5 Peste des Petits Ruminants Virus

Muhammad Munir
The Pirbright Institute, Compton Laboratory, Compton, UK

5.1 Introduction

Peste des petits ruminants (PPR) is a highly contagious, fatal and economically important disease of both domestic and wild small ruminants, and camels. Owing to high morbidity (100%) and mortality (90%), PPR was included in the OIE (Office International des Epizooties) list of notifiable terrestrial animal diseases. The disease is currently spreading rapidly in most countries of the sub-Saharan and North Africa, the Middle East and Indian sub-continent and as far as into Tibet, China.

During the rinderpest virus (RPV) eradication campaign, there have been significant improvements in understanding the biology of viruses; however, the primary focus has remained developing and improving efficient vaccines. The present chapter aims to provide an overview of all known features of the PPRV genome, structure and biology. The structural and non-structural proteins are described comprehensively. Additionally, available diagnostic tests and potent PPRV vaccines are discussed and finally current challenges and future possibilities for disease eradication are highlighted.

5.2 PPRV Identification and Historical Perspective

Officially, PPR was first described in the Republic of Côte d'Ivoire in West Africa in 1942 (Gargadennec and Lalanne, 1942), however, there are indications that the disease existed much earlier. Since PPR and RP are clinically related diseases and the viruses are antigenically similar, it is believed that PPR remained undiagnosed due to the high prevalence of RP and the inability of the available diagnostic tests to differentiate PPR from RP (Baron *et al.*, 2011). Furthermore, it is likely that, owing to cross-neutralization between PPRV and RPV, small ruminants infected with RPV would have developed protective antibodies suppressing the clinical outcome of PPRV infection (Taylor, 1979). Nevertheless, the disease gained attention when a severe rinderpest-like disease was observed in sheep and goats, which was unable to transmit to the cattle reared in the same herd or in the close vicinity. Initially, different names such as 'kata', 'pseudo rinderpest', 'syndrome of stomatitis-pneumoenteritis' and 'ovine rinderpest' were used to describe the disease. Later, a French name, 'peste des

 Mononegaviruses of Veterinary Importance Vol. I: Pathobiology and Molecular Diagnosis (ed. M. Munir)

petits ruminants', was suggested because of its clinical, pathological and immunological similarities with RPV. At the time of first PPRV recognition, it was considered a variant of RPV. However, Gibbs *et al.* (1979) revealed that PPRV is biologically and physico-chemically distinct and is therefore a new member in the genus *Morbillivirus*, along with RPV, canine and phocine distemper viruses (CDV and PDV), measles virus (MV) and morbilliviruses of porpoises, dolphins and cetaceans (PMV, DMV and CMV).

5.3 Geographical Distribution

After first identification, PPRV spread to sub-Saharan Africa, the Middle East, Turkey and the Indian subcontinent. During the last decade, the disease has been reported for the first time in China, Kenya, Uganda, Tanzania, Morocco and Tunisia (Banyard *et al.*, 2010; Munir *et al.*, 2013). This demonstrates that the virus is highly infectious, and is of emerging transboundary nature. Initially, PPRV was characterized and phylogenetically analysed based on the fusion gene (F), which classified all the strains of PPRV into four distinct lineages (Shaila *et al.*, 1996; Dhar *et al.*, 2002). Later, it appeared that phylogenetic analysis based on the nucleoprotein gene (N) presented a better molecular epidemiological pattern (Kwiatek *et al.*, 2007) and is currently preferred over F gene-based phylogenetic analysis. However, all the PPRV strains remained in the same group regardless of what gene was used as basis for classification, except that the F gene-based lineage I (i.e. Nig/75) became lineage II on the N gene-based tree. Recently, Balamurugan *et al.* (2010) suggested that the use of the haemagglutinin-neuraminidase (HN) gene, in addition to the F and N genes, could give better resolution and permit tracing of virus transmission within outbreaks. Nevertheless, it is still unclear whether differences between lineages merely reflect geographical speciation or if they are also correlated with variability in pathogenicity between isolates (Banyard *et al.*, 2010).

PPRV belonging to lineages I and II have exclusively been isolated from the countries in West Africa, where PPRV once originated. Lineage III is restricted to the Middle East and East Africa. Though lineage IV was strictly considered an Asian lineage, it is now overwhelming the other lineages in African countries, while still being predominant in Asia (Kwiatek *et al.*, 2011; Munir *et al.*, 2013) (Fig. 5.1). Most recent reports of PPRV in previously PPRV-free countries belong to lineage IV, which suggests that lineage IV is a novel group of PPRV and may replace the other lineages in the near future. It is also likely that only lineage IV is currently causing outbreaks. Moreover, it is crucial to note that countries once exclusively carrying a single lineage are now simultaneously reporting the presence of several lineages, i.e. Sudan and Uganda (Kwiatek *et al.*, 2011; Luka *et al.*, 2012). In the majority of cases, the newly introduced lineage is lineage IV (Kwiatek *et al.*, 2011; Luka *et al.*, 2012; Cosseddu *et al.*, 2013).

5.4 Economic Impact of PPR Disease

PPR is generally considered a major constraint for small ruminant production; however, the economic impact of the disease has not been fully evaluated (Ezeokoli *et al.*, 1986; Rossiter and Taylor, 1994; Nanda *et al.*, 1996). The economic importance of PPR is primarily due to its highly contagious nature, with a case fatality rate as high as 100%. This is of particular concern for the economics of small rural farms, where sheep and goats are reared as the sole source of income. Moreover, PPR is most prevalent in countries that rely heavily on subsistence farming of small ruminants for trade and food supply.

The disease consequences can be prevented by the use of highly efficacious vaccine. It has been calculated that an investment of US$2 million can bring a return of US$24 million. This estimation has been made on 1 million animals (Stem, 1993). These facts lead to the perception that PPR is one of the top ten diseases in sheep and goats that are having a high impact on the poor rural small ruminant farmers (Perry *et al.*, 2002). Collectively, it was estimated that PPR causes a loss of US$1.5 million annually in

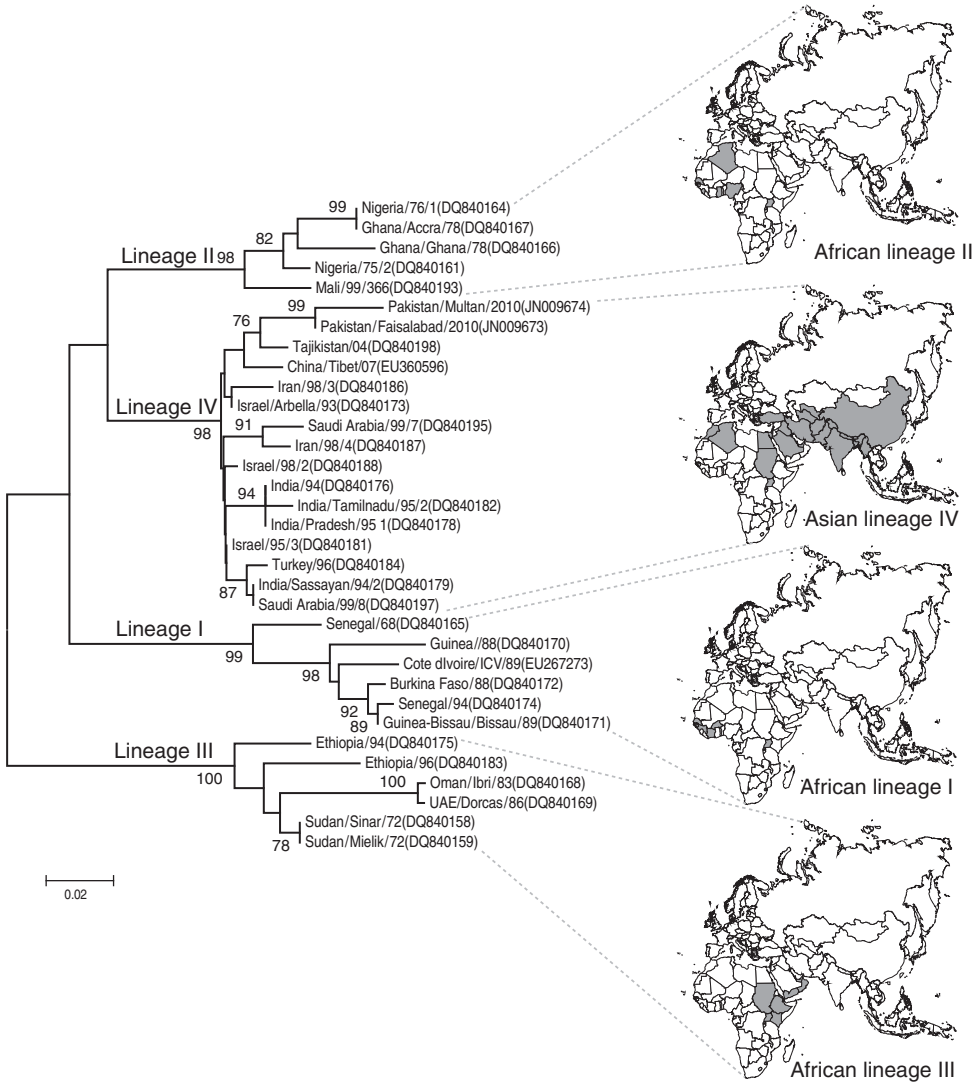

Fig. 5.1. Phylogenetic analysis of PPR isolates based on the N gene. The distribution of different lineages is detailed by shading the maps for each lineage.

Nigeria (Hamdy *et al.*, 1976), US$39 million in India (Bandyopadhyay, 2002) and at least US$1.5 million in Iran (Bazarghani *et al.*, 2006) and US$15 million in Kenya (Thombare and Sinha, 2009). Besides these figures, the worldwide economic impact of PPR largely remains elusive, and a well-planned cost-benefit analysis of PPR versus policy responses that includes both the direct and indirect impacts associated with PPR is required.

5.5 Virion Morphology, Structural and Accessory Proteins

5.5.1 PPR virions

Like other paramyxoviruses, PPR virions are enveloped, pleomorphic particles (Fig. 5.2A) and are comprised of single-stranded RNA genome with negative polarity. The length of the entire genome of PPRV is 15,948

nucleotides, which is the second longest among all morbilliviruses after a recently characterized feline morbillivirus (Bailey *et al.*, 2005; Woo *et al.*, 2012). The diameter of PPR virions ranges from 400 to 500 nm. The phosphoprotein (P) acts as a co-factor of large protein (L), which is the viral RNA dependent RNA polymerase (RdRp). There are three proteins associated with the host cell membrane-derived viral envelope. The matrix (M) protein acts as a link, which associates with the nucleocapsid and the two external viral proteins, the fusion (F) protein and the HN protein. The thickness of the PPRV envelope varies from 8 to 15 nm and the length of the surface glycoproteins ranges from 8.5 to 14.5 nm (Durojaiye *et al.*, 1985).

5.5.2 Viral ribonucleoprotein

The N protein surrounds the genomic RNA along with two other viral proteins, the L protein and the P protein to form the ribonucleoprotein (RNP). This RNP core encloses the entire genome of PPRV and protects from endonuclease digestion. The RNP strands appear as a herring bone with a thickness of ~14–23 nm (Fig. 5.2B) (Durojaiye *et al.*, 1985). Each molecule of N protein is associated with the six nucleotides of the genome, which explains the requirement of 'the rule of six' for paramyxoviruses including PPRV (Lamb and Kolakofsky, 2001). Contrary to this strongly accepted belief, it was revealed that PPRV obey the rule of six but carry a degree of flexibility. By a still unknown mechanism, transcription and replication in PPRV minigenome can accommodate some deviation in genome length, such as +1, +2 and –1 nucleotides (Bailey *et al.*, 2007). Given the fact that the PPRV genome contains 15,948 nucleotides (multiple of six bases), 2650 copies of N proteins are required to completely wrap up the genome. Electron microscopic analysis of nucleocapsid-confirmation in other morbilliviruses (Bhella *et al.*, 2004) indicates that approximately 13 copies of the N protein constitute a single helix, and therefore a genome would involve around 200 turns of the nucleocapsid helix. As is indicated in Fig. 5.2B, the individual cell may contain several copies of the encapsidated RNA PPR virus.

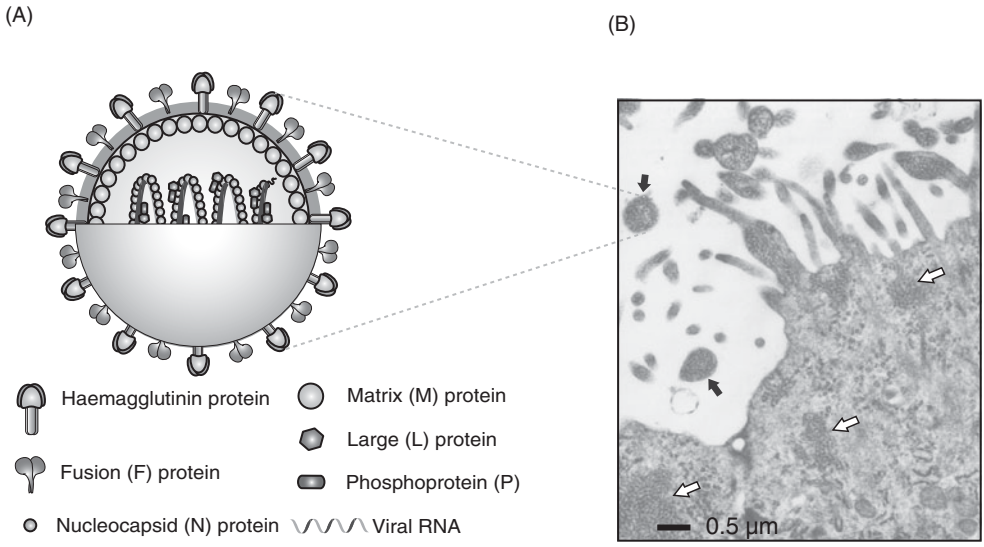

Fig. 5.2. (A) Schematic structure of PPRV, along with a key to different viral proteins. (B) Uranyl acetate and lead citrate staining of the microvilli collected from experimentally infected goats. Herringbone-like cytoplasmic nucleocapsids are labelled with white arrows and the extracellular virions extruding from cytoplasm are labelled with black arrows. (Second part of the figure is reproduced from Bundza *et al.* (1988) with permission.)

5.5.3 Genome organization, replication and transcription

The PPRV genome carries six transcriptional units; each encodes for a contiguous and non-overlapping protein except the P gene, which also expresses C and V nonstructural proteins by an alternative open reading frame and RNA editing, respectively (Mahapatra *et al.*, 2003). All the genes in PPRV are arranged in an order of 3′-N-P/C/V-M-F-HN-L-5′ (Bailey *et al.*, 2005) (Fig. 5.3). An intergenic region of variable lengths separates one gene from the other (Barrett *et al.*, 2006). Notably, owing to variable lengths of the intergenic region between the M and F genes (without having an effect on the protein lengths), the genome varies among morbilliviruses. So far, no obvious role for this variable and high GC content intergenic region has been observed in the replication of the morbilliviruses. The sequence between two consecutive genes is AAAACTTAGGA and is highly conserved throughout the morbilliviruses, including PPRV, indicating that this stretch of sequence is important for viral replications. Data from other paramyxoviruses demonstrate that nucleotides before CTT (underlined in the intergenic sequence) indicate the end of one gene (GE) and are essential polyadenylation sites, whereas the sequence after CTT is the start of next gene (GS).

It has been demonstrated that the 3′ and 5′ untranslated regions (UTRs) at both ends of the paramyxovirus genome, known as genome promoter (GP) and anti-genome promoter (AGP) respectively, are crucial for viral transcription and replication (Lamb and Kolakofsky, 2001) (Fig. 5.3). In PPRV, the 3′-genome terminus, a seat for the attachment of the RdRp polymerase complex, is a stretch of 107 nucleotides, which includes the 52-nucleotide leader region, and 3′ UTR of the N gene, both separated by a trinucleotide (GAA). This stretch of GP before the N gene's open reading frame (ORF) start codon acts as a promoter for the synthesis of viral RNA (Bailey *et al.*, 2007). The gene start and polyadenylation signal are located 52 nucleotides downstream of the N ORF stop in PPRV, which is highly conserved among the morbilliviruses. Recently, an *in vitro* transcription complex was synthesized for PPRV and it was shown that a RNP complex, collected from infected insect cells, is active in synthesizing RNA (Yunus and Shaila, 2012). As with other paramyxoviruses, the N gene present at the 3′ end of the genome was the highest transcribed gene, whereas the L gene present at

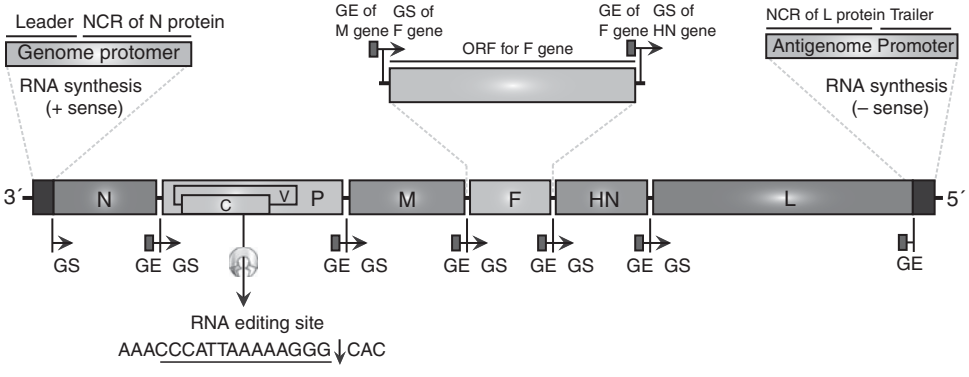

Fig. 5.3. Genome organization of typical PPRV. All the genes are organized in the order of 3′-N-P/C/V-M-F-HN-L-5′, flanked by 3′-genome and 5′-antigenome promoters responsible for the synthesis of + sense RNA and – sense RNA, respectively. Each of the promoters carries sequence for the leader or trailer along with sequence (non-coding region, NCR) from respective genes. Between two genes, there is a stretch of sequence divided into the gene start (GS) of first gene and gene end (GE) of the next gene. It is only P gene that encodes for C and V proteins via alternative open reading frame and RNA editing (insertion of G at the place of arrow after three Gs), respectively.

the end of the genome was the least, owing to attenuation at each gene junction. Quantitative analysis indicated that the level of P mRNA synthesis is 50%, if mRNA for the N gene is taken as 100%, which means that the P gene is transcribed only once out of two attempts of polymerase. Similar analyses show that the synthesis of the L gene mRNA occurs only once out of 75 attempts. Collectively, it was shown that 50% of the total transcripts consist of N mRNA only, and the remaining 50% of all other genes (Yunus and Shaila, 2012). Using this system, it is likely that the post-transcriptional modification activities associated with the L protein of PPRV will also be explored in the near future.

The AGP, which is responsible for the synthesis of genome-sense RNA, is the complement of the 5′ UTR after the L protein stop codon, including the trailer region that becomes the 3′ end of the antigenome. The conserved 3′ and 5′ termini in the entire family reflect the similarity in their promoter activities lying in these regions. A nucleotide stretch of 23–31 at the 3′-terminus of both the GP and the AGP in PPRV is highly conserved and is considered to be an essential domain required for promoter activity. This region is believed to interact with a conserved area comprising a succession of three hexamer motifs (CNNNNN). Although the exact mechanism of domains interaction is unclear, a model has been proposed that predicts that the three hexamer motifs in the second promoter element lie on the same face of the helix, exactly above the first three hexamers at the 3′ terminus (Lamb and Kolakofsky, 2001). It is therefore more likely that these two regions in the GP and AGP interact directly with each other to form a functional promoter unit. A similar assembly is also presented in the promoters of the other paramyxoviruses (Murphy and Parks, 1999). At the junction of the GP and N gene start, a conserved intergenic triplet sequence (CTT) is also considered necessary for transcription (Mioulet *et al.*, 2001). In an effort to construct a minigenome for PPRV, Bailey *et al.* (2007) demonstrated the role of GP and AGP by using chimeric minigenomes of PPRV and RPV. They showed that the use of PPRV-AGP decreased the ability of RPV to rescue the chimeric minigenome, which predicts the difference in closely related viruses. Moreover, it was shown that AGP is a very strong promoter and is responsible for the production of the full-length negative sense genome, whereas the GP is responsible for both transcription of virus mRNAs and transcription of the full-length positive sense virus genome.

5.5.4 Structural proteins

Nucleocapsid (N) protein

Owing to its location at the 3′ end of the genome, the gene that encodes for the N protein is the most transcribed among all genes for both structural and nonstructural proteins of PPRV. The length of the N protein of both PPRV and RPV is 525 amino acids. However, the mobility pattern on SDS-PAGE varies between different strains of PPRV and RPV. It has been observed that the N protein from African isolates (e.g. Nig/75/1) of PPRV moves faster (~55 kDa) than N proteins from the Arabian Peninsula (DORCAS_87) (60 kDa), but migrated slower than the RPV (66 kDa) (Taylor *et al.*, 1990). Therefore, the mobility pattern was considered a biochemical marker for the differentiation of PPRV from RPV and among different strains of PPRV (Lefevre and Diallo, 1990). This difference can be attributed to the post-transcriptional modifications such as glycosylation. Now, due to the availability of the sequences, it is possible to *in Silico* demonstrate that the Arabian isolate DORCAS_87 only has one glycosylation site at the ^{65}NGSK position, whereas the African strain Nig/75/1 has two glycosylation sites at ^{65}NGSK and ^{444}NGSE positions. This difference could contribute to the difference in the mobility of respective strains on SDS-PAGE. Moreover, it is conceivable that N protein is highly susceptible to proteolysis and degradation products may vary between the two viruses and hence differ in molecular weight. However, such predictions require experimental confirmation.

As a common property of paramyxoviruses, the N protein of PPRV is likely to interact with other N proteins (N–N interaction), with the P protein (N–P interaction) and with polymerase units (P–L interaction) to take part in the replication complex. However, only sequences required for the self-assembly of N proteins are mapped for PPRV (Bodjo *et al.*, 2008). It has been demonstrated that two domains, one at the N-terminus (1–120) and one in the central region (146–241), are responsible for the PPRV N–N self-assembly. Additionally, a short fragment in the N protein at amino acid 121–145 is essential for the stability of the resultant nucleocapsid structure.

The N protein plays an essential role in the replication of PPRV (Servan de Almeida *et al.*, 2007). It has been demonstrated that silencing of the N mRNA can block the production of N transcripts and the expression of N protein. Additionally, such shutting down of the N protein indirectly inhibits the production of M protein. Collectively, the shut down of these proteins results in the inhibition of PPRV progeny by 10,000-fold (Keita *et al.*, 2008). A region for efficient siRNA inhibition has now been identified, which is 5′-RRWYYDRNUGGUUYGRG-3′ (where R is A or G, W is A or U, Y is C or U, D is G, A or U and N is any of the four bases). Although this region is found to be common in most of the morbilliviruses, targeting a single region may not prevent the risk of escape mutants. Therefore, in case of therapeutic application of this technique, multiple targets need to be used.

Besides the essential role of the N protein in viral replication and transcription, it regulates host cell protein 72 (hsp72), interferon regulatory factor 3 (IRF3) and cell surface receptors in several morbilliviruses to indirectly promote viral RNA transcription (Zhang *et al.*, 2002; Laine *et al.*, 2003). However, such functions are not described for the N protein of PPRV. The N protein is the most accumulated protein in infected cells and is antigenically most conserved among morbilliviruses (Libeau *et al.*, 1995). Being most abundant, N is a highly immunogenic protein. However, the immune responses generated against the N protein are non-protective due to intra-viral location of the protein. Given its abundance and antigenic stability, the N protein has extensively been targeted for diagnostic assays (Munir *et al.*, 2013). Apart from its diagnostic application, the genetic diversity of the N gene has been the basis for the classification of PPRV into four lineages. This classification better represents the geographical origin than the classification based on the variation of the external glycoprotein, the F protein (Diallo *et al.*, 2007; Kwiatek *et al.*, 2007).

Phosphoprotein (P)

The phosphoprotein of PPRV, as other morbilliviruses, is acidic in nature and undergoes intensive post-translational phosphorylation (hence the acronym phosphoprotein), owing to richness in serine and threonine (Diallo *et al.*, 1987). Due to this, the P protein migrates more slowly (79 kDa) than its predicted molecular weight (60 kDa). The phosphoprotein of PPRV (strain Turkey/00) has a high serine (Ser), threonine (Thr) and tyrosine (Tyr) content (Ser: 38, Thr: 8, Tyr: 5). Approximately 50% of the potential phosphorylation residues in P proteins have high prediction scores (Pred values >0.6); however, potential phosphorylation sites vary between different strains of PPRV.

The length of P proteins varies from 506 to 509 amino acids between different morbillivirus members and the P protein of the PPRV is the longest among all. Despite the essential role of the P protein in viral replication and transcription, it is one of the least conserved proteins, which is demonstrated by the fact that the P proteins from PPRV and RPV share only 51.4% amino acid identity (Mahapatra *et al.*, 2003). Moreover, the region from 21 amino acid to 306 amino acid contains the majority of unconserved residues. Given the fact that the C-terminus of the P protein is involved in the N–P interaction, this terminus is more conserved compared with the N-terminus of the P protein.

In morbilliviruses, the P protein plays crucial roles at multiple levels in both viral

replication and immune regulation. For instance, the N–P interaction is required for key biological processes such as cell cycle control, transcription and translation regulation (Johansson *et al.*, 2003). The motifs required for the interaction of RPV P protein with N protein (N–P interaction) are conserved in the P protein of PPRV. Moreover, the P protein is the vital element of the viral L–polymerase complex, and it is assumed to be a key determinant of cross-species morbillivirus pathogenicity (Yoneda *et al.*, 2004). Despite these crucial roles of the P protein in the replication of morbilliviruses, its function in PPRV replication and pathogenicity remains elusive, which warrants future investigations.

Matrix (M) protein

The ORF for the M protein of PPRV is located at nucleotide position 3438–4442, which is translated to a protein of 335 amino acids with a predicted molecular weight of 37.8 kDa. It is therefore considered one of the smallest proteins among all the structural proteins of morbilliviruses. The protein is highly conserved and a 92.5% and 85.0% similarity and identity have been calculated between PPRV and RPV, respectively. This high degree of conservation may reflect the essential role of the M protein in the formation of progeny viruses and interaction with the surface glycoprotein in the cell membrane. Three ATG repeats (^{956}tctATGATGATGtca^{970}) are identified in the gene for the M protein of PPRV, RPV and MV, while the M gene of CDV, PDV, DMV lacks this domain (Muthuchelvan *et al.*, 2005).

The M protein constitutes the inner coat of the viral envelope and acts as a bridge to connect the surface glycoprotein (F and HN) with that of ribonucleoprotein core (genome, N, P and L) (Fig. 5.2). In an effort to construct a marker vaccine candidate, it was noticed that if the M protein of RPV was replaced with the corresponding protein of PPRV, it did not affect the growth of RPV in cell culture (Mahapatra *et al.*, 2006). Although the mechanism behind compatibility remains to be determined, it at least indicates the high level of M protein conservation among morbilliviruses.

The M protein mediates the viral budding process preferentially at specialized regions of the host membrane. For instance, the budding of MV occurs at the epical microvilli in epithelial cells due to highly concentrated actin filaments, which are required for the cellular transport (Riedl *et al.*, 2002). Electron microscopic images of intestinal epithelial cells from a goat experimentally infected with the Malig-Yemen strain of PPRV indicated that viral particles were released from the microvilli and shed in faeces (Bundza *et al.*, 1988). Moreover, the motif (FMYL) at amino acid position 50–53 required for the localization of the M protein in the cell membrane to facilitate the budding process in Nipah virus, a member of the same family, was found to be identical with that in the M protein of PPRV (Ciancanelli and Basler, 2006). However, it is not known whether these viruses share functional homologies in their M proteins.

Fusion (F) protein

The F protein (59.137 kDA) is one of the highly conserved proteins not only between PPRV and RPV but also among all the morbilliviruses. This conservation probably reflects the cross-protection between PPRV and RPV (Taylor and Abegunde, 1979). In all paramyxoviruses, the F protein is embedded in the viral lipid bilayer envelope and protrudes as spikes on the viral surface (Fig. 5.2). The cleavage of the F protein is a key mechanism of paramyxovirus virulence. The naïve form of the F protein (F0) undergoes post-translational proteolytic cleavage and results in two active subunits, F1 and F2. This mechanism is not well understood for PPRV. However, it has been shown that PPRV carries RRTRR at position 104–108 (Chard *et al.*, 2008), which is recognizable by the *trans*-Golgi associated furin endopeptidase consistent to the cleavage site RRX1X2R (X1 indicates any amino acid, but X2 must be either arginine or lysine) proposed for the morbilliviruses. It has been shown by Rahaman *et al.* (2003) that the membrane-anchoring subunit of F_1

of PPRV contains four well-described conserved motifs: an N-terminus fusion peptide (FP), heptade repeat 1 (HR1), HR2 and a transmembrane (TM) domain. The 3D structure of the HR1–HR2 complex has revealed that the heterodimer between HR2 and HR1 covers the inner core of the HR1 trimer, resulting in a six-helix bundle. The molecular mechanism of PPRV budding is not known, but it is likely based on identical structure of heptade repeats, which have a common fusion mechanism. It has further been shown that on anchoring the FP domain in the membrane, dimerization of the HR domains leads to fusion between the host cell membrane and the viral envelope by bringing them close to each other (Rahaman *et al.*, 2003). A leucine zipper motif, present in the F protein of all the morbilliviruses, is responsible for facilitating the oligomerization and fusion function of the F protein through an unknown mechanism (Plemper *et al.*, 2001). In PPRV, this motif is located at position 459–480 and is conserved among all PPRV strains characterized so far.

In all morbilliviruses, the membrane-associated proteins are glycosylated and hence are known as glycoproteins. This post-transcriptional modification is critical for the transport of the protein to the cell surface, and to maintain its fusogenic ability and integrity. All members of the morbillivirus genus contain a conserved NXS/T (X indicates any amino acid) glycosylation site in the F2 subunit of the mature protein (Meyer and Diallo, 1995). In PPRV, the three N-linked glycosylation sites include ^{25}NLS^{27}, ^{57}NIT^{59} and ^{63}NCT^{65}; however, their specific functions still need to be revealed.

Haemagglutinin-neuraminidase (HN) protein

The ORF for the HN protein gene starts from 7326 and ends at 9152 nucleotide (Nigeria 75/1) and results in a 67 kDa HN protein. The HN protein is the least conserved. While both PPRV and RPV have 609 amino acid residues in their respective HN proteins, the proteins share only 50% amino acid identity. This variation probably reflects the viral specificity for cell tropism and therefore determines the host range. Most of the viral neutralizing antibodies are mainly directed against the HN protein. Hence it is under continuous increased immunological pressure (Renukaradhya *et al.*, 2002). The fundamental roles of the HN proteins in progression of viral infection and specific binding to host cell membrane are not defined in PPRV. However, the findings that the H protein is a major determinant of cell tropism in MV and is the main cause of cross-species pathogenesis in lapinized RPV (Yoneda *et al.*, 2002) indicate that H is the vital antigenic determinant of the morbilliviruses. However, it has been determined that the HN protein of PPRV required a homologous F protein for proper functioning in virus replication (Das *et al.*, 2000).

In some paramyxoviruses, surface proteins can cause haemagglutination and can carry neuraminidase activities. Interestingly, among morbilliviruses it is only MV and PPRV that have haemagglutination capabilities (Varsanyi *et al.*, 1984; Seth and Shaila, 2001). In addition to haemagglutination (viral attachment to cell surfaces and agglutination of erythrocytes), PPRV is unique for its neuraminidase activity (cleaves sialic acid residues from the carbohydrate moieties of glycoproteins). Therefore, it is the only member of the morbilliviruses that has HN protein (Seth and Shaila, 2001), which was previously thought to be absent. RPV, which as already mentioned is very closely related to PPRV, has limited neuraminidase activity but cannot act as a haemagglutinating agent for the erythrocytes (Langedijk *et al.*, 1997). Based on these results, it is suggested to use the more descriptive term HN protein instead of the currently used H protein, as has been used for PPRV in this chapter.

Large (L) protein

The L protein of PPRV is 2183 amino acids long and is regarded as the largest protein in PPR virions. However, due to natural attenuation at each gene-junction in all mononegaviruses, the mRNA encoding for the L protein is the least abundant (Flanagan *et al.*, 2000; Yunus and Shaila, 2012).

Notably, the L protein is conserved among morbilliviruses: PPRV has an identity with RPV and CDV of 70.7% and 57.0%, respectively (Bailey *et al.*, 2005). The protein is rich in leucine and isoleucine, which can be as high as 18.4% (Muthuchelvan *et al.*, 2005). The L protein of PPRV carries a length (2183 amino acids) and molecular weight (247.3 kDa) identical to that of RPV, MV and DMV; however, the protein charge +14.5 is different from those of RPV (+22.0) and PDV (+28.0).

In all morbilliviruses, the L protein acts as RNA-dependent RNA polymerase and performs transcription and replication of the viral genomic RNA. Additionally, the L protein is also responsible for capping, methylation and polyadenylation of viral mRNA. All these steps are crucial for efficient replication of the viruses. Although the direct actions of L proteins are not investigated for PPRV, it is possible to make speculations owing to high sequence identity among morbilliviruses. Three motifs in the L protein have been identified, which are directly linked to the functions of this protein. The corresponding sequences at all these sites are found to be identical in the L protein of PPRV (Munir *et al.*, 2013). The L gene start motif (AGGAGCCAAG) in PPRV, in accordance with the motif found in other morbilliviruses [AGG(A/G)NCCA(A/G)G], is responsible for the generation of viral L gene mRNA and signal for the capping. In PPRV, the corresponding motif required for the binding of L protein with the RNA in morbilliviruses is KETGRLFAKMTYKM at amino acid position 540–553. The sequence ILYPEVHLDSPIV at positions 9–21 can act as a binding site for P and L proteins (Horikami *et al.*, 1994). This sequence for P–L interaction is conserved in paramyxoviruses: in PPRV it is totally conserved except the first amino acid, which is valine instead (Chard *et al.*, 2008). Although most of the important functions of L protein are not defined yet, it is expected that with the current establishment of the reconstituted system for PPRV, it will be possible to demonstrate the multifunctional activities of the L protein of PPRV (Yunus and Shaila, 2012).

5.5.5 Accessory proteins

C protein and V protein

It is only the P gene among all the genes of PPRV that encodes for more than one protein, known as C and V proteins, through alternative open reading frame and RNA editing, respectively, only in virus-infected cells (Mahapatra *et al.*, 2003; Barrett *et al.*, 2006). Apart from the role of C protein in viral replication, recently it has been shown that C protein in RPV inhibits interferon beta (IFN-β) production (Boxer *et al.*, 2009). The molecular mechanism of inhibition still needs to be investigated, but it is likely that the C protein blocks the activation of transcription factors which are required to make up the IFN-β enhanceosome. Whereas the C protein is known to be a virulence factor in MV infection (Patterson *et al.*, 2000) and RPV growth (Baron and Barrett, 2000), the biological function of the C protein in PPRV biology is not known and needs to be examined.

The length of the V protein of PPRV is highly variable among morbilliviruses (Table 5.1). The predicted molecular mass and iso-electric point of the V protein of PPRV is 32.28 kDa and 4.68, respectively. By virtue of having the same initial gene frame, the V protein shares the N-terminus to the P protein, but due to RNA editing, the cysteine-rich C-terminus is different (Mahapatra *et al.*, 2003). The V protein, in contrast to the C protein, undergoes phosphorylation and ~60% of the serine residues are revealed to have a high score for phosphorylation as predicted by Netphos 2.0 (Blom *et al.*, 1999). In the majority of the paramyxoviruses, the V protein antagonizes interferon actions. Studies are required to investigate the functions of the V protein of PPRV, and its relation to other morbilliviruses. My preliminary results indicate that both C and V proteins are associated with IFN regulations at ISRE level in an *in vitro* reporter system. However, the molecular mechanisms of inhibition might differ in both proteins (M. Munir, unpublished data).

Table 5.1. Nucleotide (nt) and amino acid (aa) comparison of open reading frames between genes of PPRV (Turkey/00, AJ849636) and other morbilliviruses.

	Virus/strain (Accession no.)								
	Rinderpest/Kabete 'O' (X98291)		Measles virus/9301B (AB012948)		Canine distemper virus/ Onderstepoort (AF305419)		Dolphin morbillivirus/ CeMV (AJ608288)		Post-translational modification (predicted)
Protein/level	nt	aa	nt	aa	nt	aa	nt	aa	–
Nucleocapsid protein	66.2%	72.9%	66.8%	73.5%	62.5%	68.5%	66.2%	72.9%	Glycosylation
Phosphoprotein	62.4%	50.5%	60.4%	45.1%	56.6%	45.3%	61.6%	49.1%	Phosphorylation
C protein	58.8%	41.8%	53.2%	40.3%	53.1%	35.0%	58.5%	37.2%	–
V protein	61.9%	45.1%	59.0%	41.3%	54.2%	40.4%	59.0%	43.5%	Phosphorylation
Matrix protein	72.2%	66.1%	74.0%	68.2%	73.1%	60.3%	69.1%	64.0%	–
Fusion protein	68.0%	73.8%	67.0%	71.7%	50.2%	54.2%	65.9%	73.3%	Glycosylation
Haemagglutinin/ neuraminidase	55.5%	39.4%	53.5%	34.5%	47.2%	28.4%	52.9%	37.3%	Glycosylation
Large protein	68.1%	75.6%	68.1%	75.1%	64.8%	71.1%	67.3%	73.7%	Glycosylation
Complete genome[a]	63.7%	–	63.4%	–	58.5%	–	62.0%	–	–

[a] Full-length comparison among morbilliviruses. Similarity scores were calculated using BioEdit version 7.0.9.0.

5.6 *In vitro* Cultures and Animal Model

RPV was first successfully grown on bovine kidney cells, but Gilbert and Monnier (1962) were also able to isolate PPRV on primary lamb kidney cells. However, later, because of the problematic quality and considerable variations in primary cultures, an African green monkey kidney (Vero) cell line was used for PPRV isolation (Lefevre and Diallo, 1990). To further improve the isolation method and to reduce the problems associated with the Vero cell line (i.e. low virus isolation, unsuccessful attempts and blind passages) (Abu Elzein *et al.*, 1990; Lefevre and Diallo, 1990), monkey CVI cells expressing sheep–goat signaling lymphocyte activation molecule (SLAM) has been investigated. It was shown that the monkey cell line, designated CHS-20, is highly sensitive for isolation of wild-type PPRV from clinical specimens (Adombi *et al.*, 2011). Studies have shown that SLAM can be a co-receptor for PPRV, which was first confirmed using the small interfering RNA (siRNA) technique (Pawar *et al.*, 2008). Under silenced SLAM receptor in B95a cells (a marmoset lymphoblastoid cell line), PPRV replication was observed to be reduced by 12- to 143-fold, while the virus titre ranged from $\log_{10}$ 1.09 to 2.28 (12–190 times). Taken together, expression and distribution of SLAM was directly proportional to that of PPRV cell tropism, indicating that SLAM may act as a receptor for PPRV infectivity. The mRNA level of SLAM was determined to be higher in lymph nodes and was detectable in the digestive system; however, despite the fact that PPRV also replicates in the lungs, colon and rectum, the SLAM receptors were not activated, which partially demonstrates that SLAM is not the major receptor for PPRV infectivity, and that PPRV additionally relies on other receptors for viral pathogenesis (Meng *et al.*, 2011).

Recently, in an experimental study it was shown that alpine goats are highly susceptible to Morocco strains of PPRV (Hammouchi *et al.*, 2012). The results of this and a corresponding study from the same group concluded that alpine goats can be used for both vaccine and pathogenesis studies in order to consistently reproduce PPR clinical signs in experimentally infected animals (El Harrak *et al.*, 2012).

5.7 Determinants of Virulence

There are several factors that contribute significantly in disease pathology and virus dissemination, some contributed by the host while others are physical factors.

5.7.1 Host factors

Although only domestic and wild small ruminants are considered as the main natural host, PPRV can infect other species such as cattle, pigs, buffalo, camels, and as recently reported, also the Asiatic lion (Balamurugan *et al.*, 2012a). There is little information available about susceptibility, occurrence and severity of the disease in wild ungulate species; however, current literature indicates that wild small ruminants may have a crucial role in the epidemiology of PPR (Munir, 2013). In small ruminants, the severity of the disease may vary depending on age, sex, breed and seasons (Amjad *et al.*, 1996; Brindha *et al.*, 2001; Dhar *et al.*, 2002; Munir *et al.*, 2009; Meng *et al.*, 2011). Generally, it is believed that goats show more severe clinical signs than sheep in the same environmental conditions. This is supported by the fact that the level of PPRV antibodies is higher in sheep than goats, which may render sheep resistant to the disease (Munir *et al.*, 2009). Wosu (1994) has also shown that the rate of recovery is lower in goats than in sheep. The information regarding viral preference for sheep over goats has not been investigated, but it is likely that sheep show higher natural resistance to the disease. Notably, PPRV infection can spread between goats without affecting nearby sheep (Animal Health Australia, 2009), but mixed raising of both sheep and goats is considered to be a main risk factor for seropositivity in sheep flocks (Al-Majali

et al., 2008). It is also plausible that owing to the high fertility rate in goats there may be larger flock replacement by goat offspring, which are more susceptible to the disease than adults due to decrease in maternal antibodies after 4 months (Srinivas and Gopal, 1996; Ahmed *et al.*, 2005). Furthermore, it has been demonstrated that age is the main factor for seropositivity in small ruminants (Waret-Szkuta *et al.*, 2008). The case fatality rate is higher in young goats than in adults (Shankar *et al.*, 1998; Atta-ur-Rahman *et al.*, 2004). Since the males are sold earlier and females are kept for longer, the sex-based distribution of antibodies is usually biased. Goat species from West Africa are more susceptible than European goats (Couacy-Hymann *et al.*, 2007). The dwarf varieties of goats are the most susceptible among African breeds. The disease rate (morbidity) increases with environmental stress such as confinement of animals during winter and rainy seasons (Amjad *et al.*, 1996; Brindha *et al.*, 2001; Dhar *et al.*, 2002). However, the effects of environment on the occurrence of PPR are solely based on the nature of animal husbandry conditions and socio-economic status of the farm owner. Although there have been significant contributions in understanding the risk factors, the genetic marker of disease predisposition are not determined.

5.7.2 Non-host factors

PPRV is highly contagious and in most cases the virus is spread from infected to healthy animals via close contact (Abubakar *et al.*, 2012). However, PPRV is commonly shed in all secretion and excretions, such as from the mouth, eye and nose, and in faeces, semen and urine. Shedding starts after approximately 10 days of pyrexia. Since the virus is also secreted in sneezing and coughing, it is likely that transmission may occur through inhalation or contact with inanimate objects. The survival of PPRV in the dam's milk has not been investigated; however, based on its similarity with RPV, it is likely that PPRV is also secreted in milk 1–2 days before signs appear and as late as 45 days after onset of disease. It has been observed that PPRV-infected animals start virus transmission before the onset of clinical signs (Couacy-Hymann *et al.*, 2007). However, Ezeibe *et al.* (2008) studied the shedding of virus during the post-recovery state of the animal, and realized that goats infected with PPRV can shed virus antigens in faeces for 11 weeks after complete recovery. Little is known about the fragility of PPRV in the external environment. Comparison with RPV is likely to be reliable because there are many features in common. Although transmission is not impossible through fomites, it is not common either, because of the short life of the virus in dry environments (above 70°C) and in acidic (>5.6) or basic (<9.6) pH. Moreover, PPRV cannot exist for a long time outside the host because of its short half-life, which is estimated to be 2.2 minutes at 56°C and 3.3 hours at 37°C (Rossiter and Taylor, 1994). No convalescent carrier or chronic form of PPR has been reported.

5.8 Pathophysiology and Clinical Presentation

Primarily PPRV gains entry into the host via the epithelial lining of the oral cavity, respiratory and digestive tract. Based on this, PPR is also called stomatitis pneumoenteritis complex. Due to its high lymphotropic nature, PPRV replicates in the regional lymph node after internalization. The resultant viraemia facilitates virus dissemination to the surrounding susceptible epithelial tissues of the host. Further replication of the virus in these organs leads to establishment of lesions and clinical signs. The severity of these clinical signs depends on the age, breed, body condition and innate immunity of the host and the virulence of the virus. Moreover, concurrent bacterial and parasitic infections can further aggravate the disease.

Based on these factors, the clinical outcome of the disease is divided into peracute, acute, subacute or subclinical (Braide, 1981; Obi *et al.*, 1983; Kulkarni *et al.*, 1996). However, the acute form of the disease is the most common in both sheep and goats.

Peracute disease is commonly observed in kids and lambs soon after depletion of protective passive immunity. Although this form of disease is less clinically characterized, pyrexia may develop and animals may die 4–5 days post-pyrexia.

In the acute form of the disease, a short incubation period (3–4 days) is followed by pyrexia and severe diarrhoea, which ends in emaciation and prostration. Catarrhal discharges around nostrils can lead to severe dyspnoea, sneezing and coughing (Fig. 5.4A,B). Crusting and congestion of conjunctiva at the medial canthus and conjunctival sac may eventually cause complete closure of the eyelids. Rough necrosis

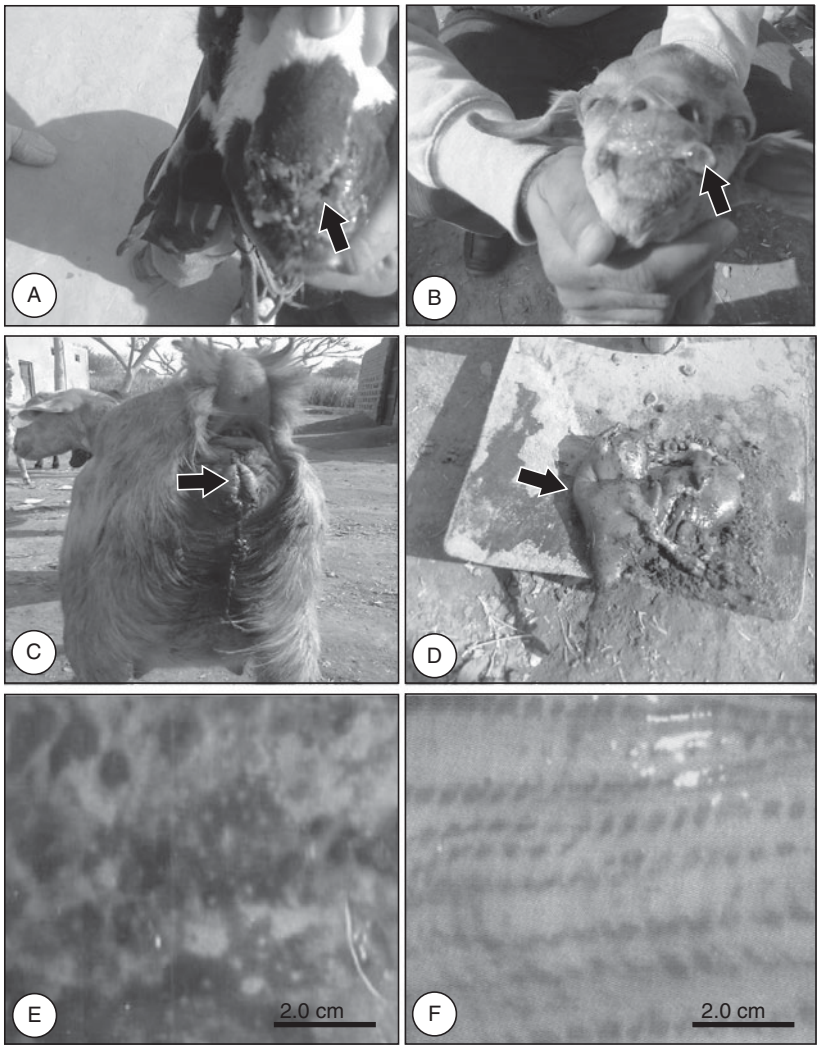

Fig. 5.4. Clinical picture of animals naturally infected with PPRV. (A) The oculo-nasal discharges become catarrhal with disease progression, occlude the nostrils and predispose victim to severe dyspnoea. (B) A serous discharge from the oral cavity and crust on the lips. (C) The pregnant animals may abort. (D) Aborted fetus from the goat shown in Fig. 5.4C. (E) Haemorrhagic erosions on the soft and hard palate. (F) Zebra stripes in the rectum. (A–D were obtained by the author after an outbreak of PPRV in district Multan, Pakistan. The samples were confirmed serologically (cELISA) and genetically (real-time PCR) for PPRV. E and F were modified from Bundza *et al*. (1988) with permission.)

is common on the dental pad, hard palate, inner side of cheek and dorsal part of the tongue, and around the commissures of the mouth. Because of these lesions, animals are reluctant to open their mouth and thus become anorexic. Occasionally, lesions may also develop in the mucous membrane of vulva and the vagina in female animals, which may cause abortion in pregnant animals (Fig. 5.4C,D). The lungs are affected in PPRV-infected animals, causing dyspnoea and productive cough. Severe signs of pneumonia such as noisy respiration with extended head and neck, nostril dilation, protruded tongue and painful cough are indications of poor prognosis. The affected animals then gradually become dehydrated, with sunken eyeballs, and often die 10–12 days post-pyrexia. The case fatality rate ranges from 70 to 80%, while survivors recover after weeks of convalescence.

The subacute form of the disease has a longer incubation period (>6 days) and animals are not as severely affected as in the acute form of the disease. Symptoms similar to contagious ecthyma, such as oral crusts due to mucosal discharges, may appear (Diallo, 2006). After low-grade pyrexia (39–40°C), animals usually recover in 10–14 days, but are immunoprotected enough to prevent re-infection and to protect the offspring for at least the first 3 months.

Subclinical disease is only observed in unusual hosts such as buffalo, as demonstrated by seropositivity against PPRV.

5.9 Gross and Histopathological Lesions

Gross lesions are very typical in the digestive and respiratory systems. In the oral cavity, the major affected sites include the dental pad, hard palate, buccal papillae and the dorsal surface of the tongue. However, ulcerative and necrotic lesions are common on the surface of the oral mucosa, pharynx, upper oesophagus, abomasum and small intestine (Fig. 5.4E). Severe congestions along the longitudinal folds of the caecum, proximal colon and rectum may lead to development of 'zebra striping' (Fig. 5.4F). Oedema and congestion of the lymph nodes may also develop in mesenteric, retropharyngeal and gut-associated lymphoid tissue. In the respiratory system, both anterior and cardiac lobes of the lungs can show severe congestion followed by consolidation and fibrinous or suppurative pneumonia. Hyperaemia, accompanied by frothy exudate, leads to erosions and multifocal ulceration in the mucosa of the nares and trachea. Rarely, focal degenerative lesions are also noticeable in the liver. Bronchitis, tracheitis, atelectasis and interstitial pneumonia may be severe due to secondary bacterial infection.

Some features of PPRV pathology such as atrophy of the villi, reduction of the lymphoid cells in Peyer's patches, dilatation of the cystic crypts of Leiberkuhn with cellular casts and infiltration of the lamina propria with macrophages and lymphocytes have been observed in both naturally and experimentally infected small ruminants. In the liver, hepatomegaly may cause narrowing of the sinusoids. Congestion and hyperplasia of the reticulo-endothelial cells is common in the spleen, whereas coagulative necrosis is common in the kidneys.

In the respiratory tract, histopathological lesions such as multifocal degeneration, ulceration and necrosis are followed by alveolar type II pneumocytes hyperplasia. These lesions lead to syncytial cell formation, which is a prominent feature in the lungs (Aruni *et al.*, 1998; Yener *et al.*, 2004). Multinucleated epithelial giant cells with intranuclear inclusion bodies are also very common among PPRV-infected animals. Notably, this pathology has not been found in the lungs of RPV-infected animals. Some of the most common pathological lesions are shown in Fig. 5.5.

As with several other morbilliviruses, the presence of PPRV in the ependymal cells clearly provides evidence that PPRV has the potential to reach and pass the blood–brain barrier and cause neurovirulence (Kul *et al.*, 2007). The clinical picture of the neurotropic form of PPR in kids and lambs is expected to be severe, especially in the presence of concurrent infections (Kul *et al.*, 2008). Although knowledge about the

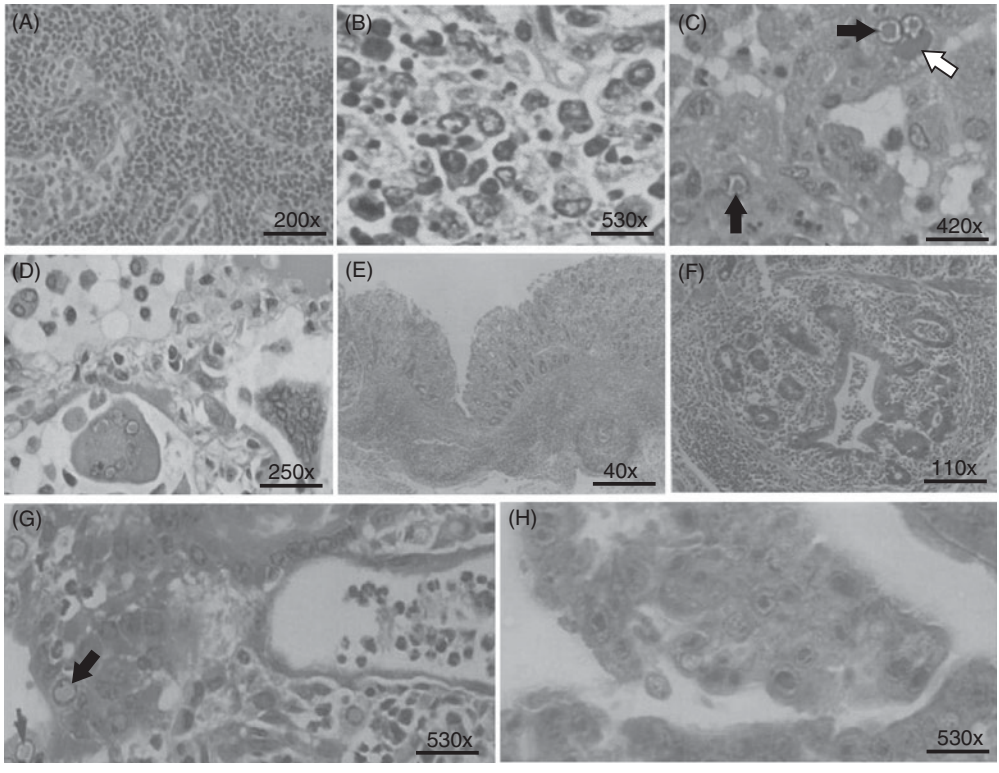

Fig. 5.5. Histopathological lesions of PPRV-infected organs of goats. (A) Macrophages in the germinal centre of the lymph node. (B) Necrosis with pycknosis of the nuclei of the lymph node. (C) Inclusions and oedema in the nucleus (black arrow) and cytoplasm (white arrow) of the lung cells. (D) Formation of oedema, macrophages and alveolar giant cells. (E) Atrophy of the intestinal villi, cellular casts in the crypts, depletion of lymphoid cells in the Peyer's patches. (F) Inflammation of intestinal crypt of PPRV-infected goat. (G) Degeneration of the epithelial cells of the mucosa of the ileum with nuclear inclusions (arrow) and distension of crypts with neutrophils. (H) PPRV antigen in both cytoplasm and nucleus as demonstrated by indirect immunoperoxidase staining. (Modified from Bunza *et al*. (1987) with permission.)

neurotropism of PPRV is in its infancy, it has been shown that the PPRV antigen is detectable in neurons and neuronal processes in the temporal, frontal and olfactory cortices in both hemispheres, and dendrite processes in the telencephalon layer of the hippocampus (Galbraith *et al.*, 1998, 2002).

5.10 Host Responses Against PPRV

5.10.1 Immune responses

Cytokine responses: All eukaryotes have the ability to block the replication of viruses, mediated by several arms of the immune systems such as neutralizing antibodies, the complement system and cytokine production. Animals infected with PPRV show increased expression of type II IFN (IFN-γ), a potent cytokine and crucial player in the direct inhibition of viral replication, in the majority of the epithelial lining the oral cavity, lung and tongue (Atmaca and Kul, 2012). A coordination between IFN-γ and interleukin-4 (IL-4) was observed to be inversely proportional in both vaccinated and infected goats, in which unique biphasic response of IL-4 expression was observed with an up-regulation of IFN-γ (Patel *et al.*, 2012). Besides epithelial cells, an elevated level of IFN-γ can also be observed in intravascular monocytes, syncytial cells, mononuclear

cells and the submucosa of the salivary glands. These results indicate that PPRV has high dissemination capacity and broad tissue tropism. The lungs, interstitial lymphocytes, syncytial cells and alveolar macrophages of PPRV-infected animals also show high expression of TNF-α (Atmaca and Kul, 2012). Due to affinity of the PPRV for the epithelial cells, it is likely that TNF-α plays an active role in stimulation of the cell-mediated immune responses, which warrants further investigations (Opal and DePalo, 2000). It is also likely that an elevated level of inducible nitric oxide synthetase (iNOS) during PPRV infection, in association with TNF-α, may be responsible for the induction of inflammation. Such interpretations have been made for MV in children (Moussallem *et al.*, 2007), whereas infection of ferrets with canine distemper virus showed no induction of cytokine expression in peripheral blood leukocytes (Svitek and von Messling, 2007). The level of IL-4 and IL-10 in PPRV-infected animals can be comparatively high (statistically non-significant) in bronchi, bronchial and interalveolar septum. IL-4 cytokine is responsible for the inhibition of IFN-γ-induced monocytes, whereas IL-10 is primarily involved in the suppression of the production of TNF-α and IL-1. This means that high levels of produced TNF-α and IFN-γ were not significantly affected by the individual or combined effect of IL4 and IL-10. Collectively, the results indicated that TH-2/type-2 cytokines are a critical determinant in deciding the survival of the PPRV-infected animal.

Passive immunity: Passive immunity, the transfer of readymade antibodies from dam to offspring, has been evaluated in naturally infected or vaccinated dams. The newborn kids and lambs acquire these antibodies in colostrum, which are enough to protect them against PPRV for 3–5 months. Different techniques were used to estimate the level of antibodies sufficient to neutralize PPRV and so the times suggested for when kids and lambs should be first vaccinated may vary. Libeau *et al.* (1992) have detected sufficient maternal antibodies until 4 months of age using virus neutralization test but only until the third month using competitive ELISA. Awa *et al.* (2000) demonstrated that protective maternal immunity against PPRV lasts until age of 3.5 to 4.5 months. However, later studies indicated that vaccination of newborns should be started at the age of 3 months in both kids and lambs in PPRV-endemic areas (Bodjo *et al.*, 2006). Although, further support is required, first vaccine at the age of 4 months is accepted in most countries (Balamurugan *et al.*, 2012b).

Cellular and humoral immunity: The nature of the cellular and humoral immunity in recovery of PPRV is not clear yet; however, it was experimentally proven that infection by and vaccination against PPRV generates effective cellular and humoral immunity. Results showed that goats developed both humoral and cell-mediated immune responses against the HN protein of PPRV, and the generated antibodies could neutralize both PPRV and RPV *in vitro* (Sinnathamby *et al.*, 2001). On the other hands, the antibodies exclusively against the F protein of PPRV or RPV are virus neutralizing and are protective against clinical disease (Romero *et al.*, 1994; Berhe *et al.*, 2003).

The mapping of T- or B-cells epitopes (the shortest immunodominant sequence that maintains stimulatory capacity for T- or B-cells) on the viral protein is crucial in designing efficient recombinant vaccines. Studies in both mice and in small ruminants identified a conserved T-cell epitope in the N-terminus (amino acids 123–137) and C-terminus (amino acids 242–609) of HN protein, which is highly conserved between PPRV and RPV (Mitra-Kaushik *et al.*, 2001; Sinnathamby *et al.*, 2001). Using a set of monoclonal antibodies, at least four and two B-cell epitopes were mapped on the N and HN protein of PPRV, respectively (Renukaradhya *et al.*, 2002; Choi *et al.*, 2005). Mitra-Kaushik *et al.* (2001) have demonstrated that the N protein of both PPRV and RPV induces class I restricted, antigenic-specific, cross-reactive strong CD8+ T-cell responses and induces the proliferation of splenic lymphocytes. It was further shown that HN protein of PPRV induces bovine leukocyte antigen (BoLA)

class II restricted helper T-cell responses and BoLA class I restricted cytotoxic T-cell (CTL) responses. The epitope was mapped at amino acid position 408–416 (Sinnathamby *et al.*, 2004). Although the immune responses against the F protein of PPRV remain largely unknown, it is possible to conclude that most of the protective immunity (cellular or humoral) is directed against the surface glycoproteins of PPRV.

5.10.2 Immune suppression

All morbilliviruses cause immunosuppression and therefore predispose the host to secondary bacterial infection, which contributes significantly to the high mortalities associated with morbilliviruses (Beckford *et al.*, 1985). The mechanism of immune suppression in PPRV-infected animals is poorly defined. However, basic studies have shown that a virulent PPRV strain Izatnagar/94 can cause severe leukopenia, lymphopenia and reduction in antibody response (Rajak *et al.*, 2005). Such immunosuppression is evident in the acute phase (4–10 days) of the infection, which coincides with the prominence of clinical disease. The lymphotropic nature of PPRV renders lymphopenia as an important indicator of immune suppression (Raghavendra *et al.*, 1997; Kumar *et al.*, 2001; Rajak *et al.*, 2005). This tropism is linked to the presence of a protein receptor on the cell surface, the SLAM also known as CD150, which is used preferentially by wild-type PPRV to bind to the host (Adombi *et al.*, 2011). It is interesting to note that the vaccine strain of PPRV (Nigeria/75/1) showed profound inhibition of freshly isolated, mitogen-stimulated bovine and caprine peripheral blood lymphocytes (PBL). The level of PBL inhibition was found to be more profound in PPRV (50%) than in a vaccine strain of RPV (30%) in caprine PBL, especially at the high multiplicity of infection (MOI) value of 5 (Heaney *et al.*, 2002). This suppression is of special interest because Nigeria/75/1 is currently being extensively used as live attenuated vaccines. The effect of such immunosuppression on viral secretion and shedding may be of interest for future investigations.

Immune-compromised animals are prone to secondary bacterial and other concurrent infections. In order to investigate the pathology and dissemination of PPRV in immune-compromised animals, Jagtap *et al.* (2012) infected steroid-induced immune-compromised animals with PPRV. These animals not only show an aggravated form of the disease, but interestingly the virus also managed to infect atypical organs such as the liver, kidney and heart, as well as infecting the typical organs. The immunocompromised animals showed viraemia for a short time. However, the rate and extent of disease severity and mortality rate were significantly higher than in non-compromised animals.

In investigations made so far (Rajak *et al.*, 2005; Jagtap *et al.*, 2012), specific antibodies against PPRV were not detected and experiments were only limited to the first 10 days of infections. It is therefore tempting to postulate that PPRV interferes in the induction of humoral immune response. Moreover, host immune responses in the latter stages (>10 days) will be interesting to monitor because mortality usually occurs before seroconversion in the infected animals. Taken together, early detection of PPRV antigens in immunocompromised animals explains the role of these animals in the rapid spread of the disease from sick to healthy susceptible animals in the case of an outbreak. Therefore, the immunosuppressed animals may play a significant role in disease transmission, and can display a severe form of the disease.

The exact mechanism behind PPRV's ability to suppress immunity is not clearly understood. However, based on other antigenic and immunological similarities between morbilliviruses, it is likely that immune-suppression is a multigenic trait. The non-structural proteins of many of paramyxoviruses are associated with immune regulations. For instance, the V protein of MV impairs the production of IFN-α/β, which is mainly mediated by the nuclear factor kappaB (NF-κB) pathway (Caignard *et al.*, 2009). C protein of RPV, in contrast to MV, has been shown to block

the induction of type I IFN (Boxer *et al.*, 2009). In PPRV, the roles of these non-structural proteins (C and V) are not ruled out in antagonizing immune responses and their contribution to viral pathogenesis. The V protein of PPRV has a very similar amino acid identity to the V protein of MV, and therefore it is likely that the IFN inhibitory character of PPRV lies in the V protein. Our preliminary results indicate that both termini of the V protein are involved in the inhibition of IFN-α/β and NF-κB signaling (Munir *et al.*, unpublished data). However, these findings need to be confirmed in both *in vivo* and *in vitro* systems.

5.10.3 Apoptosis

Several viruses cause either inhibition or induction of apoptosis as means of successful replication. Inhibition of apoptosis prevents premature death of the host cells, which results in viral persistence and increases the virus progeny from infected cells. On the other hand, induction of apoptosis helps to release the progeny viruses and the dissemination to neighbouring cells to spread infections. Additionally, induction of apoptosis helps to establish cytotoxicity that facilitates viral pathogenesis (Roulston *et al.*, 1999). PPRV, like other members of the genus, has been shown to induce apoptosis. Infection of goat peripheral blood mononuclear cells (PBMC) with PPRV resulted in peripheral condensation of chromatin, blebbing of plasma membrane, fragmentation of the nucleus and cell leading to formation of apoptotic bodies (Fig. 5.6A,B,C) (Mondal *et al.*, 2001). All these deformities are typical for the induction of apoptosis. Although the mechanism associated with PPRV-induced apoptosis is not well understood, it has been noticed that induction of apoptosis was directly proportional to that of virus replication, which indicates that PPRV exploited the programmed cell death in favour of its replication (Fig. 5.6D). The roles of different viral proteins in regulating apoptosis have been investigated for MV, a member of morbilliviruses that shares several features of PPRV. Recently, it has been shown that the nucleoprotein of MV induces apoptosis (Bhaskar *et al.*, 2011). Owing to the high genetic similarity between N proteins of PPRV and MV (Table 5.1), it is plausible that the N protein of PPRV is also responsible for regulating apoptosis. It is also highly likely that induction of apoptosis might be associated with immune suppression, as in the case of MV (Schnorr *et al.*, 1997). Since the molecular mechanisms of immune suppression in PPRV infection have not been investigated so far, such speculations require future research to underpin the mechanisms involved in these pathways.

5.10.4 Haematological responses

Natural infection by PPRV causes severe haemorrhages in the alimentary canal and in the liver. These haemorrhages and severe diarrhoea in kids lead to decrease in erythrocytes and haematocrit values (Sahinduran *et al.*, 2012). Since PPRV replicates in lymphoid organs, it can also cause leukopenia, characterized by monocytopenia, and lymphopenia (Aikhuomobhogbe and Orheruata, 2009; Sahinduran *et al.*, 2012). It has also been demonstrated that PPRV infection in kids causes significant thrombocytopenia (decrease in thrombocytes) and increases the activated partial thromboplastin time (APLTT) and prothrombin time (PT). It has been suggested that decreased production of thrombocytes or platelets (PLT) from bone marrow, increased consumption of PLT, loss of PLT due to peripheral destruction, or a combination of these factors can lead to increase in both APLTT and PT, markers that determine the clotting tendency of blood. However, trauma and disseminated intravascular coagulation in the PPRV-infected liver can also significantly delay APLTT and PT. Infection by PPRV can also cause an increase in globulin (part of the immune system) and decrease in albumin (which regulates the colloidal osmotic pressure of blood) compared with non-infected animals (Yarim *et al.*, 2006).

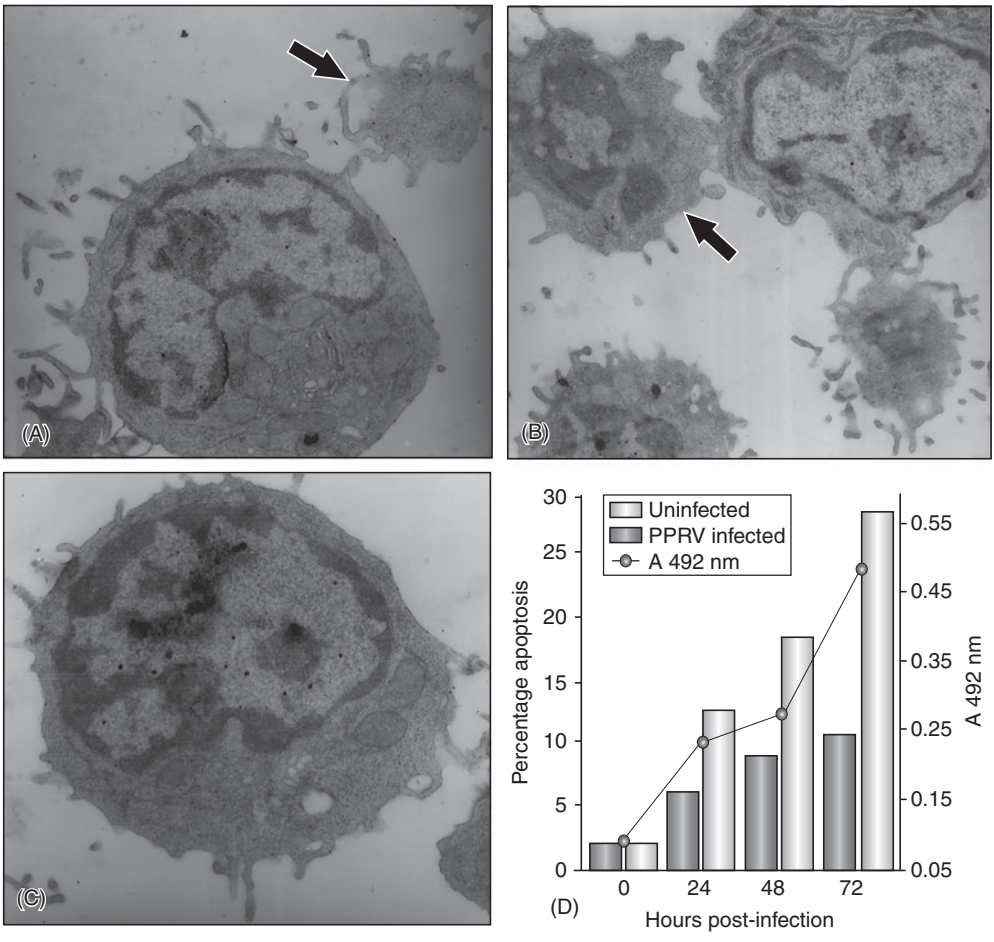

Fig. 5.6. Apoptosis induction by PPRV infection. (A) Goat cells show deformities in PPRV-infected cells, such as margination of chromatin and blebbing of the plasma membrane. (B) The infected cells showing the formation of apoptotic bodies. (C) Non-PPRV infected normal cells without any deformity. (D) The level of apoptosis is correlated with the replication of PPRV. (Modified from Mondal *et al.* (2001) with permission.)

5.10.5 Biochemical responses

PPRV has a high tendency to replicate in the kidney cells and therefore cause increase in blood urea nitrogen and creatinine, both of which are considered markers of renal function (Sahinduran *et al.*, 2012). There are several enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and gamma glutamyl-transferase (GGT), that are considered markers of liver function, all of which are significantly increased in PPRV-infected animals except GGT (Yarim *et al.*, 2006; Sahinduran *et al.*, 2012). Bilirubin is processed first in the spleen (direct bilirubin) and then in the liver (indirect bilirubin). Infection of PPRV can cause significant increase in the elevated levels of both direct and indirect bilirubin, and hence the total bilirubin in the blood serum. However, the cholesterol level (another indicator of devastating diseases) is unaffected in PPRV-infected or non-infected animals. Disturbed levels of sialic acid, a component of cell membrane and receptor for PPRV, indicate the presence of acute phases of the disease. An elevated level of sialic acid was observed in PPRV-infected animals compared with non-infected animals.

Cell-mediated immune responses may also lead to an increase in sialic acid in serum. Regardless of the cause of this induction, Yarim *et al.* (2006) suggested that serum sialic acid can be used as a marker for the diagnosis of PPRV infection in small ruminants.

5.11 Genetics and Reverse Genetics

The production of live virus entirely from cloned DNA (reverse genetics) is a fundamental step in understanding not only the biology of any virus but also to construct marker and DIVA vaccines. The greatest hurdle in the progression of PPRV research has probably been the lack of a reverse genetic system. The reverse genetics for RPV, a virus that is most closely related to PPRV, was established in 1997 using an approach that was similar to that used for rabies virus, another member of family paramyxoviridae (Schnell *et al.*, 1994; Baron and Barrett, 1997). Adapting such strategies for PPRV remained unsuccessful. However, several studies have led to a better understanding of the essential role of PPRV genes. It was shown that RPV has the capacity to accommodate several viral genes of PPRV, including HN, F and M or a combination of these. Additionally, it was shown that a compatible set of HN and F genes from a homologous system is required for satisfactory replication of RPV, which can further be improved with the inclusion of the M gene (Das *et al.*, 2000; Mahapatra *et al.*, 2006; Parida *et al.*, 2007). Using these results, a functional minigenomic replication system for PPRV was generated (Bailey *et al.*, 2007). Now, with the availability of complete genome sequences of several PPRV strains and necessary information for viral replication, a reverse genetic system has been successfully reported (Hu *et al.*, 2012). The major attempt was made to replace T7-RNA-polymerase with RNA-pol-II promoter to initiate transcription. It was thought that due to the presence of sequence elements in the PPRV genome (e.g. cryptic transcription termination signals), the transcription might have terminated prematurely. This system, which is based on the PPRV strain Nig/75/1 (vaccine strain), has the capacity to carry a foreign gene (green fluorescent gene) without having a significant effect on viral replication. This suggests that the infectious clone has the potential to be used for both DIVA and to construct recombinant multivalent vaccines by replacing the GFP ORF with the coding sequence for an immunogenic antigen from another virus, or the creation of tagged viruses for use in fundamental research on the growth and spread of PPRV in its hosts. However, the effect of knockout of a specific gene on both viral replication and its virulence remains to be determined. Additionally, it would be interesting to repeat this reverse genetic strategy for any field strains of PPRV to understand the contribution of individual genes in viral pathogenesis.

5.12 Diagnosis

Earliest possible diagnosis of PPR is crucial in implementing control measures, to contain outbreaks and minimize economic losses. Initially, the majority of PPR outbreaks were diagnosed based on typical clinical signs. However, the signs of PPR are often difficult to distinguish from those caused by a number of other diseases, such as foot-and-mouth disease and bluetongue disease (Munir *et al.*, 2013). This situation becomes even more complicated when these diseases are circulating in areas where PPR is endemic. Thus, it is necessary to confirm the clinical diagnosis through laboratory testing (Munir *et al.*, 2013). Currently, the diagnosis of PPRV is made based on demonstration of antibodies, which is a good indication because an animal infected with PPRV carries antibodies for life, with the development of a sustained antibody response.

5.12.1 Serological detection

Most of the available diagnostic assays have been developed based on the N protein. Owing to the presence at the 3′ end of the genome of PPRV, the N protein produced in quantities

higher than any other structural proteins because attenuation occurs at each intergenic region between two genes (Lefevre *et al.*, 1991; Yunus and Shaila, 2012). The antibodies produced against the N protein don't protect the animals from the disease. Due to abundance of the N protein it remains the most acceptable target for the design of PPRV diagnostic tools (Diallo *et al.*, 1994). Moreover, because the HN protein is the most diverse among all the members of morbilliviruses, RPV and PPRV share only 50% similarity in their HN proteins. The HN protein determines cell tropism; most of the protective host immune response is raised against HN protein. Therefore, serological assays have also been developed targeting HN protein (Munir *et al.*, 2012a, 2013). Commercial ELISAs are available based either on the HN (Saliki *et al.*, 1993; Anderson and McKay, 1994; Singh *et al.*, 2004) or N proteins (Libeau *et al.*, 1995) for specific detection of antibodies against PPRV, in any susceptible host. The sensitivity and specificity of these assays can be as high as 90% and 99%, respectively.

5.12.2 Antigen detection

Immunocapture (Libeau *et al.*, 1994) and sandwich ELISAs (Saliki *et al.*, 1994) are available to efficiently detect antigens in the tissues and secretions of PPRV-infected animals. Both these assays utilize monoclonal antibodies (MAbs) directed against the N protein of PPRV. Both assays are rapid, sensitive and specific with a detection limit of $10^{0.6}$ $TCID_{50}$/well. Since the MAbs used in these assays are raised against the non-overlapping domains of the N protein of PPR and RP viruses, this assay can be used to differentiate PPRV- from RPV-infected animals (Libeau *et al.*, 1994).

5.12.3 Genome detection

To overcome several shortcomings of the serological and antigen detections, such as the requirement of sera in well-preserved format, several PCRs have been developed for PPRV with wide range of sensitivities, specificities and detection limits (Munir *et al.*, 2013). Despite the high sensitivity and specificity of these assays, and their validity to detect both vaccine and field viruses, none of the assays is a formally approved OIE method. For this they need further extensive validation. None of the assays is field applicable since they require thermocycler and electrophoresis apparatus for RT-PCR, and real-time PCR for probe or SYBR-Green-based assays. However, with the development of LAMP assay, on-site detection can be proposed. It is highly plausible to combine the simple procedures for RNA extraction using Whatman FTA card (Munir *et al.*, 2012b, 2012c) and using the RT-LAMP assay for isothermal amplification. This could possibly be applied for field diagnosis of PPRV. Recently, a novel and non-amplification strategy was proposed in which two probes complementary to the target sequences (one conjugated to magnetic microparticles, the second to gold nanoparticles labelled with horseradish peroxidase) were used (Tao *et al.*, 2012). On specific binding to the target, the system allows magnetic separation and substrate detection. It was proposed to be quick (45 minutes), cheap and sensitive (17.6 ng/µl) for PPRV detection. This method holds great potential, especially when it is multiplexed for the detection of several pathogens in the same clinical sample.

5.13 Vaccines Against PPRV

The immunosuppression, caused by PPRV, predisposes the animals to secondary infections. However, through an unknown mechanism, recovery from the infection is usually followed by the establishment of a strong, specific and long-term protective immune response (Cosby *et al.*, 2005). Post-infection or vaccination immunity provided solid foundations for implementation of effective control strategies. The reverse genetic system has recently been established, which is

expected to revolutionize the recombinant vaccines with added value of DIVA. Owing to structural, genetic and antigenic similarities with RPV, significant improvements have been made in the vaccine developments against PPRV. All available PPRV vaccines can be divided into the following categories.

5.13.1 Serum immunization

Inoculation of hyperimmune serum, collected from recovered or infected animals, to susceptible animals at the fever stage could protect them from PPRV for at least 10 days, before the infection reappears (Ihemelandu *et al.*, 1985). However, this short-term passive immunization can be prolonged for to up to 9 months when hyperimmune serum is inoculated along with a virulent strain of PPRV (Adu and Joannis, 1984). This method of protection is now not often practised because of the high cost of obtaining hyperimmune serum, the unavailability of a virulent strain of PPRV and the short shelf life of virulent blood (<10 days).

5.13.2 Heterologous vaccines

After first recognition of PPRV, efforts were made to establish attenuated PPRV for immunization purposes. However, when such efforts failed until 65th cell-culture passages (Gilbert and Monnier, 1962), a RP vaccine was applied in the field to protect animals against PPRV (Bourdin *et al.*, 1970; Bonniwell, 1980). Ultimately, owing to cross-protection between RPV and PPRV, an attenuated Plowright's tissue culture RP vaccine (TCRPV) was used in many countries. This vaccine was considered safe in pregnant goats (Adu and Nawathe, 1981), and the upcoming kids carried passive immunity for at least 3 months. The vaccinated animals are protected against PPRV for at least 3 years (Rossiter, 2004), which is a consequence of strong cross-cellular immune responses. However, because of the intense effort to eradicate RPV to attain the status of RP-free countries, the use of such vaccine was discouraged. The global eradication of RP on 25 May 2011 necessitated the use of only homologous PPRV vaccine in small ruminants.

5.13.3 Homologous vaccines

In an effort to establish a homologous vaccine system for PPRV, Gilbert and Monnier (1962) successfully grew PPRV in primary cell culture, where they observed large syncytia formation as a cytopathic effect (CPE). However, despite early virus isolation, Diallo *et al.* (1989) reported a PPRV that is attenuated in cell culture, and established the bases for a homologous vaccine against PPRV. The PPRV isolated by Taylor and Abegunde (1979) from Nigerian goats that had died from PPRV infection in 1975 (Nig/75) was adapted to Vero cells at 37°C. The isolate was proved to be a powerful substitute for the heterologous vaccines. Currently, in most PPRV-endemic countries, Nig/75 is being used in the production of live attenuated vaccines (Taylor and Abegunde, 1979). The effective dose was calculated to be $10^{0.8}$ TCID50/animal; however, a dose of 10^{3} TCID50/animal also proved to be safe (Martrenchar *et al.*, 1997). Pregnant animals remained safe and were able to pass passive immunity to their offspring, which remained protected for 3–5 months. Later, the vaccine in the field was shown to be protective against wild-type PPRV virus, and immunized animals were also protected against RPV. Beside Nig/75/1, three vaccines of Indian-origin PPRV (Sungri/96, Arasur/87 and Coimbatore/97) have also been assessed as efficient in protecting against field strains of PPRV (Saravanan *et al.*, 2010). Another strain of PPRV, Egypt/87, has been attenuated and has been commercialized by the Veterinary Serum and Vaccine Research Institute, Egypt (Nahed *et al.*, 2004).

Although all the above-mentioned vaccines are highly efficacious, they are susceptible to thermodegradation. For complete immunization, these vaccines need to be

shipped at 2–8°C and should be stored at –20°C, which is difficult in the generally tropical and subtropical countries where PPR is endemic. There have been serious efforts to improve the thermostability of these vaccines (Sen *et al.*, 2010; Munir *et al.*, 2013), but with limited success, which is why the production of recombinant vaccines is still needed.

5.13.4 Recombinant marker vaccines

The surface glycoproteins (F and HN) of PPRV and RPV have been used to cross protect against the viruses. To establish heterologous marker vaccines, it has been shown that a recombinant vaccinia virus, expressing H and F proteins of RPV, is protective against both PPRV and RPV (Jones *et al.*, 1993). Similarly, a recombinant capripox virus containing either the H or F genes of RPV established complete protection against challenged PPRV (Romero *et al.*, 1995).

Again targeting the surface glycoproteins (F protein), a multivalent capripox virus-based vaccine has been reported. A capripox expressing the PPR F protein was protective for both PPRV (Guinea-Bissau/89) and capripox (Yemen isolate) (Berhe *et al.*, 2003). Moreover, a reduced dose for effective immunization was further suggested as an economical way of protecting against both diseases. Animals immunized with a baculovirus expressing HN protein construct produced a high level of neutralizing antibody responses, bovine leukocyte antigen (BoLA) class II restricted helper T cell responses and BoLA class I restricted cytotoxic T cell (CTL) responses (Sinnathamby *et al.*, 2004). Collectively, these responses protected the immunized animals against both PPRV and RPV. Baculoviruses can infect a wide variety of mammalian cells, without being able to replicate, and can therefore be used as an efficient system for delivering recombinant baculovirus-based vaccines to antigen-presenting cells for better immune responses (Ghosh *et al.*, 2002). Intraperitoneal immunization of BALB/c mice with a recombinant *Bombyx mori* nucleopolyhedrovirus (BmNPV), having ability to express antigenic epitopes of the F protein of PPRV and the H protein of RPV, lead to a high level of neutralizing antibodies against respective genes (Rahman *et al.*, 2003). It was further concluded that the use of *B. mori* larvae for large-scale production of recombinant antigens in lieu of the cell-culture system is the most economical means of protection against PPRV. The HN protein of PPRV maintained its immunodominant epitopes in its natural confirmation when expressed in peanut plants (*Arachis hypogea*). The inoculated sheep remained immunogenic and were able to express anti-PPRV-HN protein-specific cell-mediated immune responses without addition of any adjuvant (Khandelwal *et al.*, 2011).

Recently, a replication-competent recombinant canine adenovirus type-2 (CAV-2) expressing the HN gene of PPRV (China/Tibet strain) was generated (Qin *et al.*, 2012). Immunization of goats with this construct provided sterile immunity in term of protective antibodies and PPRV neutralizing antibodies even after primary infection and a profound response was observed with the booster dose. The level of antibodies remained in the goats for at least 7 months and the inoculated virus was not detected in urine or in faeces until as late as 35 days post-vaccination. Although this directly indicates the efficacy of the vaccine, animal sterility and virus shedding for longer period remain to be determined. Taken together, these are successful heterologous marker vaccines, and can be used while there are no efficient live attenuated vaccines specifically designed for PPRV.

5.13.5 Subunit vaccines

Because a PPRV infectious clone was not available until recently, efforts were made to construct subunit vaccines based on the available reverse genetic system for RPV. In the initial studies, to create a RPV in which either the F or HN gene were replaced with the corresponding genes of PPRV, it was revealed that homolog surface glycoproteins

are crucial for viral replication (Das *et al.*, 2000). However, the replacement of both genes allowed the RPV to grow comparatively better. Nevertheless, the construct provided protective immunity in vaccinated animals against challenged wild-type PPRV. To improve the replication efficacy, a triple chimeric RPV expressing the M gene, in addition to F and HN, was constructed (Mahapatra *et al.*, 2006). Interestingly, the chimeric virus grew as high as unmodified PPRV, but comparatively lower than the parental RPV. However, it remained as protective as dual chimeric virus. In the following year, the same group created a chimeric RPV that expressed the N protein derived from PPRV and suggested its use as a marker vaccine (Parida *et al.*, 2007). Recently, Buczkowski *et al.* (2012) have described a novel mechanism of marking morbillivirus vaccines, using RPV as a proof of concept, and they discuss the applicability of this method to the development of marked vaccines for PPRV. Although an efficient DIVA system is still lacking, results described above provide enough information to suggest an efficient system to discriminate infected from vaccinated animals.

5.13.6 Multivalent vaccines

Given the fact that PPRV has been reported concurrently with bluetongue virus (BTV) (Mondal *et al.*, 2009), sheep poxvirus (SPV), goat pox virus (GPV) (Saravanan *et al.*, 2007) and pestivirus (Kul *et al.*, 2008), it is essential to design multivalent (bi- or trivalent) vaccines to support economic vaccination infrastructures in developing countries. Considerable effort has been made to develop multivalent vaccine again targeting surface glycoproteins (F and HN proteins). Recombinant capripox viruses expressing HN protein (Diallo *et al.*, 2002) or F protein (Berhe *et al.*, 2003) were protective for both goat pox and PPR. However, for complete protection, a higher dose of capripox expressing HN protein was required compared to the one expressing F protein. The ability to neutralize PPRV and subsequent inhibition of viral secretion were not determined in any of these vaccines. Recently, a study conducted by Chen *et al.* (2010) showed that recombinant capripoxviruses expressing HN protein (rCPV-PPRVHN) were high inducers of virus-neutralizing antibodies than capripoxviruses expressing F protein (rCPV-PPRVF). The results have provided evidence that PPRV and goatpox viruses don't interfere in each other's immunogenicity, and that these may provide suitable bivalent vaccines in regions where both these diseases are prevalent. However, the duration of immunity conferred by the combined vaccine and field application of these vaccines still remain to be determined.

5.13.7 PPRV replication inhibition by RNA interference (RNAi)

A trend in establishing novel means of PPRV control led to a novel technique derived from molecular genetics. Silencing the expression of N protein by the use of RNAi can lead to reduction in the virus replication *in vitro* by 80% (Servan de Almeida *et al.*, 2007). Although field application and use of such control strategies *in vivo* is questionable, it is expected that investment in novel control strategies will open the way and provide foundations for understanding the biology of PPRV.

5.14 Control and Challenges

After successful eradication of RP, efforts are now being made to control and eradicate PPR, which appeared to be the most appropriate target owing to identical features of the virus, disease mechanisms and epidemiological patterns. Having this aim in mind, there are factors that can favour control and eradication of PPRV. Efficient vaccines are available for immunization and sensitive assays are available to detect the virus in all possible clinical samples. Significant efforts are now being made to improve thermostability of the vaccines and field applicability of the assays. Although

there appear to be four lineages, there is only one serotype for PPRV. The host spectrum of PPRV is relatively narrow with small ruminants as the dominant host, compared with several other infectious diseases. However, this range is now extended to most wild small ruminants and camels. The requirement of close contact for disease transmission, short incubation period (2–6 days) and life-long seropositivity further favour the control and eradication of the disease. With the availability of a reverse genetic system, novel recombinant, multivalent and also DIVA vaccines, the global eradication of PPR is feasible and achievable. On the other hand, there are factors that constrain global eradication. PPR eradication cannot be completely realized without evoking its spearhead role in animal health. The full economic consequences of the disease have not been determined. Vaccines need to be cost-effective and available in developing and PPRV-endemic countries. The overall prevalence of PPR, especially in unusual hosts such as wild small ruminants and camels, and their role in disease epizootiology, need to be investigated before promising any effort for control and eradication of the disease. Recent identification of PPRV in Asiatic lion (*Panthera leo persica*) added another dimension in the host-spectrum of PPRV (Balamurugan *et al*., 2012a). Therefore, monitoring of several wildlife species for presence of the PPRV needs to be considered.

On the other hand, due to the fact that small ruminants have a lower value compared with cattle, it is a matter of relevance when comparing the success of the RP campaign with a future PPR eradication. Moreover, because goats have a shorter lifespan and thus shorter generations, they are feeding the population with naïve individuals at a higher rate than cattle. Generally, women keep goats, whereas men manage cattle. The challenge is therefore not only biological or logistical, but also depends on societal and economic aspects. Nevertheless, a unified framework, as was in place for the RP eradication programme, is currently lacking and needs the urgent attention of international organizations, such as FAO and OIE.

5.15 Conclusions

In conclusion, despite some advances, the mechanism of infection and pathogenesis of PPRV warrants future investigation. The mechanisms of host–pathogen interaction require proper investigations to not only estimate the disease outcome, but also to provide foundations for the future DIVA and marker vaccines. Our current understanding is that PPRV differs to certain degrees from members of the same genus, which highlights the need to investigate the differences. These observations will help to estimate the host-range spectrum of the viruses in unusual hosts. Collectively, the evaluation of its economic impact, improvement and commercialization of diagnostic tests and vaccines, and coordination and integration for planning eradication are key elements to be considered in the global eradication of PPR.

References

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

Abubakar, M., Arshed, M.J., Zahur, A.B., Ali, Q. and Banyard, A.C. (2012) Natural infection with peste des petits ruminants virus: a pre and post vaccinal assessment following an outbreak scenario. *Virus Research* 167, 43–47.

Adombi, C.M., Lelenta, M., Lamien, C.E., Shamaki, D., Koffi, Y.M., Traore, A., Silber, R., Couacy-Hymann, E., Bodjo, S.C., Djaman, J.A., Luckins, A.G. and Diallo, A. (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 specimens. *Journal of Virological Methods* 173, 306–313.

Adu, F.D. and Joannis, T.E. (1984) Serum-virus simultaneous method of immunisation against peste des petits ruminants. *Tropical Animal Health and Production* 16, 119–122.

Adu, F.D. and Nawathe, D.R. (1981) Safety of tissue culture rinderpest vaccine in pregnant goats. *Tropical Animal Health and Production* 13, 166.

Ahmed, K., Jamal, S.M., Ali, Q. and Hussain, M. (2005) An outbreak of peste des petits ruminants in goat flock in Okara, Pakistan. *Pakistan Veterinary Journal* 25, 146–148.

Aikhuomobhogbe, P.U. and Orheruata, A.M. (2009) Haematological and blood biochemical indices of West African dwarf goats vaccinated against Pestes des petit ruminants (PPR). *African Journal of Biotechnology* 5, 743–748.

Al-Majali, A.M., Hussain, N.O., Amarin, N.M. and Majok, A.A. (2008) Seroprevalence of, and risk factors for, peste des petits ruminants in sheep and goats in Northern Jordan. *Preventive Veterinary Medicine* 85, 1–8.

Amjad, H., Qamar Ul, I., Forsyth, M., Barrett, T. and Rossiter, P.B. (1996) Peste des petits ruminants in goats in Pakistan. *Veterinary Reccord* 139, 118–119.

Anderson, J. and McKay, J.A. (1994) The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes. *Epidemiology and Infection* 112, 225–231.

Animal Health Australia (2009) *Disease strategy: Peste des petits ruminants (Version 3.0)*. Australian Veterinary Emergency Plan (AUSVETPLAN). Primary Industries Ministerial Council, Canberra, Australia.

Aruni, A.W., Lalitha, P.S., Mohan, A.C., Chitravelu, P. and Anbumani, S.P. (1998) Histopathological study of a natural outbreak of peste des petitis ruminants in goats of Tamilnadu. *Small Ruminants Research* 28, 233–240.

Atmaca, H.T. and Kul, O. (2012) Examination of epithelial tissue cytokine response to natural peste des petits ruminants virus (PPRV) infection in sheep and goats by immunohistochemistry. *Histology and Histopathology* 27, 69–78.

Atta-Ur-Rahman, Ashfaque, M., Rahman, S.U., Akhtar, M. and Ullah, S. (2004) Peste des petits ruminants antigen in mesenteric lymph nodes of goats slaughtered at D.I. Khan. *Pakistan Veterinary Journal* 24, 159–160.

Awa, D.N., Njoya, A. and Ngo Tama, A.C. (2000) Economics of prophylaxis against peste des petits ruminants and gastrointestinal helminthosis in small ruminants in north Cameroon. *Tropical Animal Health and Production* 32, 391–403.

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

Bailey, D., Chard, L.S., Dash, P., Barrett, T. and Banyard, A.C. (2007) Reverse genetics for peste-des-petits-ruminants virus (PPRV): promoter and protein specificities. *Virus Research* 126, 250–255.

Balamurugan, V., Sen, A., Venkatesan, G., Yadav, V., Bhanot, V., Riyesh, T., Bhanuprakash, V. and Singh, R.K. (2010) Sequence and phylogenetic analyses of the structural genes of virulent isolates and vaccine strains of peste des petits ruminants virus from India. *Transboundary and Emerging Diseases* 57, 352–364.

Balamurugan, V., Sen, A., Venkatesan, G., Bhanot, V., Yadav, V., Bhanuprakash, V. and Singh, R.K. (2012a) Peste des petits ruminants virus detected in tissues from an Asiatic lion (*Panthera leo persica*) belongs to Asian lineage IV. *Journal of Veterinary Science* 13, 203–206.

Balamurugan, V., Sen, A., Venkatesan, G., Rajak, K.K., Bhanuprakash, V. and Singh, R.K. (2012b) Study on passive immunity: time of vaccination in kids born to goats vaccinated against Peste des petits ruminants. *Virologica Sinica* 27, 228–233.

Bandyopadhyay, S.K. (2002) The economic appraisal of PPR control in India. In: *14th Annual Conference and National Seminar on Management of Viral Diseases with Emphasis on Global Trade and WTO Regime*. Indian Virological Society, New Delhi, India.

Banyard, A.C., Parida, S., Batten, C., Oura, C., Kwiatek, O. and Libeau, G. (2010) Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. *Journal of General Virology* 91, 2885–2897.

Baron, M.D. and Barrett, T. (1997) Rescue of rinderpest virus from cloned cDNA. *Journal of Virology* 71, 1265–1271.

Baron, M.D. and Barrett, T. (2000) Rinderpest viruses lacking the C and V proteins show specific defects in growth and transcription of viral RNAs. *Journal of Virology* 74, 2603–2611.

Baron, M.D., Parida, S. and Oura, C.A. (2011) Peste des petits ruminants: a suitable candidate for eradication? *Veterinary Record* 169, 16–21.

Barrett, T., Ashley, C.B. and Diallo, A. (2006) Molecular biology of the morbilliviruses. In: Taylor, W.P., Barrett, T. and Pastoret, P.-P. (eds) *Rinderpest and Peste des Petits Ruminants Virus Plagues of Large and Small Ruminants*. Elsevier, Academic Press, London, pp. 31–67.

Bazarghani, T.T., Charkhkar, S., Doroudi, J. and Bani Hassan, E. (2006) A review on peste des petits ruminants (PPR) with special reference to PPR in Iran. *Journal of Veterinary Medicine Series B* 53 (Suppl 1), 17–18.

Beckford, A.P., Kaschula, R.O. and Stephen, C. (1985) Factors associated with fatal cases of measles. A retrospective autopsy study. *South African Medicine Journal* 68, 858–863.

Berhe, G., Minet, C., Le Goff, C., Barrett, T., Ngangnou, A., Grillet, C., Libeau, G., Fleming, M., Black, D.N. and Diallo, A. (2003) Development of a dual recombinant vaccine to protect small ruminants against peste-des-petits-ruminants virus and capripoxvirus infections. *Journal of Virology* 77, 1571–1577.

Bhaskar, A., Bala, J., Varshney, A. and Yadava, P. (2011) Expression of measles virus nucleoprotein induces apoptosis and modulates diverse functional proteins in cultured mammalian cells. *PLoS ONE* 6, e18765.

Bhella, D., Ralph, A. and Yeo, R.P. (2004) Conformational flexibility in recombinant measles virus nucleocapsids visualised by cryo-negative stain electron microscopy and real-space helical reconstruction. *Journal of Molecular Biology* 340, 319–331.

Blom, N., Gammeltoft, S. and Brunak, S. (1999) Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. *Journal of Molecular Biology* 294, 1351–1362.

Bodjo, S.C., Couacy-Hymann, E., Koffi, M.Y. and Danho, T. (2006) Assessment of the duration of maternal antibodies specific to the homologous peste des petits ruminant vaccine 'Nigeria 75/1' in Djallonké lambs. *Biokemistri* 18, 99–103.

Bodjo, S.C., Lelenta, M., Couacy-Hymann, E., Kwiatek, O., Albina, E., Gargani, D., Libeau, G. and Diallo, A. (2008) Mapping the Peste des Petits Ruminants virus nucleoprotein: identification of two domains involved in protein self-association. *Virus Research* 131, 23–32.

Bonniwell, M.A. (1980) The use of tissue culture rinderpest vaccine to protect sheep and goats against peste des petits ruminants in the Ashanti region of Ghana. *Bulletin de l'Office Internationale des Epizooties* 92, 1233–1238.

Bourdin, P., Rioche, M. and Laurent, A. (1970) Emploi d'un vaccin antibovipestique produit sur cultures cellulaires dans la prophylaxie de la peste des petits ruminants au Dahomey. *Revue d'élevage et de médecine vétérinaire des pays tropicaux* 23, 295–300.

Boxer, E.L., Nanda, S.K. and Baron, M.D. (2009) The rinderpest virus non-structural C protein blocks the induction of type 1 interferon. *Virology* 385, 134–142.

Braide, V.B. (1981) PPR – a review. *World Animal Review* 39, 25–28.

Brindha, K., Raj, G.D., Ganesan, P.I., Thiagarajan, V., Nainar, A.M. and Nachimuthu, K. (2001) Comparison of virus isolation and polymerase chain reaction for diagnosis of peste des petits ruminants. *Acta Virology* 45, 169–172.

Buczkowski, H., Parida, S., Bailey, D., Barrett, T. and Banyard, A.C. (2012) A novel approach to generating morbillivirus vaccines: negatively marking the rinderpest vaccine. *Vaccine* 30, 1927–1935.

Bundza, A., Afshar, A., Dukes, T.W., Myers, D.J., Dulac, G.C. and Becker, S.A. (1988) Experimental peste des petits ruminants (goat plague) in goats and sheep. *Canadian Journal of Veterinary Research* 52, 46–52.

Caignard, G., Bourai, M., Jacob, Y., Tangy, F. and Vidalain, P.O. (2009) Inhibition of IFN-alpha/beta signaling by two discrete peptides within measles virus V protein that specifically bind STAT1 and STAT2. *Virology* 383, 112–120.

Chard, L.S., Bailey, D.S., Dash, P., Banyard, A.C. and Barrett, T. (2008) Full genome sequences of two virulent strains of peste-des-petits ruminants virus, the Cote d'Ivoire 1989 and Nigeria 1976 strains. *Virus Research* 136, 192–197.

Chen, W., Hu, S., Qu, L., Hu, Q., Zhang, Q., Zhi, H., Huang, K. and Bu, Z. (2010) A goat poxvirus-vectored peste-des-petits-ruminants vaccine induces long-lasting neutralization antibody to high levels in goats and sheep. *Vaccine* 28, 4742–4750.

Choi, K.S., Nah, J.J., Ko, Y.J., Kang, S.Y., Yoon, K.J. and Jo, N.I. (2005) Antigenic and immunogenic investigation of B-cell epitopes in the nucleocapsid protein of peste des petits ruminants virus. *Clinical Diagnosis and Laboratory Immunology* 12, 114–121.

Ciancanelli, M.J. and Basler, C.F. (2006) Mutation of YMYL in the Nipah virus matrix protein abrogates budding and alters subcellular localization. *Journal of Virology* 80, 12,070–12,078.

Cosby, S., Kai, C. and Yamanouchi, K. (2005) *Immunology of Rinderpest: An Immunosuppression but a Lifelong Vaccine Protection*. Academic Press, Elsevier, Amsterdam, The Netherlands.

Cosseddu, G.M., Pinoni, C., Polci, A., Sebhatu, T., Lelli, R. and Monaco, F. (2013) Characterization of peste des petits ruminants virus, Eritrea, 2002–2011. *Emerging Infectious Diseases* 19, 160–161.

Couacy-Hymann, E., Bodjo, S.C., Danho, T., Koffi, M.Y., Libeau, G. and Diallo, A. (2007) Early detection of viral excretion from experimentally infected goats with peste-des-petits ruminants virus. *Preventive Veterinary Medicine* 78, 85–88.

Das, S.C., Baron, M.D. and Barrett, T. (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. *Journal of Virology* 74, 9039–9047.

Dhar, P., Sreenivasa, B.P., Barrett, T., Corteyn, M., Singh, R.P. and Bandyopadhyay, S.K. (2002) Recent epidemiology of peste des petits ruminants virus (PPRV). *Veterinary Microbiology* 88, 153–159.

Diallo, A. (2006) Control of peste des petits ruminants and poverty alleviation? *Journal of Veterinary Medicine Series B: Infectous Diseases in Veterinary Public Health* 53 (Suppl 1), 11–13.

Diallo, A., Barrett, T., Lefevre, P.C. and Taylor, W.P. (1987) Comparison of proteins induced in cells infected with rinderpest and peste des petits ruminants viruses. *Journal of General Virology* 68, 2033–2038.

Diallo, A., Taylor, W.P., Lefèvre, P.C. and Provost, A. (1989) Atténuation d'une souche de virus de la peste des petits ruminants: candidat pour un vaccin homologue vivant. *Revue d'élevage et de médecine vétérinaire des pays tropicaux* 42, 311–317.

Diallo, A., Barrett, T., Barbron, M., Meyer, G. and Lefevre, P.C. (1994) Cloning of the nucleocapsid protein gene of peste-des-petits-ruminants virus: relationship to other morbilliviruses. *Journal of General Virology* 75, 233–237.

Diallo, A., Minet, C., Berhe, G., Le Goff, C., Black, D.N., Fleming, M., Barrett, T., Grillet, C. and Libeau, G. (2002) Goat immune response to capripox vaccine expressing the hemagglutinin protein of peste des petits ruminants. *Annals of the New York Academy of Sciences* 969, 88–91.

Diallo, A., Minet, C., Le Goff, C., Berhe, G., Albina, E., Libeau, G. and Barrett, T. (2007) The threat of peste des petits ruminants: progress in vaccine development for disease control. *Vaccine* 25, 5591–5597.

Durojaiye, O.A., Taylor, W.P. and Smale, C. (1985) The ultrastructure of peste des petits ruminants virus. *Zentralblatt Fur Veterinarmedizin Reihe B* 32, 460–465.

El Harrak, M., Touil, N., Loutfi, C., Hammouchi, M., Parida, S., Sebbar, G., Chaffai, N., Harif, B., Messoudi, N., Batten, C. and Oura, C.A. (2012) A reliable and reproducible experimental challenge model for peste des petits ruminants virus. *Journal of Clinical Microbiology* 50, 3738–3740.

Ezeibe, M.C., Okoroafor, O.N., Ngene, A.A., Eze, J.I., Eze, I.C. and Ugonabo, J.A. (2008) Persistent detection of peste de petits ruminants antigen in the faeces of recovered goats. *Tropical Animal Health and Production* 40, 517–519.

Ezeokoli, C.D., Umoh, J.U., Chineme, C.N., Isitor, G.N. and Gyang, E.O. (1986) Clinical and epidemiological features of peste des petits ruminants in Sokoto Red goats. *Revue d'élevage et de médecine vétérinaire des pays tropicaux* 39, 269–273.

Flanagan, E.B., Ball, L.A. and Wertz, G.W. (2000) Moving the glycoprotein gene of vesicular stomatitis virus to promoter-proximal positions accelerates and enhances the protective immune response. *Journal of Virology* 74, 7895–7902.

Galbraith, S.E., Tiwari, A., Baron, M.D., Lund, B.T., Barrett, T. and Cosby, S.L. (1998) Morbillivirus downregulation of CD46. *Journal of Virology* 72, 10,292–10,297.

Galbraith, S.E., McQuaid, S., Hamill, L., Pullen, L., Barrett, T. and Cosby, S.L. (2002) Rinderpest and peste des petits ruminants viruses exhibit neurovirulence in mice. *Journal of Neurovirology* 8, 45–52.

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

Ghosh, S., Parvez, M.K., Banerjee, K., Sarin, S.K. and Hasnain, S.E. (2002) Baculovirus as mammalian cell expression vector for gene therapy: an emerging strategy. *Molecular Therapy* 6, 5–11.

Gibbs, E.P., Taylor, W.P., Lawman, M.J. and Bryant, J. (1979) Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. *Intervirology* 11, 268–274.

Gilbert, Y. and Monnier, J. (1962) Adaptation du virus de la peste des petits ruminants aux cultures cellulaires. *Revue d'élevage et de médecine vétérinaire des pays tropicaux* 15, 321.

Hamdy, F.M., Dardiri, A.H., Nduaka, O., Breese, S.R.J. and Ihemelandu, E.C. (1976) Etiology of the stomatitis pneumcenteritis complex in Nigerian dwarf goats. *Canadian Journal of Comparative Medicine* 40, 276–284.

Hammouchi, M., Loutfi, C., Sebbar, G., Touil, N., Chaffai, N., Batten, C., Harif, B., Oura, C. and El Harrak, M. (2012) Experimental infection of alpine goats with a Moroccan strain of peste des petits ruminants virus (PPRV). *Veterinary Microbiology* 160, 240–244.

Heaney, J., Barrett, T. and Cosby, S.L. (2002) Inhibition of in vitro leukocyte proliferation by morbilliviruses. *Journal of Virology* 76, 3579–3584.

Horikami, S.M., Smallwood, S., Bankamp, B. and Moyer, S.A. (1994) An amino-proximal domain of the L protein binds to the P protein in the measles virus RNA polymerase complex. *Virology* 205, 540–545.

Hu, Q., Chen, W., Huang, K., Baron, M.D. and Bu, Z. (2012) Rescue of recombinant peste des petits ruminants virus: creation of a GFP-expressing virus and application in rapid virus neutralization test. *Veterinary Research* 43, 48.

Ihemelandu, E.C., Nduaka, O. and Ojukwu, E.M. (1985) Hyperimmune serum in the control of peste des petits ruminants. *Tropical Animal Health and Production* 17, 83–88.

Jagtap, S.P., Rajak, K.K., Garg, U.K., Sen, A., Bhanuprakash, V., Sudhakar, S.B., Balamurugan, V., Patel, A., Ahuja, A., Singh, R.K. and Vanamayya, P.R. (2012) Effect of immunosuppression on pathogenesis of peste des petits ruminants (PPR) virus infection in goats. *Microbial Pathogenesis* 52, 217–226.

Johansson, K., Bourhis, J.M., Campanacci, V., Cambillau, C., Canard, B. and Longhi, S. (2003) Crystal structure of the measles virus phosphoprotein domain responsible for the induced folding of the C-terminal domain of the nucleoprotein. *Journal of Biological Chemistry* 278, 44,567–44,573.

Jones, L., Giavedoni, L., Saliki, J.T., Brown, C., Mebus, C. and Yilma, T. (1993) Protection of goats against peste des petits ruminants with a vaccinia virus double recombinant expressing the F and H genes of rinderpest virus. *Vaccine* 11, 961–964.

Keita, D., Servan de Almeida, R., Libeau, G. and Albina, E. (2008) Identification and mapping of a region on the mRNA of Morbillivirus nucleoprotein susceptible to RNA interference. *Antiviral Research* 80, 158–167.

Khandelwal, A., Renukaradhya, G.J., Rajasekhar, M., Sita, G.L. and Shaila, M.S. (2011) Immune responses to hemagglutinin-neuraminidase protein of peste des petits ruminants virus expressed in transgenic peanut plants in sheep. *Veterinary Immunology and Immunopathology* 140, 291–296.

Kul, O., Kabakci, N., Atmaca, H.T. and Ozkul, A. (2007) Natural peste des petits ruminants virus infection: novel pathologic findings resembling other morbillivirus infections. *Veterinary Pathology* 44, 479–486.

Kul, O., Kabakci, N., Ozkul, A., Kalender, H. and Atmaca, H.T. (2008) Concurrent peste des petits ruminants virus and pestivirus infection in stillborn twin lambs. *Veterinary Pathology* 45, 191–196.

Kulkarni, D.D., Bhikane, A.U., Shaila, M.S., Varalakshmi, P., Apte, M.P. and Narladkar, B.W. (1996) Peste des petits ruminants in goats in India. *Veterinary Record* 138, 187–188.

Kumar, A., Singh, S.V., Rana, R., Vaid, R.K., Misri, J. and Vs, V. (2001) PPR outbreak in goats: epidemiological and therapeutic studies. *Indian Journal of Animal Sciences* 71, 815–818.

Kwiatek, O., Minet, C., Grillet, C., Hurard, C., Carlsson, E., Karimov, B., Albina, E., Diallo, A. and Libeau, G. (2007) Peste des petits ruminants (PPR) outbreak in Tajikistan. *Journal of Comparative Pathology* 136, 111–119.

Kwiatek, O., Ali, Y.H., Saeed, I.K., Khalafalla, A.I., Mohamed, O.I., Obeida, A.A., Abdelrahman, M.B., Osman, H.M., Taha, K.M., Abbas, Z., El Harrak, M., Lhor, Y., Diallo, A., Lancelot, R., Albina, E. and Libeau, G. (2011) Asian lineage of peste des petits ruminants virus, Africa. *Emerging Infectious Diseases* 17, 1223–1231.

Laine, D., Trescol-Biemont, M.C., Longhi, S., Libeau, G., Marie, J.C., Vidalain, P.O., Azocar, O., Diallo, A., Canard, B., Rabourdin-Combe, C. and Valentin, H. (2003) Measles virus (MV) nucleoprotein binds to a novel cell surface receptor distinct from FcgammaRII via its C-terminal domain: role in MV-induced immunosuppression. *Journal of Virology* 77, 11,332–11,346.

Lamb, A. and Kolakofsky, D. (2001) *Paramyxoviridae: the viruses and their replication. Fields Virology*. Lippincott Williams & Wilkins, Philadelphia, Pennsylvania.

Langedijk, J.P., Daus, F.J. and Van Oirschot, J.T. (1997) Sequence and structure alignment of Paramyxoviridae attachment proteins and discovery of enzymatic activity for a morbillivirus hemagglutinin. *Journal of Virology* 71, 6155–6167.

Lefevre, P.C. and Diallo, A. (1990) Peste des petits ruminants. *Revue Scientifique et Technique* 9, 935–981.

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

Libeau, G., Diallo, A., Calvez, D. and Lefevre, P.C. (1992) A competitive ELISA using anti-N monoclonal antibodies for specific detection of rinderpest antibodies in cattle and small ruminants. *Veterinary Microbiology* 31, 147–160.

Libeau, G., Diallo, A., Colas, F. and Guerre, L. (1994) Rapid differential diagnosis of rinderpest and peste des petits ruminants using an immunocapture ELISA. *Veterinary Record* 134, 300–304.

Libeau, G., Prehaud, C., Lancelot, R., Colas, F., Guerre, L., Bishop, D.H. and Diallo, A. (1995) Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. *Research in Veterinary Science* 58, 50–55.

Luka, P.D., Erume, J., Mwiine, F.N. and Ayebazibwe, C. (2012) Molecular characterization of peste des petits ruminants virus from the Karamoja region of Uganda (2007–2008). *Archives of Virology* 157, 29–35.

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

Mahapatra, M., Parida, S., Baron, M.D. and Barrett, T. (2006) Matrix protein and glycoproteins F and H of Peste-des-petits-ruminants virus function better as a homologous complex. *Journal of General Virology* 87, 2021–2029.

Martrenchar, A., Zoyem, N. and Diallo, A. (1997) Experimental study of a mixed vaccine against peste des petits ruminants and capripox infection in goats in Northern Cameroon. *Small Ruminant Research* 26, 39–44.

Meng, X., Dou, Y., Zhai, J., Zhang, H., Yan, F., Shi, X., Luo, X., Li, H. and Cai, X. (2011) Tissue distribution and expression of signaling lymphocyte activation molecule receptor to peste des petits ruminant virus in goats detected by real-time PCR. *Journal of Molecular Histology* 42, 467–472.

Meyer, G. and Diallo, A. (1995) The nucleotide sequence of the fusion protein gene of the peste des petits ruminants virus: the long untranslated region in the 5'-end of the F-protein gene of morbilliviruses seems to be specific to each virus. *Virus Research* 37, 23–35.

Mioulet, V., Barrett, T. and Baron, M.D. (2001) Scanning mutagenesis identifies critical residues in the rinderpest virus genome promoter. *Journal of General Virology* 82, 2905–2911.

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

Mondal, B., Sreenivasa, B.P., Dhar, P., Singh, R.P. and Bandyopadhyay, S.K. (2001) Apoptosis induced by peste des petits ruminants virus in goat peripheral blood mononuclear cells. *Virus Research* 73, 113–119.

