Muhammad Munir *Editor*

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Preface

It was an enchanting moment in the history of the veterinary profession when the Food and Agriculture Organization of the United Nations (FAO) announced on 28 June 2011 that rinderpest had been globally eradicated and there was no constraint to international trade due to rinderpest. At a time when research communities were gathered under the "Global Rinderpest Eradication Programme (GREP)" for the development of control and eradication strategies for rinderpest, concerns were also raised about another morbillivirus of small ruminants, peste des petits ruminants (PPRV). Since then there have been several noteworthy scientific achievements that present recent conceptual advances, and review current information on the many different facets of PPRV. In this period, recombinant and live attenuated homologous vaccines have become available, which led to a significant reduction in the occurrence of disease in PPR-endemic countries. The availability of proficient diagnostic tests has heightened awareness and importance of the epidemiological potential of the virus, in domestic and wild small ruminants, and in camels. These aspects, along with our understandings on the biology and pathogenesis of PPRV, have been reviewed in our first SpringerBriefs "Molecular Biology and Pathogenesis of Peste des Petits Ruminants Virus" (authored by M. Munir, S. Zohari and M. Berg).

In last few years, there has been a significant stimulation of research activity on several facets of the virus, primarily due to increase in the virus host and geography spectra. The availability of an increasing number of full-genome sequences from all lineages of PPRV has led to an improved taxonomic classification of the virus, enhanced our understanding of evolution, geographic variation, and epidemiology, and stimulated research activity on variation in viral virulence. Recent successful rescue of the virus using reverse genetic technology has the potential to advance our knowledge on fundamental virology, functions and properties of viral proteins, the evaluation of candidate virulence determinants, and engineering of novel and lineage-matched live attenuated vaccines. Studies on the immunobiology of PPRV have also led to the realization that the virus interacts with the host immune system in ways that are similar to other members of the genus morbillivirus. Besides these advancements, clearly a comprehensive research approach is needed to unravel the

complexities of the virus–host interactions and their exploitation for both diagnostic and therapeutic purposes.

In this edited book, *Peste des Petits Ruminants Virus*, my goal has been to assemble a team of renowned scientists who have made seminal contributions in their respective aspect of PPRV research, and to provide a comprehensive and up-to-date overview of PPRV geographical distribution, genome structure, viral proteins, reverse genetics, immunity, viral pathogenesis, clinical and molecular diagnosis, host susceptibility, concurrent infections and future challenges. The last two chapters are dedicated to comprehensively cover and to highlight the ongoing issues on the economic impact of the disease, and current control and management strategies that might ultimately lead to eradication of the disease from the planet. Each chapter is an attempt to create a stand-alone document, making it a valuable reference source for virologists, field veterinarians, infection and molecular biologists, immunologists and scientists in related fields and veterinary school libraries.

Gathering this wealth of information would not have been possible without the commitment, dedication and generous participation of a large number of contributors from all over the world. I am greatly indebted to them for the considerable amount of work and their willingness to set aside other priorities for this project. I must also acknowledge that there are many other colleagues who are active in the field, whose expertise has not been represented in this edition of the book.

Muhammad Munir

Contents

Chapter 1
Peste des Petits Ruminants: An Introduction

Muhammad Munir

Abstract Peste des petits ruminants virus (PPRV) is an acute, highly contagious, and economically important transboundary disease of sub-Saharan Africa, Middle East, Indian subcontinent, and Turkey. It is one of the World Organization for Animal Health (WHO) notifiable diseases and is considered important for poverty alleviation in PPRV-endemic regions. Significant research has been directed toward improved vaccine, diagnosis, and epidemiology of the virus in recent years; however, research on fundamental aspects of the virus is required, especially when disease spectrum and distributions patterns are increasing. This chapter is designed to provide an overview of each chapter that is describing comprehensively a specific aspect of PPRV in the book.

1.1 An Overview

Peste des petits ruminants virus (PPRV), the causative agent of peste des petits ruminants (PPR), is a member of genus *Morbillivirus* within subfamily *Paramyxovirinae* of the family *Paramyxoviridae* (Gibbs et al. 1979). PPRV is relatively recently diagnosed virus; therefore, most of our understanding on virus structure and molecular biology is based on the comparison with other morbilliviruses such as measles virus (MV), canine distemper virus (CDV), and rinderpest virus (RPV). Based on this comparison, PPR virions are pleomorphic particles and are enveloped (Fig. 1.1). The genome (15,948 nt in length) encodes sequentially for the nucleocapsid (N) protein, the phosphoprotein (P), the matrix protein (M), the fusion (F) and the hemagglutinin–neuraminidase (HN) membrane glycoproteins, and the large (L) protein (viral RNA-dependent RNA polymerase, RdRP) (Fig. 1.1) (Michael 2011; Munir et al. 2013). As with other morbilliviruses, it is only the P gene that encodes for two or three non-structural proteins, V, W, and C, through

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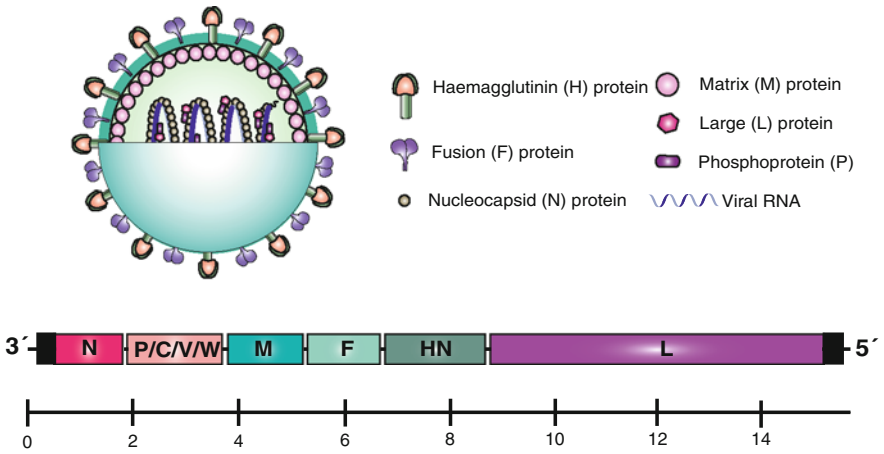

Fig. 1.1 Schematic diagrams of a Morbillivirus and its genome. Modified from Munir (2014b) with permission

"gene editing" or "alternative ORF" mechanisms. The available information on functions of each of these genes is recently reviewed by Munir (2014b), and Michael (2011) and is described compressively in the next chapter (see Chap. 2). Two essential components of PPRV life cycle, replication and transcription, are essentially regulated by genome promoter (3′ end of the genome), antigenome promoter (5′ end of the genome), and intergenic sequences between individual genes. Our understandings on the preference over replication or transcription mode are insufficient; however, different hypotheses have been proposed due to functional similarities of PPRV with other morbilliviruses (see Chap. 2). With the availability of complete genome sequences from all lineages of PPRV (Bailey et al. 2005; Muniraju et al. 2013; Dundon et al. 2014) from both vaccine strains and filed isolates, and due to the availability of reverse genetics (Hu et al. 2012), it is expected to see a surge in the research on the biology of PPRV and its pathogenic potentials in diverse hosts.

Among PPRV proteins, it is the HN protein that determines the initiation of viral infection and is the main determinant of host range selection through interaction with cellular receptors (sialic acid, signaling lymphocyte activation molecule (SLAM), and ovine Nectin-4) (Pawar et al. 2008; Birch et al. 2013). Beside presence of these receptors in several mammals, sheep and goats are remained to be the natural hosts. However, the host spectrum of PPRV has now expanded from sheep and goats to several wildlife species and to camels (Kwiatek et al. 2011; Munir 2014a). The disease can be equally severe in sheep, goats, or wild small ruminants; however, the clinical manifestation varies widely (Lefevre and Diallo 1990; Wosu 1994; Munir 2014a) (see Chap 3). Briefly, after an onset of high fever and inappetence for 1–2 days, lesion (congestion, serous to mucopurulent discharges) spread over oral and respiratory mucosa. These lesions cause *functio laesa* in these organs and lead to

cough, dyspnea, and diarrhea on third day post-infection. This clinical picture further aggravates and culminates in severe pneumonia and dehydration, and reasons 90 % mortality in immunologically naïve populations within 5–10 days. Multiple studies have revealed comprehensive disease progression, clinical scoring, and virus antigen distribution patterns in multiple organs of small ruminants (Eligulashvili et al. 1999; Munir et al. 2013; Pope et al. 2013) (see Chap. 4). Collectively, these studies indicate that the multiplication and pathogenicity of the virus are proportional to that of the host resistance or innate resistance, host's immune response, host density, the nutritional level of host, the breed, sex, and age of the animal (reviewed in (Munir et al. 2013)) (see Chap 3, 4). PPRV has high tropism for epithelial and lymphoid organs and thus leads to profound immunosuppression, which makes the infected animals vulnerable to secondary infections (Kerdiles et al. 2006). Consequently, concurrent infections aggravate the clinical outcome of PPRV by potentiating the severity of the PPR infection in immunodeficient host resulted from PPRV-induced lymphocytolysis (see Chap. 7). However, interestingly, the convalescent animals develop lifelong immunity despite immunosuppression and infection of opportunistic pathogens.

Beside its natural hosts, PPRV has been reported in cattle, domestic, and wild African buffaloes (*Synceruc caffer*) without severe consequences. Moreover, PPRV is now considered a pathogenic and emerging virus of camelids and wild small ruminants of at least *Gazellinae, Tragelaphinae, and Caprinae* subfamilies. PPRV can cause severe illness in wild small ruminants and camels; however, it is unclear whether these animals shed or transmit virus or play any role in the epizootiology of PPRV (Munir 2014a).

The disease is infectious and of emerging transboudary nature, which expanded from sub-Saharan Africa to Middle East, Turkey, and the Indian subcontinent rapidly. Up to present time, Food and Agriculture Organization (FAO 2009) has estimated that about 62.5 % of the total small ruminant population is at risk to PPR, around the globe, especially those from southern Africa, Central Asia, Southeast Asia, China, Turkey, and southern Europe. Recently, disease has been reported from previously disease-free countries such as China, Kenya, Uganda, Tanzania, Morocco, Eritrea, and Tunisia (Banyard et al. 2010; Cosseddu et al. 2013; Munir et al. 2013; Munir 2014b) (see Chap. 5). Initially, F gene-based classification was adapted for genetic characterization and for phylogenetic analysis, which was later shifted to N gene owing to its potential to depict better epidemiological patterns (Kwiatek et al. 2007). Currently, either N gene or both genes (N and F) are used for classification of PPRV strains into four distinct lineages (I, II, III, and IV). Recently, it is also suggested to use surface glycoprotein, HN, for epidemiological linking in addition to F and N gene-based analysis (Balamurugan et al. 2010). Regardless of the genes used, this classification has been only used for geographical speciation and is not indicative of stain pathogenicity or host preference. Lineages I, II, and III were considered African and the Middle East lineages, whereas lineage IV was reported exclusively from Asian countries. However, (i) this lineage (lineage IV) has been recently reported from several countries of Africa (Sudan, Uganda, Eritrea, Tanzania, Tunisia, and Mauritania) despite being still prevalent in

Asia (Banyard et al. 2010; Kwiatek et al. 2011; Cosseddu et al. 2013; Munir et al. 2013; El Arbi et al. 2014; Munir 2014b; Sghaier et al. 2014); (ii) most recent reports of PPRV in previously PPRV-free countries belong to lineage IV, (iii) countries once exclusively carrying a single lineage are now simultaneously reporting the presence of several lineages, i.e. Sudan and Uganda. In the majority of these cases, the newly introduced lineage is lineage IV (Kwiatek et al. 2011; Luka et al. 2012; Cosseddu et al. 2013) (see Chap. 5); and (iv) it is only lineage IV that is isolated from wild small ruminants (Munir 2014a) (see Chap. 6). These results indicate that lineage IV is a novel group of PPRV, has potential to replace the other lineages, and might be evolutionary more adaptive to small ruminants.

Our knowledge on current epidemiology has expanded significantly especially in small ruminants. Beside often distinct clinical picture, the availability of proficient assays for both the serology and genetic detection of the virus has contributed significantly in understanding current epidemiology of the disease. Favorably, convalescent and vaccinated small ruminants develop an early (10 days post-virus–host interaction), strong and lifelong immunity, which favor the detection of PPRV antibodies under comparatively limited resources or when sophisticated equipments for genetic detection are not available (Libeau et al. 1994). The N protein of morbilliviruses is highly conserved and is the most abundant protein owing to promoter-proximal location in the genome. Based on extensive analysis of monoclonal antibodies (mAbs) screening, selective anti-N mAbs have been used in the development of ELISAs for detection and differential diagnosis of PPRV (Libeau et al. 1994, 1995). These assays are currently in use for moderate laboratory diagnosis of PPRV (see Chap. 8). Monoclonal antibodies raised against the HN protein of PPRV have also been used in establishment of both competitive ELISA (c-ELISA) and blocking ELISAs (B-ELISA) (Saliki et al. 1994; Libeau et al. 1995; Singh et al. 2004a, b). Since antibodies against HN protein are virus-neutralizing, per se, detection of mAbs elicited against HN protein of PPRV correlates better with the virus neutralization test and immune status of the host (Saliki et al. 1993; Libeau et al. 1995). Beside antibodies detection, mAbs-based immunocapture ELISA and sandwich ELISAs (s-ELISA) have been developed and are extensively being used for the detection of antigen in both clinical and laboratory specimens (Libeau et al. 1994; Singh et al. 2004b). One of such assays, developed at Centre de Coopération Internationale en Recherche Agronomique Pour le Développement (CIRAD), France, is internationally recognized and applied for antigen detection. These assays have variable sensitivities and specificities, however, are generally at acceptable levels (Balamurugan et al. 2014). Despite availability of efficient serological assays, extensive seromonitoring has not been conducted in unvaccinated animals to estimate the prevalence of the disease. Such seromonitoring setup and information are crucial to assess the efficacy of the vaccination campaigns. However, like rinderpest eradication program, clinical surveillance will be an important marker of success in any campaign leading to disease control.

For the detection of PPRV genome, different polymerase chain reaction (PCR) chemistries, including conventional PCRs, real-time PCRs, multiplex real-time PCRs, and LAMP-PCR, have been developed to easily detect genome of PPRV,

independent of lineage variations. These assays have been designed based on the conserved sequences in the F gene (Forsyth and Barrett 1995), N gene (Couacy-Hymann et al. 2002; George et al. 2006), M gene (Balamurugan et al. 2006; George et al. 2006), and HN gene of PPRV (Kaul 2004). A conventional PCR, targeting the F gene, has extensively been used for the detection of genetic material of PPRV from clinical specimens with great success (Forsyth and Barrett 1995). Moreover, the amplified segment of F gene is long enough to draw epidemiological analysis. Owing to mismatches at the 3′ end of these primers, this PCR may not be suitable for lineages-wide detection in future. As alternatives, PCR assays targeting M and N genes have been established for specific detection of PPRV in clinical samples collected from sheep and goats (Shaila et al. 1996; Couacy-Hymann et al. 2002; Balamurugan et al. 2006; George et al. 2006) (see Chap. 8). Despite high sensitivities and specificities of these diagnostic assays, currently these assays are incapable in differentiating four lineages of PPRV strains. This is of special concern in the countries where more than one PPRV lineages are prevalent or emerging (Chaps. 5 and 9). There is also need of assays that can differentiate PPRV from diseases that show same clinical picture in animals in the event of co-infection. Currently, virus isolation is not a well-adopted model for identification of PPRV, especially for viruses that are causing new outbreaks. However, recently a new cell line that expresses SLAM/CD150 receptor has been demonstrated to be highly permissive for PPRV (Adombi et al. 2011). Moreover, an alpine goat was found to be highly susceptible to a Moroccan strain of PPRV (Hammouchi et al. 2012) and may present an experimental model in future.

Host immunological responses, in terms of innate and adaptive, are sufficiently investigated (Munir et al. 2013). Relative and definitive contributions of humoral and cell-mediated immunity in protection provided hallmarks of vaccine evaluation and provided bases of protection in both replicating and non-replicating vaccines. Our current knowledge on the immunodominant epitopes on the N and HN proteins, both for B and T cells, can be exploited for the Differentiating Infected from Vaccinated Animals (DIVA) vaccine construction. Efforts have already been started in establishing DIVA vaccine especially with the success of reverse genetic system (Hu et al. 2012) (see Chap. 10). After availability of the heterologous vaccine (RPV-based), which provided long-lasting protection, interests emerged to establish homologous vaccine for PPRV. As a result, a highly efficient vaccine, providing lifelong protection with single injection, became available in 80 (Diallo 2003). Currently, different vaccines have been developed which provide lifelong protection to reinfection and have provided foundations to establish effective control strategies. Homologous marker and subunit vaccines are proven to be effective and are now extended to build multivalent vaccines (see Chap. 12). Most of available vaccines provide lifelong immunity (6-year protection for a life span of 4–6 years in small ruminants) after even a single administration; however, the thermal stability of these vaccines is poor (half-life 2–6 h post-reconstitution at 37 °C), especially in the climatic conditions in tropical countries where disease is endemic. Current efforts have been successful in extending the thermostability (5–14 days at 45 °C in lyophilized form, whereas 21 h at 37 °C in reconstituted form) (Worrall et al. 2000;

Silva et al. 2011). Such improvements are sufficient for the shipment of PPRV vaccines in remote areas without maintaining the cold chain. However, no such vaccine has been launched in the market. Taken together, we have significant understanding of the level of protection, duration of immunity, antigenic profile, and thermostability of PPRV vaccines. While the experimentally proven vaccines are in abundance, there is still need to formulate the mechanism either for domestic production or for easy access to these vaccines especially in countries where disease is endemic.

Beside importance of disease management, availability of diagnostic assays and vaccines, it is imperative to ascertain the factual impact of the disease both at research and government levels. Comprehensive research needs to be conducted to ascertain the economic impact of the PPR on trade, export, and import of new animal breed especially out of the disease-endemic countries and into the disease-free countries. Public awareness is a central component for prioritizing the utilization of public funds in animal research. Since turnover rate of sheep and goat (natural hosts of PPR) is significantly lower than large ruminant, a well-designed cost-benefit analysis will be a critical criterion to plan the disease control program and to prioritize the research interests (see Chap. 12).

Cumulative efforts, initiated by the reference laboratories, and supported and followed on by the national laboratories and policy makers, would determine the fruitful outcome of disease control. Depending on the regional disease surveillance, individual vaccination of susceptible population (lambs and kids over 5 months) every year followed by carpet vaccination of all small ruminants every 3 years, occasional pulse vaccination, establishment of immune belt at the borders, and efficient sero-monitoring are crucial for the success of any efforts in controlling the diseases globally. Moreover, two countries each from Asian and African continents should drive the control and eradication campaign by combining their strengths and should be monitored by the international agencies such as FAO/OIE and GPRA would lead to faster accomplishment of much-needed goal of PPRV eradication (see Chap. 13).

1.2 Conclusions and Future Prospects

Molecular biology of PPRV is poorly understood and requires intensive efforts from developed laboratories to ascertain the host–pathogen interactions and to pinpoint the differences that might exist between PPRV and other morbilliviruses that might help to understand the host restrictions of the virus and its possible future expansion especially when PPRV is currently reported from a lion and when its spectrum is expanding to camels. It has now clearly been established that PPRV is an endemically important disease for poverty alleviation. However, epidemiological features such as transmission dynamics in different agro-climatic conditions require future investigations. The disease transmission has recently become important with the report of disease in wild ruminants and camels. The disease outcome is dependent on multiple factors and studies have just begun to understand any

genetics or non-genetic factors for this outcome. Epidemiologically, PPRV is expanding and this expansion is mainly contributed by the lineage IV of PPRV. Functional studies are required to understand the evolutionary mechanisms for the fitness of lineage IV over other lineages. Development and use of specific diagnostic tests that can distinguish PPR from diseases with similar signs helped unquestionably to improve our knowledge and understanding in the geographical distribution and spread of the disease in specific areas. Moreover, we are currently lacking a real-time assay that can differentiate different lineages of PPRV, which might be prevalent simultaneously in the country for proficient profiling of the lineage distribution.

In conclusion, although we have successful eradication model of rinderpest, it has to be kept in mind that "PPRV is not rinderpest and small ruminants are not large ruminants" for any initiative to be made for the control and eradication of PPRV.

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Chapter 2
The Molecular Biology of Peste des Petits Ruminants Virus

Michael D. Baron

Abstract *Peste des petits ruminants virus* (PPRV) is a negative-strand RNA virus with a monosegmented genome of length 15,948 and containing 6 genes. This chapter reviews our current knowledge of the structure and function of the genome and the six structural and three non-structural proteins produced by the virus. Although PPRV has itself been relatively little studied, the similarities between morbilliviruses allow us to deduce much about the life cycle of the virus at the molecular level. At the same time, it has become clear that there is a lot about the interaction of the virus with the host cell, and particularly the factors that restrict the host range in which the virus can cause disease, that remain to be worked-out.

2.1 Introduction

PPRV is a morbillivirus, closely related to measles virus (MV), canine and phocine (seal) distemper viruses (CDV and PDV) and rinderpest virus (RPV). The morbilliviruses form one genus within the subfamily *Paramyxovirinae* of the family *Paramyxoviridae*. Another morbillivirus is known which infects porpoise, dolphins and whales and which is sometimes referred to as separate viruses, the porpoise, dolphin and cetacean morbilliviruses (PMV, DMV, CMV). Figure 2.1 shows a phylogenetic tree of the morbilliviruses based on the nucleocapsid (N) protein gene sequence of each virus, illustrating the relationships between PPRV and the other viruses in the genus. This kind of phylogenetic analysis highlights an important point about the age of PPRV and how long it has been circulating. Although PPRV is sometimes referred to as an "emerging" virus, having only been identified as a distinct viral entity in 1979 (Gibbs et al. 1979), the PPRV branch from the presumed common ancestor of the morbilliviruses is at least as long as those showing the evolutionary distance of MV and RPV from their separation point. Given that

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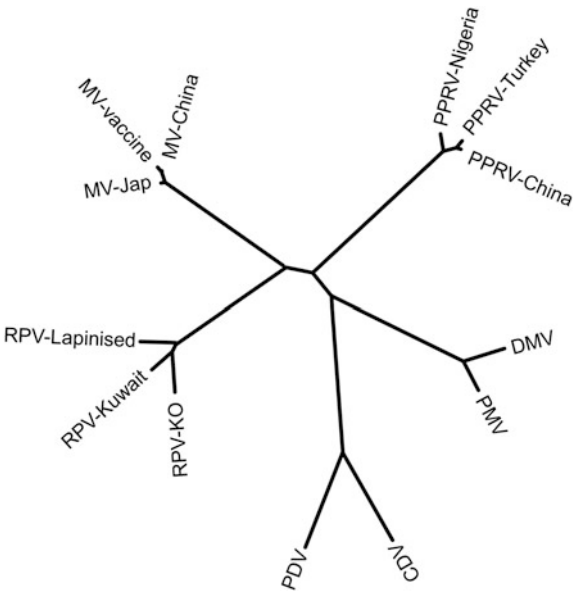

Fig. 2.1 Phylogenetic tree based on the N gene sequences of the morbilliviruses. The analysis was conducted using the program MEGA4 (Tamura et al. 2007). The evolutionary distances between each sequence were computed using the maximum composite likelihood method (Tamura et al. 2004), and the tree was drawn with branch lengths determined by the evolutionary distances. The accession numbers of the sequences used were as follows: RPV-KO (Kabete "O" strain), NC_006296; RPV-Kuwait, Z34262; RPV-Lapinized, E06018; MV-Jap, NC_001498; MV-vaccine, K01711; MV-China, EU435017; CDV, NC_001921; PDV, X75717; DMV, NC_005283; PMV, AY949833; PPRV-China, FJ905304; PPRV-Turkey, NC_006383; PPRV-Nigeria, X74443

MV is thought to have separated from this common ancestor at least 1,000 years ago (Furuse et al. 2010), it would seem that PPRV has been with us for many hundreds of years, unrecognized as a separate virus until the development of molecular techniques to distinguish it from RPV.

2.2 The Virion

Since PPRV is a relatively recently described virus, and has only become prominent as a major livestock problem in the last 15 years or so, as attention has moved from its more famous cousin, RPV, we have relatively few direct studies of the molecular biology of PPRV. However, because of the great similarity between the morbilliviruses, a lot can be deduced from studies on other members of the genus. As with all the paramyxoviruses, the morbilliviruses have a single-segmented RNA genome of negative sense. There are no good electron micrographs of PPR virions; it is

assumed that the virus resembles other paramyxoviruses, a nice schematic for which can be seen on the Viral Zone Web site (ViralZone 2010). The virions are pleomorphic enveloped structures; the size range of the diameter of RPV virions, for example, can be between 200 and 700 nm (Plowright et al. 1962; Tajima and Ushijima 1971). The nucleocapsid consists of the viral genome entirely wrapped by multiple copies of the viral nucleocapsid (N) protein, the helical packing of which in the nucleocapsid gives rise to the classic "herringbone" appearance in electron micrographs. A characteristic of these nucleocapsids is that the nucleic acid of the genome is resistant to digestion with endonucleases such as micrococcal nuclease. Given a genome length of 15,948 for PPRV (Bailey et al. 2005), and steric and genetic considerations which suggest that each N protein associates with 6 genome nucleotides (see below), each PPRV genome must associate with approximately 2,650 copies of N. Analysis of the pitch and diameter of the nucleocapsid helix (Bhella et al. 2004) suggests that each turn of the helix involves just over 13 copies of the N protein and, therefore, a genome would involve just over 200 turns of the nucleocapsid helix. Electron microscope imaging of several different paramyxoviruses shows that an individual paramyxovirus virion can hold much more than a single copy of the viral genome in the form of encapsidated RNA (Loney et al. 2009; Baron 2011), a property that has allowed recombinant measles viruses to be created where essentially two full copies of the genome have to be maintained in each infectious unit (Rager et al. 2002).

