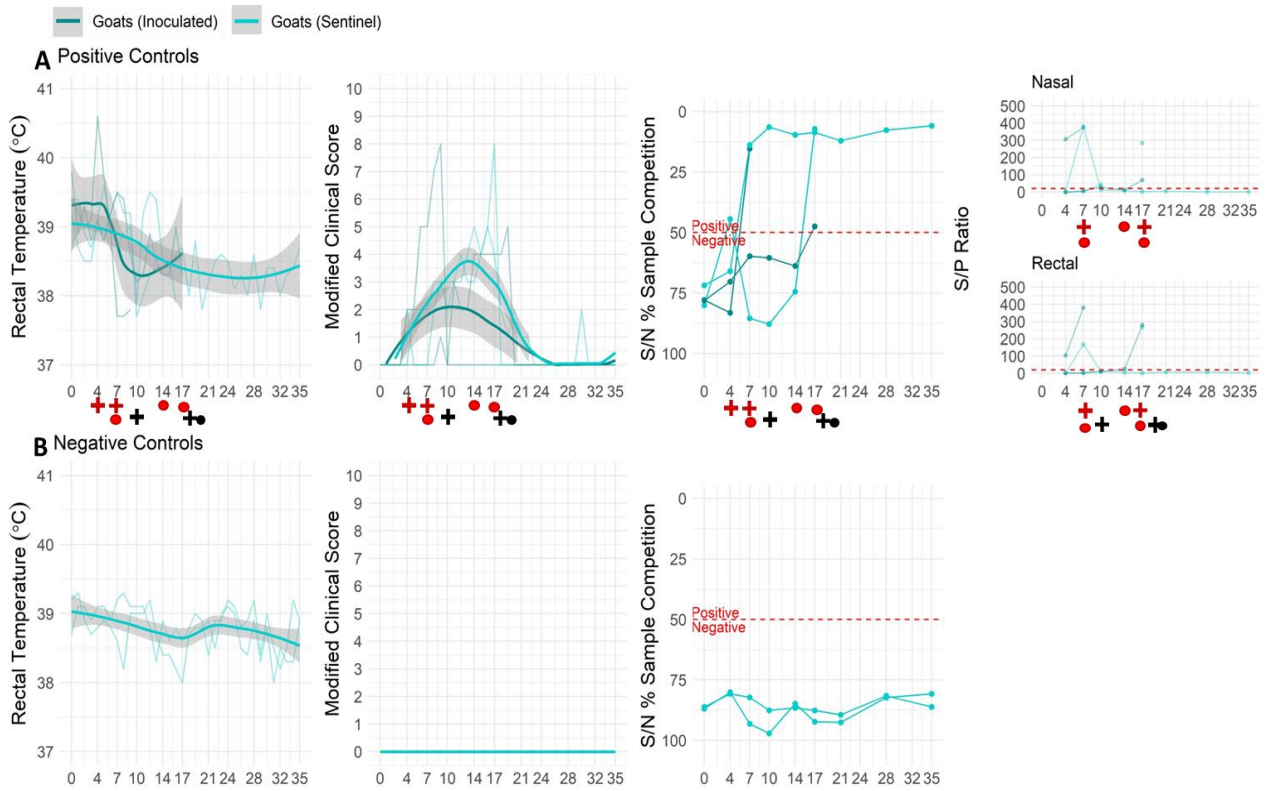


Supplemental Fig. 2.9: Clinical, serological, and molecular findings of Trial 3 goats challenged with PPRV.

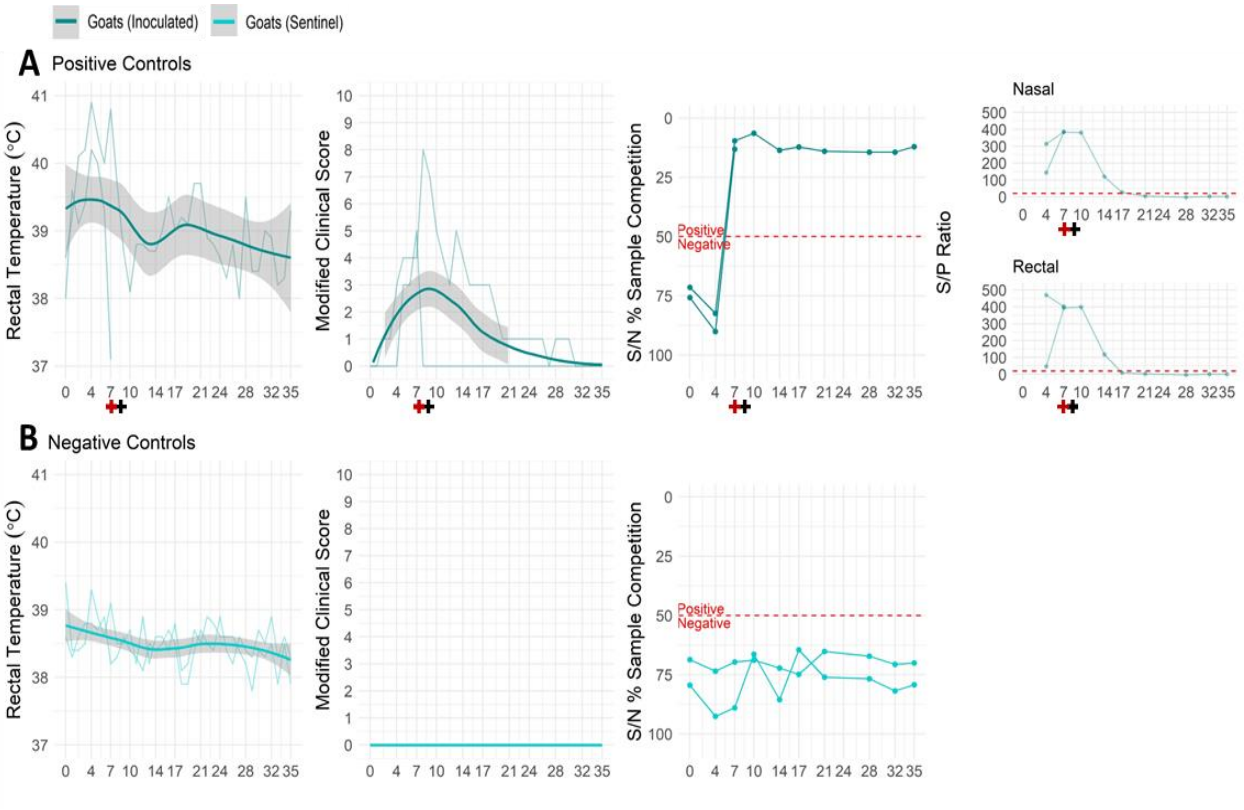
From left to right in each row there is measurements of A. daily rectal temperature, B.daily modified clinical score, C. serology (competitive ELISA), and D. viral RNA from ocular, nasal, and rectal swabs measured on antigen ELISA. No antigen ELISA was run on negative control animals as serology showed they were all negative. Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). No isolation data was collected during the challenge experiment. No animals died.



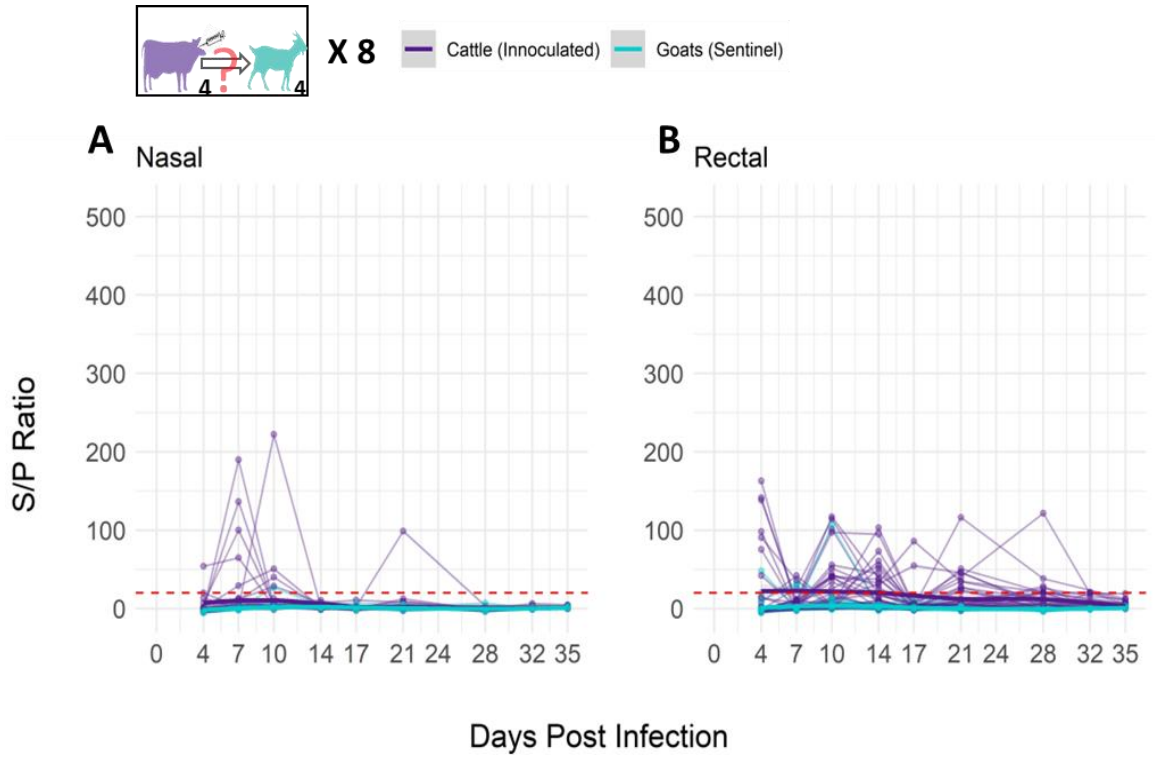
Supplemental Fig. 2.10: Trial 3 clinical, serological, and molecular results from positive control goats (**A**) and clinical and serological data from negative control cattle and goats (**B**). From left to right in each row there is measurements of daily rectal temperature, daily modified clinical score, serology (competitive ELISA), and viral RNA from ocular, nasal, and rectal swabs measured on antigen ELISA. No antigen ELISA was run on negative control animals as serology showed they were all negative. Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). When present, vertical lines indicate day post infection (dpi) of peak value and inter-peak interval (difference in dpi of peak value for each animal group) is indicated in black. Sampling days on which PPRV could be isolated (cross if isolated from inoculated animal, circle for sentinel animal) are indicated with red and dpi with deaths are indicated with black (cross for inoculated animal deaths, circle for sentinel deaths, x for euthanized) along the x-axis. Five positive controls died in total (3 inoculated [2 on dpi 10], 2 sentinel); one of the two sentinels was euthanized (dpi 17).



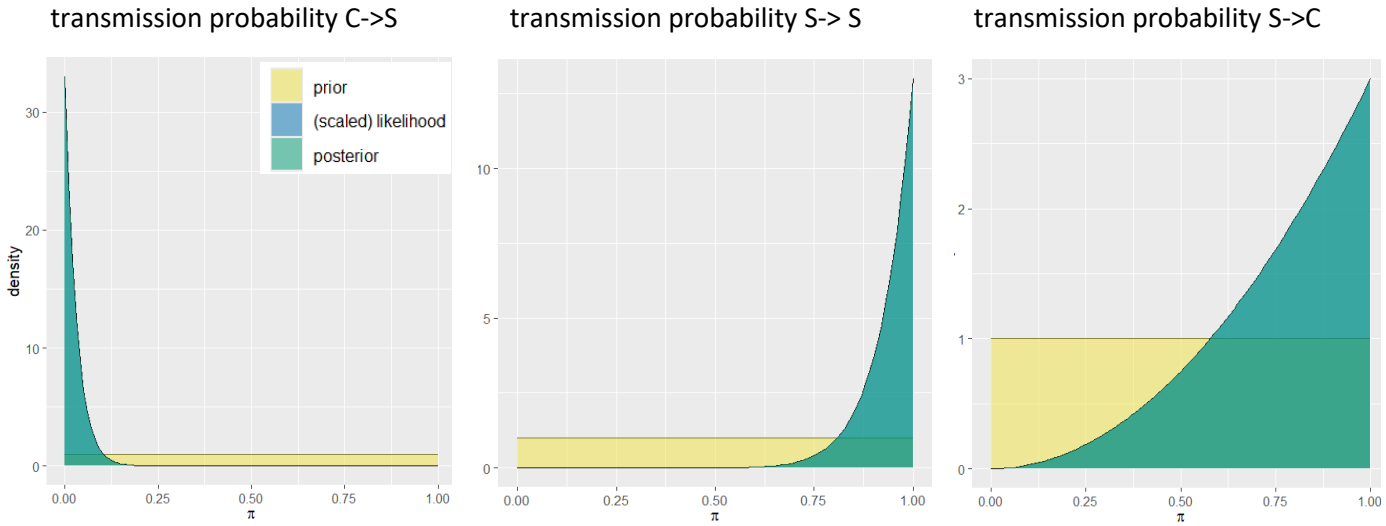
Supplemental Fig. 2.11: Trial 4 clinical, serological, and molecular results from positive control goats (**A**) and clinical and serological data from negative control cattle and goats (**B**).
From left to right in each row there is measurements of daily rectal temperature, daily modified clinical score, serology (competitive ELISA), and viral RNA from ocular, nasal, and rectal swabs measured on antigen ELISA. No antigen ELISA was run on negative control animals as serology showed they were all negative. Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). Sampling days on which PPRV could be isolated (cross if isolated from inoculated animal, circle for sentinel animal) are indicated with red and dpi with deaths are indicated with black (cross for inoculated animal deaths, circle for sentinel deaths, x for euthanized) along the x-axis. Four positive controls died in total (2 inoculated, 2 sentinel [dpi18 and dpi 20]).



Supplemental Fig. 2.12: Trial 5 clinical, serological, and molecular results from positive control goats (**A**) and clinical and serological data from negative control cattle and goats (**B**). From left to right in each row there is measurements of daily rectal temperature, daily modified clinical score, serology (competitive ELISA), and viral RNA from ocular, nasal, and rectal swabs measured on antigen ELISA. No antigen ELISA were run on negative control animals as serology showed they were all negative Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation). When present, vertical lines indicate day post infection (dpi) of peak value and inter-peak interval (difference in dpi of peak value for each animal group) is indicated in black. Sampling days on which PPRV could be isolated (cross if isolated from inoculated animal, circle for sentinel animal) are indicated with red and dpi with deaths are indicated with black (cross for inoculated animal deaths, circle for sentinel deaths, x for euthanized) along the x-axis. One inoculated positive control died.

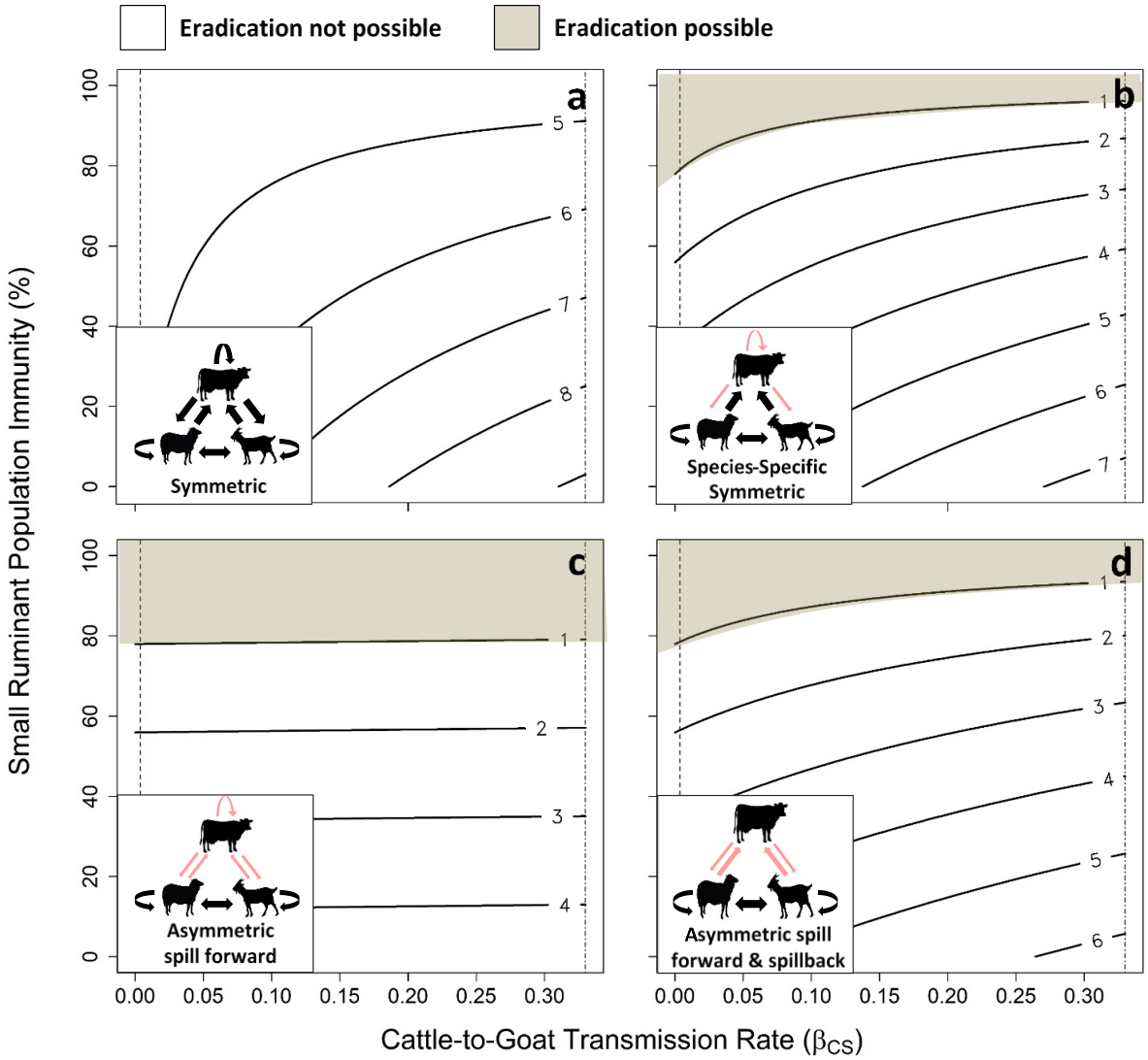


Supplemental Fig. 2.13: Trials 3 to 5 antigen ELISA results from nasal (**A**) and rectal swabs (**B**) from inoculated cattle and sentinel goats.
Thin lines represent individual animals and bold lines represent smooth local regression (LOESS) curves of all animals in the category (inoculated, sentinel). Gray shading indicates 95% confidence bands (t-based approximation).



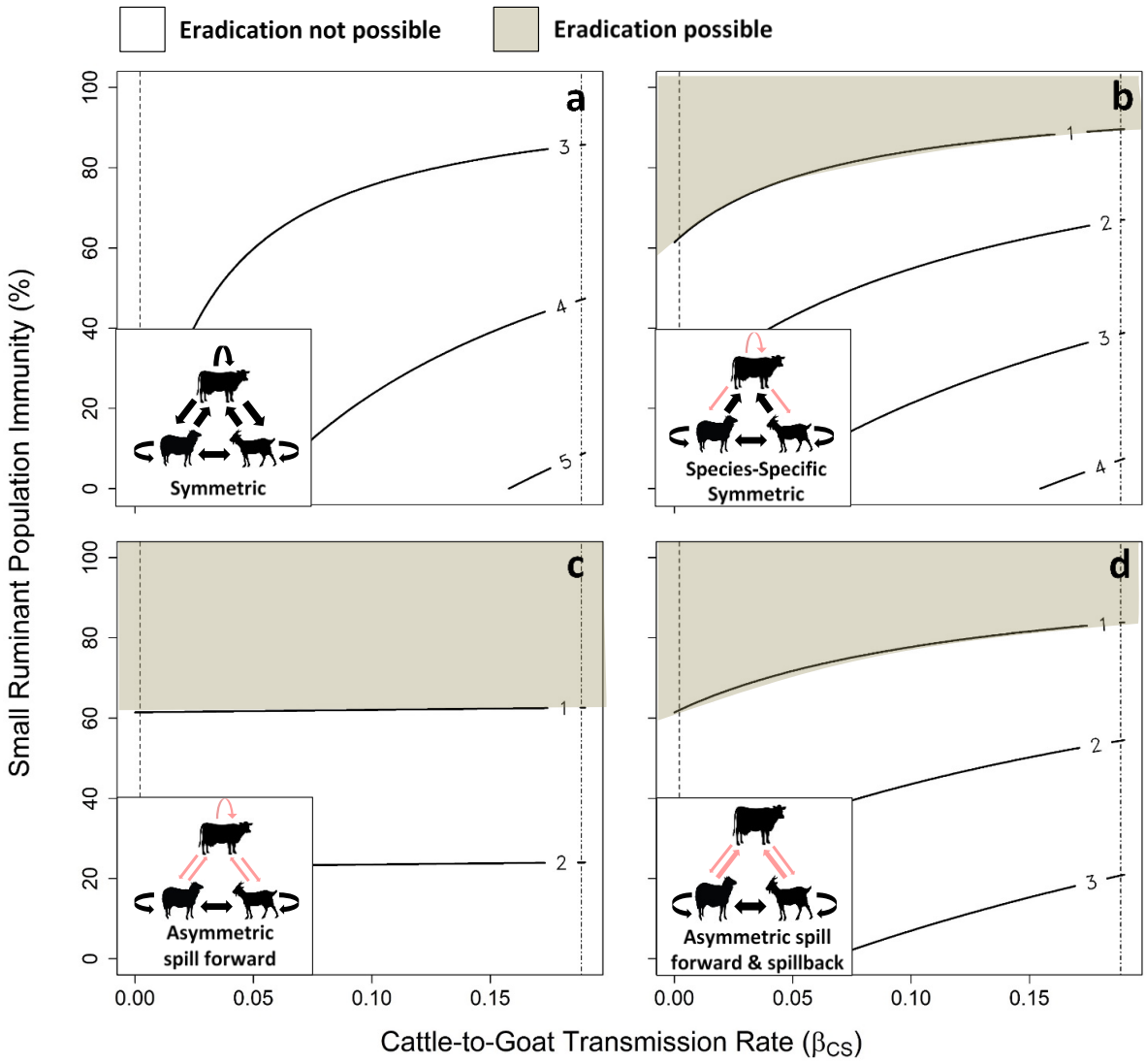
Supplemental Fig. 2.14: Posterior distributions of the transmission probabilities observed in the empirical trials, calculated from a beta binomial Bayesian model using bayesrules R package (see Methods, **Text S 2.5**). Posterior distributions are in green, uniform uninformative priors are in yellow,

and the scaled likelihood is in blue (and completely overlapped by the posterior). Cattle (C), small ruminants (S).



