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Pran Gopal Rudra

Prevalence and molecular characterization of Peste Des Petits Ruminants (PPR) in Goat

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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List of abbreviations

Abbreviation	Elaboration
µl	microliter
95% CI	95% confidence interval
BLAST	Basic Local Alignment Search Tool
CI	Confidence interval
CVASU	Chattogram Veterinary and Animal Sciences University
et al.,	and others
MUSCLE	Multiple Sequence Comparison by Log-Expectation
NCBI	The National Center for Biotechnology Information
OR	Odd ratios
p	Probability
PPR	Peste des petits ruminants
PRTC	Poultry Research and Training Centre
SAQTVH	Shahedul Alam Quadary Teaching Veterinary Hospital

Abstract

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of goat and sheep responsible for high morbidity and mortality. This study was designed to estimate prevalence and determined associated risk factors as well as molecular characterization of circulatory PPR virus strains in Chattogram Metropolitan and its surrounding areas, Bangladesh. A total of 400 samples were collected from July 2018 to June 2019. The causative agent, PPR virus (PPRV) was confirmed by clinical signs, virus isolation, N-gene based PCR and finally sequencing. Eighty-eight (22%; Confidence Interval: 18.2%-26.4%) goats diagnosed positive for PPR on the basis of clinical signs and 61 (15.3%; CI: 12.0%-19.1%) confirmed positive for PPRV by PCR. Among 88 clinically positive PPR cases, 31 were confirmed by PCR. Sex was identified as a significant risk factor where male goats (19.2%) had more PPRV infection than the female (12.2%) goats. Fever (79.5%), diarrhea (85.2%), respiratory distress (53.4%) and purulent nasal discharge (54.5%) were common clinical signs of PPR in the study. The phylogenetic analysis revealed that PPRVs circulating in this area belonged to Lineage IV and they formed a separate sub-cluster along with the recent isolates from Bangladesh, India, Pakistan and China. It also reveals that genetic character of existing PPR vaccine in this country is different from the isolates of this study.

Keywords: *Prevalence, Risk factors, Molecular characterization, PPR, Goat*

CHAPTER-I: Introduction

Goat farming is a popular business both in urban and rural areas of Bangladesh now days. Currently estimated goat population in Bangladesh is about 26.1 million (DLS, 2017-18). Goat supplies extra income to the most of rural population who are living below the poverty line and also contributes largely to the livelihood of the livestock-keeping households of low and medium-input farmers. Initial investment for a goat farm is lower than dairy, piggery and even poultry farming. Goat consumes less feed which is about one fifth of the consumption of cattle and buffalo (Das SK, 2001). However, goat farming in Bangladesh is very challenging due to many constraints. Infectious diseases are one of the most significant impediments to the commercial rearing of small ruminants especially goat (Radostits *et al.,* 2000). Among all diseases of goat, Peste des petits ruminants (PPR) have become much more important due to its devastating nature and ability to do heavy economic losses (Subir *et al.,* 2011).

PPR which literally means ‘‘plague of small ruminants’’ is also known as goat plague or kata or pseudo-enteritis complex (Otte, 1960; Rowland *et al.,* 1969; Rowland and Bourdin, 1970; Abu-Elzein *et al.,* 1990; Diallo *et al.,* 2007). It affects usually more severe in goats than sheep and considered as the strongest limiting factor for advancing goat farming in developing countries (Abubakar and Munir, 2014).

PPR is a member of genus morbillivirus of family Paramyxoviridae, sub family Paramyxovirinae and order Mononegavirales. It was first reported in West Africa particularly Ivory Coast in 1942 (Gargadennec L *et al,* 1942) and was later from other parts of the world including Arabia (Abu-Elzein EME *et al.,* 1990), the Middle East (Lefevre PC *et al.,* 1991), sub-Saharan Africa, the Arabian Peninsula, and parts of Asia (Balamurugan V *et al.,* 2010). It has 4 genetically distinct lineages; lineage I, II, III and IV (Maganga *et al.,* 2013). Lineages I and II are commonly found in Western Africa, lineage III in Eastern Africa and the Middle East and lineage IV is widely distributed in Asia and parts of the Middle East (El Arbi *et al.,* 2014). For the first time in the South Asian subcontinent, PPRV was reported in 1987 in the southern part of India (Shaila *et al.,* 1989). In Bangladesh, outbreaks of rinderpest-like disease have been occurring in goats since 1993 which was later confirmed as PPR by British reference laboratory (Sil *et al.,* 1995; Islam *et al.,* 2001). It was found that, the isolates from Bangladesh were closely related with other strains from India clustered within the Asian group of lineage IV PPR viruses (Barrett *et al*., 1997).

The disease is characterized by high fever, depression and loss of appetite followed by purulent ocular and nasal discharge, erosive and ulcerative mouth lesions, fibrino-necrotic tracheitis, pneumonia and gastro-intestinal tract inflammation which ultimately lead to severe diarrhoea (Kul O *et al.,* 2007; Gibbs *et al.,* 1979). Clinical signs of PPR are almost like other diseases such as foot-and mouth disease (FMD), caprine pox, contagious pustular dermatitis, bluetongue and contagious caprine pleuropneumonia (Singh *et al.,* 2009). As a result, differential diagnosis by appropriate laboratory tests will be needed to increase the diagnostic accuracy of the PPR.

PPR is more prevalent in Asia and Africa rather than countries of Europe and America and reported as 13.8% in Assam, 33.3% in Algeria and 15.7% in Punjab (Begum *et al.,* 2016; Nardi *et al.,* 2011; Durrani *et al.,* 2010). The overall PPR sero-prevalence in Bangladesh was reported as 21% in 2008, ranging from 6 to 49% in different geographical locations/districts (Sarkar *et al.,* 2011; Bhuiyan 2012) which was increased to 38% in 2018 (Rahman *et al.,* 2018) indicating increasing trend of spreading the disease throughout the country. An outbreak of PPR in Black Bengal goats flock was reported as 75% morbidity and 59% mortality with case fatality rate of 74% (Chowdhury *et al.,* 2014).

Although PPR has been prevailing in Bangladesh for more than a decade, epidemiology of the disease is not well studied. Limited number of studies have been conducted based on either serology or clinical signs and few recent studies studied on genetic characterization of PPRV (Islam *et al.,* 2001; Khan *et al.,* 2005; Chowdhury *et al.,* 2014; Rahman *et al.,* 2016). PPR can be diagnosed by conventional diagnostic techniques like virus neutralization test (VNT), agar gel immuno-diffusion test (AGID) and isolation of the virus in cell culture, which are time-consuming and laborious too (Libeau G *et al.,* 1994 and Wamwayi HM *et al.,* 1995). However, with the advance of molecular biological techniques like PCR, rapid and specific diagnosis of PPR has become easier (Forsyth, M. A., & Barrett, T., 1995). The viral genome can also be detected after extraction of the genomic material (Prado *et al.,* 2005) or by direct RT-PCR without extraction (Yourno and Conroy, 1992; Pitcovsky *et al.,* 1999; Kailash *et al.,* 2002).

Considering above background, the present study was aimed to estimate prevalence of PPR with associated risk factors by using clinical signs and molecular technique (PCR)

in goats at Chattogram Metropolitan area. In addition, molecular characterization and phylogenetic analysis of the circulating PPRV strain were investigated.

CHAPTER-II: Review of literature

Rearing of goat is a part and parcel of farming system in Bangladesh not only for its good meat quality but also for increasing income of the poor farmers at a minimal cost. Presently, Bangladesh government and many non-government organizations have been trying to utilize this potential resource as a tool for poverty alleviation. In many areas of Asia and Africa, small ruminant production is threatened by transboundary animal diseases (TADs) such as Peste des petits ruminants (PPR) which has a negative impact on the livelihoods of poor farmers (EMPRES, 09).

In Bangladesh, outbreaks of rinderpest-like diseases, later confirmed as Peste des petits ruminants (PPR) by British reference laboratory, have been occurring in goats since 1993 (Sil *et al.,* 1995; Islam *et al*., 2001). The disease causes severe losses to small ruminant production and is considered as one of the major threats to about 22 million small ruminant population of Bangladesh. PPR affects food security by reducing the availability of meat and milk for family consumption and also reduces genetic resources. Thus, PPR prevention and control should be essential.

2.1 Peste des petits ruminants

2.1.1 History of outbreaks and global distribution

Peste des petits ruminants is a highly contagious and infectious viral disease of domestic and wild small ruminants which has a great economic significance in case of sheep and goats (Furley *et al*., 1987 and Dhar *et al*., 2002).

Côte d'Ivoire first described it in West Africa (Gargadennec and Lalanne, 1942). In 1956, Mornet *et al*. (1956) showed in experimental animals that the causative agents of rinderpest and PPR were closely related and suggested that the second virus was a variant of the first one which is better adapted to small ruminants. PPR, also known as goat plague, kata and pseudo-enteritis complex (Otte, 1960; Rowlland *et al*., 1969; Rowlland and Bourdin, 1970; Hamdy *et al*., 1976) is similar clinically to rinderpest. The disease has been recognized as endemic in several countries like Egypt (Ismail and House, 1990; EMPRES, 2009, Sudan (El Hag Ali and Taylor, 1988; Taylor *et al*., 1990; Haroun *et al*., 2002) and Ethiopia (Roeder *et al*., 1994; Abraham *et al*., 2005). In Kenya and Uganda, officially reported first outbreaks was observed in 2007 (EMPRES, 2009). The Morocco outbreak was the first case of PPR in North Africa (EMPRES, 2009) which followed by Tunisia (Ayari-Fakhfakh *et al*., 2010). The disease has been

observed later in several countries like Jordan (Lefevre *et al*., 1991), southern India (Shaila *et al*., 1989, Nanda *et al*., 1996), Bangladesh (Sil *et al*., 1995), Pakistan (Amjad *et al*., 1996), Iraq (Barhoom *et al*., 2000), Afghanistan (Abdollahpour *et al*., 2006), Turkey (Ozkul *et al*., 2002; Toplu, 2004; Anderson and Sammin, 2005; Yesilbag *et al*., 2005), Kazakhstan (Lundervold *et al*., 2004) and Tajikistan (Kwiatek *et al*., 2007), Nepal (Banyard *et al*., 2010), China (Wang *et al*., 2009) and Bhutan (Banyard *et al*., 2010).

In the recent year gene sequence analysis has facilitated the classification of PPRV strains into four lineages which are prevailing on the different geographical location of the world (Fig.1). Lineage I is represented mainly by Western African isolates from the 1970s and recent isolates from Central Africa; lineage II by West African isolates from the Ivory Coast, Guinea and Burkina Faso; lineage III by isolates from Eastern Africa, the Sudan, Yemen and Oman; and lineage IV includes all viruses isolated from recent outbreaks across the Middle east, southern Asia and several African territories. Genetic characterization of the Moroccan and Egyptian PPR virus classified it as a lineage IV virus (EMPRES, 2009; Banyard *et al*., 2010); this marks the first time that this lineage has been detected in Africa, where all four lineages are now present (Banyard *et al*., 2010). Bhutan is the latest country being affected with PPR and samples submitted to WRLs (World Reference Lab.) from Bhutan have recently been typed as lineage IV virus (Banyard *et al*., 2010). Due to the unfamiliarity of local human populations with the disease, it may remain either unnoticed or be misdiagnosed as a different disease with similar clinical manifestations (Banyard *et al*., 2010).

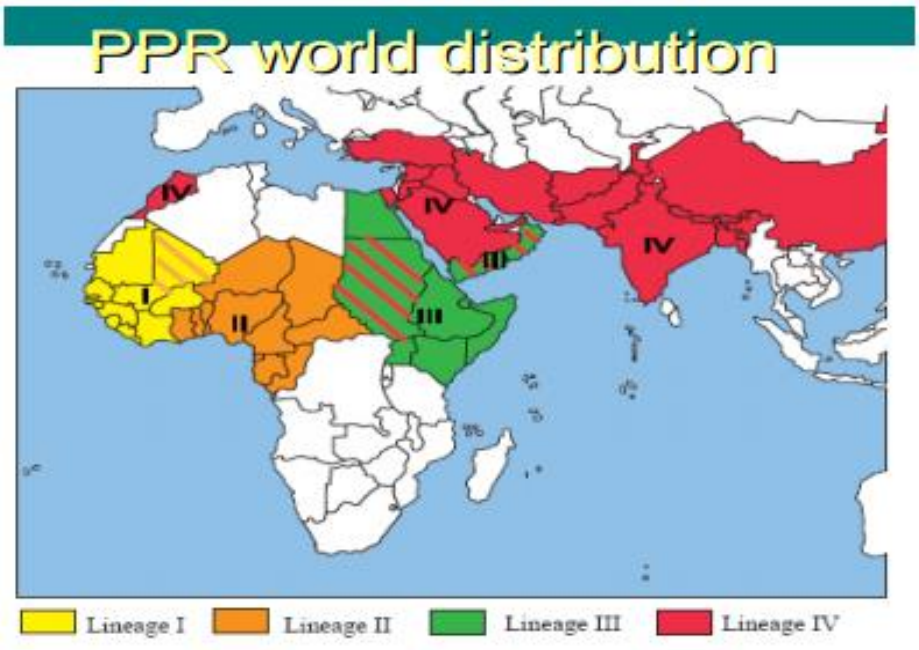


Fig.1. Geographic distribution of PPRV lineages (Minet *et al*., 2009)

2.1.2 Host range

PPR is primarily a disease of goats and sheep (Ozkul *et al*., 2002) whereas goats are usually more severely affected than sheep (Singh *et al*., 2004). In India, morbidity and case fatality reach 100% and 25%, respectively, in flocks of indigenous sheep (Shaila *et al*., 1989). Breed may affect the outcome of PPR virus infection and its epidemiology (Lefevre and Diallo, 1990).

Cattle and pigs are known to be a dead end host and all attempt to induce clinical disease in adult cattle experimentally failed but showed some silent or sub clinical infection (Gibbs *et al*., 1979; Taylor, 1984). PPR affects wildlife animals both under field condition and experimentally.

2.2 Etiology

2.2.1 Properties of the virus

PPRV is a member of the genus Morbillivirus under the family Paramyxoviridae and order Mononegavirales (Gibbs *et al*., 1979; Van Regenmortel *et al*., 2000). Paramyxoviruses are enveloped animal viruses which are found almost exclusively in nucleocapsid structures. This virus shares structural, biological, antigenic and molecular features in common with the other members of the group. It is closely related to the rinderpest virus (RPV), the measles virus (MV) of humans, the canine distemper

virus (CDV) of dogs and some wild carnivores, and the morbilliviruses of aquatic mammals. The virus is very fragile and cannot survive for a long time outside host. Its half-life has been estimated to be 22 minutes at 56°C and 3.3 hours at 37°C.

As all members of the family Paramyxoviridae, PPRV is an enveloped pleomorphic particle having diameter between 150 and 700 nm. The nucleocapsid is formed by the viral genomic RNA, a single-stranded RNA of negative sense, wrapped by the nucleoprotein (N) to which are associated two other viral proteins: the phosphoprotein (P) and the RNA polymerase (L or large protein). It appears as a tube which is long of about 1 µm with a diameter of around 18 nm (Bourdin and Laurent-Vautier, 1967; Gibbs *et al*., 1979). From the envelope protrude spikes formed by the two viral glycoproteins, the haemagglutinin (H) and the fusion (F) proteins (Fig.2) which are essential in the first steps of the host cell infection by the virus (Diallo *et al.,* 1990).

The genome of PPRV is non-segmented, negative-strand unlike other member of Morbillivirus. PPRV consist of 15948 nucleotides that encodes eight proteins: the nucleocapsid protein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin protein (H), the polymerase protein (L) and the two nonstructural proteins, C and V (Fig.2). PPRV genome is organized into six contiguous, nonoverlapping transcription units corresponding to the gene of the six structural viral proteins in the order of 3'-N-P-M-F-H-L-5' in the genome sense (Rima *et al*., 1986; Sidhu *et al*., 1993).

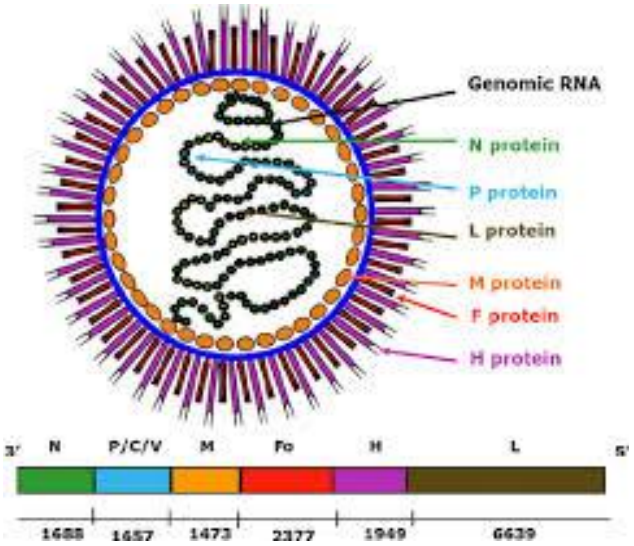


Fig.2. Genome of Morbilliviruses (image, anonym)

2.2.3 Viral proteins

2.2.3.1 Viral structural proteins

Both in the virion and in infected cells, the most abundant viral protein is the N protein (Diallo *et al*., 1987). It plays a direct role in forming the typical herring bone structure of morbillivirus nucleocapsids. Sequence data are now available for all the morbillivirus N proteins which reveal that there are 525 amino acid residues for RPV, measles virus and PPRV but 523 amino acids in the case of canine distemper virus, Phocine distemper virus (PDV), Dolphin morbillivirus (DMV) and Porpoise morbillivirus (PMV) (Diallo, 2003). Amino acid identities may vary from 67 to 74% between the different morbilliviruses.

P is a multifunctional protein which acts as a cofactor for the RNA-dependent RNA-polymerase (RdRp). It binds both the N and L proteins and acts as a chaperone to keep the N in a soluble form for binding to the RNA. RPV and PPRV sharing only 51.4% amino acid identity relating P protein (Mahapatra *et al*., 2003). Although the N protein of most morbilliviruses is found both in the cytoplasm and in the nucleus, it is found exclusively in the cytoplasm when complexed with the P protein (Huber *et al*., 1991; Gombart *et al*., 1993). The biological significance of this finding remains unknown.

The M protein is located inside the viral envelope and is the most conserved protein within the group with identities ranging from 91%, between Canine distemper virus and Porpoise distemper virus, to 76%, between RPV and Porpoise distemper virus or Canine distemper virus, while RPV and PPRV share 84% identity (Table 1).

The morbilliviruses produce two surface glycoproteins: the fusion (F) and the haemagglutinin (H) proteins. These are embedded in the viral envelope and protrude as spikes. F enables the virus to penetrate the host cell by mediating the fusion of the viral and cellular membranes at neutral pH. With the cooperation of the H protein since this H protein acts in promoting the activity of F (Moll *et al*., 2002; Plemper *et al*., 2002).

The H protein enables the virus to bind to the cell receptors to signal the lymphocyte activation molecule (SLAM, also called CD 150), a cell membrane glycoprotein of the immunoglobulin super family (Tatsuo *et al*., 2001). H cooperates with F for the fusion activity of this F protein (Wild *et al*., 1991; Das *et al*., 2000). PPRV H proteins have 609 amino acid residues. Like the F protein, maturation of H protein occurs by glycosylation, folding and oligomerization as it passes through the RER and the Golgi

complex before reaching the cell surface (Hu *et al*., 1994; Blain *et al*., 1995; Plemper *et al*., 2001). The most distantly related viruses, RPV and PDV, share only 32% amino acid identity and this only rises to about 50% when the two ruminant morbilliviruses, RPV and PPRV, are compared (Table 1). B-cell epitopes capable of inducing neutralizing antibodies have been mapped on the H protein to exposed β-sheets on the proposed three-dimensional structure (Langedijk *et al*., 1997; Sugiyama *et al*., 2002).

The L protein is the largest virus protein and is also the least abundant. It has molecular weight of about 200 kDa and is composed of 2183 amino acids for PPRV (Rima *et al*., 1986; Baron and Barrett, 1995; McIlhatton *et al*., 1997; Martha, 2001; Muthuchelvan *et al*., 2005; Bailey *et al*., 2005). The percentage identity between the L proteins of RPV and PPRV being about 83% as shown in Table 1. The L protein is assumed to carry all the activities necessary for genomic RNA replication and transcription into functional mRNA.

2.2.3.2 Viral non-structural protein

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

The first of these, the C protein is a small basic protein with a molecular weight of 19–21 kDa. For both RPV and PPRV it is composed of 177 amino acids, three residues longer than those of CDV and PDV (Barret *et al*., 1985; Curran and Rima, 1992; Baron *et al*., 1993). The phosphorylated P protein is found only in the cytoplasm with the association of the nucleocapsids. On the contrary, the C is not phosphorylated and can be detected in both the nuclear and cytoplasmic compartments of MV infected cells (Bellini *et al*., 1985; Alkhatib *et al*., 1988). RPV C protein is absent in the nucleus and distributed uniformly into the cytoplasm (Sweetman *et al*., 2001). C functions are very poorly understood in terms of their biological significance, although there is evidence that C is a virulence factor for MV (Patterson *et al*., 2000) and it was shown to be an interferon antagonist (Shaffer *et al*., 2003). Another function of C protein as infectivity factor which might be stabilized virus particles and therefore sustains the viral infection. It is therefore necessary for the efficient virus replication (Devaux and Cattaneo, 2004; Takeuchi *et al*., 2005; Von Messling *et al*., 2006).

The V protein is translated from an mRNA produced from the P gene by addition of one or more non-template G residues. V protein is shorter than P and Both have an identical N-terminal but different C-terminal protein sequences. V prorein is composed

Table 1: Percentage similarity of the RPV Kabete 'O' strain to other morbilliviruses (Berhe, 2006)

Name of the virus	**Similarity at genome**	**Similarity at protein level**							
Virus		**N**	**P**	**C**	**V**	**M**	**F**	**H**	**L**
Peste des petits ruminants	66.98	79.66	58.65	54.02	54.61	89.82	82.74	59.14	83.44
Measles	70.50	82.70	65.35	65.34	64.55	92.26	85.98	67.33	87.73
Canine distemper	63.58	75.38	53.57	52.91	44.67	83.93	78.75	46.26	81.05
Dolphin morbillivirus	65.91	79.69	57.91	55.29	50.50	89.29	84.52	57.14	83.29

of 298 amino acid residues with a predicted molecular mass of 32.3 kDa. It is now demonstrated that V protein is a highly inhibitor of interferon actions and thereby contributes to the immunosuppression induced by morbillivirus infections (Palosaari *et al*., 2003; Takeuchi *et al*., 2003; Ohno *et al*., 2004; von Messling *et al*., 2006).

2.3 Epidemiology

2.3.1 Transmission

The discharges from eyes, nose and mouth, as well as the loose feces, contain large amounts of the virus. When affected animals cough and sneeze, fine infective droplets are released into the air from these secretions and excretions (Johnson and Ritchie, 1968). For PPR to spread, close contact between infected and susceptible animals is needed (Ozkul *et al*., 2002). Inhalation of aerosols produced by sneezing and coughing of infected animals (Radostits *et al*., 2000) and direct contact with feces (Shanthikumar

et al., 1998) can transmit the virus. Infectious materials can be also contaminated by water and feed troughs and bedding and may act as additional sources of infection (EMPRES, 1999). Since the virus is enveloped, it is extremely sensitive to inactivation by environmental factors such as heat, sunlight and chemicals. The virus is very fragile and cannot survive for a long time outside host. Its half-life has been estimated to be 22 minutes at 56°C and 3.3 hours at 37°C. No carrier state is known to exist (Taylor, 1984; OIE, 2004).

The appearance of clinical PPR may be associated with introduction of recently purchased sick animals from markets (Radostits *et al*., 2000) or contact in a closed/village flock with sheep and/or goats. At markets, animals from different sources are brought into close contact with one another increases opportunities for PPR transmission (EMPRES, 1999). Changes in weather also have been suggested to contribute to outbreaks. Outbreaks were reported to be associated with the winter seasons as compared to other seasons of the year (Sil *et al*., 1995; OIE, 2004). Epidemics also reported to occur during the rainy season when goats are herded together and around Christmas when movement towards markets increases (Nawathe, 1984). The migration of animals in search of food during the hot dry summer when fodder is scarce was suggested to be one of the reasons for the higher frequency of PPR outbreaks between the months of March and June (Singh *et al*., 2004). Herd animals that are in constant contact with each other, like sheep and goats, are therefore very susceptible to serious outbreaks (Anderson and Mckay, 1994).

2.3.2 Pattern of the disease incidence

In general, morbidity is common, particularly in fully susceptible goat populations. Milder forms of the disease may occur in sheep and partially immune goat populations (Abraham, 2005). A higher affinity of the virus for the caprine species versus the ovine species and same virus being capable of inducing high mortality in goat population particularly in young goats than the susceptible sheep has been reported (Singh *et al*., 2004). There are considerable differences in the epidemiological pattern of the disease in the different ecological systems and geographical areas. Singh *et al*. (2004) observed the frequency of disease outbreaks to be greater between the months of March and June as compared to other periods of the year. The susceptibility of young animals aged 3 to 18 months was proved to be very high than adults or unweaned animals (Taylor *et al*., 1990). Mondal *et al*. (1995) noticed the prevalence was higher in Black Bengal

goats (57.34%) than in Jamunapuri breed (38.8%). Goswami *et al*. (1998) reported mortality among kids was higher (62.5%) than in adults (33.77%). In a susceptible herd, morbidity may be up to 100% with a mortality of 80-90% (Sil *et al*., 1995).

2.4 Clinical signs

The incubation period of the disease is 4-5 days in a sero-negative herd but may range between 3-10 days (Obi and Ojeh, 1989; Sil *et al*., 1995; EMPRES, 1999). During the incubation period the virus replicates in the draining lymph nodes of the oro-pharynx before spreading via the blood and lymph to other tissues and organs including the lungs causing a primary viral pneumonia. PPR occurs mainly in three forms - peracute, acute and subacute (Olaleye *et al*., 1989).

Peracute and acute forms of the disease are seen in 4 phases including incubation, prodromal, pneumonic and diarrhoea /death (Radostits *et al*., 2000). The disease usually appears in the acute form, with an incubation period of 4 to 5 days followed by a sudden rise in body temperature to 104-106° F (40-41° C) (Dhar *et al*., 2002; Abdollahpour *et al*., 2006). Affected animals appear ill and restless and have a dull coat, dry muzzle, and depressed appetite (Opasina, 1983; Taylor, 1984; Bundza *et al*., 1988). They have obvious signs of pneumonia. A clear watery discharge starts to issue from the eyes, nose and mouth, which progressively becomes mucopurulent (Taylor, 1984; Bundza *et al*., 1988; Islam *et al*., 2001; Dhar *et al*., 2002; Singh *et al*., 2004). These mucopurulent discharges tend to dry, causing matting together of the eyelids, obstruction of the nose and difficulty in breathing. Animals that survived one or two more days developed erosive and necrotising stomatitis, enteritis and anorexia (Sergany *et al*., 1992; Islam *et al*., 2001; Abdollahpour *et al*., 2006). At the height of development of oral lesions, most animals manifest severe diarrhea, often profuse but not hemorrhagic. The lining of the mouth becomes pale and coated with dying cells and, in some, cases; the normal membrane may be completely obscured by a thick cheesy material. Diarrhea commonly appears about two to three days after the onset of fever, although in early or mild cases, it may not be obvious. The feces are initially soft and then watery, foul-smelling and may contain blood streaks and pieces of dead gut tissue. As it progresses, there is severe dehydration, emaciation, and dyspnea followed by hypothermia, and death usually occurs after a course of 5 to 10 days. Pregnant animals may abort (Mornet *et al*., 1956; Hamdy *et al*., 1976; Opasina, 1983; Taylor, 1984).

2.5 Pathology

2.5.1 Pathogenesis

The PPRV has a particular affinity for lymphoid tissues and epithelial tissue of the gastro- intestinal (GI) and respiratory tracts, where it produces characteristic lesions (Susan and Aiello, 1998). It is suggested that death is a consequence of the combined effects of the pathology in the two systems (Smith *et al*., 1974). After the entry of the virus through the respiratory system, it localizes first in the pharyngeal and mandibular lymph nodes as well as in tonsil (Scott, 1981). After replicating in the upper respiratory tract, the virus spread to the local lymphatic tissues (House *et al*., 1992). Replication of virus in local lymphnodes produces primary viraemia that results in the spread of virus to other lymphoid tissues and other organs including skin, kidney and GI tract (Haffar *et al*., 1999). In these various organs, the virus replicates in the endothelial cells, epithelial cells and monocytes (Sergeny *et al*., 1992). The secondary viraemia is associated with the onset of prodromal phase of infection (Rossiter *et al*., 2000). The ability of the PPR virus-affected cells to fuse probably leads to the formation of multinucleated giant cells (Sata *et al*., 1986; Sergeny *et al*., 1992). The destructive phase of the virus on lymphocytes is suggestive as a cause of lymphopenia (Scott *et al*., 2000). Virus appeared to be released through the microvilli of the epithelial cells (Bundza *et al*., 1988).

The incubation period of the disease is 4-5 days in a sero-negative herd but may range between 3-10 days (Obi and Ojeh, 1989; Sil *et al*., 1995; EMPRES, 1999). Viremia may develop 2-3 days after infection and 1-2 days before the first clinical sign appears. Subsequently viraemia results in dissemination of the virus to spleen, bone marrow and mucosa of the gastro-intestinal tract and the respiratory system (Scott, 1981).

2.5.2 Gross lesions

The goat, infected both naturally and experimentally with PPR virus became emaciated and dehydrated at death (Khan *et al*., 2005). Erosive stomatitis is found on inside of lower lip and adjacent gum, on the cheeks near the commissures, on the ventral surface of the tongue; which in severe cases, may extend to the hard palate and pharynx (Susan and Aiello, 1998). The mucosal surfaces of the esophagus, abomasums, large intestine, rectum and cecum have congestion, severe edema, hemorrhage, necrotic plagues with erosions (Sergany *et al*., 1992; Mondal *et al*., 1995; Kumar *et al*., 2004). Rumen,

reticulum, and omasum rarely have lesions; erosions on pillars of rumen, congestion of the abomasums may be seen (Susan and Aiello, 1998; Debasis and Mousumi, 2002). Small intestine lesions are usually moderate and include extensive necrosis of Peyer's patches, resulting in severe ulceration (Saliki, 1998; Dhar *et al*., 2002). The large intestine is usually more severely affected with congestion around the ileocecal valve, at the ceco-colic junction, and in the rectum. In the posterior part of the colon and the rectum, discontinuous streaks of congestion ("zebra stripes") form on the crests of the mucosal folds (Rowland *et al*., 1971; Bundza *et al*., 1988; Sergany *et al*., 1992). Liver may be moderately enlarged and pale (Alcigir *et al*., 1996; Yener *et al*., 2004). Indented gall bladder may be found (Wadhwa *et al*., 2002). In some goat's rumen, reticulum, omasum and abomasum may be filled with foetid watery fluid and this watery fluid may also found in the small intestine (Osman *et al*., 2009). Small erosions and petechiae may be visible on nasal mucosa, turbinates, larynx and trachea, while pleuritis was seen in lungs, resulting in hydrothorax (Saliki, 1998).

Respiratory tract contained frothy exudates (Bundza *et al*., 1988; Pawaiya *et al*., 2004) and the lungs may be found to be severely congested with areas of consolidation, consistently in the apical lobes and in antero-ventral lobes (Islam *et al*., 2001; Debasis and Mousumi, 2002; Wadhwa *et al*., 2002; Kumar *et al*., 2004). Most lymph nodes throughout the body particularly mesenteric and mediastinal lymphnodes were enlarged, congested, and edematous but spleen may be either enlarged and congested or slightly atrophied (Rowland *et al*., 1971; Islam *et al*., 2001; Debasis and Mousumi, 2002; Khan *et al*., 2005). Ecchymotic and brush paint hemorrhage may be seen on the epicardium (Islam *et al*., 2001).

2.5.3 Histopathology

Microscopically, oral cavity, intestine, trachea, lungs, spleen and lymphnodes reveal most severe changes (Aruni *et al*., 1998; Pawaiya *et al*., 2004). The histopathologic findings of natural PPRV infection include erosive-ulcerative stomatitis, syncytial cells containing inclusion bodies in the tongue and the buccal, labial, soft palate mucosae; eosinophilic intracytoplasmic and/or intranuclear inclusions in epithelial cells lining of the mucosa of abomasum and ileum, multifocal coagulation necrosis and conspicuous syncytial cells in the liver (Toplu, 2004; Kul *et al*., 2007). The intestine shows atrophic villi with partial denudation of epithelial lining and intense diffusion of mononuclear cells in the lamina propria and submucosa (Aruni *et al*., 1998; Osman *et al*., 2009). The

lung shows bronchointerstitial pneumonia characterized by proliferation of bronchiolar lining epithelium, intense diffusion of mononuclear cells mainly lymphoid, macrophages and plasma cells in the periductal, the interstitial tissue and alveoli lumina (Osman *et al*., 2009). Eosinophilic intracytoplasmic and/ or intranuclear inclusions may frequently see in the cells of the syncytia (Bundza *et al*., 1988; Islam *et al*., 2001; Kul *et al*., 2007). In acute cases, lungs show fibrino-purulent pneumonia (Islam *et al*., 2001; Kumar *et al*., 2004). Chronic infection is characterized by giant cell pneumonia, which is sometimes complicated by Broncho-pneumonia (Olaleye *et al*., 1989; Islam *et al*., 2001). Eosinophilic intracytoplasmic and/or intranuclear inclusions may be observed in epithelial cells lining of the renal pelvis (Kul *et al*., 2007).

In lymph nodes, there may be edema in the cortex and medulla and infiltration of mononuclear cells and some giant cells in sub capsular areas and medullary sinuses (Osman *et al*., 2009). Depletion of lymphocytes is found in lymph nodes occasionally with syncytia formation (Islam *et al*., 2001; Kumar *et al*., 2004). In the spleen, tonsil and lymph nodes, the virus causes necrosis of lymphocytes evidenced by pyknotic nuclei and karyorrhexis (Rowland *et al*., 1971). The spleen also shows hemorrhage and hemosiderin pigment deposition (Osman *et al*., 2009).

2.7 Diagnosis

The routine diagnosis of PPR is based on clinical examination, gross pathology, histopathologic findings and laboratory confirmation which include tests for the detection of PPR antigen and PPR antibody, viral isolation, viral nucleic acid hybridization and recently polymerase chain reaction.

2.7.1 Virus isolation

PPRV may be isolated in primary lamb kidney or in African green monkey kidney (Vero) cell tissue cultures. Vero cell was found to yield very high titres and is currently used in many laboratories working on PPRV and RPV (Abraham, 2005). Samples for virus isolation include heparinized blood, eye and nasal swabs (from live animals), tonsil, mesenteric lymph nodes, spleen, section of colon and lung. For successful isolation, samples must be collected during the hyper thermic phase (Lefevre, 1987) and submitted to the testing laboratory in cold ice. The sensitivity of virus isolation technique could be increased when the virus is grown in lamb and goat kidney cells (Taylor, 1984). The characteristic cytopathic effect and pattern of its occurrence typical

to PPR virus was observed with two vaccine strains. Cytopathic effects in the form of clumping and ballooning of cells and syncytia formation was observed in PPRV-Sungri/96 at around 72 h post-infection, whereas the CPE pattern of PPRV-AR/87 was fast and entirely different and it was characterized by rounding, ballooning and degeneration of cells as early as 18 h post-infection (Hegde *et al*., 2009; Singh *et al*., 2009).

2.7.2 Antigen detection

2.7.2.1 Agar Gel Immunodiffusion Test

Agar gel immunodiffusion test (AGID) can detect up to 42.6% of positive cases from ante mortem and necropsy specimens (Obi, 1984; Abraham and Berhan, 2001). It can be used to test the presence of both antigen and antibodies (Obi, 1984). One of the important advantages of this test is that it is highly specific to morbillivirus (92%), though it cannot differentiate between PPR and RP. AGID is also relatively insensitive, and may not be able to detect small quantities of viral antigens in milder forms of PPR (Banyard *et al*., 2010).

2.7.2.2 Counter immunelectrophoresis

Counter immunoelectrophoresis (CIEP) is follwed same principle as the AGID. The only difference is the gel is electrically charged to improve the sensitivity of the test.

2.7.2.3 ELISA for antigen detection

A monoclonal antibody-based sandwich ELISA was found to be highly sensitive in detection of antigen in tissues and secretions of infected goats (Saliki *et al*., 1994; Singh *et al*., 2004). A sandwich ELISA test using PPR specific monoclonal antibody (Mab) (clone 4G6) to an epitope of nucleocapsid protein has been developed. It can give a reliable result within two hours in precoated plates and from samples maintained at room temperature for a period of seven days with no more than 50% reduction in response (Libeau *et al*., 1994).

The immunocapture ELISA was very useful in rapid differential diagnosis of PPR or rinderpest viruses, and this is of great importance as the two diseases have a similar geographical distribution and may affect the same animal species. The detecting Mab used in immunocapture ELISA are directed against two non-overlapping domains of the N protein of PPR and RP, but the capture antibody detects an epitope common to both RP and PPR (Libeau *et al*., 1994). The test is very specific and sensitive; its detect

10 0.6 TCID50 for the rinderpest virus. This discrepancy between the two viruses in the assay may be due to a difference in the affinity of the detection antibody for the different N proteins. The main advantages of this assay are: rapidity, it can be performed in a precoated plate in less than 2 hours; it can be carried out on samples which have not been kept under ideal conditions and where no viable virus is present. The immunocapture ELISA is suitable for routine diagnosis of rinderpest and PPR from field Samples such as ocular and nasal swabs (Diallo *et al*., 1995). Another modified Mab based version of ELISA the EISA (Enzyme immuno slide assay) was used in BLRI for the diagnosis of PPR from the field. Polyclonal PPR hyperimmune serum was initially used to detect PPR/Rinderpest antigen (virus infected Vero cell, its supernatants and samples from field specimens) on acetone fixed slide which was later confirmed by Mab directed against haemagglutinin protein of either PPR or RP virus (Sil *et al*., 2001).

2.7.2.4 Immunohistochemistry

Immunohistochemistry (IHC) is a method used to determine in which cells or parts of cells a particular protein or other macromolecules are located. These techniques use antibodies to bind to specific antigens, usually of protein or glycoprotein origin.

Immunohistochemical technique is performed on formalin-fixed, paraffin-embedded tissue. Both polyclonal and monoclonal antibodies can be used but monoclonal antibodies are generally considered to exhibit greater specificity (Haines *et al*., 1992; Saliki *et al*., 994). The use of Mab decreases possibilities for error caused by antigenic cross-reactivity or by nonspecific background staining. Other advantages include indefinite availability of large quantities of identical antibodies and ease of standardization of Mab based assays (Haines *et al*., 1992). Study has shown that may be a specific reagent for detection of PPRV antigen in formalin-fixed, paraffin-embedded tissues from cases of PPR. Diagnostic specimens of choice should include tongue, intestine, mesenteric lymph node, spleen, and lung (Saliki *et al*., 1994). This staining is sensitive and detects the presence of viral antigen in tissue section (Brown *et al*., 1991). This technique could be used to provide a diagnosis of PPR where fresh or frozen tissues are no longer available (Bundza *et al*., 1988) and also useful in retrospective examination of preserved specimens to verify past histological diagnosis (Jeremiah *et al*., 1994). The IHC method is very simple, precise, informative, and reproducible and therefore is very suitable to serve as an additional tool for virus

detection in combination with other laboratory procedures, such as PCR (Eligulashvili *et al*., 1999). Immunofluorescence and immunochemistry can also be used on conjunctival smears and tissue samples collected at necropsy (Banyard *et al*., 2010).

2.7.3 Molecular diagnosis

2.7.3.1 Reverse transcription polymerase chain reaction (RT-PCR)

Since the first report of PCR in 1985 (Saiki *et al*., 1985), it has been increasingly applied for research as well as for diagnosis of many diseases using a pair of specific primers. More recently for diagnostic applications, RT-PCR method has shown great promise, providing the potential of high sensitivity combined with specificity (Diallo *et al*., 1995). Among the various PCR primers developed, F gene based primers have gained wide acceptance, because of their application for specific detection as well as for molecular epidemiology of PPRV (Shaila *et al*, 1996). The PPR specific primers F1 and F2 developed by Forsyth and Barrett (1995) were used for amplification of a 372bp region between positions 777 to 1148 nucleotides of PPRV F gene. Couacy Hymann *et al*. (2002) developed a nucleoprotein (N) gene based RT-PCR for rapid and specific diagnosis of PPR. The primers NP3 and NP4 produced an amplicon of 351 bp amplifying a region of N gene between the nucleotide positions 1232 to 1583 (nucleotide positions as per the N gene sequence, accession no. X74443). This test was found 1000 fold more sensitive than the classical virus titration method based on the detection of cytopathic effect (CPE) of the virus in cell culture.

There has been development of RT-PCR test, using nucleocapsid (N), matrix (M), fusion (F), haemagglutinin (H), phosphoprotein (P) and large polymerase protein (L) genespecific primer sets to detect and differentiate RPV and PPRV (Ozkul *et al*., 2002). Sarvanan *et al*. (2004) developed an N gene-based PCR-ELISA for detection of PPRV in clinical samples. They found that the assay was more sensitive than sandwich ELISA, with percentage positivity of 66.20 percent compared to 48.60 percent for sandwich ELISA, in detecting the virus from clinical samples.

Specimens that can be collected from live animals include swabs from the eye (conjunctival sac), nasal secretions, and mouth and rectal lining, clotted and whole blood (with EDTA anticoagulant). At post-mortem samples of tonsil, tongue, spleen, lymph nodes, affected areas of the alimentary tract mucosa may be collected (OIE, 2004). Conventional transportation of the infected materials for diagnosis or virus

isolation is usually done by use of a cool chain environment. Because of poor infrastructures, transport of those samples usually takes a long time to reach the intended laboratories. So, these methods have limitations especially in remote areas where cool chain systems are unreliable or unavailable (Wambura, 2006). Filter papers have been shown to be suitable for the conservation of either DNA or RNA viruses for extended period of time (up to 411 years) at moderate or tropical temperatures (Li *et al*., 2004; Chaisomchit *et al*., 2005). The virus genome can be detected after extraction of the genomic material (Prado *et al*., 2005) or by direct RT-PCR without extraction (Yourno and Conroy, 1992; Pitcovsky *et al*., 1999; Kailash *et al*., 2002). Michaud *et al*. (2007) used filter papers for sampling of PPRV from experimentally infected goats and after long-term storage at high temperatures used in a direct PCR test without any prior extraction of nucleic acids.

RT-PCR products from both F and N gene were subjected to sequencing and phylogenetic analysis of PPR virus isolates (Shaila *et al*., 1989; 1996; Dhar *et al*., 2002; Raj *et al*., 2003; Kwiatek *et al*., 2007; Wang *et al*., 2009; Banyard *et al*., 2010; Balamuragan *et al*., 2010).

2.7.3.2 cDNA probes

For the differentiation between PPR and RP, the use of [P^{32}]-labelled cDNA probes derived from the N-protein gene of the two viruses had been described (Diallo *et al*., 1989). It could differentiate between the two viruses without need for virus isolation. cDNA directed against the matrix protein, fusion protein and phosphoprotein gene were found to cross hybridize to a much greater extent and were not suitable for use as discriminating probes (Diallo *et al*., 1989). Unfortunately, this hybridization cannot be used widely because it requires fresh specimens and in addition to the short half-life of P^{32}, there are constraints with the handling of isotopes. Therefore, probes using non-radioactive labels such as biotin (Pandey *et al*., 1992) or digoxygenin (Diallo *et al*., 1995) were developed. The biotin labeled cDNA was found to be as specific as the one using the radioactive label and more rapid in differentiation between PPR and RP (Pandey *et al*., 1992).

2.8 Serology

Many tests have been used for the demonstration of PPR antibodies in serum, such as virus neutralization test, agar gel immunodiffusion test, immune-electrophoresis and recently blocking and competitive ELISA.

2.8.1 Virus neutralization

The virus neutralization test (VNT) is sensitive and specific, but time-consuming and expensive. The standard neutralization test is carried out in roller-tube cultures of primary lamb kidney cells or Vero cells when primary cells are not available. For the detection of morbillivirus antibodies, VNT is the most reliable test (Rossitter, 1994). Serum against either PPR or RP may neutralize both viruses, but would neutralize the homologous virus at a higher titre than the heterologous virus.

2.8.2 cELISA

Competitive and blocking ELISA based on monoclonal antibodies specific for N-protein (Libeau *et al*., 1995) and H-protein (Saliki *et al*., 1993; Anderson and Mckay, 1994; Singh *et al*., 2004) were developed for detection of antibodies in animal sera. cELISA has been widely used to detect PPR antibodies in many countries (Roger *et al*., 2001; Ogunsanmi *et al*., 2003; Razzaque *et al*., 2004; Ahmad *et al*., 2005; Banik *et al*., 2008).

2.9 Sero-surveillance

Sero-surveillance is sometimes the only indicator of infection (Kwiatek *et al*., 2007). Animals once recover from PPR remain resistant to subsequent infection (Sil *et al*., 1995). The sero-prevalence rate in sheep and goats rises with age (Kwiatek *et al*., 2007). It is well documented that sheep and goats usually experienced PPRV infection at younger age and remained sero-positive for 1-2 years following exposure (Dhar *et al*., 2002; Ozkul *et al*, 2002; Singh *et al*., 2004). Animals younger than 6 months and older than 1 year had a better chance of sero-positivity to PPR (Munir *et al*., 2008). Prevalence for PPRV infection varied (range 0.87%–82.6%) and was higher in sheep (29.2%) than in goats (20%) (Ozkul *et al*., 2002). Using a competitive ELISA that used a monoclonal antibody to the N protein (Libeau *et al*., 1995) 17.6% animals were found to have antibody to PPRV in Tibet, China (Wang *et al*., 2009). The immunized goats develop both humoral and cell-mediated immune responses (Sinnathamby *et al*., 2001).

2.10 Prevention and Control

2.10.1 Vaccination

There is no specific treatment against PPR. Antibiotics may prevent secondary infections but this treatment is too costly in case of an outbreak. Therefore, the control of this disease is through the implementation of hygienic and medical prophylaxis measures (Berhe, 2006), although it is obvious that strict hygienic measures are hardly possible in developing countries (Berhe, 2006). Vaccination is the preferred method of control (Nawathe, 1984). The attenuated tissue culture Rinderpest vaccine has been used for a long time to protect small ruminants against PPR. This vaccine provides protection for over one year and is tolerated by healthy goats of all breeds (Nawathe, 1984). High vaccination coverage and vaccine efficacy are required to prevent major epidemics (Leissel and Salama, 2003). At the end of the 1980s, a PPRV strain was successfully attenuated by serial passages in Vero cells (Diallo *et al*., 1989). The PPRV homologous vaccine was found to be safe under field conditions even for pregnant animals and it induced immunity in 98% of the vaccinated animals. A single injection and the induced immunity cover at least the economic life of the animals, around three years (Diallo *et al*., 1995). Normally, homologous PPR vaccine attenuated after 63 passages in Vero cell produced a solid immunity for 3 years (Diallo *et al*., 1995). The vaccine is harmless on pregnant sheep and goats at any stage of gestation and induces the production of colostral anti-PPR antibodies that have been found in kids up to 3 months old (Diallo, 2003). It is suggested that kids and lambs from immunized or exposed dams should be vaccinated at 4 and 5 months of ages, respectively (Awa *et al*., 2002). Similarly, the antibodies due to naturally exposure to PPR infection might also interfere with the efficacy of vaccines. Therefore, monitoring of antibodies would be required before mass vaccination against PPR, especially in enzootic areas (Banik *et al*., 2008).

2.11 PPR in Bangladesh

In Bangladesh, outbreaks of Peste des petits ruminants (PPR) have been occurring in goats since 1993 (Sil *et al*., 1995; Islam *et al*., 2001). The disease causes severe losses to small ruminant production and is presently considered as one of the major threats to goats and sheep of Bangladesh. Although PPR has been prevailing in Bangladesh for a long period of time, epidemiology of the disease is not well understood (Islam *et al*., 2001; Khan *et al*., 2005). A locally developed PPR vaccine is being used to control PPR

in Bangladesh although recently PPR researches are limited to this vaccine development without characterization of the field and vaccine isolates. Disease diagnosis is mainly symptomatic and diagnostic facilities are also inadequate. No adequate laboratory facilities are available to diagnose PPR in Bangladesh. First generation tests such as virus neutralization and the agar immuno-diffusion tests are either time-consuming, labor intensive to perform or nonspecific. In the past ten years, developments in molecular biology have improved knowledge of PPR. RT-PCR is an essential tool for investigating epidemiology of the disease and phylogeny of the virus (Shaila *et al*., 1996; Ozkul *et al*., 2002; Kwiatek *et al*., 2007; Wang *et al*., 2009; Balamurgan *et al*., 2010) using adequate primers based either on the F (Forsyth and Barrett, 1995; Meyer and Diallo, 1995; Ozkul *et al*., 2002), the N (Couacy-Hymann *et al*., 2002; Kerur *et al*., 2008), the P (Forsyth and Barrett, 1995; Mahapatra *et al*., 2003) or the H (Balamurugan *et al*., 2006) protein genes. Molecular diagnosis or virus isolation from the infected materials require however a cooling chain (−20°C) which is less available for field sampling in Bangladesh.

CHAPTER-III: Materials and methods

3.1 Study area and population

The study was conducted from July 2018 to June 2019 at Shahedul Alam Quadary Teaching Veterinary Hospital (SAQTVH) of Chattogram Veterinary and Animal Sciences University (CVASU) Bangladesh. Clinical cases in and around Chattogram metropoliton city brought to the hospital for diagnosis, treatment and advisory services (Personal communication, Director of SAQTVH). On an average 30 goat patients got registration daily to this hospital. Due to availability of clinical cases and laboratory facilities, the study was undertaken at the hospital.

3.2 Study design, sample size calculation, sampling method and data collection

Relevant data such as breed, age, sex, body color, body condition, different management system and clinical signs were recorded using a close ended questionnaire (Appendix) by face to face interview with owner and close examination of goat. The required sample size for the study was determined by the formula described by Thrusfield, (2005) (The expected prevalence 50% of PPR and the 5% desired absolute precision and 95% confidence interval). The following formula was used to determine the sample size.

$$N = \frac{1.96^2 \times Pexp\ (1-Pexp)}{d^2}$$

Where, N = required sample size

P_{exp} = expected prevalence=50%

d = desired absolute precision = 5%

1.96 = the value of z at 95% confidence interval

Accordingly, the required numbers of goats were 384. However, to increase the study power the sample size was increased by 5% and total of 400 goats were included in the study. All samples were collected according to convenience sampling.

3.3 Sample collection and preservation

Blood samples (5 ml) were collected by sterile disposable needle (Appendix II) from jugular vein of goat. The area was cleaned and disinfected with 70% alcohol before puncture. Blood was transferred into vacutainer with anticoagulant (EDTA), labeled with ID number and stored at -20ºC for further analysis (Appendix II).

3.4 Extraction of genomic RNA

RNA was extracted using the Viral Nucleic Acid Extraction Kit (Addbio®, Daejeon, South Korea) according to the manufacturer's instructions. Briefly, 350 µl of lysis solution was added into 200 µl of whole blood in a 1.5 ml micro-centrifuge tube. Then 3.5 µl β-mercaptoethanol (14.2M) was added to the tube and mixed well by pulse vortexing for 10 to 15 seconds. After that, mixure was incubated at room temperature for 10 minutes and centrifuged at 3000 rpm for 5 seconds. To lysate, 150 µl of isopropanol was added and mixed well by pulse vortexing for 15 seconds. The lysate was carefully transferred into the upper reservoir of the spin column with 2 ml collecting tube and centrifuged at 13000 rpm for 1 minute. Spin column was washed 2 times with washing solution 1 and 2 by adding 500 µl of each solution into the spin column with collection tube and centrifuged at 13000 rpm for 1 minute. The spin column was dried by additional centrifugation at 13000 rpm for 1 minute to remove the residual ethanol. The column was then transferred into a new 1.5 ml micro-centrifuge tube with 70 µl elution buffer in each tube and waited for at least 1 minute. Finally, the Viral Nucleic acid was eluted by centrifugation at 13000 rpm for 1 minute. Purified RNA was stored at -20ºC for immediate use and stored at -80ºC for long term storage.

3.5 PCR amplification and visualization

PCR was performed using 2720 thermal cycler (Applied Biosystems®, USA) in a total reaction volume of 20 µl containing 10 µl of 2× RT-PCR master mix, 5 µl primer mixture and 5µl of extracted viral RNA. A set of primers (forward 5´-GAACAAAGACAAAGCGCCGA- 3´ and reverse 5´ ACCACGTGATGCAAAGGTCA-3´) with the lineage specific sequences of N-gene of PPR virus was designed to amplify 322 bp fragments for the identification of PPR positive sample. Thermal profile used for PCR specific to PPRV was 20 minutes for cDNA synthesis at 50ºC, 10-minute initial denaturation at 95ºC followed by 40 cycles including, denaturation (95ºC, 30 sec), annealing (55ºC, 30 sec) and extension (72ºC, 1 minute) with a final extension step for 5 minutes at 72ºC (Appendix II). Finally, gel electrophoresis was done using 5 µl of amplified PCR products and 1 µl of loading dye in previously stained agarose gel (1.5%) with ethidium bromide and observed under UV light for detection of positive samples and recorded (Appendix II).

3.6 RNA sequencing, processing and metadata collection

A number of 16 positive RNA samples were sent for partial gene sequencing by Sangers Dideoxy Method (Sanger and Coulson, 1975) to the commercial sequencing company (SolGent Co. Ltd. South Korea). Once the results (ABI Formatted File) of sequencing were available, the FASTA files of both forward and reverse sequences were exported from the raw files using Finch TV 1.4.0. Initially, the reverse sequences were complimented by Finch TV 1.4.0. Finally, both forward and reverse sequences of same gene were assembled by online free available tool CAP3 Sequence Assembly Program. After obtaining the assembled sequences, each of them was blast in NCBI nucleotide BLAST (The National Center for Biotechnology Information: http://blast.ncbi.nlm.nih.gov/Blast.cgi) for the conformation of the gene.

Basic Local Alignment Search Tool (BLAST) through NCBI was used to identify sequences that are homologous to the sequence of interest (Altschul *et al.*, 1997). At the same time, homologous sequences with their accession numbers were also collected from some research articles. FASTA files with related metadata of all related homologous sequences were collected from NCBI for further analysis. The accession numbers are given as follows: MG581412.1, KX421384.1, KY888168.1, MF443343.1, MF443354.1, KY967610.1, KT860065.1, KX033350.1, KT860063.1, KT860064.1, KJ867541.1, KC594074.1, NC006383, HQ197753.1, KY967608.1, KY967609.1, KJ867543.1, KJ867540.1, KJ867545.1 and KJ867544.1.

3.7 Multiple alignments and phylogenetic analysis

The multiple alignments were performed using the Multiple Sequence Comparison through Log-Expectation (MUSCLE) (Edgar, 2004) by MEGA-X. After alignments, the aligned sequences were exported as MEGA format.

A phylogenetic tree was constructed based on the PPR virus partial N gene sequences determined in the present study and those obtained from GenBank as mentioned previously by Neighbor joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.19069480 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein,1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum

Composite Likelihood method (Tamura *et al.,* 2004) and are in the units of the number of base substitutions per site. This analysis involved nucleotide sequences of thirty-seven separate partial gene sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were total of 360 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.,* 2018).

3.7 Statistical analysis

Collected information was stored in Microsoft Excel (2016). All statistical analysis was performed using STATA-13 (StataCorp, Texas, USA). For statistical analysis animals were divided into four- age groups: ≤ 5 months, 6-10 months, 13-24 months and ≥24 months old. Prevalence and corresponding 95% Confidence Interval were calculated. Association between risk factors (breed, gender, age and season) were measured using Chi square test. p value ≤ 0.05 was considered as statistically significant.

CHAPTER-IV: Results

4.1 Prevalence of PPR in goats at Chattogram Metropolitan area

Amplified cDNA fragment of 322 base pairs was considered as positive for PPRV (Figure 1). As shown in table 1, 61 (n=400) samples showed positive in PCR analysis, that is, overall prevalence of PPR was identified as 15.3% (95% CI: 12.0%-19.1%; N=400) in molecular diagnosis. Similarly, 88(n=400) goats were diagnosed as PPR on the basis of clinical signs, that is prevalence based on clinical signs was identified as 22% (95% CI, 18.2%-26.4%) (Table 1). The prevalence of PPR different between 2 diagnostic techniques (PCR and clinical signs) which was differed statistically significant ($p \leq 0.05$).

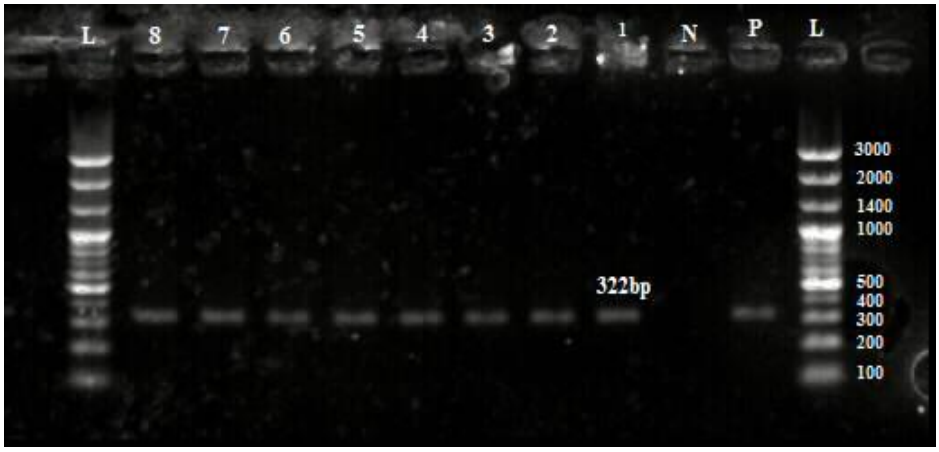


Figure 3: Amplification of the N protein gene of PPRV from genomic cDNA extracted from goat blood. Lane L is for 100 bp plus DNA ladder; Lane P is for positive control and N is for negative control; Lanes 1,3,4,5,6,7 and 8 are PPRV positive samples.

Table 2: Prevalence of PPR in goats at Chattogram Metropolitan area

Diagnostic techniques	Total (N)	Positive (n)	Prevalence (%)	95% Confidence Interval	*p*-value
PCR	400	61	15.3	12.0-19.1	0.01
Clinical signs	400	88	22	18.2-26.4	

Table 3: Comparison between the diagnostic tests used in this study to detect PPR in goat

Clinical signs	**PPR +ve**	**PCR + ve (among clinically + ve cases)**	**Negative cases**	**PCR +ve (among 312 of clinically negative cases)**
	88	31 (35.2%; 95% CI- 25.3-46.1)	312	30 (9.6%; 95% CI- 6.6-13.4)
PCR	**PPR +ve**	**Clinically positive (among PCR + ve cases)**	**Negative cases**	**Clinically positive (among PCR - cases)**
	61	31 (50.8%; 95% CI- 37.7- 63.9)	339	57 (16.8%; 95% CI- 12.9-21.2)

As shown in table 2, among clinically diagnosed 88 positive and 312 negative PPR cases, 31 (35.2%, CI 25.3- 46.1) and 30 cases (9.6%, CI 6.6-13.4) found positive for PPR in PCR, respectively. On the other hand, among 61 PCR positive, 31 (50.8%, CI 37.7-63.9) were diagnosed positive for PPR and 57 (16.8%, CI 12.9- 21.2) found positive among 339 negative cases in clinical investigation.

4.2 Risk factors analysis of PPR

Different risk factors and their association with PCR positive PPR cases were analyzed both in univariable and multivariable logistic regression method (Table 3 and 4).

In univariable analysis, the prevalence of PPR was significantly higher in male (19.8%) than female (12.2%) ($p \geq 0.05$) (Table 3). In multivariable logistic regression analysis, the ocuurence of PPR was 1.7 times more in male than female ($p \leq 0.05$) (Table 4).

Table 4: Association between PPR and each of the selected factors

Explanatory variable	Categories	Frequency no	Positive (%)	*p*- value
Season (Month)	Dry (Nov-Apr)	234	31	0.17
	Wet (May-Oct)	166	30	
Breed	Black Bengal	132	20	0.51
	Jamunapari	72	8	
	Cross breed	196	33	
Sex	Male	162	32	0.04
	Female	238	29	
Age (months)	≤ 6	103	16	0.71
	6-12	110	19	
	13-24	149	19	
	≥ 24	38	7	
Vaccination	Yes	82	11	0.60
	No	318	50	

Table 4: Multivariable logistic regression model to determine risk factors associated with PPR in goats

Explanatory variable	Categories	Odd Ratio	95% CI	*p*-value
Sex	Female	1	**Ref.**	0.05
	Male	1.7	1.1-3.0	

4.3 Description of clinical signs of PPR

Table 5: Frequency distribution of clinical signs of PPR cases in goats (N= 88)

Signs	Categories	Frequency	Percentage (%)
Fever	Yes	70	79.5
	No	18	20.5
Status of feces	Diarrhea	75	85.2
	Normal feces	13	14.8
Dehydration status	Severe dehydration	14	15.9
	Moderate dehydration	27	30.7
	Mild dehydration	47	53.4
Respiration pattern	Respiratory distress	47	53.4
	Normal	41	46.6
Nasal discharge	Purulent	48	54.5
	Serous	40	45.5

The common observable clinical signs of PPR affected goats were diarrhea (85.2%) followed by fever (79.5%), purulent nasal discharge (54.5%) and respiratory distress (53.4%) (Table 5).

4.4 Molecular characterization of PPRV

Bangladeshi isolates of PPR clustered under Lineage IV in N genes phylogeny and had a close relationship with another isolate of Bangladesh.

In case of N gene, genetic diversity among PPRV was quite evident in the phylogenetic tree (Figure-3). Within the Lineage IV several sub-clusters can be noticed. Even Bangladeshi isolates are also segregated into 3 apparent sub-clusters (Figure-3). Moreover, all isolates of PPRV are quite distinct from the PPRV used as vaccine in Bangladesh. This finding indicates that the N genes of circulating PPRV in this area are somewhat changed.

Table 6: Comparison between occurrence of PPR positive in vaccinated and non-vaccinated group in goat at Chattogram area

Explanatory variable	Categories	Frequency (n)	Positive (%)	95% CI
Vaccination	Yes	82	11 (13.4%)	6.9-22.7
	No	318	50 (15.7%)	11.9- 20.2

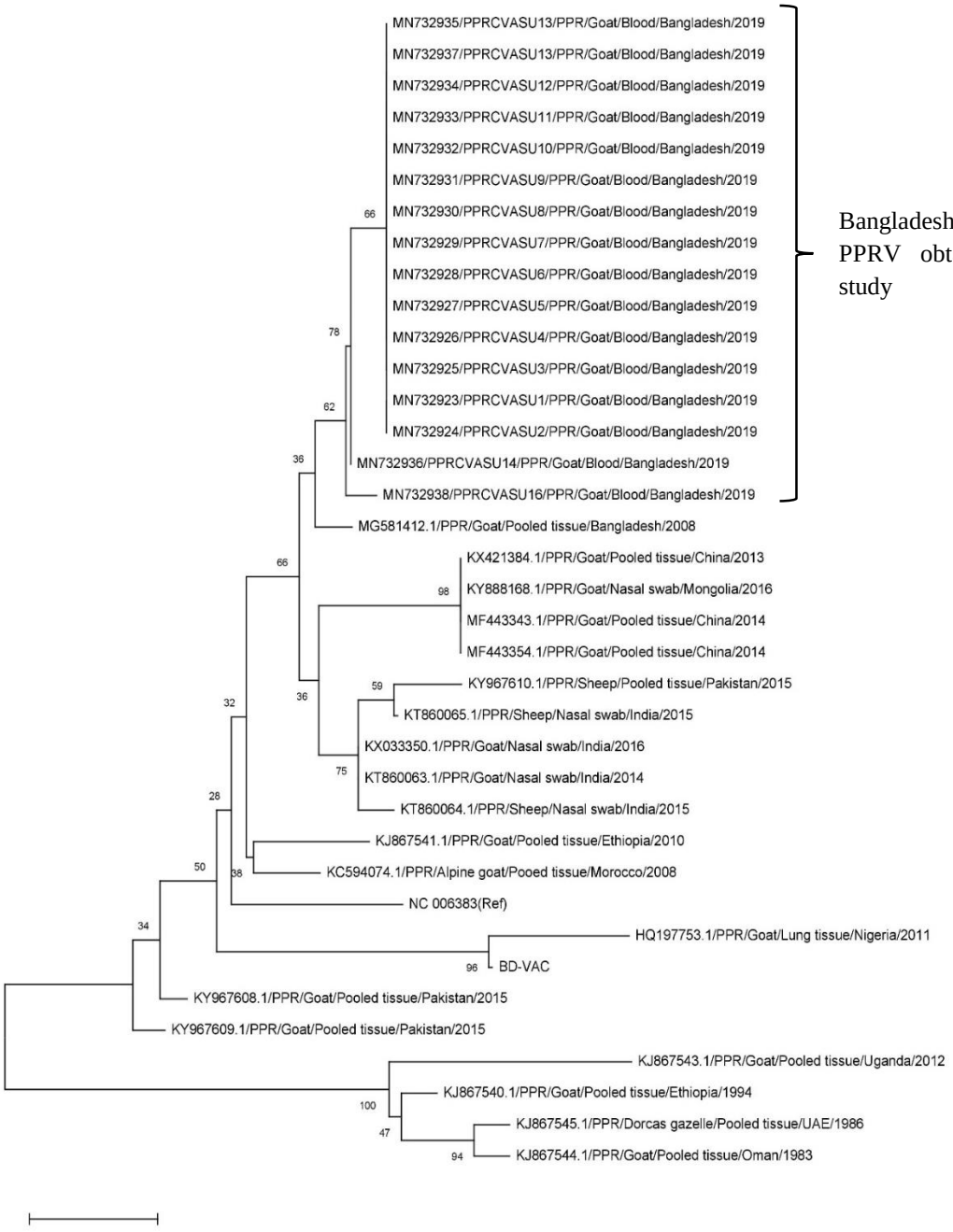


Figure 4: The phylogenetic tree of Peste des petits ruminants isolates obtained from the goats in this study and known Peste des petits ruminants in GenBank using as Peste des petits ruminants related genus and outgroups.