2.3 Genome Organization

PPRV, as other morbilliviruses, requires a genome that is a multiple of six bases in length for efficient replication (Bailey et al. 2005), an observation termed "the rule of six" when it was first observed for Sendai virus (Calain and Roux 1993), and since found to be true for most members of the subfamily *Paramyxovirinae*. There are six genes, or transcription units, in the PPRV genome, with promoter sequences [that is to say, binding sites for the viral RNA-dependent RNA polymerase (RdRP)] only at the 3′ ends of the genome [Genome Promoter (GP)] and antigenome [AntiGenome Promoter (AGP)]. The virus genes encode the nucleocapsid (N) protein, the phosphoprotein (P), the matrix protein (M), the fusion (F) and haemagglutinin (H) membrane glycoproteins and the large (L) protein, which is the viral RdRP. The P gene also encodes the three non-structural proteins V, W and C. The arrangement of transcriptional control elements and protein coding sequences in PPRV is illustrated in Fig. 2.2.

There is a relatively conserved motif at the junction between the individual genes (3′-T$^{/C}$AA$^{/TG}$TNT$^{/C}$T$^{/G}$TTTTGAATCCT$^{/C}$-5′ in genome sense), with a similar sequence at the junction of the GP with the N gene and that of the L gene with the AGP. The sequence prior to the GAA motif marks the end of one transcription unit and behaves as the polyadenylation signal for viral mRNAs, while the sequence after the GAA is the 5′ end of the next mRNA transcript. The GAA is transcribed

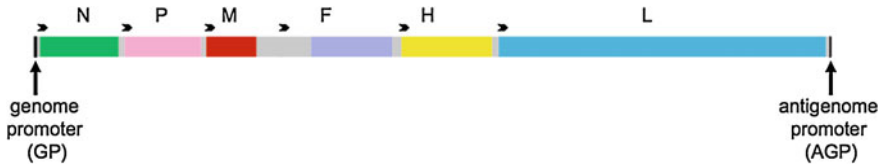

Fig. 2.2 Gene layout of PPRV. The distribution of protein coding sequences (*coloured*), promoters (*black*) and untranslated regions (UTRs) (*grey*) are shown to scale. *Arrowheads* indicate the start transcription point for each gene

only in full-length antigenome RNAs and does not appear in normal viral mRNAs. It is assumed that the viral RdRP, when in mRNA transcription mode, recognizes these sequences in some way to initiate mRNA transcription (including capping) at the start of the transcription unit and to terminate transcription at the appropriate point, where the viral RdRP adds a poly(A) tail to the mRNA. The sequences usually described as the GP and AGP are quite short, 52 and 37 bases, respectively. However, these are not the minimum sequences necessary for transcription and replication. Studies with so-called minigenomes (essentially genomes with almost all viral sequences apart from the promoters removed and replaced with a single transcription unit containing the coding sequence for a reporter gene) showed that approximately 100 bases at each end of the genome are essential for minigenome function (Bailey et al. 2007). Taken with data from other paramyxoviruses (Mioulet et al. 2001; Tapparel et al. 1998; Murphy and Parks 1999; Hoffman and Banerjee 2000; Walpita 2004), it is clear that there is another sequence element essential for transcription that lies within the first and last genes, the so-called "B-Box" (Blumberg et al. 1991) or "CRII" (Murphy and Parks 1999). This motif occupies a region 79–96 nucleotides from the 3′ end of the genome/antigenome. Given that morbillivirus nucleocapsids have around 13 N proteins per turn of the helix (Bhella et al. 2004), and probably 6 bases per N protein, each turn of the helix is about 78 nucleotides, so this motif would lie on the same side of the nucleocapsid as the highly conserved 18–20 base sequence at the 3′ end of the GP or AGP and presumably represents an extended binding site for the viral RdRP.

While most of the morbillivirus genes show a very high level of conservation in length of coding and non-coding regions, for the F genes, things are not so clear. Simple examination of the F gene sequences of the morbilliviruses would suggest that the open reading frame begins anywhere from 86 to 634 bases from the start of the gene. In addition, in some cases, the first AUG in the gene transcript is the start of a short, possibly unconserved ORF and it is the second or third AUG that is the start of the F protein ORF (Fig. 2.3a). Comparisons of PPRV with other morbilliviruses, or comparison of multiple strains of the same virus, show that the hypothetical amino terminus of the F proteins is highly variable, both in sequence and in length, up to the putative signal sequence of this class 1 membrane-anchored protein (Fig. 2.3b), downstream of which the sequence of the F protein is highly conserved. The role of this part of the F gene is not clear. Removal of the viral sequences upstream of the conserved ORF has been observed to improve F protein expression in some RPV

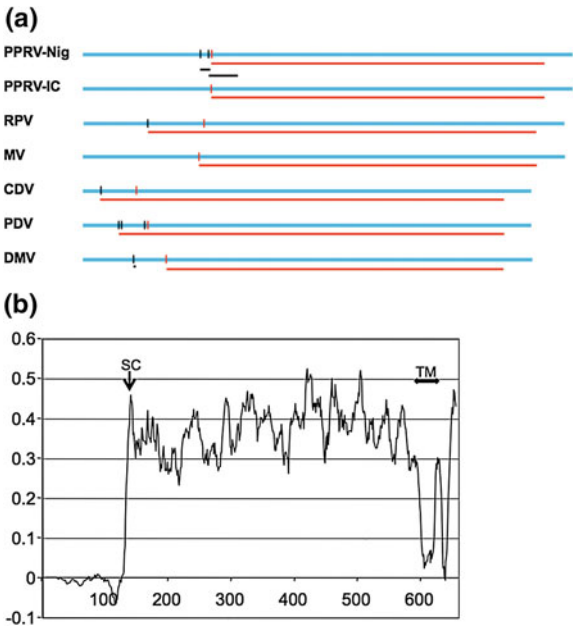

Fig. 2.3 **a** Open reading frames (ORFs) in morbillivirus F genes (shown in *blue*). Shown are the ORFs (defined as starting with an AUG codon and running to the next in-frame termination codon) for two strains of PPRV [Nigeria/75 (PPRV-Nig) and Ivory Coast/89 (PPRV-IC)] as well as examples of RPV, MV, CDV, PDV and DMV. The ORF containing the actual F protein sequence is shown in red; alternate ORFs are shown in *black*. Potential start-translation codons are marked on the gene (|). **b** Plot of sequence conservation in morbillivirus F proteins. The position of the signalase cleavage ("SC") site is shown by the *arrow*, and the position of the membrane anchor is indicated ("TM"). The degree of sequence conservation at each position was calculated using *plotcon* from the EMBOSS suite. The EBLOSUM62-12 comparison matrix was used with a sliding window of 11 amino acids

and MV (Hasel et al. 1987; Evans et al. 1990), while these sequences appear to act as a translation enhancer in PPRV (Chulakasian et al. 2013). We have found that we could replace the downstream F ORF of RPV with that of PPRV, ignoring the upstream sequence, and generate a fully viable virus (Das et al. 2000). Clearly much of the protein sequence upstream of the signal sequence is disposable, as might be expected for a peptide that is expected to be cleaved from the mature protein during synthesis. What is not clear is whether this extra protein is synthesized during viral replication, or whether F protein synthesis begins at one of the downstream start codons, or whether this varies from virus to virus.

The exact role, if any, of the genome sequences between the end of the M protein ORF and the ORF for the known functional part of the F protein, remains to be determined. The unusually long 3′ untranslated region (UTR) of the M gene, coupled with the (often) long 5′ UTR prior to the F protein coding sequence, means that there can be a block of more than 1 kb of untranslated sequence in the middle of the virus

genome. It has been suggested that this long UTR regulates the expression of F, a regulation closely related to viral virulence (Takeda et al. 2005; Anderson and von Messling 2008). For several of the morbilliviruses, this region has a very high GC content, and it might form local stem-loop or pseudo-knot structures that have an effect on transcription or translation. However, no clear difference in the organization of the M and F genes, or the function of these long UTRs, has been observed between the viruses with high GC content in this region (RPV, PPRV, MV) and those where the GC content here is no different to that found in the rest of the genome (CDV, PDV, DMV). In the case of RPV, we found that both sections of UTR could be removed from the vaccine strain without affecting viral growth or expression of the F protein (T Barrett and M D Baron, unpublished). If we accept that the main translation start point is not always the first available start codon, there must be a mechanism in some cases to cause the ribosome to skip to the start codon actually used. The F gene 5′ UTR has no internal ribosome entry site (IRES) function (Evans et al. 1990), but it might still be acting to force the ribosome to skip sequences, in the same way that sequences in the Sendai virus P gene transcript force some translation events to start internal to the P ORF to give rise to some of the C proteins generated by that virus (Latorre et al. 1998).

2.4 Viral RNA Transcription and Replication

The PPRV RdRP, as with all the viruses of the order *Mononegavirales*, has to function in two different modes, replication mode and mRNA transcription mode. When in mRNA transcription mode, the polymerase recognizes the gene start and gene end signals and initiates the synthesis of separate capped and polyadenylated mRNAs from each gene. When the RdRP is in replication mode, these signals are not recognized and transcription continues through the length of the genome (or antigenome). We have very little data as to the differences between mRNA transcription mode and replication mode. There is some overlap between the two modes: early studies on the transcription products of morbilliviruses showed that, in addition to full-length genomes and the expected mRNAs, other transcripts with the characteristics of mRNAs were observed (Barrett and Underwood 1985; Hirayama et al. 1985; Yoshikawa et al. 1986). These were deduced to be read-through transcripts, or bicistronic mRNAs, which are, in essence, places where the viral RdRP fails to stop at a gene end signal and carries on to the following gene as if in replication mode; only the upstream gene of such mRNAs is translated (Wong and Hirano 1987; Hasel et al. 1987). The two transcription modes appear to have different promoter requirements, in that the paramyxovirus GP supports transcription initiation in both modes, while the AGP only supports initiation of replicative mode transcription. The differences in these promoters and the exact sequence requirements for genome and mRNA transcription have not been defined in morbilliviruses. An early hypothesis was that replication mode required sufficient N protein to enable the co-transcriptional encapsidation that is characteristic of these

viruses (Kingsbury 1974; Lamb and Kolakofsky 1996). Although little work has been done on this subject in the case of the morbilliviruses, the balance between the two types of transcription in another paramyxovirus, respiratory syncytial virus (RSV), was found to be unaffected by the level of N protein (Fearns et al. 1997). Studies on MV and RPV showed that transcription began at the 3′ end of the genome even in mRNA transcription mode (Horikami and Moyer 1991; Ghosh et al. 1996). Our own studies have shown that the RPV C protein is essential for efficient genome transcript synthesis (Baron and Baron, unpublished), while phosphorylation of the RPV P protein has also been found to be required for replication mode RNA transcription (Kaushik and Shaila 2004; Raha et al. 2004b; Saikia et al. 2008).

One consequence of the single promoter for RdRP entry and transcription initiation is that gene transcription (mRNA synthesis) must always occur in the same order, beginning with the N gene. At the end of each gene, the polymerase has to add a poly(A) tail and then initiate the next mRNA. If this happened with 100 % efficiency each time, there would be the same amount of each virus message. However, studies of MV found that the relative levels of the viral mRNAs are not the same, but form a gradient related to the position of the gene in the genome. There are higher levels of mRNAs from genes at the 3′ end of the genome (closest to the promoter) than from those more distal (Cattaneo et al. 1987; Schneider-Schaulies et al. 1989). This led to the deduction that, at each gene end, there is a specific probability that the polymerase will detach and fail to initiate at the next gene. The frequency of reinitiation at each gene junction varies (Rennick et al. 2007) and, by analogy with data on other paramyxoviruses, the probability of reinitiation also varies with the junction sequence (Kato et al. 1999; He and Lamb 1999; Rassa and Parks 1998); this may represent one way of modulating the profile of the transcription gradient to give finer control of the relative levels of each viral protein.

Co-transcriptional editing of mRNA transcripts from the P gene occurs in PPRV, as is found for all the morbilliviruses (Cattaneo et al. 1989; Baron et al. 1993; Blixenkrone-Möller et al. 1992; Haas et al. 1995; Mahapatra et al. 2003). This mechanism, first identified for MV (Cattaneo et al. 1989), inserts one or more extra G residues into P gene mRNA transcripts at a defined editing site roughly half way along the P protein ORF. This editing process appears to be a result of the viral RdRP stuttering at the editing site in a similar way to the mechanism proposed for the addition of poly(A) tails to viral mRNAs, which is by repetitively transcribing the short run of Ts at the end of each gene (Vidal et al. 1990a, b; Hausmann et al. 1999). The extra nucleotides inserted at the editing site result in a frameshift when the mRNA is being translated, giving rise to different proteins depending on how many nucleotides are inserted: the P protein (no insertions), the V protein (insertion of one G) or the W protein (insertion of two Gs) (Fig. 2.4). Insertion of three nucleotides gives rise to a P protein once again (albeit now with an extra glycine amino acid). All three proteins share the first 231 amino acids of the P protein sequence, followed by different carboxy-terminal sequences (see below).

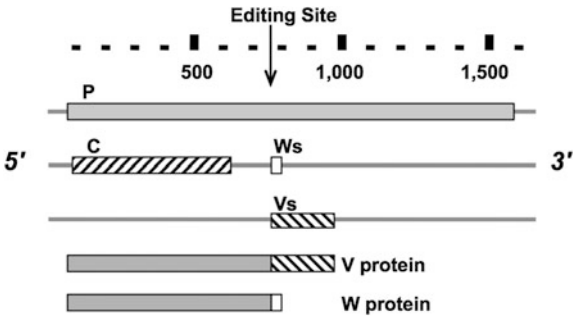

Fig. 2.4 Open reading frames used in the P gene of PPRV. The derivation of the four P gene-derived proteins is shown. The C protein is encoded in ORF +1 relative to the P protein ORF and can be translated from all P gene mRNAs. The V protein-specific (Vs) sequence is encoded in ORF +2, and the V protein is translated from mRNAs in which 1 extra G has been inserted at the editing site (*arrowed*). Insertion of 2 Gs at this site gives rise to the W protein, in which the P/V shared domain is followed by the W protein-specific (Ws) sequence, encoded in the +1 ORF

2.5 Viral Protein Structure and Function

Although PPRV has only six genes, it produces 9 different proteins. By virtue of the co-transcriptional "editing" in the P gene and by the use of two alternate reading frames in P gene transcripts, four different proteins can be produced from the P gene, a remarkable example of efficient use of genetic material. The viral proteins can be divided into roughly three functional groups, those associated with the nucleocapsid core (N, P, L), those associated with the membrane envelope (M, F, H) and the non-structural proteins (C, V, W). Most of our basic knowledge of the structure and functions of morbillivirus proteins has come from studies on MV, not surprisingly given its importance as a human pathogen, and the restrictions on handling livestock pathogens such as PPRV. The close similarity in the sequences of most of the morbillivirus proteins means that it is reasonable to assume fairly close similarity in structure and function. I have considered the proteins of the morbilliviruses as a group, highlighting where specific studies have revealed PPRV-specific details.

2.5.1 The Nucleocapsid Protein (N)

The N protein is the product of the most transcribed gene and is the most common viral protein. It accumulates to high levels in infected cells [see, e.g. (Sweetman et al. 2001)]. The PPRV N protein is 525 amino acids in length. The N protein interacts directly with other N proteins and with the P protein (Huber et al. 1991; Bankamp et al. 1996). The amino-terminal 398 amino acids are highly conserved across all morbilliviruses and appear to form the helical core of the nucleocapsid, since this

section of N, expressed alone, assembles into nucleocapsid-like structures (Bankamp et al. 1996). It is necessary to express at least the first 375 amino acids of MV N to give a protein that will self-assemble (Kingston et al. 2004a); internal deletions will prevent self-assembly (Bankamp et al. 1996). There appear to be two kinds of N–P interaction: those giving rise to stable, approximately equimolar, N–P complexes, and requiring extended regions of the N protein core as well as the carboxy terminus (Bankamp et al. 1996), and the interactions associated with replication mode transcription, where the P protein, in association with the L protein, is binding to N protein oligomers (Kingston et al. 2004a, b). In the latter case, with much of the core N protein buried in the nucleocapsid oligomer, P–N interaction appears to occur only at the carboxy terminus of N, an intrinsically disordered region that is constrained to a helix on binding to a triple-helix bundle formed in the P protein carboxy terminus (Johansson et al. 2003). Structural studies have described this N–P interaction as involving either multiple interaction sites (Longhi et al. 2003; Bourhis et al. 2005), or a single, short site (Kingston et al. 2004a, b; Yegambaram and Kingston 2010).

The carboxy terminus of the morbillivirus N protein also binds to the host cell protein hsp72 (Zhang et al. 2005), a dominant member of the hsp70 family of heat-shock proteins; this interaction appears to be essential for viral RNA transcription (Zhang et al. 2002). This role of hsp72 in part explains the observations of the increased replication of morbilliviruses in cells after a heat-shock or other cellular stress (Oglesbee et al. 1993, 1996; Parks et al. 1999; Vasconcelos et al. 1998).

2.5.2 The Phosphoprotein (P)

The P protein was first identified as a highly phosphorylated, nucleocapsid-associated protein (Robbins and Bussell 1979). Although the morbillivirus P proteins have similar sizes (506–509 amino acids), with predicted molecular weights of approx. 55 kDa, they all migrate anomalously slowly on SDS-PAGE gels (Bellini et al. 1985; Diallo et al. 1987). This is thought to be due to phosphorylation of primarily serine and some threonine residues, of which the P protein sequence is particularly rich. P is the second most common morbilliviral protein and appears to play two major roles in the virus life cycle, firstly as a structural subunit of the viral RdRP, enabling the progress of the polymerase along the genome through its interaction with the N proteins, and also as a chaperone in the assembly of nucleocapsids, preventing the N protein from random self-assembly and keeping it available for incorporation into nascent viral genomes. The P protein binds strongly to both N and L proteins as well as forming an oligomer of itself. Cross-linking studies on the RPV P protein have suggested that it forms a tetramer (Rahaman et al. 2004).

Co-expression of the P protein with N limits the self-assembly of the N protein and alters the density of the resulting nucleocapsid-like filaments (Spehner et al. 1997). The stable interaction of P with N monomers requires domains at both the

carboxyl and amino ends of P (Harty and Palese 1995; Shaji and Shaila 1999), although there is some evidence that just the N-binding site in the amino-terminal half of P is sufficient for transient interaction with monomeric N (Tober et al. 1998). It is thought that the P protein regulates the assembly of N during viral replication to limit the spontaneous oligomerization of N monomers and keep them available for assembly into proper nucleocapsids, as has been demonstrated for Sendai virus (Curran et al. 1995). Interestingly, interaction of P with nucleocapsids (N-RNA complexes) appears to require only the carboxy-terminal approximately 50 amino acids of P (Johansson et al. 2003; Kingston et al. 2004a). This domain forms a triple-helix coiled-coil structure which binds to the carboxy-terminal tail of the N protein (Johansson et al. 2003; Kingston et al. 2004a).

The amino-terminal half of the P protein is less conserved in its sequence than the carboxy-terminal half, and little is known of its function other than its potential role as a chaperone in nucleocapsid assembly. Because of the position of the editing site, this part of the P protein is common to P, V and W proteins. In addition, the coding sequence for the viral C protein is contained within this region of the P/V/W ORF in the +1 reading frame and it is possible that requirements for virus-specific C protein sequences have led to greater variation in (overlapping) P/V/W coding sequences between the different morbilliviruses.

Binding of P to L has been less well characterized. The L protein appears to be stabilized to some extent by the presence of P (Horikami et al. 1994; Chattopadhyay and Shaila 2004), and P–L complexes have to be formed during co-expression to be effective (Raha et al. 2004a). Although the P protein binds to a region in the amino-terminal 500 amino acids of L and the same domain is responsible for L–L dimerization, different residues are involved in the two processes (Cevik et al. 2004). The P protein appears to act as the "legs and feet" of the full polymerase in progressing along the nucleocapsid (Yegambaram and Kingston 2010; Kingston et al. 2004b). The details of these processes remain to be determined.

2.5.3 The Large Protein (L)

The L protein is the enzymatically functional subunit of the viral RdRP. It is a very large protein in morbilliviruses, 2,183 amino acids long with an estimated molecular weight of 250 kDa. Enzymatically, it is responsible for the essential RNA-dependent RNA polymerase activity of the virus, and all the motifs associated with such polymerases (Tordo et al. 1988; Poch et al. 1989, 1990) are found in the morbillivirus L proteins (Blumberg et al. 1988; Baron and Barrett 1995; Muthuchelvan et al. 2005). In addition, a motif associated with capping has been identified in all the viruses of the order *Mononegavirales* (Ferron et al. 2002), so it is likely that the morbillivirus L protein also caps viral mRNAs. The addition of poly(A) tails to viral mRNAs appears to be the result of "stuttering" or repeated copying by the

polymerase of a short stretch of T residues at the end of each gene. Other activities may be associated with the L protein, given its size, but these have not yet been identified.

The sequence of the morbillivirus L proteins is very highly conserved over the whole length of the protein, except for two short regions of very low conservation which have been termed "hinge" regions (McIlhatton et al. 1997), thought to be flexible connecting regions between functional domains. Insertion of the coding sequence for green fluorescent protein (GFP) while maintaining polymerase function has been possible at the second of these "hinges", but not the first (Duprex et al. 2002), showing that there are physical constraints on the flexibility or relative positioning of domains 1 and 2, but much greater flexibility allowed between domains 2 and 3. Recombinant RPV with such an inserted GFP grew normally in tissue culture, but was attenuated in vivo (Brown et al. 2005b). The major polymerase-associated sequence motifs are distributed in all three domains, suggesting that the protein functions as a single complex unit. Although the L protein homo-oligomerizes (Cevik et al. 2004), whether or to what extent the individual L proteins in the functional oligomer work together is unknown.

As mentioned above, L–L interaction and L–P interaction both map to the amino terminus of the protein. We have also found that the non-structural C protein binds L (Sweetman et al. 2001), binding specifically to domain 2 (Baron and Baron, unpublished data). The C protein of morbilliviruses has been shown to have a number of effects on viral RNA transcription; this is discussed below under *C protein*. Our own studies have also found that RPV L also binds to the host cell protein striatin (Sleeman and Baron 2005), a component of protein kinase C (PKC) complexes. The role of striatin or PKC in viral RNA transcription, or whether other host cell proteins play a role in viral transcription, is not known.

2.5.4 The Nucleoprotein Core (N–P–L)

Replication of and/or transcription from viral genomic RNA requires all three core proteins (Sidhu et al. 1995; Baron and Barrett 1997), while transcription from isolated or synthetic nucleocapsids requires co-expressed L+P and N+P complexes (Raha et al. 2004a). Interestingly, the N, P and L proteins of the morbilliviruses, although they have highly conserved sequences in general, and particularly in the defined domains involved in N–P and P–L interactions, do not function well when mixed in heterologous sets for the transcription of minigenomes (Brown et al. 2005a), suggesting that the interactions between the core proteins are complex and that sufficient virus-specific sequence changes have taken place that these viral proteins cannot work together well unless they are in a set of proteins that have co-evolved. Despite this limitation, a chimaeric virus in which the N protein of RPV was replaced by the N protein of PPRV was not only viable, it grew at the same rate in tissue culture as the parental RPV strain used (Parida et al. 2007), suggesting that viral RNA transcription is not a limiting step in viral growth. The viral transcription

complex also appears to need host proteins, since purified RPV nucleocapsids show defective RNA synthesis, requiring the addition of whole cytoplasmic extract or purified host cell proteins, either tubulin (Moyer et al. 1990) or microtubule-associated proteins (MAPs) (Baron and Baron, unpublished) as well as hsp70 family proteins (Oglesbee et al. 1996), for efficient, full-length RNA synthesis.

2.5.5 The Matrix Protein (M)

The exact role of the morbillivirus M protein is not yet entirely clear. It appears to play a complex role in the assembly of the virion, particularly virus budding. The M protein appears to associate directly with membranes even in the absence of the viral glycoproteins, though it requires full viral infection to be transported to and accumulate at the normal budding site, the apical surface of the cell (Manie et al. 2000; Riedl et al. 2002). The M protein is found in detergent-resistant membrane fractions (DRMs) (Manie et al. 2000; Vincent et al. 2000), and expression of the M protein alone can lead to budding of virus-like membrane vesicles (Pohl et al. 2007). At the same time, there is clearly interaction with the viral glycoproteins, because sorting of F and H to the apical surface of the host cell requires the presence of the M protein (Naim et al. 2000). This interaction of the M protein with the viral glycoproteins has a significant role in virus budding and subsequent fusion, so that a chimaeric RPV with the glycoproteins of PPRV instead of those of RPV was viable, but grew relatively slowly (Das et al. 2000); growth was improved by also replacing the M protein with that of PPRV (Mahapatra et al. 2006). The M protein predominantly seems to interact with the cytoplasmic tail of the F glycoprotein (Cathomen et al. 1998a; Naim et al. 2000; Moll et al. 2002), although mutations that strengthen interactions with the cytoplasmic tail of the H protein have been found on adaptation of MV to Vero cell culture (Tahara et al. 2005, 2007), simultaneously improving budding from Vero cells and decreasing infection through the normal virus receptor (SLAM). These findings indicate a complex set of interactions at the inner surface of the host cell membranes leading to budding. The M protein is currently considered to be the main driving force for the budding process, although generation of virus-like particles has also been seen when just the F protein is expressed (Pohl et al. 2007). A recombinant MV without an M protein gene grew, but was severely defective in budding, spreading mostly by cell–cell fusion (Cathomen et al. 1998a).

The M protein also binds to the N protein and inhibits RNA transcription from viral nucleocapsids (Suryanarayana et al. 1994; Iwasaki et al. 2009; Reuter et al. 2006) as well as enabling nucleocapsids to associate with host cell membranes (Iwasaki et al. 2009; Runkler et al. 2007). It thus provides a bridge between the viral envelope (glycoproteins F and H) and the ribonucleoprotein core (N, P, L). The nucleocapsids may be transported to the membranes along actin microfilaments, since disassembly of microfilaments using cytochalasin B inhibits MV budding

(Stallcup et al. 1983), while association of complete nucleocapsids with actin has been reported (Moyer et al. 1990). The M protein of morbilliviruses, unlike the orthologous VP40 of filoviruses, does not utilize the endosomal sorting complex required for transport (ESCRT) pathway in its budding process (Salditt et al. 2010).

2.5.6 The Fusion Protein (F)

As might be expected from the name of this protein, it is responsible for the fusion of the viral envelope with the plasma membrane of the infected cell, allowing the release of the nucleocapsid into the host cell cytoplasm. Expression of the viral proteins on the cell surface of infected cells leads to fusion with neighbouring cells, forming polynucleated syncytia, and allowing the virus nucleocapsid to be passed into new cells without necessarily forming a virion. Most researchers have found that effective fusion requires co-expression of the F and H proteins on the same cell (Wild et al. 1991; Heminway et al. 1994), although there is some evidence for the PPRV F protein being able to induce cell–cell fusion on its own (Seth and Shaila 2001a). Expression of the M protein as well as the two glycoproteins limits cell–cell fusion (Cathomen et al. 1998a; Tahara et al. 2007), while alterations to the cytoplasmic tails of the MV glycoproteins can increase cell–cell fusion, presumably by preventing normal interaction with the M protein (Cathomen et al. 1998b).

The F protein itself is synthesized as a type 1 membrane protein, which is glycosylated at several positions along its length. As described in Sect. 2.3, the actual start-translation point in virus infection is not always clear, but assuming the use of the first start codon in frame with the F protein ORF, these proteins have leader peptides of variable length and unconserved sequence, ending in all cases except PPRV with classical (von Heijne 1983) hydrophobic signal sequences and signal peptide cleavage sites. In the case of PPRV, there is a relatively short sequence upstream of the start of the conserved F protein and it would seem that this remains attached to the rest of the protein, as this sequence does not fit the requirements of a signalase cleavage site (von Heijne 1983). From the cleavage point on, the morbillivirus F protein sequences are highly conserved (Buckland et al. 1987; Evans et al. 1994; Meyer and Diallo 1995). The protein downstream of the signalase cleavage site, F_0, is processed to the mature protein, which exists as a disulphide-linked F_1–F_2 heterodimer following post-translational cleavage (Sato et al. 1988). Separation of the (amino-terminal) F_2 peptide by this cleavage exposes the hydrophobic fusion domain at the amino terminus of the larger F_1 peptide, which also includes the membrane anchor and cytoplasmic tail of the protein at its carboxy terminus. The cleavage site in morbillivirus F proteins has the pattern R-R-X-(R/K)-R-*cut*, which resembles the furin consensus R-X-X-R. Inhibition of cellular furin (Watanabe et al. 1995) or mutation of the last arginine in the motif to leucine (Alkhatib et al. 1994a) inhibits syncytium formation (cell–cell fusion) and release of infectious virus, suggesting that the cleavage of F_0 is normally performed

by furin and showing that this cleavage is essential for the fusion functionality of the protein.

There are three highly conserved N-linked glycosylation sites, all in the F_2 domain (Meyer and Diallo 1995), and all are required for proper folding of the protein and transport to the cell surface (Alkhatib et al. 1994b; Hu et al. 1995; Bolt et al. 1999). The F protein is further modified by attachment of palmitoyl residues, primarily to a cysteine residue in the transmembrane domain (Caballero et al. 1998). This modification is essential for fusion activity and may be directing the protein to the correct type of membrane, as the F protein, similar to the M protein, is found associated with so-called detergent-resistant membrane domains (DRMs) (Pohl et al. 2007). The mature F protein exists as a homotrimer (Zhu et al. 2002; Rahaman et al. 2003), based on crystal structure of expressed sections of the membrane-proximal core of the protein, or as a homotetramer (Malvoisin and Wild 1993), based on cross-linking of the mature protein. A leucine zipper motif, located proximal to the membrane anchor, is required for fusion activity, but not for homo-oligomerization of the F protein (Buckland et al. 1992).

2.5.7 The Haemagglutinin Protein (H)

Despite its name, the H proteins of most of the morbilliviruses do not act as haemagglutinins, there being no suitable virus receptor on red blood cells. An exception (and the partial origin of the protein's name) is the H protein of tissue culture-adapted MV, which will agglutinate rhesus monkey, but not human, red cells. The monkey erythrocytes express CD46, the protein that MV uses as a receptor after adaptation to tissue culture cells (Ono et al. 2001). An early assay for anti-measles antibodies was the ability of sera to inhibit this haemagglutination.

An interesting difference between the morbillivirus H proteins and the corresponding attachment proteins of other paramyxoviruses (usually called HN) is the lack of the "N" (for neuraminidase). Sialic acid forms, or is an important part of, the host cell receptor for many of the viruses of the *Respirovirus* and *Rubulavirus* genera of paramyxoviruses, so these viruses have maintained the neuraminidase function to enable release of progeny virus from infected cells. While sequence analysis has identified the residual signature of a neuraminidase in morbillivirus H proteins (Langedijk et al. 1997) as well as low-level enzyme activity in the expressed H proteins of RPV and PPRV (Langedijk et al. 1997; Seth and Shaila 2001b), neuraminidase activity has not generally been observed among the morbilliviruses, presumably because they have adapted to use direct protein–protein interactions with their common receptors (SLAM and nectin4) in binding to their target cells (Tatsuo et al. 2000; Noyce et al. 2011; Muhlebach et al. 2011; Adombi et al. 2011; Birch et al. 2013).

The morbillivirus H proteins are type 2 membrane proteins, with a typical signal–anchor sequence near the amino terminus, leaving 34 amino-terminal amino acids in the cytoplasm. The extracellular domain is thought to form a

membrane-proximal extended stalk-like structure with the carboxy-terminal half of the protein forming the globular "head" of the protein that contains the receptor binding sites. The mature protein forms cysteine-linked homodimers (Hu et al. 1994; Plemper et al. 2000) in which the disulphide links are in the stalk. These dimers themselves appear to dimerize to give tetrameric H proteins (Plemper et al. 2000). The globular head of MV H protein, minus its stalk, has recently been crystalized both in isolation (Colf et al. 2007) and associated with one of its receptors, CD46 (Santiago et al. 2010). The structure shows similarities to known neuraminidases, including the deep pocket that normally accommodates the glycan chain, but without the requisite active sites, thus confirming the earlier speculation that the morbilliviruses are derived from an ancestor with an HN protein, but have lost this functionality during their evolution. The fact that PPRV has retained the most neuraminidase activity suggests that it may be closer to the putative ancestral morbillivirus than any of the other known members of the genus.

Unlike their F proteins, the glycosylation patterns of morbillivirus H proteins vary with the virus. Glycosylation is required for correct folding of the H protein and transport from the endoplasmic reticulum (ER) to the cell surface. The H and F protein oligomers interact physically and functionally: adding an ER retention signal to the MV F protein leads to retention of H protein in the ER as well (Plemper et al. 2001); mutation of the H protein cytoplasmic tail decreased H–F interactions and led to a concomitant increase in infectivity and cytopathic effect (Plemper et al. 2002); various mutations in the H protein stalk have been shown to affect cell–cell fusion (Corey and Iorio 2007). Heterologous combinations of F and H proteins from different viruses show variation in their functional compatibility, so that PPRV H and F proteins only functioned as an homologous pair (Das et al. 2000), while CDV and MV H proteins could be exchanged (von Messling et al. 2001) as could RPV and CDV H proteins (Brown et al. 2005a).

2.5.8 The Non-structural Proteins V and W

The V and W proteins are the products of co-transcriptional editing of P gene mRNA transcripts, as described above. Judging by the frequency of double G insertions during editing, W-type transcripts are a very minor (1–2 %) component of the total P gene output in PPRV (Mahapatra et al. 2003), similar to findings in RPV (Baron et al. 1993), and W proteins are, therefore, probably very minor components of the infected cell. Whether they have a distinct role or are simply accidental by-products of the editing mechanism that produces the V protein is not yet known. The V protein, on the other hand, is produced from about a third of P gene transcripts and is an important factor in both viral replication and the ability of the virus to block the host innate immune response.

The production of a V protein (or, properly, co-transcriptional editing) seems to have evolved in an ancestor of the whole *Paramyxovirinae* subfamily (Jordan et al. 2000), since editing is found in almost all the members of this group of viruses,

with the exception of the human parainfluenza virus type 1 (hPIV1) (Matsuoka et al. 1991). The major part of the V protein is, of course, the same as the amino-terminal half of the P protein and is relatively poorly conserved among morbilliviruses. The V-specific sequence downstream of the editing site shows a Cys-rich motif that is highly conserved across all the paramyxovirus V proteins and has been shown to bind 2 zinc ions (Liston and Briedis 1994). The V protein is not essential for virus growth in cell culture, since recombinant PPRV (Sanz Bernardo and Baron, unpublished), RPV and MV (Schneider et al. 1997; Baron and Barrett 2000) have been made that do not express V and these viruses are viable, although they show changes in RNA transcription. Specifically, preventing V expression gave rise to a virus with increased mRNA expression and increased tendency to form syncytia (Baron and Barrett 2000; Tober et al. 1998; Schneider et al. 1997); such constructs grew at the normal rate in multistep growth curves, but to lower final titres than V+ virus when preparing stocks. Correspondingly, expression of excess V protein inhibits viral RNA transcription in either the whole virus (Tober et al. 1998) or a minigenome system (Witko et al. 2006; Parks et al. 2006).

The V protein is found uniformly distributed in infected cells (Sweetman et al. 2001) and does not specifically associate with the viral N/P/L proteins, which exist in large complexes in the infected cell, although direct interaction between RPV V and L proteins has been reported (Sweetman et al. 2001). It is possible that the V protein binds transiently to monomeric N (N_0) (Tober et al. 1998), which helps to prevent improper assembly of N during infection. The absence of V might limit the availability of N for replicative transcription, biasing the processes in the infected cells towards relatively increased mRNA synthesis, which is what is observed. However, this hypothesis implies a functional difference between V–N_0 and P–N_0 dimers, since preventing the expression of V by blocking editing will lead to a concomitant increase in the expression of the P protein, which can also bind N_0.

The other major role of the V protein is in helping the virus overcome the host's innate immune defences. The first of these to come into play in most virus infections is the type 1 interferon (IFN α/β) response, triggered through cytoplasmic and/or extracytoplasmic pathogen-associated molecular pattern (PAMP) receptors. The expression of IFN leads, in both an autocrine and a paracrine manner, to the induction of a large number of host cell proteins that can inhibit viral replication, up to and including the suicide (through apoptosis) of the infected cell. Type 1 IFN also stimulates further innate and adaptive immune responses, including the expression of type 2 IFN (IFN γ). These host cell defence systems and the ways that paramyxoviruses set out to block them have been extensively reviewed recently (see, e.g. Goodbourn and Randall 2009; Fontana et al. 2008), so I shall highlight here what we know about the mechanisms employed by PPRV.

The V proteins of PPRV and RPV block both type 1 and type 2 IFN signalling in infected cells, preventing the phosphorylation of both STAT1 and STAT2 (Chinnakannan et al. 2013; Nanda and Baron 2006). STAT1 is bound by the V protein by a sequence in the P/V/W shared domain, and all three proteins can be shown to co-immunoprecipitate this signalling protein (Nanda and Baron 2006). Interestingly, although all three proteins bind STAT1 equally, only the V protein is

efficient in blocking STAT1 and STAT2 phosphorylation (Nanda and Baron 2006) and only the V protein could prevent cells entering the type 1 IFN-induced antiviral state (Chinnakannan et al. 2013), indicating that this binding of STAT1 is not the key element in blocking IFN signalling. The V proteins of PPRV and RPV also bind STAT2 (Chinnakannan et al. 2013), but this is also not directly responsible for the blocking of type 1 IFN action. We have shown that the V proteins of all the morbilliviruses bind directly to the IFN receptor-associated Janus kinases (Chinnakannan et al. 2013), supporting previous suggestions by others about the MV V protein (Caignard et al. 2007; Yokota et al. 2003) and explaining the observation that the activation of these kinases by IFN is blocked in virus-infected cells (Chinnakannan et al. 2013). We have found that the ability to block type 2 IFN signalling correlates with the ability to co-precipitate STAT1 in various virus isolates (Chinnakannan et al. 2013).

The V protein of the rubulavirus parainfluenza virus type 5 (PIV5) was the first to be shown to have a significant effect on the induction of type 1 IFNs (He et al. 2002; Poole et al. 2002). This block on IFN induction was due to the binding of the host cell protein mda-5 (melanoma differentiation-associated protein 5*)* (Andrejeva et al. 2004), which acts as a cytoplasmic pattern recognition receptor (PRR), binding dsRNAs (Kato et al. 2006; Pichlmair et al. 2009) and inducing, though a complex pathway, the activation of transcription from the IFN-β promoter. Binding of mda-5 by the PIV5 V protein was shown to inhibit IFN induction, and these findings were extended to MV as well as other paramyxoviruses (Childs et al. 2007). We have shown that the V proteins of PPRV and RPV also bind to mda-5 and block the induction of IFN via this protein (Sanz Bernardo and Baron, unpublished). One area that is still under investigation, though, is why paramyxoviruses should block the activity of mda-5 in the first place, since these viruses do not produce detectable dsRNA during infection (Weber et al. 2006) and an alternative PRR, the product of retinoic acid-inducible gene 1 (RIG-I), has been found to be essential for IFN responses to MV and other paramyxoviruses (Hornung et al. 2006; Pichlmair et al. 2006; Plumet et al. 2007; Loo et al. 2008; Hausmann et al. 2008; Rehwinkel et al. 2010). Our own studies have found that PPRV infection and replication per se do not induce type 1 IFN (Sanz Bernardo and Baron, unpublished), suggesting that the virus is actively avoiding activating cellular PRRs that would trigger the host's defence.

2.5.9 The Non-structural Protein C

The C proteins are the least conserved among all the morbillivirus proteins. In infected cells, the RPV C and the MV C are found both in the nucleus and associated with the N/P/L complexes in the cytoplasm. When expressed from plasmids, the MV and RPV C proteins accumulate almost entirely in the nucleus (Boxer et al. 2009; Nishie et al. 2007), although no conserved nuclear localization signal (NLS) can be found in the morbillivirus C protein sequences. A "pat7"-type NLS (P, X_2,

$(K/R)_{3/4}$) identified in MV C protein (Nishie et al. 2007) is found in the C proteins of CDV and PDV, but not PPRV, RPV or DMV. No classical NLS is present in the PPRV C protein. A conserved positively charged motif is found in the short highly conserved domain near the centre of the C protein sequence, which might form a non-standard NLS. It may be that nuclear localization is an evolutionarily recent function for some of the morbillivirus C proteins and different viruses have acquired this capability through different mutational events.

The main role of the C protein appears to be in viral RNA synthesis. The RPV C protein was found to bind to the L protein (Sweetman et al. 2001), and RPV that does not express the C protein was viable in tissue culture, but grew more slowly and showed overall decreased viral mRNA synthesis (Baron and Barrett 2000). Further studies have shown that, in a cell-free viral RNA transcription assay, nucleocapsids lacking the C protein also showed a greatly decreased replicative transcription relative to viral mRNA transcription (Baron, J. and Baron, M.D., unpublished). In this respect, RPV C and MV C appear to differ slightly, since MV C has been reported to decrease all types of viral RNA transcription (Bankamp et al. 2005).

Studies on MV have shown that the absence of the C protein greatly reduces viral growth in IFN-producing cells (Escoffier et al. 1999) or those with active IFN-induced genes (Toth et al. 2009; Devaux and Cattaneo 2004), or in experimental animals (Patterson et al. 2000) (Takeuchi et al. 2005) (Devaux et al. 2008). Recent work has shown a clear role for the MV C protein in inhibiting IFN induction (Nakatsu et al. 2006; McAllister et al. 2010), although at least one group has ascribed this to an indirect effect of MV C on IFN induction through an effect on viral RNA transcription (Nakatsu et al. 2008). The PPRV C protein has no effect on IFN induction when expressed alone (Sanz Bernardo and Baron, unpublished).

2.6 Conclusions

Although only identified as a specific virus species 35 years ago, molecular evidence suggests that PPRV has been in existence for many 100s of years. Whether it has been causing disease in sheep and goats in all that time, or has only recently moved into those species from some as-yet undiscovered close relative, remains to be determined. The molecular biology of PPRV and its proteins has been relatively little studied, in part because of the restrictions most countries place on handling the virus, making other morbilliviruses such as MV or CDV more accessible ways to understand this group of viruses. What we do know of PPRV has shown us that it appears to be similar in most respects to other morbilliviruses in its replication and function. We know as yet very little of the host cell proteins that are the targets of direct interaction with viral proteins, although the virus is known to use ovine/caprine SLAM and nectin4 as receptors (Adombi et al. 2011; Birch et al. 2013) as has previously been shown for MV (Tatsuo et al. 2000; Noyce et al. 2011; Muhlebach et al. 2011). As the economic importance of the virus is recognized, it is

hoped that it will become possible to analyse further the specific interactions with sheep and goat cells that lead to the rapid growth of the virus in these hosts and the consequent disease that underlies that economic impact.

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Chapter 3
Host Susceptibility to Peste des Petits Ruminants Virus

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Abstract Peste des petits ruminants virus infects a wide range of domestic and wild small ruminants and the host spectrum has recently increased to camels. Despite this host range, the clinical manifestation varies not only between domestic and wild small ruminants but also between different species. Studies have begun to reveal different host genetics and non-genetic factors that may play significant roles in disease outcome and virus susceptibility. The role of wild small ruminants in the epizootiology of PPRV is not fully understood, however, evidences indicate that these could play a role in disease transmission. Understanding these factors may be of help in disease control and eradication campaigns.

3.1 Introduction

Small ruminants play a vital role in maintaining the sustainable livelihood of the rural poor farmers in developing and underdeveloped countries of the world. Peste des petits ruminant (PPR) is a highly contagious viral disease of domestic and wild small ruminants and is currently emerging to cause infections in camels. The PPR, being the plague of goats and sheep, poses heavy threat to the national economy of the countries, where the disease is endemic. PPR virus (PPRV) needs close contact between infected and susceptible animals to spread because of either the lability of the virus outside the host or due to low resistance of the virus in the environment (Braide 1981). The major determinants of the host range and tissue tropism of a virus are cellular receptors. Interaction of the host and virus is initiated by specific receptor binding, which is mediated by the hemagglutination (H) protein of PPRV

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and sialic acid on the host cell membrane (Munir et al. 2013). Studies on the receptors that determine the tissue tropism of PPRV are meager. However, the only established host receptor for this virus is the immune cell marker signaling lymphocyte activation molecule (SLAM) (Pawar et al. 2008). Evidences from the experimental studies on PPRV infection and records based on accurate field observations in sheep, goats, and cattle have clearly suggested that natural PPRV infection occurs by entry of the virus through the upper respiratory tract epithelium (Taylor et al. 1965). Small ruminants infected with PPRV exhibit lesions typical of epithelial infection and necrosis. The multiplication and pathogenicity of the virus are proportional to that of the host resistance or innate resistance, host's immune response, parasitic infection in the host, the nutritional level of host, the breed, sex and age of the animal, etc., as mentioned earlier (Munir et al. 2013).

For measles virus (MV), the cellular receptor is the SLAM (also known as CD150), which is a membrane glycoprotein (Tatsuo et al. 2000) expressed on some lymphocytes and dendritic cells (Cocks et al. 1995). Like other morbilliviruses, the target selective infection and destruction of SLAM positive cells for RPV are epithelial cells, activated lymphocytes, and macrophages (Rey Nores et al. 1995). The marmoset B cell line (B95a) has been shown to be sensitive to both virulent and vaccine virus of rinderpest virus (RPV) and PPRV (Lund and Barrett 2000; Sreenivasa et al. 2006). Using siRNA approaches, it has been confirmed that the SLAM could be a putative co-receptor for PPRV. Recently, Pawar et al. (2008) showed relationship between the level of SLAM mRNA and replication of PPRV in peripheral blood mononuclear cells (PBMC) of cattle, buffalo, sheep, and goats. Their findings revealed that the SLAM expression and PPRV replication are highly correlated and different levels of SLAM mRNA could influence the virus replication in different animals. Sarkar et al. (2009) studied the comparative sequence analysis of the SLAM receptors of sheep, goat, cattle, and buffalo. RPV and PPRV strains could use SLAMs of non-host species receptors, albeit at reduced efficiencies (Tatsuo et al. 2001). The other putative receptor namely ovine Nectin-4 protein, when overexpressed in epithelial cells, permits efficient replication of PPRV (Birch et al. 2013). This gene was predominantly expressed in epithelial tissues and encoded by multiple haplotypes in sheep breeds from around the world (Birch et al. 2013).

Further, different host genetics and non-genetic factors may play a significant role in variation to disease susceptibility (Munir et al. 2013). Studies on immunogenetics indicate that a significant part of individual variation in these traits of the host has a genetic component, with varying degrees of heritability. The major histocompatibility complex (MHC) provides diversity at the genetic level for capturing the antigen for processing especially the variability at peptide binding groove of the MHC region at the genetic level will result in identifying the allelic combinations and haplotypes responsible for immune response to the virus. However, vaccine response in the population has been reported to be variable for several diseases in humans as well as animals (O'Neill et al. 2006).

Transmission of PPRV either directly or indirectly from sheep or goats to cattle provides a mechanism for the virus to survive outside of the environment in the

unnatural hosts (Abraham et al. 2005). The presence of PPRV antibodies in camels and wild ruminants, besides sheep, goats, cattle, and buffaloes, suggests the natural transmission of PPRV infection among these animals under field condition (Abraham et al. 2005; Balamurugan et al. 2012a). This chapter provides comprehensive aspects on the host susceptibility to PPRV infection and its importance in the epidemiology of the disease with future perspectives.

3.2 Species Susceptibility

3.2.1 Sheep and Goats

The PPRV primarily affects small ruminants such as sheep and goats, which are the natural hosts, and occasionally, it infects some other artiodactyls including camels and small ruminant wildlife (Munir 2014). Many authors believe that although PPRV infects sheep and goats, severity of the clinical symptoms is more predominant in goats than sheep (Lefevre 1980; Wosu 1994; Tripathi et al. 1996; Singh et al. 2004). The findings of Wosu (1994) indicate that the rate of recovery is lower in goats than in sheep. The virus generally causes an acute disease in small ruminants, although sheep are often less severely affected than goats (Lefevre and Diallo 1990). Differential virulence of field strains for both species or innate resistance within the sheep have been speculated to be responsible for the said observation to development of the clinical disease (Taylor 1984). The difference in pathogenicity between sheep and goats may not be due to viral affinity, but may be due to a high recovery rate in sheep. However, such observations are yet to be proved by scientific evidences. Severe outbreaks of PPR have been noticed in regions having large sheep populations, indicating PPRV causes serious clinical disease in sheep as well (Singh et al. 2004). Curiously, in some of the PPR outbreaks attended by our investigation team found more pronounced clinical signs and severe disease in sheep than goats in flocks where sheep and goats reared together. In laboratory studies, some of the PPRVs isolated from goat from north India have shown to cause severe infection in goats as compared to sheep (Nanda et al. 1996; Singh et al. 2004). However, in another laboratory experiment, a PPRV isolated from goats did not induce apparent clinical signs in sheep in the few initial passages. This isolate induced clinical signs in sheep after few passages of adaptation in sheep (Muthuchelvan et al. IVRI, Mukteswar, personal communication). Nevertheless, it cannot be undermined that, the recovery rate in goat is comparatively less than those in sheep after infection. The higher rate of slaughter and fecundity in goats results in production of naive goat population every year in India. These newly born animals become susceptible to the infection; presumably another reason for greater susceptibility of the goat population as reported earlier (Singh et al. 2004). The studies at the molecular aspects of virus affinity and species susceptibility would unravel the greater susceptibility of goat than sheep. In tropical

areas, the fertility rate is higher in goats than sheep, which accounts for larger flock replacement by goat offspring. Further, it is well documented that some strains of RPV have higher affinity for Asian cattle and also to small ruminants in comparison with animals of African countries (Couacy-Hymann et al. 1995). Hence, it is also possible that the PPR viruses (lineage) circulating in south East Asia have a greater affinity for goats. Recently, Balamurugan et al. (2012b) attributed greater PPR positivity in clinical samples from goats to the fact most of the suspected samples were from regions, which had larger goat population. Similarly, Soundararajan et al. (2006) reported a higher mortality rate among infected goats than sheep in a large organized farm, which too has larger goat population. Thus, it is possible that species selection of strains and further amplification in these lead adaptation to a particular species resulting in more severe disease in adapted species. But nevertheless, it is also important to study virus characteristics and species susceptibility at molecular level in order to understand the underlying mechanism. In general, the prevalence of PPRV antibodies in young and adult sheep and goats indicates subclinical or in apparent or non-lethal infections, as vaccinations against the disease is limited and irregular in the endemic countries. However, prevalence of PPRV antibodies in adult sheep or goats is not always indicative of PPR infection, as there is always a high probability of these animals receiving vaccination once during a lifetime. Singh et al. (2004) stated that the higher prevalence of antibodies to PPRV in sheep (36.3 %) than in goats (32.4 %) was attributed to the greater recovery rate (lower case fatality rate) rather than increased susceptibility of sheep to PPRV and retention of sheep for meat and wool purposes for larger duration in comparison with goats while testing countrywide serum sample analyses in India. Similarly, Khan et al. (2008b) reported a greater proportion of the sheep (56.80 %) versus the goat (48.24 %) population that was infected with PPRV in several Pakistani provinces. Further, Khan et al. (2008a) in view of determining the disease situation in different geographical regions, seasons, age, sex groups, and species of sheep and goats, they reported higher numbers of positive cases in southern and western districts of Punjab province when compared to other parts of the province. Similarly, based on the higher prevalence of antibodies in sheep (41.35 %) than goats (34.91 %), Ragavendra et al. (2008) has opined that sheep are more susceptible to goats and prevalence rate is comparable to the goat population in southern states of India. Various studies by different investigators, from different parts of the world, showed various percentages of the mortality and morbidity with the involvement of different strains of PPRV in both sheep and goats populations. Thousands of outbreaks of PPR have been reported in Indian states with variable morbidity and mortality from 1994 to-date. However, this scenario is likely to change drastically once intensive vaccinations are carried in the sheep and goats population in India. Nevertheless, further experimental infection studies are required to map both viral and genetic makers of differential disease severity in sheep and goats.

3.2.2 Wildlife

There have been several reports of PPR in wild ruminant species, namely wild ungulate from three families: Gazellinae (dorcas gazelle), Caprinae (Nubian ibex and Laristan sheep), and Hippotraginae (gemsbok) (Fentahun and Woldie 2012; Munir et al. 2013). Initially, the role of wild animals in the epizootiology of PPR was realized by some of the leading investigators (Taylor 1984). Outbreaks of PPR in wild animals (Taylor 1984) or in zoological collections in the Arbian gulf (Furley et al. 1987) could be of considerable significance for virus perpetuation. In Saudi Arabia, PPR was suspected on clinical and serological grounds in gazelle and deer (Abu Elzein et al. 1990). The PPRV caused severe disease with high mortality in Dorcas Gazelles (*Gazella dorcas*), Nubian Ibex (*Capra ibex nubiana*), Laristan sheep (*Ovis orientalis laristani*), and gemsbok (*Oryx gazella*). Subclinical infection in Nilgai (*Tragelaphinae*) has also been observed. However, there is only one report of highly fatal peracute naturally occurring PPR in captive wild ungulates (Gazelles) (Abu-Elzein et al. 2004). Antelope and other small wild ruminantspecies can also be severely affected (Abu-Elzein et al. 2004). Komolafe et al. (1987) investigated the possible role of peridomestic rats in the epizootiology of PPR in goats in Nigeria. A virulent strain of PPRV induced subclinical infection in rats, which, however, could not be transmitted to healthy goats housed together with the infected rats. American white-tailed deer (*Odocoileus virginianus*) has also been found fully susceptible with experimental infections (Hamdy et al. 1976). The PPR seroprevalence in cattle, buffaloes (Anderson and Mckay 1994; Govindarajan et al. 1997; Balamurugan et al. 2012a), camels (Roger et al. 2000; Khalafallaa et al. 2010), bharals (*Pseudois nayaur*) (Bao et al. 2011), and other wild ruminants or ones in zoological collections (Furley et al. 1987) has been used to study the natural transmission of PPRV among these animals under field conditions (Abraham et al. 2005). However, the presence of infectious virus in these cases has not yet been reported except in a few hosts such as gazelles (Abu-Elzein et al. 2004), camels (Khalafallaa et al. 2010), and wild bharal (Bao et al. 2011). Recently, Balamurugan et al. (2012c) have detected the nucleic acid of PPRV in the lion tissue samples which was died of trypanosomosis and have provided new insights in the PPR host susceptibility and transmission. Such cases of infection could be due to close contact with other infected animals or contaminated fomites. In general, the wildlife, by sharing same grazing field or areas and water bodies with domestic animals, may potentially be a part of the epidemiology of the disease. Wild ruminants have been suspected to play a role in spreading of the disease. However, the actual role of wildlife on the epizootiology of the disease remains unclear for the moments and requires future investigations as described earlier (Banyard et al. 2010; Munir et al. 2013).

3.2.3 Cattle and Buffalo

The occurrence of PPR in alternate animal hosts is still a matter of debate. There have been several reports of PPR subclinical infection occurring in other ruminant species of domestic animals. Cattle and pigs are susceptible to infection with PPRV, but they do not exhibit clinical signs and do not transmit disease to other animals. There is no evidence of carrier state for PPRV. However, they may play a role in the epizootiology of PPR because they are apparently unable to transmit the disease to other animals (Furley et al. 1987). Such subclinical infections result in seroconversion and cattle are protected from challenge with virulent RPV. Cattle were considered as dead-end hosts (Gibbs et al. 1979). However, the virus was also isolated from one confirmed natural outbreak in buffaloes caused fatal syndrome (Govindarajan et al. 1997). This virus was also suspected to have been involved in a respiratory disease, which affected the camels in Ethiopia (Roger et al. 2000). There was also a report of a rinderpest-like disease in Indian buffalo, which was caused by PPRV (Govindarajan et al. 1997). Different researchers have reported seroconversion in cattle and pigs following contact with the sick sheep and goats. However, development of clinical signs in these animals has not been reported (Nawthane and Tayler 1979; Dardiri et al. 1976). Dardiri et al. (1976) reported that experimentally infected cattle developed PPRV antibody without clinical signs. Although clinical disease has not been reported in adult cattle exposed under field condition (Gargadennec and Lalanne 1942) or following experimental inoculation (Dardiri et al. 1976), the results of (Mornet et al. 1956) where pyrexia and oral lesions could be produced experimentally in calves could be of interest. In a similar study at the Central University Laboratories, Madhavaram, Chennai, India, the PPRV isolated from a confirmed natural outbreak in buffaloes caused fatal syndrome in experimentally infected buffaloes (Govindarajan et al. 1997). The PPRV may be adapted in bovine, which are subclinically infected without showing any symptoms of illness under natural condition. The circulation of PPRV in unnatural host(s) may have a positive role or may help in the control or restrict the spread of PPR in small ruminants in particular geographical area (Balamurugan et al. 2012a). This assumption may be due to the possibility of adaption and change in virulence of the virus where small and large ruminants are reared together in integrated farming systems. It is believed that, in the situation, where large and small ruminants coexist, seroconversion in cattle of cross-reacting PPRV antibodies might have also helped to eradicate RP disease. All these studies indicated an extensive endemicity of the disease, which could be attributed to the agro-climatic conditions and the migration of livestock. The results also suggested natural transmission of PPRV among cattle, buffaloes, goats, and sheep under field conditions (Singh et al. 2004; Balamurugan et al. 2011, 2012a, b, 2014). This serological evidence necessitates inclusion of cattle and buffaloes in the PPR serology (Anderson and Mckay 1994; Khan et al. 2008a, b; Balamurugan et al. 2012a). Anderson and Mckay (1994) provided serological evidence for transmission of PPRV from sheep and goats to cattle and highlighted the need to include cattle in the PPR serology in the

seromonitoring programme. Subsequent upon development of specific diagnostics, the presence of PPRV antibodies has now also been reported in cattle and buffaloes in a number of reports in India and in other countries and also potential of camel to act as a reservoir was suggested (Hinshu et al. 2001; Haque et al. 2004; Abraham et al. 2005; Balamurugan et al. 2012a). Cattle, goats, and in some cases camels grassing alongside sheep did not show any signs of the disease. Our earlier study on subclinical PPR infection in experimental cattle showed that PPRV antibody and antigen could be detected over a period of one year (Sen et al. 2014). All these report showed that, PPR could also be transmitted directly or indirectly from sheep or goats to cattle, providing a mechanism for the virus to survive outside of the environment in the unnatural host. The list of species of animals in which either PPRV antibodies or virus infection was detected by different researchers as reported earlier (Banyard et al. 2010; Munir et al. 2013) are summarized with update in Table 3.1.

3.3 Breed of Animals

Besidesspecies, breed of the animal may also have effect on the outcome of PPRV infection and disease epidemiology. Some breeds are more susceptible to disease than others (Lefevre and Diallo 1990). TheGuinean breeds (West African dwarf, Logoon, Kindi, and Djallonke) were reported to be highly susceptible (Lefevre and Diallo 1990). Similarly, El Hag and Taylor (1984) reported in an experimental study that British breedexhibited severe clinical symptoms, while the Sudanese breeds failed to develop a characteristic clinical signs. A more recent observation detected variations in breed susceptibility within goats in West Africa. The acute form of the disease was observed in WAD, goats while WALL breed developed only mild form of the disease (Diop and Sarr 2005). Nomadic goats and sheep in the Sahel area west of Africa have a high innate resistance and undergo subclinical infections, whereas settled flocks south of the Sahel and indigenous goats and sheep in the Middle East posses a low innate resistance (Munir et al. 2013). No such report is available in India on susceptibility of different breed of the sheep and goats except a study conducted by Pawar et al. (2008). In this study, peripheral blood mononucleated cells (PBMCs) from goats have been shown to express higher level of SLAM mRNA followed by sheep, cattle, and buffalo. Further, they reported different breeds of goats had different basal levels of SLAM (Pawar et al. 2008). High mortality (65 %) was also observed among crossbred Barbari-Black Bengal goats. Recently, a severe outbreak of PPR among the Tellicherry breed of goats was reported with 100 % mortality among kids and 87.5 % mortality among adults. Kanni and Salem black breed of goats have not shown disease at all, although adjacent animals of other breeds suffered. Moreover, severe outbreaks of PPR were reported in Barbari goat in India (Paritosh 1997; Rita et al. 2008). These reports indicate a certain level of host genetic involvement in determining disease severity; however, it is still required to underpin the markers of host genetic-resistance for not only better protection but also for future breeding plans.

Table 3.1 Detection of PPR virus or PPR virus antibodies in various animal species

No.	Species (Latin)	References
1.	Afghan Markhor goat (*Capra falconeri*)	Kinne et al. (2010)
2.	African Grey duiker (*Sylvicapra grimma*)	Ogunsanmi et al. (2003)
3.	Arabiangazelles (*Gazella gazella*)	Kinne et al. (2010)
4.	Arabian mountain gazelles (*Gazella gazella cora*)	Kinne et al. (2010)
5.	Arabian oryx (*Oryx leukoryx*)	Frolich et al. (2005)
6.	Asiatic Lion (*Panthera leo persica)*	Balamurugan et al. (2012c)
7.	Barbary sheep (*Ammotragus lervia*)	Kinne et al. (2010)
8.	Bharals (Pseudois nayaur)	Bao et al. (2011)
9.	Bubal hartebeests (*Alcelaphus buselaphus*)	Couacy-Hymann et al. (2005)
10.	Buffalo (*Bubalus bubalis*)	Govindarajan et al. (1997) Balamurugan et al. (2012a)
11.	Buffaloes (*Syncerus caffer*)	Couacy-Hymann et al. (2005)
12.	Bushbucks (*Tragelaphus scriptus*)	Kinne et al. (2010)
13.	Camel (*Camelus dromedarius*)	Khalafalla et al. (2010); Abraham et al. (2005)
14.	Defassa waterbuck (*Kobus defassa*)	Couacy-Hymann et al. (2005)
15.	Dorcas gazelle (*Gazella dorcas*)	Furley et al. (1987)
16.	Gemsbok (*Oryx gazella*)	Furley et al. (1987)
17.	Goat (*Capra hircus*)	Several researchers
18.	Impala (*Aepyceros melampus*)	Kinne et al. (2010)
19.	Indian cattle (*Bos indicus*)	Abraham et al. (2005); Balamurugan et al. (2012a)
20.	Kobs (*Kobus kob*)	Couacy-Hymann et al. (2005)
21.	Laristan Sheep (*Ovis gmelini laristanica*)	Furley et al. (1987)
22.	Nubian Ibex (*Capra nubiana*)	Furley et al. (1987)
23.	Pig (Sus *scrofa domesticus or Sus domesticus*)	Nawthane and Tayler (1979)
24.	Rheemgazelles (*Gazella subguttorosa marica*)	Kinne et al. (2010)
25.	Sheep (*Ovis aries*)	Several researchers
26.	Springbuck (*Antidorcas marsupialis*)	Kinne et al. (2010)
27.	Thompson's gazelle (*Eudorcas thomsonii*)	Abu-Elzein et al. (2004)

3.4 Age of the Animals

Association between age and severity of the PPR has also been reported by Obi et al. (1983) and Taylor (1984). According to these researchers, young animals aged from 6 months to 1 year old are more susceptible than adult animals. The disease results in high mortality, especially among young goats, although the frequency of the disease is higher in older goats (Toplu 2004; Gulyaz and Ozkul 2005). In Nigeria and the other parts of Africa, the disease was reported to occur in waves of infection at the intervals of 3–5 years (Bourdin et al. 1973). Serological data, however, indicated presence of antibodies in a proportion of goats of all ages varying from 4 months to 2 years. In two successive outbreaks in Nigeria, majority of aged animals were affected in the first and relatively younger ones (1–2 years) in the second outbreaks. Several disease outbreaks in India have suggested that animals of all age groups could be affected in a flock, in the first outbreak, but, in the subsequent outbreaks in the same flock, only few animals may get affected probably due to presence of protective level of antibodies. Singh et al. (2004) recorded relatively lower proportion (11.8 %) of sheep showing antibodies to PPRV than goats (18.7 %), while investigating prevalence of PPRV antibodies in different age groups of animals from organized sector in the country. Interestingly, the trend was reverse in the adult animal above one year of age. Similarly, Khan et al. (2008b) reported that the higher prevalence (72.86 %) of disease occurred in animals aged more than 2 years compared with the other age groups. A high frequency (59.24 %) of disease occurrence has been reported in females than in males (41.18 %) (Khan et al. 2008b).

3.5 Perspectives

PPR is still a poorly recognized disease, particularly with regard to epidemiological features such as transmission dynamics under different production systems in different species, species and breed susceptibility, mechanism of spread of virus to other natural and unnatural host, and the role of the wildlife on the epizootiology of disease. Understanding the environmental determinants affecting virus or vaccine response, immunobiology of vaccine response for PPR in different hosts, especially in sheep and goat, will enable us to find low and high responder animals and will also direct us regarding how to modulate these factors to obtain better protection for combating the disease during control and eradication campaigns.

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Chapter 4
Pathology of Peste des Petits Ruminants

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Abstract Peste des petits ruminants (PPR) represent an economically important plague of small ruminants. PPR is endemic across much of Africa and Asia with its geographical distribution seemingly expanding. Infection most commonly leads to a profound immunosuppression that allows opportunistic secondary infections to develop, increasing the morbidity and mortality rates observed. The pathogenesis associated with PPR virus (PPRV) has largely been assumed from that established for closely related viruses such as rinderpest virus (RPV), measles virus and canine distemper virus. Here, we overview the current thought of pathogenesis of PPR in small ruminants.

4.1 Introduction

Peste des petits ruminants (PPR) are an economically devastating disease of small ruminants caused by PPR virus (PPRV), a non-segmented negative-strand RNA virus belonging to the morbillivirus genus, within the family Paramyxoviridae. PPR

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infection is often associated with significant losses due to high morbidity and mortality rates. Alongside this, the highly contagious nature of the virus and the uncontrolled movement of animals through trade mean that PPRV is considered to be a serious transboundary problem. Following a PPRV outbreak, the disease is generally controlled through the subcutaneous administration of a live attenuated PPRV vaccine. Although the vaccine provides long-term immunity, generation of virus neutralising antibodies does not occur rapidly enough to prevent rapid spread of the disease among in-contact animals. Therefore, for disease control, a clear understanding of the mechanisms behind the development of disease needs to be defined. The pathology of a viral infection generally encompasses four distinct components of disease: (i) aetiology, (ii) mechanisms of development (pathogenesis), (iii) structural alterations of cells (morphologic changes) (iv) and the consequences of changes (clinical manifestations) (Robbins 2010). The current understanding of PPRV pathology has been heavily assumed from the closely related rinderpest virus (RPV) (Wohlsein et al. 1993, 1995) and other morbillivirus infections, and there have been very few studies performed that have focused specifically on the pathology of PPRV. As such, little is known about the mechanisms underlying establishment of the disease (pathogenesis) in susceptible animals. Since the aetiology of PPRV is detailed within this publication (Chap. 2), here we describe what is currently understood regarding PPRV-driven pathology in susceptible hosts.

4.2 Clinical Manifestation of PPR

Depending on any predisposing factors and the virulence of the infecting virus, clinical manifestation for PPR can be seen in per-acute, acute, subacute and subclinical forms. However, PPR in sheep and goats is generally observed as an acute disease. The per-acute form of disease is often seen in kids infected at the age of 4 months and older during the time frame whereupon any pre-existing maternal antibody levels wane. This per-acute form of disease has a short incubation period (2 days) with a rapid development of pyrexia with body temperature rising to 40–42 °C. Depression, congestion of mucous membranes, oculo-nasal discharge, dyspnoea and profuse watery diarrhoea lead to the death of infected animals within 4–5 days (Munir et al. 2013).

In the acute form of disease, a 3- to 4-days incubation period precedes the development of pyrexia and the onset of other clinical disease signs, including watery oculo-nasal discharge, congestion of the mucous membranes of the buccal cavity, conjunctiva of the eye and the vulva (Abubakar et al. 2008). A diarrhoeic phase follows, often resulting in the generation of bloody faecal matter leading to dehydration and ultimately death of the animal. As the disease progress, the watery oculo-nasal discharge may become mucopurulent and can occlude the nostrils, predisposing to dyspnoea.

In the subacute form of disease, the animals do not develop severe clinical disease and low mortality rates are seen. With this form of infection, the animals may develop temperatures ranging from 39 to 40 °C, but do not develop the characteristic clinical signs normally associated with PPRV infection. Animals usually recover from the disease within 10–14 days. A subclinical form of disease is also seen in large ruminants (buffalo and cattle), where the infected animals are able to clear virus in the complete absence of clinical disease, but seroconvert to PPRV, often generating strong neutralising antibody responses.

4.3 Stepwise Clinical Manifestations in PPRV-infected Goats

Our current understanding of PPR pathology has been mainly based on reports detailing field infection. As such, the assessment of pathomorphological disease progression has generally been studied during the later stages of acute disease. As a result, there is a gap in knowledge of events that occur very early on following infection. Ideally, this should be performed using a virulent virus and a route of infection that mimics that seen naturally in the field, such as experimental intranasal infection. A few experimental studies have been reported that have looked at earlier time points, although several obstacles have precluded a thorough assessment of very early time points following infection (Couacy-Hymann et al. 2007; El Harrak et al. 2012; Hammouchi et al. 2012; Pope et al. 2013). Based on both published and unpublished data available to us, we describe here the disease course during both the early and later phases of PPR infection.

As detailed above, PPR in susceptible small ruminants occurs most commonly as an acute infection (Fig. 4.1a–h). The severity of disease may be influenced by a number of factors including, but through knowledge gaps, not limited to: the genetics of the infecting virus strain; the infectious dose of the virus; the route of infection; the species and breed of infected animal; and the immunological and nutritional status of the infected animal. A key factor influencing disease progression, given the profound immunosuppression seen following PPR infection, is the presence of pathogenic organisms, as a result of pre-existing pathological processes, or environmental exposure. Evaluations of the virulence of different PPRV strains has been reported (Couacy-Hymann et al. 2007; El Harrak et al. 2012; Emikpe et al. 2013; Pope et al. 2013; Rajak et al. 2005). Couacy-Hymann and colleagues performed in vivo studies utilising representative strains of PPRV from each of the 4 defined lineages of PPRV in African dwarf goats via the subcutaneous route. The output of this experimentation is detailed in Table 4.1. Interestingly, infection with the different isolates led to a variation in the onset of clinical signs, ranging from mild infection to severe disease with some mortality. Although all the virus lineages caused clinical disease, virus isolates from lineage I and lineage IV appeared more virulent in this experimental model. Further to this study, Emikpe and colleagues infected African dwarf goats with a virulent form of the Nigeria 75/1

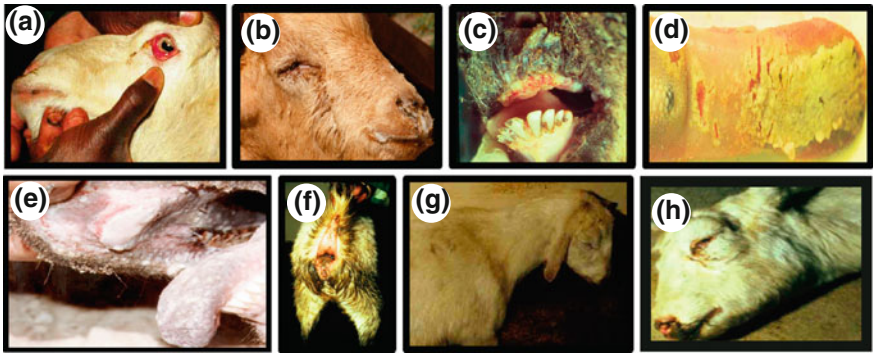


Fig. 4.1 Stepwise clinical manifestations in goats infected with PPR virus. **a** Congestion of conjunctiva on 5 days post-infection (dpi); **b** mucoid ocular and nasal discharge on 7 dpi (cited from FAO web); **c** oral (gum) lesions on 7 dpi; **d** and **e** tongue lesions and deposit of fibrins on 8 dpi; **f** diarrhoea on 10 dpi; **g** progressive weight loss and dull on 10 dpi and **h** death on 10 dpi

strain intratracheally and observed severe disease. In another study, El Harrak and colleagues infected Alpine goats with lineage IV virus via the intravenous, intranasal and subcutaneous routes. Although they demonstrated disease by all three routes, it was concluded that the intranasal route was the most suitable to mimic natural infection for pathological and immunological studies.

The progression of disease during the acute form generally results in the following sequential order:

i. The infected animals incubate the disease for 2–7 days before the development of pyrexia ranging from 39.5 to 41 °C, which lasts from 3 to 10 days
ii. 2–3 days after the onset of hyperthermia, the conjunctival (Fig. 4.1a) and orofacial mucosa becomes congested
iii. Ocular and nasal discharge occurs from days 4 to 7 post-infection (Fig. 4.1b) and lasts between 2 and 4 days; the serous clear discharge gradually becomes mucoid/mucopurulent towards the later phase (Fig. 4.1b); the congested orofacial mucosa concomitantly shows the appearance of lesions on the gum (dental pad), tongue, soft palate and nasal mucosa (Fig. 4.1c)
iv. In severe cases, oral lesions are observed on the hard palate with oral ulcerations and necrotic lesions appearing between days 5 and 9 post-infection
v. These necrotic lesions progress with the appearance of a caseous deposit of fibrin on the tongue (Fig. 4.1d, e), and at this point, halitosis is often evident due to these buccal lesions
vi. Finally, diarrhoea begins 4–10 days post-infection (Fig. 4.1f), sometimes becoming projectile, and the animal may become dyspnoeic within 8–12 days, suffering progressive weight loss and emaciation (Fig. 4.1g) that ultimately leads to death (Fig. 4.1h). In some cases, particularly in mild infection, animals may convalesce, returning to a pre-infection health status within 10–15 days of infection.

Table 4.1 Occurrence and duration of different clinical signs as reported by Couacy-Hymann et al. (2007)

Strain and animal	Temperature ≥39 °C	Nasal/ocular discharges	Oral ulceration	Diarrhoea	RT-PCR results				
					1–2	3	4	5	6–9
Côte D'Ivoire 89	a 5–8	4–9	7	7–9	−	+O/N	+O/N/S	+O/N/S	+O/N/S
	b 6–7	6–8	5	6–8	−	−	+O/N/S	+O/N/S	+O/N/S
	c 5–8	4–8	−	6–8	−	+O/N	+O/N/S	+O/N/S	+O/N/S
Guinea Conakry	a 7–8	7	5	−	−	+O	+O/N/S	+O/N/S	+O/N/S
	b 6–8	7–9	7	7–9	−	−	+O/N/S	+O/N/S	+O/N/S
	c 6–7	8–9	−	6–8	−	+O	+O/N/S	+O/N	+O/N/S
Nigeria 75/1	a 6–8	−	−	9	−	−	−	+O/N	+O/N/S
	b 7–8	8	−	−	−	−	−	+O	+O/N/S
	c 8	−	−	−	−	−	−	−	+O/N/S
Sudan – Sennar	a 7–8	6	−	−	−	−	−	+O	+O/N/S
	b 6–7	7–8	8	8–9	−	−	−	+O/S	+O/N/S
	c 8	−	−	−	−	−	−	−	+O/N/S
India – Calcutta	a 8	−	−	−	−	−	+O	+O	+O/N/S
	b 7–9	7–9	7	8–9	−	+O/N	+O/N/S	+O/N/S	+O/N/S
	c 6–8	9	8	−	−	+O/N	+O/N	+O/N/S	+O/N/S

(−) No clinical sign/negative RT-PCR results; +O/N/S, positive result on ocular, nasal and/or saliva samples

4.4 Clinical Scoring

Clinical scoring to assess the severity of disease was not clearly defined earlier in the literature. A clinical scoring method for PPRV has been developed to help assess the severity of disease following infection and to guide the human euthanasia of animals exhibiting clear clinical disease during in vivo experimentation (El Harrak et al. 2012; Pope et al. 2013). Both published clinical scoring systems recommend sacrificing animals when a defined score is reached during a defined time period. The decision to euthanase is made on ethical grounds (Hecker 1983; Smith and Sherman 2009) if the following criteria are fulfilled, in the system proposed by Pope et al. (Table 4.2):

Table 4.2 Clinical score sheet for assessment of animals infected with PPRV as reported by Pope et al. (2013)

Clinical score	General signs	Pyrexic response	Ocular/nasal discharge	Facial mucosal lesions	Faeces	Respiratory symptoms
0	Normal	<39.5 °C	None	None	Normal	Normal respiration rate (Sheep: 15–40[a]; Goats: 10–30[b])
1	Mildly inactive	>39.5 °C but <40 °C	Watery ocular discharge	Congested oronasal mucosa and buccal papillae	Soft	Slight tachypnoea
2	Mildly inactive and depressed, mild inappetence	>40 °C but <41 °C	Watery to mucoid oculonasal discharge: reddened eyes and mild conjunctivitis	Pinprick lesions within buccal cavity, with some becoming more extensive	Runny	Tachypnoea/mild cough
3	Inactive, apathetic, restless and anorexic	>41 °C or >39.5 °C for >5 days	Mucopurulent nasal discharge and/or severe conjunctivitis with mucopurulent ocular discharge	Clear erosive lesions on oronasal mucosae; severely congested/oedematous buccal papillae	Frank diarrhoea	Tachypnoea and dyspnoea/coughing present
4	Severe obtundation, reduced mobility and dehydration	>41 °C or >39.5 °C for >5 days followed by rapid fall in temperature (<38 °C[a])	Mucopurulent nasal discharge and severe conjunctivitis with profuse mucopurulent ocular discharge	Severe erosive/ulcerative lesions throughout buccal cavity, nasal mucosa and nares; oedematous lips and erosions on vulval labia	Muco-haemorrhagic diarrhoea	Marked tachypnoea/dyspnoea/cough

[a] Hecker (1983)
[b] Smith and Sherman (2009)

1. A maximal grading of 4/4 is allocated to the general clinical appearance of the animal (severe obtundation, lack of mobility and dehydration), indicating severe morbidity.
2. A score of 3/4 is achieved for the above general signs for 2 complete consecutive days, and a score of 10 or greater is achieved in other categories.
3. A score of two is achieved in the above general signs for 2 complete consecutive days, and a score of 15 or greater is achieved in other categories.
4. A cumulative score of 20 across all categories is reached.

Not only will the utilisation of such clinical score sheets aid assessment of experimental infection, it will also aid consistency of reporting when applied to field infections. Of course, the assessment of infection based on clinical observations is insufficient for diagnosis, as several other pathogens can cause clinical

disease similar to that seen with PPRV. Clearly, confirmatory laboratory diagnosis is required to satisfactorily conclude PPRV infection. The differential diagnosis of small ruminant disease is dealt with elsewhere within this publication.

4.5 Gross Pathology

Many pathological characteristics are common at post-mortem examination following PPRV infection. Ulcerative to necrotic lesions are clearly evident throughout the buccal cavity in PPRV-infected sheep and goats at post-mortem. The buccal papillae, dental pad (Fig. 4.2a), gum (Fig. 4.2b), dorsal surface of tongue, palatine tonsil (Fig. 4.2c) and hard palate are mainly affected. Congestion of the digestive tract, particularly the duodenum, abomasum, ileum, caecum and colon, is often seen. Extensive congestion along the longitudinal folds of the caecum, colon and rectum may be evident as zebra striping (Fig. 4.2d). The ileo-cecal valve can also demonstrate extensive mucosal haemorrhage. In severe cases, hyperaemic, oedematous and ulcerative mucosa are also seen throughout the intestines (Munir et al. 2013). An interesting observation in a recent study (Pope

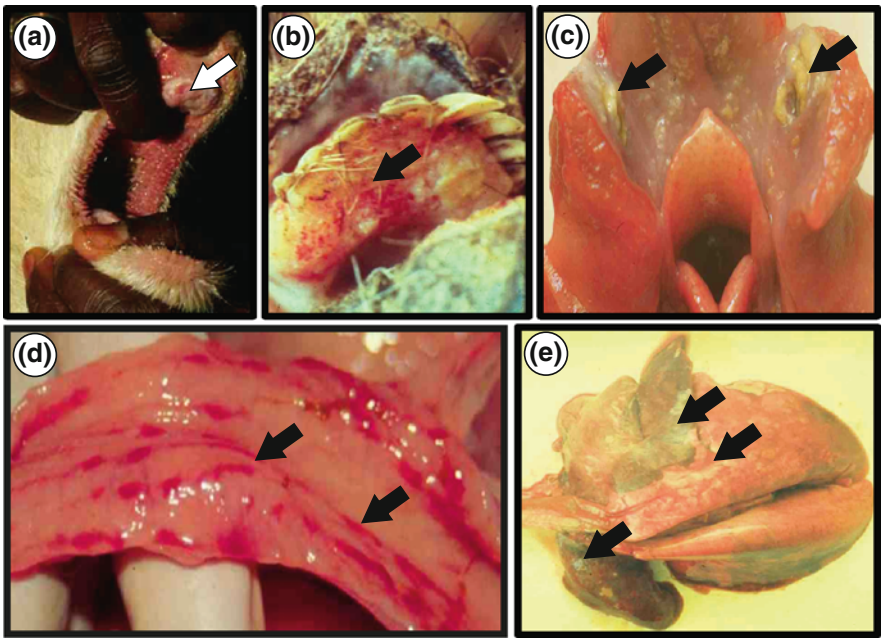


Fig. 4.2 Gross pathological lesions at post-mortem in goats infected with PPR virus. **a** Necrotic lesions on dental pad; **b** lesions on gum and oral cavity; **c** oro-pharyngeal sections showing necrotic lesions on palatine tonsils and small fibrin deposits on the base of tongue; **d** zebra striping in large intestine and **e** consolidated lungs leading to pneumonia

et al. 2013) was the relative difficulty in the detection of Peyer's patches in the ileum of virus-infected animals in the absence of obvious necrosis or haemorrhage in these areas. Previous studies have reported the association of PPRV with these lymphoid structures and extensive necrosis and collapse of the Peyer's patches has been observed in both natural and experimental infections (Kul et al. 2007; Kumar et al. 2004; Taylor 1984). In contrast to infected animals, the Peyer's patches were readily detected in uninfected tissues of control animals, which may imply that during infection, a redistribution of lymphocytes from these aggregates to sites of infection occurs as suggested following CDV infection (von Messling et al. 2006). The enlargement of lymph nodes accompanied with necrosis and haemorrhage, particularly the mesenteric lymph nodes and atrophied congested spleen are also sometimes seen in PPRV-infected goats in the field (Khan et al. 2008).

Similar to the buccal mucosa, the nasal mucosa becomes hyperaemic. The caudal part of the trachea and bronchus may contain froth (Emikpe et al. 2013). Pulmonary congestion and oedema with varying degree of red and grey consolidations can be seen with severe infections (Fig. 4.2e). The pleural surface of the consolidated lobes often shows patchy fibrin depositions (Emikpe et al. 2013). Congestion of lungs and bronchopneumonia was also seen in PPR-infected West African dwarf goats and was associated with bacterial infection (Couacy-Hymann et al. 2007).

4.6 Histopathology

Histopathological assessment of PPRV-infected tissues demonstrates many of the common characteristics of morbillivirus infection, including syncytial formation and extensive necrosis. However, observations are clearly linked to the extent of the disease as despite being seen during late stage of disease, inclusion bodies were not detected when assessing sections from animals exhibiting a mild form of disease (Pope et al. 2013). Despite this, even where mild disease is seen, large numbers of syncytiated cells, ranging in viability and size, can be detected in the paracortex of lymph nodes from day 5 and in the cortex and follicles of lymph nodes, splenic white pulp and gastrointestinal submucosal lymphoid tissue from day 7 of infection (Pope et al. 2013). In this study, necrosis/apoptosis of syncytia and individual cells was marked in paracortical areas on day 5, but declined after this point, indicating that nuclear debris is degraded very quickly within the lymph nodes. Lymph nodes can also become oedematous with mild lymphoid depletion. Moderate-to-severe lymphoid depletion is seen in the germinal centres of spleen. Cellular necrosis, as manifested by nuclear debris, was not as prominent in tonsillar and splenic tissues, as that seen in the lymph nodes. Squamous epithelial syncytia were also observed in tonsillar, facial and digestive tract epithelial tissues (Pope et al. 2013).

Large alveolar macrophages with intranuclear and intracytoplasmic inclusion bodies, numerous neutrophils, fibrin exudates and multinucleic giant cells have also been reported in severe PPRV infection (Emikpe et al. 2013), although again some

of these histopathological changes may result from secondary bacterial or parasitic infections. The alveolar lining cells become cuboidal and the interstitium is infiltrated by lymphocytes and neutrophils. Desquamation of bronchiolar epithelial lining cells is common, and the bronchiolar lumen contains purulent exudates. Occasionally coagulative necrosis of the lung parenchyma is also seen. Intestinal villi may become atrophied, mucosal glands may become necrotic, and, depending on the severity of disease, the lamina propria is infiltrated by lymphocytes and plasma cells. Furthermore, throughout the intestines and in the abomasum, marked diffuse lymphoid infiltration and oedema, causing varying levels of crypt disruption, can be observed (Pope et al. 2013). Lymphoid syncytial formation is often seen within the lamina propria with associated cellular necrosis.

4.7 Immunohistochemical Localisation of Viral Antigen

The only current study that examines distribution of PPRV antigen in experimental animals at staggered time points following infection is that described by Pope et al. (2013). Here, the authors conducted a study where 15 goats were challenged by the intranasal route with a virulent PPRV isolate, (Côte d'Ivoire'89) and sacrificed at strategically defined time points post-infection to enable pre- and post-mortem sampling. This approach enabled precise monitoring of the progress and distribution of virus throughout the infection from the time of challenge, through peak viremia and into a period of convalescence (Pope et al. 2013).

In this study, the lymphotropic nature of the virus was clearly evident from the distribution of virus antigen in lymphoid tissues at both early and late time points post-infection (Table 4.3). Examples of antigen detection within lymphoid tissues at different time points post-infection by immunohistochemistry (IHC) are shown in Fig. 4.3. The earliest detection of PPRV by IHC was seen in regions of lymph nodes and tonsillar tissue sampled at day 5 post-infection, with the paracortical areas of lymph nodes and diffuse lymphoid tissue of tonsils affected most significantly, before infection, as judged by the degree of immunolabelling spreads to the cortical/follicular areas of nodes/tonsils (Table 4.3).

Often, virus antigen was present in the paracortex, medullary cords and some regions of the non-follicular cortex and subcapsular area of lymph nodes, with large numbers of necrotic and apoptotic cells (Fig. 4.3a–c). Positive immunolabelling was seen in abundance in all sampled lymph nodes and tonsillar sections by IHC (Fig. 4.3d, e) from day 7 post-infection. Syncytia formation is evident within paracortical areas of the left pre-scapular lymph node (Fig. 4.3e, arrows) at 5 days post-infection and at day 7 post-infection (Fig. 4.3f). Virus antigen can also be seen within pharyngeal tonsil, advancing from day 5 (Fig. 4.3g) to 7 days post-infection (Fig. 4.3h). Virus antigen was detected most readily within the retropharyngeal lymph node, taken as a representative of the facial lymph nodes, with the tonsil also exhibiting high levels of virus antigen, especially at 7–9 days post-infection (Table 4.3).

Table 4.3 Antigen detection within lymphoid tissues at different days post-inoculation following challenge with the Côte d'Ivoire'89 strain of PPRV [from Pope et al. (2013)]

		Antigen detection in post-mortem tissues		
		Day 5	Day 7	Day 9
RPLN	Subcapsular area	+/++	+++	++
	Follicle/mantle	0/+	++	+/++
	Germinal centre	+	++	+
	Paracortex	++/+++	++/+++	+/++
	Medulla	+/++	++	0/+
Mesenteric lymph node	Subcapsular area	+	+++	++/+++
	Follicle/mantle	0	++/+++	+/++
	Germinal centre	0/+	++	+
	Paracortex	++	++/+++	++
	Medulla	+/++	++	+
Left pre-scapular lymph node	Subcapsular area	++	++/+++	++/+++
	Follicle/mantle	+	+/++	+/++
	Germinal centre	+	+/++	+
	Paracortex	+/++	++	++
	Medulla	++	+	+
Right pre-scapular lymph node	Subcapsular area	+	++	++
	Follicle/mantle	0	+/++	+/++
	Germinal centre	0/+	+	+
	Paracortex	++	++	++
	Medulla	++/+	+/++	+
MSLN	Subcapsular area	+	++/+++	+++
	Follicle/mantle	0/+	+/++	++/+++
	Germinal centre	0/+	++	++
	Paracortex	+/++	++	++
	Medulla	+	+	+/++
Tonsil	Follicle/mantle	0/+	++/+++	++
	Germinal centre	0/+	+++	+/++
	Diffuse lymphoid tissue	++	+++	++
	Crypt epithelium	+	++/+++	+++

(continued)

Table 4.3 (continued)

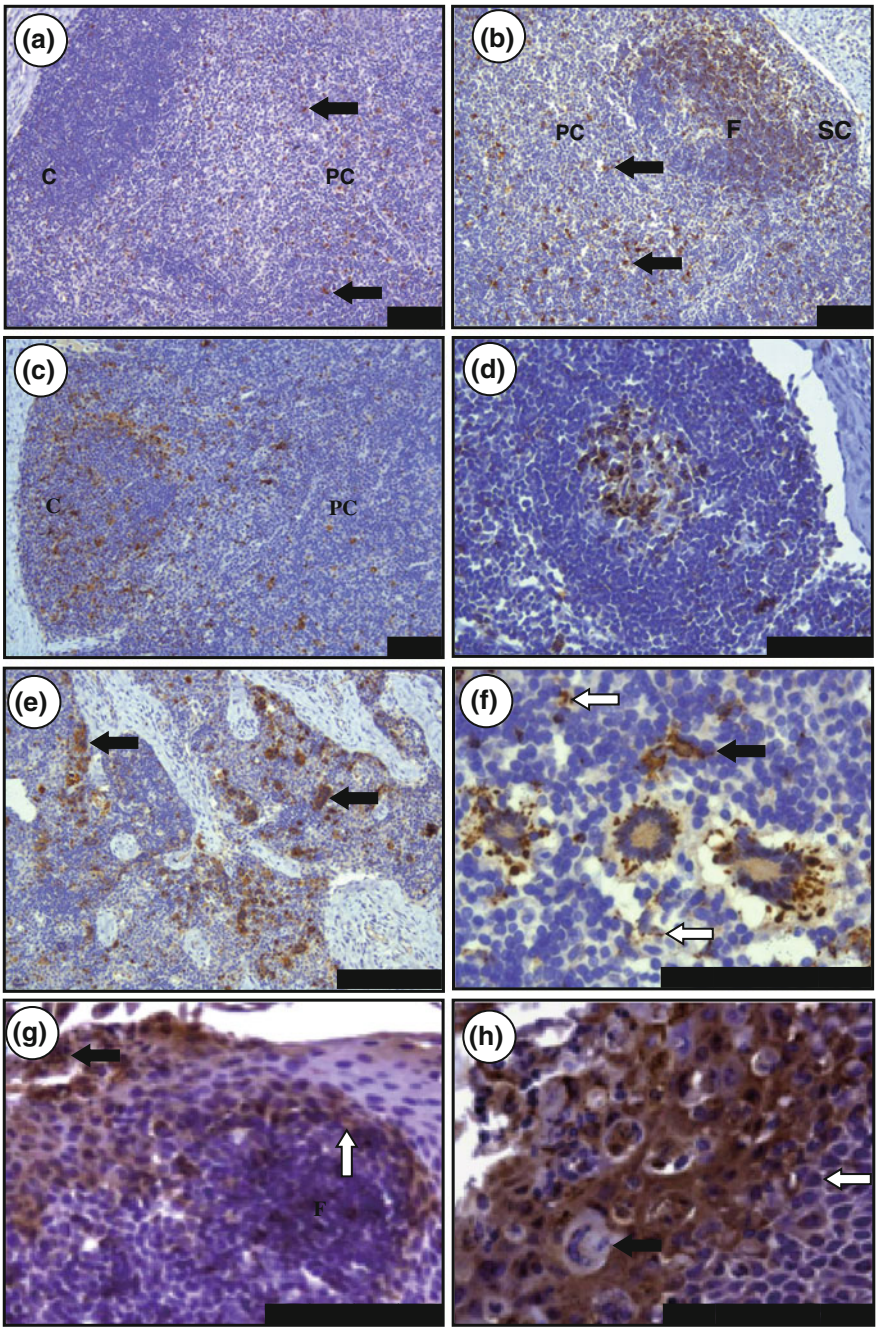
		Antigen detection in post-mortem tissues		
		Day 5	Day 7	Day 9
Spleen	PALS	0	+	+/++
	Follicle/mantle	0	/	/
	Germinal centre	0	/	/
	Red pulp	0	0/+	+

Tissues were taken on days 2, 5, 7, 9 and 21, and antigen detection was assessed by immunohistochemistry (*IHC*). Results for days 2 and 21 are omitted as all tissues analysed were negative for virus antigen. Average immunolabelling grades are given following analysis of tissues from 3 animals euthanased at each time point. Grades are formulated on a result of viral antigen density throughout a uniform tissue type. Sections were graded on three separate occasions, without referring to previous recorded results to help standardise the classification. Immunolabelling grades are defined as follows: 0 = No immunolabelling seen; + = Mild immunolabelling; + + = Moderate immunolabelling; +++ = Marked immunolabelling. Intermediate grades exist between the above four categories to give the analysis a greater degree of flexibility. **/** = Tissue type not present within section

No virus antigen was detected in any of the facial tissues taken on day 2 and day 5 post-infection by histochemical techniques. Mucosal erosions in nasal sections were seen at 7 days post-infection, with mild epidermal cellular swelling and increased numbers of lymphocytes and reticular type cells seen migrating through the lamina propria and mucosa. IHC detected viral antigen within the epithelium and lymphoid cells of the lamina propria in nasal skin/mucosal samples at 7 days post-infection (Pope et al. 2013). A greater antigen burden was detected in nasal, labial and conjunctival mucosal cell types at 9 days post-infection with antigen also being detected in the epithelium and proprial lymphoid tissues of the tongue (Pope et al. 2013). Detection of antigen throughout the intestinal tract did not occur until late stage of infection from day 9. A full immunohistochemical analysis of the distribution of virus antigen throughout infected animals can be found in Pope et al. (2013).

4.8 Proposed Pathogenesis for PPR

The current literature postulates that for PPRV and the other morbilliviruses, the initial virus replication occurs within the nasopharyngeal/respiratory epithelium (Borrow and Oldstone 1995; McChesney et al. 1997; Yanagi et al. 2006), prior to infection of regional lymphoid organs, where a second round of replication occurs (Esolen et al. 1993; Osunkoya et al. 1990). von Messling et al. (2006) speculated that lymphocytes were the primary target for an initial massive burst of replication within the oral cavity and these disseminated the infection to distant organs. Studies by Farina et al. (2004) proposed that the primary targets for MeV are SLAM


(a)
C
PC
(b)
PC
F
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(c)
C
PC
(d)
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F
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◀**Fig. 4.3** PPRV IHC on sections of lymphoid tissue taken at PME showing pertinent features of PPRV infection. **a** A greater degree of immunolabelling (*arrows*) is seen in the paracortex (*PC*) (*arrows*) of the RPLN than in the cortex (*C*) (5 dpi); **b** antigen distribution in the subcapsular layer and the follicles at 7 dpi in the MLN. Paracortical virus antigen still remains (*arrows*); **c** primarily cortical immunolabelling within the RPLN (9 dpi) with antigen also remaining within the PC; **d** in contrast to **b,** the germinal centre of this follicle within the MLN contains virus antigen that is absent from the follicular mantle (9 dpi); **e** intense immunolabelling within the LPSLN medulla (5 dpi) with extensive syncytia formation (*arrows*); **f** predominately peripheral paracortical immunolabelling of syncytia within the LPSLN (7 dpi). Dendritic-type cells also present and positive for virus antigen (*arrow*) with an infected lymphocyte also present (*open arrow*); **g** immunolabelling within pharyngeal tonsil (5 dpi) indicating early epithelial infection noted both basally (*open arrow*) adjacent to an infected lymphoid follicle (*F*) and apically, abutting the crypt lumen (*solid arrow*); **h** advanced epithelial infection of the pharyngeal tonsil (7 dpi) with syncytia formation. All scale bars represent 100 μm. Figure cited from Pope et al. (2013)

(signalling lymphocytic activation molecule)-positive monocytes, DC's and lymphocytes within the respiratory tract using SLAM/CD150 as a receptor for cellular entry. SLAM is a key regulator of leucocyte activation and differentiation (Veillette 2006) and has been shown to be a receptor for morbillivirus cellular entry (Tatsuo et al. 2001). SLAM is normally expressed on immature thymocytes, memory T cells, a proportion of B cells, activated monocytes/macrophages and mature, but not immature dendritic cells (Romero et al. 2004). It is not expressed in epithelial cells. Pope et al. assessed virus antigen distribution during the prodromal period at 2 and 5 days post-infection and observed that whilst no viral antigen was detected in any tissues at 2 days post-infection, by 5 days, there was a significant amount of viral antigen detected within lymphoid tissues, which also included nodes not involved in the drainage of the nasopharyngeal mucosa. The inability to detect virus antigen in non-lymphoid tissues early in infection following intranasal inoculation, coupled with the highly cell associated nature of morbillivirus infection (Osunkoya et al. 1990; Esolen et al. 1993), leads to the conclusion that virus reached these distant lymphoid tissues within lymphoid or reticular cells, via the blood and high endothelial venules (HEVs). The study concluded that immune cells such as macrophages and/dendritic cells present in the intraepithelial space and the lamina propria of the respiratory mucosa take up PPR virions from the lumen of the respiratory tract and migrate to the T cell-rich areas of local lymphoid organs, where SLAM-mediated replication occurs within lymphoid cells prior to virus entering circulation. This study implies that a productive viraemia occurs within the first 5 days of infection, supported by PCR positivity of PBLs and conjunctival swab samples at 5 days post-infection, but from when exactly this viraemia was initiated is difficult to pinpoint with the data available.

SLAM mRNA expression and PPRV replication in PBMCs of different species appear to be highly correlated (Pawar et al. 2008a, b). Levels of SLAM mRNA expression were found to be the highest in goats followed by sheep, cattle and buffalo. However, when SLAM was blocked by anti-SLAM antibodies, the PPRV titre was reduced 100-fold but not neutralised completely. This suggested that PPRV might use alternate receptors (Pawar et al. 2008a, b), and as such, nectin-4

has been proposed as a second receptor for PPRV (Birch et al. 2013). This adherens junction protein is present in the basolateral border of epithelial cells. This cellular localisation pattern fits well to our proposed model, that after the general viraemia, PPRV utilises nectin receptors which are present in respiratory and gastrointestinal epithelial cells where secondary replication of virus occurs and further disease is observed. However, the exact role of nectin-4 still needs to be clarified, as expression levels within tissues often do not correlate with the severity of infection within these tissues (Birch et al. 2013; Pope et al. 2013).

It is established that the morbilliviruses display a strong tropism for lymphoid tissues and during infection destruction of leucocytes often causes a profound immunosuppression (Rajak et al. 2005). During the acute phase of PPRV infection, i.e. 4–10 days post-infection, animals become markedly leucopaenic. Even following a mild viral infection, the generation of viraemia occurs within first 5 days and by 7 days post-infection the total circulating peripheral lymphocyte count (PBL) count may be reduced to as little as 40 % of the pre-infection count (Pope et al. 2013). PBL counts in animals that survive infection often exhibit a recovery in lymphocyte count by 12 days post-infection that has often normalised by 16 days post-infection. In a study by El Harrak et al., goats infected with a virulent isolate (Morocco/2008) via the intravenous, subcutaneous and intranasal routes, exhibited total white blood cell (WBC) counts that had decreased from an average count of 12,575–4,725/μl blood within 2–4 days post-infection (Fig. 4.4). In this model, the circulating WBC count appeared to be affected differently according to route of inoculation. The subcutaneously inoculated group dropped to a minimal circulating WBC level by day 4 and remained low between days 4 and 8 post-infection before

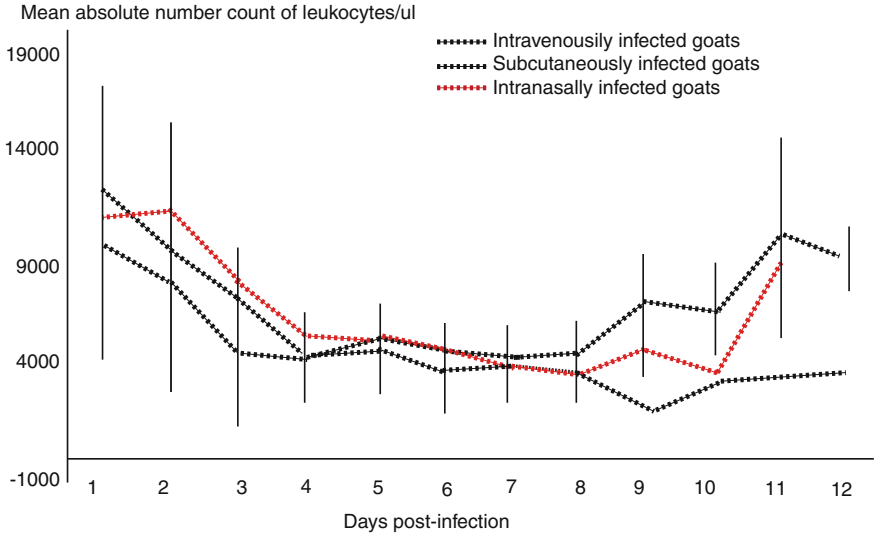

Fig. 4.4 Comparison of mean leucocyte counts in Alpine goats infected with PPR virus by subcutaneous, intravenous and intranasal routes

increasing in animals that survived infection. Interestingly, the group inoculated intravenously had a reduced WBC count longer than the other groups studied (El Harrak et al. 2012). This mechanism is closely associated with the outcome of infection, as where a significant leucopaenia occurs, the development of secondary infections exacerbates the clinical disease associated with infection. A recent study reiterated the finding that immunosuppressed animals developed more extensive and severe disease advancement with higher mortality rates than those that did not experience such a pronounced leucopaenia (Jagtap et al. 2012)

During the leucopaenic phase, secondary bacterial, viral or protozoal infections can occur. An important example of this is seen in the respiratory system. Although pneumonia is a common clinical feature observed in late stage of disease following PPRV infection in the field, its aetiology is most commonly associated with secondary bacterial infection, such as *Pasteurella haemolytica,* and the lesions observed are typical of pneumonic pasteurellosis (FAO 2008). Using a virulent strain of PPRV in Biosafety Level 3 at The Pirbright Institute, Pope et al. (2013) demonstrated a mild form of disease in British White goats in the absence of pneumonia (Pope et al. 2013). The reasons for the mild disease seen despite intranasal inoculation with the virulent strain of PPRV Coˆte d'Ivoire 89/1 are unclear. However, previous studies have observed similar outcomes (Mahapatra et al. 2006). Indeed, it is postulated that breed susceptibility may play an important role in the development of clinical disease although mechanisms that dictate this remain unclear and differential breed susceptibility has not been explored beyond isolated observations (Couacy-Hymann et al. 2007; Diop et al. 2005). Other factors such as nutritional status, environmental factors and co-infection with pre-existing parasitic organisms may also contribute to exacerbated disease courses that lead to high morbidity and mortality rates (Couacy-Hymann et al. 2007; Ugochukwu and Agwu 1991).

4.9 Conclusions

Clearly, there are several forms (mild to severe) of disease seen following PPRV infection. However, the mechanisms of infection and events very early on following experimental infection require further analysis to understand the molecular mechanisms behind infection and dissemination of virus within the host. Histochemical and histopathological investigations have demonstrated that the initial site for virus replication is not within the epithelial cells of the respiratory mucosa, as has been previously reported, but is within the tonsillar tissue and lymph nodes draining the site of inoculation. Pope et al. (2013) proposed that the virus is taken up by immune cells within the respiratory mucosa which then serve to transport the virus to lymphoid tissues where primary virus replication occurs and from where virus enters circulation. Although it has been hypothesised that the initial site of PPRV replication is not within the epithelial cells of the respiratory mucosa, further studies are required to address these questions by assessing the sites of viral replication very

early on following infection. The profound immunosuppression that follows infection clearly also contributes to the outcome of infection with secondary infections often contributing significantly to the high mortality rates associated with infection. Clearly, extensive investigation into the mechanisms of virus induced pathology is required to fully understand this economically important viral pathogen.

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Chapter 5
Molecular Epidemiology of Peste des Petits Ruminants Virus

Ashley C. Banyard and Satya Parida

Abstract Peste des petits ruminants virus (PPRV) causes an economically important plague of small ruminants. The virus is endemic across much of the developing world and has even spread into the developed world through the spread of the infection into sheep and goat populations within European Turkey. Where the virus is present, it is often seen to disproportionately affect small ruminant production, often causing increased poverty in what are already the poorest areas of the globe. PPR is considered to be a transboundary disease of great significance through its effect on the development and maintenance of sustainable agriculture in developing countries, most notably in Western Africa and South Asia. Here, we review reporting to the World Organization for Animal Health (OIE) and World Reference Laboratories (WRLs) over the last 16 years and comment on reporting systems. Furthermore, we discuss the utility of molecular tools to genetically type PPRV infection across Africa, Asia and Europe.

5.1 Introduction

The morbilliviruses constitute an important group of pathogens of great significance to both medical and veterinary sectors. From a human standpoint, measles virus (MV) continues to cause outbreaks in the human population that, where immunological status and nutrition are poor, causes significant deaths within the paediatric sector. From a veterinary perspective, alongside canine distemper virus (CDV) and related infections of cetaceans, this group of viruses includes one of the most feared veterinary viral diseases known to mankind, rinderpest virus (RPV) (Roeder 2011).

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Whilst the spectre of rinderpest has been removed through an extensive eradication campaign, the story of which spans over 40 years, peste des petits ruminants virus (PPRV), a closely related virus that infects small ruminants, continues to plague the farming industry of the developing world. Following the eradication of RPV, a shift in attention has been made to the study of PPRV, and this shift has started to highlight just how extensive the problem of PPRV is in areas where the virus in endemic, in particular, across the developing world. Alongside those viruses already mentioned, novel morbillivirus-like viruses have been described from various different hosts that awaiting classification into the genus. These include a virus termed feline morbillivirus (Woo et al. 2012) as well as a whole host of viruses derived from different rodent and bat species that genetically cluster within the morbillivirus genus (Drexler et al. 2012). Further characterisation of these novel isolates may give insight into the evolutionary mechanisms of morbillivirus host specificities and differentiation.

Within the field of virology, the subject of molecular epidemiology can be interpreted in many ways, often to encompass the evolutionary background to disease and its geographical occurrence. In its simplest form the study of molecular epidemiology encompasses, 'the various biochemical and molecular techniques used to type and subtype pathogens'. It is in this way that this chapter addresses the distribution and molecular differentiation of PPRV isolates across areas where the virus exists. From an epidemiological perspective, the genetic typing of different morbillivirus isolates has enabled lineage differentiation of different viruses within a species and as such has highlighted movement of virus geographically as well as highlighting knowledge gaps in our understanding of cross-species transmission. This latter feature has been particularly important in the study of canine distemper virus, a virus initially thought to be restricted to the infection of members of the *Canidae* which has, over a number of years been found to infect a very wide range of hosts including members of *the Felidae, Viverridae, Mustelidae* and *Cetacea* (Barrett et al. 2006). Here, we discuss the tools used to type PPRV isolates and detail the distribution of these isolates across areas where the virus remains endemic.

5.2 Reporting Systems for PPRV

Historically, the reporting of cases of PPRV has been viewed with some suspicion due to the circulation of the closely related rinderpest virus in livestock populations. Indeed, acute, subacute and inapparent rinderpest reactions were described in goats and sheep (Anderson et al. 1996) that could easily be confused following observation alone as being PPRV. However, following the development of tools that could more conclusively diagnose infection with PPRV, reporting by different countries in regions where the virus is endemic has enabled a picture of the distribution of the disease to be built. The submission of PPRV suspect samples to the World Reference Laboratories (WRLs) was recently reviewed by us (Banyard et al. 2010), and here,

we overview the reporting to the World Organization for Animal Health (OIE) alongside that within the scientific literature to detail the molecular epidemiology of PPRV.

The official reporting of outbreaks of PPRV to the OIE has been conducted for over 15 years now, and data for individual annual and monthly disease reports by both country and region are available online through the Handistatus (1996–2004) and WAHID (2005–Present) interfaces (www.oie.int). Between 1996 and 2005, information was reported according to observation of clinical disease consistent with that expected following infection with PPRV, and data were collected as the number of outbreaks of disease as detailed within each annual country report. Interestingly, between 1996 and 2004 of the 55 defined African countries/territories, only 42 % ($n = 23$) were reporting outbreaks of PPRV to the OIE. Of those that were reporting outbreaks of PPRV, the majority were West African (Fig. 5.1a). In contrast, by 2011, 96 % of the 55 African countries were reporting the presence or absence of PPRV although 31 % of those appear to remain free of PPRV either being genuinely free or having not reported to the OIE. Interestingly, over the last 6 years, the reporting of PPRV has increased dramatically with disease being present across much of the African continent (Fig. 5.1b). Of course, reporting systems in developing countries are far from ideal and this is highlighted by countries apparently experiencing consistently high numbers of outbreaks whilst neighbouring countries remain free from disease.

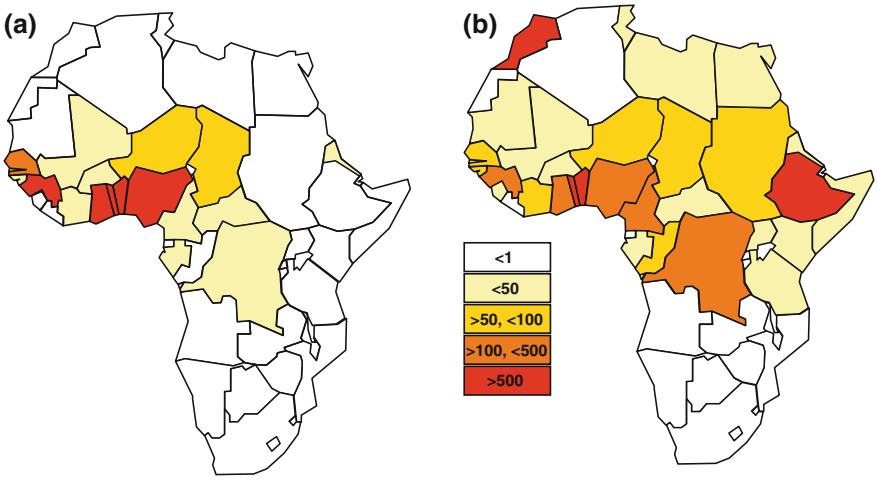


Fig. 5.1 Countries reporting outbreaks of PPRV across Africa to the OIE between **a** 1996–2004 (Handistatus II resource) and **b** 2005–2012 (WAHID Interface). Frequency of outbreaks are denoted over each time period as shown in the key

5.3 The Historical Emergence of PPRV

Historically, the inability to differentiate between infection with PPRV and RPV led to inconsistencies in the reporting of PPRV. The initial detection of PPRV in West Africa led to the belief that the virus originated in the region, with the virus being considered to be a variant of RPV. Further attempts to characterise the virus eventually led to the important conclusion that PPRV was a distinct entity from RPV and was declared as the fourth member (Gibbs et al. 1979) of the morbillivirus genus joining RPV, MV and CDV. From this stage onwards, and with the development of molecular assays that used sequence data to genetically type virus isolates, the reporting of PPRV became more reliable. Alongside this, the successes seen with the RPV eradication programme highlighted the significance of this disease of small ruminants to subsistence farmers in areas where RPV was no longer present.

For many years, PPRV was considered to be a disease endemic within regions of Africa but then, during the 1980s, the virus was discovered in the Sultanate of Oman on the Arabian peninsular (Taylor 1984) and was then later, and significantly found to be circulating in India (Shaila et al. 1989). It was the discovery and subsequent spread of PPRV across India and surrounding countries that highlighted PPRV as a disease of significant economic burden across much of Asia and the Middle East. This period of emergence across vast areas with significant morbidity and mortality rates reminds us of the devastation caused by RPV across populations of large ruminants in Africa. However, serological screening of small ruminant populations across these regions suggest that the virus may have been present for a significant period of time prior to the increased disease inflicted by what is assumed to be the emergence of a highly virulent strain in India during the 1980s. Currently, the true origin of PPRV remains a mystery to science although it is clear that the virus undoubtedly has a long history within small ruminant populations.

5.4 Molecular Methods for the Characterisation of PPRV

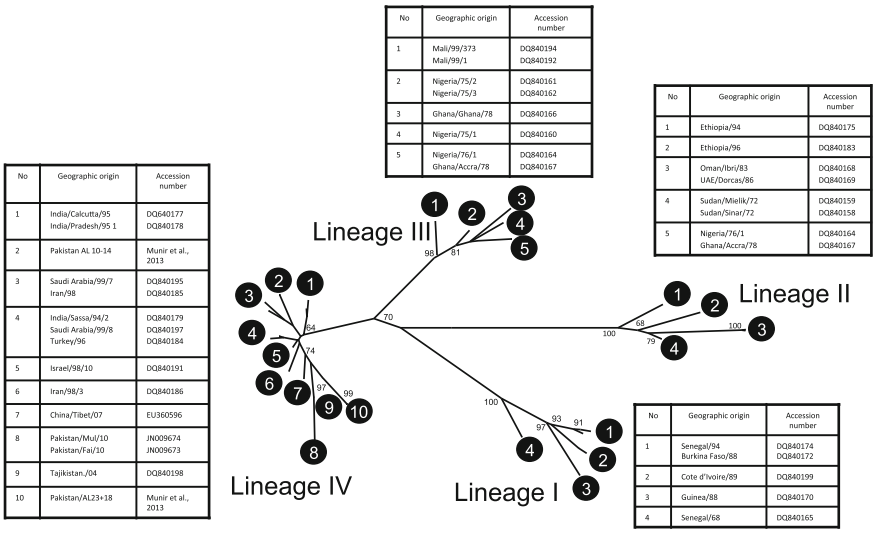
The development of molecular tools with which to genetically type PPRV has shaped our knowledge of the virus, its different lineages and their distribution across the globe. The molecular biology of the virus is dealt in Chap. 2, so here, we will only describe aspects of the molecular biology of the virus that are of significance for the development of tools for genetic typing of different isolates. The morbilliviruses all contain non-segmented negative-strand genomes that encode eight proteins; the nucleocapsid protein (N); the phosphoprotein (P); the matrix protein (M); the fusion protein (F); the haemagglutinin protein (H); the polymerase protein (L) and the three non-structural proteins, C, V and W. Importantly, the negative-sense nature of the genome requires that genes are transcribed into messenger RNA species that are then translated by host cell machinery to generate viral proteins. The morbilliviruses, as

with all other members of the Order *Mononegavirales*, have evolved to synthesise mRNA transcripts according to a ratio required for efficient replication. To this end, each gene is expressed to a different level according to the position of genes from the genome promoter at the 3′ end of the negative-sense genome RNA. The gene order for the morbilliviruses is 3′-N-P-M-F-H-L-5′. When transcribing mRNAs, the transcriptase complex initiates transcription at the 3′ end of the genome and generates a short 'Leader' RNA before moving along the template to transcribe an N gene RNA. Following transcription of the N gene, the transcriptase complex reaches a gene stop sequence where the mRNA is polyadenylated, and an intergenic trinucleotide is reached before transcription of the next gene occurs. During transcription, the transcriptase complex falls off the template at each gene boundary meaning that in effect, a transcriptional gradient is generated whereby 3′ proximal genes are transcribed, and subsequently expressed, at higher levels that those distal to this promoter. This means that very high levels of N are produced, whilst in contrast the L protein is produced in catalytic amounts. This transcriptional gradient is believed to be an evolutionary adaptation to regulate gene synthesis and is important in the way molecular tools have developed to detect viral nucleic acid.

Following the development of PCR techniques for the study of PPRV, several targets have been used to detect PPRV genomic material using PCR. Initially, the fusion protein gene was used as the target due to the conserved nature of the gene and the lack of sequence data available to perform phylogenetic analysis. Forsyth and Barrett (1995) reported the first molecular PCR assays used to differentiate between PPRV and RPV using an F gene PCR, and subsequently, this region of the genome was used for several years to phylogenetically type isolates (Forsyth and Barrett 1995). However, with the reporting of greater amounts of sequence data, new assays were developed to improve the sensitivity of PCR assays, and as such, the N gene was chosen as the most suitable target due to the relative abundance of N gene mRNA over that of other genes (Couacy-Hymann et al. 2002). Furthermore, the region of the N gene targeted was conserved at primer binding sites across a large number of isolates but contained divergent regions across the length of the amplicon and as such enabled a more in depth phylogenetic analysis to be performed (Kwiatek et al. 2007; Kerur et al. 2008). Regardless, across different laboratories, lineage differentiation is determined by the sequence comparison using either the F gene or the N gene PCR depending on the reaction components used by the testing laboratory. Lineage differentiation based on phylogenetic typing historically numbered African isolates of the virus as lineage I–III according to the proposed spread of the virus from West Africa to East Africa. Following this nomenclature, N gene-based amplicons genetically classify as follows: viruses from West Africa (including Senegal, Guinea, Guinea-Bissau, Ivory Coast and Burkina Faso) as belonging to lineage I; viruses detected in Ghana, Mali and Nigeria as lineage II; and samples that clustered together from Ethiopia and Sudan were from lineage III. Interestingly, data derived from F gene material reversed the classification of lineage I and II isolates and historically, and somewhat confusingly, this difference has been maintained (Shaila et al. 1996; Banyard et al. 2010).

The most recent molecular characterisation of PPR virus isolates, based on the N gene, divides them into four genetically distinct lineages (Fig. 5.2). Here, lineage I isolates are represented mainly by Western African isolates from the 1970s and recent isolates from Central Africa; lineage II isolates include West African isolates from the Ivory Coast, Guinea and Burkina Faso; lineage III isolates include isolates from Eastern Africa, the Sudan, Yemen and Oman; and lineage IV includes all samples genetically typed from outbreaks across the Arabian Peninsula, the Middle East, Southern Asia and most recently across several African territories (Banyard et al. 2010; Dhar et al. 2002; Munir et al. 2013). This genetic data are routinely used to construct phylogenetic trees for PPR and ascribe different isolates to each of the different lineages (Shaila et al. 1996; Dhar et al. 2002; Ozkul et al. 2002; Banyard et al. 2010). Although previously postulated, it appears unlikely that this lineage differentiation has any relationship to virulence of isolates and is more likely a result of geographical speciation.

Having briefly outlined the history of PPRV and the molecular tools applied to type virus, the remainder of this chapter will detail reports of PPRV across the developing world focussing on data reported to the OIE alongside that published in scientific journals.



No	Geographic origin	Accession number
1	India/Calcutta/95 India/Pradesh/95 1	DQ640177 DQ840178
2	Pakistan AL 10-14	Munir et al., 2013
3	Saudi Arabia/99/7 Iran/98	DQ840195 DQ840185
4	India/Sassa/94/2 Saudi Arabia/99/8 Turkey/96	DQ840179 DQ840197 DQ840184
5	Israel/98/10	DQ840191
6	Iran/98/3	DQ840186
7	China/Tibet/07	EU360596
8	Pakistan/Mul/10 Pakistan/Fai/10	JN009674 JN009673
9	Tajikistan./04	DQ840198
10	Pakistan/AL23+18	Munir et al., 2013

No	Geographic origin	Accession number
1	Mali/99/373 Mali/99/1	DQ840194 DQ840192
2	Nigeria/75/2 Nigeria/75/3	DQ840161 DQ840162
3	Ghana/Ghana/78	DQ840166
4	Nigeria/75/1	DQ840160
5	Nigeria/76/1 Ghana/Accra/78	DQ840164 DQ840167

No	Geographic origin	Accession number
1	Ethiopia/94	DQ840175
2	Ethiopia/96	DQ840183
3	Oman/Ibri/83 UAE/Dorcas/86	DQ840168 DQ840169
4	Sudan/Mielik/72 Sudan/Sinar/72	DQ840159 DQ840158
5	Nigeria/76/1 Ghana/Accra/78	DQ840164 DQ840167

No	Geographic origin	Accession number
1	Senegal/94 Burkina Faso/88	DQ840174 DQ840172
2	Cote d'Ivoire/89	DQ840199
3	Guinea/88	DQ840170
4	Senegal/68	DQ840165

Fig. 5.2 Phylogenetic analysis of PPRV isolates. The tree was constructed using sequence data derived from the N gene (Couacy-Hymann et al. 2005) using MEGA4 (v4). The Kimura two-parameter model was used to construct the neighbour joining tree. (Adapted from Munir et al. 2013). Where multiple sequences exist that are close in geographical origin, the most divergent has been included. Bootstrap values at significant nodes are also shown

5.5 PPRV, Small Ruminant Populations and Reporting Systems

Historically, the spark that initiated the formation of the OIE in the 1920s was the detection of RPV in Belgium and so it is fitting that we briefly outline the history of reporting of PPRV to the OIE from the inception of the organization in 1924. Reports to the OIE that detail the detection of PPRV within an area started in 1988, via the Food and Agriculture Organization (FAO) registry. Following this, in 1996 the HandiStatus II reporting system was developed and was utilised for disease reporting between 1996 and 2004. In 2005, the OIE launched a new reporting system to member countries under the heading the 'World Animal Health Information Database' (WAHID). This system of reporting was initiated to encourage transparency between different governments in disease reporting and to promote recognition of, and training towards, the diagnosis of animal diseases. Importantly, the density of small ruminants within an area is of great importance for the transmission of PPRV, as dealt with elsewhere within this article. The FAO estimates the following small ruminant populations across areas where PPRV is known to circulate: Central Asia, 43, 118, 821; Africa, 264, 275, 400; Near East, 171, 997, 500; and Far East, 647, 518, 989. With an estimated global small ruminant population of 1, 801, 434, 4416, this means that approximately 62.5 % of the global domestic small ruminant population is potentially at risk from infection with PPRV (FAO, http://www.fao.org/ag/againfo/resources/documents/AH/PPR_flyer.pdf).

For our analysis of the epidemiology of PPRV, we have chosen to start our data analysis from 1996 to both coincide with initiation of the HandiStatus II reporting system and to have greater confidence in the reports made. By 1996, several sensitive and specific serological and molecular tools to diagnose PPRV had been developed, and with these tools, our confidence in accurate reporting increases. However, when viewing the analysis, caution must still be taken to not over interpret the data. Importantly, this analysis does not consider several important factors including the following: the scale of the outbreaks including the morbidity and mortality rates observed; the species involved; the level of impact of small ruminant populations; the small ruminant populations generally present within a country; and most importantly, the availability of reporting systems within a region. This final caveat is of great importance as clearly, if veterinary services are not implemented within a region, then accurate diagnosis and reporting cannot be made. Alongside these features, the presence of other pathogens within an area that might exacerbate the morbidity and mortality rates seen following infection with a highly immunosuppressive virus such as PPRV is also not known. However, despite these drawbacks to the data set, the analyses give an interesting overview of the burden of PPRV in different regions where reporting has occurred and suitably illustrate the increase in reporting of PPRV in different regions that have led to the perceived emergence of the disease across the developing world.

5.6 Molecular Epidemiology of PPRV in Africa

The African continent is currently divided into 57 countries (or independent territories), many of which rely heavily on livestock for fuel, motor power and sustenance. Within this section, we detail what is known regarding the distribution of PPRV across the African continent through both submissions to the OIE and WRLs as well as scientific reports in published peer-reviewed literature. For the purpose of this chapter, countries reporting a heavy burden of disease that is attributable to PPRV will be analysed in more detail. Island nations that are distributed in the seas surrounding Africa will not be discussed as such island nations have been able to maintain freedom from PPRV. However, rare importations of PPRV to islands will be mentioned where they have occurred. Of the 57 countries currently listed as existing within Africa, ten are island nations and as such are not included within analyses performed.

Indeed, between 1996 and 2004, reports to the OIE from each of the regions within Africa vary considerably with several countries within West Africa suffering a high burden of disease whilst reports from other regions were scarce with only Ethiopia in the East, and Cameroon and the Democratic Republic of Congo in Central Africa reporting outbreaks. During this time, no PPRV was reported across North Africa. Of course, whilst OIE reports are of great utility in determining the epidemiology of disease, they also highlight areas where disease reporting may have been absent most often through lack of resources and facilities. One example of this is the endemic nature of PPRV across much of West Africa alongside the apparent complete absence of disease in areas surrounded by countries suffering regular epidemics. Of course, vaccination campaigns within specific areas may also be responsible for the reduced reporting seen although where serological reports have been published the presence of potentially vaccinated animals can confuse the situation as the current vaccines do not enable serological differentiation between naturally infected and vaccinated animals. A comparison of the number of outbreaks reported to the OIE during the periods 1996–2004 and 2005–2011 accompany details of each region described below. Alongside these representations of this data, a continental comparison can be found in Fig. 5.2, highlighting the disease incidence as reports to the OIE between these periods.

5.7 West Africa

Historically, reports of PPRV to the OIE across Africa have been most frequently made from countries within West Africa where between 1996 and 2004, 93.6 % of the total number of PPRV outbreaks from the entire continent reported to the OIE originated. The cluster of countries lining the ‘Gold Coast’ represents countries suffering the highest burden of disease whilst neighbouring countries also reported disease outbreaks.

From the literature, the presence of PPRV in Africa appears to have started with the detection of PPRV in the West, from Cote d'Ivoire in 1942. The virus continues to be present within West Africa with sustained outbreaks occurring at a high frequency in Benin, Ghana and Nigeria where during 2011, a total of 188, 177 and 95 outbreaks were reported, respectively (Fig. 5.3a). The virus continues to be endemic across the region and the differences seen in outbreak reporting must be a consequence of a lack of reporting systems and facilities within which to conduct molecular tests. Interestingly, despite outbreaks being reported to the OIE, from these areas over multiple years, the generation of genetic data from samples taken in these regions to conclusively genetically type the viruses causing these outbreaks remains rare. Only following submission to World Reference Laboratories (WRLs) and the recent review of samples characterised therein (Banyard et al. 2010) has

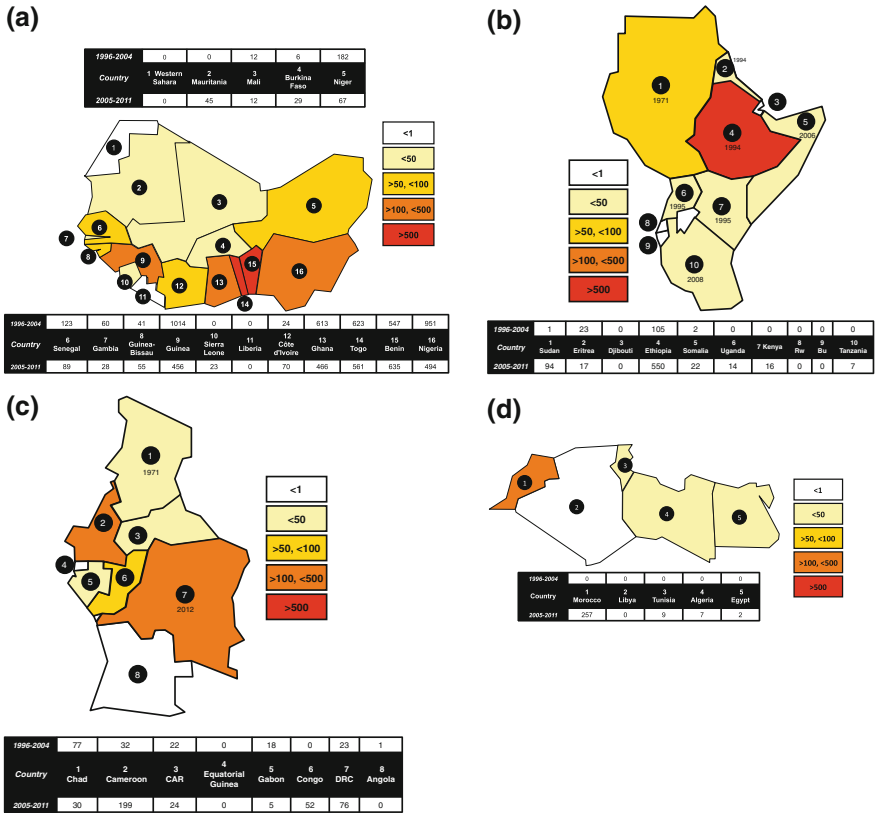


Fig. 5.3 **a** Number of outbreaks recorded across West Africa. Frequency of outbreaks over each reporting period are coloured according to the key. Total number of outbreaks reported for each time period are tabulated below the map. **b** Number of outbreaks recorded across East Africa. The map and outbreak data are as detailed in (**a**). **c** Number of outbreaks recorded across Central Africa. The map and outbreak data are as detailed in (**a**). **d** Number of outbreaks recorded across North Africa. The map and outbreak data are as detailed in (**a**)

highlighted genetic data available for phylogenetic studies. Interestingly, where genetic data have been made available, the time frames between genetic analyses can be often quite long, although based on areas targeted for analysis; the viruses have not diverged significantly within regions.

Historically, following the initial detection of PPRV in Cote d'Ivoire in 1942, the virus was next detected in Senegal (1955- (Mornet et al. 1956), Nigeria (1967-(Hamdy et al. 1976); (Whitney et al. 1967), Togo and Benin (1972-(Benazet 1973; Bourdin 1973) and most recently in Sierra Leone in 2009 (Munir et al. 2012b). The early isolation of virus in Nigeria served as the seed virus for the development of the tissue culture adapted live attenuated Nigeria strain vaccine that is used to limit outbreaks across Africa today. As can be seen, the reporting of PPRV in the literature differs considerably from the reports made to the OIE where between 1996 and 2004, all countries in West Africa reported PPRV with the exception of Liberia, Mauritania and Sierra Leone. From the latter data, it is probable that the virus was actually endemic across the entire region, especially when considering that the only country to have not reported the disease to date is Liberia.

The samples received by the World Reference Laboratories for PPRV during the last 16 years have confirmed either antibodies to the virus or the detection of viral nucleic acid in samples from Burkina Faso (2008), Ghana (2010), Nigeria (2007) and Senegal (2010). From the genetic perspective, both lineage I and lineage II strains of PPRV continue to circulate within West Africa although clearly, molecular characterisation of circulating strains is rare. Alongside official reports to the OIE, descriptions of PPRV in different species have been published in scientific literature. Nigerian cases of PPRV have been described in sheep, goat and camels (Obidike et al. 2006; El-Yuguda et al. 2010). Most interestingly, the study by Obidike et al. (2006) suggested that excretion of virus by healthy animals may serve to transmit the virus between different groups of animals, a hypothesis that remains of great interest today (Abubakar et al. 2012). Otherwise, the only other study reporting PPRV in the scientific literature details serological positivity in the Soaum province in the northern region of Burkina Faso (Sow et al. 2008). Despite the high incidence of PPRV in Benin, Ghana, Guinea, Niger, Nigeria, Senegal and Togo, full details of outbreaks and molecular characterisation of circulating strains remain to be reported.

5.8 East Africa

The region defined as East Africa includes 10 countries, namely Burundi, Djibouti, Eritrea, Ethiopia, Kenya, Rwanda, Somalia, Sudan, Tanzania and Uganda. PPR is endemic across the majority of these countries with only Rwanda, Djibouti and Burundi having not reported PPRV although reports to the OIE in 2008 and 2010 suspected its presence within Rwanda. From a phylogenetic perspective, the earliest genetic typing of PPRV in East Africa was in 1996 when a lineage III virus was detected. Historically, this lineage had previously only been detected in Sudan

(1972), and within wildlife on the Arabian Peninsula in Oman (1983) and the UAE (1986). Alongside this, a further lineage III isolate was detected in Southern India as discussed further below. Submissions to the World Reference Laboratories have detected serological evidence of PPRV in Kenya in (1999 and 2009) and Uganda (2005 and 2007). Further genetic data have characterised viruses as belonging to lineage III (Sudan (2000), Uganda (2007), and Tanzania (2010)), whilst lineage IV viruses have also been isolated (Sudan in 2000, 2004, 2008 and 2009) (Saeed et al. 2010). Reports to the OIE highlight the presence of PPRV in Ethiopia between 1996 and 2004 with occasional disease in Eritrea during this time period also (Fig. 5.3b). Historically, it seems that Ethiopia has suffered the heaviest burden of disease having originally been suspected as having PPRV in 1977 (Pegram and Tereke 1981) with confirmatory diagnosis in 1984 (Taylor 1984) and characterisation of a quite extensive outbreak 10 years later (Roeder et al. 1994). Further reports in the scientific literature have confirmed the widespread distribution of PPRV across Ethiopia and the impact of the virus on a country whose economy relies heavily on agriculture (Waret-Szkuta et al. 2008). A further important feature of PPRV in Ethiopia is the detection of PPRV antibodies in camels (Ismail et al. 1992; Haroun et al. 2002; Abraham et al. 2005; Albayrak and Gur 2010). The virus detected in camels was found to belong to lineage III, being closely related to isolates circulating in sheep and goats within the same region (Roger et al. 2001b). This finding suggests either a spillover of virus from small ruminant populations into camels causing high morbidity with low mortality or, perhaps controversially, a role for camels in the maintenance and transmission of PPRV between naïve flocks of small ruminants. Either way, further research is required to understand the epidemiology of PPRV in different species. Whilst not confirmed by laboratory diagnosis, PPR was also seen in Ethiopia in 2008 and 2009 and vaccination was carried out alongside CCPP vaccination programmes (Munir et al. 2013).

In contrast, reporting between 2005 and 2012 shows extensive outbreaks across Ethiopia and Sudan with outbreaks also reported in Kenya, Somalia, Tanzania, Eritrea and Uganda albeit at a lesser frequency (Fig. 5.3b). Clearly, both lineages III and IV are circulating in the Sudan, and further, serological reports from the country have confirmed outbreaks of PPR in Sudan (Osman et al. 2009). Recent genetic analyses have clearly demonstrated the division of Sudanese isolates into either lineage III or lineage IV with a more recent increase in lineage IV detections over lineage III (Saeed et al. 2010; Kwiatek et al. 2011). Whether this reflects a dominance of virus isolates from one lineage over another is unclear although the detection and lack of spread of lineage III in southern India is hypothesised to be the result of lineage IV virus being more efficiently spread. Further genetic and epidemiological analyses are required to accept or refute this hypothesis.

The presence of PPRV in the regions encompassing Kenya, Uganda and Tanzania has also historically been poorly characterised with no reports to the OIE of these viruses being present within this triad of countries between 1996 and 2004 (Fig. 5.3b). However, the existence of PPRV within this area probably exceeds the reports known. Most critically, in 2006, 10 outbreaks of PPRV were reported in Kenya where following initial suspicion of disease in the Turkana district led to a

wave of PPRV across 16 districts causing severe socio-economic consequences for food security and impacting significantly on the livelihoods of farmers across the affected area. Mortality rates reached 100 % mortality in kids, with 40 and 10 % mortality in young and adult animals, respectively (Munir et al. 2013). Estimates predict that the epidemics seen between 2006 and 2008 affected more than 5 million animals across sixteen Kenyan districts with an economic impact in excess of 1 billion Kenyan shillings (US$15 million; £10.5 million) each year. Successful implementations of vaccination and quarantine methods have prevented the continued spread off PPR in Kenya. However, several factors including, but not limited to, inadequate funding, availability of vaccine, shortage of trained staff to coordinate vaccination programmes, tribal clashes, drought and the mobility of the pastoral communities involved, have made the task more problematic (Anonymous 2008).

In Uganda, significant outbreaks during 2007 led to mass vaccination of animals during 2008 to avoid a potential humanitarian food shortage crisis (RO-CEA 2008). Serological evidence of PPRV circulation has been reported with an overall prevalence of >50 %. However, for such serological surveys, the inability to differentiate between naturally infected and vaccinated animals is highlighted by the division of serological profiles of animals reported. Indeed, in a study that addressed the serological status of known vaccinated, known-unvaccinated and animals with an unknown vaccination status serological profiles were 55.3, 11.7 and 53.3 %, respectively. This feature highlights a potential benefit of application of a DIVA vaccine to PPRV (Banyard et al. 2010). From a genetic standpoint, multiple lineages of PPRV circulate in Uganda with lineages I, II and IV all having been detected (Luka et al. 2012)

In Tanzania, PPRV appears to be absent for long periods of time despite the detection of the virus in neighbouring countries with no outbreaks reported for several years. A study by Swai et al. (2009) confirmed natural transmission of PPR and circulation of virus within herds in Tanzania with a further study demonstrated 40 and 50 % seroconversion in herds of sheep and goats, respectively (Munir et al. 2013). Importantly, the detection of PPRV in Tanzania raised the possibility of the virus moving south towards areas that have never seen the disease and as such exist as highly susceptible naïve herds. This potential further spread and threat to the South African Development community led to the FAO advising that countries in the region be on alert for possible incursions of disease and that Tanzania perform mass vaccination to contain the pathogen (FAO 2012). Currently, only lineage III virus has been detected within Tanzania although with other lineages being present within Uganda, the potential for transmission of other lineages remains possible.

Alongside the extensive outbreaks of PPPRV in Kenya in 2006, Somalia was also affected with the central regions being most seriously affected. Fortunately, the geological topology of Somalia prevented transmission of PPRV across the entire country. Regardless, ring vaccination was implemented in 2009 in Somalia to prevent further spread (Nyamweya et al. 2008). Genetic characterisation of Somali virus isolates remains to be performed although it is likely to be lineage III as this lineage predominates in neighbouring countries.

One other interesting occurrence of PPR is a region considered part of East Africa was a report of PPRV on the Island of Mauritius reported in 1999 and between 2001 and 2003. The virus present on Mauritius must has been carried onto the island from the mainland although molecular characterisation of these outbreaks has not been conducted to enable the transmission chain to be elucidated.

5.9 Central Africa

Historically, PPRV was first reported from Central Africa when the question was asked, 'Does PPRV circulate in Central Africa?' (Provost et al. 1972). The conclusion was that it was present with detection of infection in Chad. Later, in 1995, isolation of the virus and experimental induction of disease was demonstrated in Chad, confirming that PPRV was present in the region (Bidjeh et al. 1995). Apart from this, reporting of PPRV in Central Africa has been infrequent with only post vaccination and colostral PPRV antibody dynamics being investigated by researchers in North Cameroon. Reports to the OIE have also been rare with only Cameroon, Gabon, the Central African Republic and the Democratic Republic of Congo (DRC) reporting PPRV between 1996 and 2004 (Fig. 5.3c). In the last 8 years, however, reporting has increased with virus detected across much of Central Africa with only Angola seemingly free from disease. Interestingly, Equatorial Guinea has reported suspicion of disease between 2009 and 2011 although a confirmatory diagnosis remains lacking. Most recently, the DRC suffered a large outbreak of PPRV with a high mortality rate with over 75,000 goats succumbing to the disease with over 1 million small ruminants at risk (FAO 2012). Fortunately, during this outbreak, the FAO implemented a rapid vaccination campaign and the outbreak was stopped. Emergency funds were made available to support: vaccination of more than 500,000 sheep and goats in surrounding, unaffected areas; limiting animal movements, by preventing them from moving to communal grazing areas and temporarily interrupting sale and transport of animals; raising awareness via rural radio and village-level meetings to educate farmers about steps, they can take to prevent PPR; increasing active surveillance for PPR throughout the area; and the training of field veterinarians and para-veterinarians in the clinical diagnosis of PPR and field investigation techniques. As during the outbreaks seen in 2008 in Tanzania, The Southern Africa Development Community, including Angola, Botswana and Zambia, which are at high risk should the disease spread in a southerly direction, has awareness and prevention of PPRV a major animal health priority. However, outbreaks such as this highlight the continuing threat posed by this virus to agricultural communities. From a genetic standpoint, the only lineage demonstrated to be present in Central Africa is lineage IV. However, with lineages I, II and IV, all being present in Uganda; lineage II present in Nigeria; and lineage III present in the Sudan and Tanzania, further evaluation of isolates circulating in this region is likely to indicate further areas in which multiple lineages circulate.

5.10 North Africa

North Africa represents the band of countries places across the north of the continent linked by the Sahara to sub-Saharan Africa and includes seven countries or territories; Algeria, Egypt, Libya, Morocco, Sudan, Tunisia, and Western Sahara. Historically, PPRV was absent from these countries until in 2008 when the virus was responsible for an extensive outbreak in Morocco (Fig. 5.3d). During 2008, local veterinary services reported more than 250 outbreaks across 36 of Morocco's 61 provinces. Despite low mortality and morbidity rates, this outbreak was of great significance due to commercial trade between Morocco and both Algeria and Spain. The perceived threat from the virus, due to these trade routes, heightened interest in the Moroccan outbreaks, and resources were made available to enable enforcement of vaccination campaigns. These campaigns enabled vaccination of Morocco's sheep and goat population with approximately 20.6 million individual animals being vaccinated rapidly in response to the outbreak (EMPRESS 2008). Genetic characterisation of the Moroccan virus classified it as a lineage IV virus (Khalafalla et al. 2010). The true source of the Moroccan outbreak remains unidentified although since 2008, other studies have presented evidence for PPR across other North Africa countries including Tunisia (Ayari-Fakhfakh et al. 2011) and Algeria (De Nardi et al. 2012), so the virus may well be present across other as yet unknown regions of North Africa.

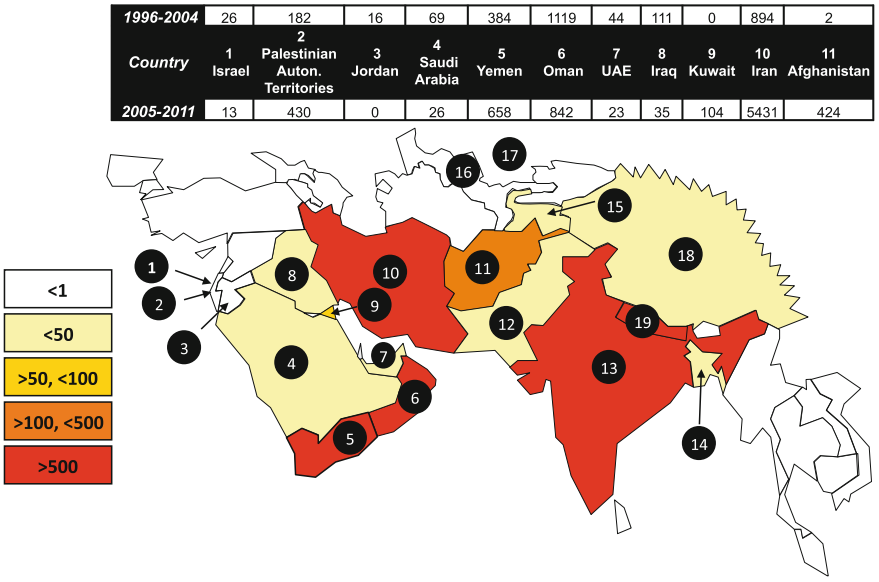
The situation in Egypt is of historical relevance as PPRV has been infrequently detected since 1987 (Ismail and House 1990; Ismail et al. 1990). Indeed, there have been only two outbreaks reported to the OIE in the last 16 years. However, in the scientific literature, the presence of PPRV has been reported in the Aswan province with variable morbidity and mortality being reported (El Hakim 2006). Genetically, the Egyptian isolates have been typed as belonging to lineage IV, and this suggests that perhaps Egypt represents the gateway from the predominantly Asian lineage of PPRV into Africa. Certainly, Egypt's transcontinental geographical position and the established trade routes through the Maghreb make this route of transmission feasible. However, with lineage IV now detected across several areas in Africa, conclusive determination of the movement of lineage IV virus into Africa is problematic.

5.11 Molecular Epidemiology of PPRV in Asia and the Middle East

The distribution of PPRV across Asia and the Middle East is extensive although, as with across Africa, the true burden of the disease has not been quantified. Alongside this, as documented elsewhere, substantial variation in clinical disease means that the virus can circulate unnoticed in animal populations whilst sporadically causing extensive outbreaks. As in Africa, many countries across Asia and the Middle East

are reliant on livestock for fuel, motor power, and sustenance, and as such, PPR has a huge impact on livelihoods. Within this section, we detail what is known regarding the distribution of PPRV across the Asian continent through both submissions to the OIE and World Reference Laboratories as well as scientific reports in published peer-reviewed literature. We comment on historic detection of virus and reporting over the past 20 years.

Historically, PPRV was detected in the Middle East in Saudi Arabia (Asmar et al. 1980) and the United Arab Emirates (UAE) (Furley et al. 1987) with the first reports from Asia being made in India in the late 1980s where the virus was first detected in Tamil Nadu (Shaila et al. 1989). However, during the 1990s, the virus was detected across much of Asia, an expansion in geographical range that appears to be ongoing today. Between 1996 and 2004, only a handful of countries reported the presence of PPRV to the OIE with the highest burden of disease being reported across India, whilst in the Middle East, extensive disease outbreaks were reported in Iran, Saudi Arabia, Yemen and Oman (Fig. 5.4). Alongside this, reports in the scientific literature detailed outbreaks in Pakistan and Bangladesh although precise reporting to the OIE did not occur. Below we detail reports of PPRV across Asia



1996-2004	26	182	16	69	384	1119	44	111	0	894	2
Country	1 Israel	2 Palestinian Auton. Territories	3 Jordan	4 Saudi Arabia	5 Yemen	6 Oman	7 UAE	8 Iraq	9 Kuwait	10 Iran	11 Afghanistan
2005-2011	13	430	0	26	658	842	23	35	104	5431	424

1996-2004	7	2291	7	1	1	1	0	2165
Country	12 Pakistan	13 India	14 Bangladesh	15 Tajikistan	16 Uzbekistan	17 Kazakhstan	18 China	19 Nepal
2005-2011	14	2928	5	3	0	0	6	1075

Fig. 5.4 Number of outbreaks recorded across Asia and the Middle East. Frequency of outbreaks over each reporting period is coloured according to the key. Total number of outbreaks reported for each time period are tabulated below the map. The most recent data set is used for the colouration (WAHID interface)

and the Middle East over the last 20 years. As detailed for PPRV in Africa, a comparison of the number of outbreaks reported to the OIE during the periods 1996–2004 and 2005–2011 accompany details of each region described below. Alongside these representations of this data, a continental comparison can be found in Fig. 5.4 highlighting the disease incidence as reports to the OIE between these periods.

5.11.1 India

Globally, India continues to be one of the highest livestock holding countries and is estimated to farm an estimated combined sheep and goat population of 190.2 million animals. This constitutes approximately 10 % of the global small ruminant population. This population can be divided into areas principally concerned with either sheep of goat farming, and as such, the majority of the goat population is found across seven states, namely West Bengal, Rajasthan, Uttar Pradesh, Maharashtra, Bihar, Tamil Nadu and Madhya Pradesh, whilst the majority of sheep farmed is present within four states: Andhra Pradesh, Rajasthan, Karnataka and Tamil Nadu. Importantly, as previously stated, the majority of the goat and sheep in the country are reared by small and marginal farmers and landless labourers (Metha 2011). India is estimated to have 126 million goats and 58.2 million sheep, with the husbandry of these animals generally being the responsibility of landless labourers and small farms. The result of this agricultural division is that the impact of PPR is greater on the poorer section of society, due to this intrinsic dependence on sheep and goat farming. From an economic perspective, the financial burden of PPR in India has been estimated at approximately 1800 million Indian Rupees (US$ 39 million, UK £21 million) per year (Singh et al. 2004). Alongside the loss of animals to the disease, and where animals survive the subsequent reduction in milk and meat yield, the cost of vaccination to protect animals is often of great significance.

Historically, PPRV has been reported in India since 1987 although it is likely that the virus had been in circulation across India for many years prior to its initial detection. The utilisation of PCR technologies typed virtually all cases of PPRV across India as being of lineage IV (Shaila et al. 1996; Dhar et al. 2002). The sole exception to this is the detection of a lineage III PPRV in the Tamil Nadu region in 1992. However, this detection remains largely unexplainable although it is hypothesised that small ruminant trade may have brought this lineage to the region where it was perhaps unable to spread within local populations of small ruminant or that it was possibly replaced by lineage IV virus, the epidemiology of which is described elsewhere (Dhar et al. 2002; Banyard et al. 2010).

More recent epidemiological studies of PPR in India have characterised a number of closely related lineage IV viruses being present (Kerur et al. 2008). Reports of PPRV in the north (Kataria et al. 2007), the east (Saha et al. 2005), the south-west (Chavran et al. 2009; Santhosh et al. 2009) and across the southern peninsula (Raghavendra et al. 2008) reflect the endemicity of the disease across the

entire country. Between 1996 and 2004, over 7,000 reports were made from across Asia to the OIE of PPRV with more than 30 % of the outbreaks being reported in India. Where the virus is endemic, it is considered to be a major obstacle to the development of a sustainable small ruminant industry. Interestingly, recent studies have documented a high degree of seroconversion to PPRV antigen in large ruminants including cattle and buffalo (Balamurugan et al. 2012). The significance of this observation remains unclear.

5.11.2 Pakistan

Clinical suspicion of PPR in Pakistan was first reported in the Punjab province (Pervez et al. 1993) and was soon confirmed by RT-PCR as belonging to lineage IV (Amjad et al. 1996). During this time, the suspicion and clinical observation of PPR was reported to the OIE, but apparently veterinary services were lacking as no details surrounding outbreaks were reported and between 1996 and 2004. As a result, only the presence, rather than the number and extent of outbreaks, was reported. Despite this paucity of data, the existence of PPR in Pakistan has been well documented in the scientific literature with numerous reports of serological and genetic detection. Serological detection has been confirmed following clinical suspicion of disease in several areas (Khan et al. 2008; Zahur et al. 2008; Rashid et al. 2010; Abubakar et al. 2011; Munir et al. 2013). Most recently, genetic analyses have been used to characterise a panel of isolates from Pakistan and all were found to be lineage IV (Munir et al. 2012a). Critically, a more thorough epidemiological analysis of PPRV is needed across Pakistan and neighbouring countries.

5.11.3 The Near East

The detection of PPR in Afghanistan, Tajikistan, Kurdistan and neighbouring countries has highlighted the emergence of the virus in areas previously thought to be free of PPRV. Prior to 2005, Afghanistan merely noted the suspicion of PPRV. However, between 2005 and 2012, there have been 424 outbreaks of PPRV reported to the OIE although, despite this figure, reports are often incomplete, and as such, this is likely to be an underestimate of disease outbreaks occurring in the area. Of course, the ability to report infectious diseases in small ruminants in the face of international conflict is highly problematic. Historically, PPRV may have been present within this region during the mid-1990s although only serological evidence of infection exists from this time. It was not until 2003 that high levels of seropositivity was detected following clinical suspicion of disease although in the absence of DIVA tools, serological status in response to vaccination cannot be confirmed (Martin and Larfaoui 2003). Certainly, more extensive surveillance is

required to establish the current status of PPRV in the country. In neighbouring Tajikistan, PPR has been reported (Kwiatek et al. 2007) and it is also thought that the virus circulates in Kazakhstan although limited data support this observation (Lundervold et al. 2004). The presence of PPRV in this region, including Uzbekistan who reported PPRV in 2000, also requires further confirmation.

5.11.4 The Far East

The detection of PPR in the Far East is currently restricted to outbreaks in Nepal and in China where the virus was first detected in small ruminants in the Ngari region of western Tibet in July 2007 (Wang et al. 2009). This initial detection was genetically confirmed, and the virus detected was related to viruses previously typed from neighbouring countries including India, Nepal and Tajikistan. The presence of PPRV in Nepal and India has been reported to the OIE for many years, and together, India and Nepal have shared the major burden of disease based on outbreak data reported to the OIE (Fig. 5.4). However, the burden of disease reported in Nepal and India appears disproportionate to neighbouring countries, a feature that again questions the reliability of the available data and the need for enhanced veterinary surveillance. Emergence of PPRV in Tibet is thought to have occurred through a knowledge gap in veterinary expertise where local veterinarians were unaware of the clinical disease signs associated with PPRV infection. Having established the presence of PPRV in China, the authorities moved quickly to vaccinate susceptible populations and successfully restricted spread of the disease across China. The detection and genetic characterisation of PPRV in free living wild Himalayan Blue Sheep or bharals (*Pseudois nayaur*) has since furthered an interest in infection of wildlife species (Bao et al. 2011). Bharals are known to be present across China, Tibet, Nepal, India, Pakistan and Bhutan. In Nepal, population densities of this species have reached 0.9–2.7 animals per km^2 although currently the infection dynamics of bharals with PPRV is poorly understood and further investigations into the role of this species and infection with PPRV is of interest. However, in this case, it is likely that PPRV outbreaks in the local area preceded infection of the bharals (Wang et al., 2009; Bao et al., 2011). A further interesting observation was the detection of mortalities of both bharals and Mongolian gazelles in the area although it is thought, as reported previously that the infection of wildlife species represents spillover from small ruminant populations rather than a reservoir of disease (Elzein et al. 2004; Couacy-Hymann et al. 2005). The recent whole-genome analysis of PPRV from bharals will serve as a useful tool as more sequence data become available from this and surrounding areas.

Further suggestions of PPRV in the Far East have come from Laos, who reported an outbreak in 1998 although the virus has not been reported since. Serological detection of antibodies to PPR has also been detected in samples from Vietnam (Maillard et al. 2008) and genetic data have shown lineage IV PPRV to be present in Bhutan. These findings suggest that the virus may be present across an even

greater area than currently thought. It is possible that PPR has spread into many other bordering countries, but the unfamiliarity of local human populations with the disease means that it may remain either unnoticed or be misdiagnosed as a different disease with similar clinical manifestations.

5.11.5 The Arabian Peninsula and the Middle East

Reports to the OIE have highlighted the presence of PPRV on the Arabian Peninsula for many years with Saudi Arabia, Oman, Yemen, the UAE and more recently Qatar all reporting the disease. In Saudi Arabia, the virus was known historically with reports in the literature in the 1980s although it was not until the 1990s that virus was isolated from infected goats (Abu Elzein et al. 1990). In April 2002, an outbreak with a case mortality rate of 100 % was reported in sheep and goats (Housawi et al. 2004), and since then, further serological surveys and outbreaks have been reported (Al-Dubaib 2008, 2009). Importantly for Saudi Arabia, to date there has been no reports of PPRV in camels. The role played by camels in the maintenance and transmission of PPRV is unclear although PPRV has been shown to completely decimate camel populations in other areas (Roger et al. 2001; El-Hakim 2006).

Also on the Arabian Peninsula, the infection of a number of wildlife species has been reported with lineage IV virus being detected in a game reserve in the United Arab Emirates (Kinne et al. 2010) as well as in Qatar (2010). Interestingly, in Qatar, both lineage III and lineage IV are circulating with both lineages being isolated from goats in 2010 (Banyard et al. 2010). The situation in Qatar is further complicated by the recent detection of PPR in wild deer although, as with the situation reported in bharals, the role of sylvatic PPR and potential transmission to domestic species remains unknown. Within Yemen, the most southerly region of the peninsula, lineage III virus continues to circulate with no introduction of lineage IV having been reported in Yemen or Oman. Interestingly, detection of PPRV in Oman was the first detection of the virus outside of Africa in the late 1970s (Hedger et al. 1980). This discovery paved the way for the development of differential assays for the specific detection of PPRV and RPV (Taylor et al. 1990), a key feature of tests that later led to the eradication of rinderpest (Roeder 2011). Alongside these reports, serological detection of anti-PPRV antibodies has been reported in small ruminant populations in both North Jordan (Al-Majali et al. 2008) and in the Lebanon with seroprevalence of up to 48.6 % being reported (Attieh 2007). Further characterisation of strains circulating in these regions will further enable an epidemiological understanding of PPRV in this region.

Across the remainder of the Middle East, the epidemiology of PPR is not well understood. Historically, detection of PPR in Iraq dates back to 2000 where a virus causing high morbidity and low mortality rates was characterised (Barhoom et al. 2000) whilst retrospective seroanalysis has shown that the virus was circulating in

1994 (Banyard et al. 2010). However, compared to neighbouring Iran, few reports have been made to the OIE for PPRV in Iraq, although as with Afghanistan, the provision of veterinary services during times of international conflict is no doubt highly problematic. Iran has reported 32 % of the outbreaks reported to the OIE over the last 16 years with over 5000 outbreaks being recorded between 2005 and 2011 (Fig. 5.4). Vaccination of small ruminant populations has been attempted to limit spread of disease, but often such campaigns are limited. For example, in 2011, just over 3.5 million small ruminants received vaccine from an estimated National population of just fewer than 80 million animals. During this time, over 2000 outbreaks of PPRV were reported across the country. Clearly, a more substantial vaccination programme is required to limit disease. Historically, PPRV has been recognised in Iran since 1995 and since that time, the virus has spread across much of the country (Bazarghani et al. 2006). Further outbreaks of PPRV in Iran have been reviewed in (Abdollahpour et al. 2006). Genetically, all data from Iranian PPRV have belonged to lineage IV being most closely related to viruses genetically typed from the surrounding area.

5.12 Does PPRV Pose a Risk to the European Union?

PPRV has been reported in Turkey since 1996 (Ozkul et al. 2002). The presence and continued outbreaks of PPRV in Turkey have enhanced the profile of the virus in the scientific community, especially following the eradication of RPV. PPRV continues to be reported in Turkey with extensive outbreaks in western Turkey being reported from Bursa province (Yesilbag et al. 2005) and Mugla and Aydin provinces (Toplu 2004). Extensive outbreaks of PPRV were documented in Turkey during 2005 where both quarantine and vaccination efforts were coordinated to contain the disease. During the 12-month period of 2011, over 200 outbreaks were reported to the OIE with the vaccination of more than 13 million small ruminants being undertaken to try and limit the continued spread of disease (WAHID, www.oie.int). Certainly with disease outbreaks of this extent and the presence of PPRV in both Anatolia and Thrace, the threat of PPRV to neighbouring countries within Eastern Europe remains high. All Turkish isolates of PPRV that have been genetically typed to date fall within lineage IV.

Alongside the threat of PPRV spreading from Turkey into the European Union, the emergence of PPRV across North Africa represents a further potential avenue of disease transmission into the European Union via trade route through Morocco into Southern Spain. The trade of both sheep and goats across the Mediterranean Sea is of importance, and as such, it is of great importance that the regions involved are aware of the risk of importation of PPRV and the consequences should importation occur (Minet et al. 2009).

5.13 Conclusions

PPRV continues to decimate small ruminant populations across much of the developing world. Furthermore, the virus continues to be reported in areas where it has not previously detected and as such is considered by some to be an emerging pathogen. However, the increased detection of the virus in the light of rinderpest eradication and the lack of awareness of the disease in areas where the virus has been detected for the first time in recent years must also contribute to this emergence. From a molecular perspective, clearly the paucity of genetic data currently available suggest that increased molecular investigations are required to truly define the genetic variation within outbreaks and following infection of different species.

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Chapter 6
Peste des Petits Ruminants in Unusual Hosts: Epidemiology, Disease, and Impact on Eradication

P. Wohlsein and R.P. Singh

Abstract Peste des petits ruminants (PPR) is primarily a highly contagious morbillivirus infection of small domesticated ruminants. However, it can also infect and cause disease in buffaloes, camels, and a variety of wild ungulates. In camels, it can cause serious disease, although experimental infection often failed to reproduce the natural situation. Clinical disease in wildlife has been observed in various locations of the Arabian Peninsula and in Asia. In numerous cases, there is epidemiological evidence of a spillover of PPR virus from sheep or goats to unusual hosts. Whilst there is clear evidence that a number of wildlife species are susceptible to infection, the actual role of wildlife in the epidemiology of PPR virus remains largely undetermined. Although an enzootic occurrence of PPR in unusual hosts has not been observed up to now, the epidemiological role of these hosts including wildlife populations should not be underestimated and is therefore important in PPR control and final eradication for reaching to PPR zero level.

6.1 Introduction

Peste des petits ruminants (PPR) virus is regarded as the fourth member of the genus *Morbillivirus* in the family *Paramyxoviridae* (Gibbs et al. 1979) in the order Mononegavirales (Tober et al. 1998). Other members of the genus morbillivirus include rinderpest virus (RPV), which has now been eradicated globally, measles virus (MV) of humans, canine distemper virus (CDV), feline morbillivirus (Woo et al. 2012), and

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several morbilliviruses of marine mammals (de Swart et al. 1995). The morbilliviruses are pleomorphic in nature having a lipoprotein membrane enveloping a ribonucleoprotein core, which contains the RNA genome (Haffar et al. 1999). The genome is a single-stranded RNA, about 16 kilobases (Kb) long with negative polarity (Haas et al. 1995). The genome is divided into six transcriptional units encoding two non-structural proteins (V, C) and six structural proteins, i.e. the surface glycoproteins which include the fusion (F) and haemagglutinin (H) proteins, the matrix (M) protein, the nucleoprotein (N), and the phosphoprotein (P) which forms the polymerase complex in association with the large (L) protein (Diallo et al. 1994). The genome sequences of nine isolates of PPR viruses have been fully elucidated. They include one isolate from lineage 1 (Côte-d´Ivoire/89) two isolates from lineage 2 (Nigeria/76/1 and Nigeria/75/1), one isolate from lineage 3 (Ethiopia/94), five isolates from lineage 4 (Turkey/2000, Tibet/30/2007, Tibet/2007, Tibet/Bharal/2008, and Morocco/2008). Furthermore, a nearly complete genome sequence of an Indian vaccine strain (Sungri/96) is available. Interestingly, out of the nine viruses, one of the isolates from a wild Bharal has been sequenced completely (Bao et al. 2012).

The surface glycoproteins, the H and F proteins, mediate virus attachment and penetration of susceptible host cells (Scheid et al. 1972). Antibodies to these surface proteins probably play a key role in the development of a protective immunity against morbilliviruses (Norrby et al. 1986). The M protein of paramyxoviruses forms an inner coat to the viral envelope and thus serves as a bridge between the surface viral glycoproteins and the ribonucleoprotein core. By virtue of its position, the M protein appears to play a central role in the formation of new virions, which are liberated from the infected cells by budding. The N protein is the most abundant viral protein and represents the major component of the nucleocapsid core. It is believed to play a major role in virus transcription and replication (Kingsbury 1990). Due to its abundance, there is a great interest in utilizing N protein and the N gene for the diagnosis of a morbillivirus infection in a host. Monoclonal antibodies specific to N protein of PPR virus have been used for the detection of viral antigens (Libeau and Lefevre 1990; Singh et al. 2004a).

The P gene of the morbilliviruses is expressed to give rise to three protein products. The full-length mRNA is translated to give the P protein. A smaller polypeptide, called C protein, is encoded in an alternative open reading frame (ORF). The V protein is encoded by a mRNA that differs from the primary transcript by having an additional non-templated G residue (Haas et al. 1995) (see Chap. 2).

6.2 Epidemiology and Geographic Distribution of Peste des Petits Ruminants

PPR virus is the causative agent of an acute highly contagious viral disease of small ruminants, primarily domesticated goats and sheep, which are highly susceptible between the ages of 4 months and 1 year. The disease is widespread in western,

central, eastern and northern Africa, the Near and Middle East, Arabian Peninsula, and wide parts of Asia (Banyard et al. 2010; Munir et al. 2013). More than 60 % of the global domestic small ruminant population is considered to be at risk to get infected with PPR virus. The definite host range of PPR virus is still unknown particularly with respect to wild animals, because there is very limited information on species susceptibility and the occurrence of disease. Due to some recent outbreaks in wild ruminants, PPR has to be considered as an emerging viral disease in non-domesticated species. However, it is sometimes difficult to detect illness and mortality in free-living wild animal populations, and therefore, it is highly likely that many potential PPR cases might have remained undetected.

Before rinderpest eradication, the epidemiology of PPR was more complicated by the fact that goats and sheep are also susceptible to rinderpest virus, which is antigenically very similar to PPR virus (Forsyth and Barrett 1995). Both rinderpest and PPR have been reported to be endemic in the same geographical areas of Africa, Asia, and Middle East. Nowadays, large-scale vaccination programmes are being established to control PPR outbreaks in several countries, both in Asia and Africa, where vaccine is available with the help of national or international agencies such as the World Organisation for Animal Health (OIE) (Singh 2011; Office International des Epizooties 2012). Organized mass vaccination campaigns will further change the epidemiology of PPR disease in endemic regions.

Phylogenetic relationship of PPR virus isolates from different geographical areas of the world have been determined, and four genetically distinct lineages (namely, 1–4) of PPRV have been recognized based on a partial analysis of the fusion (F) protein (Dhar et al. 2002; Özkul et al. 2002) and nucleoprotein (N) genes (Kwiatek et al. 2007) that have a specific geographic distribution (Fig. 6.1). Four distinct groups, three in Africa and one in Asia, were identified. These include lineage 1 in West Africa, lineage 2 in Nigeria and Cameroon, lineage 3 in East Africa, as well as lineage 4 in Asia. Based on the F gene sequence, there is a solitary report of lineage 3 PPR virus (India/TN/92) from southern India (Shaila et al. 1996). There have been no further reports of lineage 3 virus from India after this occasion. Recent reports indicate that lineage 4 viruses have been spread fast touching Morocco and Tibet (Wang et al. 2009; Kwiatek et al. 2011).

In spite of genetic variation, there is only one serotype of the PPR virus. This affords the opportunity for the use of a single vaccine to control PPR without any restriction, provided national authorities have no objection on the use of different lineages of vaccine viruses. Based on available literature, it is obvious that the virus tends to spread more often to new areas in neighbouring regions (Banyard et al. 2010). The genetic relatedness of lineage 4 virus isolated from wild animals with recently characterized Chinese isolates provided clues that importation of domesticated or wild small ruminants may play a role in transmission of the disease from Asia to the UAE. Alternatively, based on current reports, it may be that lineage 4 is now overwhelming the other lineages (Albina et al. 2013; Munir 2014).

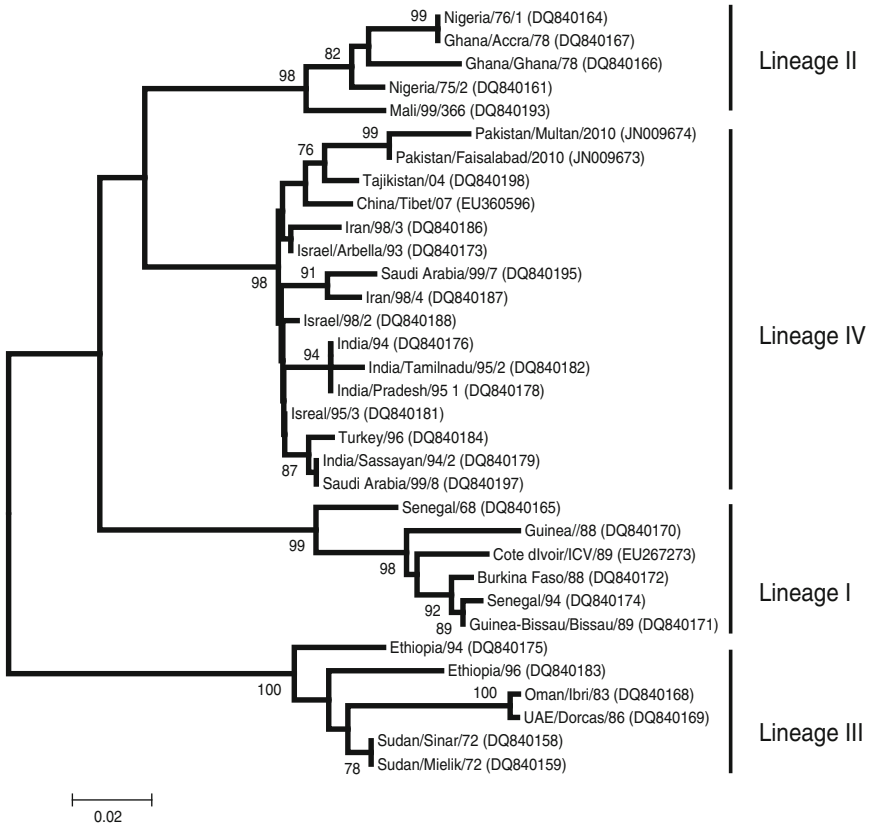

Fig. 6.1 Phylogenetic tree of PPR virus isolates from different parts of the world based on part of F gene sequence analysis

6.3 Hosts of Peste des Petits Ruminants Virus

PPR virus is highly adapted to goats and sheep (Wohlsein and Saliki 2006). Asides these domesticated small ruminants, the full host range is unknown (Barrett 1994). Particularly, taxonomically related species are susceptible to natural or experimental infections resulting in a range of symptoms from subclinical course to fatal disorders. Natural transmission to domesticated large ruminants and disease signs of PPR has not been reported except for domesticated Asian water buffaloes, synonymous to Indian buffalos (*Bubalus bubalis*) (Govindarajan et al. 1997). Domesticated cattle from various African countries were shown to be infected naturally as demonstrated by the presence of antibodies without showing overt disease (Anderson and McKay 1994; Haroun et al. 2002). Cattle experimentally inoculated with PPR virus or infected by in-contact goats with clinical PPR exhibit no disease. However, they develop antibodies against the virus which are protective against

rinderpest (Mornet et al. 1956; Dardiri et al. 1976; Gibbs et al. 1979) and they excrete viral antigen in their lachrymal secretions (Nanda et al. 1996). Domesticated and wild African buffaloes (*Syncerus caffer*) do not show overt clinical signs, but may seroconvert in about 10 % in regions where PPR is enzootic (Couacy-Hymann et al. 2005). Pigs experimentally inoculated with PPR virus or infected by in-contact goats with clinical PPR show only seroconversion. Virus transmission neither to goats nor to pigs was observed (Nawathe and Taylor 1979). These findings indicate that cattle and pigs represent dead-end hosts for PPR virus.

The susceptibility of single-humped camels (*Camelus dromedarius*) to PPR virus infection has been established through various serological surveys in different African and Near East Asian countries including Ethiopia, Nigeria, Sudan, Egypt, and Turkey (Ismail et al. 1992; Roger et al. 2001; Haroun et al. 2002; Abraham et al. 2005; Abubakar et al. 2008; Albayrak and Gür 2010; Saeed et al. 2010). However, camels may also show overt disease after natural infection (Roger et al. 2000; Khalafalla et al. 2010; Kwiatek et al. 2011) or mild clinical signs subsequent to experimental inoculation (El-Hakim 2006).

PPR can also affect wild ungulates of the family *Bovidae* including members of the subfamilies *Caprinae*, *Bovinae*, *Antilopinae*, *Hippotraginae*, *Cephalophinae*, *Reduncinae,* and *Aepycerotinae* as well as of the family *Cervidae* including *Capreolinae*. However, there is very limited information on species susceptibility and the occurrence of disease. Phylogenetic analysis has shown that all PPR virus isolates from wild ungulates belong to lineage 4 (Munir 2014). Although in relation to the population biology and densities, numerous species of African, Arabian, and Asian wildlife species are potential candidates for natural PPR virus infections (Anderson 1995), only a limited number of natural PPR virus infections in wild ruminants have been recorded in areas corresponding to the geographic distribution of PPR in domesticated goats and sheep.

The species, the area, and the results of serological and virological investigations are summarized in Table 6.1. However, it is interesting that no cases of clinical PPR have been recorded amongst wild ruminants in West African wildlife. In some species, there is only serological evidence of natural PPR virus infection including African grey duiker (*Sylvicapra grimma*), Nilgai antelope (*Boselaphus tragocamelus*), Defassa waterbuck (*Kobus defassa*), buffaloe (*Syncerus caffer*), and goitered gazelle (*Gazella subgutturosa subgutturosa*). In Bubal hartebeest (*Alcelaphus buselaphus*) and kob (*Kobus kob*), PPR virus-specific genome fragments have been identified. Other ruminant species, particularly gazelles, appear highly susceptible to PPR and show overt clinical signs. These species include Dorcas gazelle (*Gazella dorcas*), Rheem gazelle (*Gazella subgutturosa marica*), Arabian mountain gazelle (*Gazella gazella cora*), Arabian gazelle (*Gazella gazella*), Thomson's gazelle (*Eudorcas thomsonii*), gemsbok (*Oryx gazella*), Laristan sheep (*Ovis gmelini laristanica*), Nubian ibex (*Capra nubiana*), bushbuck (*Tragelaphus scriptus*), impala (*Aepyceros melampus*), springbuck (*Antidorcas marsupialis*), Barbary sheep (*Ammotragus lervia*), Afghan Markhor goat (*Capra falconeri*), Bharal or Himalayan blue sheep (*Pseudois nayaur*), Sindh ibex (*Capra aegagrus blythi*), and the wild goat (*Capra aegagrus*) (Munir 2014; Munir et al. 2013). According to the reported

Table 6.1 Natural infection of PPRV confirmed by serology and/or virus isolation in wildlife species of different countries

Area and illustration of affected species	Species (common name)	Species (taxonomic name)	Serology	Virus identification	Reference
Africa 	African Grey duiker	*Sylvicapra grimmia*	+	n.d.	Ogunsanmi et al. 2003
	Bubal hartebeest	*Alcelaphus buselaphus*	−	yes	Couacy-Hymann et al. (2005)
	Buffaloe	*Syncerus caffer*	+	yes	Couacy-Hymann et al. (2005)
	Defassa waterbuck	*Kobus defassa*	+	yes	Couacy-Hymann et al. (2005)
	Kob	*Kobus kob*	−	yes	Couacy-Hymann et al. (2005)

(continued)

Table 6.1 (continued)

Area and illustration of affected species	Species (common name)	Species (taxonomic name)	Serology	Virus identification	Reference
Arabian Peninsula 	Nilgai	*Boselaphus tragocamelus*	+	n.d.	Furley et al. (1987)
	Laristan sheep	*Ovis gmelini laristanica*	n.d.	yes	Furley et al. (1987)
	Nubian Ibex	*Capra nubiana*	n.d.	yes	Furley et al. (1987)
	Gemsbok	*Oryx gazella*	n.d.	yes	Furley et al. (1987)
	Dorcas gazelle	*Gazella dorcas*	+	yes	Furley et al. (1987) Abu Elzein et al. (2004)
	Thompson's gazelle	*Eudorcas thomsonii*	n.d.	yes	Abu Elzein et al. (2004)
	Springbuck	*Antidorcas marsupialis*	n.d.	yes	Kinne et al. (2010)
	Arabian mountain gazelle	*Gazella gazella cora*	n.d. −	yes	Kinne et al. (2010) Sharawi et al. (2010)
	Arabian gazelle	*Gazella gazella*	n.d.	yes	Kinne et al. (2010)
	Rheem gazelle	*Gazella subgutto-rosa marica*	n.d.	yes	Kinne et al. (2010)
	Barbary sheep	*Ammotragus lervia*	n.d.	yes	Kinne et al. (2010)
	Bushbuck	*Tragelaphus scriptus*	n.d.	yes	Kinne et al. (2010)
	Impala	*Aepyceros melampus*	n.d.	yes	Kinne et al. (2010)
	Afghan Mark-hor goat	*Capra falconeri*	n.d	yes	Kinne et al. (2010)

(continued)

Table 6.1 (continued)

Area and illustration of affected species	Species (common name)	Species (taxonomic name)	Serology	Virus identification	Reference
Asia 	Indian buffalo	*Bubalus bubalis*	n.d.	yes	Govindrajan et al. Govindarajan et al. (1997)
	Goitered gazelle	*Gazella subguttu-rosa subgutturosa*	+	no	Gür and Albayrak (2010)
	Bharal	*Pseudois nayaur*	+	yes	Bao et al. (2011)
	Sindh Ibex	*Capra aegagrus blythi*	n.d.	yes	Abubakar et al. (2011)
	Wild goat	*Capra aegagrus*	n.d.	yes	Hoffmann et al. (2011)
	Asiatic lion	*Panthera leo persica*	n.d.	yes	Balamurugan et al. (2012.)

n.d. = not done
+ = demonstration of PPRV-specific antibodies
− = demonstration of PPRV-specific antibodies failed

circumstances of the outbreak in free-living Bharals, also Mongolian gazelles (*Procapra gutturosa*) and Tibetan antelopes (*Pantholops hodgsoni*) were possibly infected with PPR virus and succumbed to the disease (Bao et al. 2011).

Experimental infection of the American white-tailed deer (*Odocoileus virginianus*) with PPR virus resulted in seroconversion and clinical disease (Hamdy and Dardiri 1976). Infection of non-ruminant wild species with PPRV was shown experimentally in peridomestic rats but without evidence of virus spread and clinical disease (Komolafe et al. 1987). Furthermore, PPR virus was detected in tissues from an Asiatic lion (*Panthera leo persica*) that had died of trypanosomiasis (Balamurugan et al. 2012).

6.4 Sero-epidemiology of Peste des Petits Ruminants

Seroprevalence and sero-epidemiology is one of the important indicators for judging the presence and the endemic nature of any infectious disease agent. Prevalence of PPR virus antibodies has been reported in several species of animals other than small ruminants. This information provides basic input for launching any disease control programme and their final eradication from a defined geographic area. Several factors including vaccination campaigns, type of animal husbandry practices, and geography of the place may affect the epidemiology of a contagious disease such as PPR. Information on sero-epidemiology and seroprevalence of PPR virus for different countries is known during recent studies using state-of-the-art diagnostic techniques (Singh et al. 2004b; Libeau et al. 1995). Most of these reports indicate an overall seroprevalence of around 30–40 %, showing that about one-third of the small ruminants had been infected and recovered (Singh et al. 2004c). There has been no organized investigation on the prevalence of PPR antibodies in wild animals except solitary reports of virus isolation and presence of antibodies as given in Table 6.1. Findings from several wildlife species indicate that the virus replicates to some extent in these animals and also produces clinical disease. Therefore, these animal species are important for disease eradication at local as well as at global level.

6.5 Clinical Disease of Peste des Petits Ruminants in Unusual Hosts

Clinical signs of PPR in unusual hosts share common features with the disease in sheep and goats (Wohlsein and Saliki 2006). However, there is variation of the outcome of PPRV infection of hitherto unknown reasons (Wernery 2011). Possible factors influencing the clinical course include the virulence of the respective strain and the species and individual susceptibility. In addition, age, season, immune status of the host, concurrent infection(s), stress, and previously existing parasitism have to be considered (Munir et al. 2013).

Domesticated Indian water buffaloes (*Bubalus bubalis*) show fever, congested conjunctivae, profuse salivation, and depression. The case fatality rate adds up to 96 %, and mortalities are not age-related. Deaths have been preferentially observed in females (Govindarajan et al. 1997). Experimental infection in Indian Murrah calves results only in a temporary febrile response without other clinical signs (Govindarajan et al. 1997).

The clinical disease in dromedary camels (*Camelus dromedarius*) appears to be variable, possibly depending on the strain involved. There are reports about a spontaneous contagious acute respiratory disease characterized by respiratory distress and abdominal breathing (therefore originally termed "camel contagious pneumonia" or "trekking fever"), lacrimation, fever, and finally depression and recumbency with a high morbidity rate of over 90 % and a mortality rate of about 5 % in which lineages 2 and 3 PPR virus strain were involved (Roger et al. 2000; Kwiatek et al. 2011). *Streptococcus equi* ssp. *equi* and *Mannheimia haemolytica* have been also isolated from affected camels, most likely as secondary pathogens. In a different outbreak of PPR caused by a strain belonging to lineage 4, the disease was characterized by sudden death of apparently healthy animals. Affected animals showed yellowish to bloody diarrhoea and abortion. Additional findings included subcutaneous oedema and submandibular swelling, chest pain, and seldom coughing. There was decreased milk yield, weight loss, and increased water consumption in some cases which persisted up to two weeks. The mortality rate ranged between 0 and 50 % and all age, sex, and breed groups were affected. However, adult animals, mostly recently delivered and pregnant females died preferentially (Khalafalla et al. 2010).

After experimental infection of dromedary camels in Saudi Arabia and Dubai with PPR virus, only subclinical infections or mild respiratory disease with coughing, nasal discharge, and fever were observed, although PPR virus was transmitted to other camels and goats but not to sheep (El-Hakim 2006). However, an inoculation of a camel bull with a lineage 4 strain did neither cause clinical signs nor seroconversion (Wernery 2011).

Descriptions of clinical disease in wild ungulates are rare, because it is difficult to detect illness in free-living wild animal populations and animals are often found dead. Therefore, many potential PPR cases might have been undetected.

Naturally, PPR virus-infected Dorcas (*Gazella dorcas*) and Thomson's gazelles (*Eudorcas thomsonii*) show after an incubation period of 3–4 days (Munir et al. 2013) a peracute to acute clinical course, in contrast to the subacute reactions observed in gazelles infected with rinderpest (Scott 1981). They exhibit initially anorexia and dullness followed by fever up to 41.5 °C. