CLINICAL INVESTIGATION OF *PESTE DES PETITS RUMINANTS* (PPR) VIRUS IN SMALL-RUMINANTS CIRCULATING IN BANGLADESH

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CERTIFICATE

This is to certify that the thesis entitled ***"Clinical Investigation of Peste Des Petits Ruminants (PPR) Virus in Small-Ruminants Circulating in Bangladesh"*** *has been submitted to the Department of Pathology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in PATHOLOGY****, embodies the results of a piece of bona fide research work carried out by* ***MD. JISAN AHMED****, Registration No.* ***15-06605*** *under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.*

I further certify that any help or sources of information received during this investigation have duly been acknowledged.

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DEDICATION

This piece of achievement is dedicated to my beloved father, mother, sibling, respected teachers who offered me unconditional sacrifices, blessings, love, and support, and to all the valiant freedom fighters who fearlessly fought against oppression and tyranny, dedicating their lives to the noble cause of securing our nation's freedom, your legacy of bravery and determination will forever be cherished.

ABBREVIATIONS

GDP	Gross Domestic Product
PPR	Peste des Petits Ruminants
SRMV	Small Ruminant Morbillivirus
OIE	World Organization for Animal Health
PPRV	Peste des Petits Ruminants Virus
RNA	Ribonucleic Acid
N	Nucleocapsid
P	Phosphoprotein
M	Matrix
F	Fusion
L	Large Polymerase
H	Haemagglutinin
3D	Dehydration, Diarrhea, and Death
FMD	Foot and Mouth Disease
ELISA	Enzyme-Linked Immunosorbent Assay
PCR	Polymerase Chain Reaction
FAO	Food and Agriculture Organization
GEP	Global Eradication Programme
RNP	Ribonucleoprotein
WOAH	World Organization for Animal Health
RT-PCR	Reverse Transcription Polymerase Chain Reaction
DRC	Democratic Republic of the Congo
UAE	United Arab Emirates
KSA	Saudi Arabia
SR	Small Ruminants
CDV	Canine Distemper Virus
MV	Measles Virus
PDV	Phocine Distemper Virus
DMV	Dolphin Morbillivirus
PMV	Porpoise Morbillivirus
CMV	Cetacean Morbillivirus
RdRP	RNA-dependent RNA polymerase

GP	Genome Promoter
AGP	Anti-Genome Promoter
ORF	Open Reading Frame
RPV	Rinderpest Virus
mRNA	Messenger RNA
kDa	Kilo Dalton
IFN	Interferon
aa	Amino Acid
nt	Nucleotide
SH	Small Hydrophobic
CPE	Cytopathic Effect
CSNT	Cross-Serum Neutralization Test
MHC	Major Histocompatibility Complex
GI	Gastrointestinal
MLN	Mesenteric Lymph Nodes
LAMP	Loop-mediated isothermal amplification
cDNA	Complementary DNA
DNA	Deoxyribonucleic Acid
RT-LAMP	Reverse Transcription-Loop-Mediated Isothermal Amplification
PEI	Polyethyleneimine
LFD	Lateral Flow Devices
QD	Quantum Dot
AGID	Agar Gel Immunodiffusion
IHC	Immunohistochemistry
Mab	Monoclonal Antibody
sELISA	Sandwich ELISA
RT-qPCR	Real-Time Polymerase Chain Reaction
VNT	Virus neutralization Test
LIPS	Luciferase Immunoprecipitation system
RT-RPA	Reverse Transcriptase Recombinase Polymerase Amplification Assay
LFIAS	Lateral-flow Immunoassay Strip
IPMA	Immunoperoxidase Monolayer Assay
CCPP	Contagious Caprine Pleuropneumonia

DIVA	Differentiation of Infected and Vaccinated Animals
CAV	Canine Adenovirus
BLRI	Bangladesh Livestock Research Institute
SAU	Sher-e-Bangla Agricultural University
VNE	Viral Nucleic Acid Extraction
bp	Base Pair
TAE	Tris Acetate EDTA
NA	Nutrient Agar
MSA	Mannitol Salt Agar
EMB	Eosine Methylene Blue
SS Agar	Salmonella Shigella Agar
MHA	Muller Hinton Agar
LB Broth	Luria Bertani Broth
NCCLS	National Committee for Clinical Laboratory Standards
AML	Amoxicillin
AMP	Ampicillin
AZM	Azithromycin
ERY	Erythromycin
NA	Nalidixic acid
CN	Gentamicin
TE	Tetracycline
LEV	Levofloxacin
CIP	Ciprofloxacin
E. coli	*Escherichia coli*
R	Resistance
I	Intermediate
S	Susceptible
mm	Millimeter
µg	Microgram

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Md. Jisan Ahmed

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ABSTRACT

Peste des petits ruminants (PPR) is an acute, contagious, and economically important, viral transboundary disease of small-ruminants endemic in nearly more than 70 countries across the world including Bangladesh. The WOAH and FAO have jointly developed the PPR global control and eradication strategies (PPRGCES) and have set the goal of successful implementation of the eradication program of PPR by 2030. The current study aimed to assess the seroprevalence of PPRV with associated risk factors and the molecular characterization of circulating virus from the selected areas in the Dhaka division. Moreover, it determined bacterial co-infection associated with PPR with antimicrobial susceptibility. The study was conducted from July, 2023 to March 2024. A total of 368 (n=368) [Dhaka =196, Gazipur =98, Narsingdi=74] serum samples were collected randomly from apparently healthy and unvaccinated sheep (n=36) and goats (n=332) for seroprevalence using c-ELISA and 100 (n=100) samples (nasal and/or oral swabs and tissues) were also collected from clinically suspected animals for molecular characterization of circulating PPRV strain by RT-PCR based on complete N-gene amplification. In the results, the overall seroprevalence of Anti-PPRV antibodies was 52.45% (193/368) with 51.51% (171/332) and 61.11% (22/36) found in sheep and goats, respectively. The risk factors significantly associated with PPRV seropositivity include species, gender, age, area, feeding system, ecological zone, and the introduction of new animals. The detected virus strain's nucleotide sequence and phylogenetic analysis indicated that the circulating PPRV was clustered genetically in Lineage-IV. The results demonstrated in this study suggested that a higher seroprevalence of PPRV in unvaccinated and apparently healthy animals confirmed the presence of the virus in the study area. The isolated virus strain clustering the Lineage-IV was highly linked to Indian (Tripura) and Chinese strains, and other BD PPRV strains. Regarding bacterial co-infection, *staphylococcus* spp. and *Escherichia coli* were found from PPRV positive animals and were sensitivity against most of antibiotics tested. Regular surveillance studies and characterization of circulating viruses are crucial to implement global PPR control and eradication programs recommended by the WOAH and FAO.

Keywords: Seroprevalence, PPRV, genetic characterization, small-ruminants, risk factors, Bangladesh

CHAPTER I
INTRODUCTION

Bangladesh is a densely populated, low-lying country with over 180 million people, 75% of whom reside in rural areas facing a rural poverty rate of 18.7%, with 5.6% classified as extremely poor (Schutter, 2021). The nation hugely relies on agriculture and livestock plays a crucial role in the agricultural economy. Currently, the agricultural industry contributes 16.52% to the Gross Domestic Product (GDP) while the livestock sub-sector contributes about 1.85% to the overall economy (DLS, 2022). The livestock industry of Bangladesh is primarily centered around cattle, goats, sheep, buffalo, and poultry reflecting its significant contribution to the national economic landscape. The current population of ruminants in livestock is about 57.14 million including 24.86 million Cattle, 26.95 million goats, 3.83 million sheep, and 1.52 million buffalo (DLS, 2022). Sheep and goats display remarkable adaptability to diverse climates and geographical conditions making them valuable assets in agriculture worldwide, more resilient than other livestock, and widely distributed (Islam *et al.*, 2021). Their resilience enables them to thrive in environments ranging from arid regions to mountainous terrains, contributing significantly to global livestock management (Islam *et al.*, 2021).

In Bangladesh, sheep and goat farming are popular compared to large ruminants because of multifaceted factors. Firstly, most rural poor people in Bangladesh cannot afford to raise large ruminants, so they mostly depend on raising small ruminants like goats and sheep to supplement their livelihoods (Prank *et al.*, 2023). Secondly, sheep and goats are more adaptable to the environmental conditions in Bangladesh including hot and humid climates, and can thrive on a variety of dietary regimens such as in uncultivable lands and grazing areas (Haque *et al.*, 2020). Additionally, sheep and goats have proven to be hardy animals and are resistant to many tropical diseases making them a more suitable choice for farmers (Rout *et al.*, 2021). Moreover, these animals consume less feed which is about one-fifth of the consumption of cattle and buffalo (Das, 2001). Finally, sheep and goat farming require less capital and management practices compared to large ruminants making them more accessible for small-scale farmers (Hossain, 2021).

However, the major constraints that hampered the optimum production in sheep and goat farming in Bangladesh because of different types diseases, insufficient feed and fodder supply with high prices, low or no management practices, high kid mortality, and a poor marketing channel with lower popularity of sheep meat than goat meat (Rakib *et al.*, 2022).

Among these constraints, infectious diseases are among the most impactful barriers to rearing small ruminants, especially sheep and goats (Radostits *et al.*, 2000). PPR is one of the major concerns for small ruminants because of its severe impact and ability to cause significant economic losses. PPR, which is also called Ovine rinderpest currently known as small-ruminant morbillivirus (SRMV), came from the French language that means plague of small-ruminants. Additionally, it is also known as the Goat Plague, Kata, syndrome of stomatitis-pneumoenteritis, or pseudo-enteritis complex (Parida *et al.*, 2015). PPR is a highly contagious, list-A disease according to the World Organization for Animal Health (OIE) and an economically highly significant transboundary infectious viral disease of small-ruminants especially in sheep and goats because of high morbidity (about 100%) and mortality rate (about 90%), resulting huge socio-economic impacts (Schmitz *et al.*, 2023). PPR threatens more than 1.74 billion sheep and goats in approximately 70 countries globally including Asia, Africa, and the Middle East (Zhao *et al.*, 2021). PPR causes approximately $2,972.5 million in annual losses in the SAARC region (Kumar *et al.*, 2014). Though the actual annual losses due to PPR in Bangladesh are not known it is predicted to be $25 million (Rahman *et al.*, 2021). PPR causes an annual economic loss of $1.2–1.7 billion due to factors like mortality and production loss (Rahman *et al.*, 2021). Eradicating PPR globally could bring a net benefit of $74.2 billion, with a benefit-cost ratio of 33.8 (Jones *et al.*, 2016).

The causal agent of PPR is the *peste des petits ruminants* virus (PPRV) under the genus *Morbillivirus* belongs to the *Paramyxoviridae* virus family (Gibbs *et al.*, 1979). PPRV exhibits pleomorphism, featuring a negative-sense, single-stranded RNA genome spanning 15,948 nucleotides. The genome encodes six (6) structural proteins including nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), haemagglutinin (H), and large polymerase (L) (Munir *et al.*, 2013). The N-gene is suggested as the optimal gene for molecular characterization of closely related isolates based on sequence analysis of PPRV strains (Kwiatek *et al.*, 2007). Based on the molecular characterization, PPRV strains can be classified into four distinct lineage groups namely lineage-I, -II, -III, and -IV each with a specific geographical distribution pattern that has been changed in recent years (Wang *et al.*, 2009).

Lineage-I identified from Western Africa and Central Africa, Lineage-II includes those from West African countries like the Ivory Coast, Guinea, and Burkina Faso, and Lineage-III represents strains from Eastern Africa and some parts of Middle East including Sudan, Yemen, and Oman (Munir *et al.*, 2013). Moreover, lineage-IV PPRV strains were reported from the Arabian Peninsula, the Middle East, and South Asia (Munir *et al.*, 2013). The first

identification of PPR was reported in Ivory Coast, West Africa in 1942. In the South Asian subcontinents, PPRV was first reported in 1987 located in the southern part of India (Shaila *et al.*, 1996). PPR was first identified in Bangladesh in 1993 and reported in goats and since then it has become endemic in the country (Islam *et al.*, 2001). Many studies revealed that Lineage-IV of PPRV is prevalent in Bangladesh which is similar to the strains found in Nepal, Bhutan, and China (Rahman *et al.*, 2018).

PPRV enters the host body through the nasal and oral passages, with viral replication occurring in the nasopharyngeal/respiratory epithelium (Yanagi *et al.*, 2006). The virus exhibits affinity to lymphoid and epithelial tissues, disseminating through blood and lymph, causing clinical signs and cytolytic changes (Vinayagamurthy, 2017). PPRV antigens are detected in various tissues, including the oral cavity and lymphoid organs (Vinayagamurthy, 2017). The incubation period is 4-5 days with viremia leading to the dissemination of the virus. PPRV is highly contagious and transmitted through close contact, infected materials, and airborne droplets (Chauhan *et al.*, 2009). Animal movement, live animal market interactions, and nomadic practices significantly contribute to PPRV transmission, emphasizing the role of diverse host populations and mobility networks (Abubakar *et al.*, 2015).

Though the primary host of PPR is small-ruminants, especially sheep and goats, it can infect many other species as well such as camels, wild ruminants, cattle, and buffalo (Jones *et al.*, 2016). PPR is clinically manifested by fever, oculo-nasal discharge, pneumonia, severe diarrhea, and inflammation of the mucus lining of the respiratory and digestive system, followed by death (Schmitz *et al.*, 2023). PPR is sometimes called a “3D” disease due to nasal discharge, diarrhea, dehydration, and Death (Islam *et al.*, 2001). Based on the onset of severity, PPR can be classified into different categories such as Peracute, Acute, Subacute, and Subclinical (Kabir *et al.*, 2019). In regions affected by epidemics, the morbidity rate of PPR is approximated to be between 80% and 90% with a mortality rate ranging from 50% to 80% (Ezeibe *et al.*, 2008).

PPR can be diagnosed through the important clinical manifestations, pathological lesions, and identification of virus antigens and antibodies in clinical samples using molecular techniques and a variety of serological tests, respectively (Balamurugan *et al.*, 2014). The most commonly practiced diagnostic techniques are cell culture isolation and RT-PCR, agar gel immunodiffusion, hemagglutination tests, and ELISA (Mahmoud *et al.*, 2022). PPR is endemic in Bangladesh however most of the previous studies that were conducted were based on clinical signs or serology and a few studies were done on the genetic characterization of

PPR (Rahman *et al.*, 2016). Therefore, the molecular epidemiological linkages and transmission of PPRV strains remain poorly understood with the occurrences in sheep and goats in Bangladesh necessitating research into molecular characterization and phylogenetic analysis of spreading virus strains circulating at the field level. Since the importance of sheep and goats in the livelihood of the poor and marginal farmers, PPR is highly concerned with security and poverty alleviation. RPV is another *Morbillivirus*, which has already been globally eradicated through mass vaccination (Almeshay *et al.*, 2017). Thus, the World Organization for Animal Health (WOAH) and the Food and Agriculture Organization (FAO) have collaboratively developed a strategy to control and eliminate PPR by the year 2030.

The PPR is caused by only one serotype and there is not much evidence of either a carrier or a sustainable-reservoir species outside of domestic small-ruminants. A high-quality vaccine is available against PPRV but the instability due to thermo-sensitivity, and unavailability of adequate doses provides low coverage, the implementation of PPR control and eradication programs in this sub-continent, including Bangladesh (Rahman *et al.*, 2021) has not been a great success. Alongside vaccination, a regular basis of active surveillance practice of PRV in domestic and wildlife hosts might be a useful indicator for detecting the outbreaks and locally circulating virus strains, which is crucial for the development of effective PPR control and/or elimination strategies. Additionally, a better understanding of PPR seroprevalence and associated factors would lead to improve the disease control measures. Therefore, the current study aimed to carry out the following major objectives -

- Assessment of sero-prevalence of PPRV in non-vaccinated small-ruminants especially in sheep and goats in the selected areas of Dhaka division.
- Isolation and Identification of PPRV strains in small-ruminants circulating locally using PCR technique.
- Genetic characterization of the isolated PRRV compared to the other circulating virus strains across the prevalent countries and vaccine strain using phylogenetic analysis.
- Assessment of co-infections associated with PPRV infection and antimicrobial-resistance (AMR) profiling.

CHAPTER II
REVIEW OF LITERATURE

2.1. Introduction

Small-ruminants especially sheep and goats rearing play a crucial role not only in the production of high-quality meat but also raise the income of poor farmers at low cost. Currently, both governmental and non-governmental organizations in Bangladesh have actively been trying to utilize this resource for poverty alleviation initiatives and women's empowerment (Ogolla *et al.*, 2022). However, the sustainability of this practice faces threats from transboundary animal diseases (TADs) like *Peste des petits ruminants* (PPR), which significantly jeopardize the livelihoods of small-scale farmers across Asia and Africa (Getachew & Hamid, 2019). PPR is alternatively known as goat plague, kata, syndrome of stomatitis-pneumoenteritis, and ovine rinderpest (Parida *et al.*, 2015). In Bangladesh, the emergence of PPR outbreaks, rinderpest-like diseases, has been identified since 1993 despite the PPR was first reported in the Ivory Coast, West Africa in 1942 (Devi *et al.*, 2016). These outbreaks have inflicted severe losses on small-ruminant production, posing a huge threat to the small ruminant population in the country (Islam *et al.*, 2001). PPR not only diminishes food security by reducing meat and milk availability for household consumption but also undermines genetic resources crucial for sustaining livestock populations (Balamurugan *et al.*, 2023). Consequently, effective prevention and control measures against PPR are imperative to safeguard Bangladesh's small-scale farmers' economic viability and food security.

2.2. Etiology

2.2.1. Properties of PPR Virus

PPR virus (PPRV) known as small ruminant morbillivirus (SRMV), is an enveloped RNA virus under the genus Morbillivirus, which belongs to the family *Paramyxoviridae*, subfamily *Paramyxovirinae* and order *Mononegavirales* (Gibbs *et al.*, 1979). PPRV is closely related to other morbilliviruses including the Rinderpest virus (RPV), Canine distemper Virus (CDV) in dogs, Measles Virus (MV) in humans, Phocine Distemper Virus (PDV), Dolphin, Porpoise and Cetacean Morbillivirus (DMV, PMV, CMV) (Barrett *et al.*, 1993). PPRV, similar to the other members of the *Paramyxoviridae* virus family, is a pleomorphic entity with a lipoprotein membrane to envelop a ribonucleoprotein core, which contains an RNA genome

(Vinayagamurthy, 2017). PPRV is an RNA virus that is a single-stranded RNA of negative sense. The virus displays protruding spikes on its envelope, which consist of two viral glycoproteins: haemagglutinin (H) and fusion (F) proteins. These proteins play a vital role in initiating the initial stages of host cell infection by the virus (Diallo *et al.*, 1987).

2.2.2. PPRV Genome

PPR virions are enveloped, pleomorphic containing ssRNA as the genome, enclosed in a ribonucleoprotein (RNP) and core. PPRV has a genome length of 15,948 nucleotides, considered the longest of all of the morbillivirus members (Bailey *et al.*, 2005). However, the longest genome is 16,050 bases for feline morbillivirus (Woo *et al.*, 2012). Similar to other morbilliviruses, efficient replication of PPRV requires a genome length that is a multiple of six bases (Bailey *et al.*, 2005). This principle, first identified with the Sendai virus and recognized as "the rule of six," has been confirmed in the majority of *Paramyxovirinae* subfamily members (Calain & Roux, 1993). The PPRV genome comprises six genes or transcription units. Promoter sequences, which serve as binding sites for the viral RNA-dependent RNA polymerase (RdRP), are exclusively located at the 3' ends of both the genome (Genome Promoter or GP) and the antigenome (Anti-Genome Promoter or AGP) (Munir, 2014). The genes of the virus contain the nucleocapsid (N) protein, the phosphoprotein (P), the matrix protein (M), the fusion (F) and haemagglutinin (H) membrane glycoproteins, and the large (L) protein, which functions as the viral RNA-dependent RNA polymerase (RdRP). Additionally, within the P-gene, there are three non-structural proteins: V, W, and C (Munir, 2014).

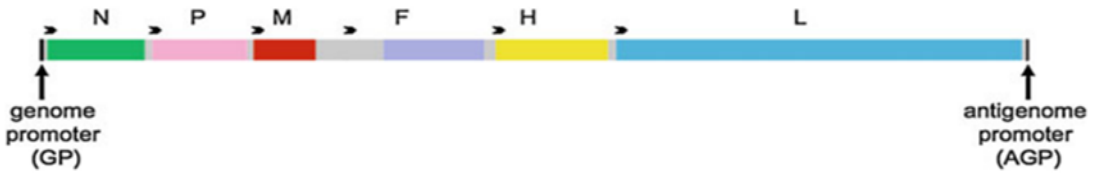


Figure-1: PPRV Genes/genome layout. Protein distribution coding sequences (colored), promoters (black), and untranslated regions (grey) are shown to scale. Arrowheads indicate the start transcription point for each gene.

2.2.3. Viral Proteins

2.2.3.1. Viral Structural Proteins

The PPRV genome has a length of 15,948 bp (nucleotides) although a variant virus with an additional 6 nucleotides was identified in a recent Chinese epizootic (Bao *et al.*, 2014). The genome is organized into six transcription units, encoding the nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin (H), and large (L) proteins in sequential order. The L protein in combination with the P protein constitutes the viral RNA-dependent RNA-polymerase (Munir, 2014).

2.2.3.2. Nucleocapsid Protein (N)

The PPRV N-protein is abundant, exhibits strong antigenic conservation, and serves as a primary constituent in the nucleocapsid core of the virion though it does not induce protective immunity against the virus (Sweetman *et al.*, 2001). Its essential functions encompass pivotal roles in both transcription and replication processes (Vinayagamurthy, 2017). The N-protein has been widely used in the development of diagnostic tests and has been classified into four lineages based on the N-gene, which reflects geographical origin (Munir *et al.*, 2012). The N-protein plays a crucial role in various stages of the virus life cycle, including virus assembly, encapsidation of genomic RNA, and replication and transcription. The length of the N-protein is 525 amino acids and consists of 1578 nucleotides. The initial 398 amino acids at the N-protein's amino terminal are conserved in all morbilliviruses and likely form the nucleocapsid's helical core.

2.2.3.3. Phosphoprotein (P)

The P-protein, the second most prevalent morbilliviral protein, serves two significant roles in the virus life cycle. Firstly, it acts as a structural component of the viral RNA-dependent RNA polymerase (RdRP), facilitating the polymerase's movement along the genome by interacting with N-proteins. Secondly, it functions as a chaperone during nucleocapsid assembly, preventing random self-assembly of the N-protein and maintaining its availability for incorporation into emerging viral genomes. The P-protein exhibits robust binding to both N and L proteins, forming oligomers of itself, and cross-linking studies on the RPV P-protein suggest the formation of a tetramer (Rahaman *et al.*, 2004).

2.2.3.4. Matrix Protein (M)

The M-protein is a small and highly conserved component located within the viral envelope, possessing an intrinsic ability to bind to lipid membranes (Haffar *et al.,* 1999). The open reading frame (ORF) for the PPRV M-protein spans nucleotide positions 3,438–4,442, resulting in a 335-amino acid protein with a predicted molecular weight of 37.8 kDa, it plays a crucial role in virus assembly, virion formation, and their release through budding (Wang *et al.*, 2017). The M-gene starts with the characteristic AGGA sequence at nucleotide (nt) 3,406 and ends with AAACAAAA, a conserved gene end in MV, RPV, and all PPRV strains (Muthuchelvan *et al.*, 2005). The M-protein's conservation is vital for progeny virus formation, and it interacts with glycoproteins in the cell membrane.

2.2.3.5. Fusion Protein (F)

F-protein adds viral envelope with the plasma membrane of the infected cell allowing the nucleocapsid to be released into the host cell cytoplasm. Viral protein expression leads to join neighbor cells. Without forming a virion, polynucleated syncytia is formed. Expression of F and H proteins is required for fusion effectively (Heminway *et al.*, 1994) although only F can do this fusion (Seth & Shaila, 2001). The F-gene in PPRV encodes a highly conserved GC-rich protein of 546 amino acids, with a predicted molecular weight of 59.137 kDa. This conservation enables extensive cross-protection among different morbilliviruses, allowing vaccines designed for one virus, like RPV, to immunize against PPRV. The shared sequence homology among paramyxoviruses underlies their common fusion property driven by the conserved F protein-based biological activity. PPRV's 5’ UTR, like other morbilliviruses, contains secondary stem-loop structures and potential initiation codons.

2.2.3.6. Haemagglutinin Protein (H)

The HN (or H) protein is among the least conserved proteins in morbilliviruses, with closely related ruminant viruses RPV and PPRV exhibiting only 50% amino acid identity despite having 609 amino acid residues in their proteins. The HN protein is a disulfide-linked homodimer, with its N-terminus as a signal peptide on the cytoplasmic side of the membrane and the C-terminus protruding on the outer side. Due to epitopic sites indicating immunodominance, the HN protein faces continuous immunological pressure from neutralizing antibodies (Vongpunsawad *et al.*, 2004).

2.2.3.7. Large Protein (L)

The L-protein in morbilliviruses, a massive 2,183 amino acid (6,949 nt) enzymatic subunit, serves as the essential RNA-dependent RNA polymerase (RdRP) responsible for viral replication. It contains conserved motifs associated with polymerases and likely participates in capping viral mRNAs (Poch *et al.,* 1990). The L protein is the most substantial among morbilliviruses, and owing to attenuation at every gene junction, the mRNA that encodes the L protein is notably the scarcest (Flanagan *et al.*, 2000).

2.2.4. Viral non-structural protein

In addition to the six structural proteins, Paramyxoviruses can also produce a range of non-structural proteins in infected cells. These are encoded by the P transcription unit in alternative reading frames.

2.2.4.1. Proteins V and W

The V and W protein arises from the P-gene through a frameshift mechanism, involving the insertion of a single G residue at a specific mRNA editing site. W-type transcripts, constituting only about 1–2% of the overall P-gene output in PPRV (Mahapatra *et al.,* 2003) and showing similar patterns in RPV (Baron *et al.,* 1993), are likely to be negligible components within infected cells. This conserved editing site (-5' -TTAAAAGGGCACAG-3') is present in the P-gene of morbilliviruses, and in PPRV, it is situated at positions 742–756. The length of the V-protein varies among morbilliviruses, with PPRV having 298 amino acids, CDV and RP having 299, and MV and DMV having 300 and 303 amino acids, respectively. The predicted molecular mass of the V-protein is 32.28 kDa, with a predicted iso-electric point of 4.68.

2.2.4.2. Protein C

The C-protein, translated from the P-gene's second initiation codon, is a short protein with 117 residues and a predicted molecular weight of 20.11 kDa in PPRV. While it lacks phosphorylation, it can be found in both cytoplasmic and nuclear compartments in MV-infected cells (Barrett *et al.,* 2006). The C-protein's role in viral RNA synthesis involves binding to the L-protein, impacting viral mRNA synthesis and replicative transcription.

Although the RPV C-protein positively influences viral RNA transcription, MV C has been reported to decrease all types of viral RNA transcription (Patterson *et al.*, 2000).

Table-1: Nucleotide (nt) and amino-acid (aa) comparison for open reading frames of each gene of PPR

Virus strain	**Rinderpest**		**Measles Virus**		**Canine Distemper Virus**		**Dolphin Morbillivirus**		**Post-translational modification**
Protein/ level	nt	aa	nt	aa	nt	aa	nt	aa	-
N	66.2	72.9	66.8	73.5	62.5	68.5	66.2	72.9	Glycosylation
P	62.4	50.5	60.4	45.1	56.6	45.3	61.6	49.1	Phosphorylation
C	58.8	41.8	53.2	40.3	53.1	35.0	58.5	37.2	-
V	61.9	45.1	59.0	41.3	54.2	40.4	59.0	43.5	Phosphorylation
M	72.2	66.1	74.0	68.2	73.1	60.3	69.1	64.0	-
F	68.0	73.8	67.0	71.7	50.2	54.2	65.9	73.3	Glycosylation
H/N	55.5	39.4	53.5	34.5	47.2	28.4	52.9	37.3	Phosphorylation
L	68.1	75.6	68.1	75.1	64.8	71.1	67.3	73.7	Glycosylation
Complete Genome	63.7	-	63.4	-	58.5	-	62.0	-	-

Note: aa = amino acid, nt = nucleotide

2.2.5. Comparative Genome Analysis

The genetic arrangement of Peste des Petits Ruminants Virus (PPRV) corresponds to that of fellow morbilliviruses in the same genus. Although there is a slight diversity in genome length, all genera members, including PPRV, possess genomes shorter than 16 kb. Morbilliviruses are classified in the subfamily Paramyxovirinae within the family Paramyxoviridae. In this subfamily, all genera exhibit a shared genome structure, except for the Rubulavirus genus, which includes an extra SH (small hydrophobic) gene (Munir *et al.*, 2012).