Supplemental Figure 2.15: Increasing cattle-to-small ruminant transmission increases the amount of small ruminant vaccination needed in some but not all transmission scenarios. Eradication is possible in scenarios **B**, **C**, and **D** when R_E <1 (i.e. area above the contour labelled "1" in B, C & D and shaded in gray), but is not possible in scenario **A**. Community R_E values are indicated in the numeric values on labelled contours. For an infectious period of 8 days, the estimated transmission rates for cattle-to-small ruminant and small ruminant-to-small ruminant are shown as dashed (farther left) and dot dashed (farther right) vertical lines, respectively. In scenarios **B** and **D**, increasing levels of small ruminant vaccination is indicated, as the rate of cattle-to-small ruminant transmission increases from the observed cattle-to-goat transmission rate to slightly above the goat-to-goat transmission rate observed in the trials. For each transmission scenario, cattle (C) transmission rate to small ruminants (S) varies from 0 to 0.1 (x-axis, gray arrow). In panels B, C, D changes in transmission scenarios relative to panel A are indicated with red arrows or removal of an arrow in the inset figure. Scenario 1 (**A**) explores a symmetric transmission rate for all species ($\beta_{SS} = \beta_{SC} = \beta_{CC} = 3.3 \times 10^{-1}$; β_{CS} varies 0-0.26). Scenario 2 (**B**) explores a symmetric transmission rate within each species ($\beta_{SS} = \beta_{SC} = = 3.3 \times 10^{-1}$; $\beta_{CC} = 3.7 \times 10^{-3}$; β_{CS} varies 0-0.33).).

Scenario 3 (**C**) explores asymmetric transmission rate between species (β_{SS} = 3.3 x 10^{-1}; β_{SC} = β_{CC} =3.7 x 10^{-3}; β_{CS} varies 0-0.33).). Lastly, scenario 4 (**D**) explores asymmetric transmission rate within and between species (β_{SS} = 3.3 x 10^{-1}; β_{SC} = 1.7 x 10^{-1}; β_{CC} = 0; β_{CS} varies 0-0.33).). See Methods, Text S5, Text S6, Table S2 for more information on parameter values selected and model code.



Supplemental Figure 2.16: Increasing cattle-to-small ruminant transmission increases the amount of small ruminant vaccination needed in some but not all transmission scenarios.

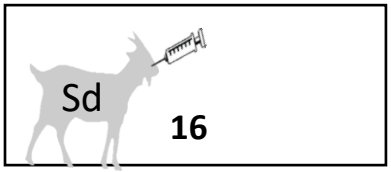
Eradication is possible in scenarios **B**, **C**, and **D** when R_E <1 (i.e. area above the contour labelled "1" in B, C & D and shaded in gray), but is not possible in scenario A. Community R_E values are indicated in the numeric values on labelled contours. For an infectious period of 14 days, the estimated transmission rates for cattle-to-small ruminant and small ruminant-to-small ruminant are shown as dashed (farther left) and dot dashed (farther right) vertical lines, respectively. In scenarios **B** and **D**, increasing levels of small ruminant vaccination is indicated, as the rate of cattle-to-small ruminant transmission increases from the

observed cattle-to-goat transmission rate to slightly above the goat-to-goat transmission rate observed in the trials. For each transmission scenario, cattle (C) transmission rate to small ruminants (S) varies from 0 to 0.1 (x-axis, gray arrow). In panels **B**, **C**, **D** changes in transmission scenarios relative to panel **A** are indicated with red arrows or removal of an arrow in the inset figure. Scenario 1 (**A**) explores a symmetric transmission rate for all species ($\beta_{SS} = \beta_{SC} = \beta_{CC} = 1.9 \times 10^{-1}$; β_{CS} varies 0-0.19). Scenario 2 (**B**) explores a symmetric transmission rate within each species ($\beta_{SS} = \beta_{SC} = 1.9 \times 10^{-1}$; $\beta_{CC} = 2.1 \times 10^{-3}$; β_{CS} varies 0-0.19).). Scenario 3 (**C**) explores asymmetric transmission rate between species ($\beta_{SS} = 1.9 \times 10^{-1}$; $\beta_{SC} = \beta_{CC} = 2.1 \times 10^{-3}$; β_{CS} varies 0-0.19).). Lastly, scenario 4 (**D**) explores asymmetric transmission rate within and between species ($\beta_{SS} = 1.9 \times 10^{-1}$; $\beta_{SC} = 9.9 \times 10^{-2}$; $\beta_{CC} = 0$; β_{CS} varies 0-0.19).). See Methods, Text S2.5, Text S2.6, Table S2.2 for more information on parameter values selected and model code.

Supplemental Text 3.1: Experimental animals veterinary careAll experimental animals were dewormed using broad spectrum anthelmintic drugs (Albendazole 2500mg bolus, one bolus for 300kg body weight for cattle; 300mg bolus, one bolus for 40kg body weight for sheep and goats); long acting oxytetracycline was used as deemed necessary.

Supplemetal Test 3.2: Study/Experimental design

16 experimental goats were used



PPR virus used: 2014 Ethiopian Isolate

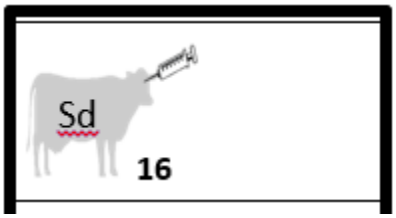
(Goat, Lineage IV)

Passage 4, $TCID_{50} \sim 10^{\wedge 5.3}$

Sampling:

- Daily 0-8 dpi:
    - Clinical signs and rectal temperature
    - Serology
    - Swabs for Ag ELISA and qRT-PCR
    - Isolation in VDS cell culture of qRT-PCR positive swabs or tissues
- Sacrifice:
    - 2 goats/day, 1 per barn, starting 1 dpi
    - 14 tissues collected for qRT-PCR and histopathology (retropharyngeal LN, mediastinal LN, bronchial LN, mesenteric LN, tracheal LN, lung cranial, lung caudal, colon, jejunal/ileal peyers patch, spleen, tongue (apex), tonsil, nasal mucosa, third eyelid)

16 experimental cattle were used



PPR Virus used: 2014 Ethiopian Isolate

(Goat, Lineage IV)

Passage 4, $TCID_{50} \sim 10^{\wedge 5.3}$

Sampling:

- Daily 0-28 dpi:
    - Clinical signs and rectal temperature

- Other:
    - Serology: 0, 2, 4, 6, 8, 10, 17, 24, 28
    - Swabs for AgELISA: 0, 2, 4, 6, 8, 10, 17, 24, 28
    - Swabs for qRT-PCR: 0, 2, 4, 6, 8, 10, 17, 24, 28
    - Isolation in VDS cell culture of qRT-PCR positive swabs or tissues
- Sacrifice:
    - 2 cattle/day, 1 per barn, starting 2 dpi

14 tissues collected for qRT-PCR and histopathology (retropharyngeal LN, mediastinal LN, bronchial LN, mesenteric LN, tracheal LN, lung cranial, lung caudal, colon, ileal &cecal peyers patch, spleen, tongue (apex), tonsil, nasal mucosa, third eyelid)

Supplementary Table 3.1:- PPRV gross and histopathology lesion scoring matrix

a) Gross lesion scoring

Grade	Category	Lesion description
Grade 0	All inspected tissues and organs	No gross lesion typical of PPR
Grade 1	Buccal cavity, nasal cavity and conjunctiva	No lesion in the buccal cavity, nasal mucosa with mucus crust;
	Head LN's (including tonsils)	Swelling and inflammation of the head LNs; Tonsils slightly inflamed;
	Mesentery (both LN's and bowel track)	Swelling and inflammation of mesenteric LNs
	Lungs including associated LNs	Swelling and inflammation of lung and its associated LNs;
	Visceral organs (liver, spleen, kidneys)	Apparently normal
Grade 2	Buccal cavity, nasal cavity and conjunctiva	1-3 ulcerative to necrotic lesions with 1-2mm in diameter on the lower lip, mucus crust and /or congestion in the nasal mucosa; Hyperemic nasal mucosa;
	Head LN's (including tonsils)	Swelling and hemorrhagic inflammation of the head LNs; Tonsils swollen, hemorrhagic and inflamed;

	Mesentery (both LN's and bowel track)	Inflammation of the Peyer's patches; enlarged and /or hemorrhagic mesenteric lymph node;
	Lungs including associated LNs	Bronchopneumonia;
	Visceral organs (liver, spleen, kidney)	Atrophied and /or congested spleen, petechial hemorrhage in liver and kidneys
Grade 3	Buccal cavity, nasal cavity and conjunctiva	Conjunctivitis, stomatitis, extensive necrotic lesions (lower lip and adjacent gum on the checks near commissures and the ventral surface of the tongue);
	Head LN's (including tonsils)	Enlarged and/or edematous head associated lymph nodes and the tonsils;
	Mesentery (both LN's and bowel track)	Enlarged mesenteric lymph nodes; Congestion and/or hemorrhage of the digestive tract, particularly the duodenum, abomasum, ileum, caecum, colon and rectum resulting the characteristic Zebra-striped appearance, and severely affected Payer's patches
	Lungs including associated LNs	Severely congested and/or consolidated firm lung parenchyma; Bronchopneumonia, purulent exudative areas and patchy fibrin depositions on pleural surface of the consolidated lobes;
	Visceral organs (liver, spleen, kidneys)	- Enlarged (congested and edematous) spleen; - Focal degenerative areas in liver; extensive hemorrhage in kidneys

B) Histopathology lesion scoring

Grade	Category	Lesion description
Grade 0	All inspected tissues and organs	No histological alterations typical of PPR
Grade 1	Buccal cavity, nasal cavity and conjunctiva	Small area of mucosal sloughing of the nasal mucosa and tongue epithelia; few depleted lymphocytes in the third eyelid;

	Head LN's (including tonsils)	Few depleted lymphocytes in the tonsil; Few lymphocyte depletion in LNs of the head; edematous LNs of the head and/ or tonsil;
	Mesentery (both LN's and bowel track)	Few lymphocyte depletion in mesentery; edematous mesenteric LNs
	Lungs including associated LNs	Congestion of the lung; Few lymphocyte depletion in lung associated LNs; edematous lung associated LNs;
	Visceral organs (liver, spleen)	Apparently normal histology
Grade 2	Buccal cavity, nasal cavity and conjunctiva	Lymphocyte depletion primarily in the cortical lymphatic nodules; numerous lymphocytes with pyknotic or karyorrhexic nuclei in third eyelid;
	Head LN's (including tonsils)	Prominent lesions in the palatine tonsils, which included necrosis of surface and crypt epithelium with infiltration of neutrophils, formation of syncytial cells and scattered intra-nuclear inclusion bodies; Lymphocyte depletion primarily in the cortical lymphatic nodules; numerous lymphocytes with pyknotic or karyorrhexic nuclei in Tonsil; Numerous multinucleated syncytial cells and apoptotic cells in all section of the head LNs;
	Mesentery (both LN's and bowel track)	Numerous multinucleated syncytial cells and apoptotic cells in all section of the mesenteric LNs; Lymphocyte depletion primarily in the cortical lymphatic nodules; numerous lymphocytes with pyknotic or karyorrhexic nuclei in Peyer's patches and mesentery lymph nodes;
	Lungs including associated LNs	Bronchopneumonia, prominent peribroncheal gland, thickening of the alveolar septa; Numerous multinucleated syncytial cells and apoptotic cells in all section of the lung and its associated LNs,
	Visceral organs (liver, spleen, kidneys)	Lymphocyte depletion primarily in the cortical lymphatic nodules; numerous lymphocytes with pyknotic or karyorrhexic nuclei in, spleen white pulp; kidneys congestion in few portion
Grade	Buccal cavity, nasal cavity and	Multifocal erosions in nasal mucosa, nasal cavity, syncytial

3	conjunctiva	cells in the upper cell layers, necrosis and/or loss of epithelium with replacement by edema fluid and neutrophils;
	Head LN's (including tonsils)	Moderate lymphocytes depletion in the head LNs and tonsil; Pyknotic lymphocytes in the head LNs;
	Mesentery (both LN's and bowel track)	Pyknotic lymphocytes in the mesenteric LNs; Intestine characterized by shortened villi, lymphocyte depletion in the Peyer's patches; hemorrhagic lamina propria with infiltration by mononuclear cells;
	Lungs including associated LNs	Moderate lymphocytes depletion in the lung and associated lymph nodes; Bronchopneumonia or Interstitial pneumonitis;
	Visceral organs (liver, spleen, kidneys)	In the spleen, depleted splenic syncytial cells in the white pulp area, and hyper cellular red pulp, and presence of hemosiderin; hemorrhagic and congested kidneys
Grade 4	Buccal cavity, nasal cavity and conjunctiva	Sloughed nasal mucosa and tongue;
	Head LN's (including tonsils)	Severe lymphocytes depletion in head associated LNs and tonsil;
	Mesentery (both LN's and bowel track)	Intestine manifested by blunted villi, degeneration of surface and crypt epithelial cells, expansion of lamina propria by primarily mononuclear infiltration, hemorrhage and necrosis in the mucosa with scattered syncytial cells and severe depletion of lymphocytes within Peyer's patches;
	Lungs including associated LNs	Severe lymphocytes depletion in the lungs including associated LNs; Bronchopneumonia or Interstitial pneumonia; Large alveolar macrophages with intra-nuclear and intra-cytoplasmic inclusion bodies, numerous neutrophils, fibrin exudates and multi-nucleic giant cells;

	Visceral organs (liver, spleen, kidneys)	Periportal hepatic fibrosis and focal necrosis of hepatocytes; Eosinophilic intranuclear inclusion bodies in hepatocytes, hepatic cells with pyknotic nuclei, presence of inclusion bodies; Severely depleted splenic syncytial cells in the white pulp area, and hyper cellular red pulp, and presence of hemosiderin, hemorrhagic and congested kidneys

Supplementary Table 3.2: A summary of observed clinical signs following PPR virus infection from 4 dpi through 8 dpi (end date of goats experiment) since no clinical signs observed till 3 dpi. The clinical data was recorded and summarized as per (Pope et al., 2013).

dpi	General signs	Fever/Pyrexia	Diarrhea	ODND	CMM	Coughing	MPND	Oral lesion
4	5/10	3/10	0/10	5/10	0/10	0/10	0/10	0/10
5	7/8	4/8	1/8	6/8	5/8	0/8	0/8	0/8
6	6/6	2/6	1/6	5/6	2/6	2/6	1/6	0/6
7	4/4	2/4	0/4	2/4	3/4	0/4	2/4	0/4
8	2/2	2/2	2/2	1/2	2/2	1/2	1/2	1/2

dpi= days post inoculation; General signs:- any or combination of depression, loss of appetite, rough coat; Fever/Pyrexia=rectal temperature≥39.5^{o}C; ODND=ocular and/or serous nasal discharge; CMM=Congested Mucous Membrane; MPND=Muco-Purulent Nasal Discharge; Oral lesions= necrosis and/or sloughing of oral mucosa.

Supplementary Table 3.3: Molecular detection of viral RNA by RT-qPCR test on different swab samples (Ocular, Nasal, Rectal) of experimental goats collected at different dpi and their positivity percentage per swab type. Since 1 and 2 dpi's collected samples generated negative results the below result only included 3 dpi and onwards collected swab samples.

Day Post Infection (DPI)	Swab sample type	No. of animals positive for viral RNA/number of samples tested (P/T)	Positivity percentage and Cycle threshold (Ct) range
3	Ocular (OC)	(0/12)	0%
	Nasal (NS)	(1/12)	8.33% (Ct=26.23)
	Rectal (RC)	(0/12)	0
4	Ocular (OC)	(4/10)	40% (Ct range 23.31-29.91)
	Nasal (NS)	(0/10)	0
	Rectal (RC)	(0/10)	0
5	Ocular (OC)	(3/8)	37.5% (Ct range 21.02-26.68)
	Nasal (NS)	(6/8)	75% (Ct range 21.90-34.50)
	Rectal (RC)	(0/8)	0
6	Ocular (OC)	(3/6)	50% (Ct range 28.33-34.29)
	Nasal (NS)	(5/6)	83.3% (Ct range 22.26-23.21)
	Rectal (RC)	(3/6)	50% (Ct range 25.76-29.78)
7	Ocular (OC)	(4/4)	100% (Ct range 20.16-27.53)
	Nasal (NS)	(4/4)	100% (Ct range 19.15-27.14)
	Rectal (RC)	(3/4)	75% (Ct range 21.38-26.04)
8	Ocular (OC)	(2/2)	100% (Ct range 19.51-21.56)
	Nasal (NS)	(2/2)	100% (Ct range 18.98-20.34)
	Rectal (RC)	(2/2)	100% (Ct range 19.44-19.90)

OC=Ocular; NS=Nasal; RC=Rectal

Samples produced Cycle threshold (Ct) value of ≤35 were considered positive while Ct>35 considered negative in our study. Number of processed samples progressively decreased day by day due to sacrificed (i.e 2 goats per day) experimental goats at predetermined time points. Generally as indicated in the above table lower Ct values (high concentration of viral RNA) were recorded as the dpi increased this implies concentration of viral RNA was increased from 3 dpi to 8 dpi collected and tested samples. Percent positivity of nasal and ocular swabs were higher

than rectal swab samples and detection of viral RNA was consistently observed in nasal and ocular swabs than rectal swabs.

Supplementary Table 3.4: Test agreement results (Cohen's Kappa values) of different methods used for virological diagnosis in goats group after experimental intranasal infection with PPRV lineage-IV strain Ethiopia/Habru/2014. Different sample matrices i.e., swabs of ocular, nasal, rectal and sera were analyzed. Antigen Enzyme Linked Immunosorbent Assay (AgELISA), cELISA (Competition Enzyme Linked Immunosorbent Assay), RT-qPCR (real time quantitative Polymerase Chain Reaction), and V-Culture (Virus Culture) methods were compared.

Compared test methods	Interpretation of Cohen's kappa	
	Kappa coefficient (95% CI)	Level of agreement
AgELISA vs cELISA	0.18 (-0.084-0.45)	Slight
AgELISA vs RT-qPCR	0.75 (0.3-1)	Substantial
AgELISA vs V-Culture	0.25 (-0.082-0.58)	Fair
RT-qPCR vs cELISA	0.29 (-0.049-0.62)	Fair
RT-qPCR vs V-Culture	0.38 (-0.0035-0.77)	Fair

If value of kappa is "< 0", No agreement; "0-0.20", Slight agreement; "0.21-0.40", Fair agreement; "0.41-0.60", Moderate agreement; "0.61-0.80", Substantial agreement; "0.81-1.00", Almost perfect agreement.

Supplemental Table 3.5: Observed Cytopathic Effect in Positive Goats Swab Samples Across Various Culture Days

ID	DPI	Sample type	Culture Day				
			1	2	3	4	5
253	4	Ocular swab	-	-	+	+++	+++
241	5	Nasal swab	-	+	+++	+++	+++
254	5	Ocular swab	-	-	-	-	++
254	5	Nasal swab	-	+	++	+++	+++
196	5	Nasal swab	-	+	++	+++	+++
253	5	Ocular swab	-	-	+	++	+++
253	5	Nasal swab	-	+	++	+++	+++
206	6	Nasal swab	-	++	++	+++	+++
206	6	Rectal swab	-	-	-	++	++
254	6	Ocular swab	-	+	++	+++	+++
254	6	Nasal swab	-	++	+++	+++	+++
254	6	Rectal swab	-	+	++	+++	+++
178	6	Nasal swab	-	++	++	+++	+++
196	6	Nasal swab	-	++	+++	+++	+++
253	6	Ocular swab	-	++	++	+++	+++
253	6	Nasal swab	-	++	+++	+++	+++
253	6	Rectal swab	-	++	++	++	+++
206	7	Nasal swab	-	-	++	+++	+++
206	7	Rectal swab	-	++	+++	+++	+++
254	7	Ocular swab	-	++	++	+++	+++
254	7	Nasal swab	-	++	+++	+++	+++
254	7	Rectal swab	-	++	++	+++	+++
196	7	Nasal swab	-	++	+++	+++	+++
253	7	Ocular swab	-	++	++	+++	+++
253	7	Nasal swab	-	+	+++	+++	+++
253	7	Rectal swab	-	++	+++	+++	+++
254	8	Ocular swab	-	++	++	+++	+++
254	8	Nasal swab	-	+	+++	+++	+++
254	8	Rectal swab	-	+	++	+++	+++
253	8	Ocular swab	-	++	++	+++	+++
253	8	Nasal swab	-	++	++	+++	+++
253	8	Rectal swab	-	+	++	+++	+++

The table presents the cytopathic effect (CPE) observed in positive swab samples from experimental goats over various culture days. Symbols indicate CPE intensity: "-" (none), "+" (mild), "++" (moderate), "+++" (strong), and "ID" (Animal Identification). Only positive swab samples are shown, swabs collected from all other animals were negative based on culture and observed CPE.