CHAPTER-V: Discussion

PPR is the most devastating and economically important disease of goats in Bangladesh. This study determined the prevalence of PPR in Chattogram metropolitan area on the basis of clinical signs and through molecular technique PCR. On the basis of clinical signs the prevalence of PPR in goat in this area was 22%. This finding was supported by previous studies conducted by Sarkar *et al.* (2011) and Ozkul *et al.* (2002) where they estimated prevalence as 20.6% and 20% respectively indicating that, the diagnosis of PPR on the basis of clinical findings were almost similar in different areas of the country. Based on PCR, prevalence of PPR found as 15.3% which was almost similar with the prevalence rate of Durrani *et al.* (2010) (15.7%) but less than the prevalence reported as 38% by Rahman *et al.* (2018). This variation might be due to sample size, geographical location and above all studies that reported prevalence rather than proportionate prevalence.

The only risk factor related with PPRV infection in goat in this study was sex of the animal. The association between sex and prevalence of PPR in this study was statistically significant ($p \leq 0.05$) and male animals were more at risk to PPR infection than female which was supported by many earlier studies (Parvez *et al.,* 2014; Sarkar *et al.,* 2011; Rahman *et al.,* 2004 and Mahajan *et al.,* 2011). The reason behind this might be genetic factors (Sarkar *et al.,* 2011). Moreover, males get frequent contact with females of different flocks during mating which might increase the infection rate of male animals (Mahajan *et al.,* 2012).

Season, breed, age and vaccination status were not found as significant risk factors related with PPRV infection in goat in this study. However, there were previously determined as potential risk factors (Abubakar *et al.,* 2009; Mahajan *et al.,* 2012; Islam *et al.,* 2014; Parvez *et al.,* 2014; Saminathan *et al.,* 2016; Rony *et al.,* 2017; Rahman *et al.,* 2016, 2018). The reason of non-significance of these factors might be due to large variation of sample size in different categories within same explanatory variables as well as small sample size.

Clinical signs associated with PPR infection in goat were recorded in this study and found that, the most significant clinical signs were fever (79.5%), diarrhea (85.2%), respiratory distress (53.4%) which were supported by Islam *et al.,* (2014) and Mahajan *et al.,* (2017). Moreover, animals suffered from mild to severe dehydration which was supported by Gurcay *et al.* (2013). All of these signs found more prevalent in the terminal stage of PPR infection in goat (Ahmad *et al.,* 2005; Mahajan *et al.,* 2017 and Gurcay *et al.*, 2012).

There are four reported distinct genetic lineages (Lineages I, II, III, and IV) of PPRV circulating in the world (Shaila *et al.,* 1996; Dhar *et al.,* 2002). In Asia only Lineage IV virus is circulated (Banyard *et al.,* 2010). Phylogenetic analysis based on partial N gene sequences of 16 Bangladeshi PPRV isolates shows that within lineage IV, Bangladeshi isolates formed separate well defined sub-groups along with another isolate from Bangladesh.

Some degree of divergence also existed within the circulating Bangladeshi isolates. The recent Bangladeshi isolates are somewhat divergent from the earlier Bangladeshi isolate specially the PPRV strain used for vaccination, indicating that the PPRV are in continuous evolution. This finding of the present study indicates further detailed molecular epidemiological study, preferably with full-length gene sequences.

CHAPTER-VI: Conclusion

This study gives an overall view of the prevalence of PPR in goats of Chattogram and associated risk factors which were closely related with it. In addition, it provides molecular characteristics of circulating PPRVs in Chattogram area. Both clinical signs and PCR were used to detect PPRVs. The proportionate prevalence was found 22% by clinical sign and 15.3% by PCR. Male animals were found affected more than female animals. The phylogenetic analysis revealed that PPRVs circulating in this area belonged to Lineage IV and closely related with the isolates of Bangladesh, India, Pakistan and China. The genetic character of existing PPR vaccine in this country formed different cluster from the isolates of this study which might be one of the reasons of vaccine failure.

CHAPTER-VII: Limitations and Recommendations

There are some limitations in this study. The study was conducted on the goats of Chattogram Metropolitan and surroundings areas which came to SAQTVH for treatment. This didn't reveal the true prevalence of PPR infection in goat in whole Chattogram division as well as in Bangladesh. There might be some information bias in this study. Further research at a large scale is needed to identify prevalence of PPR infection in goat of whole Chattogram division as well as in Bangladesh.

References

Abdollahpour, G.A., J. Najafi Roofi, F. Sasani and E. Sakhaie. (2006). Clinical and paraclinical findings of a recent outbreak of Peste des petits ruminants in Iran. J. Vet. Med., 53: 14-16.

Abraham, G. (2005). Epidemiology of Peste des petits ruminants virus in Ethiopia and Molecular studies on Virulence. Online version of PhD thesis. (EMVT, France).

Abraham, G. and A. Berhan. (2001). The use of antigen capture enzyme linked immunosorbent assay (ELISA) for the diagnosis of rinderpest and Peste des petits ruminants in Ethiopia. Trop. Anim. Hlth. and Prod., 33: 423-430.

Abraham, G., A. Sintayehu, G. Libeau, E. Albina, F. Roger, Y. Laekemariam, D. Abayneh and K.M. Awoke. (2005). Antibody sero-prevalences against Peste des petits ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia. Prev. Vet. Med., 70: 51-57.

Abu Elzein, E.M.E., M.M. Hassanien, A.I.A. Alfaleg, M.A.Abd Elhadi and F.M.T. Housawi (1990). Isolation of PPR virus from goats in Saudi Arabia. Vet. Rec., 127: 309-310.

Abu Elzein, E.M.E., F.M.T. Housawi, Y. Bashareek, A. A. Gameel, A. I. Al- Afaleq and E.C. Anderson. (2004). Severe PPR infection in Gazelles kept under semi-free range conditions in Saudi Arabia. J. Vet. Microbiol., 51: 68–71.

Abubakar, M. and Munir, M., (2014). Peste des Petits Ruminants (PPR) virus: An emerging threat to goat farming in Pakistan. Transbound. Emerg. Dis., 61(Suppl. 1): 1-4.

Abubakar, M., Jamal, S. M., Arshed, M. J., Hussain, M., & Ali, Q. (2009). Peste des petits ruminants virus (PPRV) infection; its association with species, seasonal variations and geography. *Tropical animal health and production*, *41*(7), 1197.

Abu-Elzein, E. M. E., Hassanien, M. M., Al-Afaleq, A. I., Abd-Elhadi, M. A., & Housawi, F. M. I. (1990). Isolation of peste des petits ruminants from goats in Saudi Arabia. *Veterinary Record*, *127*(12), 309-310.

Adombi, C.M., M. Lenenta, C.E. Lamien, D. Shamaki, Y.M.Koffi, A. Traore, R.Silber, E. Couacy-Hymann, S.C. Bodjo, J.A. Djaman, A.G. Luckins and A.Diallo. (2011). Monkey CV1 cell line expressing the sheep-goat SLAM protein: A highly sensitive cell line for the isolation of Peste des petits ruminants virus from pathological specimen. J.Virol. Methods.173:306-313

Ahmad, K., S.M. Jamal, Q. Ali and M. Hussain. (2005). An outbreak of Peste des petits ruminants (PPR) in a goat flock in Okara, Pakistan. Pakistan Vet. J., 25: 146-148.

Alkahatib, G., B. Massie and D.J. Briedis. (1988). Expression of bicistronic measles virus P/C mRNA by using hybrid adenoviruses: levels of C protein synthesised in vivo are unaffected by the presence or absence of the upstream P initiator codon. J. Virol., 62: 4059-4069.

Al-Tarazi, Y.H.M. and Daghall, G.J.K. (1997). Nasal carriage goats of Pasteurella hemolytica serotypes by the sheep and goats in the Jordan *Trop. Anim. Health and Prod.*, 29:177-179.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ., (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acid Res. 25: 3389–3402.

Amjad, H., M. Qamar-ul-Islam, A.M. Forsyth, T. Barrett and P.B. Rossiter. (1996). Peste des petits ruminants in goats. Pakistan Vet. Rec., 139: 118-119.

Anderson, J. and McKay. (1994). The detection of antibodies against PPR virus in cattle, sheep and goats and the possible implications to RP control programs. Epid. And Infect., 112: 225-231.

Anderson, J. and D. Sammin. (2005). Peste des petits ruminants in the Thrace region of Turkey. EMPRES Trans-boundary Animal Diseases Bulletin, 27: 12-15.

Aruni, A.W., P.S. Lalitha, A.C. Mohan, P. Chitravelu and S.P. Anbumani. (1998). Histopathological study of a natural outbreak of Peste des petits ruminants in goats of Tamilnadu. Small Rum. Res., 28: 233-240.

Awa, D.N., A. Ngagnou, E. Tefiang, D. Yaya and A. Njoya. (2002). Post vaccination and colostral Peste des petits ruminants antibody dynamics in research flocks of Kirdi goats and Foulbe sheep of north Cameroon. Prev. Vet. Med., 55: 265-271.

Ayari-Fakhfakh, E., A.Ghram, A. Bouattour, I. Larbi, L. Gribaa-Dridi, O. Kwiatek, M. Bouloy, G. Libeau, E. Albina and C. Cetre-Sossah. (2010). First serological investigation of Peste-des- etits-ruminants and Rift Valley fever in Tunisia. Vet J., 187: 402-404.

Bailey, D., A. Banyard, P. Dash, A. Ozkul and T. Barrett. (2005). Full genome sequence of Peste des petits ruminants virus, a member of the Morbillivirus genus. Virus Res., 110: 119–124.

Balamurugan V., Sen A., Saravanan P., Rasool T.J., Yadav M.P., Bandyopadhyay S.K. & Singh R.K. (2006) Development and characterization of a stable vero cell line constitutively expressing Peste des petits ruminants virus (PPRV) hemagglutinin protein and its potential use as antigen in enzyme-linked immunosorbent assay for serosurveillance of PPRV. Clinical and Vaccine Immunology 13, 1367–1372.

Balamurugan, V., A. Sen, P. Saravanan, R.P. Singh, R.K. Singh, T.J. Rasool and S.K. Bandyopadhyay. (2006). One-step Multiplex RT-PCR Assay for the Detection of Peste des petits ruminants virus in Clinical Samples. J. Vet. Res. Commun., 30: 655-666.

Balamurugan, V., Saravanan, P., Sen, A., Rajak, K. K., Venkatesan, G., Krishnamoorthy, P., ... & Singh, R. K. (2012). Prevalence of peste des petits ruminants among sheep and goats in India. *Journal of veterinary science*, *13*(3), 279-285.

Banik S.C., S.C. Podder, M.A. Samad and M.T. Islam. (2008). Sero-surveillance and immunization in sheep and goats against Peste des petits ruminants in Bangladesh. Bangl. J. Vet. Med., 6: 185–190.

Banyard, A.C., S. Parida, C. Batten, C. Oura, O. Kwiatek and G. Libeau. (2010). Global distribution of Peste des petits ruminants virus and prospects for improved diagnosis and control. J. Gen. Virol., 91: 2885–2897.

Barhoom, S. S., W.A. Hassan and T.A.R. Mohammed. (2000). Peste des petits ruminants in sheep in Iraq. Iraqi J. Vet. Sci., 13: 381-385.

Baron, M.D., M.S. Shaila and T. Barrett. (1993). Cloning and sequence analysis of the phosphoprotein gene of rinderpest virus. J. Gen. Virol., 74: 299-304.

Baron, M.D. and T. Barrett. (1995). Sequencing and analysis of the nucleocapsid (N) and the polymerase genes and the terminal extragenic domains of the vaccine strain of rinderpest virus. J. Gen. Virol., 76: 593-602.

Barrett, T., S.B. Shrimpton and S.E.H. Russell. (1985). Nucleotide sequence of the entire protein coding region of Canine Distemper Virus polymerase-associated (P) protein messenger RNA. Virus Res., 3: 367-372.

Begum, S. S., Mahato, G., Sharma, P., Sharma, K., Hussain, M., Das, B. C., ... & Muthuchelvan, D. (2016). Seroprevalence of peste des petits ruminants in goats in Assam, India. *Asian J. Anim. Vet. Adv*, *11*(3), 210-212.

Bellini, W.J., G. Englund, S. Rozenblatt, H. Arnheiter and C.D. Richardson. (1985). Measles virus P gene codes for two proteins. J. Virol., 53: 908-919.

Berhe, G., C. Minet, C. LeGoff, T. Barrett, A. Ngangnou, C. Grillet, G. Libeau, M. Fleming, D.N. Black and A. Diallo. (2003). Development of a dual recombinant vaccine to protect small ruminants against Peste-des-petits-ruminants virus and capripoxvirus infections. J. Virol., 77: 1571-1577.

Berhe, G. (2006). Development of Dual vaccines for the control of Peste des petits ruminants and Capripox infections of Small Ruminants. On line version of PhD thesis. (EMVT, France).

Bhuiyan, A. R. (2012). Epidemiology and pathology of Peste des Petits Ruminants (PPR) in Bangladesh and molecular characterization of the virus. *Department of Pathology,. Bangladesh Agricultural University,, Mymensingh, Bangladesh*.

Blain, F., P. Liston and D.J. Briedis. (1995). The carboxy-terminal 18 amino acids of the measles virus hemagglutinin are essential for its biological function. Bio. Biotech Commun., 214: 1232–1238.

Blumberg, B. and D. Kolakofsky. (1981). Intracellular vesicular stomatitis virus leader RNAs are found in nucleocapsid structures. J. Virol., 40: 568–576.

Bourdin, P. and A. Laurent-Vautier. (1967). Note sur la structure du virus de la Peste des petits ruminants. Rev. Elev. Méd. Vét. Pays Trop., 20: 383-386.

Braide, V.B. (1981). Peste des petits ruminants. World Anim. Review, 39: 25-28.

Brown C.C., J.C. Mariner and H.J.Olander. (1991). An immunohistochemical study of the pneumonia caused by Peste des petits ruminants virus. Vet. Pathol., 28: 166-170.

Bundza, A., A. Afshar, T.W. Dukes, D.J. Myers, G.C. Dulac and S.A. Becker . (1988). Experimental Peste des petits ruminants (goat plague) in goats and sheep. Can. J. Vet. Res., 52: 46-52.

Cartee, T.L., A.G. Megaw, A.G. Oomens and G.W. Wertz. (2003). Identification of a single amino acid change in the human respiratory syncytial virus L protein that affects transcriptional termination. J. Virol., 77: 7352–7360.

Chaisomchit, S., R. Wichajarn, N. Janejai and W. Chareonsiriwatana. (2005). Stability of genomic DNA in dried blood spots stored on filter paper. South Asian J. Trop. Med. Pub. Hlth, 36: 270–273.

Chowdhury E.H., Bhuiyan A.R., Rahman M.M., Siddique M.S. & Islam M.R. (2014) Natural peste des petits ruminants virus infection in Black Bengal goats:

virological, pathological and immunohistochemical investigation. BMC Veterinary Research 10, 263.

Coronel, E.C., T. Takimoto, K.G. Murti, N. Varich and A. Portner. (2001). Nucleocapsid incorporation into parainfluenza virus is regulated by specific interaction with matrix protein. J. Virol., 75: 1117–1123.

Couacy-Hymann, E., F. Roger, C. Hurard, J.P. Guillou, G. Libeau and A Diallo. (2002) . Rapid and sensitive detection of Peste des petits ruminants virus by a polymerase chain reaction assay. J. Virol. Meth., 100: 17-25.

Crowley, J., P. Dowling., J. Mennoma., J. Silvermann., D. Schuback., S. Cook and B. Blumberg. (1988). Sequence variability and function of measles virus 3' and 5' ends and intercistronic regions. Virol., 164: 498-506.

Curran, M.D. and B.K. Rima. (1992). The genes encoding the phosphoprotein and matrix proteins of phocine distemper virus. J. Gen. Virol., 73: 1587-1591.

Das, K.K., N.K. Shil and M.R. Islam. (2007). Sero-epidemiological investigation on Peste des petits ruminants in black Bengal goats. Bangl. J. Microbiol., 24: 143–145.

Das, S. K. (2001). Prospect and potentiality for goat farming in north eastern region of India–a review. *Agricultural Reviews*, *22*(3and4), 228-233.

Das, S.C., M.D. Baron and T. Barrett. (2000). Recovery and Characterization of a Chimeric Rinderpest Virus with the Glycoproteins of Peste des petits ruminants virus: Homologous F and H proteins are required for Virus Viability. J. Virol., 74: 90399047.

Debasis, J. and G. Mousumi. (2002). Incidence of an explosive outbreak of Peste des petits ruminants (PPR) in Black Bengal goats in Bankura District of West Bengal. Ind. Vet. J., 79: 739-740.

Devaux, P. and R. Cattaneo. (2004). Measles virus phosphoprotein gene products: conformational flexibility of the P/V protein amino-terminal domain and C protein infectivity factor function. J. Virol., 78: 11632-11640.

Dhar, P., B.P. Sreenivasa, T. Barrett, M. Corteyn, R.P. Singh and S.K. Bandyopadhyay. (2002). Recent epidemiology of Peste des petits ruminants virus (PPRV). Vet. Microbiol., 88: 153-159.

Diallo, A., T. Barrett, P.C. Lefevre and W.P Taylor. (1987). Comparison of proteins induced in cells infected with RP and PPR viruses. J. Gen. Virol., 68: 2033-2038. Diallo, A., T. Barrett., M. Barbron, G. Meyer and P.C. Lefèvre. (1994).

Cloning of the nucleocapsid protein gene of Peste des petits ruminants virus: relationship to other morbilliviruses. J. Gen. Virol., 75: 233-237.

Diallo, A., T.Barrett. M. Barbron, S.M. Subbarao and W.P. Taylor. (1989). Differentiation of rinderpest and Peste des petits ruminants viruses using specific cDNA clones. J. Virol. Meth., 23: 127-136.

Diallo, A. (1990). Morbillivirus group: genome organisation and proteins. Vet. Microbiol., 23: 155-163.

Diallo, A., G. Libeau, E. Couacy-Hymaun and M. Barbron. (1995). Recent developments in the diagnosis of rinderpest and Peste des petits ruminants. Vet. Microbiol., 44: 307-317.

Diallo A, Minet C, Le Goff C, *et al.*, (2007). The threat of peste des petits ruminants: progress in vaccine development for disease control. Vaccine;25(30):5591–7.

Diop, M.J. Sarr and G. Libeau. (2005). Evaluation of novel diagnostic tools for Peste des petits ruminants virus in naturally infected goat herds. Epid. and Infect., 133: 711717.

Durojaiye, O.A., W.P. Taylor and C. Smale. (1985). The ultastructure of Peste des petits ruminants virus. Zentral. für Veterinärmedizin, 32: 460-465.

Durrani, A. Z., Kamal, N., Mehmood, N., & Shakoori, A. R. (2010). Prevalence of peste des petits ruminants (KATA) in sheep and goats of Punjab. *Pakistan Journal of Zoology*, *42*(3).

Edgar RC (2004). "MUSCLE: multiple sequence alignment with high accuracy and high throughput". Nucleic Acids Research. 32 (5): 1792–97.

El Arbi A.S., El Mamy A.B., Salami H., Isselmou E., Kwiatek O., Libeau G. et al. (2014) Peste des petits ruminants virus, Mauritania. Emerging Infectious Diseases 20, 333–336.

El Hag Ali, B. and W.P. Taylor. (1988). An investigation of rinderpest virus transmission and maintenance by sheep, goats and cattle. Bull. Anim. Hlth. and Prod. Afr., 36: 290-294.

EMPRES, (Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases). (1999). Recognizing Peste des petits ruminants: a field manual. Rome, Italy: FAO (Food and Agriculture Organization of the United Nations).

EMPRES, (Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases). (2008). Recognizing Peste des petits ruminants: a field manual. Rome, Italy: FAO (Food and Agriculture Organization of the United Nations).

EMPRES, (Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases). (2009). Recognizing Peste des petits ruminants: a field manual. Rome, Italy: FAO (Food and Agriculture Organization of the United Nations).

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791.

Food and Agricultural Organization(FAO), a.I.A.E.A. (2010). Joint FAO/IAEA Programme: Nuclear Technique in Food and Agriculture: Livestock: Black Bengal - A Promising goat genetic resource of Bangladesh Joint FAO and IAEA Programme Accessed on June 09, 2011.

Forsyth, M. A., & Barrett, T. (1995). Evaluation of polymerase chain reaction for the detection and characterisation of rinderpest and peste des petits ruminants viruses for epidemiological studies. *Virus research*, *39*(2-3), 151-163.

Furley, C.W., T.W. Taylor and T.U. Obi. (1987). An outbreak of PPR in a zoological collection. Vet. Rec., 121: 443-447.

Gargadennec, L and A. Lalanne. (1942). La Peste des petits ruminants. Bulletin des Services Zoo Technique et des Epizootie de l'Afrique Occidentale Française, 5: 16 –21.

Gibbs, E. P. J.; Taylor, W. P.; Lawman, M. J. P. (1979). Classification of Peste des petits

ruminants (PPR) virus as the fourth member of the genus Morbilivirus Intervirology. 15:35-41.

Gombart, A.F., A. Hirano and T.C. Wong. (1993). Conformational maturation of measles virus nucleocapsid protein. J. Virol., 67: 4133–4141.

Goswami, K.B., R. Saha and S. Chakrabarty. (1998). Studies on age-specific and monthwise mortality pattern and sequele to an onslaught of Peste des petits ruminants on reproductive behaviour of goat. Indian J. Anim. Hlth., 37: 29-31.

Gurcay, M., Kizil, O., & Baydar, E. (2013). Peste Des Petits Ruminants (PPR) Virus Infections in Goats in the Eastern Anatolia of Turkey. *Kafkas Univ Vet Fak Derg*, *19*, 93-98.

Haffar, A., G. Libeau, A. Moussa, M. Cecile and A. Diallo. (1999). The matrix protein gene sequence analysis reveals close relationship between Peste des petits ruminants virus (PPRV) and dolphin morbillivirus. Virus Res., 64: 69–75.

Haines D.M., E.G. Clark and E.J. Dubovi. (1992). Monoclonal antibody based immunohistochemical detection of bovine viral diarrhea virus in formalin-fixed, paraffin- mbedded tissues. Vet. Pathol., 29: 27–32.

Hamdy, F.M., A.H. Dardiri, O. Nduaka, S.S. Breese and E.C. Ihemelandu. (1976). Etiology of the stomatitis pneumoenteritis complex in Nigerian dwarf goats. Can. J. Comp. Med., 40: 276-284.

Haroun, M., I. Hajer, M. Mukhtar and B.E. Ali. (2002). Detection of antibodies against Peste des petits ruminants virus in sera of cattle, camels, sheep and goats in Sudan. Vet. Res. Commun., 26: 537-541.

Hasan, M. (2012). Seroprevalence of Peste des petits ruminants (PPR) in goat and sheep in selected areas of Bangladesh (Doctoral dissertation).

Horikami, S.M., S. Smallwood and S.A Moyer. (1996). The Sendai virus V protein interacts with the NP protein to regulate viral genome RNA replication. Virol., 222: 383– 390.

House, J.A., A.E. Castro and W.P. Heuschele. (1992). Peste des petits ruminants. Vet. Diagn. Virol. Pract. guide, P 212-215.

Hu, A.H., R. Cattaneo., S. Schwartz and E. Norrby. (1994). Role of N-linked oligosaccharide chains in the processing and antigenicity of measles virus hemagglutinin protein. J. Gen. Virol., 75: 1043–1052.

Huber, M., R. Cattaneo, P. Spielhofer, C. Orvell, E. Norrby, M. Messerli, J.C. Perriard and M.A. Billeter. (1991). Measles virus phosphoprotein retains the nucleocapsid protein in the cytoplasm. Virol., 185: 299–308.

Islam, M.R., M. Shamsuddin, P.M. Das and M.L. Dewan. (2001). An outbreak of Peste des petits ruminants in Black Bengal goats in Mymensingh, Bangladesh. The Bangl. Vet., 18: 14-19.

Islam, M.R., M. Giasuddin, M.M. Rahman and M.A Kafi. (2003). Antibiotic combined hyperimmune serum therapy for PPR infected goats. Bangl. J. Vet. Med., 1: 49-51.

Islam, K., Ahad, A., Altaf Mahmood, M., Rahman, M., Islam, M. Z., Bin, M. H., ... & Biswas, P. K. (2014). Prevalence and Clinico–Pathological Features of Peste des Petits Ruminants in Different Breeds of Goats and their Response to Antimicrobials. *Journal of Infection and Molecular Biology*, *2*, 43-48.

Ismail, T.H., M.K. Yamanaka, J.K. Saliki, A. Elkhoy, C. Mebus and T. Yilma. (1995). Cloning and expression of the nucleoprotein of PPR virus in baculovirus for use in serological diagnosis. Virol., 208: 776-778.

Jeremiah, T., J.T. Saliki, C. C. Brown, J.A. House and E.J. Dubovi. (1994). Differential immunohistochemical staining of Peste des petits ruminants and rinderpest antigens in formalin-fixed, paraffin-embedded tissues using monoclonal and polyclonal antibodies. J. Vet. Diagn. Invest., 6: 96-98.

Johnson, R.H. and J.S. Ritchie. (1968). A virus associated with pseudorinderpest in Nigerian dwarf goats. Bull. Epizoot. Dis. Afr., 16: 411-417.

Kailash, U., S. Hedau, V. Gopalkrishan, S. Katiyar and B.C. Das. (2002). A simple 'paper smear' method for dry collection, transport and storage of cervical cytological specimens for rapid screening of HPV infection by PCR. J. Med. Microbiol., 51: 606-683.

Kataria, A.K., N. Kataria and A.K. Gahlot. (2007). Large scale outbreaks of Peste des petits ruminants in sheep and goats in Thar desert of India. Slov. Vet. Res., 44: 123-132.

Kerur, N., M.K. Jhala and C.G. Joshi. (2008). Genetic characterization of Indian Peste des petits ruminants virus (PPRV) by sequencing and phylogenetic analysis of fusion protein and nucleoprotein gene segments. Res. Vet. Sci., 85: 176–183.

Khan H.A., M. Siddique, M.J. Arshad, Q.M. Khan and S.U. Rehman. (2005). Sero-revalence of Peste des petits ruminants (PPR) virus in sheep and goats in Punjab province of Pakistan. Pakistan Vet. J., 27: 109-112.

Kozak, M. (1986). Point mutations define a sequence flanking the AUG initiation codon that modulates translation by eukaryotic ribosomes. Cell, 44: 283-292.

Kul, O., Kabakci, N., Atmaca, H. T., & Özkul, A. (2007). Natural peste des petits ruminants virus infection: novel pathologic findings resembling other morbillivirus infections. *Veterinary pathology*, *44*(4), 479-486.

Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547–1549.

Kumar, P., B.N. Tripathi, A.K. Sharma, R. Kumar, B.P. Sreenivasa, R.P. Singh, P. Dhar and S.K. Bandyopadhyay. (2004). Pathological and immunohistochemical study of experimental Peste des petits ruminants virus infection in goats. J. Vet. Med. B. Infect. Dis. Vet. Pub. Hlth., 51: 153-159.

Kwiatek, O., C.G. Minet, C.C. Hurardy, E. Carlssonz, B.Karimovz, E. Albina, A. Diallo and G. Libeau. (2007). Peste des Petits Ruminants (PPR) Outbreak in Tajikistan. J. Comp. Path. 136: 111-119.

Langedijk, J., F. Daus and J. von Oirschot. (1997). Sequence and structure alignment of Paramyxoviridae attachment proteins and discovery of enzymatic activity for a morbillivirus hemagglutinin. J. Virol., 71: 6155–6167.

Laurent, A. (1968). Aspects biologiques de la multiplication du virus de la Peste des petits ruminants sur les cultures cellulaire. Rev. Elev. Méd. Vét. Pays trop., 21: 297-308.

Lecouturier, V., J. Fayolle, M. Caballero, J. Carabana, M.L. Celma, R. Fernandez-Muñoz., T.F. Wild and R. Buckland. (1996). Identification of two amino acids in the hemagglutinin glycoprotein of measles virus (MV) that governs hemadsorption, HeLa cell fusion, and CD46 downregulation: phenotypic markers that differentiate vaccine and wild-type MV strains. J. Virol., 70: 4200–4204.

Lefevre, P.C. (1987). Peste des petits ruminants et infection bovipestique des ovins et caprins (Synthèse bibliographique), Institute d'Elevage et de Médecine vétérinaire des pays tropicaux, 94704 Maison-Alfort, France, P 99.

Lefevre, P.C. and A. Diallo. (1990). Peste des petits ruminants virus. Rev. Sci. Tech. Off. Int. Epiz., 9: 951-965.

Lefèvre, P. C., Diallo, A., Schenkel, F., Hussein, S., & Staak, G. (1991). Serological evidence of peste des petits ruminants in Jordan. *The Veterinary Record*, *128*(5), 110.

Leissel, T. and P. Salama. (2003). Assessing field efficacy for measles in famine – affected rural Ethiopia. The American J. Trop. Med. and Hyg., 68: 545-546.

Li, C.C., I.A. Beck., K.D. Seide and L.M. Frenkel. (2004). Persistence of Humanimmun odeficiency virus type 1 subtypes B DNA in dried-blood samples on FTA filter paper. J. Clin. Microbiol., 42: 3847–3849.

Libeau, G., A. Diallo, D. Calvez and P.C. Lefevre. (1992). Competitive ELISA using antiN monoclonal antibodies for specific detection of RP antibodies in cattle and small ruminants. Vet. Microbiol., 31: 147-160.

Libeau, G., A. Diallo, F. Colas and L. Guerre. (1994). Rapid differential diagnosis of rinderpest and Peste des petits ruminants using an immunocapture ELISA. Vet. Rec., 134: 300-304.

Livestock Economy at a Glance, DLS (2017-18).

Lundervold, M., E.J. Milner-Gulland, C.J. O'Callaghan, C. Hamblin, A. Corteyn and A. Macmillan. (2004). A serological survey of ruminant livestock in Kazakhstan during post-soviet transitions in farming and disease control. Acta. Vet. Scand., 45: 211– 224.

Maganga G.D., Verrier D., Zerbinati R.M., Drosten C., Drexler J.F. & Leroy E.M. (2013). Molecular typing of PPRV strains detected during an outbreak in sheep and goats in south-eastern Gabon in 2011. Virology Journal 10, 82

Mahajan, S., Agrawal, R., Kumar, M., Mohan, A., & Pande, N. (2012). Risk of seroconversion to peste des petits ruminants (PPR) and its association with species, sex, age and migration. *Small ruminant research*, *104*(1-3), 195-200.

Mahajan, V., Filia, G., Bal, M. S., Kaur, G., Sharma, S., Dantotia, A., & Singh, C. K. (2017). Outbreaks of peste des petits ruminants (PPR) in goats in Punjab, India. *Int. J. Curr. Microbiol. App. Sci*, *6*(8), 3705-3710.

Mahapatra, M., S. Parida, B.G. Egziabher, A. Diallo and T. Barrett. (2003). Sequence analysis of the phosphoprotein gene of Peste des petits ruminants (PPR) virus: editing of the gene transcript. Virus Res., 96: 85-98.

Maillard, J. C., K.P. Van, T. Nguyen, T.N. Van, C. Berthouly, G. Libeau and O. Kwiatek. (2008). Examples of probable host-pathogen coadaptation/ co-evolution in isolated farmed animal populations in the mountainous regions of North Vietnam. Ann. NY. Acad. Sci., 1149: 259– 262.

Malur, A.G., S.K. Choudhary, B.P. De and A.K. Banerjee. (2002). Role of a highly conserved NH (2)-terminal domain of the human parainfluenza virus type 3 RNA polymerase. J. Virol., 76: 8101–8109.

Martha, Y. (2001). Séquence et analyse du gène de la polymérase (L) et de la séquence extragénique terminale du virus de la Peste des petits ruminants. Mémoire de Maîtrise -es- Sciences Vétérinaires ; Ecole Nationale vétérinaire de Toulouse,

McIlhatton, M.A., M.D. Curran and B.K. Rima. (1997). Nucleotide sequence analysis of the large (L) genes of phocine distemper virus and canine distemper virus (corrected sequence). J. Gen. Virol., 78: 571–576.

Meyer, G. and A. Diallo. (1995). The nucleotide sequence of the fusion protein gene of the PPR virus: The long untranslated region in the 5-end of the F-protein gene of morbilliviruses seems to be specific to each virus. Virus Res., 37: 23-38.

Michaud, A., P. Gil, O. Kwiatek, S. Prome, L. Dixon, L. Romero, M.F.Le Potier, M. Arias, E. Couacy-Hymann, F. Roger, G. Libeau and E. Albina. (2007). Long-term storage at tropical temperature of dried-blood filter papers for detection and genotyping of RNA and DNA viruses by direct PCR. J. Virol. Meth., 146: 257265.

Mitra-Kaushik, S., R. Nayak and M.S. Shaila. (2001). Identification of a cytotoxic Tcell epitope on the recombinant nucleocapsid proteins of Rinderpest and Peste des petits ruminants viruses presented as assembled nucleocapsids. Virol., 279: 210– 220.

Moll, M., H.D. Klenk and A. Maisner. (2002). Importance of the cytoplasmic tails of the measles virus glycoproteins for fusogenic activity and the generation of recombinant measles viruses. J. Virol., 76: 7174–7186.

Mondal, A.K., A.P. Chottopadhyay, S.D. Sarkar, G.R. Saha and M.K. Bhowmik. (1995). Report on epizootiological and clinicopathological observations on Peste des petits ruminants (PPR) of goats in West Bengal. Indian J. Anim. Hlth., 34: 145-148.

Mornet, P., J. Orue, Y. Gillbert, G. Thiery and S Mamadou. (1956). La Peste des petits ruminants en Afrique occidentail francaise; ses rapports avec la peste bovines. Rev. Elev. Med.Vet. Pays Trop., 9: 313-342.

Munir, M., M. Siddique, A. Shehzad, S. Zohari and S. Tah. (2008). Seroprevalence of Antibodies to Peste des petits ruminants at various Government Livestock Farms of Punjab, Pakistan. A. J. Epide., 1: 82-90.

Munir, M., Zohari, S., Suluku, R., LeBlanc, N., Kanu, S., Sankoh, F. A. R., ... & Ståhl, K. (2012). Genetic characterization of peste des petits ruminants virus, Sierra Leone. *Emerging infectious diseases*, *18*(1), 193.

Muthuchelvan, D., A. Sanyal, R.P. Singh, D. Hemadri, A. Sen, B.P. Sreenivasa, R. K. Singh and S.K. Bandyopadhyay. (2005). Comparative sequence analysis of the large polymerase protein (L) gene of Peste des petits ruminants (PPR) vaccine virus of Indian origin. Arch. Virol., 150: 2467-2481.

Nanda, Y.P., A. Chatterjee, A.K. Purohit, A. Diallo, K. Innui, R.N. Sharma, G. Libeau, J.A. Thevasagayam, A. Bruning, R.P. Kitching, J. Anderson, T. Barrett and W.P Taylor. (1996). The isolation of Peste des petits ruminants virus from northern India. Vet. Microbiol., 51.

Nawathe D.R. 1984. Control of Peste des petits ruminants in Nigeria. Prev. Vet. Med., 2:147–155. Nigeria, 20-24 July (1987). Pudoc (Centre for Agricultural Publishing and Documentation), Wageningen., The Netherlands. P 29-37.

Obi, T.U. (1983). Studies on the epidemiology of PPR in Southern Nigeria. *Trop. Vet.*, 1:209-217.

Obi, T. U. (1984). The detection of PPR virus antigen by agar gel precipitation test and counter-immunoelectrophoresis. J. Hyg., 93: 579-586.

Obi, T.U. and C.K. Ojeh. (1989). Dot enzyme immunoassay for detection of PPR virus antigen from infected caprine tissues. J. Clin. Microbiol., 27: 2096-2099.

Ogunsanmi, A., E. Awe, T.Obi and V Taiwo. (2003). Peste des petits ruminants virus (PPR) antibodies in African Gray Duiker (Sylvicapra grimmia). African J. Biom. Res., 6: 59–61.

Ohno, S., N. Ono, M. Takeda, K. Takeuchi and Y Yanagi. (2004). Dissection of measles virus V protein in relation to its ability to block alpha/beta interferon signal transduction. J. Gen. Virol., 85: 299–2999.

OIE (Office International des Epizooties/ World Organization for Animal Health). (2004). Peste des petits ruminants. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chap. 2.1.5.

Olaleye, D.D., B.O. Ikede and O.A. Durojaiye. (1989). Fluid electrolyte and hematological changes in experimental PPR virus infection in West African Dwarf goats. Cytobiol., 58: 39-51.

Opasina, B.A. (1983). Epidemiology of PPR [pest of small ruminants] in the humid forest and the derived savanna zones [Nigeria] in Peste des petits ruminants (PPR) in sheep and goats. Hill, D.H. (Ed.). Proceedings of the international workshop held at IITA, Ibadan, Nigeria, 24-26 September 1980. P 14-21.

Osman, N.A., A.S. Ali, M.E.A. Rahman and M.A Fadol. (2009). Pathological, serological and virological findings in goats experimentally infected with Sudanese Peste des petits ruminants (PPR) virus isolates. J. Gen. and Mol. Virol., 1:1-6.

Otte, E. (1960). Clinical studies on abu nini in the Sudan: a contagious disease of goats and sheep possibly caused by pleuropneumonia-like organism. Vet. Record, 72.

Ozkul, A., Akca, Y., Alkan, F., Barrett, T., Karaoglu, T., Dagalp, S.B., Anderson, J., Yesilbag, K., Cokcaliskan, C., Gensay, A. and Burgu, I. (2002). Prevalence,

distribution, and host range of Peste des petits ruminants virus, Turkey. *Emerg. Infect. Dis.*, 8:708-712.

Palosaari, H., J.P. Parisien, J.J. Rodriguez, C. M. Ulane and C.M. Horvath. (2003). STAT protein interference and suppression of cytokine signal transduction by measles virus V protein. J. Virol., 77: 7635-7644.

Pandey, K.D., M.D. Baron and T. Barrett. (1992). Differential diagnosis of rinderpest and PPR using biotinylated cDNA probes. Vet. Rec., 131: 199-200.

Parvez MA, Khatun R, Al–Noman MA (2014). Prevalence and associated risk factors of peste des petits ruminants in goats in Chittagong district, Bangladesh. Res. J. Vet. Pract. 2 (1S): 14 – 17.

Pawaiya, R.V.S., N. Mishra, P.S.K. Bhagwan and S.C. Dubay. (2004). Pathology and distribution of antigen in goats naturally infected with PPRV. Ind. J. Anim. Sci., 74: 35-40.

Peeples, M.E. (1991). Paramyxovirus M proteins: pulling it all together and taking it on the road. In: David W. Kingsbury (Ed.). The Paramyxoviruses. New York: Plenum Press, 427–456.

Peter, B. (2001). Vaccination. Van Roitt, Jonathan brostoff and David male. Immunology 6 the dn, P 277-87. Mosby Edinburgh.

Pitcovski, J., E. Shmueli. S. Krispel and N. Levi. (1999). Storage on filter paper for genetic analysis. J. Virol. Meth., 83: 21-26.

Plemper, R.K., A.L. Hammond and R. Cattaneo. (2000). Characterization of a region of the measles virus hemagglutinin sufficient for its dimerization. J. Virol., 74: 6485– 6493.

Plemper, R.K., A.L. Hammond and R. Cattaneo. (2001). Measles virus envelope glycoproteins hetero-oligomerize in the endoplasmic reticulum. J. Biol and Chem,. 276: 44239–44246.

Plemper, R.K., A.L. Hammond, D. Gerlier, A.K. Fielding and R. Cattaneo. (2002). Strength of envelope protein interaction modulates cytopathicity of measles virus. J. Virol., 76: 5051–5061.

Prado, I., D. Rosario, L. Bernardo, M. Alvarez, R. Rodriguez, S. Vazquez and M.G. Guzman. (2005). PCR detection of dengue virus using dried whole blood spotted on filter paper. J. Virol. Meth., 1: 75–81.

Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff. (2000). Veterinary Medicine. 9th Edn. W.B. Saunders Company Limited. London. P 1059-1063.

Rager, M., S. Vongpunsawad., W. P. Duprex and R. Cattaneo. (2002). Polyploid measles virus with hexameric genome length.The EMBO J., 21: 2364–2372.

Rahman, M. M., Alam, K. J., Alam, M. S., Hasan, M. M., & Moonmoon, M. (2016). A study on prevalence of peste des petits ruminant (PPR) in goat at Bagmara upazilla at Rajshahi district in Bangladesh. *Research in Agriculture Livestock and Fisheries*, *3*(2), 339-344.

Rahman, M. M., Parvin, R., Bhuiyan, A. R., Giasuddin, M., Chowdhury, S. M. Z. H., Islam, M. R., & Chowdhury, E. H. (2016). Genetic characterization of Peste des petits ruminants virus circulating in Bangladesh. *British Journal of Virology*, *3*(4), 115-122.

Raj, G.D., A.S.S. Kumar, M.S. Shaila, K. Nachimuthu and K.S. Palaniswami. (2003). Molecular epidemiology of Peste des petits ruminants viruses from southern India. Vet. Rec., 152: 264-266.

Randall, R.E. and A. Bermingham. (1996). NP-P and NP-V interactions of the paramyxo virus simian virus 5 examined using a novel protein-protein capture assay. Virol., 224: 121–129.

Razzaque, M.A., M.B. Rahman, M.A. Kafi, M.R. Islam, M.F.R. Khan and K.A.M.N.H. Nazir. (2004). Application of C-ELISA for detection of PPRV-specific antibodies in domestic ruminants in different areas of Mymensingh, Bangladesh. Mol. Biol. And Biotech J., 2: 40–43.

Rima, B.K., K. Baczko, D.K. Clarke, M.D. Curran, S.J. Martin, M.A. Billeter and V. Ter Meulen. (1986). Characterization of clones for the sixth (L) gene and transcriptional map for morbilliviruses. J. Gen. Virol., 67: 1971-1978.

Roeder, P.L., G. Abraham, Kenfe and T. Barrett. (1994). PPR in Ethiopian goats. Trop. Anim. Hlth. Prod., 26: 69-73.

Roger, F., M. G. Yesus, G. Libeau, A. Diallo, L. M. Yigezu and T.Yilma. (2001). Detection of antibodies of rinderpest and Peste des petits ruminants viruses (Paramyxoviridae, Morbillivirus) during a new epizootic disease in Ethiopian camels (Camelus dromedarius). Rev. Med. Vet., 152: 265–268.

Romero, C.H., T. Barrett, R.W. Chamberlain, R.P. Kitching, M. Fleming and D.N. Black. (1994). Recombinant capripoxvirus expressing the hemagglutinin protein gene of rinderpest virus: protection of cattle against rinderpest and lumpy skin disease viruses. Virol., 204: 425-428.

Rony M.S., Rahman A.K., Alam M.M., Dhand N. & Ward M.P. (2017) Peste des Petits Ruminants risk factors and space-time clusters in Mymensingh. Transboundary and emerging diseases: Bangladesh.

Rossiter, P.B. (1994). Rinderpest. In: Coetzer, J.A.W., Thompson, G.R., Tustin, R.C. and Kriek, N.P. (Eds.), Infectious Diseases of Livestock with Special Reference to South Africa, vol. 2, Oxford University Press, Cape Town, P 735-757.

Rossiter, P.B. and W.P. Taylor. (1994). Peste des petits ruminants. In infect. Dis. livestock, 2: 758.

Rowland, A. C., G.R. Scott and D.H. Hill. (1969). The pathology of an erosive stomatitis and enteritis in West African dwarf goats. J. Path., 98: 83-87.

Rowland, A.C. and P. Bourdin. (1970). The histological relationship between Peste des petits ruminants and kata in West Africa. Revue d'Elevage et deMe£ decineVe£ te£ rinaire des Pays Tropicaux, 23 : 301-307.

Rowland, A.C., G.R. Scott and S. Ramachandran. (1971). A comparative study of Peste des petits ruminants and Kata in West African dwarf goats. Anim. Hlth. Prod., 3: 241247.

Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich and N. Arnheim. (1985). Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Sci., 230: 1350-1354.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, *4*(4), 406-425.

Saliki, J.T., C.C. Brown, J.A. House and E.J. Dubovi. (1994). Differential immunohistochemical staining of Peste des petits ruminants and rinderpest antigens in formalinfixed, paraffin embedded tissues using monoclonal and polyclonal antibodies. J. Vet. Diagn. Invest., 6: 96-98.

Saliki, J.T. (1998). Peste des petits ruminants. In: US Animal Health Association, Committee on Foreign Animal Disease. Foreign Animal Diseases: the Gray Book. Ed 6. Part IV. Richmond, VA: US Animal Health Assoc.

Samad, M.A. (2008). Animal Husbandry and Veterinary Science. Volume 2, 1st Pub., LEP, Mymensingh, Bangladesh, p 1512-1514.

Saminathan, M., Rana, R., Ramakrishnan, M. A., Karthik, K., Malik, Y. S., & Dhama, K. (2016). Prevalence, diagnosis, management and control of important

diseases of ruminants with special reference to Indian scenario. *J Exp Biol Agric Sci*, *4*(3S), 338-367.

Sanger F and Coulson AR. (1975). A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J. Mol. Biol*. 94 (3): 441–8.

Saravanan, P., R.P. Singh, V.Balamurugan, P. Dhar, B.P. Sreenivas and D. Muthuchelvan. (2004). Development of a N gene-based PCR-ELISA for detection of Peste des petits ruminants virus in clinical samples. Acta Virol., 48: 249-255.

Sarker, S., & Islam, M. H. (2011). Prevalence and risk factor assessment of Peste des petits ruminants in goats in Rajshahi, Bangladesh. *Veterinary world*, *4*(12), 546.

Sata, T., T. Kurata, Y. Aoyama, M. Sakaguchi, K. Yamanouchi and K. Takeda. (1986). Analysis of viral antigens in giant cells of measles pneumonia by immunoperoxidase method. Virchows Archiv.: Pathol. Ana. and Histopathol., 410: 133-138.

Scott, G. R. (1981). Rinderpest and Peste des petits ruminants. In Gibbs, E.P.J. (Ed.). Virus Diseases of Food Animals. Vol. II Disease Monographs. Academic Press, New York. P 401– 425.

Seifert, H.S.H. (1996). Tropical animal health Kluwer Academic publishers, London.

Sergeny, M.A., I. M. Reda, E. E. A. Sarker and A. A. Abourowash. (1992). Pathology of PPR in sheep in Egypt. J. Comp. Pathol., 5: 25-39.

Seth, S. and M.S. Shaila. (2001). The hemagglutinin-neuraminidase protein of Peste des petits ruminants virus is biologically active when transiently expressed in mammalian cells. Virus Res., 75: 169–177.

Shaffer, J.A., W.J. Bellini and P.A. Rota. (2003). The C protein of measles virus inhibits the type I interferon response. Virol., 315: 389-397.

Shaila M.S., Purushothaman V., Bhavasar D., Venugopal K. & Venkatesan R.A. (1989) Peste des petits ruminants of sheep in India. The Veterinary Record 125, 602.

Shaila, M. S., D. Shamaki, A.M. Forsyth, A. Diallo, L. Goatley, R.P. Kitching and T. Barrett. (1996). Geographic distribution and epidemiology of Peste des petits ruminants viruses. Virus Res., 43: 149-153.

Shanthikumar, S.R., S.A. Malachi and K.A Majiyagbe. (1998). Rinderpest like outbreak in free living wild-life in Nigeria. Vet. Rec., 117: 469-470.

Shukla, N., Singh, K.C.P., Hirpurkar, (2008). Seroprevalence of peste des petits ruminants in sheep in Chhattishgarh. Indian J. Small Rum. 14 (1), 131–132.

Siddiqui, M. S. I., Ahasan, A., Islam, N., Kundu, P., Munshi, M. N., & Chowdhury, E. H. (2014). Peste des Petits Ruminants (PPR) virus antibodies in goats and cattle of the Saint Martin s Island in Bangladesh. *Bangladesh Veterinarian*, *31*(2), 55-59.

Sidhu, M.S., W. Husar., S.D. Cook., P.C. Dowling and S.A. Udem. (1993). Canine distemper terminal and intergenic non-protein coding nucleotide sequences: completion of the entire CDV genome sequence. Virol., 193: 66-72.

Sil, B. K., Rahman, M. M., Taimur, M. J. F. A., & Sarker, A. J. (1995, December). Observation of outbreaks of PPR in organized goat farms and its control strategy. In *Annal Conference of the Bangladesh Society of Veterinary Education and Research, BARC, Dhaka*.Barrett, T., Pronob, D., Sreenivasa, B.P. and Corteyn, M. (1997). Recent epidemiology of PPRV. *Vet. Microbiol.*, 88:125-130.

Singh R.K., Balamurugan V., Bhanuprakash V., Sen A., Saravanan P. & Pal Yadav M. (2009) Possible control and eradication of peste des petits ruminants from India: technical aspects. Veterinaria Italiana 45, 449–462.

Singh, R.P., P. Saravanan, B.P. Sreenivasa, R.K. Singh and S.K Bandyopadhyay. (2004). Prevalence and distribution of Peste des petits ruminants virus infection in small ruminants in India. Rev. Sci. Tech. Off. Int. Epiz., 23: 807-819.

Sinnathamby, G., G.J. Renukaradhya, M. Rajashekhar, R. Nayak and M.S. Shaila. (2001). Immune responses in goats to H-N glycoprotein of PPRV: identification of a T- cell determinant. Vac., 19: 4816-4823.

Smith, H.A., T.C. Jones and R.D. Hunt. (1974). Veterinary Pathology. Lea & Febiger, Philadelphia. 4th edn. P 1224.

Sugiyama, M., N. Ito, N. Minamoto and S. Tanaka. (2002). Identification of immunodominant neutralizing epitopes on the hemagglutinin protein of rinderpest virus. J. Virol., 76: 1691–1696.

Susan, E. and B.S. Aiello. (1998). The Merck Veterinary Manual. 8th edn. Merck and Co., INC. Whitehouse Station, N.J., U.S.A. P 539-541.

Swai, E.S., A.Kapaga, F. Kivaria, D. Tinuga, G. Joshua and P. Sanka. (2009) . Prevalence and distribution of Peste des petits ruminants virus antibodies in various districts of Tanzania. Vet. Res. Commun., 33: 927–936.

Sweetman, D.A., J. Miskin and M.D. Baron. (2001). Rinderpest virus C and V proteins interact with the major (L) component of the viral polymerase. Virol., 281: 193–204.

Takeuchi, K., M. Takeda, N. Miyajima, Y. Ami., N. Nagata, Y. Suzaki, J. Shahnewaz, S. Kadota and K. Nagata. (2005). Stringent requirement for the C protein of wild-type measles virus for growth both in vitro and in macaques. J. Virol., 79: 7838-7844.

Takeuchi, K., S. Kadota, M. Takeda, N. Miyajima and K. Nagata. (2003). Measles virus V protein blocks interferon (IFN)-alpha/beta but not IFN-gamma signalling by inhibiting STAT1 and STAT2 phosphorylation. FEBS Lett., 545: 177-182.

Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor–joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030–11035.

Tatsuo, H., N. Ono and Y. Yanagi. (2001). Morbilliviruses use signaling lymphocyte activation molecules (CD150) as cellular receptors. J. Virol., 75: 5842-5850.

Taylor, W. P. $(1979)_a$. Serological studies with the virus of Peste des petits ruminants in Nigeria. Res. Vet. Sc., 26: 236-242.

Taylor, W.P. $(1979)_b$. Protection of goats against PPR with attenuated RP virus. Res. Vet. Sci., 27: 321-324.

Taylor, W.P. (1984). The distribution and epidemiology of PPR. Prev. Vet. Med., 2: 157166.

Taylor, W.P., S. Abusaidy and T. Barret. (1990). The epidemiology of PPR in the sultanate of Oman. Vet. Micro., 22: 341-352.

Thrusfield M. (2005). Veterinary Epidemiology, 2nd edition, Black well scientific, London. 225–228.

Toplu, N. (2004). Characteristic and non-characteristic pathological findings in Peste des petits ruminants (PPR) of sheep in the Ege district of Turkey. J. Comp. Pathol., 131: 135- 141.

Van Regenmortel, M.H.V., C.M. Fauquet, and D.H.L. Bishop. (2000). Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of

Viruses. Academic Press, San Diego-San Francisco-New York-Boston-London-SydneyTokyo.

Von Messling, V., N. Svitek and R. Cattaneo. (2006). Receptor (SLAM [CD150]) recognition and the V protein sustain swift lymphocyte-based invasion of mucosal tissue and lymphatic organs by a morbillivirus. J. Virol., 80: 6084-6092.

Wadhwa, D.R., B. Prasad, V. Subash, A.K. Sharma and S. Verma. (2002). Occurrence of Peste des petits ruminants (PPR) like outbreak among migratory goats and sheep in Himachal Pradesh. Ind. J. Vet. Med., 22: 105-106.

Wambura, P.N. (2006). Impregnation and storage of Newcastle disease virus on to filter papers and detection of viral RNA by a single tube RT-PCR assay. African J. Biotech., 5: 21-24.

Wamwayi, H. M., Rossiter, P. B., Kariuki, D. P., Wafula, J. S., Barrett, T., & Anderson, J. (1995). Peste des petits ruminants antibodies in East Africa. *Veterinary record: journal of the British Veterinary Association*.

Wang, Z., J. Bao, X.Wu, U. Liu, L. Li, C. Liu, L. Suo, Z. Xie, W. Zhao, W. Zhang, N. Yang, J. Li, S. Wang and J. Wang. (2009). Peste des petits ruminants virus in Tibet, China. Emerg. Infect. Dis., 15: 299–301.

Watanabe, M., A. Wang, J. Sheng, A F. Gombart, M. Ayata, S. Ueda, A. Hirano and T.C. Wong. (1995). Delayed activation of altered fusion glycoprotein in a chronic measles virus variant that causes subacute sclerosing panencephalitis.J Neurovirol. 1:177-88. Corrected and republished in: J. Neurovirol., 1: 412-423.

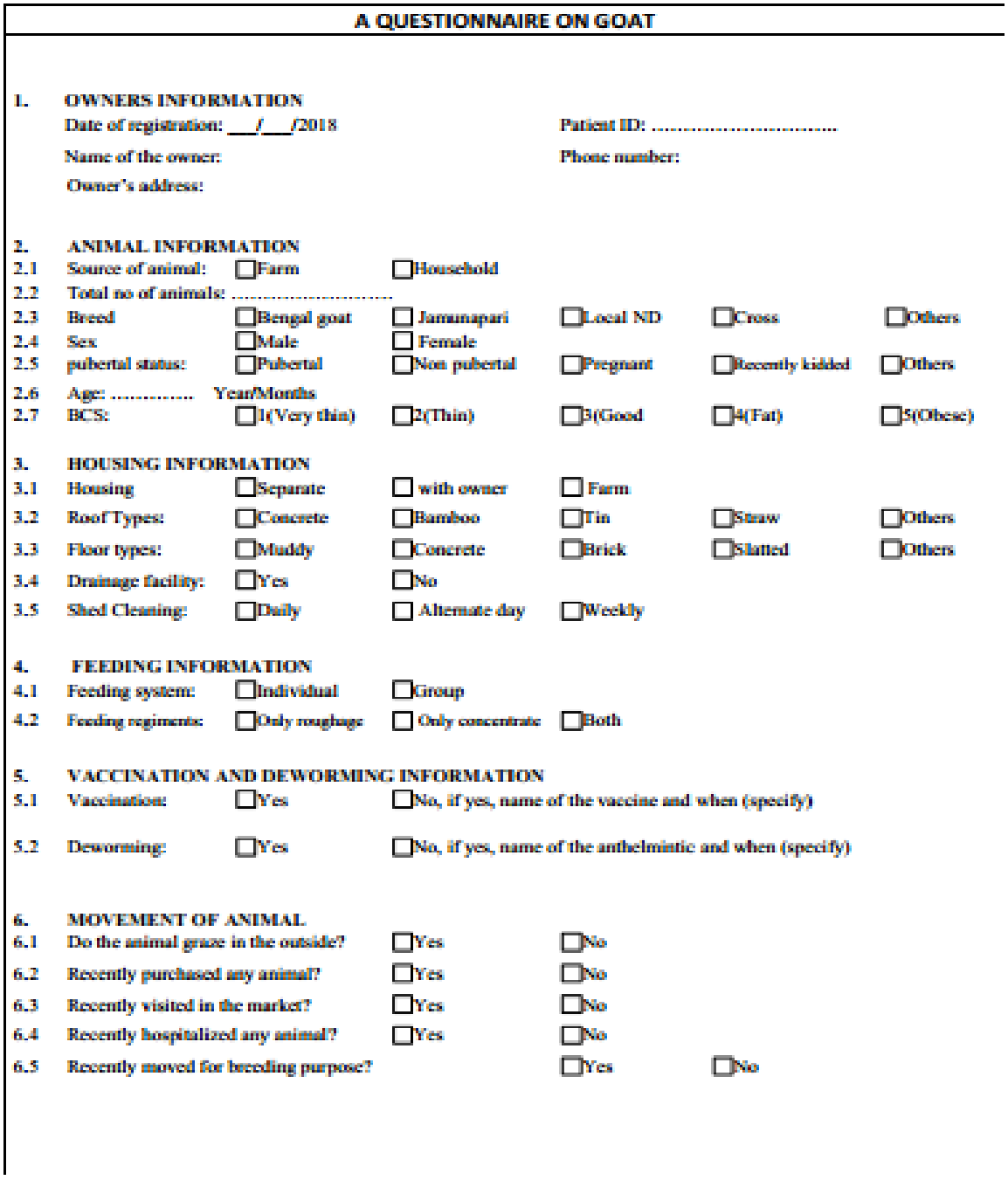
Watanabe, N., M. Kawano, M. Tsurudome, S. Kusagawa, M. Nishio, H. Komada, T. Shima and Y. Ito. (1996). Identification of the sequences responsible for nuclear targeting of the V protein of human parainfluenza virus type 2. J. Gen. Virol., 77: 327–338.

Wild T. F., E. Malvoisin and R. Buckland. (19910. Measles virus: both the hemagglutinin and fusion glycoproteins are required for fusion. J. Gen. Virol., 72: 439-442.

Wosu L.O. (1985). Agglutination of red blood cells by Peste des petits ruminants (PPR) virus. Nigeria Vet. J., 14: 56-58.

Yourno, J. and J. Conroy. (1992). A novel polymerase chain reaction method for detection of human immunodeficiency virus in dried blood spots on filter paper. J. Clin. Microbiol., 30: 2887–2892.

Appendix I



A QUESTIONNAIRE ON GOAT

1. OWNERS INFORMATION

Date of registration: ___/___/2018 Patient ID: .....

Name of the owner: Phone number:

Owner's address:

2. ANIMAL INFORMATION

2.1 Source of animal: ☐Farm ☐Household

2.2 Total no of animals: .....

2.3 Breed ☐Bengal goat ☐ Jamunapari ☐Local ND ☐Cross ☐Others

2.4 Sex ☐Male ☐ Female

2.5 pubertal status: ☐Pubertal ☐Non pubertal ☐Pregnant ☐Recently kidded ☐Others

2.6 Age: ..... Year/Months

2.7 BCS: ☐1(Very thin) ☐2(Thin) ☐3(Good ☐4(Fat) ☐5(Obese)

3. HOUSING INFORMATION

3.1 Housing ☐Separate ☐ with owner ☐ Farm

3.2 Roof Types: ☐Concrete ☐Bamboo ☐Tin ☐Straw ☐Others

3.3 Floor types: ☐Muddy ☐Concrete ☐Brick ☐Slatted ☐Others

3.4 Drainage facility: ☐Yes ☐No

3.5 Shed Cleaning: ☐Daily ☐ Alternate day ☐Weekly

4. FEEDING INFORMATION

4.1 Feeding system: ☐Individual ☐Group

4.2 Feeding regiments: ☐Only roughage ☐ Only concentrate ☐Both

5. VACCINATION AND DEWORMING INFORMATION

5.1 Vaccination: ☐Yes ☐No, if yes, name of the vaccine and when (specify)

5.2 Deworming: ☐Yes ☐No, if yes, name of the anthelmintic and when (specify)

6. MOVEMENT OF ANIMAL

6.1 Do the animal graze in the outside? ☐Yes ☐No

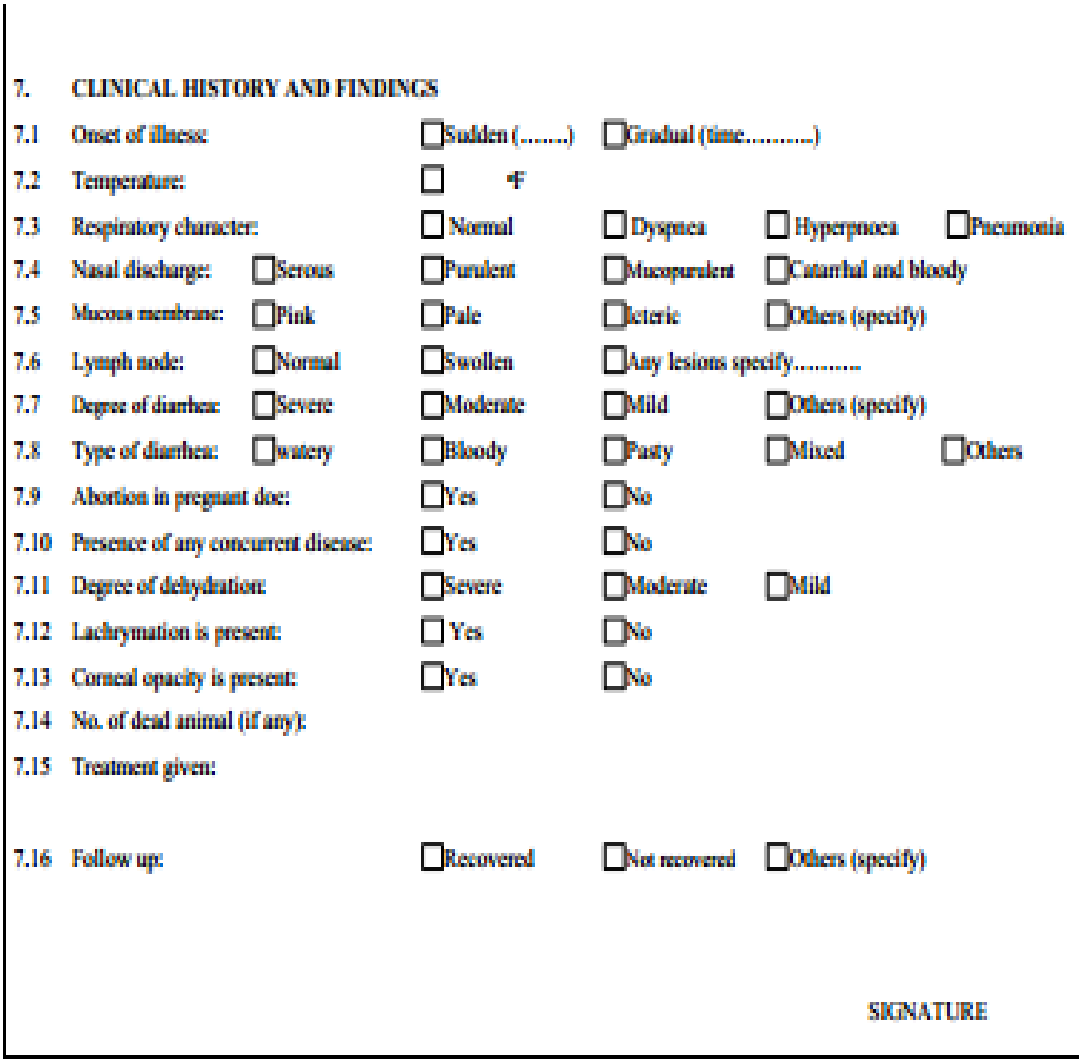
6.2 Recently purchased any animal? ☐Yes ☐No

6.3 Recently visited in the market? ☐Yes ☐No

6.4 Recently hospitalized any animal? ☐Yes ☐No

6.5 Recently moved for breeding purpose? ☐Yes ☐No

Figure 5. A questionnaire on PPR in goat (Page-1)



7. CLINICAL HISTORY AND FINDINGS

7.1	Onset of illness:		☐Sudden (.....)	☐Gradual (time.....)		
7.2	Temperature:		☐ °F			
7.3	Respiratory character:		☐ Normal	☐ Dyspnea	☐ Hyperpnoea	☐Pneumonia
7.4	Nasal discharge:	☐Serous	☐Purulent	☐Mucopurulent	☐Catarrhal and bloody	
7.5	Mucous membrane:	☐Pink	☐Pale	☐Icteric	☐Others (specify)	
7.6	Lymph node:	☐Normal	☐Swollen	☐Any lesions specify.....		
7.7	Degree of diarrhea:	☐Severe	☐Moderate	☐Mild	☐Others (specify)	
7.8	Type of diarrhea:	☐watery	☐Bloody	☐Pasty	☐Mixed	☐Others
7.9	Abortion in pregnant doe:		☐Yes	☐No		
7.10	Presence of any concurrent disease:		☐Yes	☐No		
7.11	Degree of dehydration:		☐Severe	☐Moderate	☐Mild	
7.12	Lachrymation is present:		☐ Yes	☐No		
7.13	Corneal opacity is present:		☐Yes	☐No		
7.14	No. of dead animal (if any):					
7.15	Treatment given:					
7.16	Follow up:		☐Recovered	☐Not recovered	☐Others (specify)	

SIGNATURE

Figure 5. A questionnaire on PPR in goat (Page-2)

Appendix II

Blood collection **(A)**, Sample preservation **(B)**, Loading blood sample into eppendorf tube **(C)**, Centrifugation during DNA extraction **(D)**, Setting of PCR tube into PCR machine **(E),** Loading of PCR product into gel electrophoresis machine **(F)**

Biography

I, Pran Gopal Rudra, son of Pravat Kusum Rudra and Swapna Rudra, was born in Hathazari Upazilla at Chattogram, Bangladesh. I completed my Secondary School Certificate (SSC) Examination in 2008 with GPA-5.00 and Higher Secondary Certificate (HSC) Examination in 2010 with GPA-4.70. I completed Doctor of Veterinary Medicine (DVM) from Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh in 2017 with CGPA 3.67 out of 4.00. I have been studying Masters of Science in Medicine at the Department of Medicine and Surgery of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. At present I am doing my thesis work which is compulsory for the degree of Master of Science (MS) in Medicine. In future, my immense interest push toward the higher studies as well as research in the field of companion animal medicine.