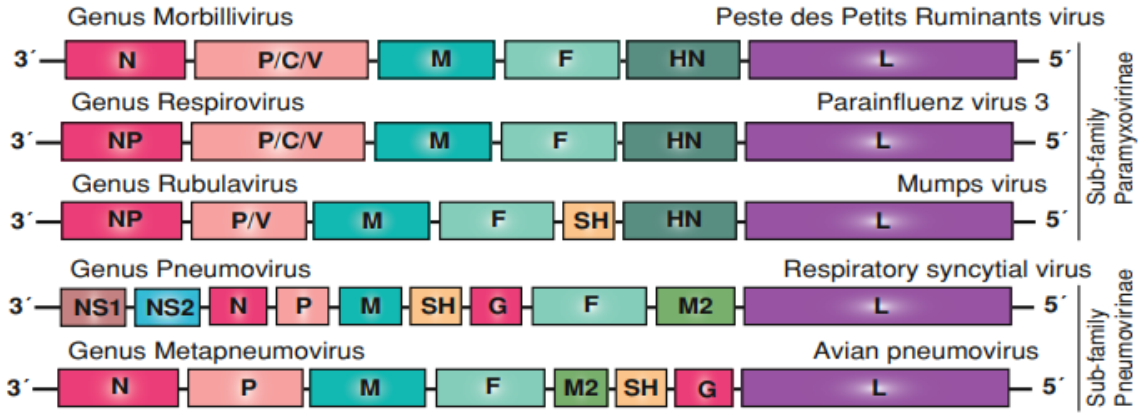


Figure-2: The genome organization of representative members of each genus of two subfamilies within the family Paramyxoviridae

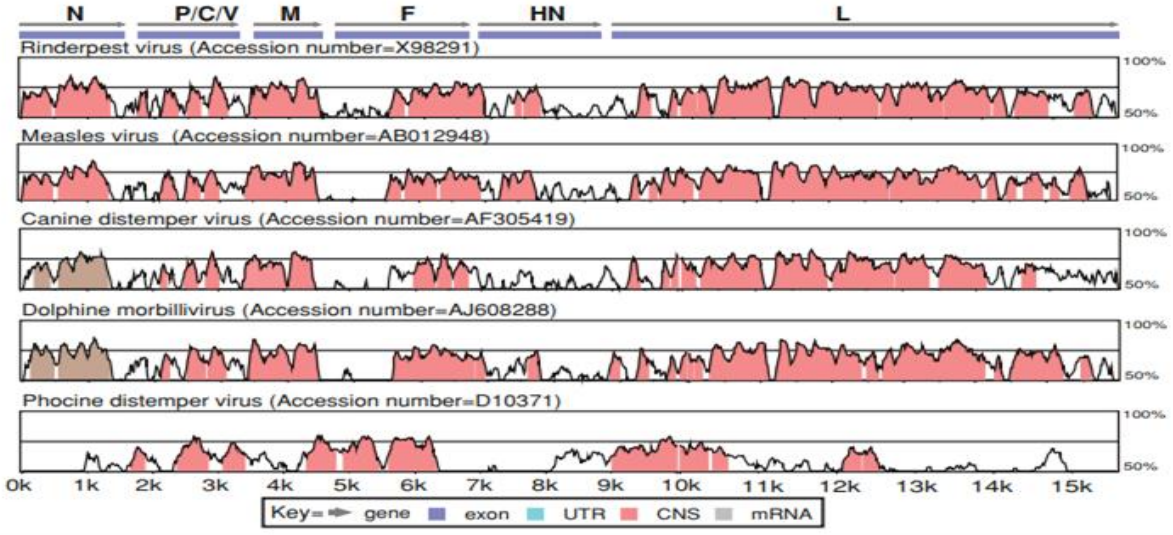


Figure-3: Global pairwise sequence comparison of PPRV with selected strains of rinderpest, measles virus, canine distemper virus, dolphin morbillivirus, and phocine distemper virus.

2.2.6. Molecular Biology of PPRV

Upon infection, PPRV induces classical cytopathic effects (CPE) within 3–5 days, leading to cell rounding, grape-bunch clusters, vacuolation, granulation, and syncytia formation. The virus exhibits sensitivity to ether but demonstrates stability within a pH range of 5.8–10.0 and is inactivated at 50 °C for 60 minutes (Vinayagamurthy, 2017).

The replication cycle begins with viral attachment to the cell membrane, facilitated by the H-protein's interaction with sialic acid receptors. Subsequent fusion, mediated by the F-protein,

releases genetic material into the cell. Replication takes place in the cytoplasm, with the viral genome encapsulated by the N-protein to form ribonucleoproteins (RNPs). The polymerase complex, comprising P-and L-proteins, transcribes the viral genome, producing a full-length anti-genome RNA, ultimately leading to viral egress from the host cell (Parida *et al.*, 2015).

Molecular characterization and phylogenetic analyses have identified four PPRV lineages, each with distinct geographical distributions. Lineages I and II are prevalent in West Africa, while lineages III and IV are found in Ethiopia, Yemen, Sudan, Oman, Burundi, the Arabian Peninsula, the Middle East, and Southeast Asia. Co-circulation of multiple lineages has been observed in Nigeria and China, indicating complex viral dynamics (Altan *et al.*, 2019). The prevalence of PPRV lineages, with specific strains and isolates varies across continents and regions, contributing to the understanding of the virus's global epidemiology (Altan *et al.*, 2019). In India, PPRV primarily involves lineage IV strains/isolates, with sporadic reports of lineage III (PPR TN/92) (Balamurugan *et al.*, 2010).

In Bangladesh, the prevalence of Peste des petits ruminants virus (PPRV) lineage IV is notable, resulting in substantial infections among goats and sheep (Rahman *et al.*, 2018). A unique sub-cluster within lineage IV has been observed in recent isolates (Rahman *et al.*, 2016). Molecular epidemiological investigations have substantiated the prevalence of lineage IV PPRV strains inside the nation, which exhibit a similar resemblance to strains identified in adjacent areas (Rahman *et al.*, 2023). Furthermore, it has been verified by molecular identification and sequencing that the PPRV strains obtained from outbreaks in Bangladesh between 2012 and 2015 are classified as lineage IV (Nooruzzaman *et al.*, 2021).

2.3. Pathogenesis

The PPRV enters animals' bodies through their oral and nasal passages by air droplets. After entering the organism, the virus replicates within the nasopharyngeal/respiratory epithelium initially (Yanagi *et al.*, 2006). The viral replication and the pathogenicity of this virus depend on many factors such as host resistance, immune response, parasitic infection of the host, nutritional level, breed, sex, and age of the host animal (Munir *et al.*, 2012). Additionally, host genetics and non-genetic factors may play a role in determining the immune response to the virus (Vinayagamurthy, 2017).

The PPRV has an affinity to lymphoid and epithelial tissue of the gastrointestinal and respiratory tracts (Haffar *et al.*, 1999). Through the blood and lymph (Primary viremia), the

virus is disseminated throughout the body including the skin, kidney, and GI tract (Vinayagamurthy, 2017). Clinical signs of PPRV infection emerge synchronously with the virus replication in the target tissues (Brown & Torres, 1994).

Dramatic cytolytic changes occur in primary lymphoid organs and epithelial tissues. The virus is detected in the cytoplasm of bronchial and bronchiolar epithelium with the highest concentration in the respiratory epithelium, including the trachea, bronchi, and bronchioles (Vinayagamurthy, 2017). The virus is also found in desquamated bronchial and bronchiolar epithelial cells, pneumocytes, macrophages, and other leukocytes in the respiratory tract lumen (Kumar *et al.*, 2004). PPRV antigens are detected in various tissues and organs of infected goats, including the oral cavity, sebaceous glands, cryptal and villous epithelium of the small and large intestines, Peyer's patches, mesenteric lymph nodes (MLNs), and lymphoid follicles (Vinayagamurthy, 2017). B-cells in circulation and lymphoid organs may undergo polyclonal activation during secondary viremia. The response to challenge is likely mediated by interferon and other cytokines rather than humoral antibodies (Olaleye *et al.*, 1989). Neutralizing antibodies appear with the development of clinical signs and tissue damage. Secondary viremia is associated with the onset of the prodromal phase of infection (Parida *et al.*, 2015). The ability of the PPR virus-affected cells to fuse probably leads to the formation of multinucleated giant cells (Rudra, 2019). The destructive phase of the virus on lymphocytes is suggestive of a cause of lymphopenia (Scott, 1981). The virus appeared to be released through the microvilli of the epithelial cells (Rudra, 2019). The incubation period of the disease is 4-5 days in a seronegative herd but may range between 6-10 days (Diop *et al.*, 2005). Viremia may develop 2-3 days after infection and 1-2 days before the first clinical sign appears. Subsequently viremia results in the dissemination of the virus to the spleen, bone marrow, and mucosa of the gastrointestinal tract and the respiratory system (Chauhan *et al.*, 2009).

2.4. PPR Outbreak History and Global Distribution

PPR is known as Goat plague, a highly contagious viral disease of small ruminants including sheep and goats (Ahaduzzaman, 2020). It is widely distributed to sub-Saharan Africa, the Arabian Peninsula, the Middle East, and major parts of the Indian subcontinent (Banyard *et al.*, 2010). PPRV has caused major outbreaks and is considered endemic across these regions (Dhar *et al.*, 2002). PPR is free in Europe, Australia, the USA, and many other countries

including Canada, and Brazil (WOAH, 2023). However, it is an emerging threat to European countries (Parida *et al.*, 2016).

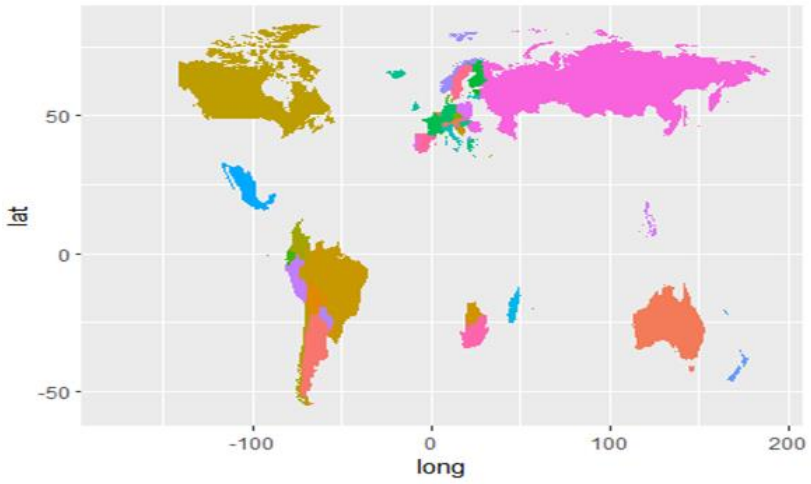


Figure-4: Countries free of PPR around the world (WOAH, 2023).

The PPRV genome is a unique negative single-strand RNA molecule with six structural proteins that elicit protection against the disease in infected or vaccinated animals (Diallo *et al.*, 2002). The viral genomic RNA and N, P, and L-proteins form the ribonucleoprotein (RNP), which is the minimal essential structure for viral replication in cells (Bailey *et al.*, 2007). However, PPRV lineage differentiation is based on comparing the genetic sequences of the F or N-genes depending on reaction components (Couacy-Hymann *et al.*, 2002). Historically, African PPRV isolates were classified into three lineages: lineage-I in West Africa, lineage-II in Ghana, Mali, and Nigeria, and lineage-III in Ethiopia and Sudan (Dhar *et al.*, 2002). Today, PPRV strains can be divided into four different lineages (Parida *et al.*, 2015). The three first lineages (lineage-I,-II, and-III) were isolated in Africa based on the spread of the virus from West to East Africa (Banyard *et al.*, 2010). Lineage-I is detected in Western Africa, Lineage-II is isolated from the Ivory Coast, Guinea, and Burkina Faso, and Lineage-III is found in Sudan, Yemen, Qatar, Oman, and East Africa. The fourth lineage (Lineage-IV) was recently detected in Asia, including the Arabic peninsula, India, Southern Asia, African territories, and Turkey (Munir, 2011).

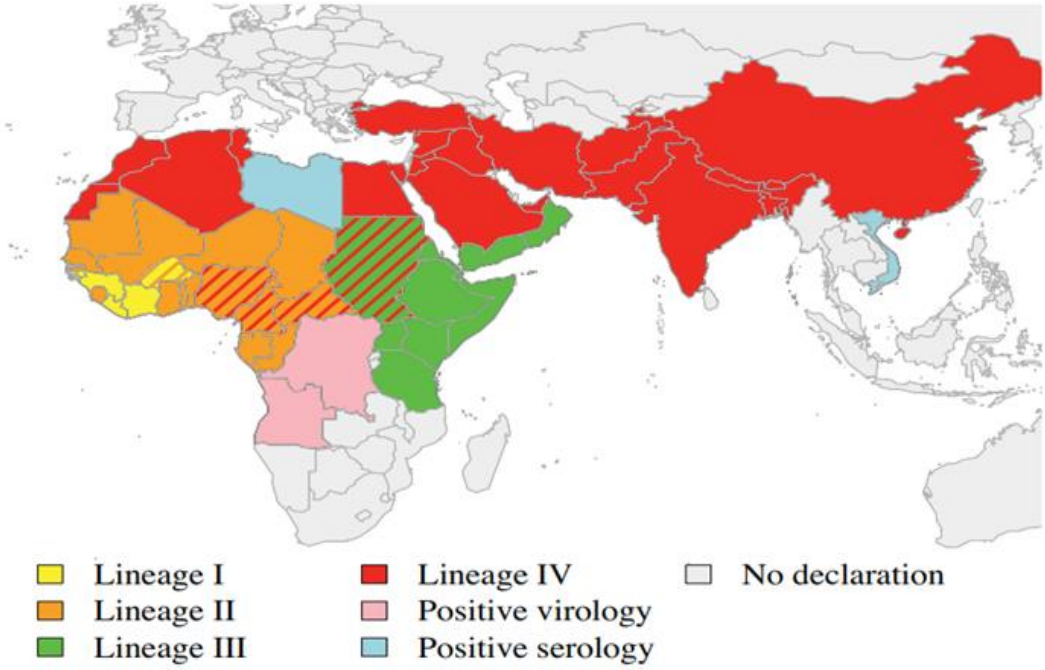


Figure-5: This map depicts the global distribution of the four distinct lineages of the Peste des Petits Ruminants (PPR) virus. Each lineage is represented by a unique color, and hatched bars identify the most recently identified lineage within each country (Albina *et al.*, 2013).

2.4.1. West Africa

West Africa comprises 16 countries with 5 million square kilometers of area (Banyard *et al.*, 2010). Several countries in West Africa have faced a significant number of outbreaks of PPRV. The first PPRV case was reported in the Ivory Coast in 1942 (Ahaduzzaman, 2020). It is currently believed to be endemic across much of West Africa (Banyard *et al.*, 2010). In Faso, RT-PCR testing of goats and sheep showed 53.84% positive for PPRV and genetic analysis revealed that the PPRV is from lineage-IV, which is closely related to samples from Nigeria and Cameroon (Couacy-Hymann *et al.*, 2023). This is the first identification of lineage-IV in Burkina Faso in 2021, suggesting its expansion from Nigeria to Burkina Faso, Niger, and Mali (Biguezoton *et al.*, 2022). However, the Virus from an outbreak in Burkina Faso in 1999 fell into the lineage-1 group (Dhar *et al.*, 2002). A high seroprevalence, almost 50%, for PPRV in small-ruminants was reported in the North East zone of Nigeria. The phylogenetic tree showed the presence of lineage-I, II and-IV. Lineage-I is similar to the vaccine strain Nigeria 75/1 based on the F-gene sequence. However, if it is based on N-gene sequencing it could be lineage-II (Esonu *et al.*, 2022). In Ghana, the prevalence of PPR was 6.84% between 2005 and 2013 in small-ruminants. The highest number of outbreaks of PPR

occurred in 2011, followed by 2012 and 2010 (Folitse *et al.*, 2017). The full genome of a PPRV isolated from a sheep lung sample collected in Ghana, Western Africa, in 2010, has been sequenced. Phylogenetic analysis demonstrated that the virus clustered within the lineage-II (Dundon *et al.*, 2014). In Benin, PPR was identified in early 1940s and it was called *peste des espèces ovine et caprine* (Plague of ovine and caprine). In 2011, PPRV was detected by using a cell culture technique and genetic analysis of the partial N-gene sequence showed that it was lineage-II (Dundon *et al.*, 2020). The positive result of PPRV detection was confirmed between 1988 and 1989 while rinderpest vaccination was conducted in Gambia. Then, PPR in goats was identified as belonging to lineage-II which was shown by N-and F-gene sequencing (Dundon *et al.*, 2020). In Guinea-Bissau, there are four nucleotide sequences available in GenBank between 1988 and 1991 and the isolated strains are under lineage-I (Dundon *et al.*, 2020). Lineage-II was isolated from Liberia and the first characterization of PPRV was reported in 2015 (Boussini *et al.*, 2016).

2.4.2. East Africa

East Africa mainly consists of Ethiopia, Sudan, Tanzania, Somalia, Uganda, and a few more countries (UN, 2013). PPRV was suspected for the first time in 1977 in goats and the clinical and serological evidence of PPRV was reported by Taylor (1990), it was confirmed in Ethiopia in 1991 (Abraham *et al.*, 1994). The survey conducted in 1994, 1996, and 1997 showed that the PPRV was present in both sheep and goats and the genetic typing of PPRV was lineage-III isolated in 1996 (Banyard *et al.*, 2010). In Sudan, PPR is considered an endemic disease, similar to other countries in East Africa, with a high prevalence of 58.1% to 93.8%. In 1971, the first report of PPR was recorded in Sudan followed by 1972 in Central Sudan and Khartoum (Salih *et al.*, 2014). In Tanzania, samples collected in 2004 showed that there were antibodies against PPRV, indicating the presence of PPRV during Rift Valley Fever and PPR surveys (Wambura, 2000). Later, it was officially confirmed in Tanzania in 2008 and was restricted to the northern zone in districts bordering Kenya (Kivaria *et al.*, 2013).

2.4.3. Central Africa

Central Africa includes Burundi, the Central African Republic (CAR), Angola, Cameroon, Chad, Gabon, the Democratic Republic of the Congo (DRC) and Rwanda. In Central Africa, as in other parts of the continent, it is also considered endemic (Abraham *et al.*, 2005). The

first outbreak report received in Angola in 2012 (OIE, 2012), Burundi in 2017 (Niyokwishimira *et al.*, 2019), Cameroon in 1997 (Banyard *et al.*, 2010), Chad in 1995 (Bidjeh *et al.*, 1995), Central African Republic in 2004 (Dundon *et al.*, 2020), DRC in 2012 (OIE, 2012), and Gabon in 2007 (Banyard *et al.*, 2010). Genome sequences of PPRV in these nations revealed that Lineage-IV was found in Angola, Cameroon, Central African Republic, Gabon, Lineage-III in Burundi (close to Kenya, Uganda, and Congo), Congo (Lineage-II and Lineage-III) (Dundon *et al.*, 2020).

2.4.4. North Africa

The first outbreak was recorded in Egypt, a North African country in 1990 (Ismail & House, 1990). Egypt also experienced outbreaks in 1993 and 2010 (Abd El-Rahim *et al.*, 2010). Gene sequence revealed that the lineage-IV was found in 2010, followed by 2012, 2014, and 2015 (Elhaig *et al.*, 2018). Until 2008 Morocco was considered one of the PPR-free countries in the world. However, the first report of PPR was filed in 2008 (Kwiatek *et al.*, 2011). The complete genome sequence explaining the PPRV found in Morocco was Lineage-IV (Muniraju *et al.*, 2013). During 2008-2011, Morocco actively worked to eliminate the virus through three successive vaccinations there. However, later in 2015, another lineage-IV PPR outbreak was recorded which is close to Algeria, and Tunisia, suggesting that the possible reason is transboundary transmission (Fakri *et al.*, 2016).

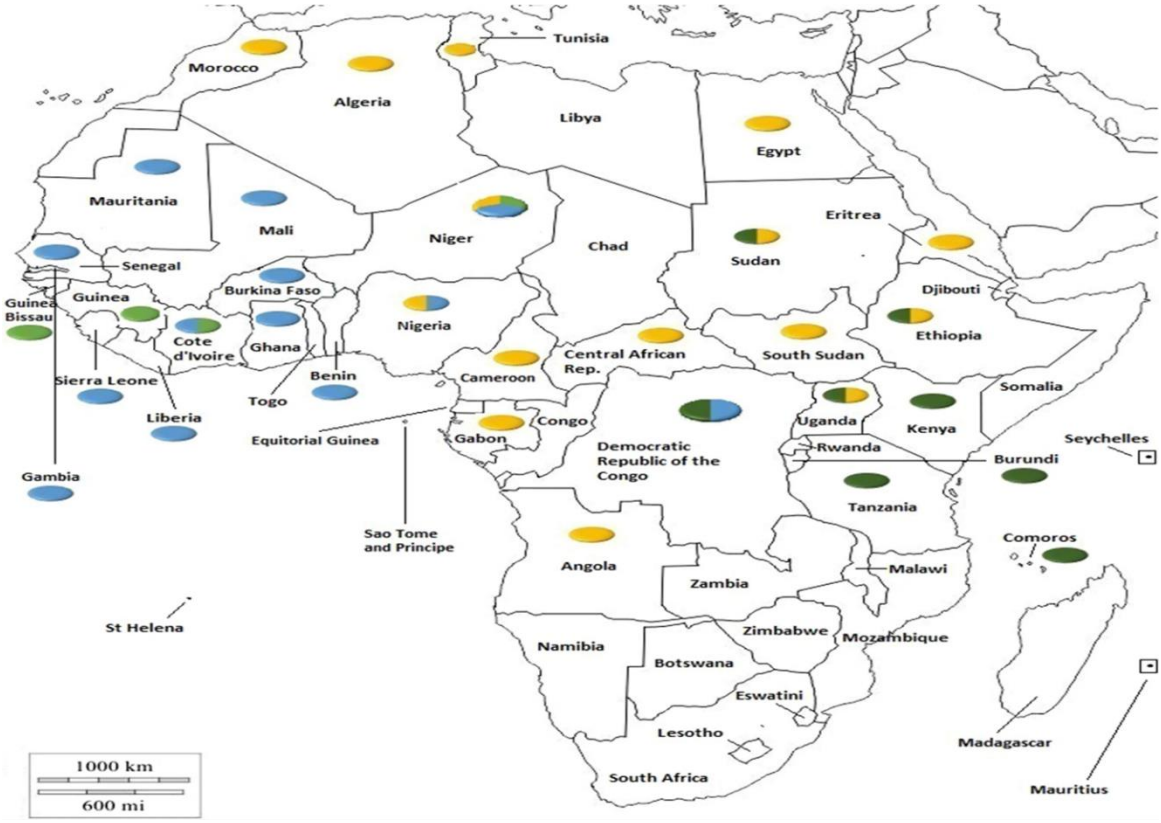


Figure-6: Distribution of PPRV lineages in Africa. Colors in the circles indicate the viral lineages reported in the country: lineage-I, light green; lineage-II, blue, lineage-III, dark green; lineage-IV, yellow (Dundon *et al.*, 2020).

2.4.5. Asia

2.4.5.1. The Middle East

In Iraq, the presence of Peste des Petits Ruminants (PPR) in sheep and goats was first documented in 1997 with high seroprevalence rates (Saoud, 1997). The first official outbreak of PPR in Iraq occurred in 1998 (Khoran *et al.*, 2021). Despite vaccination campaigns and efforts to control the disease, PPR outbreaks continued, and the virus was found in various parts of the country (Benfield *et al.*, 2023). PPR also affected wild goats and other species including deer, cattle, and buffalo (Benfield *et al.*, 2023). In 2019, Iran had the fifth largest sheep population globally with over 41 million sheep and 15 million goats (FAOSTAT, 2022). Since the first documented outbreak of PPR in 1995, PPR has posed a significant economic and health threat in the country (Bazarghani *et al.*, 2006). PPR isn't confined to domestic small-ruminants; it has also impacted Iranian wildlife including vulnerable wild sheep and goats (Marashi *et al.*, 2017). Multiple outbreaks in national parks between 2014 and 2016 led

to over 1,000 wild sheep and goat deaths with suspicions of PPR involvement dating back to 2000 (Marashi *et al.*, 2017). Additionally, a PPR outbreak in a camel herd was also documented in 2013 (Bazarghani *et al.*, 2006).

In Kuwait, PPRV was reported in 1989 for the first time and until 2007 it stayed unreported for this long time (Benfield *et al.*, 2023). Genomic analysis showed that the virus that was identified in 1999 belongs to the Lineage-IV (Dhar *et al.*, 2002). PPR was reported in Lebanon in 1978 and it was reported also in the 1990s (Attieh, 2007). Several reports in 2004-2006 displayed that prevalence was high in sheep and goats and present in cattle at the rate of 13.5% (Hilan *et al.*, 2006). The first report of PPR presence in Oman dates back to 1978 (Hedger *et al.*, 1980). In 1983, strains belonging to lineage-III were detected both in goats and wildlife (Muniraju *et al.*, 2014). In Palestine, PPR is considered an endemic disease with 850 outbreaks reported there between 2005 and 2017 (Alzuheir, 2019). PPR has never officially been reported in Qatar. However, the first was in 2010, when viruses belonging to lineage-III and-IV were identified in goats (Banyard *et al.*, 2010). Peste des Petits Ruminants (PPR) is legally classified as a notifiable disease in the Syrian Arab Republic, but no confirmed cases have been reported for several decades.

PPRV strains in the UAE have been classified as lineage-III in 1986 and later as lineage-IV in more recent sequencing studies conducted by various institutions (Muniraju *et al.*, 2014). PPRV was initially isolated in the eastern part of Saudi Arabia (KSA) in 1988, although suspicions of its presence date back to the late 1970s based on clinical and serological evidence in sheep, deer, and gazelles (Abu-Elzein *et al.*, 1990).

2.4.5.2. South East and East Asia

Between July and November 2007, a series of outbreaks of Peste des Petits Ruminants (PPR) were observed in domestic small-ruminants (SR) in southwestern Tibet, China (Wang *et al.*, 2009). Strict control measures were implemented leading to no evidence of PPRV circulation in Tibet by 2010. However, PPR re-emerged in Xinjiang province in late 2013 and quickly spread throughout the country in 2014, affecting more than 22 provinces, autonomous regions, and municipalities (Liu *et al.*, 2018). This rapid spread was attributed to the transportation of infected sheep and goats from Xinjiang to various markets across China in the spring of 2014 (Liu *et al.*, 2018). Phylogenetic analysis indicated that the Tibetan strains from 2007-2008 were more closely related to viruses found in India in 2014-2016 than to

those circulating in China since 2013 (Bao *et al.*, 2017). Additionally, PPRV infections in Chinese wildlife have been reported over the years. During the Tibetan outbreak, several wild animals, including bharals, ibexes, argali sheep, and goitered gazelles, tested positive for PPRV.

2.4.6. Europe

Europe is considered a PPR-free continent while the first PPR outbreak was reported in Bulgaria on 23rd June 2018 (Altan *et al.*, 2019). In Turkey, PPRV infection was first officially reported in southern and eastern Anatolia in 1999, and approximately 1,000 (997) PPR outbreaks in Turkey from 1999 to 2018 (Yesilbag *et al.*, 2005). These outbreaks reached a climax in 2007 and 2011, especially in the Marmara and Aegean regions of Turkey. In Thrace, Turkey PPRV infection was reported in Istanbul in 2000 followed by Edirne (bordering Greece) in 2004 and Kırklareli (bordering Bulgaria) in 2006. The last outbreak was reported in 2014 on the European border in the Thrace region, and a total of 55 outbreaks have been reported in this region since the first report of the disease (Altan *et al.*, 2019).

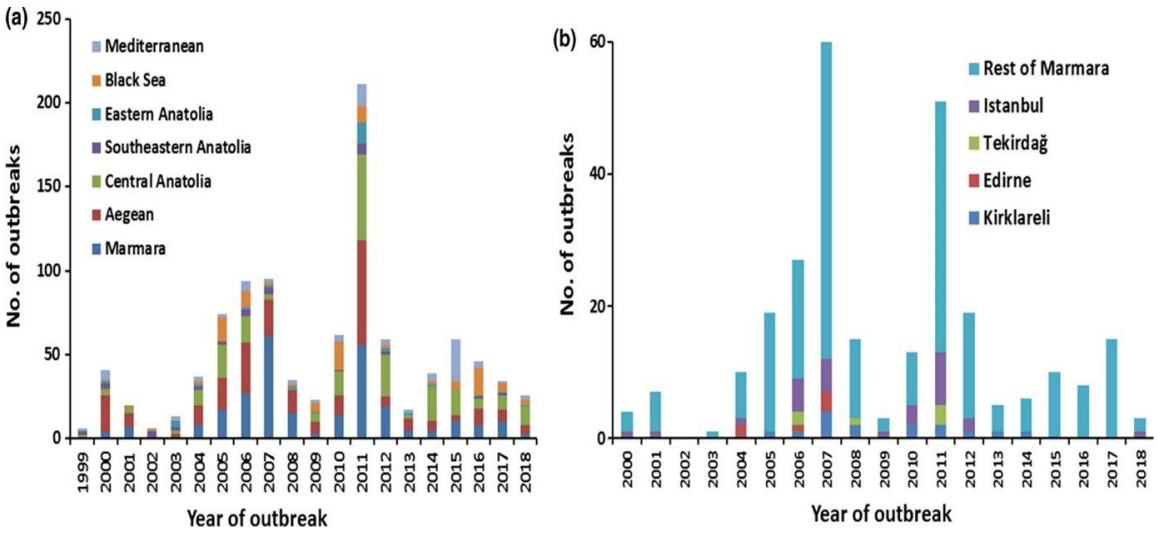


Figure-7: (a) PPR outbreaks reported in different regions of Turkey from 1999 to 2018 (2018: January to June). All data were taken from OIE's official website (http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail). (b) PPR outbreaks were reported in different provinces in the Thrace region and the rest of Marmara of Turkey from 2000 to 2018 (2018: January to June).

Source: (http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail).

2.4.7. South Asia

The first PPR outbreak was documented in 1994, and several outbreaks have been faced in Nepal (Khakural, 2003). First 5 years after recording the 1st outbreak, it became rampant such that ended up being endemic in 68 out of 75 districts of Nepal (Acharya *et al.*, 2018).

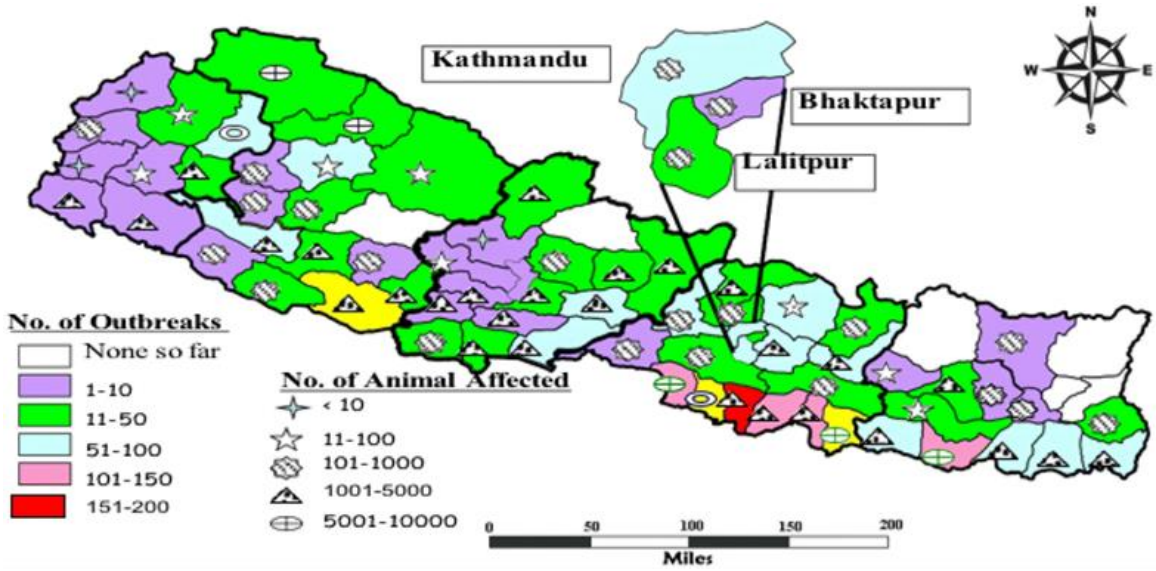


Figure-8: Spatial distribution of PPR outbreaks and number of animals affected, in Nepal during 2000–2015 (Acharya *et al.*, 2020).

In Pakistan, PPRV was suspected by observing clinical signs and post-mortem lesions without performing any laboratory diagnosis procedure in 1991 when Rinder pest-like disease was reported in Punjab (Khokhar *et al.*, 2003), a phylogenetic tree analysis of the N-gene revealed that in Pakistan all the PPRV identified are under Lineage-IV (Anees *et al.*, 2013). Another study showed that all Pakistani PPRV strains regardless of the gene used either the F-gene or N-gene are grouped in lineage-IV, which is the most prominent and prevalent lineage in Asia (Abubakar *et al.*, 2018). In Pakistan, PPR is endemic and increasingly affected newer areas since the first outbreak (Ullah *et al.*, 2022).

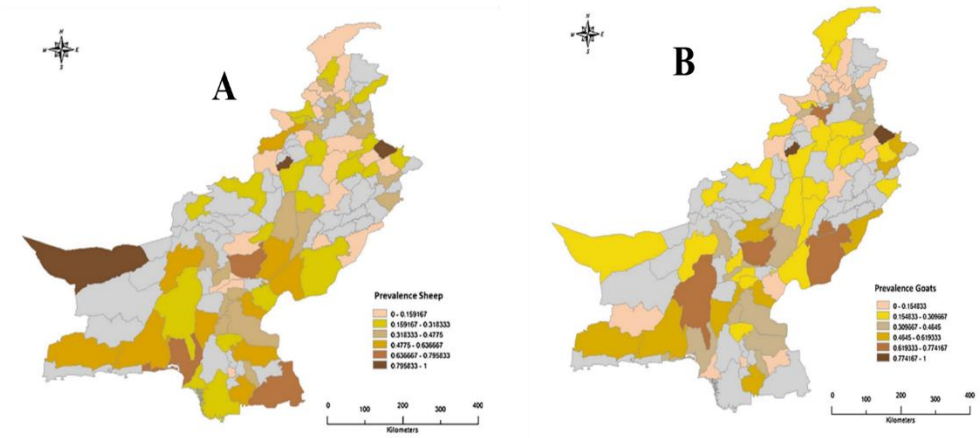


Figure-9: Geographical distribution of PPR in sheep (A) and Goat (B) populations of Pakistan. Regions in gray were not included in the study (Abubakar *et al.*, 2017).

In India, PPR was first identified in Tamil Nadu state in 1987 and thought that the virus was restricted to southern India until severe epidemics affected the rest of India in 1994 (Kulkarni *et al.*, 1996). Then, the disease became endemic in many states in India (Balamurugan *et al.*, 2014).

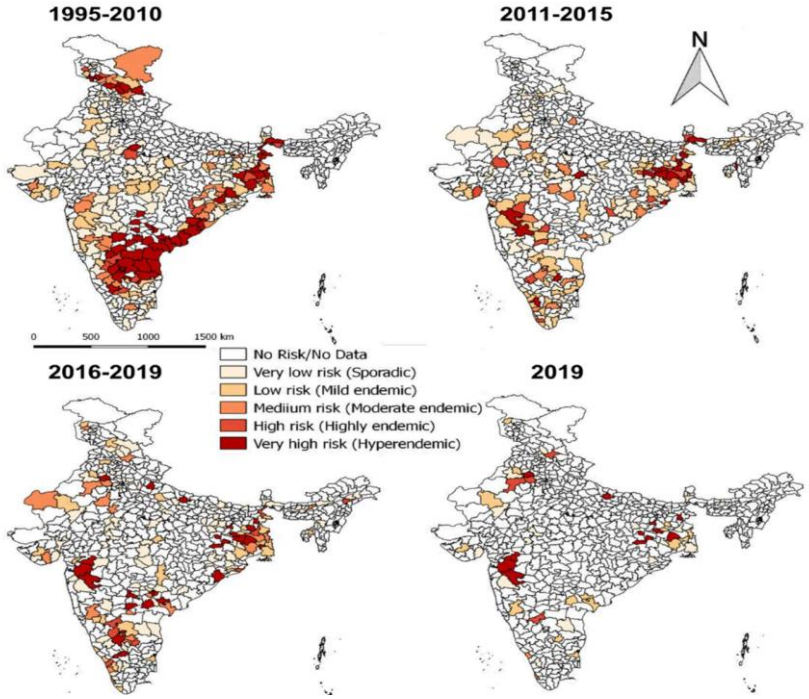


Figure-10: Endemic districts of PPR in different states of India (1995–2019). The endemicity categorization is based on the scale of the cumulative outbreaks, that occurred in the districts per year in the given period of analysis and classified into the categories of risk, if the outbreak numbers in the district as 0 –no risk, 1- very low, 2- low, 3- medium, 4- high and >4 very high-risk districts depicted in the map (Balamurugan *et al.*, 2021).

In India, it is shown that a high degree of genetic similarity among isolates/strains within Asian lineage-IV, with nucleotide sequence identities ranging from 97.7% to 100% and amino acid sequence identities ranging from 97.7% to 99.8% (Balamurugan *et al.*, 2010). The phylogenetic analysis based on amino acid sequences revealed that all the PPRV isolates belonged to lineage-IV, consistent with earlier observations that only lineage-IV has been circulating in India since the disease was first reported (Mishra *et al.*, 2020).

In Bangladesh, PPR, was first observed in goats in 1993 and has been considered endemic in the country since then (Islam *et al.*, 2001). Previous studies on PPR in Bangladesh primarily focused on serology or clinical symptoms, with only a few recent publications involving genetic characterization (Rahman *et al.*, 2016). In 2008, the overall sero-prevalence of PPR was 21%, with variations between 6% and 49% in different geographic locations or districts within Bangladesh (Bhuiyan, 2012). A study conducted in 2009-2010, which confirmed PPR outbreaks in Black Bengal goats showed high morbidity and mortality rates with 75% and 59%, respectively, and a case fatality rate of 74% (Chowdhury *et al.*, 2014). Genome sequencing of three PPRV isolates from 2008 (BD2), 2015 (BD12), and 2017 (BD17) along with full-length sequencing of the nucleocapsid (N), matrix (M), and fusion (F) genes from seven more samples collected between 2015 and 2020 revealed through phylogenetic analysis that all ten PPRVs from Bangladesh are belonging to the lineage-IV. Additionally, these strains were found to be closely related to PPRV variants identified in China and Tibet during 2007-2008 and in India during 2014-2018 as well (Nooruzzaman *et al.*, 2021).

2.5. Sero-surveillance

2.5.1. Seroprevalence of Bangladesh

PPR was initially identified in goats in Bangladesh in 1993, becoming endemic after that with most studies relying on serology or clinical signs while recent publications include genetic characterization (Rahman *et al.*, 2016). Phylogenetically, PPRV strains in Bangladesh fall within lineage-IV, forming a distinct subgroup closely related to the China-Tibet/07 and Indian/TN/VEL/2015 PPRV isolates. The overall sero-prevalence of PPR in 2008 was 21%, varying from 6% to 49% across different geographical locations or districts in Bangladesh (Bhuiyan, 2012). In 2010, Bangladesh recorded around 84,000 veterinary clinical cases of Peste des Petits Ruminants (PPR). Yet, this figure may not accurately reflect the true burden of the disease since not all cases were laboratory-confirmed. Presumptive diagnoses by veterinarians alone carry only a moderate predictive value (Chowdhury *et al.*, 2014). Rahman

et al., (2023) showed that phylogenetic analysis and nucleotide sequencing of the PPR virus based on F-and H-gene. The strains were related to Lineage-IV with genetic divergence (Rahman *et al.*, 2023). A study revealed that the mortality and morbidity rate was 75% and 59%, respectively with a case fatality rate was 74%, and sequencing results showed that the strains resembled Asian isolates (Chowdhury *et al.*, 2014). From 2012 to 2015, several outbreaks in Bangladesh were detected where partial and whole genome sequencing was done, which showed that 98.2% - 99.99% was similar to the Indian isolates. These findings depict that transmission of this virus was held between both countries (Clarke *et al.*, 2018). RT-PCR test confirmed PPRV circulation in various regions. Sero-prevalence analysis revealed that high susceptibility in areas like Chuadanga (47.81%), Satkhira (56.92%), and Jhenaidah (33.33%), emphasizing the urgent need for targeted PPR vaccination to align with global control strategies and contribute to Sustainable Development Goals (Yousuf *et al.*, 2019). Another study showed the seroprevalence was 68.4% in goats and 79.7% in sheep with an overall average of approximately 55.1% of cases testing seropositive within vaccinated flocks. However, unvaccinated flocks showed a lower seropositivity of about 16.8%. These findings suggest a previous PPRV attack in the region (Hasan, 2012).

Between October 2014 and March 2017, 2420 sheep serum samples were collected in Bangladesh to detect antibodies against PPRV by cELISA showing a 44.3% seropositivity rate. Factors like crossbreeds (60%), males (69.8%), imported sheep (74.3%), and winter season (57.2%) were associated with increased seroprevalence (Rahman *et al.*, 2023).

2.5.2. PPR in Wild animals

Wild artiodactyl species show susceptibility to PPRV infection, evidenced by serological detection of antibodies, and outbreaks in Asia are often attributed to spillover from infected domestic small-ruminants causing sporadic wildlife epidemics. However, there's no reported spillback from wildlife to livestock (Aguilar *et al.*, 2020). Studies from Sudan identified the PPRV in Nile lechwe in 2008, and in dorcas gazelles in 2016–2017 though the clinical, pathological, epidemiological, and laboratory details related to these are incomplete (Asil *et al.*, 2019). A study conducted in Kenya and Tanzania revealed that the prevalence of PPRV in 11 species was 19.7% though the body condition of these animals was from good to very good (Jones *et al.*, 2021). In Tanzania, having a high prevalence of African buffalo, Topi, Kongoni, and Grant’s gazelle is associated with 61.1% prevalent overall (Jones *et al.*, 2021). In 2018-2019, seroprevalence of Peste des Petits Ruminants (PPR) was detected in African

buffalo (12.0%), Grant's gazelle (1.1%), and Thomson's gazelle (25.2%) based on cELISA results in Kenya and Tanzania. Additionally, a percentage of animals in each species showed doubtful results (Jones *et al.*, 2021).

2.6. Epidemiology

2.6.1. Host Range

The primary host for PPRV is small ruminants including goats and sheep, which are the main natural hosts for the virus (Özkul *et al.*, 2002). Though PPRV causes infection in sheep and goats, the clinical symptoms are more severe in goats than sheep (Singh *et al.*, 2004). It affects other species including Camels and wild small ruminants. Serological evidence of PPRV in Camel was reported in Nigeria (Woma *et al.*, 2015). PPRV was also reported in Camel in Ethiopia, Egypt, Turkey, and Sudan (Saeed *et al.*, 2010). PPRV antibodies were observed in both small and large ruminants in the field conditions and experimentally (Abubakar *et al.*, 2017). However, in camel, the disease was not successful to produce experimentally (Wernery, 2011). PPRV is not as pathogenic in Cattle, domestic, and wild African buffalo (*Syncerus caffer*) as in sheep and goats (Abraham *et al.*, 2005).

In Senegal, the seroprevalence of PPRV in cattle and small-ruminants was as high as 80% without showing any clinical signs in cattle (Baron *et al.*, 2016). However, a high fatality rate was observed in India in domestic buffalo (*Bubalus bubalis*) while the virus was inoculated experimentally (Baron *et al.*, 2016). A study found that when pigs were exposed to the PPR virus or infected through contact with sick goats, they only developed antibodies (seroconversion) but didn't show any clinical signs of the disease. Additionally, the virus didn't spread from pigs to other pigs or from goats to pigs (Nawathe & Taylor, 1979). Therefore, these findings suggest that both cattle and pigs do not play a significant role in the transmission of the PPR virus; they are considered dead-end hosts for the virus (Wohlsein & Singh, 2014).

Peste des Petits Ruminants (PPR) can also impact various wild ungulates belonging to the Bovidae family, such as members of several subfamilies, including *Caprinae, Bovinae, Antilopinae, Hippotraginae, Cephalophinae, Reduncinae,* and *Aepycerotinae*. Additionally, it can affect certain members of the Cervidae family within the Capreolinae subfamily. However, there is limited available information regarding which specific species are susceptible to the disease and the extent of its occurrence in these wild ungulates (Wohlsein

& Singh, 2014). In West African wildlife, there have been no documented cases of clinical Peste des Petits Ruminants (PPR) among wild ruminants.

Additionally, there have been reports of PPR outbreaks in free-living Bharals, and it's possible that Mongolian gazelles and Tibetan antelopes were also infected with the PPR virus and suffered from the disease (Wohlsein & Singh, 2014). Moreover, some species such as Asiatic lions and dogs are susceptible to PPRV. The PPRV genomes were detected from the pooled tissue samples in Asiatic lions and nasal swabs in dogs (Rahman *et al.*, 2020). The infected dogs showed gastroenteritis whereas another dog was suspected of having Canine distemper infection. These animals were infected because of PPRV-infected sheep/ goat meat or contaminated fomites (Rahman *et al.*, 2020).

Table-2: Identification of PPR in Wildlife

Common name	Scientific name	Country	References
Wild species from which the PPR virus has been isolated in cell culture			
Water deer	*Hydropotes inermis*	China	(Zhou *et al.*, 2018)
Wild ibex	*Capra ibex*	China	(Zhu *et al.*, 2016)
Bushbuck	*Tragelaphus scriptus*	UAE	(Kinne *et al.*, 2010)
Springbuck	*Antidorcas marsupialis*	UAE	(Kinne *et al.*, 2010)
Arabian gazelle	*Gazella gazella*	UAE	(Kinne *et al.*, 2010)
Arabian mountain gazelle	*Gazella gazella cora*	UAE	(Kinne *et al.*, 2010)
Dorcas's gazelle	*Gazella dorcas*	UAE. KSA	(Elzein *et al.*, 2004)
Thomson's gazelle	*Eudorcas thomsonii*	KSA	(Elzein *et al.*, 2004)
Goitered gazelle	*Gazella subgutturosa*	UAE	(Kinne *et al.*, 2010)
Impala	*Aepyceros melampus*	UAE	(Kinne *et al.*, 2010)
Gemsbok	*Oryx gazella*	UAE	(Wensman *et al.*, 2018)
Afghan markhor goat	*Capra falconeri*	UAE	(Kinne *et al.*, 2010)
Nubian ibex	*Capra nubiana*	UAE	(Wensman *et al.*, 2018)
Wild species from which PPR virus antigen or nucleic acid has been identified using ELISA/ PCR/ sequencing			
Water deer	*Hydropotes inermis*	China	(Zhou *et al.*, 2018)
Chowsingha	*Tetracerus*	India	(Jaisree *et al.*, 2018)

	quadricornis		
African buffalo	*Syncerus caffer*	Côte d'Ivoire	(Couacy-Hymann *et al.*, 2005)
Saiga antelope	*Saiga tatarica*	Mongolia	(Wensman *et al.*, 2018)
Blackbuck	*Antilope cervicapra*	Pakistan	(Wensman *et al.*, 2018)
Goitered gazelle	*Gazella subgutturosa*	Mongolia, China	(Li *et al.*, 2017)
Grant's gazelle	*Nanger granti*	Tanzania	(Mahapatra *et al.*, 2015)
Kob	*Kobus kob*	Côte d'Ivoire	(Couacy-Hymann *et al.*, 2005)
Nile lechwe	*Kobus megaceros*	Sudan	(Wensman *et al.*, 2018)
Defassa waterbuck	*Kobus ellipsiprymnus*	Côte d'Ivoire	(Couacy-Hymann *et al.*, 2005)
Bubal hartebeest	*Alcelaphus buselaphus*	Côte d'Ivoire	(Couacy-Hymann *et al.*, 2005)
Wild goat	*Capra aegagrus*	Kurdistan, Iran	(Marashi *et al.*, 2017)
Sindh ibex	*Capra aegagrus blythi*	Pakistan	(Wensman *et al.*, 2018)
Siberian ibex	*Capra sibirica*	Mongolia	(Wensman *et al.*, 2018)
Wild ibex	*Capra ibex*	China	(Zhu *et al.*, 2016)
Nubian ibex	*Capra nubiana*	UAE, Israel	(Kinne *et al.*, 2010)
Bharal	*Pseudois nayaur*	China	(Bao *et al.*, 2011)
Argali	*Ovis ammon*	China	(Li *et al.*, 2017)
Wild species in which PPRV antibodies have been found using ELISA			
African buffalo	*Syncerus caffer*	Côte d'Ivoire, Tanzania	(Couacy-Hymann *et al.*, 2005)
Goitered gazelle	*Gazella subgutturosa*	Turkey	(Gür & Albayrak, 2010)
Dorcas's gazelle	*Gazella dorcas*	Sudan, Nigeria	(Bello *et al.*, 2016)
Grant's gazelle	*Nanger granti*	Tanzania	(Mahapatra *et al.*, 2015)
African gray duiker	*Sylvicapra grimmia*	Nigeria	(Ogunsanmi *et al.*, 2003)

Defassa waterbuck	*Kobus ellipsiprymnus*	Côte d'Ivoire	(Couacy-Hymann *et al.*, 2005)
Impala	*Aepyceros melampus*	Tanzania	(Mahapatra *et al.*, 2015)
Blue wildebeest	*Connochaetes taurinus*	Tanzania	(Mahapatra *et al.*, 2015)
Bharal	*Pseudois nayaur*	China	(Bao *et al.*, 2011)
Wild species in which PPRV antibodies have been found using cross-serum neutralization tests (CSNT)			
Dorcas's gazelle	*Gazella dorcas*	KSA	(Elzein *et al.*, 2004)
Thomson's gazelle	*Eudorcas thomsonii*	KSA	(Elzein *et al.*, 2004)
Wild species infected experimentally with PPRV			
White-tailed deer	*Odocoileus virginianus*	USA	(Wensman *et al.*, 2018)

2.6.2. Transmission

PPR virus is enveloped and sensitive to inactivation by heat, sunlight, and chemicals. The virus cannot survive for a long time outside the host or has low resistance in the environment (Chauhan *et al.*, 2009). So, it needs close contact to spread (Özkul *et al.*, 2002). Being an acute and highly contagious disease in small-ruminants, healthy animals can be infected by the virus through direct contact with infected animals and contaminated materials including ocular and nasal discharges, oral discharges, and feces, which are the significant source of the virus (Vinayagamurthy, 2017). Goats infected with PPRV can continue to excrete the virus in their feces for a minimum of two months following a natural infection. Infected animals release infectious droplets in the air through coughing and sneezing (Abubakar *et al.*, 2012). Animals inhale the air droplets while in close contact. The infectious droplets also can contaminate water, feed troughs, and bedding materials, and these become sources of infection of PPRV (Dilli *et al.*, 2011). The movement of animals plays a crucial role in the transmission of PPRV. Pursuing small ruminants at the animal market, where animals from various sources come into close contact, significantly heightens the chances of PPRV transmission (Colenutt *et al.*, 2022). Additionally, animal migration over the border between India and Bangladesh mostly involves the transportation of cattle and buffalo. There is a limited quantity of sheep that is imported, but there is no information on goats being imported

from India (Khatun *et al.*, 2016). Buffalo and sheep imported from India, which are sub-clinically infected, might potentially heighten the risk of PPR transmission in the affected regions. Nevertheless, the genetic data from PPRV samples in Bangladesh and India also indicates the occurrence of transboundary transmission (Ahamed *et al.*, 2019). Cattle and pigs are also brought to the market where these animals can be infected by PPRV (Colenutt *et al.*, 2022). The detection of PPRV antibodies in camels, cattle, and wild ruminants, in addition to sheep and goats, indicates the natural transmission of PPRV infection among these animals in real-world conditions (Rahman *et al.*, 2020). Additionally, nomadic animals frequently encounter local sheep and goat populations, potentially leading to virus transmission (Abubakar *et al.*, 2015). Subsequently, infected migratory animals can transmit the virus to other susceptible local sheep and goats (Abubakar *et al.*, 2015). Small ruminant populations are additionally divided by village or ecosystem, forming a complex meta-population. This structure comprises basic host populations linked through an intricate mobility network, which includes local and regional trade as well as transhumance (Vinayagamurthy, 2017). In such a scenario, the transmission of the virus varies significantly, and PPRV could persist for an extended duration compared to a straightforward, uniform host population (Nkamwesiga *et al.*, 2023). Nutritional deficiencies and a weakened immune system in animals could also contribute to the rapid transmission of the PPRV leading to severe outbreaks (Abubakar *et al.*, 2015).

2.7. Risk Factors

The Peste des petits ruminants (PPR) virus mainly affects small-ruminants such as sheep and goats. However, it rarely infects camels and other wildlife. Many scholars cited that despite infecting small-ruminants severely, it has a higher affinity for the caprine species than the ovine species (Ejigu *et al.*, 2023). Despite the lack of a carrier state, infected animals exhibit excretion of PPRV in their secretions and excretions. This excretion occurs from the 3rd day to the 26th day post-infection, and in some instances, it may persist for up to 16 weeks after infection (Wasee *et al.*, 2016). This finding emphasizes the crucial role of infected animals as a source of silent infection in the epizootiology of PPR. The age of animals, specifically those between 6 months to 1 year old, is identified as a critical factor influencing the severity of the disease compared to adults (Gitonga, 2015). The passive immunity in offspring is noted to fade away after 4–5 months in endemic environments (Balamurugan *et al.*, 2012). Additionally, the mortality rate is higher in kids rather than adults. Furthermore, the presence of maternal antibodies has an impact on vaccination response (Gowane *et al.*, 2016).

The migratory pattern, involving seasonal movement from hilly areas through diverse routes to avoid severe winters, significantly intensifies the potential for PPR dissemination within these flocks. Several factors contribute to the heightened susceptibility of migratory flocks to PPR. The combination of stress induced by transportation, underfeeding, and underlying parasitic infections acts synergistically to compromise the animals' immune defenses, thereby increasing their vulnerability to diseases (Mahajan *et al.*, 2012).

Season is a key factor in PPR outbreaks and the incidence of PPR is associated with the movement of small ruminants and climate factors (Zhang *et al.,* 2022). In enzootic conditions, PPR occurs throughout the year with higher frequency during the lean period (Balamurugan *et al.,* 2016). Small ruminants, typically raised by small or marginal farmers in free-range grasslands, shrubs, and forest areas, tend to travel long distances during the lean period in search of fodder and water. This movement contributes significantly to the increase in PPR incidence (Munibullah *et al.,* 2022). However, with the onset of the monsoon, nutrition improves, and animal migration is restricted due to the availability of local fodder, resulting in a substantial decrease in the frequency of PPR outbreaks (Malik *et al.,* 2020).

The introduction of new animals from unknown sources to flocks is identified as a major risk factor for outbreaks (Babaoglu *et al.,* 2022). The purchase of new animals from the market and grazing in the same field with infected goats were the source of the present outbreak (Das *et al.,* 2007).

Vaccination is an important tool for controlling PPR. Several studies have highlighted the effectiveness of vaccination campaigns in reducing the PPR burden and preventing outbreaks. The response to the PPR vaccine in sheep and goats is highly variable, influenced by both environmental and genetic factors including the MHC class-II (Gowane *et al.,* 2018).

Breed is identified as a risk factor for PPR in several studies. In Bangladesh, the Black Bangle breed had a higher susceptibility to PPR compared to Jamunapari and other crossbreeds (Solaiman *et al.*, 2022). In Algeria, the within-flock prevalence of PPR was significantly higher in mixed flocks compared to sheep flocks (Kardjadj *et al.,* 2015).

One study found that the overall seroprevalence of PPR was significantly higher in males compared to females (Mahajan *et al.,* 2012). Another study observed that disease prevalence was higher in male goats compared to females, although it was not statistically significant (Parvez *et al.,* 2014). Additionally, a study conducted in Algeria found that the mean within-flock prevalence of PPR was significantly higher in females compared to males (Cao *et al.,*

2018). These findings suggest that gender may play a role in the risk of PPR, with males potentially being more susceptible to the disease. However, further research is needed to fully understand the relationship between sex and PPR risk.

Feeding practices represent a potential risk factor influencing the prevalence of PPRV infection in sheep in Bangladesh with grazing systems posing a higher likelihood of infection compared to stall-feeding systems (Rahman *et al.*, 2023). In Bangladesh, where open grazing is common, the mixing of sheep with those from other farms may create favorable conditions for PPRV transmission. Additionally, inadequate adherence to isolation and quarantine protocols in open grazing setups exacerbates this risk. A study highlights that animals grazing freely in open environments exhibit higher rates of PPRV infection than those in confined stall-grazing systems (Bwihangane *et al.*, 2016). Farms utilizing a grazing feeding system need to be consistently monitored to prevent the spread of diseases among animals and from one farm to another, as infected animals can transmit illnesses to healthy ones.

Moreover, herd size has been identified as a risk factor for PPR reported in several studies (Ejigu *et al.*, 2023; Rahman *et al.*, 2021). Larger flock sizes were found to be significantly associated with higher PPRV sero-positivity in sheep and goats (Ejigu *et al.*, 2023). Small-ruminants from small herds were more likely to be seropositive for PPRV compared to those from medium-sized herds (Rahman *et al.*, 2021). These findings suggest that herd size plays a role in the transmission and spread of PPRV. It is important to consider herd size as a risk factor when implementing control measures for PPR such as vaccination campaigns.

There is a substantial correlation between road length and the cumulative prevalence of PPR in Bangladesh. Animal movement is the primary catalyst for the dissemination of transboundary disease (Balamurugan *et al.*, 2012; Munir *et al.*, 2015). Animals often migrate via supply chain networks, which consist of herds that travel between local minor markets and larger central markets. Bangladeshi people have a strong preference for goat meat. Approximately 15 million goats are yearly slaughtered in Bangladesh, with 40% of them being sacrificed during the religious holiday called "Eid-ul-Adha" (DLS, 2007). A significant number of both local and major livestock markets are organized before the celebration of "Eid-ul-Azha", which also enables the spread of PPRV across the supply chain. Animals are mostly moved by road and river networks. Vehicles may serve as vectors for the transmission of PPRV, which can be present in the excretions of infected animals, thus facilitating the spread of the disease across different locations (Rahman *et al.*, 2021).

2.8. Clinical Signs

Peste des Petits Ruminants (PPR) is a contagious viral infection that affects both wild and domestic cloven-hoofed small ruminants. It's characterized by symptoms including fever, pneumonia, severe diarrhea, and inflammation in the mucous linings of the respiratory and digestive systems, followed by either death or recovery of the animals (Munir *et al.*, 2012). PPR is called 3D disease, which is characterized by Discharge, Diarrhea, Dehydration, and Death (Islam, 2011). Additionally, sometimes Bronchopneumonia is considered as the 4th component of PPR (Munir *et al.*, 2012). PPR shares clinical and pathological similarities with rinderpest (RP) such as frequent pneumonia and scabby crusts around the lips (Mahapatra *et al.*, 2021).

PPR can be classified into different stages: first, a short incubation period (5-7 days); then, a febrile phase with fever; next, a mucosal phase with symptoms such as eye and nasal discharges, mouth lesions, and redness in the eyes and nose; followed by a stage with diarrhea, pneumonia, dehydration, and possible death. In cases that don't end fatally, there's a "recovery stage" where survivors gain long-lasting immunity to PPR (Munir *et al.*, 2012). The severity of Peste des Petits Ruminants (PPR) can vary based on predisposing factors, and virus virulence. It can be classified as peracute, acute, subacute, or subclinical (Kabir *et al.*, 2019).

2.8.1. Peracute form: The per acute form of PPR often affects young goats, typically around 4 months old, shortly after they lose their maternal immunity. The disease starts very quickly, with an incubation period of less than 2 days, followed by a high fever of 40-42°C that lasts for several days. Due to the fever, the animal loses its appetite and becomes increasingly depressed. During this period, the mucous membranes in the mouth may become congested, and sometimes, erosion is visible. This, along with eye and nasal discharges, can lead to breathing difficulties. Initially constipated, the animals then develop severe, watery diarrhea. Unfortunately, most animals do not survive this phase, and they typically die within 4 to 5 days of developing the fever (Munir, 2015).

2.8.2. Acute form: Animals that survive the initial peracute phase of Peste des Petits Ruminants (PPR) with nonspecific symptoms, progress to a more characteristic stage of the disease. The acute form is characterized by serous ocular and nasal discharges after the onset of fever. As the disease advances, ocular and nasal discharges become catarrhal, blocking the

nostrils and leading to breathing difficulties, sneezing, and coughing (Munir, 2015). Oral lesions start as necrosis on the lower gum and can extend to cover various parts of the mouth, causing pain and reluctance to open the mouth. Female animals may also develop similar issues in their genital mucous membranes, potentially leading to abortion in pregnant animals (Abubakar *et al.*, 2008). Severe pneumonia signs, such as noisy breathing, extended head and neck, nostril dilation, and painful cough, indicate a poor prognosis. Dehydration sets in, with sunken eyeballs, and affected animals often succumb to the disease 10-12 days after the onset of fever. Mortality rates range from 70-80%, while survivors require weeks of recovery (Parida *et al.*, 2015).