Mondal, B., Sen, A., Chand, K., Biswas, S.K., De, A., Rajak, K.K. and Chakravarti, S. (2009) Evidence of mixed infection of peste des petits ruminants virus and bluetongue virus in a flock of goats as confirmed by detection of antigen, antibody and nucleic acid of both the viruses. *Tropical Animal Health and Production* 41, 1661–1667.

Moussallem, T.M., Guedes, F., Fernandes, E.R., Pagliari, C., Lancellotti, C.L., De Andrade, H.F. Jr and Duarte, M.I. (2007) Lung involvement in childhood measles: severe immune dysfunction revealed by quantitative immunohistochemistry. *Human Pathology* 38, 1239–1247.

Munir, M. (2013) Role of wild small ruminants in the epidemiology of peste des petits ruminants. *Transboundary and Emerging Diseases* doi: 10.1111/tbed.12052.

Munir, M., Siddique, M. and Ali, Q. (2009) Comparative efficacy of standard AGID and precipitinogen inhibition test with monoclonal antibodies based competitive ELISA for the serology of Peste des Petits Ruminants in sheep and goats. *Tropical Animal Health and Production* 41, 413–420.

Munir, M., Abubakar, M., Zohari, S. and Berg, M. (2012a) Serodiagnosis of Peste des Petits Ruminants Virus. In: Al-Moslih, M. (ed.) *Serological Diagnosis of Certain Human, Animal and Plant Diseases*. InTech, Croatia, pp. 37–58.

Munir, M., Zohari, S., Saeed, A., Khan, Q.M., Abubakar, M., Leblanc, N. and Berg, M. (2012b) Detection and phylogenetic analysis of peste des petits ruminants virus isolated from outbreaks in Punjab, Pakistan. *Transboundary and Emerging Diseases* 59, 85–93.

Munir, M., Zohari, S., Suluku, R., Leblanc, N., Kanu, S., Sankoh, F.A., Berg, M., Barrie, M.L. and Stahl, K. (2012c) Genetic characterization of peste des petits ruminants virus, Sierra Leone. *Emerging Infectious Diseases* 18, 193–195.

Munir, M., Zohari, S. and Berg, M. (2013) Current advances in molecular diagnosis and vaccines for peste des petits ruminants. In: Munir, M., Zohari, S. and Berg, M. (eds) *Molecular Biology and Pathogenesis of Peste des Petits Ruminants Virus*. Springer, Berlin, Germany, pp. 105–133.

Murphy, S.K. and Parks, G.D. (1999) RNA replication for the paramyxovirus simian virus 5 requires an internal repeated (CGNNNN) sequence motif. *Journal of Virology* 73, 805–809.

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

Nahed, A.K., Hanan, S.A., Hanan, M.S., Laila, A.S. and Fatma, S.M. (2004) The production and evaluation of a standard diagnostic peste des petits ruminants (PPR) hyperimmune serum prepared from the Egyptian antigen (Egypt 87). *Egyptian Journal of Immunology* 11, 9–14.

Nanda, Y.P., Chatterjee, A., Purohit, A.K., Diallo, A., Innui, K., Sharma, R.N., Libeau, G., Thevasagayam, J.A., Bruning, A., Kitching, R.P., Anderson, J., Barrett, T. and Taylor, W.P. (1996) The isolation of peste des petits ruminants virus from northern India. *Veterinary Microbiology* 51, 207–216.

Obi, T.U., Ojo, M.O., Durojaiye, O.A., Kasali, O.B., Akpavie, S. and Opasina, D.B. (1983) Peste des petits ruminants (PPR) in goats in Nigeria: clinical, microbiological and pathological features. *Zentralblatt Für Veterinarmedizin B* 30, 751–761.

Opal, S.M. and Depalo, V.A. (2000) Anti-inflammatory cytokines. *Chest* 117, 1162–1172.

Parida, S., Mahapatra, M., Kumar, S., Das, S.C., Baron, M.D., Anderson, J. and Barrett, T. (2007) Rescue of a chimeric rinderpest virus with the nucleocapsid protein derived from peste-des-petits-ruminants virus: use as a marker vaccine. *Journal of General Virology* 88, 2019–2027.

Patel, A., Rajak, K.K., Balamurugan, V., Sen, A., Sudhakar, S.B., Bhanuprakash, V., Singh, R.K. and Pandey, A.B. (2012) Cytokines expression profile and kinetics of peste des petits ruminants virus antigen and antibody in infected and vaccinated goats. *Virologica Sinica* 27, 265–271.

Patterson, J.B., Thomas, D., Lewicki, H., Billeter, M.A. and Oldstone, M.B. (2000) V and C proteins of measles virus function as virulence factors in vivo. *Virology* 267, 80–89.

Pawar, R.M., Raj, G.D., Kumar, T.M., Raja, A. and Balachandran, C. (2008) Effect of siRNA mediated suppression of signaling lymphocyte activation molecule on replication of peste des petits ruminants virus in vitro. *Virus Research* 136, 118–123.

Perry, B.D., Randolph, T.F., McDermott, J.J., Sones, K.R. and Thornton, P.K. (2002) *Investing in Animal Health Research to Alleviate Poverty*. International Livestock Research Institute, Nairobi, Kenya.

Plemper, R.K., Hammond, A.L. and Cattaneo, R. (2001) Measles virus envelope glycoproteins hetero-oligomerize in the endoplasmic reticulum. *Journal of Biological Chemistry* 276, 44,239–44,246.

Qin, J., Huang, H., Ruan, Y., Hou, X., Yang, S., Wang, C., Huang, G., Wang, T., Feng, N., Gao, Y. and Xia, X. (2012) A novel recombinant Peste des petits ruminants–canine adenovirus vaccine elicits long-lasting neutralizing antibody response against PPR in goats. *PLoS ONE* 7, e37170.

Raghavendra, L., Setty, D.R.L. and Raghavan, R. (1997) (2000) Clinico-biochemical changes in sheep and goats experimentally infected with Vero cell adapted peste-des petits ruminants (PPR) virus. *International Journal of Animal Sciences* 15, 171–173.

Rahaman, A., Srinivasan, N., Shamala, N. and Shaila, M.S. (2003) The fusion core complex of the peste des petits ruminants virus is a six-helix bundle assembly. *Biochemistry* 42, 922–931.

Rahman, M.M., Shaila, M.S. and Gopinathan, K.P. (2003) Baculovirus display of fusion protein of peste des petits ruminants virus and hemagglutination protein of rinderpest virus and immunogenicity of the displayed proteins in mouse model. *Virology* 317, 36–49.

Rajak, K.K., Sreenivasa, B.P., Hosamani, M., Singh, R.P., Singh, S.K., Singh, R.K. and Bandyopadhyay, S.K. (2005) Experimental studies on immunosuppressive effects of peste des petits ruminants (PPR) virus in goats. *Comparative Immunology, Microbiology and Infectious Diseases* 28, 287–296.

Renukaradhya, G.J., Sinnathamby, G., Seth, S., Rajasekhar, M. and Shaila, M.S. (2002) Mapping of B-cell epitopic sites and delineation of functional domains on the hemagglutinin-neuraminidase protein of peste des petits ruminants virus. *Virus Research* 90, 171–185.

Riedl, P., Moll, M., Klenk, H.D. and Maisner, A. (2002) Measles virus matrix protein is not cotransported with the viral glycoproteins but requires virus infection for efficient surface targeting. *Virus Research* 83, 1–12.

Romero, C.H., Barrett, T., Chamberlain, R.W., Kitching, R.P., Fleming, M. and Black, D.N. (1994) Recombinant capripoxvirus expressing the hemagglutinin protein gene of rinderpest virus: protection of cattle against rinderpest and lumpy skin disease viruses. *Virology* 204, 425–429.

Romero, C.H., Barrett, T., Kitching, R.P., Bostock, C. and Black, D.N. (1995) Protection of goats against peste des petits ruminants with recombinant capripoxviruses expressing the fusion and haemagglutinin protein genes of rinderpest virus. *Vaccine* 13, 36–40.

Rossiter, P.B. (2004) Peste des petits ruminants. In: Coetzer, J.A.W. and Tustin, R.C. (eds) *Infectious Diseases of Livestock*, 2nd edn. Oxford University Press, Cape Town, South Africa, pp. 660–672.

Rossiter, P.B. and Taylor, W.P. (1994) Peste des petits ruminants. In: Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C. (eds) *Infectious Diseases of Livestock*, vol. 2. Oxford University Press, Cape Town, South Africa.

Roulston, A., Marcellus, R.C. and Branton, P.E. (1999) Viruses and apoptosis. *Annual Review of Microbiology* 53, 577–628.

Sahinduran, S., Albay, M.K., Sezer, K., Ozmen, O., Mamak, N., Haligur, M., Karakurum, C. and Yildiz, R. (2012) Coagulation profile, haematological and biochemical changes in kids naturally infected with peste des petits ruminants. *Tropical Animal Health and Production* 44, 453–457.

Saliki, J.T., Libeau, G., House, J.A., Mebus, C.A. and Dubovi, E.J. (1993) Monoclonal antibody-based blocking enzyme-linked immunosorbent assay for specific detection and titration of peste-des-petits-ruminants virus antibody in caprine and ovine sera. *Journal of Clinical Microbiology* 31, 1075–1082.

Saliki, J.T., House, J.A., Mebus, C.A. and Dubovi, E.J. (1994) Comparison of monoclonal antibody-based sandwich enzyme-linked immunosorbent assay and virus isolation for detection of peste des petits ruminants virus in goat tissues and secretions. *Journal of Clinical Microbiology* 32, 1349–1353.

Saravanan, P., Balamurugan, V., Sen, A., Sarkar, J., Sahay, B., Rajak, K.K., Hosamani, M., Yadav, M.P. and Singh, R.K. (2007) Mixed infection of peste des petits ruminants and orf on a goat farm in Shahjahanpur, India. *Veterinary Record* 160, 410–412.

Saravanan, P., Sen, A., Balamurugan, V., Rajak, K.K., Bhanuprakash, V., Palaniswami, K.S., Nachimuthu, K., Thangavelu, A., Dhinakarraj, G., Hegde, R. and Singh, R.K. (2010) Comparative efficacy of peste des petits ruminants (PPR) vaccines. *Biologicals* 38, 479–485.

Schnell, M.J., Mebatsion, T. and Conzelmann, K.K. (1994) Infectious rabies viruses from cloned cDNA. *EMBO Journal* 13, 4195–4203.

Schnorr, J.J., Seufert, M., Schlender, J., Borst, J., Johnston, I.C., Ter Meulen, V. and Schneider-Schaulies, S. (1997) Cell cycle arrest rather than apoptosis is associated with measles virus contact-mediated immunosuppression in vitro. *Journal of General Virology* 78, 3217–3226.

Sen, A., Saravanan, P., Balamurugan, V., Rajak, K.K., Sudhakar, S.B., Bhanuprakash, V., Parida, S. and Singh, R.K. (2010) Vaccines against peste des petits ruminants virus. *Expert Review of Vaccines* 9, 785–796.

Servan De Almeida, R., Keita, D., Libeau, G. and Albina, E. (2007) Control of ruminant morbillivirus replication by small interfering RNA. *Journal of General Virology* 88, 2307–2311.

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

Shaila, M.S., Shamaki, D., Forsyth, M.A., Diallo, A., Goatley, L., Kitching, R.P. and Barrett, T. (1996) Geographic distribution and epidemiology of peste des petits ruminants virus. *Virus Research* 43, 149–153.

Shankar, H., Gupta, V.K. and Singh, N. (1998) Occurrence of peste des petits ruminants like diseases in small ruminants in Uttar Pradesh. *Indian Journal of Animal Sciences* 68, 38–40.

Singh, R.P., Sreenivasa, B.P., Dhar, P., Shah, L.C. and Bandyopadhyay, S.K. (2004) Development of a monoclonal antibody based competitive-ELISA for detection and titration of antibodies to peste des petits ruminants (PPR) virus. *Veterinary Microbiology* 98, 3–15.

Sinnathamby, G., Renukaradhya, G.J., Rajasekhar, M., Nayak, R. and Shaila, M.S. (2001) Immune responses in goats to recombinant hemagglutinin-neuraminidase glycoprotein of Peste des petits ruminants virus: identification of a T cell determinant. *Vaccine* 19, 4816–4823.

Sinnathamby, G., Seth, S., Nayak, R. and Shaila, M.S. (2004) Cytotoxic T cell epitope in cattle from the attachment glycoproteins of rinderpest and peste des petits ruminants viruses. *Viral Immunology* 17, 401–410.

Srinivas, R.P. and Gopal, T. (1996) Peste des petits ruminants (PPR): a new menace to sheep and goats. *Livestock Advisor* 21, 22–26.

Stem, C. (1993) An economic analysis of the prevention of peste des petits ruminants in Nigerian goats. *Preventive Veterinary Medicine* 16, 141–150.

Svitek, N. and Von Messling, V. (2007) Early cytokine mRNA expression profiles predict Morbillivirus disease outcome in ferrets. *Virology* 362, 404–410.

Tao, C., Li, G., Wang, Y. and Huang, H. (2012) Enzymatic reporting of peste des petits ruminants virus genes ligating two specific probes on nanoparticles. *Biotechnology Letters* 35, 613–618.

Taylor, W.P. (1979) Protection of goats against peste-des-petits-ruminants with attenuated rinderpest virus. *Research in Veterinary Science* 27, 321–324.

Taylor, W.P. and Abegunde, A. (1979) The isolation of peste des petits ruminants virus from Nigerian sheep and goats. *Research in Veterinary Science* 26, 94–96.

Taylor, W.P., Al Busaidy, S. and Barrett, T. (1990) The epidemiology of peste des petits ruminants in the Sultanate of Oman. *Veterinary Microbiology* 22, 341–352.

Thombare, N.N. and Sinha, M.K. (2009) Economic implications of Peste des petits ruminants (PPR) disease in sheep and goats: a sample analysis of district Pune, Maharastra. *Agricultural Economics Research Review* 22, 319–322.

Varsanyi, T.M., Utter, G. and Norrby, E. (1984) Purification, morphology and antigenic characterization of measles virus envelope components. *Journal of General Virology* 65, 355–366.

Waret-Szkuta, A., Roger, F., Chavernac, D., Yigezu, L., Libeau, G., Pfeiffer, D.U. and Guitian, J. (2008) Peste des petits ruminants (PPR) in Ethiopia: analysis of a national serological survey. *BMC Veterinary Research* 4, 34.

Woo, P.C., Lau, S.K., Wong, B.H., Fan, R.Y., Wong, A.Y., Zhang, A.J., Wu, Y., Choi, G.K., Li, K.S., Hui, J., Wang, M., Zheng, B.J., Chan, K.H. and Yuen, K.Y. (2012) Feline morbillivirus, a previously undescribed

paramyxovirus associated with tubulointerstitial nephritis in domestic cats. *Proceedings of the National Academy of Sciences of the USA* 109, 5435–5440.

Wosu, L.O. (1994) Current status of peste des petits ruminants (PPR) disease in small ruminants. A review article. *Stud. Research in Veterinary Medicine* 2, 83–90.

Yarim, G.F., Nisbet, C., Yazıcı, Z. and Gumusova, S.O. (2006) Elevated serum total sialic acid concentrations in sheep with peste des petits ruminants. *Medycyna Weterynaryjna* 62, 1375–1377.

Yener, Z., Saglam, Y.S., Temur, A. and Keles, H. (2004) Immunohistochemical detection of peste des petits ruminants viral antigens in tissues from cases of naturally occurring pneumonia in goats. *Small Ruminant Research* 51, 273–277.

Yoneda, M., Bandyopadhyay, S.K., Shiotani, M., Fujita, K., Nuntaprasert, A., Miura, R., Baron, M.D., Barrett, T. and Kai, C. (2002) Rinderpest virus H protein: role in determining host range in rabbits. *Journal of General Virology* 83, 1457–1463.

Yoneda, M., Miura, R., Barrett, T., Tsukiyama-Kohara, K. and Kai, C. (2004) Rinderpest virus phosphoprotein gene is a major determinant of species-specific pathogenicity. *Journal of Virology* 78, 6676–6681.

Yunus, M. and Shaila, M.S. (2012) Establishment of an in vitro transcription system for peste des petits ruminant virus. *Virology Journal* 9, 302.

Zhang, X., Glendening, C., Linke, H., Parks, C.L., Brooks, C., Udem, S.A. and Oglesbee, M. (2002) Identification and characterization of a regulatory domain on the carboxyl terminus of the measles virus nucleocapsid protein. *Journal of Virology* 76, 8737–8746.