Supplemental Table 3.6: Observed Cytopathic Effect in Positive Goats Tissue Samples Across Various Culture Days

ID	DPI	Sample type	Culture Day				
			1	2	3	4	5
234	3	Retropharyngeal LN	-	+	+++	+++	+++
200	3	Spleen	-	+	++	+++	+++
198	4	Retropharyngeal LN	-	+	+	+++	+++
198	4	Tracheal LN	-	+	++	+++	+++
198	4	Spleen	-	+	+++	+++	+++
195	4	Retropharyngeal LN	-	+	+	+++	+++
195	4	Spleen	-	+	+++	+++	+++
195	4	Palatine Tonsil	-	-	-	+	+++
241	5	Retropharyngeal LN	-	+	+++	+++	+++
241	5	Mediastinal LN	-	+	+	+++	+++
241	5	Bronchial LN	-	+	++	+++	+++
241	5	Mesenteric LN	-	+	+++	+++	+++
241	5	Tracheal LN	-	+	+	+	+++
241	5	Spleen	-	++	+++	+++	+++
241	5	Palatine Tonsil	-	+	++	+++	+++
241	5	3rd Eyelid	-	-	+	++	++
209	5	Retropharyngeal LN	-	+	+	+++	+++
209	5	Mediastinal LN	-	-	+	++	+++
209	5	Tracheal LN	-	-	+	++	+++
209	5	Spleen	-	-	+	+	+++
209	5	Palatine Tonsil	-	-	+	++	+++
150	6	Bronchial LN	-	-	++	+++	+++
150	6	Mesenteric LN	-	+	+	+++	+++
150	6	Spleen	-	+	+	++	+++
150	6	Palatine Tonsil	-	+	+	+++	+++
150	6	3rd Eyelid	-	+	+	+	++
178	6	Spleen	-	-	+	++	+++
178	6	Palatine Tonsil	-	++	++	+++	+++
196	7	Mediastinal LN	-	+	+	+	++
196	7	Lung Cranial	-	-	+	++	+++
196	7	Lung Caudal	-	-	++	++	+++
196	7	Colon	-	++	++	+++	+++
196	7	Palatine Tonsil	-	++	+++	+++	+++
196	7	3rd Eyelid	-	-	+	++	+++
254	8	Colon	-	++	+++	+++	+++

The table presents the cytopathic effect (CPE) observed in positive tissue samples from experimental goats over various culture days. Symbols indicate CPE intensity: "-" (none), "+" (mild), "++" (moderate), "+++" (strong), "ID" (Animal Identification), and "LN" (lymph node). Only positive samples are shown, tissues collected from all other animals were negative based on culture and observed CPE.

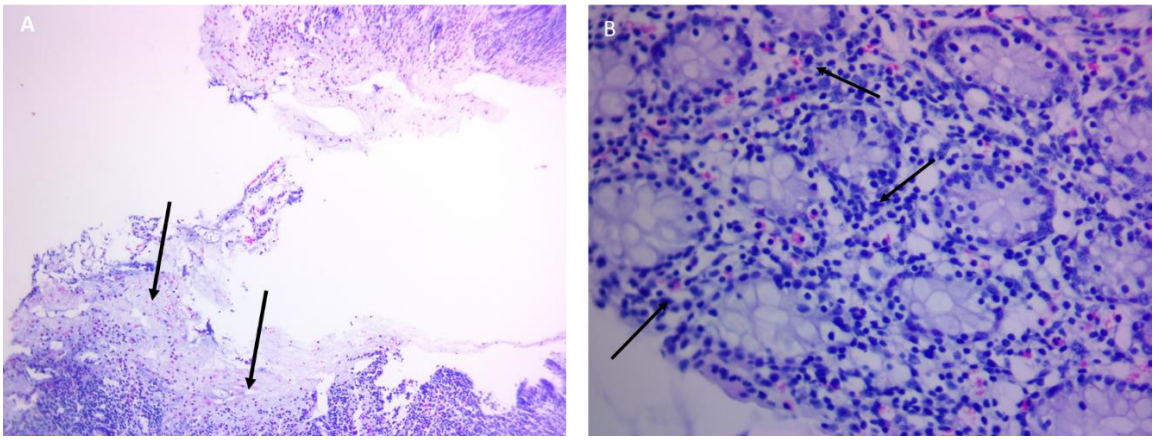
Supplementary Table 3.7: Virus culture results of all tested tissues and a swab sample from cattle Across Various Culture Days

ID	DPI	Sample type	Culture Day						
			1	2	3	4	5	6	8
220	4	Retropharyngeal LN	-	-	-	-	-	-	ND
220	4	Tracheal LN	-	-	-	-	-	-	ND
221	6	Retropharyngeal LN	-	-	-	-	-	-	ND
221	6	Mediastinal LN	-	-	-	-	-	-	ND
221	6	Bronchial LN	-	-	-	-	-	-	ND
221	6	Palatine Tonsil	-	-	-	-	-	-	ND
229	6	Retropharyngeal LN	-	++	+++	+++	+++	+++	+++
229	6	Mediastinal LN	-	-	-	++	++	++	ND
229	6	Bronchial LN	-	-	-	-	-	+	++
229	6	Palatine Tonsil	-	-	+	+++	+++	+++	ND
212	8	Palatine Tonsil	-	-	-	-	-	-	ND
212	8	3rd Eyelid	-	-	-	-	-	-	ND
212	8	Retropharyngeal LN	-	-	-	-	-	-	ND
212	8	Mediastinal LN	-	-	-	-	-	-	ND
212	8	Bronchial LN	-	-	-	-	-	-	ND
212	8	Tracheal LN	-	-	-	-	-	-	ND
225	8	Mediastinal LN	-	-	-	-	-	-	ND
225	8	Bronchial LN	-	-	-	-	-	-	ND
225	8	Palatine Tonsil	-	-	-	-	-	-	ND
225	8	3rd Eyelid	-	-	-	-	-	-	ND
217	10	Palatine Tonsil	-	-	-	-	-	-	ND
217	10	3rd Eyelid	-	-	-	-	-	-	ND
219	10	Tongue Apex	-	-	-	-	-	-	ND
216	17	Retropharyngeal LN	-	-	-	-	-	-	ND
216	17	Palatine Tonsil	-	-	-	-	-	-	ND
211	24	Palatine Tonsil	-	-	-	-	-	-	ND
230	28	Tongue Apex	-	-	-	-	-	-	ND
223	28	Retropharyngeal LN	-	-	-	-	-	-	ND
223	28	Mediastinal LN	-	-	-	-	-	-	ND
223	28	Bronchial LN	-	-	-	-	-	-	ND
223	28	Tracheal LN	-	-	-	-	-	-	ND
223	28	Palatine Tonsil	-	-	-	-	-	-	ND
223	28	3rd Eyelid	-	-	-	-	-	-	ND
211	8	Nasal swab	-	-	++	+++	+++	ND	ND

The table presents all results including the cytopathic effect (CPE) observed in tissue samples from animals 229 and 211 over various culture days. Symbols indicate CPE intensity: "-"(none), "+" (mild), "++" (moderate), "+++" (strong), "ID" (Animal Identification), "ND" Not determined, and "LN" (lymph node). Only four tissue samples collected from animal 229 at 6 days post infection (DPI), and a swab sample from animal 211 collected at 8 DPI were positive. Tissues and swabs collected from all other animals were negative based on culture and observed CPE.



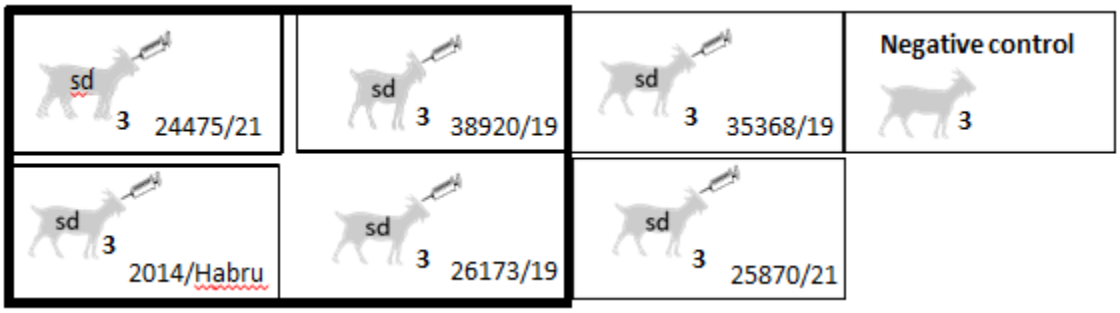
Supplemental Figure 3.1: Clinical signs observed at various days post-infection (dpi) in experimental goats after infection with the Ethiopia/Habru/2014 Lineage-IV PPRV strain **(Fig S 3.1A)** Serous nasal discharge at 4 dpi; **(Fig S 3.1B)** Soft feces (diarrhea) at 6 dpi; (**Fig S 3.1C)** Necrosis, sloughing and erosions of oral mucosa and gums at 8 dpi.



Supplemental Figure 3.2: Nonspecific histopathological lesions were documented in Ilium and Colon of experimental cattle cohort. **(A)** Necrotized mucosa in the Ilium (Black arrow); **(B)** Mononuclear cells and eosinophil's infiltrations in the lamina propria of Colon (Black arrow), ear tag no. 214. Hematoxylin-eosin stains 10 x magnifications.

Supplementary File

Supplementary Text 4.1: Research design



Sampling:

Daily 0-12 dpi

- Clinical signs and rectal temperature were monitored and recorded.

Other

- Serology : 0, 1, 3, 5, 7, 10, and 12 dpi
- Swabs for AgELISA: 1, 3, 5, 7, 10, and 12 dpi
- Swabs for RT-qPCR : 1, 3, 5, 7, 10, and 12 dpi

Sacrifice:

- 1 goat from each barn/isolate on 7, 10, and 12 dpi
- 14 tissues and LNs were collected for RT-qPCR and histopathology analysis that included:- retropharyngeal LN, palatine tonsil, third eyelid, nasal mucosa, tongue apex, tracheal LN, bronchial LN, mediastinal LN, lung cranial, lung caudal, mesenteric LN, spleen, jejunal and ilial Peyer's patch and colon.
- For the virus isolate/strain information (refer to Table 1)

Text S 4.2: Veterinary attention/care

All goats received deworming treatment using a broad-spectrum anthelmintic (Albendazole 300mg bolus, administered at a rate of one bolus per 40kg body weight of the goat). Long-acting oxytetracycline was administered as required.

Text S 4.3: Summarized procedure of RT-qPCR test

The reaction mixture included components such as Express Universal SuperScript mix, Express SuperScript enzyme mix, ROX reference dye (Invitrogen, Thermo Fisher Scientific), primers, probe, and RNase-free water, with the extracted RNA, making up a total reaction volume of 20µl. Amplification was carried out using an Applied Biosystems 7500 FAST thermal cycler with reverse transcription at 50°C for 15 minutes, DNA polymerase activation at 95°C for 20 seconds, and subsequent cycling comprising denaturation at 95°C for 3 seconds and annealing/extension at 60°C for 30 seconds, following the protocol outlined by (Batten et al., 2011, Aklilu et al., 2024).

Text S 4.4: Virus isolation summarized procedures and origin of isolates

For virus isolation, VDS cells were prepared by washing with PBS and then inoculated with 100-200µl of supernatants from ocular, nasal, and rectal swabs or processed tissues, which had been previously homogenized and centrifuged at 1650g for 20 minutes at 4°C. These cells, with over 70% confluence in 24-well plates, underwent a pre-adsorption process, followed by an hour of incubation at 37°C with 5% CO2 and 96% humidity, with intermittent agitation every 15 minutes to facilitate virus adsorption. After removing the inoculum and washing with serum-free DMEM, 500µl of DMEM with 2% serum was added to each well. The cultures were then examined under an inverted microscope for non-specific reactions before a final incubation of five to seven days under the same conditions to detect CPE indicative of viral replication, following the protocol outlined by (Gomes et al., 2016, Aklilu et al., 2024).

Regarding the origin of the virus isolates, in the present study, two isolates (26173/19 and 25870/21) were obtained during our recent outbreak investigations. The remaining four isolates (from 2014, 38920/19, 24475/21, and 35368/19) were retrieved from our repository of PPR-positive samples collected during previous outbreaks. All isolates, regardless of origin, were propagated in Vero Dog SLAM (VDS) cells and confirmed as PPRV-positive by RT-qPCR prior to sequencing and lineage-level characterization.

Text S 4.5: Euthanasia method

Goats were euthanized at pre-determined periods i.e. at 7, 10, and 12 dpi's, with one randomly selected from each barn/group. In this experiment, we utilized captive bolt stunning as the chosen method for animal euthanasia due to its humane nature. Immediately after stunning, the animals were bled efficiently in AHI, postmortem room under biosecured condition to reduce any undue stress or pain during the post-mortem procedure. The Bolt Stunner Model KS, manufactured by Karl Schermer GmbH in Germany, was selected for its effectiveness with goats and sheep.

Text S 4.6: Histological analysis and grading

Histopathological examination was conducted on a total of 252 tissue samples to evaluate cellular changes induced by PPRV infection. For the specimens collected from each individual animal (see sampling section). Standard operating procedures (SOPs) of the AHI pathology laboratory were followed. In summary, formalin-fixed tissue samples were dehydrated in graded ethanol and clarified with xylene using the Shandon Citadel 1000 tissue processor (Thermo Fisher Scientific, Waltham, MA, USA) before embedding in paraffin with the TEC2900 modular Tissue Embedding Centre (Histo-Line Laboratories, Milan, Italy). Tissue sections with a thickness of 4-5 μm were prepared and stained with Hematoxylin and Eosin. These sections were then examined under light microscopy at magnifications of 4x, 10x, and 40x, and histopathological lesions were scored using a grading system ranging from 0 (no lesions) to 4 (severe lesions), as outlined in (Aklilu et al., 2024).

Text S 4.7: Clinical signs observed for each individual strain

Experimental goats infected with lab code 24475/21, lineage IV PPRV, showed some disease signs started from 4 days post-infection (dpi) through 12 dpi. Some of the observed signs including:- muco-purulent nasal discharge (MPND) (**Figure S 4.1B**) congested mucous membrane (CMM), (**Figure S 4.1D**), and ocular discharge (OD) (**Figure S 4.1A**) and erosive mouth lesion (**Figure S 4.1C**).

Infection with lineage IV 2014 Ethiopian isolate

Typical clinical signs were observed started from 4 dpi to 12 dpi, including fever, nasal discharge (ND) and congested mucous membrane (CMM) diarrhea and coughing.

Infections with strain lab code 38920/19, lineage-IV Ethiopian isolate

Experimental goats infected with this virus showed only one clinical sign from 6 to 9 dpi, i.e slight nasal discharge (ND). The maximum temperature recorded in this group was 39.4°C at 9 dpi, which was below the cutoff of 39.5°C.

Infection with strain lab code 26173/19, lineage-III PPRV

Some clinical signs were observed started from 4 dpi to 12 dpi, including nasal discharge (ND), congested mucous membrane (CMM), fever coughing, and diarrhea.

Infection with strain lab code 35368/19, lineage-III PPRV

Evident signs including fever, serous nasal discharge (ND) that later became muco-purulent nasal discharge (MPND), congested mucous membrane (CMM), cough, and later-stage diarrhea were recorded started from 4 dpi through 12 dpi.

Infection with strain lab code 25870/21, Lineage –IV PPRV

Typical clinical signs were observed started from 4 dpi until 12 dpi, including fever, serous nasal discharge (ND), muco-purulent nasal discharge (MPND), congested mucous membrane (CMM), cough, diarrhea, and erosive mouth lesion.

Text S 4.8: Detail results of antigen detection of individual strains

Specifically goats infected with strain 24475/21 produced 5/12 (41.6%); strain 2014 7/12 (58.33%); strain 38920/19, 1/12 (8.3%); strain 26173/19 produced 6/12 (50%); strain 35368/19 produced 9/12 (75%); and strain 25870/21 produced 9/12 (75%) antigen positive result from nasal swabs. While from rectal swabs strain 24475/21 produced 4/12 (33.3%); strain 2014/Habru, 5/12 (41.66%); strain 38920/19, 1/12 (8.3%); strain 26173/19 produced 1/12, (8.3%); strain 35368/19 produced 6/12 (50%); and strain 25870/21 produced 9/12 (75%) antigen positive result from rectal swabs.

Text S 4.9: **Detail RT-qPCR result of swab samples for individual strains**

Goats infected with the 24475/21 strain had 5/9 swabs positive at 5 dpi, with all swabs testing positive by 7 dpi. By 10 dpi, all 6 tested swabs were positive, and at 12 dpi, all 3 swabs from the last goat were positive. Goats infected with the 2014 Ethiopian strain showed increasing positivity: 2/9 swabs positive at 5 dpi, 8/9 at 7 dpi, and all swabs positive at 10 and 12 dpi. Goats infected with 38920/19 strain, had 2/9 positive at 5 dpi and 3/9 positive at 7 dpi, but all swabs tested negative at 10 and 12 dpi. Goats infected with the 26173/19 strain, showed 8/9 positive at 5 dpi, all positive at 7 dpi, 5/6 positive at 10 dpi, and 2/3 positive at 12 dpi. Goats infected with the 35368/19 strain had 7/9 swabs positive at 5 dpi, 8/9 at 7 dpi, and all 6 swabs positive at 10 dpi, but only 1/3 was positive at 12 dpi. Goats infected with the 25870/21 strain, had all tested swabs (ocular, nasal, and rectal) positive at 5, 7, 10, and 12 dpi.

Text S 4.10: Detail individual strain gross pathology results

Gross pathological findings were evaluated as per the criteria outlined by (Aklilu et al., 2024). Accordingly, goats infected with strain 24475/21 and sacrificed at 7, 10 and 12 dpi's manifested some PPRV characteristics grade 2 gross lesions. Including: swelling with edema, congestion and hemorrhage in most of the inspected tissues and lymph nodes (LNs) were observed. Particularly a goat sacrificed by 10 dpi showed distinct signs on lung tissue like hepatization and meaty appearance (**Figure S 4.2**), thickened mucosa of jejunum and congested Peyer's patch were also observed. From the same lineage IV, a goat infected with 2014/Habru strain showed grade 2 gross pathological lesions, like congested lungs and jejunum mucosa and catarrhal enteritis. By 10 dpi, lungs became emphysematous, hemorrhagic and hepatized. At 12 dpi, showed hepatized and hemorrhagic cranial lung and meaty and congested middle lung. In addition, thickened mucosa of jejunum and congested Peyer's patch were also noticed.

From the same lineage IV, a goat infected with 38920/19 strain and sacrificed at 7 dpi, showed grade 1 gross lesion, like slight enlargement in some of inspected LNs and slightly hepatized cranial lung. While goats sacrificed at 10 and 12 dpi's from the same group showed no typical gross lesions observed on inspected tissues and LNs.

Moreover, goats inoculated with same lineage IV, 25870/21 strain produced grade 2 gross lesions at 7 dpi, including hepatized cranial and caudal lungs. By 10 dpi, meaty cranial lung and emphysematous caudal lung and redness of ileum Peyers' patch was observed. At 12 dpi, hepatized, hemorrhagic and emphysematous cranial lung and catarrhal enteritis were observed.

Experimental goats inoculated with lineage III, 26173/19 virus strain showed grade 2 gross lesions, particularly hepatization on cranial lung. By 10 dpi, hepatizations of the cranial lung along with redness of the jejunal mucosa were also observed. At 12 dpi, cranial lung atelectasis, hemorrhage in the middle lung, and hepatization of the caudal lung and catarrhal enteritis were observed. A goat infected with the same lineage III, 35368/19 strain manifested grade 2 gross lesion starting from 7 dpi. By 10 dpi, cranial as well as caudal lungs were hepatized. Similarly at 12 dpi, hepatized (meaty) cranial lung and emphysematous caudal lung were observed.

Text S 4.11: Histopathological findings

Histopathological lesions of virus inoculated goats were evaluated as per the methods outlined in (Aklilu et al., 2024). In the current study, the consistently observed histological lesions were characterized as grade 2 histopathological lesions except (38920/19 strain from lineage IV), and were observed beginning at 7 dpi in sacrificed goats, included lymphocyte cell depletion (characterized by pyknosis and karyolysis), congestion, and hemorrhage of the head, lung-associated, and mesenteric lymph nodes, as well as in other inspected non-lymphoid organs. Different degrees of histological lesions were recorded in the tonsils of most of the sacrificed goats, specifically in 83.33% (15/18) of the goats, particularly tonsil was characterized by lymphoid tissue cells depletion and syncytial cell formation (**Figure S 4.3A**). In lung tissue both the caudal and cranial lobes of the lungs exhibited various typical histological lesions of the PPR virus in nearly all experimental groups, with an occurrence rate of 88.8%% (16/18), particularly hemorrhage and emphysema, (**Figure S 4.3B**) aggregation of lymphocytes, thickening of lobal septa, Broncho-interestitial pneumonia were commonly observed. Among the inspected GIT tissues, the colon was commonly affected organ by the inoculated viruses, showing pathology characterized by necrosis, sloughed mucosa (**Figure S 4.3E**) and lamina propria infiltrated with mononuclear cells (**Figure S 4.3F**). This was observed in 83.33% (15/18) of the inspected goats. In the small intestine, the jejunum and ileum were consistently affected tissues by the virus and

histopathological examinations revealed the presence of lymphoid depletion, eosinophils and syncytial cells (**Figure S 4.3C**), short villi, congestion of lamina propria and mononuclear cells in the lamina propria (**Figure S 4.3D**), necrosis and sloughing also commonly observed. The Peyer's patches in the jejunum and ileum were also affected, characterized by cell depletion in 77.7% (14/18) of the inspected goats. The spleen was also affected and exhibited various pathologies, including white pulp cell depletion, red pulp hyper cellularity, and hemosiderin deposition in 83.3% (15/18) goats. Upon inspection, the third eyelid was characterized by cell depletion, congestion, and sometimes the presence of syncytial cells. Pathological changes in the third eyelid were observed in 77.7% (14/18) of goats. Histologically, 16.6% (3/18) of nasal mucosa and 11.1% (2/18) of tongue tissues were affected by the infected virus.



Figure S 4.1: **Typical clinical signs resulting from PPR virus infection (24475/21 strain)**
(**A**) Muco-purulent ocular discharge (**B**) Muco-purulent nasal discharge (**C**) Erosive mouth lesion and (**D**) Congested mucous membrane of the conjunctiva (Taken by GoPro Camera, Animal ID: 286 at 9 dpi).

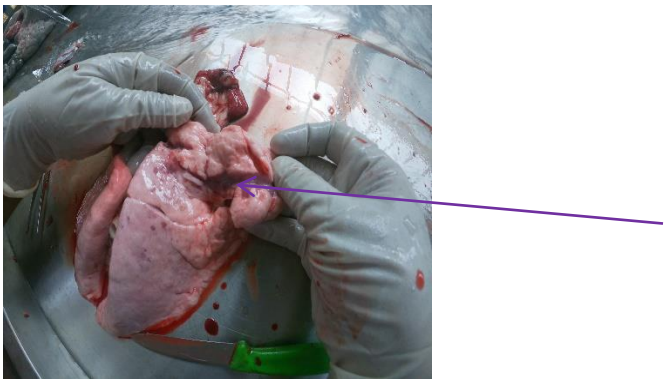


Figure S 4.2: Lung hepatization and meaty appearance

The gross pathology of the lung tissue from the sacrificed goat (strain code 24475/21, at 10 dpi) shows a lesion with a hepatized and meaty appearance in the right cranial lobe (indicated by the yellow arrow).

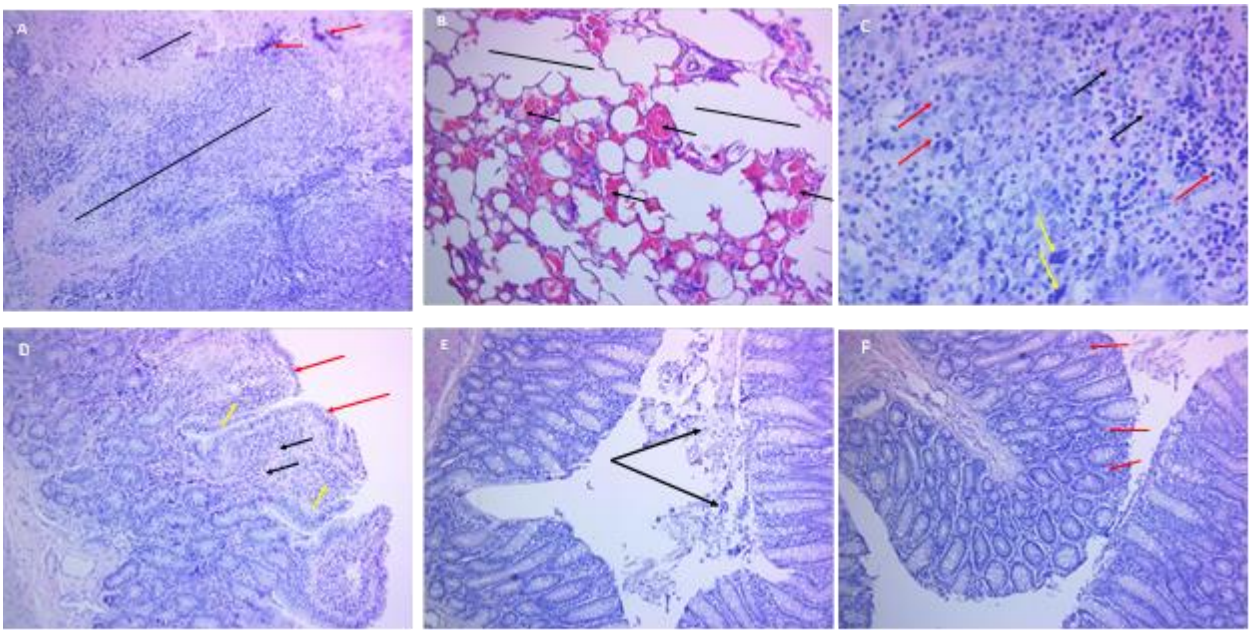


Figure S 4.3: Histopathological findings resulted due to different PPR virus strains (**A**) In Tonsil tissue lymphoid tissue cells depletion (black line) and syncytial cell formation (red arrow), ID: 277, 7 dpi (24475/21 strain); (**B**) Caudal lung, hemorrhage (black arrow) and emphysema (black line) due to PPR virus, ID: 280, 12 dpi (25870/21 strain) ; (**C**) Ileum manifested depleted cells (red arrow) and eosinophils (black arrow) and syncytial cells (yellow arrow), ID: 276, 7 dpi (25870/21 strain); (**D**) Ileum short villi (red arrow) and congestion of lamina propria (black

arrow) infiltrated with mononuclear cells (yellow arrow), ID, 276, 7 dpi (25870/21 strain); (**E**) Colon characterized by sloughed and necrotized mucosa (black arrow) (**F**) lamina propria infiltrated with mononuclear cells (red arrow) ID: 293, 12 dpi (26173/19 strain). All subfigures (**A**) through (**E**) stain with hématoxyline- éosine, 10 x magnifications.

Table S 4.1: Summary of the range of days post infection for which clinical scores were above the lowest category for 18 experimentally infected goats inoculated with six different PPRV strains.

Strain	Animal ID	Temp (≥39.5)[(a)]	General signs [(a)]	Nasal/Ocular Discharges [(a)]	CMM [(a)]	Diarrhea [(a)]	Respiratory Signs [(a)]
24475/21	277	5-7	4-7	5-7	6-7	-	7
	286	4-8	4-12	5-12	5-12	7-11	7-12
	296	4-10	4-10	4-10	4-10	6-10	4-10
2014/Habru	279	4-7	4-7	5-7	5-7	-	-
	289	5-7	4-12	4-12	5-12	5-12	7-12
	292	4-9	4-10	4-10	5-10	5-10	7-10
38920/19	299	-	-	6-9	-	-	-
	300	-	-	7	-	-	-
	301	-	-	6-9	-	-	-
26173/19	281	4-7	4-7	4-7	4-7	-	-
	291	8	4-10	5-10	5-10	-	8
	293	8	4-12	4-12	6-12	7-9	-
35368/19	278	5-8	4-10	5-10	4-12	-	-
	294	4-12	4-12	4-12	4-12	8-12	5-12
	298	4-7	4-7	4-7	4-7	-	-
25870/21	276	-	4-7	4-7	5-7	7	-
	280	4-11	4-12	4-12	5-12	8-12	9
	283	4-10	4-10	4-10	4-10	8-9	6-10

-, negative; CMM, Congested mucous membrane; General signs, including loss of appetite, inactivity, rough coat, dullness/depression; [(a)], Duration of clinical signs.

Table S 4.2: RT-qPCR positivity and virus isolation results from swabs and tissue samples from experimental goats inoculated with six different PPRV strains at various days post-inoculation (dpi).

Strain/ Isolate	RT-qPCR				Virus culture/isolation			
	Total swabs (of ocular, nasal, rectal) collected at 5, 7, 10 and 12 dpi		Total tissue and LNs collected at sacrifice days i.e. 7, 10 and 12 dpi		Proportion and percentage of virus culture positive swabs and tissues at various dpi's			
	Positivity	Percentage	Positivity	Percentage	dpi 7		dpi 10 and 12 combined	
					swabs	tissues	swabs	tissues
24475/21	23/27	85.1	25/42	59.5	(7/9, 77.77)	(5/12, 41.66)	(0/3, 0)	(0/13, 0)
2014/Habru	19/27	70.3	32/42	76.16	(8/8, 100)	(7/12, 58.33)	(0/3, 0)	(0/20, 0)
38920/19	5/27	18.5	15/42	35.7	(2/3, 66.66)	(8/12, 66.66)	NA	(0/3, 0)
26173/19	24/27	88.8	28/42	66.6	(2/9, 22.22)	(5/11, 45.45)	(0/3, 0)	(0/17, 0)
35368/19	22/27	81.4	29/42	69	(3/8, 37.5)	(7/14, 50.0)	(0/6, 0)	(0/15, 0)
25870/21	27/27	100	34/42	80.9	(5/9, 55.55)	(5/13, 38.46)	(0/7, 0)	(0/21, 0)

NA= Not Applicable

Table S 4.3: Detail RT-qPCR results of the 14 individual tissue/LN samples on different days of post-infection inoculated with six different PPRV strains

Tissue type	Strain used, LIV			Strain used, LIV			Strain used, LIV			Strain used, LIV			Strain used, LIII			Strain used, LIV		
	38920/19			24475/21			2014/Habru			25870/21			26173/19			35368/19		
	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID	ID
	300	299	301	277	296	286	279	292	289	276	283		281	291	293	298	278	
	dpi7	dpi10	dpi12	dpi7	dpi10	dpi12	dpi7	dpi10	dpi12	dpi7	dpi10	dpi12	dpi7	dpi10	dpi12	dpi7	dpi10	dpi12
3rdEyelid	18.1	UD	UD	15.4	UD	UD	18.1	15.4	29.6	19.3	25.1	28.8	20.5	27.8	UD	20.9	26.7	UD
NasMuc	UD	UD	UD	31.7	24.5	22.3	UD	31.7	27.2	33.1	30.2	26.1	26.3	UD	UD	31.5	UD	UD
Tongue	UD	UD	UD	26.5	UD	UD	UD	26.5	UD	26.1	UD	UD	26.6	UD	UD	25.8	UD	UD
Tons	18.7	UD	UD	15.7	32.4	30.1	18.7	15.7	26.2	14.7	24.7	24.8	UD	25.0	29.3	17.5	21.8	28.4
Retroph LN	15.7	UD	UD	18.3	28.9	23.1	15.7	18.3	23.8	16.1	21.1	21.5	18.7	23.9	24.8	18.3	19.5	25.9
TrachLN	17.2	UD	UD	25.1	UD	23.9	17.2	25.1	31.1	17.9	23.7	24.5	21.9	30.8	26.5	20.9	21.5	32.9
BronchLN	22.6	UD	24.8	23.8	33.4	24.1	22.6	23.8	UD	16.9	22.9	23.4	19.0	23.9	26.3	18.7	22.4	UD
LungCran	20.4	UD	28.8	UD	UD	UD	20.4	UD	UD	UD	UD	UD	UD	UD	UD	24.2	UD	UD
LungCaud	25.3	UD	UD	UD	UD	UD	25.3	UD	UD	26.8	UD	UD	UD	UD	UD	30.1	UD	UD
MediLN	20.1	UD	UD	20.4	UD	22.7	20.1	20.4	25.2	16.6	23.1	25.1	18.1	34.5	25.3	18.4	21.7	24.8
MesLN	15.7	UD	UD	21.8	UD	22.5	15.7	21.8	23.5	16.9	28.5	25.3	17.7	24.2	23.9	18.1	18.7	30.2
Spln	19.1	UD	UD	18.1	UD	24.7	19.1	18.1	27.2	16.5	19.6	26.4	17.9	26.7	32.2	17.7	22.5	32.9
Colon	18.2	UD	UD	19.6	UD	UD	18.22	19.6	UD	19.1	25.1	31.3	18.9	26.4	UD	23.8	22.8	UD
PP	17.6	UD	UD	18.2	29.4	UD	17.6	18.2	UD	17.3	34.3	UD	17.1	UD	28.7	17.8	UD	UD

DPI", days post-infection ; NasMuc - Nasal Mucosa; Tons - Tonsil; 3rdEyelid - Third Eyelid; RPLN - Retropharyngeal Lymph Node; Tongue - Tongue (Apex); TrachLN - Tracheal Lymph Node; BronchLN - Bronchial Lymph Node; LungCran - Lung Cranial; LungCaud - Lung Caudal; MediLN - Mediastinal Lymph Node; Spln - Spleen; PP - Ileal & Cecal Peyer's Patches; Colon - Colon; MesLN - Mesenteric Lymph Node.; "UD" Undetermined with RT-qPCR CT >35.

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- PhD Fellow – Addis Ababa University (2020 – Present)
- MSc in Transboundary Animal Disease Management – Addis Ababa University, 2012
- DVM – Addis Ababa University, Ethiopia, 2007
- Diploma – Addis Ababa University, Ethiopia, 1990

Professional Experience

- Animal Health Institute, Ethiopia (2012 – Present): Researcher in Veterinary Microbiology; Project involvement in Role of Cattle in PPRV Transmission Dynamics in collaboration with Penn State University.
- October 2007 to July 2012: Pastoral Development Department Head in Gambella Agricultural Bureau.
- September 1999 to January 2002 Animal and Fishery Resource Development Coordinator in Gambella Agricultural Bureau at Gog and Jor Wereda.
- March 1993 to September 1998 senior Assistant Veterinarian in Gambella Agricultural Bureau at Gog and Jor Wereda.
- September 1991 to March 1993 Junior Assistant Veterinarian in Oromia (Bale Zone) Agriculture Department at Genale Awraja.

Peste des Petits Ruminants (PPR) Related Publications

1. Herzog, C.M., **Aklilu, F.,** Sibhatu, D. et al. Empirical and model-based evidence for a negligible role of cattle in peste des petits ruminants virus transmission and eradication. Commun Biol 7, 937 (2024). https://doi.org/10.1038/s42003-024-06619-2.

2.Aklilu, F., Ashenafi, H., Kassa, T. *et al*. Comparative pathogenesis of Ethiopia/Habru/2014 Lineage-IV peste des petits ruminants virus in goats and cattle. *BMC Vet Res* **20**, 473 (2024). https://doi.org/10.1186/s12917-024-04313-3.

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4. Simon King, Michael D. Baron, Menbere Kidane, **Fasil Aklilu**, Vivek Kapur, Catherine M. Herzog, Carrie Batten (2023): Complete genome of a 2014 isolate of peste des petits ruminants virus from Ethiopia. ASM Journals/Microbiology Resource Announcements. Vol.12, No.9 https://doi.org/10.1128/MRA.00242-23.

5.Abde Aliy Mohammed, Tesfaye Rufael Chibssa , Waktole Terfa, **Fasil Aklilu**, Delesa Damena, Redeat Belayneh, and Menbere Kidane: Isolation and Molecular Characterization of Peste des Petits Ruminants Virus from Outbreaks in Southern Ethiopia. Hindawi, Advances in Virology Volume 2022, Article ID 5329898, 7 pages https://doi.org/10.1155/2022/5329898.

Other Publications

1. Aklilu Fasil, Asfaw Yilkal, Baumann Maximillan and Abie Getnet (2015): Epidemiological study of Contagious Caprine Pleuropneumonia (CCPP) in selected districts of Gambella Region, Western Ethiopia. African Journal of Agricultural Research. Vol. 10, No. 24, 2470-2479 ref. 40.

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3. Bashitu L, Afera B, Tuli G, **Aklilu F** (2015): Sero-Prevalence Study of Bovine Brucellosis and its Associated Risk Factors in Debrebirhan and Ambo Towns. J Adv Dairy Res 3: 131. http://dx.doi.org/10.4172/2329-888X.1000131.

4. Tolesa Ebisa, Eyob Hirpa, and **Fasil Aklilu** (2015)**:** Study on Sero-prevalence and Risk factors of Contagious Pleuropneumonia (CBPP) in Southern Nation Nationalities People of Ethiopia Regional State in Amaro Special District. Sci. Technol. Arts. Res. J., Oct-Dec 2015, 4(4):106-112. http://dx.doi.org/10.4314/star.v4i4.15.

5. Abde Aliy, Kumela Lelisa**, Fasil Aklilu and** Delesa Damena (2017): Contagious Bovine Pleuropneumonia: Serological Prevalence in Derashe District, Southern Ethiopia. Global Veterinaria, 19 (5): 616-621, 2017. DOI: 10.5829/idosi.gv.2017.616.62.

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7.Azeb Gebretensay, Gezahegn Alemayehu, Mourad Rekik, Biruk Alemu, Aynalem Haile, Barbara Rischkowsky**, Fasil Aklilu,** Barbara Wieland **( ):** Risk factors for reproductive disorders and major infectious causes of abortion in sheep in the highlands of Ethiopia. Small Ruminant Research Vol. 177, August 2019, Pages 1-9 https://doi.org/10.1016/j.smallrumres.2019.05.019.

8. Beheru Sultan, Demeke Sibhatu, **Fasil Aklilu**, Melesse Yilma (2020)**:** Seroprevalence and Associated Risk factors of Maedi-Visna in Amedguya Sheep breed Multiplication Center,

amhara Region, Northern Ethiopia. Global scientific Journals, Vol. 8, Issue 8, August 2020 Edition.

9.Tamirat Haile, Mulugeta Abera, Tsegaye Teklemariam, Demeke Sibhatu & **Fasil Asres** (2022) Seroprevalence of Bluetongue Virus Antibodies in Ovine in Maji District of West Omo Zone, Southwest Ethiopia, Veterinary Medicine: Research and Reports, , 257-264, DOI: 10.2147/VMRR.S375482.

10.Demeke Sibhatu, Gezahagne Mamo, **Fasil Aklilu**, Demeke Zewde, Elias Walelign, Ayelech Muluneh, Abdi Aliey, Tadele Mirkena, Nega Tewolde, Getachew Gari, Gijs van 't Klooster,Ihab Elmasry, Sophie VonDobschuetz, Malik Peiris,Daniel Chu, Ranawaka APM Perera, Yilma Jobire (2020): Cross Sectional Study of Middle East Respiratory Syndrome (Mers-Cov Infection) in Camels at Selected Sites of Amibara District, Afar Region, Ethiopia. bioRxiv, 2020. https://doi.org/10.1101/2020.10.25.353227.

11. Demeke Sibhatu, **Fasil Aklilu**, Gezahagne Mamo (2020): A Review on MERS-CoV Disease Globally and Ethiopian context. GSJ: Volume 8, Issue 8, August 2020 ISSN 2320-9186.

12. L. Yizengaw, N. Belayneh, A. Zegeye, **F. Aklilu** and A. Kefale (2020): Sero-prevalence and associated risk factors of Maedi-visna virus in sheep population of selected area of eastern Amhara, Ethiopia. Indian J. Anim. Hlth. (2020), 59(2): 150-158 DOI:10.36062/ijah.59.2.2020.150-158.

Trainings and conferences:

Fasil Aklilu, Hagos Ashenafi, Tesfu Kassa, Hassen Chaka, Demeke Sibhatu, Dereje Shegu, Abde Aliy Mohammed, Redeat Belainehe, Menbere Kidane, Hagos Asgedom, Tesfaye Chibssa, Getnet Mekonnen, Asegedetch Sirak, Solomon Gebredufe, Claudia Schulz, Catherine M. Herzog and Vivek Kapur (2024): Comparative pathogenesis of Ethiopia/Habru/2014 Lineage-IV peste des petits ruminants virus in goats and cattle. **Conference**, Research Week at Aklilu Lemma Institute of Pathobiology, Addis Ababa University, May 13-17, 2024. Oral presentation.

The same work was also presented in an **International Conference,** on 7th PPR Global Research and Expertise Network (PPR-GREN). Annual Meeting in ADAFSA, ABU DHABI, UAE, 20–22 January 2025. Oral presentation.

Attended a two weeks **Advanced Training** on" Mathematical Models for Infectious Disease Dynamics (VIRTUAL) hold in Wellcome Genome Campus, Hinxton, Cambridge, CB101RQ, 08 Mar 2021-19 Mar 2021. During the training, I learned the latest techniques for modelling infectious disease dynamics and control in humans, animals or plants. The programme will cover introductory and advanced concepts in mathematical modelling of infectious diseases, including: Mathematical review (calculus, probability, etc); Population dynamics; Deterministic and stochastic models; Modelling structured populations: age-structured, spatial and network models; Applied programming with R; Statistical estimation of models and Computer-based simulations. This virtual training was sponsored by Penn State University.