2.8.3. Subacute form: This form of the disease has a longer incubation period of 6 days. It is characterized by low-grade pyrexia, lack of signs, low mortality, presence of mucosal discharge, and oral crust. Animals usually recover from this phase within 10-14 days (Maina *et al.*, 2015).

2.8.4. Subclinical form: Occasionally, not only sheep and goats but also large ruminants such as buffalo, cattle, and Pigs can naturally be infected with the subclinical form of the disease (Bwihangane, 2018). Viruses can be cleared from the animals without causing any clinical manifestation. However, PPRV tend to undergo seroconversion, a process where their immune system produces antibodies against the virus. This often results in the generation of potent neutralizing antibodies, which play a crucial role in fighting the infection (Parida *et al.*, 2015).

2.8.5. Clinical Score: A clinical scoring method has been developed to assess the severity of PPRV infection to lead to the humane euthanasia of animals showing clear clinical disease during experimental studies (Pope *et al.*, 2013). This scoring system provides specific criteria for euthanizing animals based on a defined score reached within a specified time frame (Parida *et al.*, 2015). The decision to euthanize is made on ethical grounds, as outlined in the system proposed (Pope *et al.*, 2013).

Table-3. Clinical Score of PPR

Clinical Score	General Signs	Pyrexia response	Ocular/ Nasal Discharge	Facial mucosal lesions	Faces	Respiratory symptoms
0	Normal	<39.5	None	None	Normal	Normal respiration rate
1	Mildly inactive	>39.5 but <40.0	Watery ocular discharge	Congested oronasal	Soft	Slight tachypnea

				mucosa and buccal papillae		
2	Mildly inactive, depressed, inappetence	>40.0 but <41.0	Watery to mucoid oculonasal discharge, reddened eyes, mild conjunctivitis	Pinprick lesions within the buccal cavity	Runny	Tachypnea/ mild cough
3	Inactive, apathetic, restless, and anorexic	>41 or >39.5 for >5 days	Mucopurulent nasal discharge, severe conjunctivitis with mucopurulent ocular discharge	Clear erosive lesions on oronasal mucosa, severely congested/ edematous buccal papillae	Frank Diarrhea	Tachypnea and dyspnea/ coughing present
4	Severe obtundation, reduced morbidity, and dehydration	>41 C or >39.5 C for >5 days followed by rapid fall in temperature (<38C)	Mucopurulent nasal discharge, severe conjunctivitis with profuse mucopurulent ocular discharge	Severe ulcerative lesions in the buccal cavity, nasal mucosa, nares, edematous lips, and erosions on vulval labia	Muco-bloody diarrhea	Tachypnea/ dyspnea/ cough

2.9. Pathology

2.9.1. Gross Pathology

Retrogressive and necrotic changes in lymphoid tissues and epithelial cells of gastrointestinal and respiratory systems characterize the pathology of PPR (Balamurugan *et al.*, 2010). The severity of the disease varies depending on the species, breed, and immune status of the host (Chowdhury *et al.*, 2014). The primary histomorphologic diagnosis for PPR was acute pneumonia, accompanied by severe gastroenteritis (Chowdhury *et al.*, 2014). Various gross pathological changes were observed in different parts, tissues, or organs, as described below. The respiratory system exhibits prominent signs of PPR, characterized by lung consolidation and color changes, with the lungs transitioning to a dark red to purple color (Ugochukwu *et al.*, 2018). Frothy mucus is observed when the lung is compressed, and the lung texture becomes notably firm to the touch (Kumar *et al.*, 2004). Secondary bacterial pneumonia often complicates the respiratory manifestations, accompanied by congested alveolar borders,

mucopurulent exudates in the trachea, multifocal bronchitis, bronchiolitis, and hemorrhage in the tracheal ring. Bronchopneumonia, frequently coupled with pleuritis and hydrothorax, is a common feature (Balamurugan *et al.*, 2010). Erosion and ulceration in the nasal mucosa and petechiae on the nasal mucosa, turbinate, larynx, and trachea (Ishag *et al.*, 2023). Within the lymphatic system, PPR induces enlargement, edema, and congestion of lymph nodes associated with the lung and intestine. Mesenteric lymph nodes become edematous and congested (Chowdhury *et al.*, 2014), while the spleen exhibits signs of congestion and engorgement (Kumar *et al.*, 2002). Additionally, ecchymotic and brush-paint hemorrhages may manifest on the epicardium, with the presence of serosanguinous fluid in the pericardial sac of the heart. Minor hemorrhages on the cortico-medullary junction of the kidney are also observed (Ugochukwu *et al.*, 2019). The gastrointestinal system is profoundly affected by PPR, presenting severe congestion and necrotic lesions in the rumen, reticulum, and abomasum (Balamurugan *et al.*, 2010). The small intestine may exhibit black discoloration of the mucosal lining, accompanied by hemorrhage and erosion in the ileum (Ishag *et al.*, 2023). Lesions in the liver and other internal organs are notable, often indicating necrotic enteritis with extensive necrosis (Obi *et al.*, 1983). Severe ulceration of Peyer's patches and sloughing off of the intestinal wall are additional gastrointestinal signs (Balamurugan *et al.*, 2010). Around the cecal-colic junction, rectum, and the posterior part of the colon, discontinuous streaks of congestion are visible, which are recognized as characteristic "zebra" stripes or "zebra markings" on the mucosal folds, a distinctive feature of PPR (Ugochukwu *et al.*, 2018). Oral cavity pathology is a significant aspect of PPR, with widespread erosions, ulcerations, and necrosis in various parts of the oral cavity (Ishag *et al.*, 2023). Deposition of necrotic materials on palates, tongue, and oral mucosa along with erosion in the gums, palates, tongue, cheeks, and esophagus are present in the Oral cavity (Balamurugan *et al.*, 2010). Lips are swollen and often eroded, while emaciation, conjunctivitis, and erosive stomatitis may be present. Severe cases can exhibit lesions on the hard palate, pharynx, and upper third of the esophagus (Wohlsein & Saliki, 2006). Furthermore, clinical signs of PPR may include emaciation, soiled hindquarters with soft and watery feces, eyes and nose containing crusty discharges, and necrotic or orf-like lesions in the lips (Ugochukwu *et al.*, 2017).

2.9.2. Gross Pathology in Unusual Host

The clinical manifestations of PPRV infection exhibit considerable variability across different wild and domesticated animal species. In Wild Asian ungulates, the impact is primarily pulmonary, marked by pulmonary effects, and occasional erosion and ulceration of the upper digestive tract (Wohlsein & Singh, 2014). Indian water buffaloes infected with PPRV experience abomasitis accompanied by edema and hemorrhagic gastroenteritis (Govindarajan *et al.*, 1997). Dromedary camels show a diverse range of symptoms, including pulmonary congestion, pneumonia, liver paleness, keratoconjunctivitis, lymph node enlargement, and hemorrhagic ulcers on the tongue (Zakian *et al.*, 2016). Dorcas's gazelles exhibit severe abomasitis, mucosal congestion in the rumen, rectal and colonic mucosal hemorrhage, and erosions in the oral mucosa. Wild ruminants demonstrate suppurative pneumonia, occasional fibrinous pleuritis and peritonitis, spleen hemorrhage, and erosions in the tongue, lips, and soft palate. Gazelles experience mucoid esophagus, congested papillae in the rumen, hemorrhagic abomasitis, erosions throughout the gut, and congestion in multiple organs, including the lung, liver, kidney, pancreas, and brain. Gemsbok displays focal fibrinous pleuritis and a mottled liver with a distended gall bladder.

2.9.3. Histopathology

In lung histopathology, necrosis and sloughing of surface epithelium, bronchial epithelium, infiltration of mononuclear cell in bronchi and bronchiole. Bronchointerstitial pneumonia characterized by bronchiolar lining proliferation, and diffusion of lymphoid, macrophages, and plasma cells is shown in the Lung (Osman *et al.*, 2016). Bronchointerstitial pneumonia is often complicated by purulent and sero-fibrinous exudates (Kumar *et al.*, 2002). Hyperplasia and desquamation of bronchial and bronchiolar epithelium are the characteristics. One of the most consistent findings is hypertrophy and hyperplasia of alveolar lining cells. Inclusion bodies including intranuclear and intracytoplasmic are usually seen in alveolar macrophages. Additionally, syncytial and bronchiolar epithelial cells contain inclusion bodies (Vinayagamurthy, 2017). Moreover, fibrino-purulent pneumonia was observed in the lungs in acute cases (Kumar *et al.*, 2004) and giant cell pneumonia was found in chronic infection (Islam *et al.*, 2001). Sloughing of surface epithelium and infiltration of mononuclear cells in alveoli, bronchi, and bronchiole are common findings (Islam *et al.*, 2021). Syncytial cells in alveoli are the pathognomic character in PPR (Kul *et al.*, 2015). Hyperplastic type-II pneumocytes expanse the alveolar wall (Truong *et al.*, 2014). In Trachea, there are not many

changes; but the presence of desquamation and necrosis in the tracheal lumen. Vacuolation and hyperplasia of the tracheal epithelium and presence of eosinophilic intranuclear and intracytoplasmic inclusions. Tracheal degeneration and cystic lesions in the tracheal gland and infiltration of mononuclear inflammatory cells in the tracheal sub-mucosa are observed (Ugochukwu *et al.*, 2019). Epithelial cell degeneration, characterized by ballooning, is succeeded by necrotic processes within the oral mucosa. Extensive necrosis and lymphocyte depletion are evident in the spleen, lymph nodes, tonsils, and gut-associated lymphatic tissues. Concurrently, reticuloendothelial cells manifest hyperplasia and sporadically harbor nuclear inclusion bodies (Parida *et al.*, 2015).

In the liver, hepatocytes displayed swelling characterized by pale and finely granular cytoplasm. Sinusoids exhibited variable dilation, and hepatic cords experienced disruption. Epithelial hyperplasia was observed in the bile ducts within the portal areas (Kul *et al.*, 2015). In the kidneys, the epithelial lining cells of the proximal convoluted tubules exhibited swelling, occasionally occluding the lumen. Additionally, the cytoplasm demonstrated an increased granularity compared to the typical state (Kumar *et al.*, 2002).

Lamina propria of the Intestine showed infiltration of mononuclear cells and plasma cells with moderate vascular congestion (Wohlsein & Saliki, 2006). The goblet cells were conspicuously prominent, characterized by their filled mucin content. Villi displayed a reduction in height, presenting a blunt morphology. Peyer's patches consistently demonstrated areas of lymphoid depletion, akin to observations in lymph nodes. Periodically, typical syncytia were discerned in the Peyer's patches of the ileum (Ugochukwu *et al.*, 2019). The cortex and medulla of lymph nodes show edema and infiltration of mononuclear cells and giant cells. Lymphocytes are depleted in lymph nodes occasionally with syncytia formation (Jagtap *et al.*, 2012).

Within the spleen, tonsils, and lymph nodes, the viral infection induces lymphocyte necrosis, as manifested by the presence of pyknotic nuclei and karyorrhexis. Additionally, the spleen exhibits hemorrhage and the deposition of hemosiderin pigment (Osman *et al.*, 2016). Hemorrhages and lymphoid cell depletion in both the cortex and medullary cords are apparent in the spleen and lymph nodes (Patel *et al.*, 2017). Lymphoid cell depletion in the cortex and medullary cords of the spleen and lymph nodes was found (Patel *et al.*, 2017). A recurring hematological occurrence in PPR is leucopenia, specifically lymphocytopenia. This

is attributed to lymphoid necrosis in the reticuloendothelial organs, aligning typically with the onset of fever during the prodromal phase.

2.9.4. Histopathology and Immunology in Unusual Host

In Camel, degeneration of bronchiolar epithelium with peribronchial infiltration of mononuclear cells. Congestion and infiltration of mononuclear cells lead to distended alveolar septa. In the lung, pulmonary edema and emphysema are observed and lymphocytes are depleted from Lymph nodes (Khalafalla *et al.,* 2010). In wild ungulates affected by PPR, there is a range of pathological findings. This includes necrotizing to hemorrhagic enteritis/colitis, lymphoid necrosis, and depletion in gut-associated lymphoid tissue. Lymphoid depletion and necrosis are observed in the spleen and body lymph nodes. The lungs exhibit subacute broncho-interstitial pneumonia with syncytial cells, hyperplasia of type II pneumocytes, and bronchiolar epithelial cells. Occasional suppurative to fibrino-purulent bronchopneumonia, likely due to secondary bacterial pathogens, is found. The liver shows multifocal hepatocellular coagulation necroses with macrophage infiltration. Eosinophilic cytoplasmic and nuclear inclusion bodies are detected in various cell types, including gastrointestinal epithelial cells, macrophages/reticular cells of lymphoid tissues, bronchial and bronchiolar epithelial cells, syncytial cells, and biliary epithelial cells. Pneumonia, necrosis, and lymphatic tissue depletion are common features of PPR affecting small domesticated ruminants (Wohlsein & Singh, 2014). In Arabian wild ungulates, immunohistochemistry demonstrated the detectability of morbillivirus antigen in the cytoplasm and nucleus of intestinal epithelial cells, bronchial and bronchiolar epithelial cells, syncytial cells, bile duct epithelia, hepatocytes, and cells of intestinal lymphoid follicles, as illustrated (Kinne *et al.,* 2010).

2.10. Diagnosis

2.10.1. Penside (Field) test for PPRV antigen

2.10.1.1. RT-LAMP

Loop-mediated isothermal amplification (LAMP) is a powerful innovative gene amplification technique emerging as a simple rapid diagnostic tool for the early detection and identification of microbial diseases (Parida *et al.,* 2008). LAMP is a molecular biology method distinguished by its utilization of six specific primers designed to identify eight unique regions on the target gene. In contrast to traditional PCR techniques, LAMP operates at a

consistent temperature, employing a strand displacement reaction for gene amplification with a high level of efficacy (Notomi *et al.*, 2000). The entire process, encompassing both amplification and gene detection, is streamlined into a single step. This involves placing a mixture of samples, primers, DNA polymerase with strand displacement activity, and substrates in a constant temperature environment, typically at around 63°C.

2.10.1.2. Lateral Flow Devices (LFD)

Veterinarians face a challenge in disease management, as timely identification is crucial for appropriate action. The traditional process involves either relying on experience for on-site diagnosis or sending samples to distant laboratories, causing delays of several days. Such delays contribute to disease spread due to postponed control measures. The separation between field sampling and final laboratory results also diminishes the motivation for timely sample submission, resulting in inefficient monitoring and delayed diagnosis in the veterinary field. Alleviating the delay in disease diagnosis for PPRV in veterinary settings could be achieved by enabling on-site field or pen-side testing (Notomi *et al.*, 2000).

2.10.1.3. Oxford nanopore MiniION

The FAO and OIE initiated a control and eradication program for PPRV in 2015, aiming for eradication by 2030 (Legnardi *et al.*, 2022). Successful implementation relies on vaccination efforts and rapid diagnosis to trace outbreaks. PPRV is prevalent in regions lacking infrastructure, hindering quick diagnosis and epidemiological investigations (Torsson *et al.*, 2020). The program's success is hindered by long sample transports that risk degrading viral nucleic acid. Bringing the laboratory closer to outbreaks minimizes risks, significantly reducing the time from clinical signs recognition to molecular epidemiological investigations (Parida *et al.*, 2015). Employing a portable miniPCR and MinION technology not only brings the laboratory to the field but also enables the generation of a complete genome within 24 hours of sample collection. The procedure has proven effective with virus isolates from cell cultures and field isolates derived from tissue samples of naturally infected goats (Torsson *et al.*, 2020).

2.10.2. Antigen detection

2.10.2.1. Agar gel immunodiffusion Test

The agar gel immunodiffusion test, known as AGID test, is recommended by OIE for the detection of antigens is used for the identification of PPRV, and is used for tentative diagnosis (Balamurugan *et al.*, 2014). The AGID technique was widely used for many years in endemic regions and is still used in many developing countries where facilities do not exist for undertaking more complex tests (Anderson *et al.*, 2006). AGID has relative diagnostic sensitivity and specificity with cELISA of 59.3% and 98.3% (Munir *et al.*, 2009). Additionally, the AGID test has a 42.6% detection rate for both antemortem and post-mortem specimens, offering results within 24 hours (Appel, 1981). Notably, it can evaluate the presence of both antigens and antibodies, demonstrating a commendable specificity of 92% (El-Yuguda *et al.*, 2010).

2.10.2.2. Immunohistochemistry

Immunohistochemistry (IHC) is a technique employing antibodies to identify the cellular location of specific proteins or macromolecules, commonly in formalin-fixed, paraffin-embedded tissues (Yener *et al.*, 2004). Monoclonal antibodies, known for their heightened specificity, are preferred in IHC, minimizing errors from antigenic cross-reactivity (Saliki *et al.*, 1994). They offer advantages such as consistent antibody availability and easy standardization of assays. IHC, particularly with monoclonal antibodies, has been shown effective in detecting PPRV antigens in various tissues like the tongue, intestine, lymph nodes, spleen, and lung (Saliki *et al.*, 1994). This method, sensitive and suitable when fresh tissues are unavailable, aids in retrospective examinations.

2.10.3. Molecular and serological diagnosis

2.10.3.1. Real-time RT-PCR (qRT-PCR)

Real-time PCR (RT-qPCR) is a precise, expeditious, and dependable technique that may be used to identify and quantify individual DNA molecules. A real-time RT-PCR assay targeting the N-gene of PPRV has been developed for the specific detection of the PPR virus. This assay is capable of detecting representatives from all four lineages of PPRV found in Africa and Asia (Batten *et al.*, 2011). The rt RT-PCR test has many benefits compared to the standard approach including sensitive, reproducible, enhanced laboratory throughput, the capacity to analyze multiple samples simultaneously, a much lower chance of contamination,

and the capability to measure the viral load in clinical specimens (Abera *et al.*, 2014). In addition, conventional RT-PCR is technically challenging and takes 4-8 hours to provide a complete diagnosis, whereas real-time PCR only takes 3-4 hours for the same results (Batten *et al.*, 2011). Furthermore, real-time PCR (qPCR) is performed in a closed tube, eliminating the need for additional manipulations. This makes the fluorogenic PCR assay valuable for studying the causes of diseases (Balamurugan *et al.*, 2012). The rt RT-PCR assay demonstrated exceptional sensitivity in comparison to conventional RT-PCR, which was 10 times less sensitive than the TaqMan rt RT-PCR (Balamurugan *et al.*, 2010). The rt RT-PCR's sensitivity surpassed that of the TaqMan rt RT-PCR and conventional RT-PCR by 1000 and 10 times, respectively (Balamurugan *et al.*, 2010). Real-time PCR techniques targeting the N- or M-gene, employing TaqMan hydrolysis probes and SYBR Green with melt curve analysis, provide rapid, highly sensitive, and specific detection and quantitation of PPRV (Balamurugan *et al.*, 2014). Various approaches have been optimized for specific detection and quantification of PPRV in clinical samples including N gene-based one-step TaqMan real-time RT-PCR (Bao *et al.*, 2008), M-gene-based two-step TaqMan hydrolysis probe (Balamurugan *et al.*, 2010), one-step real-time RT-PCR with SYBR Green (Balamurugan *et al.*, 2012), and two-step SYBR Green I-based real-time RT-PCR (Abera & Thangavelu, 2014; Abera *et al.*, 2014). Duplex real-time RT-PCR assays and detection in formalin-fixed tissues have been developed for simple and rapid PPR diagnosis, even using samples stored in formalin for extended periods (Balamurugan *et al.*, 2014).

2.10.3.2. Conventional RT-PCR

Polymerase Chain Reaction (PCR) was reported for the first time in 1985 (Saiki *et al.*, 1985). Since then, the application of PCR has increased rapidly for research and diagnosis of many diseases. For functionating, PCR needs to use specific primers. As it has the potential for high sensitivity and specificity, its usage shows great promise in this field (Diallo *et al.*, 1995). Additionally, PCR is more sensitive than nucleic acid hybridization, competitive and immunocapture ELISAs, and differential immuno-histochemical staining using MABs for detecting viruses (Balamurugan *et al.*, 2014). Furthermore, RP and PPR from clinical samples were detected and identified by using RT-PCR.

In 1995, a study revealed that various known morbilliviruses can be differentiated using designed genus-specific and universal primers (Forsyth & Barrett, 1995). Moreover, to

identify and differentiate RP and PPR, different primers based on N, F, and P genes (Couacy-Hymann *et al.*, 2002). PCR techniques targeting various genes such as F (Forsyth & Barrett, 1995), N (George *et al.*, 2006), M (Balamurugan *et al.*, 2006), and H (Balamurugan *et al.*, 2010) have been developed for specific detection of PPRV from clinical samples. However, the PCR using PPR virus-specific external protein F gene primers may not always be suitable for all strains, variants, or isolates due to potential variations in the immunogenic protein coding region (Balamurugan *et al.*, 2006). Further, PCR strategies focusing on the M and N genes have been developed to detect and differentiate PPRV in sheep and goats. Additionally, an RT-PCR assay based on the M and N genes has been successfully employed for the direct detection of PPRV in clinical samples (George *et al.*, 2006). A highly sensitive N-gene-based RT-PCR for PPRV detection and differentiation, utilizing DIG-labelled RT-PCR product, demonstrates superior sensitivity, detecting viral RNA at a titer as low as 0.01 TCID50/100 µl, and is 10,000 times more sensitive than traditional RT-PCR, aiding in both early and late-phase diagnosis and differential diagnosis of PPR (Saravanan *et al.*, 2004).

2.10.3.3. ELISA

Monoclonal Antibody (MAb)-based ELISA methods, including immunocapture ELISA and sandwich ELISA (sELISA), are widely used to detect Peste des Petits Ruminants Virus (PPRV) in clinical samples in field conditions (Singh *et al.*, 2004). This may serve as a method and a cheap source of reagents for the clinical surveillance of PPR virus infection in other Asian countries where PPR is endemic (Singh *et al.*, 2004). A specific Monoclonal Antibody (MAb) against the nucleocapsid protein, unique to Peste des Petits Ruminants (PPR) was used for detection. The binding of the clinical material's antigen to the capture antibody was monitored using a PPR-specific monoclonal antibody (Singh *et al.*, 2004). Rinderpest hyperimmune serum served as the capture antibody, expected to contain antibodies against nucleocapsid epitopes shared by both PPR and rinderpest viruses (Singh *et al.*, 2004). The advantage of cross-reactive epitopes in the nucleocapsid protein support binding to either RP or PPR virus, with specificity confirmed by the detection antibody (PPR-specific monoclonal antibody). The internationally accepted immunocapture ELISA employs a biotinylated anti- ‘N′ MAb, developed at the World Reference Laboratory in France. sELISA is effective, and comparable to the immunocapture ELISA with diagnostic sensitivity and specificity of 89% and 93%, respectively (Baron *et al.*, 2016). Additionally, the

abundance of nucleoprotein which is targeted in sandwich-ELISA may result in relatively higher sensitivity of the ELISA as compared to F-gene-based RT-PCR (Mahajan *et al.*, 2013).

Mahajan *et al.*, (2012) revealed that clinical samples from suspected cases of Peste des Petits Ruminants (PPR) were examined using sandwich ELISA to detect the PPR virus antigen. Results indicated that nasal swabs exhibited the highest positivity for PPRV antigen detection in live animals, followed by ocular swabs, oral swabs, blood samples, and rectal swabs. Tissue samples had the highest positivity in deceased animals, followed by nasal, oral, and rectal swabs.

2.10.3.4. cELISA

For large-scale screening of samples, ELISA has widely been used for monitoring and surveillance of disease or even for diagnosis (Balamurugan *et al.*, 2014). Competitive and blocking ELISA based on monoclonal antibodies specific for N-protein (Libeau *et al.*, 1994) and H-protein (Singh *et al.*, 2004) were developed for the detection of antibodies in animal sera. MAb-based PPR c-ELISA for the detection of PPRV antibodies assay is currently being employed extensively throughout India for sero-monitoring and sero-surveillance of PPR (Singh *et al.*, 2004). Further, a polyclonal antibody-based indirect ELISA was also developed for the detection of antibodies to PPRV in the serum samples of goats and sheep, which may be a good alternative tool to c-ELISA for sero-epidemiological surveys (Balamurugan *et al.*, 2007). Though this technique is rapid, sensitive, and specific for PPR diagnosis (Mahmoud *et al.*, 2022), it is costly for countries where it is endemic (Sharma *et al.*, 2015). Mehmood *et al.*, (2009) studied 4548 serum samples by performing cELISA to detect PPRV (Mehmood *et al.*, 2009) where more than 18% sample was identified as positive for the PPR Virus.

2.10.3.5. cDNA probes

A quick and effective approach for distinguishing between infections caused by Rinderpest Virus (RPV) or PPRV involves the utilization of specific cDNA probes. These probes are derived from the mRNAs encoding the nucleocapsid proteins of each virus, enabling a rapid and unequivocal differentiation between the two virus types, and these probes were labeled with ^{32}**P** nucleotide, facilitating the detection process (Diallo *et al.*, 1989).

2.10.3.6. Virus neutralization

The Virus neutralization Test (VNT) was developed for the first time for a morbilliviral disease, Rinderpest (RP) (Rioche, 1969). Later, the PPR test was refined and established as a screening test (Rossiter *et al.*, 1985). VNT was one of the earliest serological assays to determine the protective immunity. For international trade, VNT was prescribed by the OIE Terrestrial Manual (OIE, 2013). The method operates on the principle of titrating serum antibodies by assessing their neutralization impact on virus infectivity in cells. In this process, serum dilutions are mixed with a viral suspension and distributed onto a cell culture in tubes or plates. Following one to two weeks of incubation, the presence of neutralizing antibodies will impede visible cytopathic effects (CPE) compared to the virus alone. Stocks of sensitive cells and vaccine viruses will be needed for this. For confirmation and differentiation of RP and PPR virus, VNT was performed. Differential neutralization test is one of the important means to distinguish RP and PPR viruses (Rossiter *et al.*, 1985) and a micro-VNT has been employed to measure the infectivity titer of RPV and PPRV in calf kidney, sheep kidney, and Vero cells (Rossiter *et al.*, 1985). Although PPRV is clinically and antigenically related to RPV, PPRV is distinguishable serologically not only from RPV but also from MV and CDV (Gibbs *et al.*, 1979).

2.10.3.7. Luciferase Immunoprecipitation System (LIPS)

In the LIPS technology, antibodies are measured using luciferase-tagged antigens created in mammalian cells (Burbelo *et al.*, 2010). The method utilizes the 30-kDa luciferase gene from Renilla reniformis, known for its highly precise output. Creating Renilla luciferase (Ruc) chimeric genes involves standard techniques with mammalian expression vectors like pREN2. This allows the fusion of the antigen with Ruc, enabling the use of various recombinant protein targets in LIPS. Notably, LIPS is not suitable for nonprotein targets such as phospholipids, DNA, and RNA (Burbelo *et al.*, 2009). Therefore, the Luciferase Immunoprecipitation System (LIPS) is for specifically detecting antibodies against PPRV.

