SEROPREVALENCE OF PESTE DES PETITS RUMINANTS (PPR) IN SHEEP AT DINAJPUR DISTRICT

A THESIS

BY

AFSANA SIDDIKA

REGISTRATION NO.: 1605471

SEMESTER: JANUARY-JUNE, 2018

SESSION: 2016-2017

MASTER OF SCIENCE (M.S.)

IN

PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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Approved as to style and contents by

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Dinajpur-5200

JUNE, 2018

Dedicated
To
My Beloved Parents
&
Honorable
Teachers

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January to June, 2018 *The author*

ABSTRACT

The present study was aimed to determinate the seroprevalence of Peste des petits ruminants (PPR) of sheep in Dinajpur district. The study was conducted at the Department of Pathology and Microbiology lab, Faculty of Veterinary and Animal Science, Hajee Mohammed Danesh of Science and Technology University (HSTU), Dinajpur for a period of 6 months from January to June, 2018. Blood samples were collected from sheep by jugular venipuncture method from sheep by using 5 ml syringe in a test tube without any anticoagulant without previous history of peste des petits ruminants PPR vaccination. The total blood samples were 66 and the samples were collected according to area, aged group, sex breed, lactation stage and parity. For serum collection the samples were kept for an hour at room temperature in a slightly inclined position to facilitate clotting, after clotting serum was separated and stored at -20 ^{0}C until use. The result showed that the overall prevalence in sheep was 24.24%. The present study revealed that the prevalence of PPR was not significantly (P= 0.869) differed based on area and the result showed that the highest seropositive (27.27%) was observed in Chirirbandar upazila whereas the lowest seropositivity (15.38 %) was detected from Sadar, Dinajpur. However the other locations were intermediate. In respect to sex, there was no significant variation (P= 0.935), and it was found that the seropositive percentage was not so higher in male sheep (25%) than female sheep (24%). According to the aged group, there was no significant effect among aged group (P= 0.561) and the result exhibited that the highest PPRV seropositive in sheep (38.46%) was in the first group (<1 year) while lowest seropositive (16.66%) were found from the third group (>3-6 years). According to lactation stage prevalence of PPR was higher in lactating stage or post partum stage (33.33%) than lamb (30%) and non lactating stage (13.63%) respectively. The prevalence of PPRV was not significantly (P=0.308) differed according to lactation stage. Based on parity, the prevalence of PPR was insignificantly higher in third parity (50.00%) where the other parity were no parity (30.00%), 1st parity (23.08%), 2nd parity (9.09%), 4th parity (40.00%) and there was no significant (P = 0.302) effect on prevalence of PPR in sheep based on parity. Competitive enzyme-linked immunosorbent assay (cELISA) was detected and measured antibody level against the PPR virus in the sera and the study was suggested that the PPRV was widely distributed in the study area. Therefore, it is recommended to control PPR virus such as by using regular and routine homogeneous vaccination should intensify in the study area.

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LIST OF ABBREVIATION

A.A	-	Amino acids
Abs	-	Antibodies
Ag	-	Antigen
AGDT	-	Agar gel diffusion test
AGID	-	Agar gel immunodiffusion test
AGPT	-	Agar gel precipitation test
BCC	-	Buffy coat cell
B-ELISA	-	Blocking-enzyme linked immunosorbent assay
BKC	-	Bovine kidney cells
BT	-	Blue tongue
C	-	C-protein
C	-	Control negative serum
C+	-	Control weak positive serum
C++	-	Control strong positive serum
ºC	-	Degree Celsius
Cc	-	Conjugate control
CCPP	-	Contagious caprine pleuropneumonia
cDNA	-	complementary DNA
CDV	-	Canine distemper virus
CE	-	Contagious ecthyma
C-ELISA	-	Competitive-enzyme linked immunosorbent assay
CIEP	-	Counter immunoelectrophoresis
cm	-	Centimeter
Cm	-	Monoclonal control
CNT	-	Cross Neutralisation test
CPE	-	Cytopathic effect
Da	-	Dalton
DDW	-	Deionized distilled water
DMS	-	Dimethyl sulp
DMV	-	Dolphin morbillivirus
DNA	-	Deoxyribonucleic acid
DPX	-	Distrene 80, plasticizer, xylene.
DW	-	Distilled water
EDTA	-	Ethylene diamine tetra acetic acid
ELISA	-	Enzyme linked immunosorbent assay
EM	-	Electron microscope/microscopy
F	-	Fusion protein

LIST OF ABBREVIATION (Contd.)

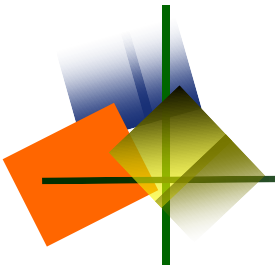
ºF	-	Degree Fahrenheit
FAO	-	Food and Agriculture Organization
FCS	-	Foetal calf serum
FLKC	-	Foetal lamb kidney cells
FMD	-	Foot and mouth disease
g	-	Gram
g	-	gravity unit
G. acetic acid	-	Glacial acetic acid
GKC	-	Goat kidney cells
GMEM	-	Glasgow minimum essential medium
GREP	-	Global Rinderpest Eradication Programme
H	-	Haemagglutinin protein
h	-	Hour (s)
H & E	-	Haematoxylin and Eosin
HA	-	Haemagglutination
HBSS	-	Hank's balanced salts solution
HI	-	Haemagglutination inhibition test
HRPO	-	Horseradish peroxidase
HS	-	Hyperimmune serum
i/m	-	Intramuscular
i/n	-	Intranasal
i/v	-	Intravenous
IAEA	-	International Atomic Energy Agency's
IAH	-	Institute for Animal He
IC-ELISA	-	Immunocapture-enzyme linked immunosorbent assay
IFAT	-	Immunofluorescence antibody test
Ig (s)	-	Immunoglobulin (s)
IHC	-	Immunohistochemistry
IP	-	Immunoperoxidase
IU/iu	-	International unit
Kb	-	Kilo bases
KDa	-	Kilo Dalton
kg	-	Kilogram
L	-	Large protein
LKC	-	Lamb kidney cells
log	-	Logarithm
LTC	-	Lamb testis cells

LIST OF ABBREVIATION (Contd.)

M	-	M-protein
M	-	Molar
ma	-	Milliamps
MAb (s)	-	Monoclonal antibody (s)
MDBK	-	Madin-Darby bovine kidney cells
mg	-	Milligram
min	-	Minute(s)
ml	-	Millilitre
mm	-	Millimeter
mM	-	millimolar
mRNA	-	Messenger RNA
MV	-	Measles virus
µg	-	Microgram
µl	-	microlitre
µm	-	micrometer
NC	-	Nucleocapsid
ng	-	Nanogram
nm	-	Nanometer
NP, N	-	Nucleoprotein
NS	-	Normal saline or Not significant
NT	-	Neutralisation test
OD	-	Optical density
OIE	-	Office International des Epizooties
OPD	-	Orthophenylene diamine
P	-	Phosphprotein
P.	-	Passage
P.D	-	Phosphate diluent
Per os	-	Per oral route/orally
p.i	-	Post infection/ Post inoculation
P.I.T	-	Precipitinogen inhibition test
P.M	-	Post –mortem
PAGE	-	Polyacrylamide gel electrophoresis
PBS	-	Phosphate buffer saline
PC/PCV	-	Packed cell/Packed cell volume
PDV	-	Phocine distemper virus
PFU	-	Plaque forming unit
pH	-	Hydrogen ion concentration

LIST OF ABBREVIATION (Contd.)

PI	-	Percentage inhibition
PMV	-	Porpoise morbillivirus
PPR (V)	-	Peste des Petits Ruminants (Virus)
r.p.m	-	Revolutions per minute
RBC(S)	-	Red blood cell (s)
RNA	-	Ribonucleic acid
RNase	-	Ribonucleases
RP (V)	-	Rinderpest (Virus)
RPTCV	-	Rinderpest tissue culture vaccine
RT-PCR	-	Reverse transcriptase-polymerase chain reaction
s/c	-	Subcutaneous
SDC	-	Sodium deoxycholate
S-ELISA	-	Sandwich-enzyme linked immunosorbent assay
SNT	-	Serum Neutralisation test
SPC	-	Stomatitis pneumo-enteritis complex
TC	-	Tissue culture
TCID	-	Tissue culture infective dose
Temp	-	Temperature
TPB	-	Tryptose phosphate broth
U/u	-	Unit
V	-	V-protein
v/v	-	Volume to volume
Vero	-	African green monkey cells
VNT	-	Virus Neutralisation test
volts	-	Voltage
w/v	-	Weight to volume



Chapter 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Peste des petits ruminants (PPR) is an acute contagious viral disease. It affects small ruminants, especially sheep and goats, which are highly susceptible and occasionally some wildlife species. PPR was first emerged in the Ivory Coast in West Africa in 1942 (Gargadennec and Lalanne, 1942). It continued to cause serious economic losses in these species in Africa, the Middle East, the Arabian Peninsula and South-West Asia (Dhar *et al.,* 2002). PPR is a disease of major economic importance. It is regarded as the biggest constraint to large-scale intensive production of sheep and goats in the West African sub-region. It is acknowledged as the most destructive disease and the number one killer disease of small ruminants in Bangladesh. The disease is grouped within the List A of the Office International des Epizooties (OIE) due to its highly contagious nature and consequent capacity for rapid spread.

Peste des petits ruminants (PPR) virus, member of the genus morbillivirus of the family Paramyxoviridae, is closely antigenically related to rinderpest virus (RPV) which can also cause a disease in small ruminants. Four distinct genetic lineages of PPRV were identified using polymerase chain reaction amplification (RT-PCR) and sequence analysis. Lineage four is restricted in Asia whereas the other three are prevalent in Africa (Shaila *et al.,* 1996; Dhar *et al.,* 2002; Banyard *et al.,* 2010).

Clinically, the disease is characterized by pyrexia, ocular and nasal discharges, erosive stomatitis, conjunctivitis, gastroenteritis, diarrhoea, respiratory distress and pneumonia (Jilo, 2016). Peste des petits ruminant is having affinity for lymphoid tissues where it replicates. It is an immunosuppressive disease and thus enhances chances for secondary bacterial infections and other diseases which complicate the clinical picture. PPR closely resembles rinderpest both clinically and pathologically. In goats and sheep, the two viruses produce clinical disease and pathology that are indistinguishable. However, for the diagnosis of rinderpest in small ruminants, it is essential to differentiate it clearly from PPR.

The infection is transmitted by close contact between infected and susceptible animals (Ozkul *et al.,* 2002). There are chances of outbreaks by the introduction of new sick

animals into the flock or migration of flocks to the endemic areas (Radostits *et al.,* 2000). PPR disease can quickly disseminate whole flocks either by itself or in combination with other diseases, such as pneumonia or gastro-intestinal parasitism. The disease appears with a higher incidence in the rainy seasons (OIE, 2004). The disease is usually acute causing great losses in sheep and goats in Bangladesh, the morbidity rate was recorded to be 100% and mortality varied from 50 to 90% (Sil *et al.,* 1995). Infection rates in sheep and goats rise with age. The disease is most prevalent in small ruminants less than one year of age and is rapidly fatal in young animals. Surviving animals usually develop a dual immunity to both PPR and RP viruses.

The diagnosis of PPR is done based on clinical, pathological and epidemiological findings). For serological diagnosis, commercially available diagnostic ELISA with high specificity and sensitivity that detect antibodies to either the N or the H proteins of the virus are available to assess seropositivity within a population (Balamurugan *et al.,* 2014). To control outbreaks of PPR disease in epizootic areas, small ruminants were vaccinated with an effective live attenuated PPR vaccine which is used in immunoprophylaxis against PPR.

Recently, PPR has become endemic across Sub-Saharan Africa, the Middle East, the Arabian Peninsula, Turkey, Iran, Iraq, Pakistan, India, Bangladesh, Tajikistan and Kazakhstan in Central Asia (Taylor and Barrett, 2007). The presence of virus has also been reported in China (Wang *et al.,* 2009). Bangladesh is a subtropical country with subsistence agro-based economy. The agriculture of Bangladesh is almost entirely based on livestock. Livestock play an important role in the national economy and the sheep is a very important species of livestock in Bangladesh. There are about 3.34 million (DLS, 2016) sheep populations are found in Bangladesh. Sheep rearing is an economically important aspect of the agriculture sector, as most of the people live in rural areas (Tariq *et al.,* 2010). PPR can cause a serious economic loss. Although PPR has been prevalent in Bangladesh since early 1990, very limited studies have been done (Islam *et al.,* 2001; Khan *et al.,* 2005). In the present study a systematic and detailed investigation of PPR was conducted in sheep of Chirirbandar, Parbatipur, Birol and Sadar at Dinajpur district.

Therefore this study was carried out under the following objectives:-

i. To detect the PPRV antibodies in the serum sample of sheep.
ii. To correlate the seroprevalence of affected sheep with area, age, sex, lactation stage and parity at four upazila in Dinajpur district.
iii. To estimate and investigate the distribution, the status and the sero-prevalence of PPR virus in different upazila of Dinajpur district.

Chapter 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

The literature pertinent to the present study on investigation of a PPR outbreak is reviewed in this part of thesis.

2.1 Definition

Peste des petits ruminants (PPR) is a highly contagious, infectious and fatal viral disease of domestic and wild small ruminants (Furley *et al.,* 1987; Roeder and Obi, 1999). Goats are usually more severely affected than sheep (Lefevre, 1987; Roeder *et al.,* 1994; Diallo, 2000). The clinical disease is similar to rinderpest in cattle and characterized by clinical signs and lesions of respiratory and alimentary systems involvement (Losos, 1986).

Peste des petits ruminants (PPR) has various synonyms including kata (Whitney *et al.,* 1967), stomatitis pneumoentritis complex (Nduaka and Ihemelandu, 1973), goat plaque, pest of small ruminants, Rinderpest like disease or syndrome pseudorinderpest of small ruminants (Johnson and Ritchie, 1968; Durtnell and Eid, 1973), contagious pustular stomatitis and contagious vulvovaginitis (Wosu, 1992). Kata is a pidgin-English word for catarrhal used locally in Western Nigeria for a pseudorinderpest of goats first recognized in1965 (Whitney *et al.,* 1967).

2.2 History of the Disease

In the early 1940's, a fatal disease of sheep and goats with high mortality was first described as Peste-des-petits-ruminants (PPR) in the Ivory Coast (Cote d'Ivoire) in West Africa (Gargadennec and Lalanne, 1942). In the same period, a similar syndrome was described in Dahomy (Benin) by Cathou (1944) under the name of "Peste des especes ovine et caprine". The disease has since been described in Senegal (Mornet *et al.,* 1956) and Ghana. Afterwards, it was quickly recognized in other French West African colonies. The early work by Mornet and colleagues showed that the virus was related to rinderpest virus (RPV) (Mornet *et al.,* 1956). They concluded that it actually was a rinderpest virus (RPV) with adapted pathogenicity for goats and sheep. The first isolation of the PPRV was recorded in Senegal in 1962 (Gilbert and Monnier, 1962). They adapted the virus on lamb kidney cell cultures and studied the serological relationship of the virus with the RP virus.

In 1950s, a disease affecting mainly goats was studied in Nigeria by Johnson and Ritchie (1968). This disease was given a variety of names including Stomatitis- pneumoenteritis complex (SPC), Pseudorinderpest and Kata. Johnson and Ritchie (1968) isolated a virus from cases of SPC in goats in Ibadan in Nigeria which showed similar cytopathic effect (CPE) in goat embryo kidney-cell culture to those produced by the PPRV of Gilbert and Monnier and differing in characteristics from that of measles virus and RPV. For the second time, Whitney and co-workers observed a Stomatitis and enteritis in Nigerian dwarf goats that closely simulated RP and locally termed "Kata" (Whitney *et al.,* 1967). Originally this disease was considered distinct from PPR for two reasons. Firstly, this disease was seen mainly in goats and sheep, secondly, its lesions consisted mainly of crusted labia (Whitney *et al.,* 1967; Johnson and Ritchie, 1968; Isoun and Mann, 1972). Further studies have proved that PPR and SPC are the same diseases (Rowland and Bourdin, 1970; Hamdy *et al.,* 1976). Rowland and co-workers proved that the experimental infection of West African dwarf goats with PPR and Kata were indistinguishable clinically and pathologically (Rowland *et al.,* 1971). Hamdy *et al.* (1976) were able to isolate the causative agent of SPC and the virus was identified immunologically and morphologically as identical with the PPRV.

Since then the original name of peste des petits ruminants (PPR) has been designated as the official name of the disease beside many synonyms.

For many years it was thought that PPR was restricted to West Africa, until a disease of goats in the Sudan, which had originally diagnosed as RP in 1972 (El Hag Ali, 1973) was confirmed to be PPR 10 years later (El Hag Ali and Taylor, 1984). As a result, it is possible that cases of severe rinderpest diagnosed in small ruminants in the past may have in fact been PPR. Gibbs and co-workers (1977) reported a mixed viral infection when isolated adenoviruses from goats that died from PPR in Nigeria.

For the first time PPR was reported in wild ruminants including gazelles, ibex and gemsbok at Al in in the Arabian Gulf by Furley *et al.* (1987).

2.3 Etiology

The etiologic agent of PPR is an envelope, pleomorphic particle containing single stranded RNA virus, originally considered a variant of rinderpest virus specifically adapted for small ruminants. Later molecular studies showed that it was distinct from, but closely

related to RPV (Defra,2001). The PPRV is antigenically related to canine distemper (CDV), RPV and the Measles virus of humans (Gibbs *et al.,* 1979; Hamdy, 1980). The biological and physiochemical characteristics of the virus indicate that it is closely related to them. All the four viruses are closely related serologically when analyzed using polyclonal antisera (Imagawa, 1968; Orvell and Norrby, 1974; Gibbs *et al.,* 1979; Hall *et al.,* 1980; Appel *et al.,* 1981).

2.3.1 Classification

Peste des petits ruminants is caused by a virus that belongs to the genus morbillivirus of the family Paramyxoviridae. The genera morbillivirus together with respirovirus, rubulavirus and equine morbillivirus form Paramyxovirinae subfamily in the Paramyxoviridae which is a member of the order Mononegavirales (Murphy *et al.,* 1995; Murphy *et al.,* 1999).

The morbilliviruses are a closely related group of important human and animal pathogenic viruses (Gibbs *et al.,* 1979; Barrett, 1987). The members of the morbillivirus genus are RPV of cattle and buffaloes, the Measles virus (MV) of human, PPRV of domestic and wild small ruminants, canine distemper virus (CDV) of dogs and some wild carnivores, phocine distemper virus (PDV) of seals, dolphin and porpoise morbilliviruses of aquatic mammals (Kingsbury *et al.,* 1978; Gibbs *et al.,* 1979; Losos, 1986; Barrett *et al.,* 1993; Visser *et al.,* 1993; Murphy *et al.,* 1995; Anderson *et al.,* 1996; Murphy *et al.,* 1999; Roeder and obi, 1999).

Losos (1986) based this classification on the morphology of the virus, growth in tissue culture, the nucleic acid composition antigens and the physiochemical properties. The virus has been shown to be similar in ultrastructure and morphogenesis to RPV, CDV and MV (Plowright *et al.,* 1962; Bourdin and Laurent-Vautier, 1967; Gibbs *et al.,* 1979). PPRV is sufficiently distinct from these three viruses to justify considering it as the fourth member of the morbillivirus genus in the Paramyxoviridae (Gibbs *et al.,* 1979; Nduaka and Ihemelandu, 1973) reported that the PPRV has been classified in the morbillivirus on the basis of reciprocal cross-neutralisation, pathogenicity and degree of cross protection between RPV and PPRV. PPRV has been differentiated biochemically from RPV on the basis of a very marked difference in the apparent molecular weight of the nucleoprotein (Diallo *et al.,* 1987).

2.3.2 Virus Properties

2.3.2.1 Morphology

Generally morbillivirus virions are enveloped pleomorphic particles with a size ranging from 350 to 600 nm. The size of PPRV varies between 150 and 700 nm (around500 nm) and of RPV is about 300 nm (Bourdin and Laurent-Vautier, 1967; Durojaiye *et al.,* 1985; Diallo, 1990). The particles have a lipoprotein membrane covered with large peplomers (8-20 nm in length) and contain a herring-bone shaped helically symmetrical nucleocapsid. It is formed by the genomic RNA associated with three viral proteins: the nucleocapsid (N), the phosphoprotein (P) and the polymerase (L) protein. The genome consists of non-segmented single-stranded RNA molecule of negative sense and about 15-16 kilo bases (kb) in length (Diallo, 1990; Murphy *et al.,* 1999; Diallo, 2003).

2.3.2.2 Genomic Structure and Gene Expression

The genome is organized in six transcriptional units or genes encoding two non- structural proteins (V and C) and six structural proteins: the surface glycoproteins (The fusion (F) and the haemagglutinin (H) proteins); the nucleocapsid (N); the phosphoprotein (P); the matrix (M) protein; and the polymerase or large (L) protein which forms the polymerase complex in association with the (P) protein (Crowley *et al.,* 1988; Diallo, 1990; Rima, 1993; Sidhu *et al.,* 1993; Sharma and Adlakha, 1994; Diallo *et al.,* 1994; Haffar *et al.,* 1999; Diallo, 2003). The RNA has to be transcribed by their own polymerase into at least six mRNAs translated into six structural proteins separately while a part of P mRNA is translated into P, C and V proteins (Murphy *et al.,* 1999; Diallo, 2003). The nucleocapsid protein (N) is a major viral protein. It folds and protects the RNA genome against degradation by RNase (Diallo, 1990; Barrett *et al.,* 1993). It is very sensitive to intracellular proteolysis (Diallo, 1990). Diallo *et al.* (1994) determined the sequence of the N protein of PPRV Nigerian 75/1 vaccine strain which consists of 1662 nucleotides while that of RPV and MV consists of 1683 nucleotides. It contains one long open reading frame encoding a protein of 525 amino acids with molecular weight of 58,008Da which is close to the value 60,000 Da estimated by the polyacrylamide gel electrophoresis pattern of PPR induced proteins in infected cells (Diallo *et al.,* 1987). N protein is considered to be the major cross-reacting antigen of morbilliviruses (Orvell and Norrby, 1974). In monoclonal antibodies some differences have been demonstrated between the N proteins of these

closely related viruses (Libeau and Lefevre, 1990). Therefore, it has been suggested that RPV might be the archevirus from which the othershave originated (Norrby *et al.,* 1985; Meyer and Diallo, 1995). Comparisons of the nucleic acid and protein sequences of all the morbillivirus nucleoproteins indicated two major subgroups: one included CDV and PDV and the other RPV, MV and PPR which was found to be slightly more related to CD and PDV than MV and RPV (Diallo *et al.,* 1994).

The phosphoprotein (P) is very susceptible to proteolysis. It is the second protein involved in the ribonucleo-capsid complex (Diallo, 2003). It is associated with the L protein as a non catalytic co-factor in the RNA polymerase complex and play a function in mRNA transcription and replication (Al Khatib *et al.,* 1988; Diallo, 2003). The P gene of PPRV was sequenced and found to encode a protein of 507 amino acids with molecular weight of 54,000 Da (Diallo, 2003). This is much lower than 70,000 Da value estimated by its electrophoretic migration on a polacrylamide gel (Diallo, 2003).

The polymerase or large protein (L) is the third viral protein involved in the ribonucleoprotein structure. It is the minor viral protein (Diallo, 1990). Its gene is the last transcribed by the virus and it is 6,643 nucleotides long and is capable of encoding a protein of 2,183 amino acids residues (Yami, 2001). Because of its large size, it's expected to exhibit the majority of the RNA polymerase activity. Therefore, there are different enzymatic activities associated with the L protein including: RNA polymerase, mRNA capping, methylation, polyadenylation and protein kinase (Banerjee, 1987; Diallo, 2003). Sequence analysis of PCR-derived P gene fragments, together with serological data have shown that DMV and PMV are more closely related to PPRV than to other members of the genus (Barrett *et al.,* 1993; Visser *et al.,* 1993).

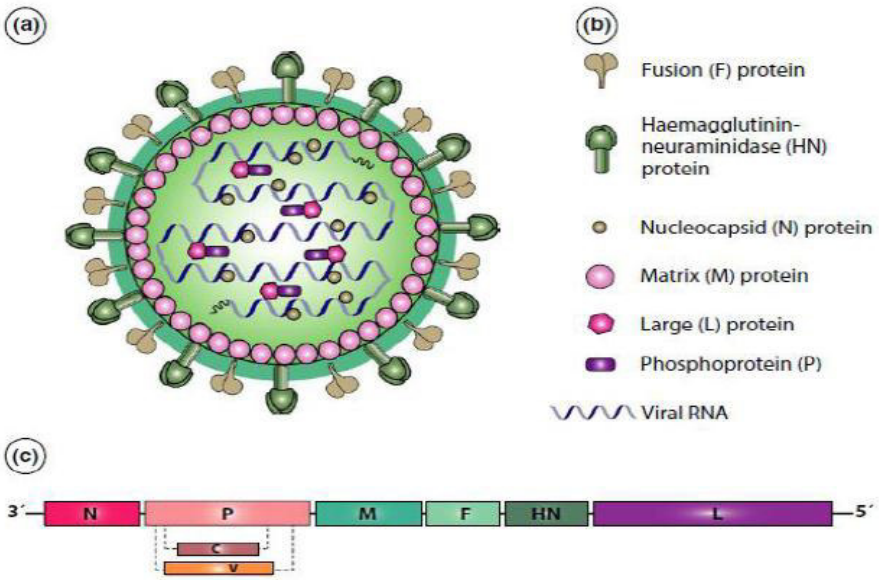


Figure 1: (a) Schematic structure of a typical morbillivirus (PPRV). (b) The structural components of PPR. (c) The genome organization of all known genes of PPRV.

The matrix protein (M) of a PPRV forms an inner coat to the viral envelope and thus serves as a bridge between the surface viral glycoproteins and the ribonucleoprotein core (Diallo, 1990; Diallo, 2003). By virtue of its position, M protein appears to play a central role in the formation of the new virions which are liberated from the infected cell by budding (Peeples, 1991). Defects in the M protein prevent the virus from completing its infectious cycle leading to a persistent, non-productive virus infection that was observed in MV and CDV (Barrett *et al.,* 1993; Haffar *et al.,* 1999). The mRNA corresponding to the M gene of morbilliviruses has been cloned and sequenced and that of PPRV is the longest (1466 nucleotides) long with an open reading frame capable of encoding basic protein of 335 amino acids (Haffar *et al.,* 1999). The apparent molecular weight of the PPR M gene is 38,057 Da is in a good agreement with its apparent molecular weight (39,000 Da) estimated by polyacrylamide gel electrophoresis (Diallo, 1990). The nucleotide sequence of the PPR M protein when compared with those of the other morbilliviruses shows that the most closely related to PPRV was DMV (Haffar *et al.,* 1999).

The fusion protein (F) mediates fusion between the virus envelope and the infected cell or between infected and adjacent cells giving an essential role in spreading the virus within the host by inducing cyncytia (Diallo, 1990). It is synthesized on newly formed virions as precursor F 0 which is activated by proteolytic cleavage to two proteins: F 1 and F 2 linked by disulphide bonds. This cleavage is essential for virus infectivity and virulence

(Diallo, 1990; Murphy *et al.,* 1999). The F gene of PPRV was found to be 2321 nucleotides long excluding poly (A) tail (Meyer and Diallo, 1995) with molecular weight of 57, 155 Da (Meyer and Diallo, 1995; Diallo, 2003).

The haemagglutinin (H) is the second viral glycoprotein. It serves attaching the virus to the host cell. It elicits neutralizing antibodies that inhibit adsorption of the virus to cellular receptors (Diallo, 1990; Diallo, 2003). It is 609 amino acid long. H and F proteins are playing a role in the pathogenesis of all Paramyxovirus infections. They are highly immunogenic and confer protective immunity (Diallo, 2003). Norrby *et al.* (1975) found that anti-F-antibodies seem to provide more protection than anti- H- antibodies. C protein is the smallest viral protein with molecular weight of 19.5 KDa and it contains only 186 amino acids. It is found only in the infected cells. C and V proteins were involved in the transcription and replication processes (Diallo, 1990).

2.3.3 Ultrastructure of PPRV

The morphology of PPRV observed by negative staining electron microscopy was typical of Paramyxoviruses and indicated that the genome was ribonucleic acid (Bourdin and Laurent-Vautier, 1967; Gibbs *et al.,* 1979). The virions of RPV and CDV when studied by electron microscopy had three distinct morphological components: an internal helical component, an outer membrane and a layer of surface projections (Tajima *et al.,* 1971). Ultrastructure studies of PPRV revealed that the intact virus particle is pleomorphic either spherical or ovoid with a diameter varying between 130 and 390 nm (Durojaiye *et al.,* 1985). The intact particles possess an outer envelope with spikes projecting from the surface. The thickness of the envelope ranges between 8-15 nm while the length of the spikes measures 8.5-14.5 nm. The core of the particles was occupied by rod-shaped helical structures with bilateral projections presenting a herring bone-like nucleocapsid structure. The thickness of the nucleocapsid strands varied between 14-23 nm (Durojaiye *et al.,* 1985). Some filamentous forms of PPR and RP viruses were observed (Plowright *et al.,* 1962; Durojaiye *et al.,* 1985). These properties are very similar to those described for RPV; however the particles are bigger in size than those of RP (Bourdin and Laurent-Vautier, 1967). The particle of RP varies between 120 and 300 nm in diameter while the internal helical component about 17.5 nm in diameter (Bourdin and Laurent-Vautier, 1967).

2.3.4 Replication

The replication of Paramyxoviruses was described by Murphy *et al.* (1999). Paramyxoviruses replicate mainly within the cytoplasm. The initial step of replication is the attachment of the virions via their haemagglutin in protein to cellular sialoglycoprotein receptors. Viral attachment was followed by fusion of the viral envelope with the plasma membrane. Fusion which is mediated by the fusion protein occurs at physiologic pH. The liberated nucleocapsid remains intact, with its associated proteins (N, P and L). The nucleocapsid being required for transcription by the virion-associated RNA dependent RNA polymerase (transcriptase). The genome is transcribed progressively into six or ten discrete unprocessed mRNA by sequential interrupted synthesis from a single promoter. The replication of negative-sense RNA genome required synthesis of a template which is full length RNA genome of positive-sense. The control of these processes is mainly at the level of transcription. Newly synthesized negative-sense RNA associate with the N protein and transcriptase to form the nucleocapsides. Finally, virions maturation occurs through several processes:

- The incorporation of viral glycoproteins into patches on the host cell plasma membrane.
- The association of matrix protein (M) and other non-glycolysated proteins with this altered host cell membrane.
- The alignment of the nucleocapsid beneath the M protein.
- The formation of the mature virions which is released via budding.

2.3.5 Physiochemical Properties

It is assumed that the survival characteristics of PPRV are similar to those for RPV. Since these viruses are enveloped they are fragile and can easily be destroyed by heat, desiccation, light, ultraviolet (UV) radiation, pH extremes and common disinfectants (Rossiter and Taylor, 1994; Diallo, 2003).

2.3.5.1 Temperature

PPR viruses are not very resistant. The half-life in infected tissues at56ºC is measured in minutes, at 25ºC in hours, at 5ºC in days and at -20ºC in months (Scott and Brown, 1961). At 56ºC the half-life is short but a small proportion may survive for 50 to 60 minutes

(Plowright and Ferris, 1961) or 60ºC for 30 minutes (DeBoer and Barber, 1964; Andrews and Pereira, 1972). The virus looses its infectivity within 30 minutes at 50ºC (Sharma and Adlakha, 1994). The virus is rapidly inactivated at temperatures above 70ºC. PPRV is rapidly inactivated at 56ºC. Some viruses may resist 60ºC for 60 min (OIE, 2002). In lymph node homogenate the virus is killed within 1-4 hours at 56ºC, between 1 and 3 days at 37ºC, within 10 days at 25ºC and within 56 days at 7ºC. Scott (1955) demonstrated viable virulent virus in infected spleens stored for three years at -25ºC. The infectivity of cell culture preparations of RPV has a half-life of 1-3 hours at 37ºC and only few minutes at 56ºC (Sharma and Adlakha, 1994). Survival in cultures for at least 4 months at -20ºC, 8 weeks at 4ºC, 1 week at 20-25ºC and less than 2.6 days at 37ºC. PPR and RP viruses survive for long periods in chilled and frozen tissues (OIE, 2002). The virus preparations are best preserved by lyophilisation or by addition of 2% dimethyl sulphoxide (DMS) and stored at 4ºC. Dried virus is much more resistant to heat than hydrated virus (Sharma and Adlakha, 1994).

2.3.5.2 Sunlight

Peste des petits ruminants virus is susceptible to sunlight. It is rapidly inactivated by ultraviolet light and desiccation within 4 days (Scott and Brown, 1961; OIE, 2002).

2.3.5.3 Hydrogen Ion Concentration (pH)

The optimal pH for survival of PPRV is 7.0-8.0 (Scott and Brown, 1961). At 4ºC, the virus is most stable at pH values 7.2 and 7.9 but can survive between pH 4 - 10.2 (Scott and Brown, 1961; liess and Plowright; 1963; Sharma and Adlakha, 1994; OIE, 2002). The virus is sensitive to low pH and rapidly inactivated at pH values less than 5.6 or greater than 9.6 (Laurent, 1968; OIE, 2002). The virus is rapidly inactivated by autolysis, putrefaction and lactic acid fermentation in meat which destroyed the virus. But in lymph nodes the virus is protected from pH changes after death (Sharma and Adlakha, 1994). The inactivation rate in alkaline hydrogen ion concentrations is even greater. The optimal molarity for survival is 0.1 M (Scott and Brown, 1961).

2.3.5.4 Chemicals and Disinfectants

Peste des petits ruminants virus is sensitive to a wide range of disinfectants due to its large size, lipid containing virus envelope and sensitivity to both acid and alkali conditions

(Scott and Brown, 1961). The virus is found to be ether sensitive (Scott and Brown, 1961; Gibbs *et al.,* 1979). It is also sensitive to chloroform, alcohol and trypsin (Sharma and Adlakha, 1994). Most lipid solvent disinfectants and detergents are virucidallike phenol, cresol and 2% sodium hydroxide for 24 hours. Various chemicals like β-propiolactone, hydroxylamine, phenol and formalin inactivate the virus. The virus also gets inactivated with prolonged treatment with glycerin (OIE, 2002).

2.3.5.5 Antibiotics

Antibiotics and sulphanamides have no effect on RP and PPR viruses (Scott and Brown, 1961).

2.4 Epidemiology of PPR

2.4.1 Geographical Distribution

Peste des petits ruminants was first described in Cote d'Ivoire in West Africa (Gargadennec and Lalanne, 1942). For many years PPR was considered an African disease localized mainly in West and Central Africa (Mornet *et al.,* 1956; Isoun and Mann, 1972; Bourdin, 1980; Durojaiye, 1980; Scott, 1981; Obi, 1984; Lefevre, 1987). The disease has since been described in Senegal (Mornet *et al.,* 1956), Nigeria (Johnson and Ritchie, 1968) and Ghana (Roeder and Obi, 1999). PPR-like disease has also been reported in Benin (Bourdin, 1973) and Chad (Provost *et al.,* 1972).

The first recorded isolation of PPR was made by Gilbert and Monnier (1962) in Senegal. Serological evidence of PPR has been reported in Nigeria (Zwart and Rowe, 1966) and Senegal (Bourdin and Bernard, 1967). PPR was reported several times in Nigeria (Whitney *et al.,* 1967; Johnson and Ritchie, 1968; Hamdy *et al.,* 1976). Although the disease was early reported in Nigeria the virus was isolated in the late 1970's (Taylor and Abegunde, 1979). Elhag for the first time isolated PPR viruses from Sudan (Elhag Ali, 1973; Elhag Ali and Taylor, 1984).

In Cameroon, the first evidence of PPR was confirmed by Saliki *et al.* (1987). Ismail and House (1990) confirmed the first isolation of PPRV in Egypt. Roeder *et al.* (1994) confirmed the presence of PPR in Ethiopia by using specific cDNA probes. Clinical PPR was reported in Eritrea (Anon, 1994), Israel (Perl *et al.,* 1994) and Pakistan (Amjad *et al.,*

1996). Bidjeh *et al.* (1995) recorded the first isolation of PPRV in Chad and induced the disease experimentally.

In Sultanate of Oman, Hedger *et al.* (1980) recorded neutralizing antibodies against PPRV in sheep and goats. Also, Ata and Al Sumry (1989) diagnosed PPRV using AGPT while Taylor *et al.* (1990) isolated and identified the virus by animal inoculation, pathogenicity and by specific hybridization with nucleic acid probes. PPR was first described in wild ruminants in a zoo (Furley *et al.,* 1987) and in small ruminants (Moustafa, 1993) at Al Ain in the Arabian Gulf. PPR was reported in sheep in Saudi Arabia (Asmar *et al.,* 1980) and also in gazelles and deer (Hafez *et al.,* 1987). Abu Elzein and others confirmed the first isolation of PPRV in Saudi Arabia (Abu Elzein *et al.,* 1990). Lefevre *et al.* (1991), by ELISA and virus neutralisation (VN) tests, confirmed the seroprevalence of PPR in Jordan. There is serological evidence for PPR from the Syrian Arab Republic (Roeder and Obi, 1999). PPR was reported in Turkey in 1999 (Roeder and Obi, 1999; Diallo, 2000; Diallo, 2004) and Turkish isolate was compared at the genomic level with other PPRV isolates (Ozkul *et al.,* 2002).

In India the first confirmation of the presence of PPRV through virus isolation was reported by Shaila and others in the State of Tamil Nadu (Shaila *et al.,* 1989). Then PPR was diagnosed by using cDNA probes (Shaila *et al.,* 1990). Subsequent several outbreaks were reported from different parts of the country (Mondal *et al.,* 1995; Shaila *et al.,* 1996; Kulkarni *et al.,* 1996; Nanda *et al.,* 1996; Dhar *et al.,* 1997; Shankar *et al.,* 1998; Taylor *et al.,* 2002). Nanda *et al.* (1996) isolated PPR viruses and confirmed them by Immunocapture ELISA, RT-PCR and IF with specific monoclonal antibodies (MAbs).

Presently, PPR occurs in most African countries situated in a wide belt between the Sahara and the equator, the Arabian Peninsula and in most of the Middle Eastern countries and in the India and South-West Asia (Diallo, 2000; Diallo, 2004). Roeder and Obi (1999) reported PPR in countries including: Islamic Republic of Iran, Iraq, Kuwait, Lebanon, Yemen, Nepal, **Bangladesh** and Afghanistan.

2.4.1.1 Lineages of PPRV

The genetic relationship between PPR viruses of distinct geographical origin was studied through sequencing of a selected region of the fusion (F) protein gene of the virus using reverse transcription (RT) followed by polymerase chain reaction (PCR) amplification

(Shaila *et al.,* 1996). Shaila and co-workers (1996) compared nineteen PPRV isolates using phylogenetic analysis and identified four separate lineages of PPR virus three from Africa and one from Asia. Recently, Dhar used the same analysis for 27 PPR isolates (Dhar *et al.,* 2002). Lineage one (I) have been found in West Africa and included: Senegalese strain, Nigeria 75/1, 75/2, 75/3, 76/1 and Burkina Faso/99. Lineage two (II) found also in West Africa and include the isolates: Guinea Biseau/91 and Ivory Coast/89. Lineage three (III) includes viruses which have been isolated from East Africa and Asia: Sudan/72, Oman/83, India/TN/92, Ethiopia/96 and Yemen/01. Lineage four (IV) found only in Asia and includes viruses whose origins are in the Middle East, Saudi Arabia and South Asia: 15 Indian isolates, Bangladesh/93 and Bangladesh/00, Nepal/95, Turkey/96 and Turkey/00, Israel/94, Pakistan/94 and Pakistan/98, Saudi Arabia/94, Iran/94, Iraq/00a and Iraq/00 and Kuwait/99.sThe existence of both lineages III and IV in the Middle East and Asia are probably due to animal importation from Africa and South Asia (Diallo, 2003).

2.4.2 PPR in Bangladesh

Since 1993 PPR has been endemic in sheep and goats in Bangladesh. In BD the morbidity was recorded to be 100% and mortality varied from 50 to 90% (Sil *et al.,* 1995).A seroprevalence study of PPR specific antibody conducted different area of BD that the seroprevalence in sheep, goat and cattle was 36%,49.17% and 19.05%, respectively (Rahman *et al.,* 2005).

A seroprevalence study was recorded of PPR antibodies in sheep and cattle at St. Martin's Island in Bangladesh from July 2012 to June 2013. There was no previous history of PPR outbreak, and no PPR vaccination. Blood samples were collected from 192 sheep and 132 cattle randomly. Serum antibody titre (CP value) was determined by a commercially available c-ELISA kit. The overall seroprevalence of PPR in sheep was 37.5%. No serum samples from cattle were positive. In view of the high risk of PPR, a control strategy is proposed (Emdadul *et al.,*2014) . The other epidemiological study was conducted to find out the incidence of Peste des Petits Ruminants (PPR) in goat and sheep at Upazilla Veterinary Hospital, Rangpur sadar, Rangpur during the period of January to April, 2014. In this period, 236 clinically infected goat and sheep were examined in which 22 (9.32%) PPR cases were diagnosed on the basis of history, clinical signs and gross pathological lesions. This present study reveals that about 7-12 months aged group of sheep were more

prone (40.91%) to PPR compare to adult (above 1 year) and where the occurrence of PPR disease was more in goat (81.82%) than sheep (18.18%)(copright © Md mamunur Rahman).

2.4.3 Host range of the disease

Kihu *et al.* (2012) carried out a study in Turkana Kenya and confirmed that crowding of sheep and goats at watering points during the dry season was found to be a significant risk factor for outbreaks in 2009 while in 2010, sick adult goats and sheep sharing of grazing and water with lambs and kids was found to be significant source of outbreaks. Al-Majali *et al.* (2008) conducted a study in Jordan and found that sheep and goat flocks, large flock size, visiting live animals market and inadequate veterinary services were identified as risk factors for seropositivity. Mixed (sheep and goats) raising was identified as a risk factor for seropositivity in sheep flocks only.

Peste des petits ruminants are a disease of sheep and goats, in general goats are more susceptible than sheep; with sheep undergoing a milder form of the disease (Lefevre and Diallo, 1990). Other domestic animals such as camels, cattle and pigs are known to undergo subclinical infection of PPR (Taylor, 1984). The disease has been reported in wild small ruminants in a zoo (Furley *et al.,* 1987) and those living in the wild (Ogunsanmi *et al.,* 2003; Sharawi *et al.,* 2010; Kinne *et al.,* 2010).

2.4.4 Transmission

2.4.4.1 Natural Transmission

Like other Paramyxoviruses, PPR is a fragile virus and it can not survive for long time outside the host (Rossiter and Taylor, 1994; Diallo, 2003). Abegunde and Adu (1977) reported that the virus is excreted in nasal, oral and ocular discharges at the onset of pyrexia and in faeces at the onset of diarrhoea. They suggested that an animal in the incubation stage can infect other animals (Abegunde and Adu, 1977; Diallo, 2003). Durojaiye (1980) reported that the virus is naturally transmitted through the mouth and the respiratory tract. The spread of the disease is by contact between infected and susceptible animals. Lefevre and Diallo (1990) mentioned that the disease is transmitted between animals living in close contact by inhalation of aerosols. Aerosols which contain fine

infectious droplets are produced by sneezing and coughing. Also the disease can be spread by indirect contact through water, feed troughs and bedding contaminated with the virus.

2.4.4.2 Experimental Transmission

Experimentally, the virus has been transmitted paranterally through different routes: nasal, oral, subcutaneous, intraocular, intratracheal and intravenous or by contact (Durtnell, 1972; Durojaiye, 1980). Durtnell (1972) used infective lymphoid tissue suspension to infect goats and sheep by PPRV after the propagation of the virus through three serial passages in goats. The tissue suspension failed to infect inoculated sheep and in contact sheep and goats. The transmission study carried by Whitney *et al.* (1967) indicated that the syndrome SPC could pass from sheep to sheep by contact or by inoculation of susceptible sheep with the infectious agent. They also reported that the disease could not be transmitted to cattle and rabbits inoculated via subcutaneous and cerebral routes respectively. Isoun and Mann (1972) indicated that PPR is contagious due to the nature of the spread of the disease from kids to adult sheep. Nduaka and Ihemelandu (1973) also reported that PPR is contagious in the field. Mann *et al.* (1974) transmitted the disease from sheep to sheep and goats by using crude tissue material and tissue culture isolate from animals that died from Kata and SPC. Hamdy *et al.* (1976) transmitted the disease through two passages in sheep followed by one passage in cell culture then another one in sheep. Dardiri *et al.* (1976) reported that the transmission of the disease from reacting sheep to in contact cattle which develop lesions that would be diagnosed wrongly as RP.

2.4.4.2.1 Risk factors

It is important to identify and quantify the different risk factors contributing to the spread of PPR and the emergence of new epizootic outbreaks.

In endemic areas, livestock rearing and herd management practices increase the risk of PPRV circulating. This is the case when herds are mixed, combining animals with different levels of viral susceptibility. Large herds also are environments with a high risk of PPRV. It also is the case when the movement and gathering of animals promote frequent and repeated contact between animals with unknown disease status (FAO, 2009; Libeau *et al.*, 2011).

2.5 Clinical Signs

The pathogenesis of PPR is starts after the entry of the virus through the respiratory system, then it localizes first in the pharyngeal and mandibular lymph nodes and tonsils. Subsequent viremia results in dissemination to visceral lymph nodes, spleen, bone marrow and the mucosa of the gastrointestinal and the respiratory systems. The virus is having affinity for lymphoid and epithelial tissues of gastrointestinal and respiratory tracts in which it produces lesions (Scott, 1981).

The disease has peracute, acute and subacute syndromes (Losos, 1986). In the majority of cases, PPR is an acute disease. The clinical signs in sheep are the same as in goats but generally less severe (Elhag Ali, 1973; Durojaiye, 1983; Shaila *et al.,* 1996). PPR is characterized by pyrexia, catarrhal inflammation of the ocular and nasal mucous membranes, erosive stomatitis, conjunctivitis, gastroenteritis and pneumonia (Taylor, 1984).

2.5.1 Peracute Syndrome

A peracute form occurs frequently in sheep after an incubation period of about 2 days and is characterized by fever, sudden death and high mortality. There is profuse nasalcatarrh, high fever, congested and eroded mucous membranes, dyspnea, anorexia and constipation. The diarrhoea starts after about 3 days of onset of clinical symptoms and death occur 4-6 days after illness (Sharma and Adlakha, 1994).

2.5.2 Acute Syndrome

In the majority of cases, PPR is an acute disease which appears after an incubation period of 2 to 6 days after natural infection of the virus (Roeder and Obi, 1999; Diallo, 2000; Defra, 2001; Diallo, 2004). The course of the disease takes 5-6 days which may terminates in death (Braide, 1981; Taylor, 1984; Lefevre and Diallo, 1990; Sharma and Adlakha, 1994).

The onset of illness was manifested by pyrexia with a rectal temperature that reached 40-41ºC (104-106ºC). The peak of temperature remaining for 3-8 days before slowly returning to normal (Sharma and Adlakha, 1994; Saliki, 1998; Roeder and Obi,1999; Diallo, 2000; Diallo, 2004). Deaths usually occur during the late stages of fever when temperature drops below normal. With the onset of fever the animal becomes anorexic,

develops a dry muzzle and dull coat, restless and severely depressed (Aiello and Mays, 1998; Saliki, 1998). Also there is severe leukopenia in goats (Whitney *et al.,* 1967; Nduaka and Ihemelandu, 1973; Obi and Oduye, 1985). A highly characteristic syndrome begins with inflammation and erosions of the mucous membranes lining the upper respiratory, upper alimentary and urogenital tracts 1-2 days after onset of fever (Hamdy *et al.,* 1976). This was accompanied by serous oculonasal discharges which persisted for 2-7 days and progressively becomes mucopurulent (Whitney *et al.,* 1967; Nduaka and Ihemelandu, 1973; Saliki, 1998). The exudates developed an extensive encrustation at the lip commissures and consisted of brown scab material covering patchy erosions (Whitney *et al.,* 1967; Nduaka and Ihemelandu, 1973; Hamdy *et al.,* 1976; Losos, 1986). The exudates can crust over blocking the nostrils and causing respiratory distress and give a putrid odor to the breath (Aiello and Mays, 1998). Conjunctivitis with discharges from the eyes causes matting of the eyelids (Nduaka and Ihemelandu, 1973; Roeder and Obi, 1999). Most animals develop severe profuse non haemorrhagic diarrhoea 2-3 days after the development of mouth lesions. Diarrhoea is usually accompanied by severe dehydration, emaciation, hypothermia and death within 5-10 days (Nduaka and Ihemelandu, 1973; Ezeokoli *et al.,* 1986; Saliki, 1998). Pneumonia, coughing, pleural rales and abdominal breathing also occur (Diallo, 2000; Diallo, 2004). Bronchopneumonia, evidenced bycoughing is a common feature (Saliki, 1998). Necrotic stomatitis affects the lower lip and gum and it may involve the dental pad, hard palate, cheeks with their papillae and the tongue (Saliki, 1998; Roeder and Obi, 1999). A common feature in the later stages of the disease is the formation of small nodular lesions in the skin on the outside of the lips around the muzzle. These lesions cause confusion because of their similarity to the symptoms of primary contagious ecthyma or sheep and goat pox (Roeder and Obi, 1999; Diallo, 2000; Diallo, 2004). The clinical picture may be complicated by secondary latent infection (Ugochukwu, 1985). During the recovery of the disease, orf-like labial lesions develop (Obi and Gibbs, 1978; Losos, 1986). Recovered animals do not appear to be carrier. PPR causes abortion in pregnant animals (Nduaka and Ihemelandu, 1973); also vulvovaginitis has been reported as one of the lesions of PPR (Wosu, 1992). Erosive lesions have been recorded in the vulva (Ezeokoli *et al.,* 1986) producing abortion and also keratitis with corneal opacity (Diallo, 2003). PPR does not appear to qualify as a reproductive disease (Ezeibe and Wosu, 1999) because it does not cause important structural damage to the female reproductive system. All the goats that recovered from

PPR carried pregnancy successfully. Therefore abortion commonly reported in PPR may be due more to general debility (Ezeibe and Wosu, 1999).

The prognosis of acute PPR is usually poor, especially when lesions do not resolve within 2-3 days or when extensive necrosis and bacterial infection give the animals breath an unpleasant fetid odor (Aiello and Mays, 1998). Morbidity is often as high as 100% and mortality can be up to 90% in the most severe outbreaks (Losos, 1986). In milder outbreaks morbidity is still high; however the mortality rate may be closer to 50% (Diallo, 2000; Diallo, 2004).

2.5.3 Subacute Syndrome

Sheep and less frequently goats develop a subacute infection after a longer incubation period about 6 days and a longer disease course (10-15 days). The disease is manifested by slight fever, nasal catarrh, recurring erosions of the oral mucosa, respiratory distress and intermittent diarrhoea. Recovery often follows after 10-14 days (Sharma and Adlakha, 1994; Aiello and Mays, 1998).

Peracute and most acute infections are fatal, death occurring 4-10 days after the onset of illness (Defra, 2001). Convalescence in survivors is prolonged and often complicated by activated latent infections or super infections such as trypanosomiasis, dermatoplilosis and orf (Defra, 2001). The isolation of pasteurella species and *E. coli* from cases of PPR (Ezeokoli *et al.,* 1986) supports the suggestion by other investigators that bacterial pathogens may be involved in the pathogenesis of PPR (Rowland *et al.,* 1971; Nawathe, 1980; Adetosoye and Ojo, 1983; Isitor *et al.,* 1984).

Severity depends upon the susceptibility of the population. Goats are generally more susceptible to PPR than sheep. Infection rates in sheep and goats rise with age, and the disease which varies in severity, is rapidly fatal in young animals (Taylor, 1979; Obi,1982; Lefevre and Diallo, 1990; Wosu, 1994; Ozkul *et al.,* 2002). Young animals 4-8 months of age often have more severe disease (Ozkul *et al.,* 2002). Morbidity and mortality rates are higher in young animals than in adult (Nduaka and Ihemeland, 1973). Also, poor nutrition, stress of movement, and concurrent parasitic and bacterial infections worsen the clinical signs (Saliki, 1998). Although PPR infections occur under all forms of husbandry conditions, the disease produces the highest morbidity and mortality when large numbers

of goats or sheep are reared together or following the introduction of new animals into established herds (Braide, 1981; Scott, 1981; Obi *et al.,* 1983; Reoder and Obi, 1999).

2.6 Pathogenesis of PPR

The virus contaminates the animals through their oral and nasal passages. After entering into the organism, it multiplies first in the oropharynx and local lymphoid tissues. All of the immune cells (lymphocytes, macrophages, reticular cells) can be a target for virus multiplication. The newly formed virions spread throughout the host's organs and tissues with a preference for digestive, pulmonary, and respiratory mucosa and the immune system.

The resulting tissue damage, which can be observed post-mortem, is responsible for the clinical manifestations of the disease: discharge, lacrimation, diarrhea and causes.

2.7 Pathology

The principal pathological findings of PPR were confined to the alimentary and respiratory tracts (Losos, 1986; Barker *et al.,* 1993). Necropsy findings in PPR were characterized by mucosal erosions, haemorrhagic gastroenteritis and pneumonia (Rowland *et al.,* 1969). In general animals were emaciated and severely dehydrated (Whitney *et al.,* 1967; Toplu, 2004). The perineum and posterior aspects of the hind limbs were soiled with watery, sometimes blood-tinged, faeces (Whitney *et al.,* 1967). The lips were oedematous and their margins showed a progressive accumulation of golden-brown scab material particularly at the commissures (Whitney *et al.,* 1967). In the digestive system, there is usually severe erosions, necrotic stomatitis and enterocolitis (Scott, 1990). In the buccal cavity erosive areas measuring 3 to 5 mm in diameter were present in mucous membranes of the upper and lower lip, the cheek, the dental pad, hard and soft palate and severe lesions occurred in the pharynx and may extended to the oesophagus (Whitney *et al.,* 1967; Isoun and Mann, 1972; Nduaka and Ihemelandu, 1973).

Gross changes in glandular mucosa of the alimentary tract from abomasum to rectum were often inconspicuous (Rowland *et al.,* 1969). The digestive tract was usually characterized by erosions of mucosa of various parts (Nduaka and Ihemelandu, 1973). In rumen, reticulum and omasum, no abnormalitis were detected except of a single ruminal ulcer. The abomasum was oedematous, congested and showed severe haemorrhagic

inflammation and filled with foetid watery fluid (Whitney *et al.,* 1967; Rowland *et al.,* 1969; Nduaka and Ihemelandu, 1973). Also haemorrhage and ulcer were observed in the pylorus (Whitney *et al.,* 1967; Rowland *et al.,* 1969).

The contents of small intestine were limited, consisting of a bile-tinged watery fluid. In the mucosa of terminal ileum there was diffuse erythma (Whitney *et al.,* 1967; Nduaka and Ihemelandu, 1973). Payer's patches were enlarged and prominent. The duodenum showed evidence of severe inflammation (Nduaka and Ihemelandu, 1973). The ileo-cecal orifice was characterized by haemorrhagic ring around the orifice (Nduaka and Ihemelandu, 1973). In the large intestine, the contents were fluid (Rowland *et al.,* 1969). The changes, which were variable in their severity and distribution, were dramatic with "zebra-striping" of the caecum, colon and rectum (Whitney *et al.,* 1967). They were seen constantly at the caeco-colic junction which was enlarged and haemorrhagic. The caecum, colon and rectum often showed evidence of linear haemorrhages. In the rectum there were areas of ulcerations (Whitney *et al.,* 1967; Nduaka and Ihemelandu, 1973).

There was considerable variation in the gross lesions in the respiratory tract, depending on the duration of the lesions. In the nasal cavity there was an intense congestion, petechial haemorrhage and ulcers on the mucosa (Whitney *et al.,* 1967). The lungs were congested and emphysematous (Whitney *et al.,* 1967). In the acute stages of the disease, there was tracheitis which was characterized by haemorrhagic to frothy mucopurulent exudate in the trachea and bronchi (Nduaka and Ihemelandu, 1973). Pneumonia was usually observed in a few lung lobes. The one most commonly involvedwas the right apical lobe while the intermediate and cardiac lobes were involved less often (Nduaka and Ihemelandu, 1973). Bronchopneumonia with diffuse consolidation of the apical, cardiac and diaphragmatic lobes were observed (Isoun and Mann, 1972; Nduaka and Ihemelandu, 1973).

The lymphoid tissue showed little evidence of involvement (Rowland *et al.,* 1969). The spleen was congested and contracted (Whitney *et al.,* 1967; Aruni *et al.,* 1998). The lymph nodes were also congested, oedematous and slightly enlarged (Aruni *et al.,* 1998; Toplu, 2004). The carcass lymph nodes were swollen and oedematous (Whitney *et al.,* 1967).

The liver was pale and some times friable and the cut surface showed tiny, whitish- grey necrotic foci (Toplu, 2004).

No abnormalitis were observed in the urogenital system other than peticial haemorrhage or diffuse erythema of the mucosa of the bladder (Whitney *et al.,* 1967). There was no evidence, clinically or pathologically, of involvement of the central nervous system, skeletal muscle, skin and feet (Rowland *et al.,* 1969).

2.8 Diagnosis of PPR

2.8.1 Clinical Diagnosis

Clinical diagnosis is based mainly on the clinical signs and the post mortem lesions observed. However, since PPR is within the list A of the Office International des Epizooties (OIE), this diagnosis should be considered provisional until laboratory confirmation, particularly in the case of epidemic or new area (Diallo, 2003). This confirmation is important because PPR can be confused clinically with many other small ruminant diseases (Diallo, 2003).

2.8.2 Laboratory Diagnosis

2.8.2.1 Samples Required for Diagnosis

From live animals: swabs of the conjunctival discharges, and from the nasal, buccal and rectal mucosa should be taken. During the early stage of the disease, whole blood isalso collected in anticoagulant for virus isolation, PCR and haematology. Samples should be taken from animals with high body temperatures preferably before diarrhoea starts. Samples should also be collected aseptically, chilled on ice and transported under refrigeration.

At necropsy: lymph nodes especially the mesenteric and bronchial nodes, lungs, spleen and intestinal mucosa should be collected. Fragments of organs collected for histopathology are placed in 10% formalin. At the end of the outbreak blood can be collected for serological diagnosis (Diallo, 2000; Diallo, 2004).

2.8.2.2 Antigen Detection Methods

2.8.2.2.1 Agar Gel Immunodiffusion Test (AGID)

Agar gel immunodiffusion is the most frequently used technique for the detection of PPRV and RPV antigen (Appel *et al.,* 1981). It is a very simple, rapid, cheap and reliable

test that can be performed in any laboratory and even in the field (Obi and Patrick, 1984; Adu and Joannis, 1985; Diallo, 2004). The apparatus required are minimal and the reagents–except for standard antigen and anti PPR hyperimmune serum– can be readily prepared(Scott and Brown, 1961; Joshi *et al.,* 1972; Diallo, 2000; Diallo, 2004).

They found that the most suitable organs used are lymph nodes and the second best are the organs of gastrointestinal tract. Standard PPR viral antigen is prepared from mesenteric or bronchial lymph nodes, spleen or lung material and ground up as 1/3 suspensions in buffered saline and centrifuged at 500g for 10-20 minutes. Then the supernatant fluids are stored in aliquots at -20ºC and retained for 1-3 years (Diallo, 2000; Diallo, 2004). Control antigen is prepared similarly from normal tissues. Standard antiserum is made by hyperimmunizing sheep with 5 ml of PPR virus with a titer of 10 4

TCID 50 (50% tissue culture infective dose) per ml given at weekly intervals for four weeks (Diallo, 2000; Diallo, 2004). The success of the test depends largely on the preparation of potent specific hyperimmune serum (Adu and Joannis, 1985). Standard RP hyperimmune antiserum is also effective in detecting PPR antigen. Results are obtained in one day but the test is not sensitive enough to detect mild forms of PPR due to the low quantity of viral antigen that is excreted (Diallo, 2000; Diallo, 2004). The results of the test are thus meaningful only if they are positive and the negative results do not confound the provisional diagnosis (Scott, 1967). PPR antigen was detected in samples collected from goats which died or were slaughtered within nine days of the onset of fever (Obi and Patrick, 1984). They suggested that the optimal time of sampling for AGPT for PPR diagnosis would be within the first seven days of fever. PPR antigen was detected from 42.6% of ante-mortem secretions and necropsy samples and 38.2% of the post-mortem samples. It was found that about 54.5% of the ocular and buccal and 63.6% of the nasal and faecal swabs were positive by AGPT (Obi and Patrick, 1984).

2.8.2.2.2 Counter Immunoelectrophoresis Test (CIEP)

Counter immuno electrophoresis is a useful, reliable and the most rapid test for PPR viral antigen detection (Durojaiye, 1983; Majiyagbe *et al.,* 1984). Whenever a PPR specific diagnostic reagent is developed, the CIEP is the test of choice. Results can be obtained within 2-3 hours (Majiyagbe *et al.,* 1980; Majiyagbe *et al.,* 1984). It is 8 to 16 times more sensitive than the AGID for detection of PPR virus antigen. Also Elhag Ali and Lees

(1979) and Rossiter and Mushi (1980) reported that the CIEP is a more rapid and sensitive procedure for detecting RP virus antigen. With the results that it can be employed for the detection of antigen as well as antibody (Majiyagbe *et al.,* 1984; Durojaiye and Taylor, 1984). Viral antigen was detected from 80.3% of ante-mortem secretions and necropsy samples and 84.8% of post-mortem samples (Obi and Patrick, 1984). CIEP detected viral antigen in many samples when collected up to 14 days after the onset of fever (Obi and Patrick, 1984). Lines of precipitation could be seen 30 min after connecting the CIEP test samples to a power source, and were completed after 45 min. It is important to note that both the AGID and the CIEP are group-specific and may not distinguish between PPR and RP infections (Obi and Patrick, 1984).

2.8.2.2.3 Haemagglutination Test (HA)

Haemagglutination test is an easy, cheap and effective method for PPRV diagnosis (Johnson and Ritchie, 1968). PPR virus like measles virus has haemagglutination properties (Wosu, 1985; Ramachandran *et al.,* 1993; Ezeibe *et al.,* 2004). The test was able to differentiate PPRV from RPV which has no haemagglutinating properties and unable to agglutinate RBCs (Huygelen, 1960). Wosu (1985) demonstrated HA test for PPRwith homogenate antigen from mesenteric lymph nodes using piglet erythrocytes which was confirmed with known specific anti-serum to PPR virus. HA is a simple laboratory confirmatory test for diagnosis of PPR in small ruminants with samples from live animals (Wosu, 1991). It consists of haemagglutination of ocular and nasal discharges of suspected animals with piglet red blood cells (RBCs). The test diagnoses positive cases of the disease and offers a quick, accurate technique for less sophisticated laboratories. It is useful in confirming and monitoring the disease in living animals, unlike most other techniques which require samples from dead or killed animals (Wosu, 1991). The HA test represents a quick, easy, specific and reliable confirmatory diagnostic test of PPR which clinicians may find useful against PPR (Wosu, 1991). Ezeibe *et al.* (2004) investigated the different conditions which affect haemagglutination by PPR virus, namely temperature, pH, HA time and the sensitivity of RBCs of different animal species. PPR agglutinates RBCs of pig, human group 'O', chicken and goat. Human group 'O' appears to be the most sensitive. Following human RBCs in sensitivity were chicken RBC, goat RBC, and pig RBC. Dog RBC was completely insensitive and gave no HA reaction. It was recommended that to make HA test standard, it should be performed with either human

groug 'O' or chicken RBCs. The diluent for the test should be phosphate buffered saline (PBS) of pH 6.8 and the test should be performed at 4ºC (Ezeibe *et al.,* 2004).

2.8.2.2.4 Immunofluorescent Antibody Test (IFAT)

The IFAT is simple and relatively quick, and has the advantage that facilities are available in most veterinary laboratories (Last *et al.,* 1994). The use of a specific monoclonal antibody (MAb) in the IFAT can rapidly confirm PPR virus infection and differentiate it from RP. PPR virus antigen was detected in conjunctival epithelial cells obtained from goats in the early or late stages of the disease by the use of a specific monoclonal antibody to PPRV in an immunofluorescent antibody test (IFAT). The IFAT technique detected PPR antigen in conjunctival smears from suspected cases of PPR collected from a field outbreak with 100% specificity (Sumption *et al.,* 1998). The IFAT was more sensitive than staining for syncytia in the detection of viral antigen. The two tests gave 63% and 40% positive results respectively. The highest detection rate was found in animals with conjunctivitis, lesions of the oral mucosa and diarrhoea. Conjunctival slides gave a positive fluorescence with anti-PPRV MAb in 12 of the 19 animals with suspected PPR. The viral inclusions had a bright apple-green fluorescence with smooth surfaces. These inclusions were found in the cytoplasm and nucleus of the epithelia-like cells and varied in size and number. Nuclear shadows were frequently observed with a peripheral ring of cytoplasmic fluorescence (Sumption *et al.,* 1998).

2.8.2.2.5 ELISA for Antigen Detection

2.8.2.2.5.1 Immunocapture ELISA (IC-ELISA)

An immunocapture ELISA using monoclonal antibodies (MAbs) directed against the nucleocapsid (N) protein were used to detect the N protein of PPRV or RPV (Libeau *et al.,* 1994). The IC-ELISA allows a rapid differential identification of PPR or RP viruses, and this is of great importance as the two diseases have a similar geographical distribution and may affect the same animal species (Diallo, 2000; Diallo, 2004). The N protein is captured by using a virus-specific mouse MAb and detected by another specific biotinylated MAb (Libeau *et al.,* 1994). For the assay, a combination of MAbs which are specific for non-overlapping domains of the N protein of RPV and PPRV were required for adequate specificity. The capture antibody was directed against a highly conserved domain in the N protein and the detection antibodies were directed against different epitopes which were

highly specific for each virus (Libeau *et al.,* 1994). The antigen capture ELISA is more rapid and specific test, for PPR antigen detection, than the AGID (Abraham and Berhan, 2001). The test is very sensitive and specific, it can detect 55 ng of semi-purified protein or 10 2.2 TCID 50/well for RPV and 10 ng or 10 0.6 TCID/well for PPRV (Libeau *et al.,* 1994). The test can be performed in one hour on pre-coated plates. It can detect antigen in supernatants from infected cells and in field specimens even after the samples were maintained at room temperature for a period of 7 days with no more than a 50% reduction in response (Libeau *et al.,* 1994). PPR antigen was still detected in caprine lung samples kept for 4 days at room temperature even after remarkable physical deterioration of the tissue (Abraham and Berhan, 2001). The sensitivity of the IC-ELISA is equivalent to that of other techniques such as immunoperoxidase staining (Wamwayi *et al.,* 1991) but the results can be obtained more quickly. IC-ELISA test has been proved to be very simple and efficient for the diagnosis of PPR infection under field conditions (Libeau *et al.,* 1994).

2.8.2.2.5.2 Sandwich ELISA (S-ELISA)

PPR virus-specific neutralising MAb was used in a simple and rapid double- antibody Sandwich ELISA for specific detection of PPRV antigen in goat tissues and secretions (Saliki *et al.,* 1994). The double-antibody S-ELISA relies on the ability of antibody immobilized on the surface of microtiter wells to bind with antigen in a test sample. The captured antigen is then detected with a second antibody that is specific for the target antigen. Three MAbs (B2G3, B2G6 and B9G3) were designated for recognition of three nonoverlapping epitopes on the heamagglutinin protein of PPRV (Saliki *et al.,* 1994). The S-ELISA procedure can be completed in less than 7 hours compared with virus isolation and identification which takes at least 6 days to complete. The simplicity and rapidity of S-ELISA and IC-ELISA make them attractive alternatives to long, expensive and often insensitive virus isolation procedure (Saliki *et al.,* 1994). Singh and co-workers (2004) described a Sandwich ELISA test using PPR specific MAb (clone4G6) to N protein. They reported that the newly developed ELISA is suitable for PPR diagnosis under field conditions. Results can be made available within 4 hours and even earlier on antibody-coated plates. The technique which is simple, convenient, rapid and cost-effective is preferred for intensive clinical surveillance and routine diagnosis of the disease (Singh *et al.,* 2004). The antigen was captured from clinical samples by using polyclonal antisera

raised against lapinized RPV. The detection antibody is an antinucleocapsid (N) protein MAb (4G6) raised against PPRV. PPR antigen could be detected in ocular and nasal secretions between the 7^{th}-13^{th} days post infection. The S-ELISA developed could successfully detect PPR antigen in clinical material stored for more than 6 months at -20ºC (Singh *et al.,* 2004). The 4G6 S-ELISA was found to be 92.8% specific and 88.9% sensitive as compared to the IC-ELISA which was 94.8% specific and 85.1% sensitive (Singh *et al.,* 2004).

2.8.2.2.6 Virus RNA detection

2.8.2.2.6.1 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

The reverse-transcriptase polymerase chain reaction (RT-PCR) provides a method for the differential diagnosis of PPR from RP; it also produces DNA suitable for gene sequence analysis (Forsyth and Barrett, 1995). It is the most sensitive test for the specific detection of PPRV and RPV (Forsyth and Barrett, 1995; Couacy-Hymann *et al.,* 2007). This technique is very sensitive compared with other tests and results are obtained in 5 hours, including the RNA extraction (Diallo, 2000; Diallo, 2004). PCR was found 1000- fold more sensitive when compared with the titration technique on Vero cells. The viral RNA can be purified from whole blood, lymph node and tonsil, eye and mouth swabs, spleen and lung (Forsyth and Barrett, 1995). RNA is extracted using acid guanidinium thiocyanate-phenol-chloroform method. The cDNA synthesis is carried out using random hexanucleotide primers. Aliquots of the resulting cDNA are amplified using at least 3 primer sets that can detect and differentiate between the two viruses. These primer sets include two 'universal' sets based on highly conserved regions in the phosphoprotein and nucleoprotein genes that should detect all morbilliviruses, and RPV-specific and PPRV-specific sets based on sequences in the fusion protein genes of each virus (Forsyth and Barrett, 1995). The PCR products are analyzed on a 1.5% agarose gel along with a suitable DNA marker to identify the specific DNA product. Positive reaction should be confirmed either by using 'nested' primer sets based on the F gene sequences or by sequence analysis of the DNA product (Diallo, 2000; Diallo, 2004). Shaila *et al.* (1996) have reported the application of the RT-PCR to detect PPR RNA in pathological samples. To purify the RNA, they used the phenol-chloroform method which takes at least 3 hours to complete. Another RT-PCR is based on the rapid purification of RNA on glass powder. The PPRV

infected cell RNA was purified in about 30 minutes, the sample being incubated with the glass powder for 5 minutes (Couacy-Hymann *et al.,* 2007).

2.8.2.3 Virus Isolation and Identification

2.8.2.3.1 Virus Isolation

Even when diagnosis has been carried out by rapid techniques the PPRV should always be isolated from field samples in tissue cultures for further studies (Durojaiye *et al.,* 1983; Lefevre and Diallo, 1990). Virus isolation is done in primary foetal lamb or goat kidney (FLKC or GKC) cell culture (Gilbert and Monnier, 1962; Johnson and Ritchie, 1968; Taylor and Abegunde, 1979) or in Vero cells (Hamdy *et al.,* 1976; Durojaiye *et al.,* 1983; Diallo, 2000; Diallo, 2004). RPV has been cultivated in primary cultures of sheep, goat and chickembryo kidney and in primary and continuous cultures of cattle, pig, dog, hamster, monkey, rabbit and human (Plowright, 1968; Andrews and Pereira, 1972). It was established that monolayer cultures inoculated with suspected swab material, buffy coat from blood or 10% tissue suspension showed cytopathic effect (CPE) within 5-15 days (Diallo, 2000; Diallo, 2004). The CPE produced by PPRV was slow to develop (Gibbs *et al.,* 1979; Diallo, 2000; Diallo, 2004) however, Cover slip and cell suspension cultures showed the CPE more earlier and quicker (Johnson and Ritchie, 1968; Gibbs *et al.,* 1979; Diallo, 2000; Diallo, 2004). The CPE of PPRV in lamb or goat kidney cells consists of cell rounding and aggregation culminating in syncytia formation. Syncytia are recognized by a circular arrangement of nuclei giving a 'clock face' appearance. There are also intranuclear and intracytoplasmic inclusions while some cells are vacuolated (Johnson and Ritchie, 1968; Laurent, 1968; Hamdy and Dardiri, 1976; Gibbs *et al.,* 1979; Diallo, 2000, Diallo, 2004). Virus antigen appears first in the nucleus and later in the cytoplasm (liess, 1963). In Vero cells, it is difficult to see the cyncytia however when stained small cyncytia are always seen (Diallo, 2000; Diallo, 2004). It is recommended that blind passages should be carried out as CPE may take time to appear (Diallo, 2000; Diallo, 2004). The isolates of PPRV can be purified by three terminal passages in lamb kidney cells (Taylor and Abegunde, 1979) and then adapted to Vero cells with four further passages (Elhag Ali and Taylor, 1984).

2.8.2.3.2 Cross Neutralisation Test (NT)

Usually, cross neutralisation test is carried out with RP virus. PPR is normally differentiated from RP through reciprocal cross neutralisation (Taylor and Abegunde, 1979). Neutralisation of virus infectivity by specific antiserum is considered as an essential step in the identity of PPRV isolates (Scott *et al.,* 1986; Anderson *et al.,* 1996). Reciprocal cross-neutralisation test in Vero cells were used to test the antigenic relationship within groups of isolates (Taylor and Abegunde, 1979). Antisera to each virus showed more or less comparable antibody level to itself and to each of the other isolates and these results were constructed as evidence of homogeneity within the group (Taylor and Abegunde, 1979). Virus neutralisation test was also conducted for differentiating and confirming the presence of RP virus from PPR virus (Furley *et al.,* 1987; Taylor *et al.,* 1990; Lefevre and Diallo, 1990).). Reciprocal cross neutralisation is carried out in roller tube cultures of primary sheep kidney cells or Vero cells when primary cells are not available (Diallo, 2000; Diallo, 2004), using 4-fold serum dilutions mixed with an equal volume of virus containing 10 3.0 TCID/ml. The mixture was incubated overnight at 4ºC after which a 0.2 ml inoculum of it followed by 1 ml of cell suspension were added to the tubes (Elhag Ali and Taylor, 1984).

2.8.2.4 Electron Microscopy (E.M)

Electron microscopy technique was used for studying the morphology and ultrastructure of PPR virus particle (Bourdin and Laurent-Vautier, 1967; Durojaiye *et al.,* 1985). Vero cells infected with PPRV when examined by Electron microscope showed virus and virus-related morphological structures such as intranuclear and intracytoplasmic inclusion bodies (Hamdy *et al.,* 1976). Virus budding was observed at the plasma membrane of the infected cells and mature virions were seen extracellularly (Tajima *et al.,* 1964; Hamdy *et al.,* 1976).

12.8.2.5 Histopathology

Tissue samples were fixed in 10% neutral buffered formalin or in Bouins fluid, embedded in paraffin wax, sectioned at 5µm, and stained by routine methods with haematoxylin and eosin (H&E) (Rowland *et al.,* 1969; Rowland and Bourdin, 1970). Lesions of PPR in the upper alimentary tract develop within the stratum spinosum and granulosum. Affected cells show changes varying from vacculation to coagulation with nuclear pyknosis and

karyorrhexis. Syncytial aggregation also occurred. Inclusion bodies were observed, both intranuclear and intracytoplasmic in situation (Rowland *et al.,* 1969; Rowland and Bourdin, 1970; Rowland *et al.,* 1971). The respiratory tract showed mucosal necrosis and hyperplasia. Vaculation and coagulation of cell cytoplasm were present, together with pyknosis and karyorrhexis of nucleus (Rowland and Bourdin, 1970). In affected areas, the epithelial lining consisted of irregular cellular masses. Eosinophilic intranuclear and intracytoplasmic inclusion bodies were present. The lung parenchyma adjacent to affected bronchioles showed cellular infiltration (Rowland *et al.,* 1969; Rowland and Bourdin, 1970). The lymph reticular system follicles were indistinct and perifollicular lymphocytes absent. Reticulo-endothelial cells were often numerous in the sinuses and a number of the lymph nodes examined showed a scattering of pyknotic cells throughout the cortex with occasional areas of necrosis (Rowland *et al.,* 1969). In the liver there are small patches of fatty changes extending randomly across a number of lobules as a constant finding. There are foci of necrosis throughout the hepatic parenchyma (Rowland *et al.,* 1969; Rowland *et al.,* 1971). There were no abnormalities detected in the urogenital system, central nervous system, skin and skeletal muscle (Rowland *et al.,* 1969).

2.8.2.6 Serological Techniques

2.8.2.6.1 Agar Gel Diffusion Test (AGDT)

AGDT was used for the detection of antibodies against PPR in the sera of the affected goats (Durojaiye, 1982). This test is considered useful for field diagnosis of PPR. It provides a rapid serological diagnostic tool for PPR. Precipitating antibodies were detected in sera obtained in the acute phase of the disease and also in sera obtained at convalescence. Also sera which precipitated PPRV antigen did not precipitate RPV antigen (Durojaiye, 1982)

2.8.2.6.2 Precipitinogen Inhibition Test (P.I.T)

The principle of P.I.T is based on the ability of antibody in serum to inhibit diffusible virus antigen (precipitinogen) from developing a precipitin line against hyperimmune serum in AGPT. The P.I.T was found useful in the detection of PPR antibodies in convalescent sera (Durojaiye, 1987). P.I.T is therefore potentially useful in screening field sera for PPR antibodies. It was observed that this test is more sensitive (33%) as compared to NT (28%) (Durojaiye, 1987).

2.8.2.6.3 Virus Neutralisation Test (VNT)

VNT is the only available specific serological test for PPRV and RPV. Although cross virus neutralisation test can differentiate between antibodies to RP and PPR, it is laborious and difficult when the samples size is large. The test is sensitive and specific but it is time consuming (Diallo, 2000; Diallo, 2004). VN test using microplates were easy to perform and gave similar VN antibody titre for the two viruses with different cell types (Rossiter *et al.,* 1985). VN was described in sheep and goats sera for detection of PPR antibodies (Taylor, 1979) and for RP and PPR antibodies (Zwart and Rowe, 1966; Bourdin and Bernard, 1967; Obi *et al.,* 1984). There is some level of cross-neutralisation observed between antibodies against PPR and RP (Taylor, 1979; Obi *et al.,* 1984).

2.8.2.6.4 Haemagglutination Inhibition Test (HI)

Haemagglutination inhibition is a simple and rapid serological method described for PPRV specific antibody (Wosu and Ezeibe, 1992). The technique is based on adsorbing out the cross-reacting antibodies to RPV antigen from a PPR serum and leaving the specific antibodies to PPR which is determined by HI test. The adsorption technique could eliminate the cross-reactivity between PPR and RP and make a definitive diagnosis of the PPR specific antibody in a single test. HI technique eluciated the problem of cross-reactivity which exists in the diagnosis of PPR and RP (Wosu and Ezeibe, 1992). The HI test, especially in less sophisticated laboratories, is a very useful, quick and accurate method for determining the antibody levels in a given immune serum (Johnson, 1971).

2.8.2.6.5 ELISA for antibody detection

2.8.2.6.5.1 Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA)

A competitive ELISA based on a PPRV monoclonal antibodies specific for haemagglutinin (H) protein (Anderson *et al.,* 1991; Saliki *et al.,* 1993; Singh *et al.,* 2004) or nucleoprotein (N) (Libeau *et al.,* 1992; Libeau *et al.,* 1995; Choi *et al.,* 2003) was developed for detection of antibodies to PPRV in serum samples of sheep and goats. C-ELISA allowed rapid, simple, specific, sensitive and differential serodiagnosis of PPRV and RPV in sheep, goats and cattle (Anderson and Mckay, 1994). The C-ELISA involves the competition between the MAb and serum antibodies for a specific epitope on the N-B.

The C-ELISA is as sensitive and specific as the VNT with relative sensitivity of 94.5% and specificity of 99.4%. A good correlation (r = 0.94) was observed between VNT and C-ELISA (Libeau *et al.,* 1995). The C-ELISA is considered suitable for large scale testing due to its simplicity and availability of the recombinant antigen (Libeau *et al.,* 1995). C-ELISA uses MAb to a neutralizing epitope of H-protein of the virus having high relative specificity of 95.4% and sensitivity of 92.4% when compared with the VNT. It can detect successfully antibodies as early as 16-20 days and during 1 year after vaccination (Singh *et al.,* 2004). It is very convenient for large sampling frames. The C-ELISA test developed can easily replace VNT for sero-survillance, sero-monitoring following vaccination, diagnosis from paired sera samples and end-point titration of PPRV antibodies (Singh *et al.,* 2004).

2.8.2.6.5.2 Blocking ELISA (B-ELISA)

Blocking ELISA is proved to be simple, more rapid, sensitive and specific method for detection of PPR antibodies (Saliki *et al.,* 1993). Compared with the VNT, B-ELISA offers high levels of sensitivity (/90%) and specificity (/98%) besides decreasing the runtime from 7 days to less than 7 hours. Furthermore, unlike the VNT, B-ELISA may be less affected by the quality of sera such as cytotoxicity and contamination (Saliki *et al.,* 1993). The test depends on the blocking of the binding of the MAb to a specific epitope in the presence of positive serum. Serum is preincubated with a solid-phase PPRV antigen and then incubated with the MAb. B-ELISA proved to be nearly as sensitive and specific as the VNT while being simpler and more rapid. Thus it would be an adequate substitute for the VNT for assessing herd immune status and for epidemiological surveillance (Saliki *et al.,* 1993).

2.8.3 Differential Diagnosis

The differential diagnosis has to be made between PPR and diseases with stomatitis lesions, enteritis symptoms and respiratory distress (Adetosoye and Ojo, 1983; Elhag Ali and Taylor, 1984)In addition to Rinderpest, other conditions that should be considered in differential diagnosis include: Blue Tongue (BT), Pasteurellosis, Contagious Ecthyma (CE) or Orf, Foot and Mouth Disease (FMD), Heart Water, Coccidiosis, Sheep Pox, Contagious Caprine Pleuro-Pneumonia (CCPP), Plant or Mineral Poisoning, Nairobi

Sheep Disease, Salmonellosis and Gastrointestinal Helminth Infestations (Saliki, 1998; Roeder and Obi, 1999).

2.8.4 Differentiation between PPR & Rinderpest

As RP virus can cause clinical disease in small ruminants, however some times it is asymptomatic (Anderson *et al.,* 1990; Couacy-Hymann *et al.,* 1995). The differentiation of PPR from RP is often difficult in the field as the clinical signs are similar. In the laboratory, PPR is normally differentiated from RP through reciprocal cross-neutralisation (Taylor and Abegunde, 1979), reciprocal cross-protection (Hamdy *et al.,* 1975), Haemagglutination test (Wosu, 1985; Wosu, 1991), differences in the mobility of 'N' protein of PPRV and RPV in PAGE (Diallo *et al.,* 1987) and by monoclonal antibodies (MAb) directed against each protein of the virus (McCullough *et al.,* 1986). One way cross- netralisation test with RPV hyperimmune antiserum can aid in differentiation of these two related viruses (Chandran *et al.,* 1995). Similarly PPRV antibodies can be differentiated from RPV antibodies by competitive ELISA (Libeau *et al.,* 1992; Libeau *et al.,* 1995) and serum neutralisation test (Diallo *et al.,* 1995).

2.9 Excretion of PPR Virus

The virus is excreted by many routes once clinical signs appear and does not persist in the tissues of recovered animals (Losos, 1986). Infected animals shed the virus in expired air and in ocular and nasal discharges, saliva, urine, milk and semen at the onset of fever and in the faeces at the onset of diarrhoea (Johnson and Ritchie, 1968; Abegunde and Adu, 1977; Scott, 1981; Sharma and Adlakha, 1994). These secretions and excretions are infectious for at least seven days after the onset of the disease. Abegunde and Adu (1977) stated that large quantities of PPR virus were excreted by infected goats via the nasopharyngeal route. Also the quantity of virus excreted via all routes on the first day of clinical reaction was small. The high titres of the virus from the nasopharyngeal and faeces appear to originate from the organs which are most seriously affected during the course of the disease. The large intestinal mucosa and the lung generally have the highest titre of the virus (Adegunde and Adu, 1977). Virus from the oral cavity could have been derived from the buccal mucosa which has necrosis and erosions. Virus in the nasal cavities might have been derived from the nasal mucosa and nasopharynx. The titre of lung tissue in acute PPR was generally greater than 10 5.5 TCID/g (Abegunde and Adu, 1977)

2.10 Socio-Economic Impact of PPR

- Peste des petits ruminantsvirus has a widespread distribution spanning Africa and Asia (Nanda *et al.,* 1996; Shaila *et al.,* 1996). These areas encompass much of the developing world that relies heavily on subsistence farming to supply food or goods for trade, and 25 small ruminants provide an excellent supply of both. Unfortunately, in many areas of Asia and Africa, small ruminant production and therefore the livelihoods of poor farmers is threatened by PPR among other trans-boundary animal diseases (TADs). With its associated high morbidity and mortality, PPRV constitutes one of the major obstacles to subsistence farming (Banyard *et al.,* 2010).
- The socio-economic losses associated with PPR mainly result from the high mortality rate that is characteristic of the disease. This negatively affects income from production and value addition in small ruminants marketing chains. Peste des petits ruminants disease is a constraint to international trade, although this impact is mitigated in local and regional markets due to wide geographic distribution of the disease at present (Elsawalhy *et al.,* 2010).

2.11 Treatment

There is no treatment for PPR. However mortality rates may be decreased by the use of broad spectrum antibiotics and antiparasiticides which control bacterial and parasitic complications. Specifically, oxytetracycline and chlortetracycline are recommended to prevent secondary pulmonary infections (Diallo, 2000; Diallo, 2004).

Supportive care including fluid therapy can also decrease deaths loss due to dehydration and subsequent electrolyte imbalance (Wosu, 1989). Isoun and Mann (1972) found that antibiotic treatment of natural SPC in sheep was of limited value. They mentioned that pneumonia responded to Sulphadimidine and Terramycin to some extent, however, the diarrhoea and high body temperatures continue despite treatment.

Clinical cases of acute PPR can be adequately and successfully treated even in advanced cases particularly if treatment is started early (Omamegbe and Mecha, 1984). They suggested that a rapid lowering of the body temperature using anti-pyretic drugs and the suppression of coughing using antitussives enhance the chances of successful therapy. The arrest of the diarrhoea and replacement of the lost body fluids and ions would appear to be more important than the use of antibacterial agents. They mentioned that the use of broad

spectrum antibiotics, fluid therapy, gastro-intestinal sedatives, antipyretics, antitussive and good nursing resulted in a survival rate of 45%. Anene *et al.* (1987) demonstrated poor clinical response of naturally infected sheep treated with oxytetracycline, chloramphenicol 25% aqueous solution or metamerazine, thiabendazole, codeine and vitamin B complex.

Good feeding and nursing in warm draught-free pens are necessary. Wosu (1989) proved that scrubbing the orf-like labial scabs with lemon (Sour orange) fruit (Citrus oranitium) cut in half resulted in earlier healing than spraying with an iodine-antibiotic mixture. The combination of the lemon fruit treatment of mouth scabs with antibiotics and chemotherapy raised the survival rate of goats by 13.3% (Wosu, 1989). Sheep infected with pneumo- entritis syndrome were treated with norfloxacin together with oral and i/v administration of electrolytes and a liver detoxifying agent (Ayaz *et al.,* 1997). This method raises the survival rate by 20%.Adu and Joannis (1984) proved that sheep given hyperimmune serum and virulent PPR virus simultaneously developed a durable immunity without noticed clinical signs.

2.12 Immunity to PPRV

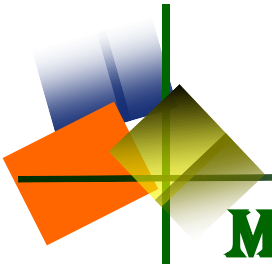
Sheep and goats that recover from PPR develop an active immunity against the disease and resist infection with PPR (Sharma and Adlakha, 1994). Antibodies have been demonstrated for four years after infection (Durojaiye, 1984; Durojaiye and Taylor, 1984), however, immunity is lifelong (Sharma and Adlakha, 1994; Diallo, 2000; Diallo, 2004). Young animals from dams with previous history of PPR are protected up to 3-4 months of age by maternal antibodies (Ata *et al.,* 1989; Bidjeh *et al.,* 1999). Clostral immunity protects kids and lambs until they are weaned (Sharma and Adlakha, 1994). Therefore, the age of three months should be considered a suitable and optimum time for effective immunization of small ruminants against PPR. The presence of high level of maternal antibodies has an immunosuppressive effect on the immune system of neonates and would interfere with the degree of immunologic response to active immunization (Trautwein, 1980 cited by Ata *et al.,* 1989). The duration of passive immunity is 120 days as estimated by the VNT compared to 90 days by C-ELISA (Libeau *et al.,* 1995). There were no differences in the length of maternal immunity in dams vaccinated with TCRP vaccine between 0 and 2 months and those vaccinated at 5 months (Bidjeh *et al.,* 1999). Sheep vaccinated with the attenuated RBOK strain of RP virus did not develop clinical disease when infected with PPR virus (Gibbs *et al.,* 1979). The Schwarz vaccine strain of measles

virus did not protect sheep against PPR virus while canine distemper virus did have some cross-protection (Gibbs *et al.,* 1979; Losos, 1986). The challenge PPR virus was found to multiply in those animals which had been immunized with RP, CD or measles but not in animals recovered from PPR (Gibbs *et al.,* 1979).

2.13 Control of PPR outbreaks

Control of PPR outbreaks relies on movement control (quarantine) combined with the use of focused 'ring' vaccination and prophylactic immunization in high risk of animal population including proper disposal of carcasses and contact fomites beside decontamination (Roeder and Obi, 1999). Peste des petits ruminants infection is mostly a result of introduction of infected animals into a herd. Thus the restriction of animal importation from affected areas is a very important part in the face of an epidemic and in prevention (Roeder and Obi, 1999). The only effective way to control PPR in endemic areas is by vaccination of the animals. Rinderpest vaccine have been used as a heterologous vaccine to protect small ruminants against PPR (Bourdin *et al.,* 1970; Bourdin, 1973; Nduaka and Ihemelandu, 1975). The attenuated RP vaccine provides protection of sheep for at least one year possibly through an antibody-mediated immune response (Taylor, 1979; Bourdin *et al.,* 1970). Nduaka and Ihemelandu (1975) successfully controlled pneumonia-enteritis complex by the use of chloroform-inactivated RPTC vaccine. This vaccine immunized sheep for 18 months (Mariner *et al.,* 1993).

Recently, a very efficient PPR homologous vaccine was developed with the attenuated PPRV (75/1 isolate (PPR 75/1 LK6 BK2 Vero70) (Diallo *et al.,* 1989). The homologous vaccine provides a life long immunity against PPR which extended for 3 years (Roeder and Obi, 1999). It was also able to protect goats against virulent RP virus (Couacy-Hymann *et al.,* 1995). The attenuated freeze-dried PPR vaccine is thermolabile which needed preservation at -20ºC. Martrenchar *et al.* (1999) evaluated the use of attenuated PPR vaccine in the field and proved its effectivity in vaccinating animals. In Bangladesh, a homologous PPR vaccine was produced successfully and used in the field and for exporting animals.



Chapter 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The research work was performed in the Pathology and Microbiology laboratory, Faculty of Veterinary and Animal Science, Hajee Mohammed Danesh University of Science and Technology (HSTU), Dinajpur for a period of 6 months since January to June 2018.

The major works of the present research includes-

1. Epidemiological survey.
2. Detect the PPRV antibodies in the serum sample of sheep by using ID.vet cELISA kit.

3.1 Study area

A cross-sectional study was undertaken in four different upazila in Dinajpur district namely Chirirbandar, Parbatipur, Birol and Sadar.

3.2 Study population

A total of 66 blood samples of sheep were collected randomly according to the animal's area, age, sex, lactation stage and parity.

3.3 Experimental layouts

Field survey was carried out and selected randomly at four upazila in Dinajpur district. The blood samples were collected directly from jugular vein by venipuncture method from sheep having no pervious history of PPR vaccination by using sterile 5 ml syringe. Then collected samples were kept for 1-2 hours in slightly inclined position after that sera were separated from blood in sterile eppendorf tube then samples were placed in ice box and transported to the Pathology lab in HSTU, Dinajpur. After that the serum samples were subjected to competitive enzyme-linked immunosorbent assay (cELISA) for the detection and measurement of antibody level against the PPR virus in the sera. Ultimately the value was calculated optical density (OD) that was taken from the cELISA plate reader machine. During the survey questionnaire was collected about the hygienic and management practice, diagnostic history, clinical signs of the animal and then finally data recorded and analyzed.

Layout of the experiment

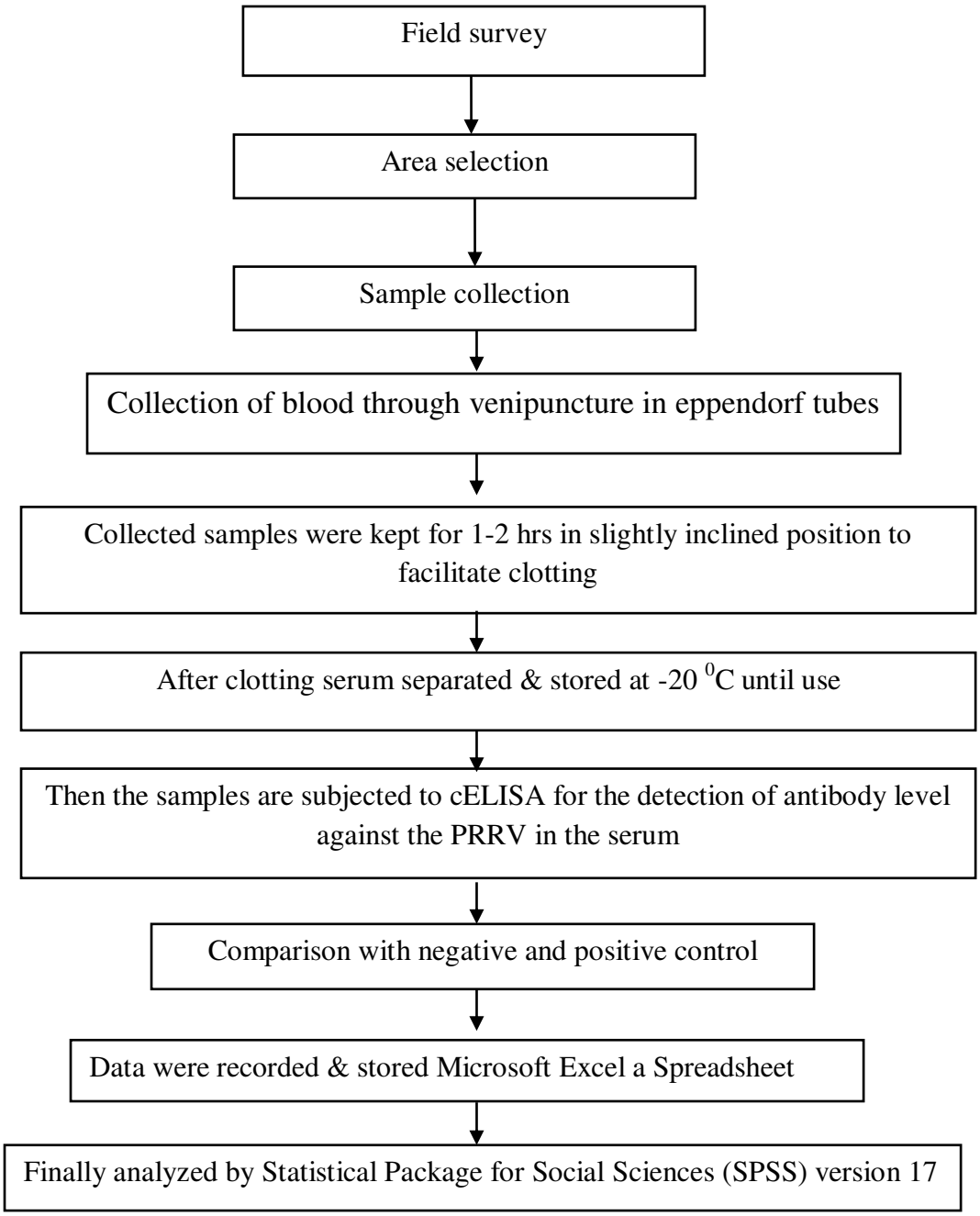


Figure 2: Schematic representation for experimental lay out

3.4 Description and Principle

The wells are coated with purified recombinant PPR nucleoprotein (NP).

The tested samples and the controls are added to the microwells. Anti-NP antibodies, if present form antibody-antigen complex which masks the NP epitopes. An anti-NP-peroxidase (HRP) conjugate is added to the microwells. It fixes to the remaining free NP epitopes, forming an antigen-conjugate-HRP complex. After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested:

- In the absence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution.
- In the presence of antibodies, no coloration appears.

The microplate is read at 450nm.

3.5 Equipments, Instruments and Reagents

3.5.1 Appliances used for blood collection

- Sterile Syringes (Vol. 5 ml)
- Ice box
- Cotton
- Hand gloves and Marker pen
- Scissors

3.5.2 Material required used for ELISA test

- ELISA plate Reader,
- ELISA Kit, Manufactured by ID. Vet, CIRAD-EMVT, Montpellier, France.
- Mono or multi-channel Micropipettes capable of delivering volumes of 10µl, 100µl, 200µl and Disposable tips of varying categories.
- Distilled water
- Foil paper
- Falcon tube

- Beaker (500 ml), Racks
- Eppendorf tubes
- Cotton and Tissue paper
- Waste disposal container
- Wash system

3.5.3 Chemicals and reagents used for cELISA

- Microplate coated with recombinant nucleoprotein
- Anti-NP-HRP concentrated conjugate (10X)
- Positive control
- Negative control
- Dilution buffer 4
- Dilution buffer 13
- Wash concentrate (20X)
- Substrate solution
- Stopping solution (0.5M)

3.6 Antibody detection by cELISA

3.6.1 ELISA kit

Serum sample were applied cELISA kit manufactured by ID.vet Innovative Diagnostics, CIRAD-EMVT, Montpellier, France. to detect of anti-PPRV nucleoprotein antibodies in sheep serum.

Figure 3: ELISA kit

3.6.2 Reagent Preparation

Preparation of Wash Solution

300µl Wash solution (1X) was prepared by diluting the wash concentrate (20X) in double distilled water as per follows

- ✓ Double distilled water : 290 µl
- ✓ Wash solution : 10 µl

Preparation of Conjugate

10 µl Conjugate (1X) was prepared by diluting Conjugate (10X) in Dilution Buffer 4 as per follows

- ✓ Dilution buffer 4 : 10 µl
- ✓ Concentrate conjugate: 1 µl

3.6.3 Sample preparation

96-well plate were added sample test and control sample, also avoided in different incubation time then transferred them into an ELISA microplate that was used multi-channel pipettes

3.6.4 ELISA Plate Layouts

1. **Positive control (Pc):** Wells A1, B1, was the positive control. They contain dilution buffer 13, positive control, conjugate, substrate and stop solution.
2. **Negative Control (Nc):** Wells C1, D1 was the negative control, they contain dilution buffer 13, negative control, conjugate, substrate and stop solution.
3. **Serum Sample:** 66 tested sera were added in the remaining wells (A3 to H12). They contain dilution buffer 13, conjugate, substrate and stop solution.

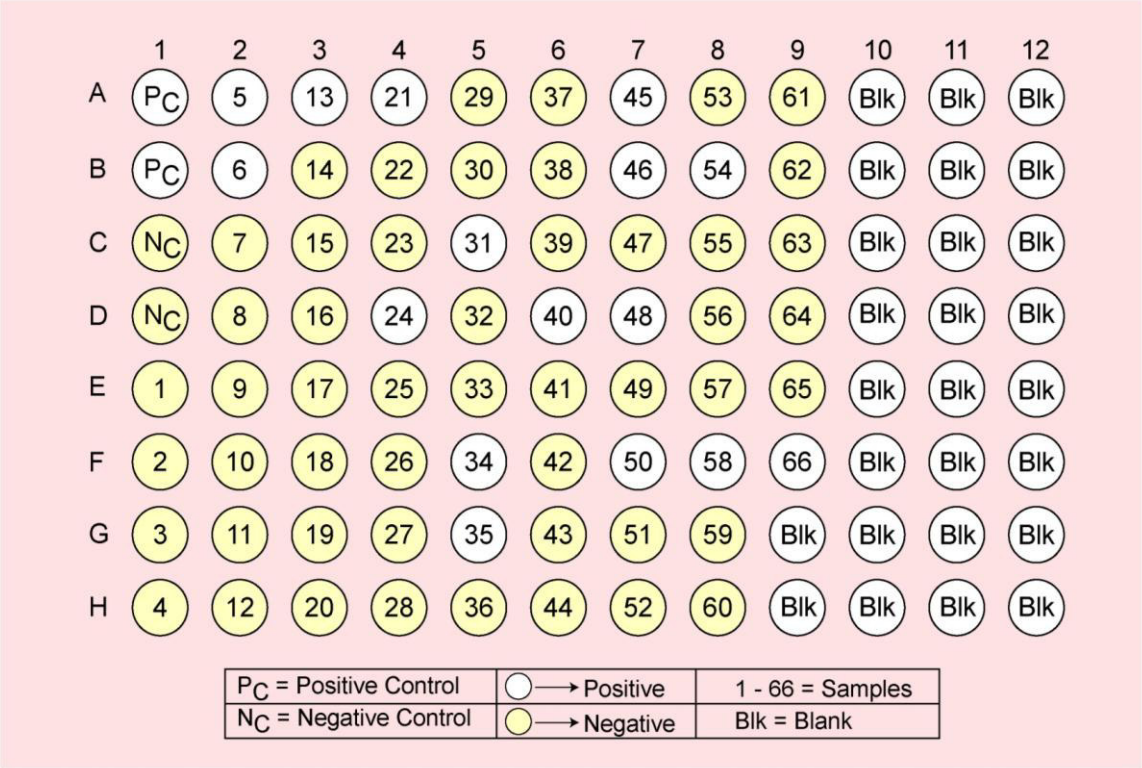


Figure 4: Layouts of ELISA kit

3.6.5 ELISA Testing Procedure

Allowed all the reagents to come at room temperature (21^0 C ± 5^0 C) before used and homogenized all the reagents by inversion or Vortex.

1. Added:
    - 25 µl of **Dilution Buffer 13** were added to each well.
    - 25 µl of the **Positive Control** were added to wells A1and B1.
    - 25 µl of **Negative control** were added to wells C1 and D1.
    - 25 µl of each sample were tested to the remained wells.
2. Incubated **45 min ± 4 min** at 37 ^{0}C (**±** 3 ^{0}C).
3. Three times were washed each well with a proximately 300µl of the **washing solution.** Avoid drying of the well between washings.
4. 100 µl of the **Conjugate 1X** were added to each well.
5. Incubated **30 min ± 3 min** at 21 ^{0}C (**± 5**^{o}C).
6. Three times were washed each well with a proximately 300µl of the **washing solution.** Avoid drying of the well between washings.
7. 100 µl of the **Substrate solution** were added to each well**.**

8. Incubated **15 min ± 2 min** at 21 ^{0}C (± 5^{o}C) in the dark.
9. 100 µl of the **Stop solution** were added to each well in order to stop the reaction.
10. Read and recorded the O.D at 450 nm.

3.6.6 Calculation of the result

For each sample were calculated by the competition percentage (S/N %).

$$S/N\ \% = \frac{ODsample}{ODNc} X100$$

Samples were presented S/N %:

- Less than or equal to ≤ 50% were considered Positive,
- Greater than 50 % and less than or equal to 60 % (50-% < SN % ≤ 60 %) were considered doubtful,
- Greater than 60 % (SN % > 60%) were considered Negative.

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

3.7 Methods

3.7.1 Field survey

A field survey was carried out and questionnaire was used to collect the data about the risk factors associated with the PPRV disease outbreak from the households.

3.7.2 Blood Collection and Serum separation

Blood samples were collected from jugular vein of goats through venipuncture method by using sterile 5 ml syringe and test tube without any anticoagulant .Collected samples were kept at least 1 hour at room temperature in a slightly inclined position to facilitate clotting and separation of serum. Then clotted blood samples with sera were transferred to

refrigerator at 4°C and kept overnight. After this period, the collected sera were decanted into eppendorf tubes then these sera were stored at -20°C until use.

3.8 Data analysis

Data were recorded and stored in Microsoft Excel a spreadsheet and the collected data were analyzed with Statistical Package for Social Sciences (SPSS) version 17.

Figure 5: PPRV reagent kit component supplied by ID.vet developed by CIRAD

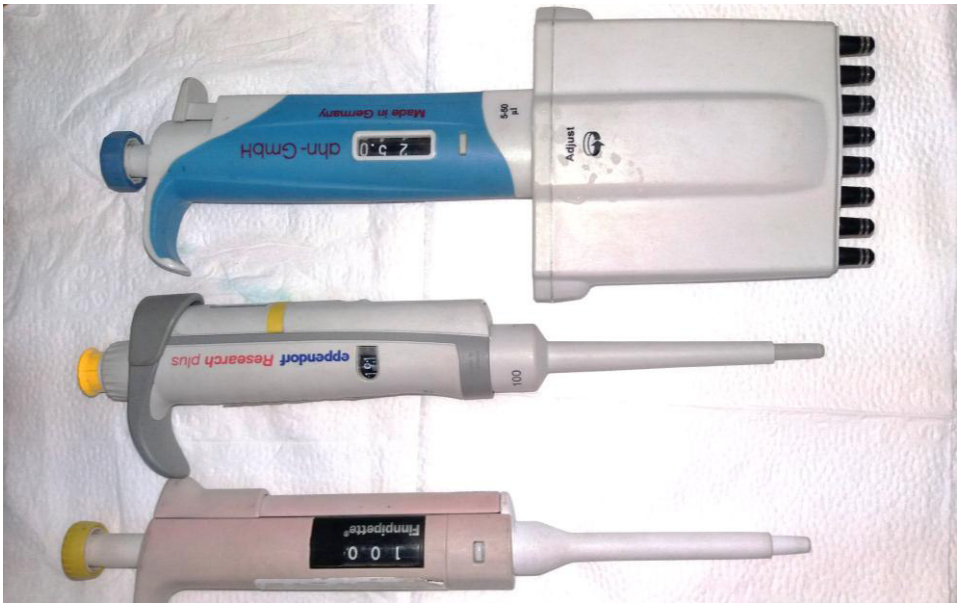


Figure 6: Different types of micropipettes

Figure 7: Collection of blood

Figure 8: Serum Seperation

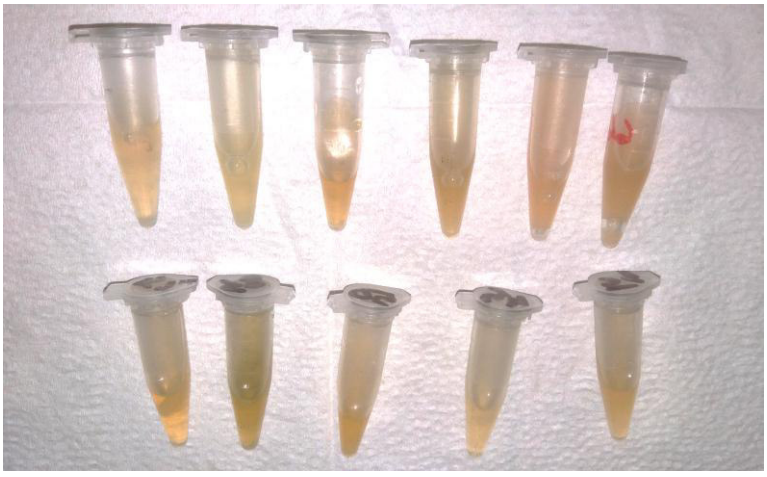


Figure 9: Seperated serum samples

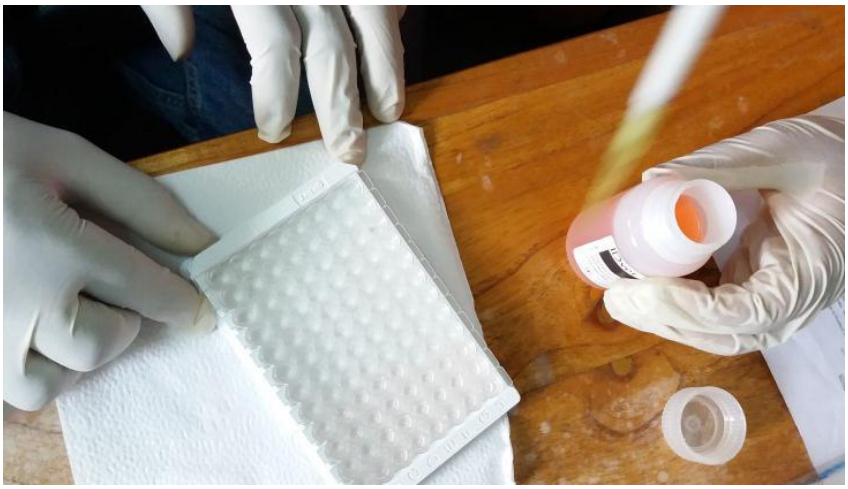


Figure 10: Addition of positive control

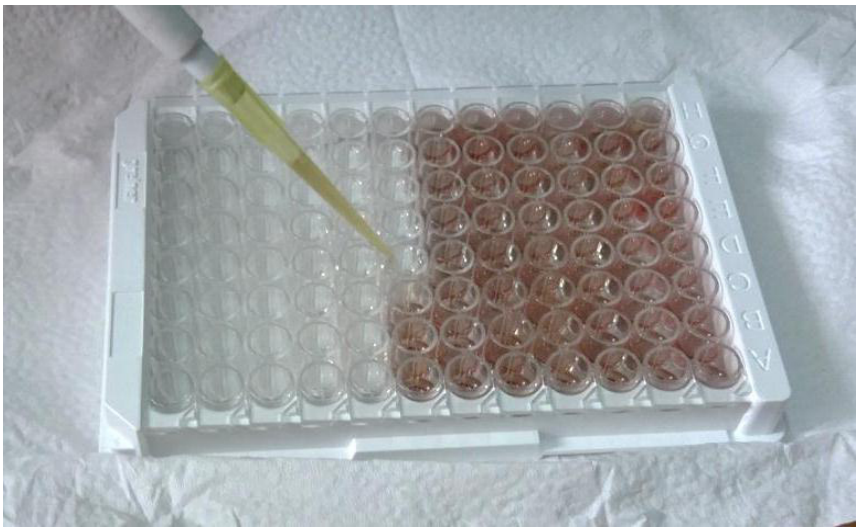


Figure 11: Addition conjugate solution

Figure 12: Addition of wash solution

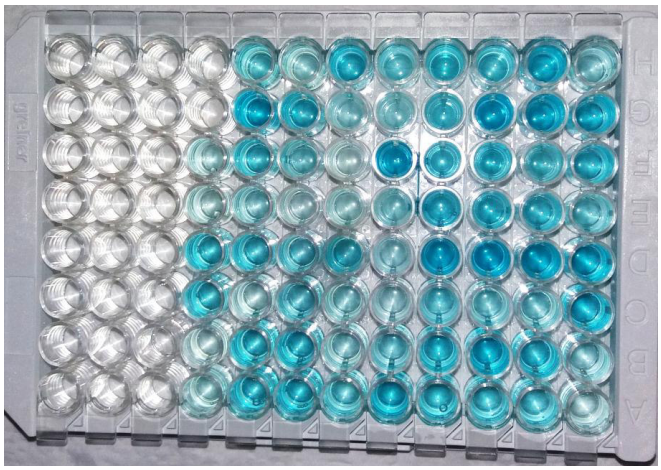


Figure 13: Addition of substrate solution

Figure 14: Addition of stoping solution

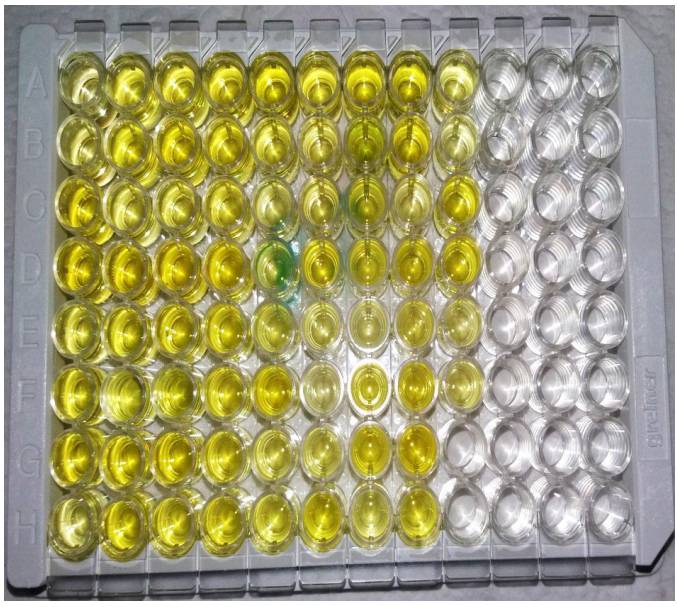


Figure 15: Reaction of stoping solution

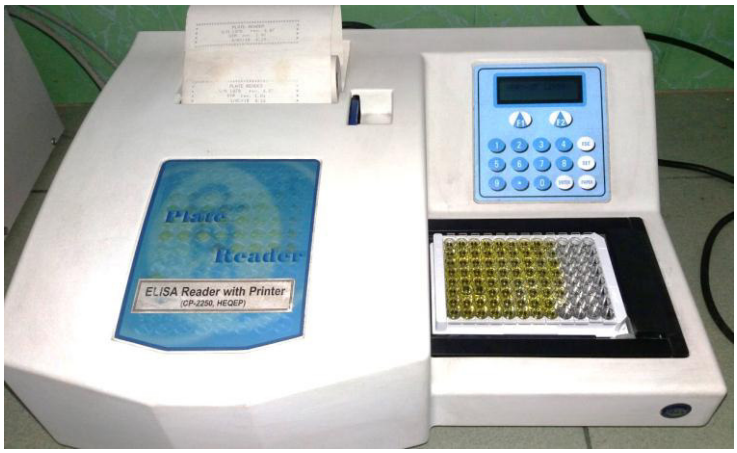
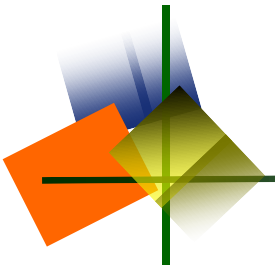


Figure 16: Examination of the samples by ELISA Reader



Chapter 4

RESULTS

CHAPTER 4

RESULTS

The research work was conducted to detect the antibody of PPRV of sheep at four different upazila namely Dinajpur Sadar, Chirirbandar, Parbatipur and Birol in Dinajpur district. In this study, A total number of 66 sheep were examined. On present of viral antibodies checked by Enzyme Linked immunosorbent Assay (ID.vet Innovative Diagnostics in France) and finally the results are presented below.

4.1 Value of Positive and Negative control

The positive and negative control has been carefully standardized represent significant amount of PPR antibody found in the test serum samples, the relative amount was calculated by reference to the negative control , the relationship is expressed as S/N % (Sample to negative ratio). The value of positive and negative control in this test presented below:

Table 1: Value of Positive and Negative control

Serial Number	Sample O.D	O.D value	Result
1.	A 1	0.001	Positive
2.	B 1	-0.003	Positive
3.	C 1	0.007	Negative
4.	D 1	0.003	Neagtive

4.2 Results of cELISA in sheep

In this study blood serum sample were collected form 66 indigenous type breed of sheep (*Ovis aries*) and checked for a presence of viral antibody by cELISA, and the result were presented in table 2.

Table 2: Result of cELISA of sheep at Dinajpur district

Sl. No.	Sample no.	OD value	S/N%	Result
1.	E1	0.003	100	Negative
2.	F1	0.006	200	Negative
3.	G1	0.004	133	Negative
4.	H1	0.003	100	Negative
5.	A2	0.001	33	**Positive**
6.	B2	0.001	33	**Positive**
7.	C2	0.007	233	Negative
8.	D2	0.002	66	Negative
9.	E2	0.007	233	Negative
10.	F2	0.004	133	Negative
11.	G2	0.008	266	Negative
12.	H2	0.011	550	Negative
13.	A3	0.001	33	**Positive**
14.	B3	0.003	100	Negative
15.	C3	0.007	233	Negative
16.	D3	0.006	200	Negative
17.	E3	0.011	550	Negative
18.	F3	0.002	66	Negative
19.	G3	0.004	133	Negative
20.	H3	0.009	450	Negative
21.	A4	0.001	33	**Positive**
22.	B4	0.006	200	Negative
23.	C4	0.015	750	Negative
24.	D4	0.001	33	**Positive**
25.	E4	0.008	226	Negative
26.	F4	0.002	66	Negative
27.	G4	0.003	100	Negative
28.	H4	0.015	750	Negative
29.	A5	0.003	100	Negative
30.	B5	0.004	133	Negative
31.	C5	0.001	33	**Positive**
32.	D5	0.002	66	Negative
33.	E5	0.003	100	Negative
34.	F5	0.001	33	**Positive**
35.	G5	0.001	33	**Positive**
36.	H5	0.003	100	Negative
37.	A6	0.003	100	Negative

38.	B6	0.004	133	Negative
39.	C6	0.006	200	Negative
40.	D6	0.001	33	**Positive**
41.	E6	0.004	133	Negative
42.	F6	0.003	100	Negative
43.	G6	0.002	133	Negative
44.	H6	0.007	233	Negative
45.	A7	0.001	33	**Positive**
46.	B7	0.001	33	**Positive**
47.	C7	0.003	100	Negative
48.	D7	0.001	33	**Positive**
49.	E7	0.006	200	Negative
50.	F7	0.001	33	**Positive**
51.	G7	0.004	133	Negative
52.	H7	0.017	850	Negative
53.	A8	0.004	133	Negative
54.	B8	0.001	33	**Positive**
55.	C8	0.005	250	Negative
56.	D8	0.003	100	Negative
57.	E8	0.004	133	Negative
58.	F8	0.001	33	**Positive**
59.	G8	0.004	133	Negative
60.	H8	0.002	66	Negative
61.	A9	0.003	100	Negative
62.	B9	0.004	133	Negative
63.	C9	0.004	133	Negative
64.	D9	0.003	100	Negative
65.	E9	0.004	133	Negative
66.	F9	0.001	33	**Positive**

- Less than or equal to ≤ 50% were considered Positive, Greater than 50 % and less than or equal to 60 % (50-% < SN % ≤ 60 %) were considered doubtful, Greater than 60 % (SN % > 60%) were considered Negative.

4.3 Prevalence of PPR in sheep

In this study, A total number of 66 sheep were examined. Here, The table shows the overall prevalence of PPR in sheep in four upazila (Dinajpur Sadar, Chirirbandar, Parbatipur and Birol) of Dinajpur district. From these sheep, 66 serum samples were

collected and tested by cELISA. Among the 66 samples, 16 were shown positive results and overall prevalence was 24.24%.

Table 3: Overall prevalence of PPR in sheep at Dinajpur District

Total tested number	Positive number	Prevalence (%)
66	16	24.24

4.4 Prevalence of PPR in sheep according to area

Prevalence of PPR in sheep based on area is presented in table. The present study revealed that the prevalence of PPR was not significantly (P > 0.05) differed based on area. The prevalence of PPR was higher in Chirirbandar (27.27%), followed by Parbatipur (26.67%), Birol (25.00%) and Dinajpur Sadar (15.38%) respectively.

Table 4: Prevalence of PPR in sheep according to area

Area	Total tested number	Positive number	Prevalence (%)	χ^2 value	P value
Chirirbandar	22	6	27.27	0.718	0.869 (NS)
Dinajpur sadar	13	2	15.38		
Parbatipur	15	4	26.67		
Birol	16	4	25.00		
Total	66	16	24.24		

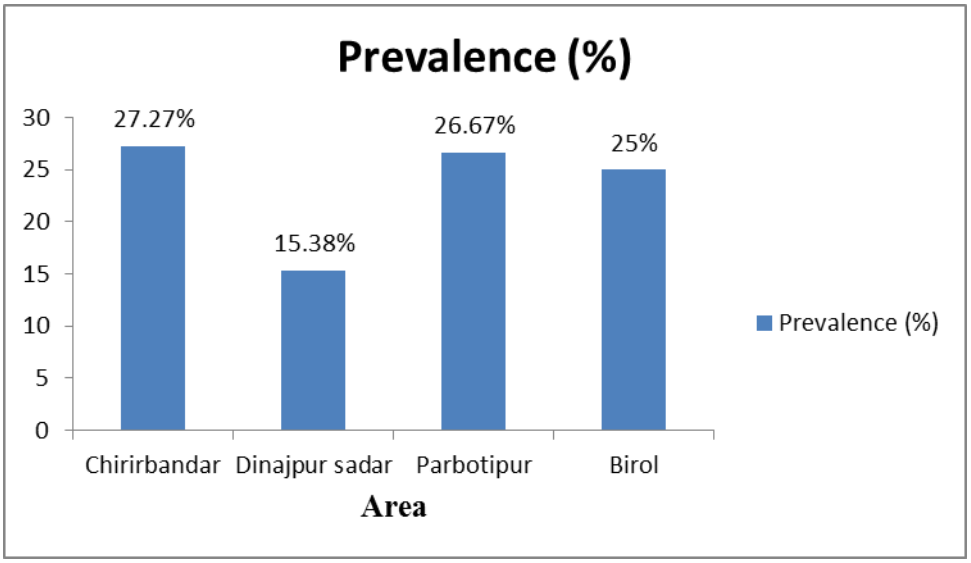


Figure 17: Prevalence of PPR in sheep according to area

4.5 Age wise prevalence of PPR in sheep

The overall prevalence of PPRV was higher in lamb (<1 year) than young or adult, but the age had no significant (P> 0.05) effects on the prevalence of PPR.

Table 5: Age wise prevalence of PPR in sheep at Dinajpur District

Age	Total tested number	Positive number	Prevalence (%)	χ^2 value	P value
< 1 year	13	5	38.46	2.056	0.561 (NS)
1-3 year	27	6	22.22		
> 3-6 year	18	3	16.66		
> 6 year	8	2	25		
Total	66	16	24.24		

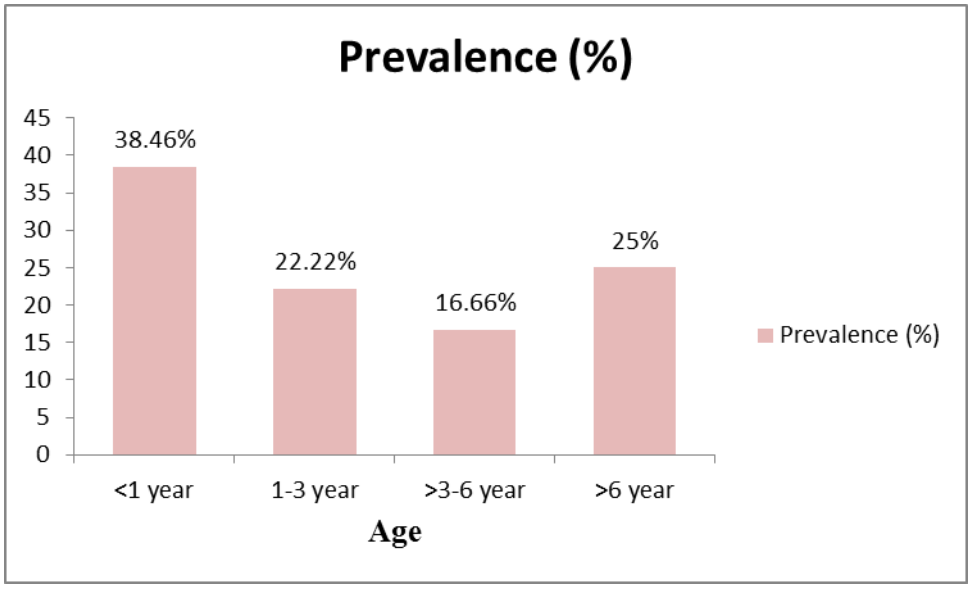


Figure 18: Age wise prevalence of PPR in sheep at Dinajpur District

4.6 Sex related prevalence of PPR in sheep

The present study showed that the prevalence of PPR insignificantly (P> 0.05) higher in male (25.00%) compared to female (24.00%).

Table 6: Sex related prevalence of PPR in sheep at Dinajpur district

Sex	Total tested number	Positive number	Prevalence (%)	χ^2 value	P value
Male	16	4	25	0.007	0.935 (NS)
Female	50	12	24		
Total	66	16	24.24		

4.7 Prevalence of PPR in sheep according to lactation stage

The present study showed that the prevalence of PPR in sheep was higher in lactating stage (33.33%) than Lamb (30%) and non-lactating stage (13.63%) respectively. But the prevalence of PPR was not significantly (P > 0.05) differed according to lactation stage.

Table 7: Prevalence of PPR in sheep according to lactation stage

Lactation stage	Total tested number	Positive number	Prevalence (%)	χ^2 value	P value
Lamb	10	3	30	2.352	0.308 (NS)
Lactating	18	6	33.33		
Non lactating	22	3	13.63		
Total	50	12	24		

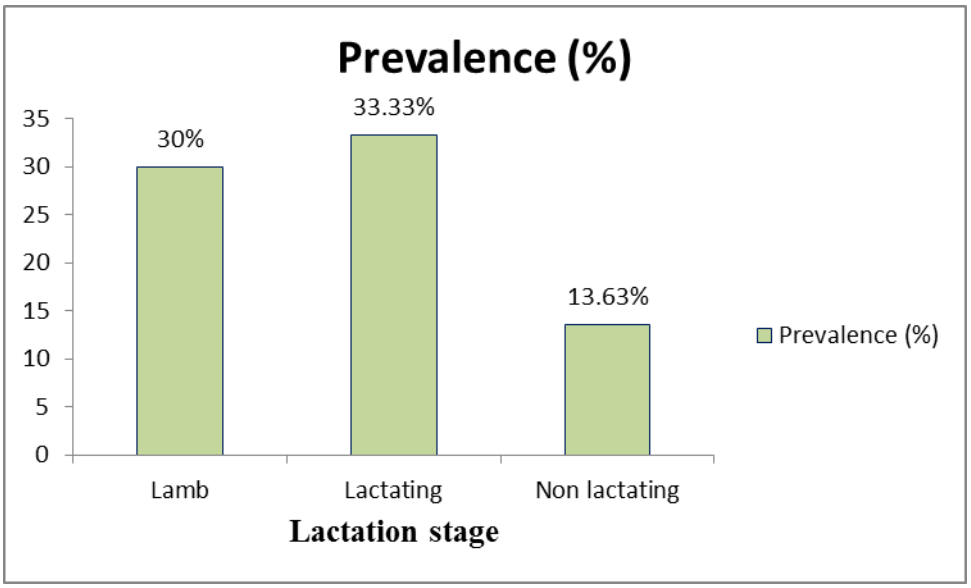


Figure 19: Prevalence of PPR in sheep according to lactation stage

4.8 Prevalence of PPR in sheep based on parity

The present study revealed that the prevalence of PPR was insignificantly higher in third parity (50.00%) where the other parity were no parity (30%), 1st parity (23.08%), 2nd parity (9.09%), 4th parity (40.00%), more than 5th parity (0%). But there was no significant (P>0.05) effect of prevalence of PPR in sheep based on parity.

Table 8: Prevalence of PPR in sheep based on parity

Parity	Total tested number	Positive number	Prevalence (%)	χ^2 value	P value
No	10	3	30	6.048	0.302 (NS)
1	13	3	23.08		
2	11	1	9.09		
3	6	3	50		
4	5	2	40		
≥5	5	0	0		
Total	50	12	24		

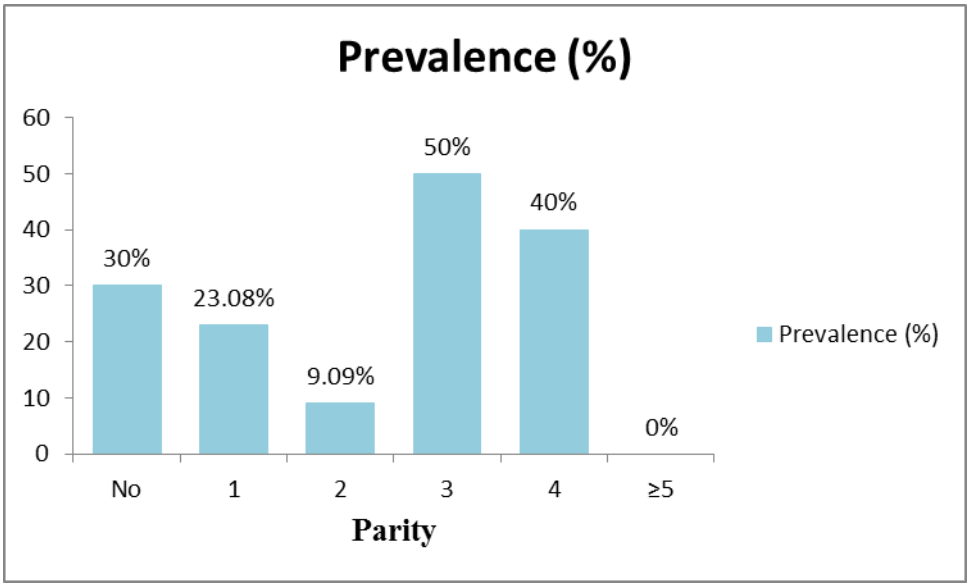
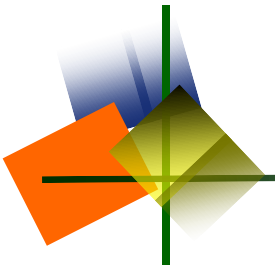


Figure 20: Prevalence of PPR in sheep based on parity



Chapter 5

DISCUSSION

CHAPTER 5

DISCUSSION

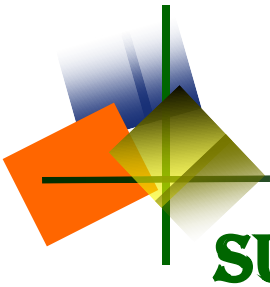
The present study was found that the overall prevalence in sheep was (24.24%). Similar findings were reported by Rahman *et al.* (2015). They revealed that the overall seoprevalence rate was 31.5%. Shamaki *et al.* (1995) found PPR prevalence rates in sheep of 40%, 40.6%, 7.7% and 38.4% were recorded from Plateau, Borno, Abia and Oyo States respectively. Luther *et al.* (2007) also reported a seroprevalence rate of 24.7% using the test. Obi *et al.* (1983) reported seroprevalence of 47.2% of PPR neutralizing antibodies in sheep. Bello (2013) also stated the overall prevalence of PPR virus antibodies in small ruminants in the study area to be 45.50%.

Based on sex, there was no significant variation (P= 0.935), and it was found that the prevalence of PPR insignificantly higher in male sheep (25%) compared to female sheep (24%). Similar results were reported by Bello *et al.* (2016) and they found that PPR seroprevalence was insignificantly higher in male 50.4% as compared to female 47.4%.

According to the aged group the result exhibited that there was no significant effect of prevalence among aged group (P=0.561). The highest PPRV Seropositive in sheep (38.46%) was the first group (<1 years) while lowest Seropositive (16.66%) were found from the third group (>3-6 years) and seronegative percentage was the contrary of the seropositive percentage. The research finding showed that the highest seropositive sheep in PPR were first group (<1 year) which is less than one year, and they are more susceptible than other ages. The present result is in accordance with the result of Sarker and Islam (2011). Regarding on age, they found that PPR was significantly higher in young (31.06%) compared to sucklers (13.14%) and adult (10.15%).

Small ruminants aged between 13-24 months had the highest prevalence of PPR virus antibodies (52.49%) followed by those aged between 6-12 months (48.46%) while the least seroprevalence rate was obtained in those animals aged above 24 months. This, to some extent agrees with the findings of Mahajan *et al.* (2012) who observed a significantly higher prevalence of PPR virus antibodies in animals aged above 12 months compared to those aged between 8-12 months.

Khan *et al.* (2008) reported a higher seroprevalence of PPRV antibodies in the >2 years age group in both sheep and goats. However, Majiyagbe *et al.* (1992) showed in their study that PPR seroprevalence increases with age. Dams infected with PPR virus can passively transfer maternal antibodies to their young ones. Although the maternal antibodies progressively decay, they remain above the protective threshold for up to 4-5 months after which PPR vulnerability increases with age (Abubakar *et al.,* 2009). This increased PPR susceptibility with age after five months in small ruminants may explain the relatively lower seroprevalence rates obtained in small ruminants aged between 6-12 months when compared with those aged between 13-24 months in this study. However, low seroprevalence of PPR was observed in those small ruminants aged above 24 months in this study.



Chapter 6

SUMMARY AND CONCLUSION

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Peste des petits ruminants (PPR) is an acute, highly contagious, viral disease of domestic and wild ruminants caused by a Morbillivirus, family paramyxovirus. It is antigenically very similar to the Rinderpest virus and other members of the genus Morbillivirus. Peste des petits ruminants is highly spread, transponder, and economic important disease in the world.

The study was conducted at Dinajpur for a period of 6 month since July to December to investigate the seroprevalence Peste des petits ruminants (PPR) in sheep. The study was consisted of two; blood sample were collected by jugular venipuncture method from sheep which is no pervious history of peste des petits ruminant (PPR) and rinderpest (RP) vaccination and the total blood sample were 66 sheep according aged group, sex, parity, lactation and location then kept for an hour at room temperature, after centrifugation serum was separated and stored at -20 ^{0}C until use while the other was survey questionnaire with sample of 90 households. The result showed that the overall prevalence in sheep was (24.24%).Based on area, the prevalence of PPR was not significantly (P= 0.869) differed and the result showed that the highest seropositive (27.27%) was observed from Chirirbandar upazila whereas the lowest seropositive (15.38 %) was detected from Dinajpur Sadar. However the other locations were intermediate. In respect to sex, there was no significant variation (P= 0.935), and it was found that the prevalence of PPR insignificantly higher in male sheep (25%) compared to female sheep (24%).

According to the aged group, there was no significant effects among aged group (P= 0.561) and the result exhibited that the highest PPRV seropositive in sheep (38.46%) was in the first group (<1 year) while lowest seropositive (16.66%) were found from the third group (>3-6 years).

According to lactation stage prevalence of PPR was higher in lactating stage (33.33%) than lamb (30%) and non-lactating stage (13.63%) respectively. But the prevalence of PPRV was not significantly (P=0.308) differed according to lactation stage.

Based on parity, the prevalence of PPR was insignificantly higher in third parity (50.00%) where the other parity were no parity (30.00%),1st parity (23.08%),2nd parity (9.09%) , 4th

parity (40.00%) and there was no significant (P = 0.302) effect on prevalence of PPR in sheep based on parity.

The present study should be concluded that the overall seroprevalence of the study area was 24.24% and based on the area, age, sex, lactation stage and parity of the disease and the animals are susceptible to this disease. Finally the following recommendations are suggested:

- To carry out further studies on isolation, molecular characterization of PPRV including pathogenesis and development of effective vaccine from local isolates of PPRV in near future to protect our sheep population.
- To develop good management practices like bio-security, to minimize the risk of the disease.

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