Table-4: Different diagnostic techniques/ assays/ tests for diagnosis of PPR in sheep and goats

Diagnostic technique/tool	Features/ Advantages	References
Agar gel immunodiffusion	Tentative diagnosis	(Obi *et al.*, 1990)

test		
Hemagglutination	Simple and inexpensive and provide results within a few hours, Total Ig detection, low specificity	(Shaila *et al.*, 1996)
Virus isolation	Gold standard test – time-consuming, tedious Primary bovine and sheep cells Vero cell line Marmoset B-Lymphoblastoid – B95a	(Sreenivasa *et al.*, 2006)
Virus or serum neutralization test	Gold standard test – time-consuming and tedious and requires cell culture facilities (Primary bovine and sheep cells, Vero cell line, marmoset B-lymphoblastoid-B95 a, VeroNectin-4 Titrated Live Virus), Detected antibodies: Anti-H and Anti-F	(Fakri *et al.*, 2016)
Indirect ELISA	PPRV antigen (Full rPPR N and rPPR H) and polyclonal antibodies (Anti-N, Anti-H)	(Zhang *et al.*, 2012)
Competitive ELISA/blocking ELISA	Neutralizing monoclonal antibodies (MAbs) – H protein MAbs – H protein	(Singh *et al.*, 2004)
Immunocapture ELISA/sandwich ELISA	Rapid differential identification and MAb – N protein	(Singh *et al.*, 2004)
Simple and aqueous phase ELISA (SNAP-ELISA)	RT-PCR/ELISA system	(Forsyth *et al.*, 2003)
Immunohistochemical detection	Detection of antigens within tissues	(Saliki *et al.*, 1994)
Dot-ELISA	Anti-M protein MAb Anti-N protein MAb	(Saravanan *et al.*, 2006)
Immunofiltration	-	(Raj *et al.*, 2008)
Antigen-competition ELISA	-	(Raj *et al.*, 2008)
Recombinant antigen-based ELISA	N protein M protein F protein H protein	(Balamurugan *et al.*, 2016)
Luciferase immunoprecipitation system (LIPS)	Specific differentiation	(Berguido *et al.*, 2016)
Nucleic acid hybridization and RT-PCR	F gene, N gene or N and M gene in either one- or two-step procedure	(Balamurugan *et al.*, 2006)

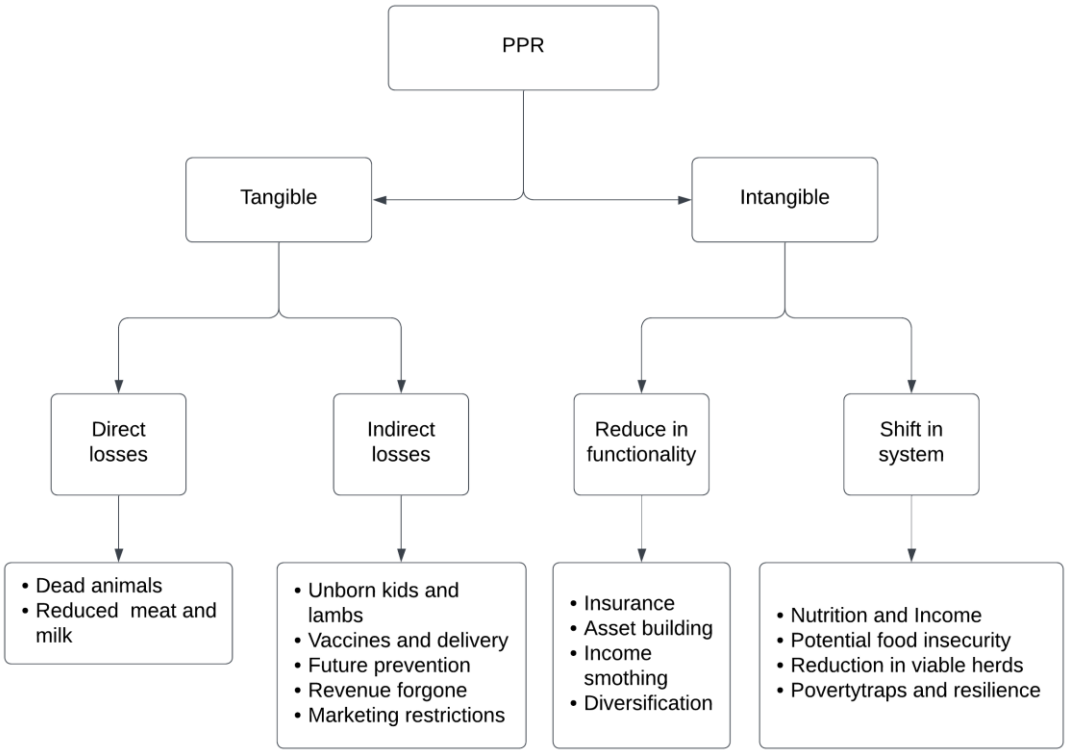
RT-PCR	F gene N gene N and M gene H gene Two-step RT-PCR One-step RT-PCR	(Balamurugan *et al.*, 2006)
PCR-ELISA	RT-PCR-ELISA	(Saravanan *et al.*, 2004)
Real-time RT-PCR	TaqMan real-time – N gene Duplex real-time – N gene M gene SYBR Green M gene	(Polci *et al.*, 2015)
Loop-mediated isothermal amplification (LAMP)	N gene	(Dadas *et al.*, 2012)
IFA	Infected monolayer, Total Ig detection, Low specificity, improved specificity with optimally infected cell monolayer	(Wang *et al.*, 2013)
Peptide-based ELISA	N synthetic peptide, Detected antibody: Anti-N	(Dechamma *et al.*, 2006)
Epitope-based C- ELISA	Epitope-based N synthetic peptide, Detected antibody: Anti-N	(Zhang *et al.*, 2012)
Reverse transcription recombinase polymerase amplification assays (RT-RPA)	Targeting N gene sequences-conventional and real-time RT-RPA	(Zhang *et al.*, 2018)
Immunohistochemical detection	Detection of antigens within tissues Detection of antigens within tissues	(Zhang *et al.*, 2016)
Real-time and lateral-flow strip RT-RPA assay	Real-time fluorescent detection by targeting the N gene of PPRV	(Yang *et al.*, 2017)
Lateral-flow immunoassay strip (LFIAS)	Fast and ultrasensitive test-strip system combining quantum dots (QDs) with a LFIAS using recombinant PPRV N protein	(Cheng *et al.*, 2017)
Immunoperoxidase monolayer assay (IPMA)	IPMA an alternative method of VNT using BHK-21 cell line stably expressing the goat SLAM	(Zhang *et al.*, 2016)

2.11. Differential Diagnosis

Rinderpest, which has been successfully eradicated worldwide, could cause clinical disease in small-ruminants, rinderpest in wildlife may have led to clinical symptoms similar to those of PPR (Hamilton *et al.*, 2017). The differential point is respiratory signs are a distinguishing feature typical for PPR where interstitial and suppurative pneumonia may occur (Wohlsein & Saliki, 2006). When distinguishing PPR from other diseases, it's important to consider specific clinical characteristics. For example, Contagious Caprine Pleuropneumonia (CCPP) can be differentiated by the absence of oral lesions (Ahaduzzaman, 2021), while Bluetongue often lacks diarrhea as a symptom (Rojas *et al.*, 2019). Diseases like Pasteurellosis and Mycoplasma infections typically do not exhibit oral lesions (Jesse *et al.*, 2014). Conditions such as Contagious Ecthyma and Foot-and-Mouth Disease rarely involve pulmonary lesions (Karki *et al.*, 2019), and Sheep and Goat pox cause distinct pox-like cutaneous lesions on the skin (Rao & Bandyopadhyay, 2000). Enterotoxaemia, Heartwater, Coccidiosis, and cases of mineral poisoning usually lack oral lesions as well (Duarte *et al.*, 2019). Sometimes, mixed infection of PPR with border disease virus, adenovirus, and other respiratory viruses such as parainfluenza virus 3, respiratory syncytial virus, bovine herpes virus -1, and bovine viral diarrhea (Toplu *et al.*, 2012). These distinctions are crucial for accurate differential diagnosis, which should be supported by consultation with a veterinarian and relevant laboratory tests.

2.12. Socioeconomic Importance of PPR

PPRV is recognized as a significant transboundary pathogen posing a substantial threat to livestock production in developing countries (Abera, 2023). In affected regions, PPR is a major obstacle to the growth of the small ruminant industry, particularly in areas of Africa and Asia where sheep and goats contribute significantly to sustainable agriculture and employment (Salih *et al.*, 2014). The economic impacts of PPR outbreaks are substantial, with a disproportionate effect on the poor of society due to their inherent reliance on sheep and goat farming (Baron *et al.*, 2011). Sheep and Goats provide social, financial, and economic balance to the landless and small farmers in underdeveloped and developing countries (Vinayagamurthy *et al.*, 2020). Direct production loss due to the high mortality (50-80%) of animals in epizootic. Additionally, weight loss, milk loss, and abortion also can be seen on the farm. The impact of PPR can be categorized in the following ways according to (Haan *et al.*, 2015).



The study reveals that in Karenga, the relative morbidity due to PPR is 14% (ranging from 4 to 27%). Given the economic constraints of the pastoralist community, allocating resources for supportive treatment of such a proportion of affected herds is challenging, especially considering that over 50% of the population in Karamoja lives on less than a dollar per day. Participants noted spending between 5000 and 25,000 UGX (~1.4 to 7.0 USD) to manage one PPR case, representing nearly 31.3% of the value of a healthy adult goat/sheep in Karenga (Akwongo *et al.*, 2022). In Bangladesh, a study showed that the economic losses due to the medication cost and loss of animals due to PPR. The total estimated medicine cost was $720.49 whereas the loss due to the dead animal was $13,800 (Islam *et al.*, 2011). In India, the estimated economic loss is $39 million annually (Bandyopadhyay, 2002), $2.6 million in Nigeria (Dilli *et al.*, 2011), and $1.5 million in Iran (Bazarghani *et al.*, 2006). According to the report by OIE and FAO, PPR has significant socio-economic implications, impacting food access, consumption patterns, and income among affected communities. The disease also affects social variables such as women's participation, employment, asset loss, and family education. The economic loss associated with PPR is substantial, estimated at USD 1.45–2.1 billion annually, affecting smallholders and extending to farm, regional, national, and global levels (OIE, 2015).

2.13. Prevention and Control

2.13.1. Control Strategies

Controlling and eradicating PPR relies on timely diagnosis, surveillance, monitoring, and vaccination in vulnerable populations. Eradication of PPR globally needs collaborative support among government, research organizations (national and international), and funding agencies. Control strategies may vary from country to country based on disease prevalence. However, choices are restricted for developing and underdeveloped countries. For both economic and sentimental reasons, infected farms cannot be stamped out. Therefore, vaccination is the recommended measure for the prevention and control of the PPRV (Vinayagamurthy, 2017). Drawing confidence from the success of the rinderpest eradication program, there is a precedent for launching a similar initiative for PPR (Vinayagamurthy, 2017). Integration of epidemiology, economics, and social science is crucial for targeting and motivating vaccination. Understanding previous eradication strategies informs strategic efforts for PPR eradication (Mariner *et al.*, 2016).

Preventive measures in PPR-free regions involve strict restrictions on importing animals or products from endemic areas. Efficient control of PPR includes isolating and sanely slaughtering infected herds, proper carcass disposal, environmental disinfection, and rigorous quarantine and movement controls. Using commercial attenuated vaccines, immunization induces a durable immune response lasting at least three years post-vaccination, providing effective prophylaxis with a solid cellular immune response and no side effects. Vaccination schedules every three years, starting at 4- to 6-months old, are crucial in PPR control to prevent fresh outbreaks, but maintaining a cold chain is essential for vaccine efficacy (Parida, 2015).

According to the DLS report, more than 90% area of the northern division was covered by mass vaccination in 2021 and 2022. However, only 18.28% and 12.25% of the rest of the areas in Bangladesh were covered in 2021 and 2022, respectively. Nevertheless, the mass vaccination under the PPR global control and eradication strategy (PPRGCES) started in October 2023, with a plan to eradicate the disease by 2030. The same program will be continued in 2024 as a part of controlling and eradicating PPR (DLS, 2023).

2.13.2. Vaccination

PPR lacks a specific treatment, and using antibiotics for secondary infections during outbreaks is prohibitively expensive. Disease control primarily relies on implementing hygienic and medical prophylaxis measures, though strict hygiene is challenging, particularly in developing countries (Gebreeziabher, 2006). Vaccination is recommended to enhance control and eradication efforts and minimize economic losses caused by the disease. Acknowledging the crucial role of small ruminants in food security and socio-economic development, FAO and OIE jointly launched a global plan in 2015 in Abidjan, Cote d'Ivoire. The objective of this plan is to control and eliminate PPR by the year 2030 (Balamurugan *et al.*, 2018).

After the emergence of PPRV, rinderpest vaccines were initially used for cross-protection, but in 1989, the live attenuated vaccine Nigeria 75/1 for PPRV was introduced and has since been widely utilized in African, Middle Eastern, and Asian countries (Bora *et al.,* 2018). To combat lineage-IV circulation in India and Asia, the Sungri 96 vaccine was developed in 1996, demonstrating effective immunity for at least 6 years after 59 generations of passage on a marmoset lymphoblastoid cell line (Sen *et al.*, 2010). Experimental studies confirm the protective efficacy of Nigeria 75/1 and Sungri 96 vaccines against all four PPRV lineages through subcutaneous and intranasal challenges, mimicking natural infection routes (Mahapatra *et al.*, 2020). A list of available PPR vaccines is listed (Dhinakar *et al.*, 2014).

Table-5: Available Peste des petits ruminants vaccines

Vaccine Strain	Lineage	Isolated from Species	Properties	References
Nigeria 75/1	I	Goat	Hemagglutinating	(Raj *et al.*, 2008)
Arasur 87 (AR 87)	IV	Sheep	Hemagglutinating, fast multiplication in cell culture	(Hegde *et al.*, 2009)
Coimbatore 97 (CBE 97)	IV	Goat	Hemagglutinating, fast multiplication in cell culture	(Raj *et al.*, 2008)
Sungri 96	IV	Goat	No hemagglutination, slow replication	(Hegde *et al.*, 2009)
Titu	IV	Goat	Attenuated vaccine Bangladesh	(Diallo *et al.*, 2019; Milovanović *et al.*, 2023)
45G37/35-K	IV	Goat	Attenuated Vaccine Russia	(Milovanović *et al.*, 2023)

The development of new-generation PPR vaccines involves diverse strategies aimed at creating effective, thermostable vaccines with the added benefit of serving as marker vaccines for the differentiation of infected from vaccinated animals (DIVA) (Herbert *et al.*, 2014). A novel strategy for developing morbillivirus DIVA vaccines involves incorporating mutations into viral proteins using reverse genetics (Buczkowski *et al.*, 2012). In this method, specific epitopes in the vaccine virus are intentionally altered. This alteration ensures that the vaccine-induced immune response lacks antibodies against the modified epitopes. Such DIVA vaccines are valuable for distinguishing between infected and vaccinated animals, enhancing control measures for morbilliviruses like PPRV (Buczkowski *et al.*, 2012).

Table-6: New generation vaccines under development for PPR

New generation vaccine approach	Details of vector	Genes expressed	Features	Reference
Virus vector vaccine	Capripoxvirus	PPRV F	Protection at a lower dose of 0.1 pfu	(Berhe *et al.*, 2003)
Virus vector vaccine	Capripoxvirus	PPRV H/F	Long-lasting immunity	(Chen *et al.*, 2010)
Virus vector vaccine	Vaccinia	PPRV H and F	Challenge protection 4 months post-vaccination	(Chandran *et al.*, 2010)
Virus vector vaccine	Canine adenovirus type 2	PPRV H/F/ H-F fusion protein	Neutralizing antibody response lasting for 21 weeks	(Euler *et al.*, 2013)
Expression in plant	Arachis hypogea	PPRV H protein	Oral immunization in sheep produced neutralizing antibodies and cell-mediated immune response	(Khandelwal *et al.*, 2011)
Reverse genetics	PPRV	GFP	Suitable for high-throughput neutralization test	(Hu *et al.*, 2012)
Reverse genetics	PPRV	Mutation in PPRV protein	An alternative approach for the DIVA vaccine, epitope altered, vaccine immune response lacks antibodies to the epitope	(Buczkowski *et al.*, 2012)

Capripoxvirus has been utilized to develop dual vaccines, expressing PPRV F-protein, providing effective protection at a low dose. Recombinant capripox virus expressing PPRV H- or F-proteins has demonstrated long-lasting immunity against PPR (Gebreeziabher, 2006). Vaccinia virus has been employed as a vector for recombinant PPRV vaccines, inducing a protective immune response in goats leading to complete protection against virulent virus challenges (Chandran *et al.*, 2010).

Vaccines against PPRV have been developed using strategies similar to other morbilliviruses due to their functional and structural similarities. Ongoing developments focus on four categories, reflecting substantial advancements in vaccine strategies to effectively control PPRV. These are i. Heterologous Attenuated PPRV Vaccines ii. Homologous Attenuated PPRV Vaccines iii. Stability of Live Attenuated PPRV Vaccines iv. Recombinant Marker PPRV Vaccines (Munir *et al.*, 2013).

PPR mass vaccination aims for 80% flock immunity, considering small ruminant dynamics, with 30–40% naïve sheep and goat population annually, influenced by farming practices and agroclimatic conditions (Woma *et al.*, 2016). Comprehensive vaccination of the entire population in a specific area, followed by vaccination of 3–4-month-old naïve animals (Singh, 2011), is crucial to prevent susceptibility in newborn animals and minimize the risk of disease spread (Balamurugan *et al.*, 2012). Vaccination strategies should target high-risk young animals, migratory or trade market flocks, and other vulnerable groups (Singh, 2011). In enzootic settings, intensive vaccinations within identified niche areas are recommended to establish "PPR-free zones." The approach involves a combination of exhaustive vaccinations, age-specific targeting, and a strategic focus on vulnerable populations to effectively control PPR.

2.13.3. Role of Wild Life

A greater understanding of the epidemiology of PPRV at the wildlife-livestock interface is required to formulate science-based management options that support eradication efforts and protect biodiversity. Knowledge gaps exist across all steps of the spillover process: susceptibility of wildlife hosts, transmission mechanisms, and the ability of new host species to maintain infection (Sokolow *et al.*, 2019).

The assessment of disease impacts on wildlife is reliant on direct counts of dead animals, which typically results in an underestimation of the true impact at the population level

(Pruvot *et al.*, 2020). To measure the actual impact of PPRV on the wildlife population species-specific surveys must be adopted and applied consistently. Utilizing dynamic models of within- and between-host transmission, the addition of information regarding the PPRV will explain several key aspects.

These models will clarify the role of wildlife in PPRV epidemiology, and the impact of wildlife hosts on eradication strategies, and predict the short- and long-term impacts of PPRV on wild ungulate communities. Insufficient species-specific data on susceptibility, viral excretion quantity and duration, and immune response dynamics hold back the interpretation of the epidemiological role played by various artiodactyl species. Monitoring the serology of vaccinated captive wildlife and atypical host species would suggest insights into the immunogenicity of existing vaccines and the dynamics of the immune response. This information can enhance the interpretation of serological data obtained through routine monitoring (Pepin *et al.*, 2017). Significant knowledge gaps exist regarding the mechanisms of transmission between livestock and wildlife, including the potential for indirect transmission via various means such as fomites, pasture, feed, water, and mechanical insect vectors. Addressing these gaps necessitates research on viral viability across diverse substrates such as water, soil, mineral licks, hair coats, feces, and carcasses, under varying environmental conditions such as temperature, humidity, ultraviolet exposure, water turbidity, and salinity. Thorough descriptions of wildlife-livestock interactions utilizing spatiotemporal analysis can enhance our understanding of the contribution of transmission routes to interspecies transmission (Pruvot *et al.*, 2014). This approach helps identify potential prevention and control measures (Sokolow *et al.*, 2019). Participants stressed the significance of monitoring wildlife species pre- and post-vaccination to learn from PPR treatments. Vaccinating livestock in crucial ecosystems allows researchers to study how in-contact animals or atypical host populations retain viruses and how livestock vaccination might reduce spillover into wild artiodactyls (Viana *et al.*, 2014).

CHAPTER III
MATERIALS AND METHODS

3.1. Description of Study area and design

The study was carried out from July 2023 to March 2024 in three (3) districts (Dhaka, Narsingdi, and Gazipur) located in the Dhaka division of Bangladesh. The study areas, Narsingdi (Monohardi) and Gazipur (Kapasia) are the central parts of Bangladesh, which surrounds the capital city of Dhaka (Figure-11). These areas are between 23.8041 to 23.9905 N latitude and 90.3877 to 90.7234 E longitude. Though Dhaka is not a pastoral city, the study area especially the Dohar and Savar Upazila depended on livestock and agriculture wherein most of the women rear sheep and goats as their major earning source. The human population of these districts is around 28 million which shares more than one-tenth of the total population of Bangladesh (HDX, 2022). The study populations were maintained through pastoral husbandry characterized by the high mobility of animals and frequent mingling with other species. The sheep and goats in these areas were subjected to an extensive farming system primarily because of the surroundings' riverside or small lakeside nature.

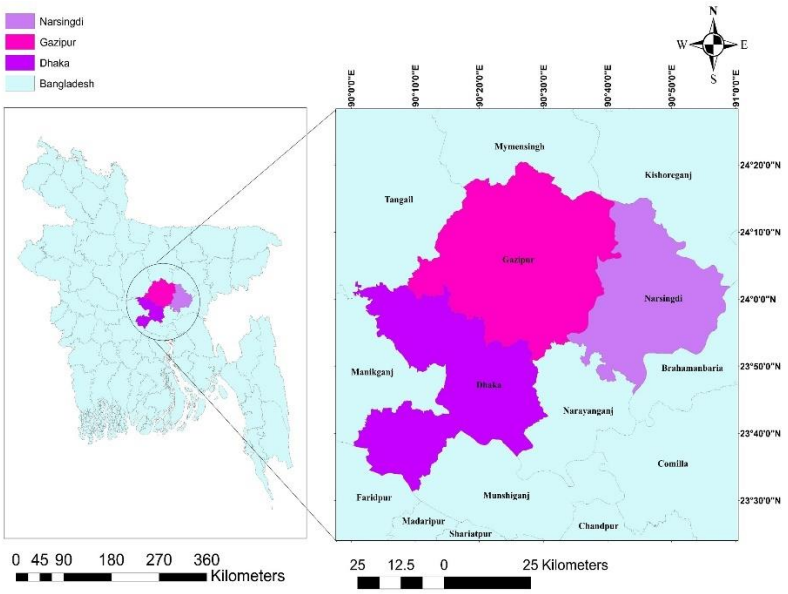


Figure-11: Map of study districts in Bangladesh. The map was generated in ArcGIS ArcMap Version 10.3

The study areas were purposefully chosen for different reasons including the study population being higher, transportation being easily accessible, and having no vaccination history within the last 2 years according to the history of the livestock office of these areas and farm owners People were eager to participate and contribute in the research study.

Flow-chart of the study design

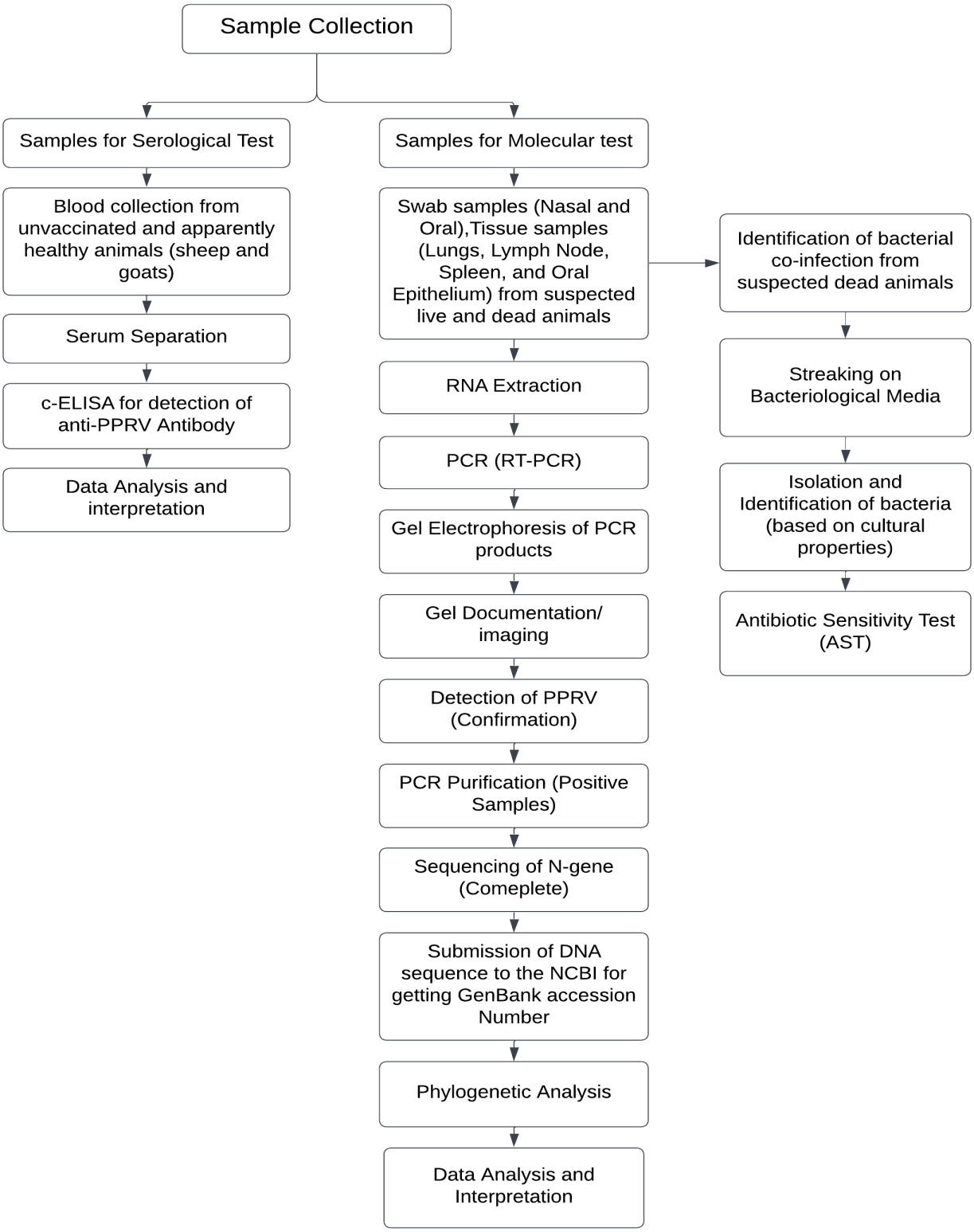


Figure-12: Sketch of the work-flow

3.2. Study population and sampling strategies

The study was conducted in small-ruminants, especially in sheep and goats aged usually over 4 months. A total of 368 (N=368; sheep, n=36 and goat, n=332) blood samples were collected from un-vaccinated and apparently healthy sheep and goats for serum separation. The animals were categorized into age, gender, and breed. Additionally, a total of 100 (n=100) clinical samples [oral and nasal swabs and different tissues (Lung, spleen, Epithelium, and Lymph node) were also collected from PPR-suspected live and dead animals (sheep and goats). This study employed a straightforward random sampling method to choose individual animals from the target population during sample collection. The current study determined the sample size following the formula provided by the previous study (Thrusfield, 2018) considering the expected prevalence was 44.3% according to the previous study conducted in Bangladesh (Rahman *et al.,* 2023). A 5% desired absolute precision and a 95% confidence interval were applied in the calculation. Despite being distinct species, sheep and goats were treated as a single population in this study. This approach was adopted because sheep and goats are the primary hosts of PPR sharing similar disease courses and being subjected to analogous management practices. Thus, the following formula was used to calculate the sample size for the study given below.

$$n = \frac{Z^2 x P_{exp}(1 - P_{exp})}{d^2}$$

Where: n = required sampling size.

Z = Multiplier from normal distribution at 95% Confidence interval (1.96).

P_{exp} = Estimated (expected) prevalence 44.3% (0.443).

1-P_{exp} = Probability of having no disease 55.7% (0.557).

d = Desired absolute precision 5% (0.05).

According to this formula, the estimated sample size is 380.

3.3. Sample collection, transportation, processing, and storage

3.3.1. Blood Sample for Serum Separation

Blood samples were collected following the previous studies with a little modification (Dubie *et al.,* 2022; Rahman *et al.,* 2023). Blood (about 3-5 ml) was aseptically collected from the jugular veins of unvaccinated and apparently healthy sheep and goats into a disposable 10 ml syringe and 19-gauge sterile needles [Getwell, Bangladesh] with the help of a local veterinarian officer. The collected blood samples in a syringe were kept at the slanting

position and left at room temperature for about 30-45 minutes (until clotting). Thereafter, the collected blood samples in a syringe were kept in an icebox while maintaining the proper cool chain until transported to the Pathology laboratory of Sher-e-Bangla Agricultural University (SAU. After transportation to the laboratory, the serum was carefully collected without disturbing the clotted blood in the syringe by using a pipette and transferred to a sterile and labeled 1.5 ml eppendorf tube [Tarsons, India]. Then, the collected serum was purified by centrifugation at 4^0C and 2500 rpm for 15 minutes and collected into 1.5 ml eppendorf tubes, aliquoted (2), and stored at -20^0C till further use.



Plate-1: Collection of Blood Samples from sheep and goats; A. Collecting blood from Goats; B, C. Collecting blood from Sheep D. Sheep farm

3.3.2 Swab and Tissue Samples Collection for virus isolation and identification

Nasal and oral swab samples were collected from PPR-suspected live and dead sheep and goats using sterile cotton swabs into 2 ml Eppendorf tubes [Tarsons] containing phosphate-buffered saline (PBS) [10X Phosphate Buffered Saline, Sisko Research Laboratories, India]

(as viral transport media) and antibiotics (penicillin-streptomycin) [MSE penicillin-streptomycin solution 100x, France]. Additionally, tissue samples (lymph node, lungs, intestine, spleen, and epithelial tissue) were also collected in a 50 ml Falcon tube (Falcon^{R}, Mexico) from PPR suspected dead animals. After collection, all samples were kept in an ice box and transported immediately (maintained with a proper cool chain) to the Pathology laboratory of Sher-e-Bangla Agricultural University (SAU) and stored at -20^{0}C until further use. All the samples were labeled accordingly.



Plate-2: Swab sample collection from PPR-suspected goat

3.4. Laboratory analysis

3.4.1. Detection of anti-PPRV antibody

Sero-prevalence was measured on the collected serum samples using a commercial ELISA kit [Green Spring®, *peste des petits ruminants* virus antibody ELISA kit, Shenzhen Lvshiyuan Biotechnology Co., Ltd., China] according to the manufacturer's instructions. The optical density (OD) values of the final plates were measured at 450 nm using an ELISA reader [BioTek 800 TS, Agilent, USA]. The OD values for each sample were transformed into S/N using the provided formula:

$$\mathrm{S/N} = \frac{\text{Sample OD value}}{\text{Average OD value of Negative Control}}$$

The results were valid as the OD value of negative control (N) ≥0.60 and the OD value of positive control (P) <0.40. The results were calculated by the Sample OD value/average OD

value of negative control (N)=S/N value), and the result was interpreted as negative if the S/N value ≥0.5 and positive if the S/N value <0.5.

3.4.1.1. Materials

i. Single-channel Pipette with tips
ii. Multichannel Pipette with tips
iii. ELISA Kit
iv. Deionized Water
v. ELISA Washer [BioTek 50 TS, Agilent, USA]
vi. ELISA Reader [BioTek 800 TS, Agilent, USA]
vii. Incubator
viii. Gloves
ix. Reservoir
x. Measuring Cylinder
xi. Disposable Tips
xii. Foil Paper
xiii. Water Bath
xiv. Disposable Box

3.4.1.2. Preparation

i. ELISA reagents were brought to room temperature (20-25^{0}C) for 30 minutes for the best results. The microplate returned to room temperature and dried before opening the package.
ii. Washing solution preparation: The concentrated (10x) washing buffer was 10 times diluted with deionized water (10 ml concentrated (10x) washing buffer + 90 ml deionized water).

3.4.1.3. Procedures

3.4.1.3.1 Microplate Preparation and Sample Loading

- The pre-coated microplate was divided according to the sample number and wells used individually.

- Two wells were designated for positive controls and two for negative controls. To these respective wells, 50 µl of positive control serum and negative control serum, respectively, were added.
- All remaining wells were considered as sample wells. To each of these, 40 µl of sample diluent was added first, followed by the addition of 10 µl of individual serum samples.
- Then, 50 µl of enzyme conjugate was added to each well. The plate was then gently shaken to ensure even mixing and covered with adhesive foil.
- Incubation was performed at 37°C for 30 minutes.

3.4.1.3.2. Washing

- Following 30 minutes of incubation, the adhesive foil was removed, and the well contents were discarded.
- Each well was then filled with 300 µl of diluted washing buffer, allowed to stand for 30 seconds, and the liquid was subsequently discarded.
- This washing step was repeated five (5) times. Lastly, the plate was patted dry using absorbent paper.

3.4.1.3.3. Substrate Addition and Reaction

- A volume of 100 µl of substrate solution was added to each well, followed by gentle mixing. The plate was then covered with adhesive foil for incubation.
- Incubation proceeded for 15 minutes at 37°C in a dark environment.

3.4.1.3.4. Reaction Termination and Measurement

- To stop the reaction, 50 µl of stop solution was added to each well.
- Absorbance measurements were taken at a specific wavelength (450 nm) within 10 minutes following the addition of the stop solution.

3.4.1.4. Interpretation

The samples with S/N value less than 0.50 were considered positive and more than or equal to 0.50 were considered Negative.

Table-7: Interpretation of ELISA Result with Positive and Negative Control

Parameter	S/N Value
Negative Sample	≥0.50
Positive Sample	<0.50

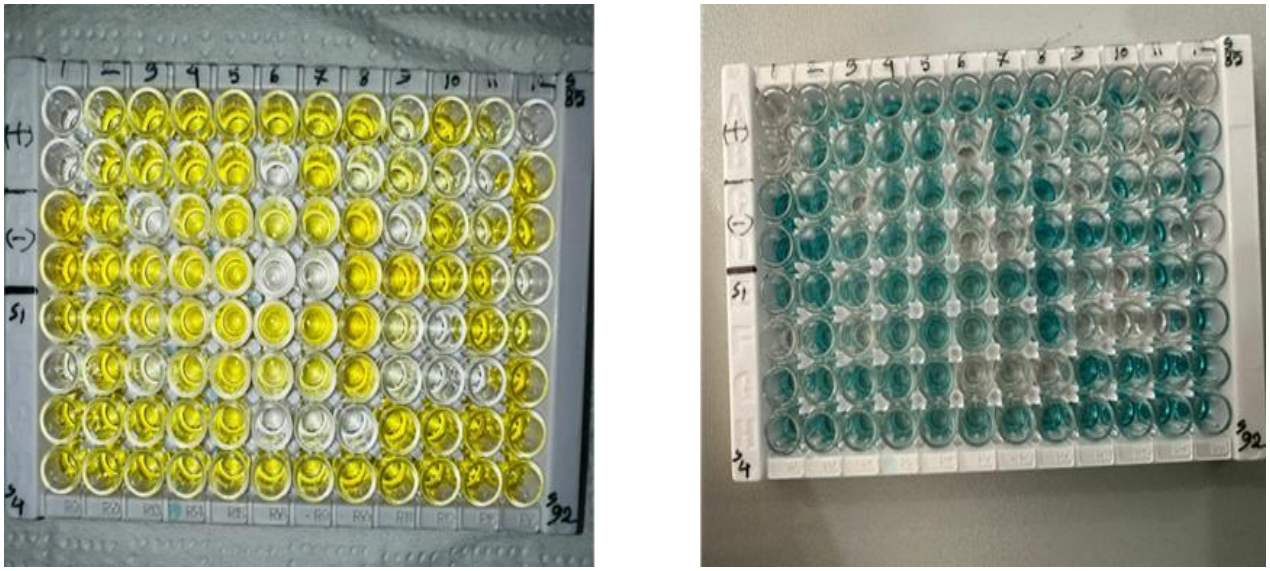


Plate-3: c-ELISA for anti-PPRV antibodies

3.4.2. RNA extraction

PPR viral RNA was extracted from the samples (oral/nasal swabs/tissues) collected from suspected live or dead animals. by using a commercial Viral RNA/Viral Nucleic Acid Extraction mini kit [FavorPrep ^{TM}, Favorgen^{R} Biotech Corp, Taiwan] as per the manufacturer's instructions. The extracted viral RNA was immediately used for PCR (RT-PCR) test and stored at -20^{0}C until further use.

3.4.2.1 Materials

- Refrigerated microcentrifuge machine
- Sterile Pipettes and tips
- Eppendorf tube
- Heating Block
- Vortex mixer
- Molecular grade Ethanol (100% pure)

3.4.2.2. Preparation of VNE-Carrier RNA Buffer

One (1) ml of VNE buffer to the tube containing lyophilized carrier RNA was added. Proper mixing was done by vortexing and transferring the mixture to the VNE buffer when it was first opened. The VNE carrier buffer was stored at 4^0C until further use.

3.4.2.3. Preparation of wash buffer-1 and wash buffer-2

Thirty-six (36) ml of 100% ethanol (MB grade) was added for wash buffer-1 to prepare 100 preps and 80 ml of 100% ethanol (MB grade) was added for wash buffer-2 to prepare 100 preps. Wash buffer-1 and wash buffer-2 were stored at 15-25^0C.

3.4.2.4. Procedure

- Firstly, the swab's transport tube was vortexed and then spun the tube to descend the drops attached to the tube wall.
- 140 µl of medium transferred to a microcentrifuge tube.
- 560 µl of VNE (carrier RNA added) buffer was added to the tube and mixed properly by vortexing. Then, the tube was incubated for 10 minutes at room temperature.
- 560 µl of 100% ethanol was added to the sample mixture and mixed well by plus-vortex.
- A VNE column with a collection tube was combined. 700 µl of sample mixture was transferred to the VNE column. Then, the sample mixture was centrifuged at 8000 g for 1 minute. Then, the flow-through was discarded. After that, the VNE column with the used collection tube was combined.
- The rest of the sample mixture (ethanol added) to the VNE column was transferred and centrifuged at 8000 g for 1 minute. Then, the flow-through and collection tube.
- The VNE column with a new collection tube was combined.
- 500 µl of wash buffer 1 was added to the VNE column and centrifuged at 8000 g for 1 minute and discarded the flow-through. Then, the VNE column with the used collection tube was combined.
- 650 µl of wash buffer 2 was added to the VNE column and centrifuged at 8000 g for 1 minute and discarded the flow-through. Then, the VNE column with the used collection tube was combined.

- Again, 650 µl of wash buffer 2 was added to the VNE column and centrifuged at 8000 g for 1 minute, and discarded the flow-through was. Then, the VNE column with the used collection tube was combined.
- The mixture was centrifuged at full speed (18000 g) for 1 minute to dry the VNE column. Then, the flow-through and collection tubes were discarded.
- The VNE column with on elution tube was combined. 60 µl of RNase-free water was added to the membrane center of the VNE column. The VNE column stood for 1 minute. For effective elution, RNase-free water was dispensed onto the membrane center and was absorbed completely.
- Finally, the elution tube was centrifuged at 18000 g for 1 minute to elute the viral RNA. Viral RNA was stored at -70^{0}C for further use.

3.4.3. RT-PCR

The N-gene (complete) of the PPR virus was amplified from extracted RNA samples with the gene-specific primer sets (Table-9) using a commercial one-step RT-PCR kit [PrimeScript™ One-Step RT-PCR Kit, Takara Bio Inc., USA] according to the manufacturer's instructions. The PCR was carried out with the final reaction volume of 50 µl containing 25 µl of 2x 1 step buffer, 2 µl of forward primer and reverse primer (20 picomoles of each primer), 2 µl of PrimeScript 1 step Enzyme mix, 1 µl of RNAse inhibitor (Ribolock) and 14 of µl of nuclease-free water, and 4 µl of RNA template. And, the reaction condition was 1 cycle of cDNA synthesis (Reverse Transcription) and predenaturation (Initial Denaturation) at 50 °C for 30 min and 94°C for 2 min, respectively, followed by 35 cycles of PCR amplification at 94°C for 15 s (denaturation), 57°C for 30 s (annealing), and 68°C for 1min/kb (extension). A final extension of 1 cycle was performed at 72^{0}c for 5 min in a Thermal cycler (BIO-RAD T100™ Thermal cycler). Then, the amplified DNA was stored at 4^{0}C until further use.

3.4.3.1. Materials

- PCR machine [BIO-RAD T100™ Thermal cycler]
- PCR Tube
- Nuclease-free Water
- PCR kit
- Forward Primer

- Reverse Primer
- RNA Template

Table-8: One-step RT-PCR Cycles with required time and temperature

Steps	Temperature	Time
Reverse Transcription	50^0C	30 Minutes
Initial Denaturation	94^0C	2 minutes
Denaturation	94^0C	15 Seconds
Annealing	57^0C	30 seconds
Extension	72^0C	1 Minute/kb
Final Extension	72^0C	5 Minutes
Hold	4^0C	Infinite

Table-9: Sequences of the primers used for PCR amplification and sequencing

Sequence region	Primer name	Nucleotide sequence (5'to 3')	Sequence length (bp)[a]	Reference
N-Gene	pN-53F	CTT AGG AGT AAA GAT CCT ACT GTC	1781	Current study
	pN-1833R	GAC ATG GTA TGC TTG TTC CTCT		
	N-740F	TGA CAA AGG GTG GCT GGA C		
	N-786R	GAT CTT CTG CAA TTC TGT TGC G		
	N-1204F	AGG AGA TGG TCA GAA GAT CTG C		

[a] Primer sequence designed based on a full-length genome of PPRV (GenBank accession number: MG581412). The subscript prefix $_{\mathbf{p}}$ indicates a primer used for both PCR amplification and sequencing. Legend: F = Forward, R = Reverse, N = Nucleoprotein (N gene).

3.4.3.2. Gel Electrophoresis

PCR amplicons (products) of 5 µl were analyzed by electrophoresis in 1% agarose gel [AddBio Inc., South Korea] in Tris-Acetate-ETDA buffer (TAE). Positive and negative controls were also used along with test samples. The agarose gel was stained with Redsafe (nucleic acid staining solution) and the DNA bands were visualized by a UV transilluminator

and the image was transferred to a computer. The procedure of gel preparation and electrophoresis is given below:

i. The gel casting tray was constructed using a gel comb that has teeth of suitable size and quantity.
ii. A solution of 1% agarose gel was produced in TAE buffer using the process of melting in a microwave oven.
iii. The casting tray was filled with melted agarose and left to harden on the bench.
iv. The gel that had solidified in its tray was moved to the electrophoresis tank, which was filled with enough amount of TAE buffer to ensure that the gel was covered by 1 mm. The hair comb was delicately extracted.
v. For each PCR product, a volume of five microliters (5µl) was combined with 1µl of 6x loading dye was poured into the respective well onto the gel
vi. In one well, a volume of five microliters (5 µl) of DNA size marker was added.
vii. The electrophoresis apparatus's leads were linked to the power source, and the electrophoresis process was conducted at a voltage of 220V.
viii. When DNA had undergone enough migration, as determined by the migration of bromophenol blue in the loading dye, the power supply was deactivated.
ix. In the dark chamber of the gel documentation system, the gel that was held in custody was positioned on the UV transilluminator.
x. The ultraviolet (UV) light of the system was activated, and the picture was seen on the display, adjusted to focus, captured, and stored on the USB flash drive.

3.4.4. PCR Purification

Purification of the PCR product was done by using a purification kit [AddPrep Fragment Purification Kit, AddBio Inc., South Korea] as per manufacturer instructions. The purified PCR products were stored at -20^{0}C and submitted to a commercially available sequencing company for further use. Laboratory procedures were conducted according to the manufacturer’s recommendation.

3.4.4.1. Materials and Solutions for PCR Purification

- ✓ PCR purification kit
- ✓ Spin Column
- ✓ Binding Solution

- ✓ Washing Buffer
- ✓ Elution Solution
- ✓ Vortex
- ✓ Centrifuge machine
- ✓ Collection Tube
- ✓ Micro-centrifuge tube

3.4.4.2. Preparation

- Ethanol was added to the washing solution
- Binding solution was checked for precipitation and was broken the precipitation by warming at 50^0C.

3.4.4.3. Procedures

i. 25 µl of Binding solution was added to 5 µl of sample and mixed properly by using Vortex.
ii. The tube was centrifuged at 13000 rpm for 30 seconds at room temperature.
iii. The mixture was transferred to a spin column.
iv. The mixture was centrifuged for 1 minute at 13000 rpm.
v. The flow-through was discarded and the spin column was put into the collection tube.
vi. 700 µl of washing solution was added to the spin column and centrifuged for 30 seconds at 13000 rpm.
vii. Again, the mixture was centrifuged for an additional 2 minutes at 13000 rpm to remove residual washing buffer in the spin column and glass fiber filter.
viii. The spin column was then transferred to a new 1.5 ml micro-centrifuge tube.
ix. 50 µl of elution solution was added to the center of the glass fiber filter in the column.
x. The mixture was let stand for 2 minutes at room temperature and centrifuge for 1 minute at 13000 rpm.

3.4.5 Sequencing and Phylogenetic Analysis

Three (3) samples of RT-PCR positive samples with N-gene amplification were submitted to a commercial sequencing company (Macrogen, Inc., South Korea) for sequencing the whole N-gene of the PPR virus. The Nucleotide sequences of the full length of N-gene were aligned

as a consensus sequence and analyzed using SeqManII and Lasergene MagAlign software (DNASTAR, Inc., Madison, WI, USA). The analyzed DNA sequences were deposited in the NCBI database under the GenBank accession number **PP534161**. Then, these sequence data were employed to construct a phylogenetic tree (the neighbor-joining method) using MEGA11 software to see the genetic distance of the circulating viruses with previous virus sequences published elsewhere and vaccine strains as well. In brief, the nucleotide sequences acquired in this study were compared with the sequence data of PPRV strains available in the NCBI GenBank database. Nucleotide sequences were aligned using the CLUSTAL W multiple sequence alignment program (Thompson *et al.*, 2003). Phylogenetic trees were constructed according to the neighbor-joining method with the Kimura 2-parameter model at the bootstrap value of 1000 replicates using the MEGA version X (Kimura, 1980; Tamura *et al.*, 2011).

3.5. Identification of bacterial co-infections

Identification of co-infections (if there were any), three (3) samples collected from dead sheep (1) and goat (2) were investigated for bacterial co-infections according to a previous study (Cheesbrough, 1981).

3.5.1. Bacterial Culture and Media Preparation

Bacteriological procedures, conducted following standard methods, were applied to swabs and tissue samples. Cultural procedures were used to diagnose bacterial co-infection. Different bacterial agar media were utilized to identify both aerobic and anaerobic bacteria in the samples.

3.5.1.1. Nutrient agar (NA)

The nutrient agar was made according to the instructions provided by the manufacturer. 23 grams of NA base [Scharlau, Spain] was dissolved in 500 ml of distilled water in a conical flask. The flask was then heated until it reached boiling point, ensuring thorough dissolution of the medium. Next, the solution underwent sterilization using autoclaving at a temperature of 121ºC and a pressure of 15 lbs per square inch for 15 minutes. Following the autoclaving process, the medium was then transferred into sterile Petri dishes and left to harden for 3-5 hours. Once the medium solidified, the plates were inverted and incubated at 37ºC overnight

to ensure their sterility. They were then stored at 4ºC in the refrigerator until they were ready to be utilized.

3.5.1.2. Mannitol salt (MS) agar

The preparation of Mannitol salt (MS) agar followed the instructions provided by the manufacturer. 111.02 grams of dehydrated MSA agar [Scharlau, Spain] was mixed with 1000 ml of distilled water in a conical flask. The mixture was then heated to boiling to fully dissolve the medium. The solution was sterilized using autoclaving at a temperature of 121ºC and a pressure of 15 lbs per square inch for 15 minutes. The liquid was thereafter put onto sterilized petri plates and left to harden for 3-5 hours. Following the solidification of the medium, the plates were inverted and subjected to overnight incubation at a temperature of 37ºC to assess their sterility. Subsequently, they were stored at a temperature of 4ºC in the refrigerator until they were needed for future use.

3.5.1.3. Eosine Methylene Blue (EMB) agar

The preparation of Eosine Methylene Blue (EMB) agar followed the instructions provided by the manufacturer. 37.5 grams of EMB agar base powder [Scharlau, Spain] was dissolved in 1000 cc of distilled water. The suspension was subjected to heat for a brief duration to achieve full dissolution of the powder with water. A thick layer of EMB agar plate was formed by pouring the sterilized medium into sterile glass Petri dishes by autoclaving at a temperature of 121ºC and a pressure of 15 lbs per square inch for 15 minutes, for 3-5 hours. Once the medium had solidified, the plates were inverted and subjected to overnight incubation at 37ºC to assess their sterility. Subsequently, they were stored at a temperature of 4ºC in the refrigerator until they were ready for use.

3.5.1.4. Salmonella Shigella (SS) agar

According to the manufacturer's instructions, Salmonella Shigella (SS) agar was made. A total of 63.02 grams of dehydrated SS agar [HiMedia, India] was suspended in 1000 ml of distilled water. Subsequently, the medium was subjected to heat until it reached a boiling point. The solution underwent autoclaving, followed by thorough mixing. Subsequently, it was transferred onto sterile petri dishes and allowed to harden for 3-5 hours. Following the solidification of the medium, the plates were inverted and subjected to overnight incubation

at a temperature of 37ºC to assess their sterility. Subsequently, they were stored at a temperature of 4ºC in the refrigerator until they were needed for future use.

3.5.1.5. Muller Hinton agar

According to the manufacturer's instructions, Muller Hinton agar was produced by suspending 38 grams of dried MH agar [HiMedia, India] in 1000 ml of distilled water. Next, the medium was heated until it reached its boiling point to fully dissolve it. The solution was sterilized using autoclaving at 121ºC under 15 lbs. pressure for 15 minutes and then cooled to 45-50^{0}C. Next, the mixture was vigorously agitated and transferred onto aseptic petri dishes for solidification (3-5 hours). Following the solidification of the medium, the plates were inverted and subjected to overnight incubation at a temperature of 37ºC to assess their sterility. Subsequently, they were stored at a temperature of 4ºC in the refrigerator until they were needed for future use.

3.5.1.6. Luria Bertani (LB) Broth, Miller

The preparation of Luria Bertani (LB) Broth [Scharlau, Spain] followed the instructions provided by the manufacturer. It included dissolving 25 grams of LB powder in 1000 cc of distilled water. Next, the substance was subjected to heat to fully dissolve the power. The solution was sterilized using autoclaving at a temperature of 121ºC and a pressure of 15 lbs for 15 minutes. It was then allowed to cool for a few minutes. Ultimately, it was held at a temperature of 4°C until further use.

3.6. Antimicrobial Sensitivity Test

In vitro testing was conducted on the recovered bacteria to assess their sensitivity and resistance to nine frequently used antibiotics. The Kirby-Bauer disc diffusion technique was used to do this, according to the protocols specified by the National Committee for Clinical Laboratory Standards (NCCLS), as previously elucidated (Bauer *et al.*, 1966). The antibiotics utilized were Gentamicin (Gen, 10µg), Azithromycin (AZM, 30µg), Levofloxacin (LE, 5µg), Tetracycline (TE, 30µg), Ampicillin (AMP, 25µg), Erythromycin (E, 15µg), Amoxicillin (AMX, 30µg), Nalidixic acid (NA, 30µg), and Ciprofloxacin (CIP, 5µg). By measuring the width of the inhibition zone generated by the diffusion of the agent into the surrounding media around the disc, this technique allowed for a quick evaluation of antimicrobial

effectiveness (Table 3.4). To summarize, the suspension of the test microorganism was produced in nutrient broth using an overnight culture method. The broth culture was transferred onto Muller Hinton agar [HiMedia, India] using a pipette, with a volume of 0.1ml. The agar was then evenly distributed using a sterilized glass spreader. Following a drying period of about 3-5 minutes, the plates were subjected to an aseptic application of antibiotic discs over the agar surface. This was done in a precise arrangement using sterile forceps. The plates were flipped and raised at a temperature of 37ºC for 24 hours. Based on the recommendations provided by the National Committee of Clinical and Laboratory Standards Institute, the zones of growth inhibition were measured in millimeters after incubation. The findings were then interpreted as susceptible/sensitive, intermediate, or resistant (CLSI, 2016).

3.6.1. Antimicrobial discs

The antimicrobial sensitivity and resistance pattern of commercially available antibacterial discs [OXOID Limited, Canada] were analyzed to assess their effectiveness against diseases. The technique enabled the prompt assessment of medication effectiveness against the test organisms by the measurement of the zone of inhibition diameter. This zone was formed as a consequence of the agent's diffusion into the surrounding media, hence impeding the development of the organisms. The sensitivity and resistance pattern of the detected bacterial pathogens (*E. coli, Staphylococcus epidermidis*, and *Staphylococcus aureus*) isolated from sheep and goats were tested using the following antimicrobial agents, along with their respective concentrations and ranges.

Table-10: List of antibiotics used in this study to assess their antibiotic sensitivity and zone of ranges of inhibition.

SL No.	Name of the Antibiotics	Disk code	Disk potency (µg)	Zone Diameter (mm)		
				Resistance (R)	Intermediate (I)	Susceptible (S)
1.	Amoxicillin	AML	10	13	14-17	20
2.	Ampicillin	AMP	10	13	14-17	17/29
3.	Azithromycin	AZM	15	13	14-17	18
4.	Erythromycin	ERY	15	13	14-22	23
5.	Nalidixic acid	NA	30	13	14-18	19

6.	Gentamicin	CN	10	< 12	13-14	15
7.	Tetracycline	TE	30	14	15-18	19
8.	Levofloxacin	LEV	5	13	14-16	17
9.	Ciprofloxacin	CIP	5	15	16-20	21

Abbreviation: µg = microgram, mm = millimeter. (CLSI, 2016)

3.7. Data analysis

The data for each sample while collecting blood samples, nasal swabs, oral swabs, and tissues was recorded by using *KoboToolBox*. Microsoft Excel spreadsheet was used for raw data entry. The Nucleotide sequences were aligned as a consensus sequence and analyzed using SeqManII and Lasergene MagAlign software (DNASTAR, Inc., Madison, WI, USA). The Chi-square (χ^2) analysis result indicated that among the associated risk factors with PPRV seroprevalence occurrence. A Chi-square was done by using the R package tidyverse, version 4.3.3 (Wickham *et al.*, 2019). The association of associated factors such as different areas, species, gender, herd size, and age to the results of seroprevalence of PPRV infection was analyzed using Univariable and multivariable logistic regression models. Then a phylogenetic tree was constructed on the analyzed nucleotide sequences using MEGA11 software. A univariable logistic regression model was done by using the lmtest package in R version 4.3.3 (Zeileis & Hothorn, 2002). The variable stepwise forward multivariable logistic regression model was used to recognize the most significant risk factors. This statistical analysis was done using the gtsummary package in R-programming version 4.3.3 (Sjoberg *et al.*, 2021). A statistically significant association between variables was said to exist if the calculated *P*-value was less than 0.05 at a 95% confidence interval (CI). GraphPad Prism 5.0.2 (GraphPad Software, Inc., CA, USA) and R programming (v4.3.3) were used to generate tables and maps. Overall seroprevalence was calculated by dividing the number of seropositive samples by the total number of animals sampled (Gebre *et al.*, 2018). The formula used to calculate the seroprevalence is given below:

$$\text{Seroprevalence} = \frac{\text{Seropositive samples}}{\text{Total number of animals sampled}} \text{ X } 100$$

CHAPTER IV
RESULTS AND DISCUSSION

4.1. Results

4.1.1. Descriptive statistics of the samples

The study delineated the demographic characteristics of the tested animal population in Table-11. A total of 368 samples were collected in this study of which 90.22% (n=332/368) were goats and 9.78% (n=36/368) were sheep from 3 districts (Dhaka, Gazipur, and Narsingdi). Of the total samples, 58.97% (n=217/368) were females while 41.03% (n=151/368) were male. Among the breeds, Cross Breed (n=196/368) and Black Bengal Breed (n=136) were for goats, and only Local Breed (n=36/368) was for Sheep. The age of the study animals was classified into four (4) categories: <6 months, 7-12 months, 13-24 months, and >24 months (approximately 17.4%, 40.2%, 32.2%, and 10.0%, respectively). Geographically, Dhaka had the highest representation (53.26%) followed by Gazipur (26.63%) and Narsingdi (20.11%). These areas were classified into two (2) ways: Plain Land (31.52%) and River Basin (68.48%) based on ecological zone. The feeding system practiced in the study areas was classified into two (2): 68.75% (n=253) of animals were practicing free-grazing while 31.25% (n=115/368) of them were practicing stall feeding. In the case of the source of animals, most animals were brought from the market (62.00%) while approximately 38.00% of animals were born on the self-farms. Approximately 55.50% of animals were introduced into the farm as a new animal while 44.50% were not introduced as new ones. Herd size was categorized into three (3) based on the number of animals; medium-sized herds being the most common (42.39%) followed closely by large herds (41.03%) and small herds (16.58%).

Table-11: The demographic characteristics of the tested animals

Variable	Category		Frequency (%)
Species	Goat		332 (90.22%)
	Sheep		36 (9.78%)
Gender	Male		151 (41.03%)
	Female		217 (58.97%)
Breed	Goat	Black Bengal	136 (36.96%)
		Cross Breed	196 (53.26%)
	Sheep	Local Breed	36 (9.78%)
Area	Dhaka		196 (53.26%)

	Gazipur	98 (26.63%)
	Narsingdi	74 (20.11%)
Age	<6 months	64 (17.39%)
	7-12 months	148 (40.22%)
	13-24 months	119 (32.34%)
	>24 months	37 (10.05%)
Ecological Zone	Plain Land	116 (31.52%)
	River Basin	252 (68.48%)
Source	Animal Market	228 (61.96%)
	Self-farm	140 (38.04%)
Feeding Systems	Grazing	253 (68.75%)
	Stall Feeding	115 (31.25%)
Introduction New Animal	Yes	204 (55.43%)
	No	164 (44.57%)
Herd Size	Small (1-5 animals)	61 (16.58%)
	Medium (6-10 animals)	156 (42.39%)
	Large (>10 animals)	151 (41.03%)

4.1.2. Seroprevalence of PPRV infection

The overall seroprevalence of PPRV antibody (anti-PPRv Ab) was 52.45% (n=193/368) while the lowest prevalence was found in goats (51.51%, n=171/332) and the highest prevalence was found in sheep (61.11%, n=22/36) (Table-12 and Figure-13). There no seropositive samples were found in the study population under six months of age while the seroprevalence from 7-12 months of age was 61.49% (n=91/148), from 13- 24 months of age was 67.23% (n=80/119) and above 2 years it was 59.46% (n=22/37). The female animals had a higher seroprevalence of 59.44% (n=129/217) compared to the lower seroprevalence of 42.38% (n=64/151) found in male animals. In different breeds, Cross Breed goats showed a slightly higher seroprevalence of 52.55% (n=103/196) compared to Black Bengal goats (50.00%, n=68/136). All the samples collected from sheep were only local breeds. Geographically, Dhaka records the highest seroprevalence of 55.61% (n=109/196) followed by Narsingdi (51.35%, n=38/74) and Gazipur (46.94%, n=46/98).

Animals that were free-grazing appeared to be more prevalent than the stall feeding with 58.10% (n=147/253) of animals free-grazing compared to 41.90% (n=46/115) in stall-feeding systems ($p = 0.001$). The seroprevalence was significantly higher in River Basin (n=148/252) than in plain land (n=45/116). Market (58.77%, n=134/228) as a source of animals exhibited a significantly higher seroprevalence compared to those from self-farms (42.14%, n=59/140). The introduction of animals into the herd was associated with a substantially elevated

seroprevalence, with 69.61% of introduced animals (n=142/204) testing positive compared to only 30.39% of non-introduced animals (n=51/164). Herd size also emerged as a significant factor, with smaller herds demonstrating the highest seroprevalence (81.97% in small herds, 51.92% in medium herds, and 66.89% in large herds) (Table-12). The findings demonstrated in this study have valuable insights into the epidemiological dynamics of PPRV infection as these animals were unvaccinated and apparently healthy animals emphasizing the need for regular surveillance of targeted host species for a successful implementation of PPR control and eradication programs locally and nationally.

Table-12: Seroprevalence of *peste des petits ruminants* (PPR) in study areas

Variable	Category	Overall	Sero-negative	Sero-positive	*p*-value
Species	Goat	332	161 (48.49%)	171 (51.51%)	0.3
	Sheep	36	14 (38.89%)	22 (61.11%)	
Gender	Female	217	88 (40.56%)	129 (59.44%)	***0.001***
	Male	151	87 (42.38%)	64 (57.62%)	
Breed	Black Bengal	136	68 (50.00%)	68 (50.00%)	0.5
	Cross Breed	196	93 (47.45%)	103 (52.55%)	
	Local Breed (Sheep)	36	14 (38.89%)	22 (61.11%)	
Area	Dhaka	196	87 (44.39%)	109 (55.61%)	0.4
	Gazipur	98	52 (53.06%)	46 (46.94%)	
	Narsingdi	74	36 (48.65%)	38 (51.35%)	
Age	<6 months	64	64 (100.00%)	0 (0.00%)	***<0.001***
	7-12 months	148	57 (38.51%)	91 (61.49%)	
	13-24 months	119	39 (32.77%)	80 (67.23%)	
	>24 months	37	15 (40.54%)	22 (59.46%)	
Feeding System	Grazing	253	106 (41.90%)	147 (58.10%)	***<0.001***
	Stall Feeding	115	69 (60.00%)	46 (40.00%)	
Ecological Zone	Plain Land	116	71 (61.21%)	45 (38.79%)	***<0.001***
	River Basin	252	104 (41.27%)	148 (58.73%)	
Source	Market	228	94 (41.23%)	134 (58.77%)	***0.002***
	Self-Farm	140	81 (57.86%)	59 (42.14%)	
Introduction of Animals	Yes	204	62 (30.39%)	142 (69.61%)	***<0.001***
	No	164	113 (68.90%)	51 (31.10%)	
Herd Size	Small	61	50 (81.97%)	11 (18.03%)	***<0.001***
	Medium	156	75 (48.08%)	81 (51.92%)	
	Large	151	50 (33.11%)	101 (66.89%)	

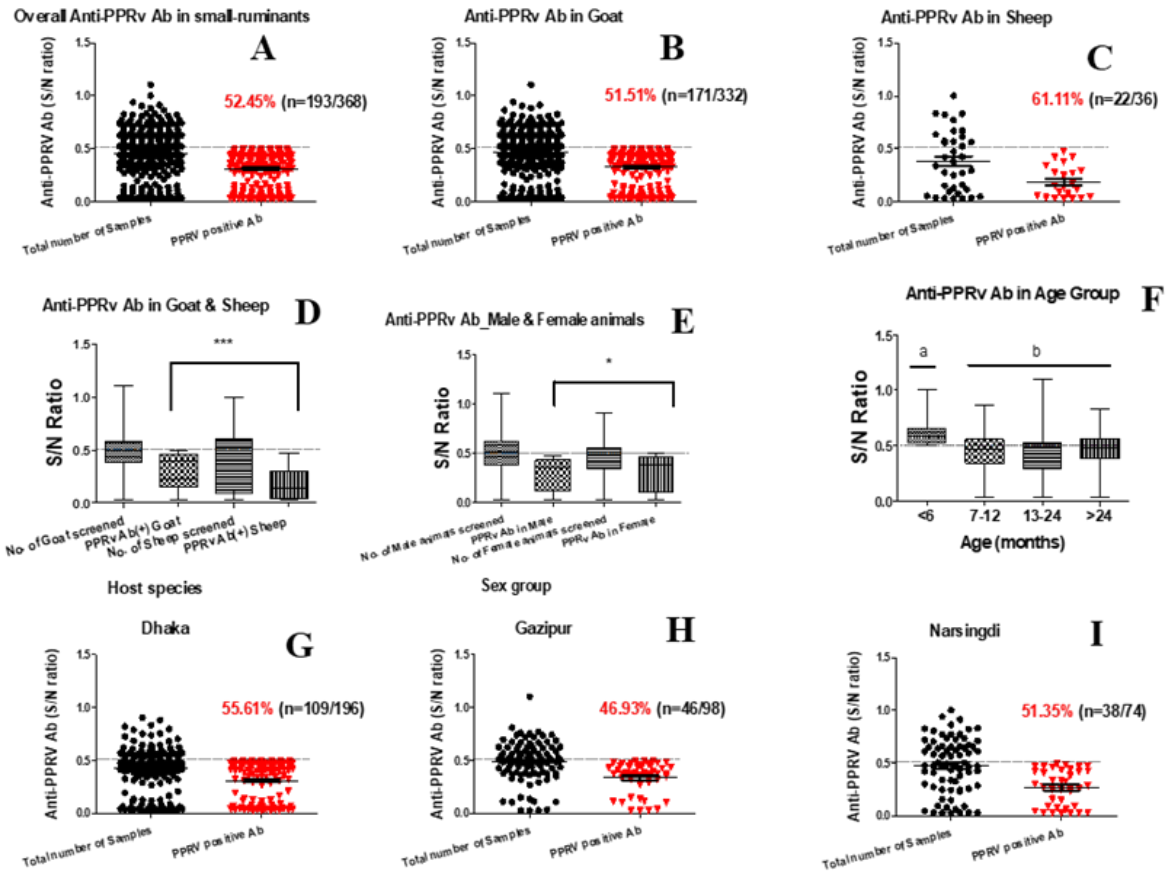


Figure-13: Seroprevalence of peste des petits ruminants virus. A) Overall seroprevalence of anti-PPRV Ab (S/N value) in small-ruminants (sheep and goat). B) Seroprevalence of anti-PPRV Ab (S/N value) in Goat. C) Seroprevalence of anti-PPRV Ab (S/N value) in Sheep. D) anti-PPRV Ab (S/N value) in sheep and goats. E) anti-PPRV Ab (S/N value) in male and female species. F) anti-PPRV Ab (S/N value) in different age groups of sheep and goats. G) Seroprevalence of anti-PPR Ab (S/N value) in the study area (Dhaka). H) Seroprevalence of anti-PPR Ab (S/N value) in the study area (Gazipur). I) Seroprevalence of anti-PPR Ab (S/N value) in the study area (Narsingdi).

4.1.3. Associated factors of PPRV infection

In assessing associated risk factors of seroprevalence for peste des petits ruminant's virus (PPRV) infection, a structured questionnaire was employed across every sampled herd to examine variables including species, gender, age, herd size, location, feeding system, ecological zone, source, and introduction of animals as described in (Table-13). In this study, the *Chi-square* test analysis results showed that among the associated risk factors with PPRV seroprevalence occurrence; gender, age, feeding system, ecological zone, source, the

introduction of new animals, and herd size were found to be statistically significant as summarized in (Table-13).

Table-13: *Chi-square* test analysis results of the associated factors of PPRV seroprevalence

Hypothesized Factors		Sample Size	Positive	*Chi-Square* (χ^2)	*p*-value
Species	Goat	332	171	1.201422	0.3
	Sheep	36	22		
Gender	Female	217	129	10.394288	***<0.001***
	Male	151	64		
Breed	Black Bengal	136	68	1.410923	0.5
	Cross Breed	196	103		
	Local Breed (sheep)	36	22		
Area	Dhaka	196	109	2.015175	0.4
	Gazipur	98	46		
	Narsingdi	74	38		
Age	<6 months	64	0	86.587911	***<0.001***
	7-12 months	148	91		
	13-24 months	119	80		
	>24 months	37	22		
Feeding System	Grazing	253	147	10.38869	***<0.001***
	Stall Feeding	115	46		
Ecological Zone	Plain Land	116	45	12.65998	***<0.001***
	River Basin	252	148		
Source	Market	228	134	9.617261	***0.002***
	Self-Farm	140	59		
Introduction of Animals	Yes	204	142	54.06048	***<0.001***
	No	164	51		
Herd Size	Small	61	11	41.60948	***<0.001***
	Medium	156	81		
	Large	151	101		

Univariable logistic regression analysis was carried out to eliminate non-significant hypothesized risk factors, using a significance threshold of p=0.30. The univariate logistic regression analysis results identified species (p=0.3), gender (p=0.001), area (p=0.2), feeding system (p=0.001), ecological zone (p<0.001), introduction of animals (p<0.001), source (p=0.002), and herd size (p<0.001) as risk factors significantly associated with *c*-ELISA seropositivity. Conversely, no significant associations were found between *c*-ELISA seropositivity and breed (p=0.6).

Table-14: Univariable analysis results of associated risk factors for PPR seroprevalence

Associated risk factors		No. of animals tested	Positive samples	OR (95% CI)	*p*-value
Species	Caprine	332	171	Ref	-
	Ovine	36	22	1.48 (0.74, 3.05)	0.3
Gender	Male	151	64	0.50 (0.33, 0.76)	***<0.001***
	Female	217	129	Ref	-
Breed	Black Bengal	136	68	Ref	-
	Cross Breed	196	103	1.11 (0.71, 1.72)	0.6
	Local Breed (Sheep)	36	22	1.57 (0.75, 3.39)	0.2
Area	Dhaka	196	109	Ref	-
	Gazipur	98	46	0.71 (0.43, 1.15)	0.2
	Narsingdi	74	38	0.84 (0.49, 1.44)	0.2
Feeding System	Grazing	253	147	Ref	-
	Stall Feeding	115	46	0.48 (0.31, 0.75)	***<0.001***
Ecological Zone	Plain Land	116	45	Ref	-
	River Basin	252	148	2.25 (1.44, 3.54)	***<0.001***
Source	Market	228	134	Ref	-
	Self-Farm	140	59	0.51 (0.33, 0.78)	***0.002***
Introduction of Animals	Yes	204	142	5.07 (3.27, 7.98)	***<0.001***
	No	164	51	Ref	-
Herd Size	Small	61	11	0.11 (0.05, 0.22)	***<0.001***
	Medium	156	81	0.53 (0.34, 0.85)	***0.008***
	Large	151	101	Ref	-

Based on the results summarized in Table-14, the variables with a *p*-value less than 0.30, along with those deemed clinically important, were selected for further analysis. Subsequently, species, age, gender, area, feeding system, ecological zone, source of animals, introduction of animals, and herd size emerged as statistically significant variables in the univariable logistic regression analysis. These variables were then incorporated into the final multivariable logistic regression model to assess their true significant contribution as associated risk factors while accounting for any potential compounding effects. The adjusted odds ratios (AOR) presented in Table-15 illustrate the impact of these variables on the occurrence of peste des petits ruminants virus (PPRV) infection in sheep and goats. Consequently, species, gender, age, feeding system, ecological zone, and introduction of the animals were identified as significant factors associated with the occurrence of PPRV infection.

The odds of seropositivity in sheep were significantly 1.14 times higher than in goats (AOR= 1.14, 95% CI = 0.01-0.27, *p*= 0.05). Males were significantly less likely to be seropositive

than females (AOR= 0.90, 95% CI= -0.19 - 0.02, p=0.012). The findings revealed that animals aged 13-24 months (AOR=1.72, 95% CI= 0.41 - 0.67, p<0.001), 7-12 months (AOR= 1.67, 95% CI= 0.39, 0.63, p<0.001), and more than 24 months (AOR= 1.67, 95% CI= 0.34 - 0.67. p<0.001) had significantly higher odds of having PPRV infection compared to the less than 6 months of age. The odds of stall-feeding (AOR= 0.81, 95% CI = -0.36 - -0.06, p= 0.006) were less likely to be seropositive than free-grazing practices. Animals in the river basin were significantly 1.35 (AOR= 1.35, 95% CI= 0.14-0.47. p<0.001) times more seropositive than plain land. The odds of the introduction of new animals in farms were 1.38 (AOR= 1.38, 95% CI= 0.19-0.45, p<0.001) times higher than those who didn't introduce new animals to their farm. Moreover, the odds of Gazipur were 1.49 (AOR= 1.49, 95% CI= 0.20-0.60, p<0.001) higher than Dhaka. However, Narsingdi (AOR: 0.97, 95% CI=-0.14- 0.08, p=0.6) had lower seropositive odds than Dhaka.

Table-15: Multivariate logistic regression analysis results of associated factors for PPR seroprevalence

Hypothesized Associated Factors		Sample Size	Seropositive	AOR (95% CI)	*p*-value
Species	Goat	332	171	Ref	-
	Sheep	36	22	1.14 (0.01, 0.27)	***0.05***
Gender	Male	151	64	0.90 (-0.19, -0.02)	***0.012***
	Female	217	129	Ref	-
Age	<6 months	64	0	Ref	-
	7-12 months	148	91	1.67 (0.39, 0.63)	***<0.001***
	13-24 months	119	80	1.72 (0.41, 0.67)	***<0.001***
	>24 months	37	22	1.67 (0.34, 0.67)	***<0.001***
Area	Dhaka	196	109	Ref	-
	Gazipur	98	46	1.49 (0.20, 0.60)	***<0.001***
	Narsingdi	74	38	0.97 (-0.14, 0.08)	0.6
Feeding System	Grazing	253	147	Ref	-
	Stall Feeding	115	46	0.81 (-0.36, -0.06)	***0.006***
Ecological Zone	Plain Land	116	45	Ref	-
	River Basin	252	148	1.35 (0.14, 0.47)	***<0.001***
Source	Market	228	134	Ref	-
	Self-Farm	140	59	1.05 (-0.05, 0.15)	0.4
Introduction of Animals	Yes	204	142	1.38 (0.19, 0.45)	***<0.001***
	No	164	51	Ref	-
Herd Size	Small	61	11	0.98 (-0.22, 0.18)	0.8
	Medium	156	81	1.11 (-0.03, 0.22)	0.14
	Large	151	101	Ref	-

4.1.4. Isolation and identification of PPRV from suspected sheep and goats

The collected swabs and/or tissue samples (n=100) from the suspected live and dead animals were analyzed by RT-PCR for amplification of N-gene (complete) (Figure-14 and Table 16). A total of 9 (9%, n=9/100) samples were found positive for N-gene of PPRV. Out of 50 samples, 5 samples (10.00%, n=5/50) were positive for PPRV found in Dhaka, followed by1 sample (3.33%, n=1/30)) were positive found in Gazipur and 3 samples (15.00%, n=3/20) were positive found in Narsingdi identified by RT-PCR. Among nine (9) positive samples, three (3) representative samples [collected from dead goat (1) and sheep (1) from Dhaka and Narsingdi, respectively, and one (1) from live goat from Gazipur] were submitted to a commercially available sequencing company (Macrogen, Korea) for sequencing. Among three (3) submitted samples, the sequencing result for one sample collected from sheep in Monohardi, Narsingdi was successful and the other two samples failed. The successful sequencing data was deposited in the NCBI database under the GenBank accession number **PP534161**.

Table-16: RT-PCR test results on the samples collected from PPR-suspected live and dead animals

Study area	Animal species		Total no. of samples tested (n)	Types of samples collected	N-gene positive by RT-PCR	Prevalence (%)
	Live (n)	Dead (n)				
Dhaka	Goat (48)	Goat (2)	50	Nasal Swab, Lung, Lymph node	5 ***s***	10.00%
Gazipur	Goat (30)	-	30	Nasal Swab	1 ***s***	3.33%
Narsingdi	Goat (19)	Sheep (1)	20	Nasal Swab	3 ***s***	15.00%
			100		**09**	**9%**

s indicated one (1) sample from each group of the study area was submitted for sequencing.

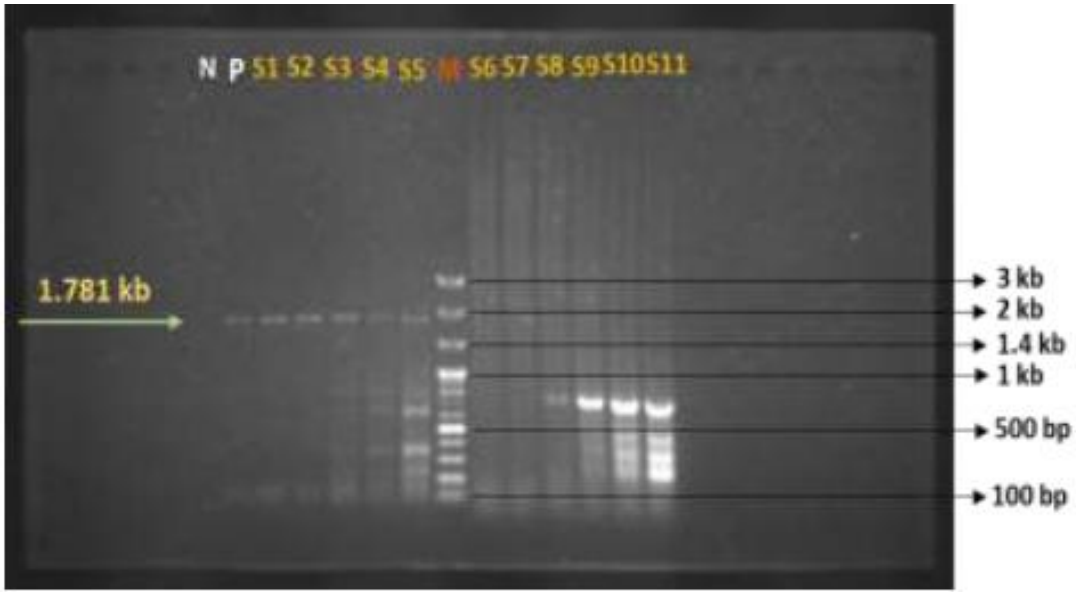


Figure-14: Amplification of full N-gene (1.781 kb) of PPRV on the samples collected from PPR-suspected sheep and goats. N: negative sample, P: positive (control) sample, S1-S11: clinical samples, M: DNA Marker

4.1.5. Phylogenetic Analysis

The sequence data of N-gene of PPRV (GenBank accession # **PP534161)** were employed to construct a phylogenetic tree (the neighbor-joining method) using MEGA11 software to see the genetic distance of the circulating viruses with previous virus sequences published elsewhere and vaccine strains as well to gain a thorough understanding of the epidemiology of *peste des petits ruminants* (PPR) and the circulating virus strains., The phylogenetic analysis was done based on the N-gene sequences (partial and complete length) as it is the best way for the phylogenetic study of PPRV (Kwiatek *et al.*, 2007). The results of phylogenetic tree analysis showed that the sequences of isolated PPRV in this study had similarities to many of the virus sequences documented in Bangladesh and China exhibited a clustering pattern consistent with lineage-IV, a lineage that was previously mostly found in Asia but has since extended its geographical range to include other continents such as Africa (Figure- 15, 16) (both phylogenetic trees)

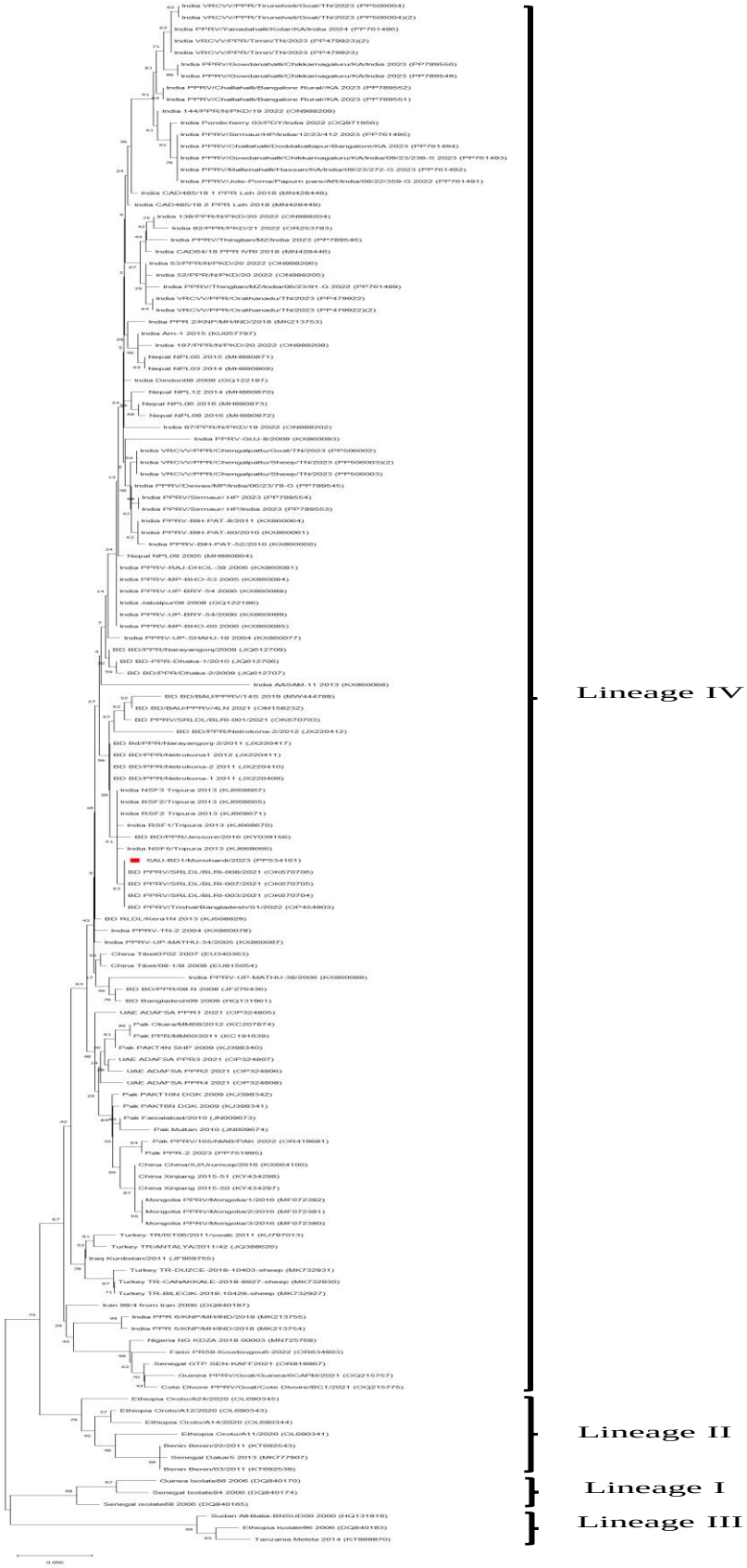


Figure-15: Phylogenic analysis of the PPRV based on the N-gene sequences (partial) of PPR virus isolates across the prevalent countries. The Neighbor-Joining method using the Kimura

2-parameter method in Mega X at bootstrap value 1000 replicates based on PPRV complete N-gene sequences isolated from in this study (Red square) and selected sequences from GenBank (NCBI).

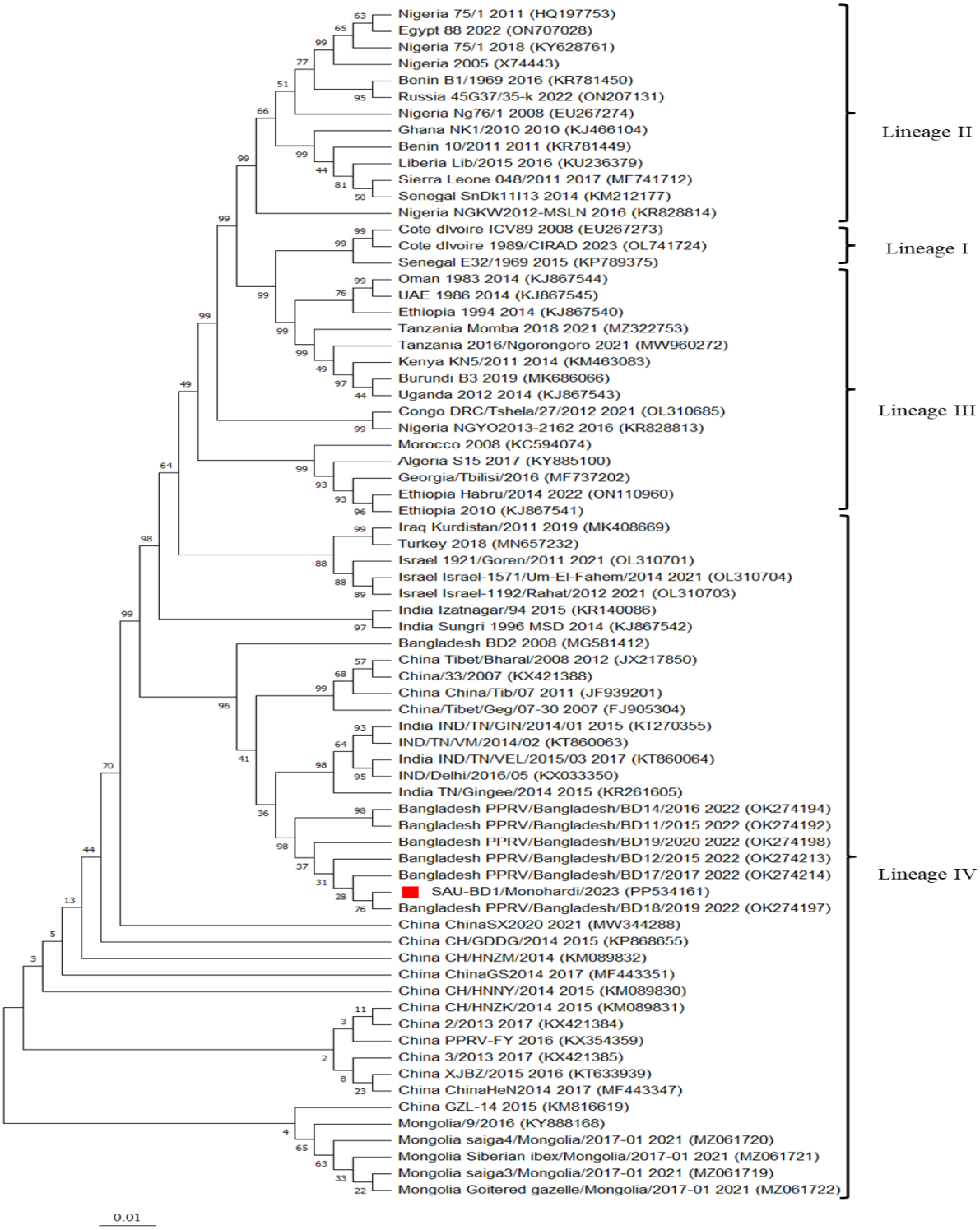


Figure-16: Phylogenic analysis of the PPRV based on the N-gene sequences (complete) of PPR virus isolates across the prevalent countries. The Neighbor-Joining method using the Kimura 2-parameter method in Mega X at bootstrap value 1000 replicates based on PPRV

complete N-gene sequences isolated from in this study (Red square) and selected sequences from GenBank (NCBI).

4.1.6. Bacterial co-infections: Isolation and Identification of Bacteria

Samples were collected from suspected dead sheep and goats to observe any bacterial coinfections with PPR. Firstly, collected samples were subjected to culture on Nutrient agar media [Scharlau, Spain] for the primary growth of bacteria. After that, the individual colonies were picked up and cultured in the Luria Bertani Broth (LB) [HiMedia, India]. Later, Subculture was done on different selective media including EMB agar [HiMedia, India] and Mannitol Salt Agar (MSA) [HiMedia, India].

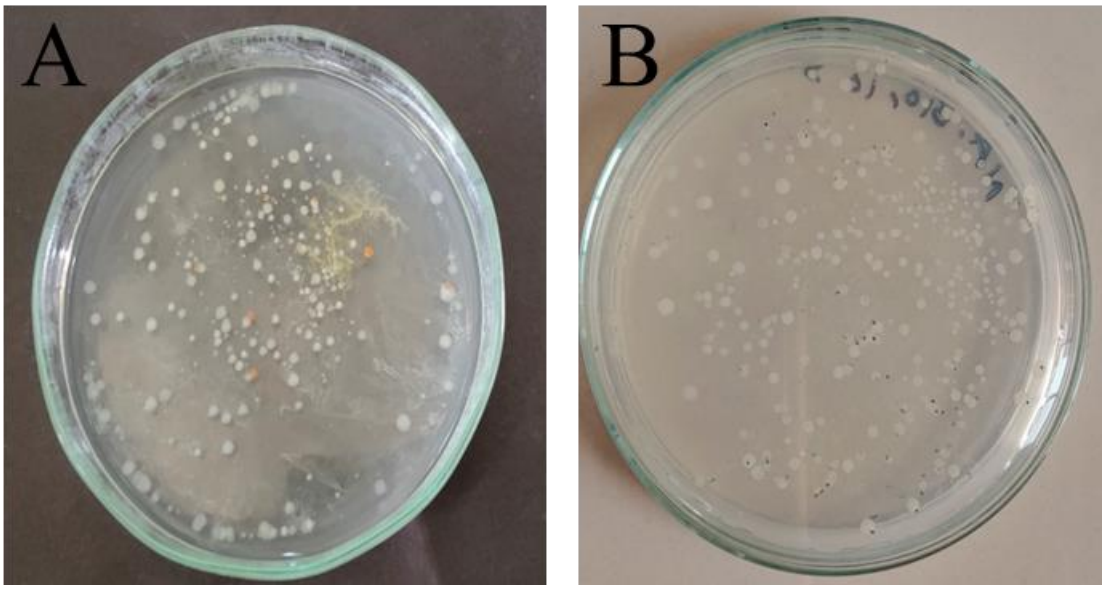


Plate-4: Bacterial colony characteristics on Nutrient Agar (NA) Media (Whitish to Yellowish Colonies)

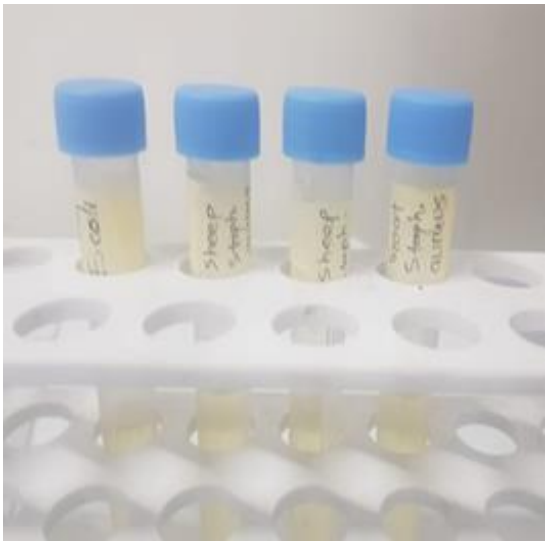


Plate-5: Bacterial isolates were picked into the LB Broth

Table-17: Different bacterial species isolated from PPR-suspected sheep and goat

Species (area)	**Source**	**Isolated Bacteria**	**Colony characteristics** (Cheesbrough, 1985).		
			Nutrient Agar (NA) Media	**Mannitol Salt Agar (MSA) Media**	**EMB Agar Media**
Goat (Dhaka)	Lung Tissue	*Staphylococcus aureus*	Yellowish colony	Yellowish colony with yellow zone	-
Goat (Gazipur)	Nasal Swabs	*Staphylococcus aureus*	Yellowish colony	Colorless colony with pink zone	-
		Escherichia coli	White Colonies	-	Greenish metallic sheen
Sheep (Narsingdi)	Nasal Swabs	*Staphylococcus aureus*	Yellowish colony	Yellowish colony with yellow zone	-
		Staphylococcus epidermidis	Cream to white	Colorless colony with pink zone	-
		Escherichia coli	White Colonies	-	Greenish metallic sheen

Among the tested samples, 3 bacterial species were isolated and identified based on cultural colony characteristics in different selective media i.e., *Escherichia coli*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.

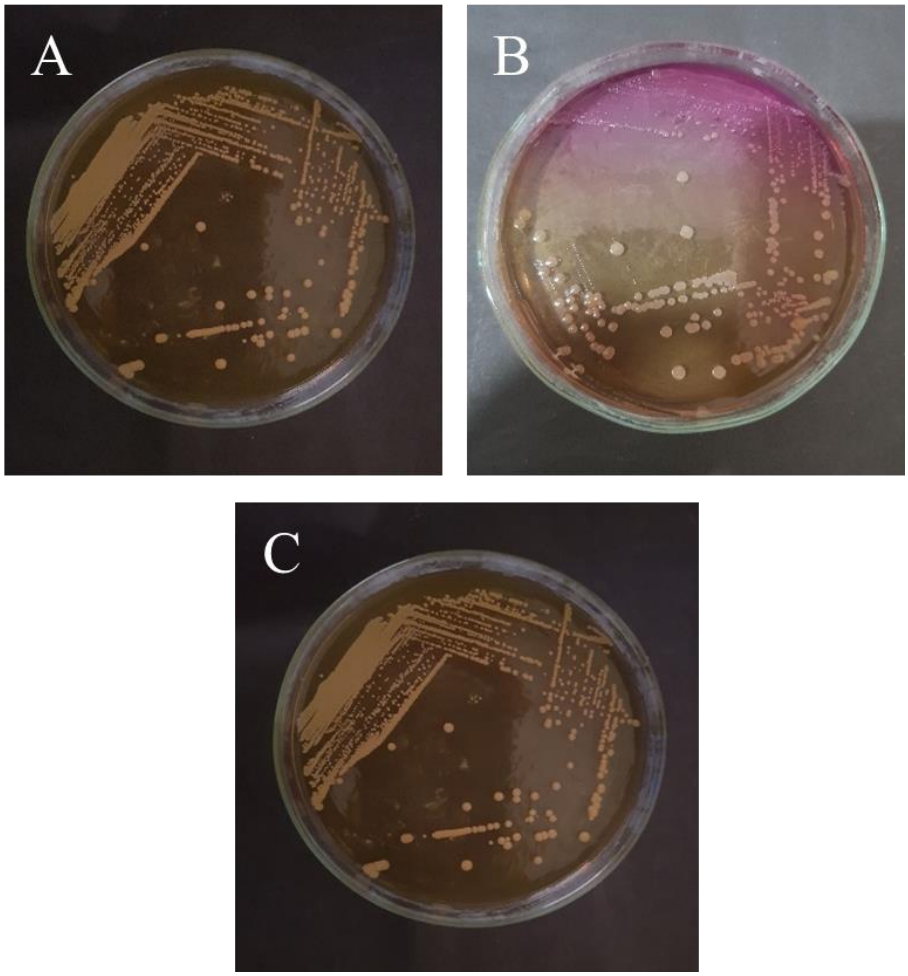


Plate-6: Colony characteristics of *Staphylococcus* spp.: *Staphylococcus aureus* was showing yellow-colored colonies with yellow zones on MSA (A, B & C), *Staphylococcus epidermidis* was showing colorless colonies with pink zones on MSA (C)



Plate-7: Colony characteristics of *Escherichia coli*: Showing Greenish metallic sheen on EMB agar media.

4.1.7. Antimicrobial Susceptibility Test

All the isolated bacterial strains were subjected to evaluate the antimicrobial resistance (AMR) profile using antibiotic disk diffusion tests against nine antibiotics [OXOID Limited, Canada], and the results are summarized in Table-10. AMR test was conducted by Kirby-Bauer Disc diffusion method as described earlier (Bauer, 1966). The results were interpreted into different categories according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2016).

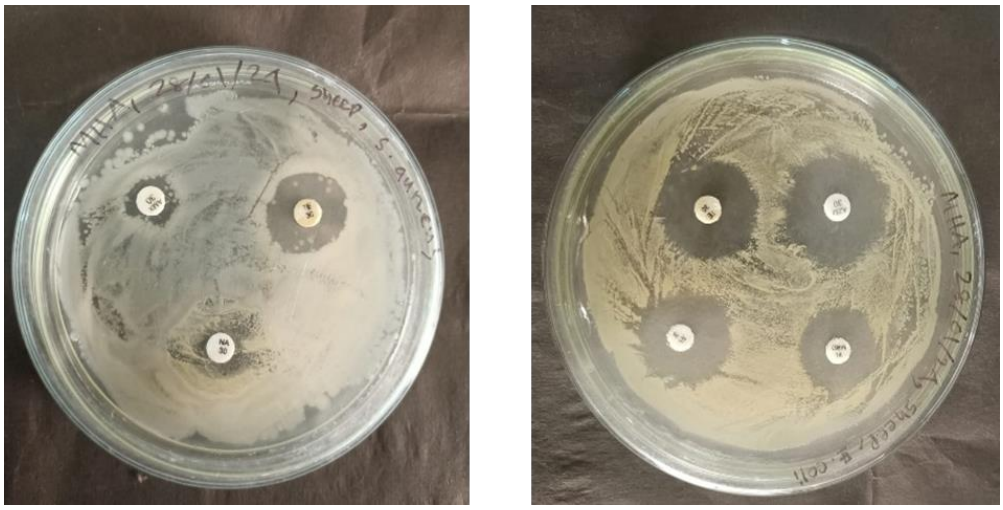


Plate-8: Antimicrobial-resistance profiling of bacterial species isolated from PPR-suspected goat and sheep

Among 2 isolated strains of E. coli, a large number of isolates showed complete resistance to ampicillin (100%) and Nalidixic acid (100%). However, a large number of strains of E. coli were also found to be completely sensitive to Amoxicillin (100%), Azithromycin (100%), Erythromycin (100%), Gentamicin (100%), Tetracycline (100%), Levofloxacin (100%), and Ciprofloxacin (100%). Similarly, among 3 isolated strains of *S. aureus*, a large number of isolates showed resistance to ampicillin (100%) and amoxicillin (100%). A few numbers of isolates showed intermediate resistance to tetracycline (100%) and nalidixic acid (100%). However, a large number of isolates were also found to be 100% sensitive to Azithromycin, Erythromycin, Gentamicin, Levofloxacin, and Ciprofloxacin. Besides, 1 isolated strain of *S. epidermidis* showed complete resistance to Amoxicillin (100) and Ampicillin (100). It also showed intermediate resistance to Erythromycin (100) and Nalidixic acid (100) (Table-18). However, the strain showed complete sensitivity to Azithromycin, Gentamicin, tetracycline, Levofloxacin, and Ciprofloxacin.

Table – 18: Antibiotics-resistance/ susceptibility profile of the isolated bacterial species from PPR-suspected sheep and goat

Antimicrobial	Disc name	Disc potency (µg)	*S. aureus* (N = 3) (%)			*S. epidermidis* (N = 1) (%)			*E. coli* (N = 2) (%)		
			R	I	S	R	I	S	R	I	S
Amoxicillin	AMX	30	3 (100)	0	0	1 (100)	0	0	0	0	2 (100)
Ampicillin	AMP	25	3 (100)	0	0	1 (100)	0	0	2 (100)	0	0
Azithromycin	AZM	30	0	0	3 (100)	0	0	1 (100)	0	0	2 (100)
Erythromycin	E	15	0	0	3 (100)	0	1 (100)	0	0	0	2 (100)
Nalidixic acid	NA	30	0	3 (100)	0	0	1 (100)	0	2 (100)	0	0
Gentamicin	GEN	10	0	0	3 (100)	0	0	1 (100)	0	0	2 (100)
Tetracycline	TE	30	0	3 (100)	0	0	0	1 (100)	0	0	2 (100)
Levofloxacin	LE	5	0	0	3 (100)	0	0	1 (100)	0	0	2 (100)
Ciprofloxacin	CIP	5	0	0	3 (100)	0	0	1 (100)	0	0	2 (100)

4.2. Discussions

Small-ruminants especially sheep and goats play an important role in Bangladesh's agricultural economy. However, PPR as an endemic disease poses a serious threat to the development of small-ruminants (sheep and goats) farming in Bangladesh (Ahamed *et al.*, 2019; Das *et al.*, 2007). The disease, PPR is considered a top economic concern for livestock farming, based on its impact on high morbidity, mortality, and treatment costs (Rahman *et al.*, 2023). Even though FAO and OIE intended to eradicate the disease by 2030, the studies revealed high seropositivity of the PPR in sheep and goats in Bangladesh (Rahman *et al.*, 2023).

In the current study, the overall seroprevalence of PPRV antibody (anti-PPRv Ab) was 52.45%, which is higher than other studies including 44.0% (Rahman *et al.*, 2023), 26% (Banik *et al.*, 2008), 8.70% (Islam *et al.*, 2016), 16.8% (Hasan, 2012), 40% (Ahamed *et al.*, 2019). Despite the PPR is endemic in Bangladesh, the extent of seropositivity among small ruminants without vaccination remains unknown. The results of this study revealed that PPR has been spreading in certain regions of Bangladesh without being officially reported to veterinary authorities. Consequently, the national disease monitoring systems aimed at controlling PPR were found to be ineffective. The seroprevalence against PPRV antibody (anti-PPRv Ab) varied across neighboring countries of Bangladesh. Some countries reported similar levels of seroprevalence, while others showed higher or lower rates found in Pakistan at 64.0% (Unar *et al.*, 2024), 45.0% (Rasheed *et al.*, 2020); in India at 48.42% (Hota *et al.*, 2018), 14.5% (Balamurugan *et al.*, 2020) and 54.2% (Balamurugan *et al.*, 2020). Similarly, the seroprevalence across the world was also found different e.g., 60.15% in Ethiopia (Dubie *et al.*, 2022), 46.7% in Libya (Almeshay *et al.*, 2017), 68.1% in Sudan (Saeed *et al.*, 2018), and 27.3% in Uganda (Nkamwesiga *et al.*, 2023). The variations in seroprevalence of PPRV antibodies across different areas in Bangladesh and different countries may be attributed to several factors including differences in management practices within dispersed geographical locations, immune status of animals, differences in agro-ecological conditions, variations in diagnostic tests and sampling procedures utilized, differences in the socio-economic status of individual farmers, variations in veterinary services such as vaccination programs, animals' movement, and frequent interactions between herds. Additionally, the migration of livestock within and between countries also plays a role in influencing these variations.

In Bangladesh, several studies have demonstrated that sheep are more susceptible to the PPR virus than goats. In this study, it was found that the seroprevalence of PPRV antibody was in

sheep (61.11%), which is higher than found in goats (51.51%). This data is supported well by previous studies (Hasan, 2012; Rahman *et al.*, 2017). However, this finding is also contrary to the other previous studies wherein they had reported that goats were more susceptible than sheep (Balamurugan *et al.*, 2020; Dubie *et al.*, 2022). These differences suggest that species-specific factors such as host immune response variations or susceptibility differences, along with regional variations, etc.

The seroprevalence of PPRV antibody in goats was found higher in crossbreed goats compared to indigenous breeds (Black Bengal). The seroprevalence of PPRV antibody was found slightly higher (52.55%) in crossbreeds compared to Black Bengal (BB) goat (50.00%) which was not statically different (ns). This might be due to in-country animal movement. This may explain the surge in PPRV in crossbreeds. Moreover, inbreeding or unethical breeding of crossbred goats for profit might increase their vulnerability to viral illnesses like PPRV infection. Lacking immunity, crossbred sheep may be less disease-resistant than native sheep (Rahman *et al.*, 2023) though no crossbred sheep were in this study.

In this study, a significantly higher seroprevalence was found in females (59.44%) than in males (57.62%), which is highly supported by the other studies as well (Nkamwesiga *et al.*, 2023; Tajpara *et al.*, 2022). Females have a higher susceptibility to infection owing to physiological differences, which arise from the stress experienced during production and reproduction (Megersa *et al.*, 2011). Furthermore, this phenomenon may also be attributed to the livestock breeding practices of Nepalese farmers, whereby females are retained for extended periods to facilitate reproduction, while the majority of males are castrated and thereafter sold for meat production. The duration of female confinement for herd maintenance directly correlates with the likelihood of increased exposure to the environment, which in turn may lead to higher seroprevalence rates (Acharya *et al.*, 2018).

Based on age groups, the seroprevalence of PPRV antibody is significantly higher at 13-24 months of age. There no seropositive of PPRV antibody was found in animals under 6 months of age, which might be the provision of passive protection by colostral antibodies up to the age of less than six months (Dubie *et al.*, 2022). The present study demonstrated that older animals are more susceptible to PPRV infection which is also supported by the previous studies reported elsewhere (Mahajan *et al.*, 2012; Salih *et al.*, 2014). The increased probability of adult animals being exposed to PPRV compared to younger animals is the

underlying cause. Furthermore, the higher seroprevalence seen in adults might be attributed to their extended lifespan, which enables greater exposure to PPRV.

Similarly, the seroprevalence of PPRV antibody in Dhaka was slightly higher (55.61%) than in two other areas (Gazipur, 46.94%, and Narsingdi, 51.35%) (ns). This variation could be due to animals coming to the capital city from different places and farmers trading their animals in the animal market of the city.

The feeding system of animals is one of the risk factors for contagious diseases such as PPR. The seropositivity of PPR in free-grazing (58.10%) is significantly higher than in stall feeding (40.00%). This finding is consistent with the previous study (Bwihangane *et al.*, 2016). A possible explanation for this might be the spread of the virus occurs when animals from different locations congregate on a shared grazing pasture. Moreover, infected animals may spread the infection to the water sources near the grazing area from where healthy animals may be infected (Rahman *et al.*, 2023).

This study only includes two out of the 30 agroecological zones that Bangladesh is divided into. The river basin zone had the highest seroprevalence (58.73%) of PPRV antibody, whereas the plain land zone showed the lowest seroprevalence (38.79%). There are several possible explanations for this variation in results. The seroprevalence of PPRV antibody varied significantly ($p < 0.05$) between ecological zones, perhaps owing to changes in agroecology and management techniques. Another possible explanation for this phenomenon might be because the grazing areas for animals were situated close to the river basin, perhaps leading to animal contamination (Fentie *et al.*, 2018).

Regarding the source of animals, animals that were brought from the market (58.77%) had a significantly higher seropositivity of PPR compared with animals of self-farm (42.14%). This could be due to infected animals being displayed in the live animal market and acting as a possible source of infection for transmitting PPRV. This study also identified that one of the reasons for the transmission of PPRV is the introduction of new animals (69.61%) in the herd without maintaining a proper quarantine period. This finding broadly supports the works of other studies (Abubakar *et al.*, 2009; Almeshay *et al.*, 2017). Most Bangladeshi sheep and goats are native; however, some farmers breed exotic ones. Farmers import them from neighboring countries or bring in new animals without quarantine. PPR may be caused by the transfer of infected animals from animal live markets to the farm or by animals coming into

close contact with sick animals that were transported to market but not sold and returned to farms.

Herd size is one of the important risk factors of transmission of PPRV. In this research finding, herd size and seropositivity were statistically significant (p<0.05). Large herd size (66.89%) had higher seroprevalence than small herd size (18.03%). This finding was supported by previous studies (Dubie *et al.*, 2022; Selvaraju, 2014), which might potentially suggest the infectious characteristics of the illness and its mechanism of transmission that can be linked to the overcrowding of animals. This overcrowding allows for increased frequency of direct contact, hence increasing the likelihood of transmission of PPRV

The current study successfully identified PPRV (9.0%) in the clinical cases (having clinical signs and symptoms, and suspected live and/or dead animals) using RT-PCR by N-gene amplification, which confirmed the circulatory of PPRV within the study areas in Bangladesh. This finding is consistent and supported by other studies (7.4%) using reports in Nigeria (Adeoye *et al.*, 2022). Nevertheless, this may not accurately reflect the prevalence of PPRV since only animals exhibiting clinical indications of PPR were included in the study. Several reports have shown that a significantly higher rate of PPR diagnosed by using RT-PCR including 44.4% in Morocco (Kwiatek *et al.*, 2011), 80.0% in Sudan (Kwiatek *et al.*, 2011), 33.3% in Algeria (Nardi *et al.*, 2012), 51.2% in Nigeria (Luka *et al.*, 2011), 29.6% in Nothern Tanzania (Kgotlele *et al.*, 2014), 31.1% in Eastern Tanzania (Kgotlele *et al.*, 2014), 29.91% in Iran (Babaoglu *et al.*, 2022), 33.33% (Nabi *et al.*, 2018) and 35.6% (Rahman *et al.*, 2023) in Bangladesh, 10.34% in Turkey (Pestil *et al.*, 2020), 77.3% in Eastern Sudan (Saeed *et al.*, 2021), 27.88% in India (Shrivastava, 2023) and 78.95% in Pakistan (Kabir *et al.*, 2020). Previous studies have identified that the prevalence of positive cases of PPR diagnosis may be affected by sample type, infection stage, and gene targeting as well (Luka *et al.*, 2012).

To get a better understanding of the genetic characteristics of the circulating PPRV in the study areas, we have analyzed phylogenetic trees using N-gene (partial and complete) of 72 different PPRV isolates across the prevalent countries. A recent study has shown that the N-gene sequences have a higher degree of accuracy in distinguishing between various strains when compared to the F and H genes (Shahriari *et al.*, 2019). The analysis of the phylogenetic tree reveals that the PPRV under investigation has a higher level of resemblance to the previously reported isolates s coming from China and India. The similarity has also been found in the isolates of the PPRV that were reported previously in Bangladesh and

India, indicating the close geographical vicinity of Bangladesh and India, and China as well. This closeness facilitates unregulated cross-border animal trade and transboundary movements, particularly those with India as Bangladesh shares three borders. Moreover, this analysis may provide empirical support for the presence of remote clustering among the sequences elucidated in the investigation, aligning with prior research conducted in Bangladesh. The findings of the genetic characteristics suggest that the PPRV isolates found in this specific geographical area have a common evolutionary lineage-IV. The first confirmation of Lineage-IV of PPRV occurred in the Indian Subcontinent, namely in North India in 1994 (Clarke *et al.*, 2018). Subsequently, Lineage-IV has been reported in neighboring countries such as Pakistan in 1994 (Amjad *et al.*, 1996), Bangladesh in 1993 (Islam *et al.*, 2001), Nepal in 1995 (Dhar *et al.*, 2002), and Bhutan (Parida *et al.*, 2015). Moreover, the predominant circulating lineage of PPRV throughout the Middle East and Asia is Lineage-IV (Banyard *et al.*, 2014). It has recently spread into China and Tibet (Wang *et al.*, 2009) as well as into North (Baazizi *et al.*, 2017), Central (Maganga *et al.*, 2013), and East Africa, reaching as far south as Tanzania (Mahapatra *et al.*, 2015). Molecular characterization of circulating isolates is crucial for understanding PPRV epidemiology, and tracking outbreaks nationwide, which further helps to understanding insight into PPRV immunopathogenesis as well. Data on PPRV diversity, animal movement, origin, and associated risk factors of introduction into the herd may assist in determining variations in virulence (Libeau *et al.*, 2014). Additionally, this finding will enhance the current control and elimination efforts recommended by WOAH.

The results demonstrated in this study as secondary bacterial co-infection was associated with PPR in sheep and goats. Up to 40% of healthy sheep have infectious pathogens in their upper respiratory tract, which might lead to respiratory illnesses under stress (Aziz & Lafta, 2021). Bacterial respiratory infections may be primary in healthy animals or secondary to immunosuppressive circumstances. Secondary bacterial infections occur when respiratory mucosa resistance decreases and bacteria growth in the upper respiratory tract expands lower (Mustafa *et al.*, 2021). In this study, *Staphylococcus* spp. was isolated from the upper respiratory tract of sheep (nasal swab) and goats (nasal swab and lungs). The possible explanation might be that *Staphylococcus* spp. is considered a major inhabitant of the upper respiratory tract mucosa and plays a pathogenic role in hosts with compromised immune systems (Azizi *et al.*, 2013). Moreover, *Staphylococcus* spp. was identified as a common pathogen in the investigation of the bacterial causes of ovine pneumonia (Srinivasan *et al.*,

2003). Nasal cavities, tonsils, tracheal swabs, and lung samples collected under aseptic conditions were examined bacteriologically in which gram-positive cocci were the predominant isolates with coagulase-negative *Staphylococcus* (CNS) being the most frequent species followed by *S. aureus* (Yimer & Asseged, 2007). *Staphylococcus aureus* emerged as the second most commonly isolated bacterium obtained from pneumonic lungs and nasal swabs (Emikpe *et al.*, 2009). *Staphylococcus* spp. was isolated from nasal swabs of pneumonic black Bengal goats, often occurring in mixed infections with *Pasteurella* spp. (Momin *et al.*, 2011). Furthermore, *Staphylococcus aureus* has zoonotic characteristics, allowing for the potential for reciprocal transmission between humans and domestic animals in situations when natural barriers are compromised (Ajuwape & Aregbesola, 2002). This finding is in good agreement with the previous observations (Doley *et al.*, 2018; Rajashekar *et al.*, 2023) wherein they found the presence of *Staphylococcus* spp. in the respiratory tract in disease and healthy conditions. Antimicrobial drugs are used without discrimination in animal production systems to prevent and control infectious diseases. This practice leads to the development of resistance to these treatments, notably in zoonotic bacteria, which can readily pass to humans via food chains (Chang *et al.*, 2015). Antibiotic resistance is a significant issue worldwide public health concern. The transmission of antibiotic resistance among those directly involved in agricultural activities may occur via several means, such as the ingestion of infected milk, meat, water, soil, direct contact with animals, or the use of environmental equipment (Keba *et al.*, 2020). In this study, *Staphylococcus* spp. showed complete resistance to Ampicillin and Amoxycillin. These microbes are well recognized for their resistance to several antimicrobials, particularly those with broad-spectrum activity (Aziz & Lafta, 2021). However, it showed high sensitivity to Azithromycin, Gentamicin, Levofloxacin, and Ciprofloxacin. This finding supports evidence from previous observations (Aziz & Lafta, 2021).

Similarly, *Escherichia coli* was the most frequently isolated bacteria from the respiratory system with pneumonic lesions in sheep and goats (Rajashekar *et al.*, 2023). The current study revealed that *E. coli* was found in the respiratory tract of sheep and goats, which is supported by previous studies (Tijjani *et al.*, 2012; Yegoraw *et al.*, 2017). While *E. coli* is often benign in its natural environment, it has been proposed to be responsible for respiratory and urogenital infections (Pelczar *et al.*, 1986). It is noteworthy that *E. coli*, along with other bacteria, emerged as the predominant aerobic microorganisms isolated from cases of fibrinous bronchopneumonia (Aziz & Lafta, 2021). *E. coli* can contribute to pneumonia in

sheep and goats, although it's not as common as other bacterial pathogens like *Pasteurella multocida* or *Mannheimia haemolytica* (Rajashekar *et al.*, 2023). However, *E. coli* was found in the gut of these animals and can cause secondary pneumonia, particularly in association with viral infections (Ganipisetti *et al.*, 2023). Regarding antimicrobial susceptibility, *E. coli* isolates showed high resistance to Ampicillin and Nalidixic acid. However, it showed a high sensitivity to antibiotics such as Ciprofloxacin, Levofloxacin, Gentamicin, Erythromycin, Azithromycin, and Amoxicillin.

There are several limitations, especially for the molecular characterization of PPRV in this study. Firstly, the study was conducted on sick animals were usually come to the veterinary hospitals which might exclude these sick animals if they did not come to the hospitals. Secondly, sequences were obtained from clinical samples with low concentrations of PPRV RNA, potentially leading to false negative results. Thirdly, the small sample size for molecular analysis of PPRV may limit the representativeness and reliability of the genetic characterization results and of course, the short duration of the study period as well. Further investigation is required to get a deeper understanding of the virus diversity and anticipated antigenic changes of PPRV by tissue culture-based isolation, and subsequent whole genome sequencing for getting a better vaccine candidate for future research. Additionally, the identification of bacteria was done using cultural properties. The limitation is there was no molecular identification of bacteria associated with PPR. Furthermore, the small sample size for determining bacterial co-infection and their antimicrobial profiling may lead to false results.

CHAPTER V
SUMMARY AND CONCLUSIONS

The findings demonstrated in this research suggest that there is a higher seroprevalence of PPRV antibodies in the study areas of Bangladesh, confirming the presence of the disease among the sheep and goat populations that are apparently healthy. This research also provides insights into the epidemic characteristics of PPR in Bangladesh, explaining possible epidemiological risk variables including geographical locations, species, breed, sex, sources, feeding pattern, introduction of new animals, and herd size. Genetically, the PPRV isolate reported in this study belongs to lineage-IV, which is very closely related to the other Bangladeshi PPRV isolates reported previously. It is suggesting that if the control and preventative measures are delayed, the national small-ruminant production may suffer. Preventing disease spread requires regulating animal trade and monitoring suspicious instances. A well-designed strategic mass vaccination program throughout the country and frequent immunization of young animals might also prevent PPRV infection in sheep and goats. More studies should be done to educate farmers about this disease. Moreover, active surveillance programs are strongly recommended to develop suitable control and eradication programs for PPR virus infection in Bangladesh.

CHAPTER VI
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APPENDICES

APPENDIX I

Activities throughout the study period



Plate-9: Blood collection from sheep and goats in the study areas



Plate-10: Sample transportation and collection tube of serum in an ice box



Plate-11: Sample collection and carrying the ice box in the study areas



Plate-12: Swab samples collection from clinically PPR-suspected animal

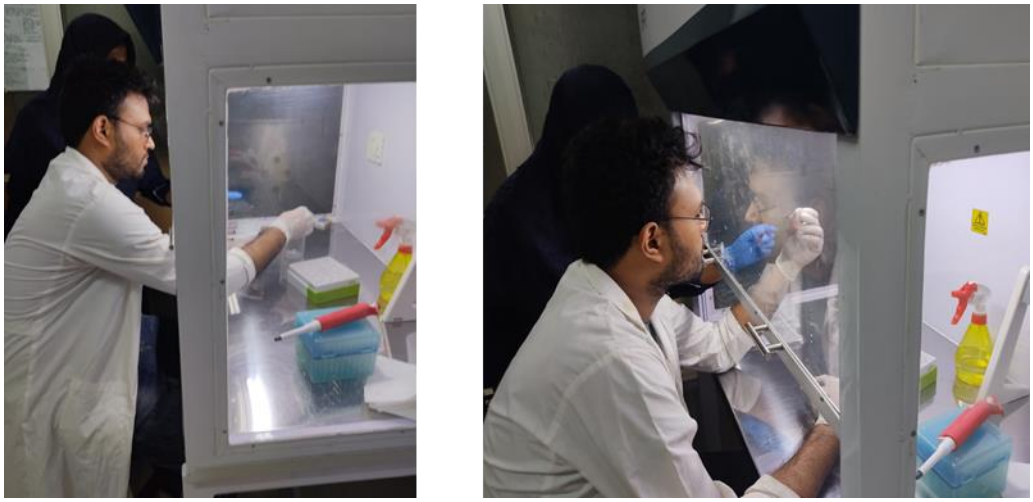


Plate-13: Processing of samples



Plate-14: Disposal of wastage in a proper way



Plate-15: Working under supervision in the pathology lab



Plate-16: Labeling the collection tubes for the sample




Plate-17: Collection of blood serum




Plate-18: Sample processing in the lab




Plate-19: c-ELISA assay (anti-PPRV antibodies)



Plate-20: FAO and WFF PPR Prize Winner 2022

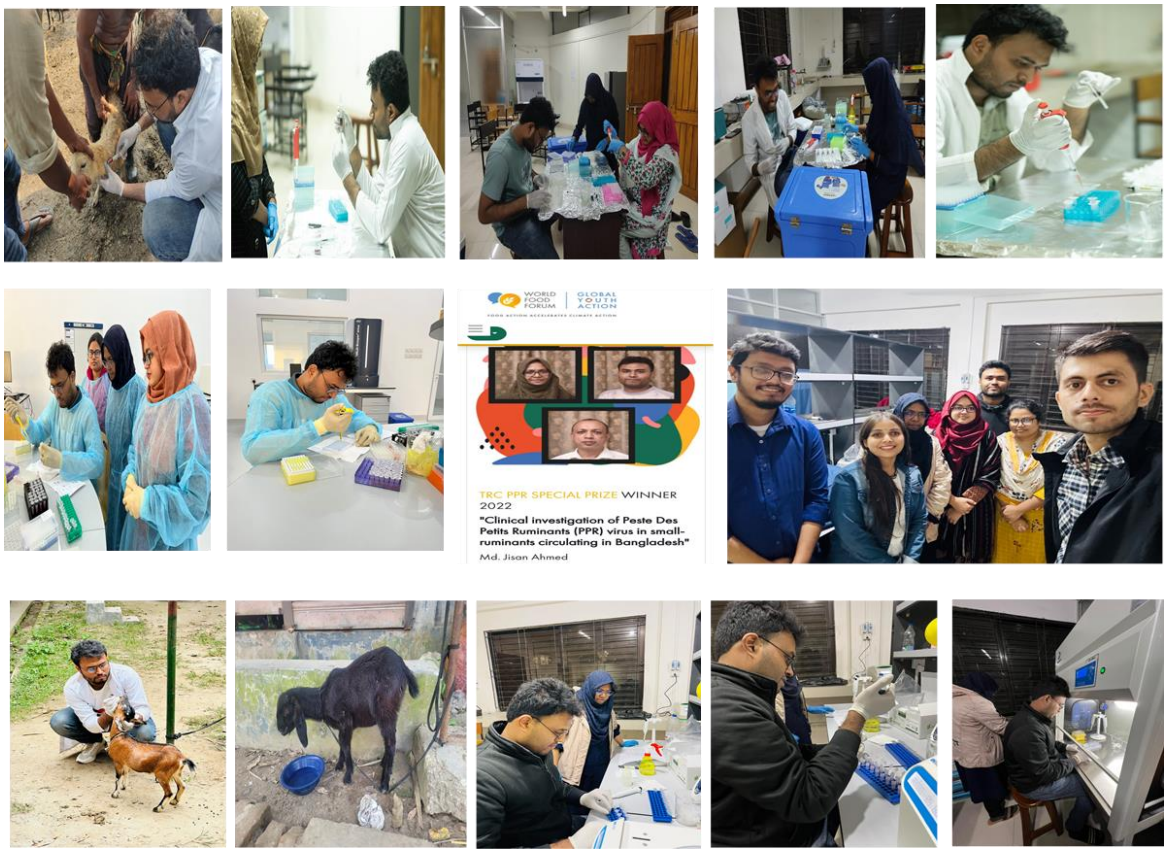


Plate-21: Fields and laboratory activities throughout the study period.





Plate-22: Upazila livestock officials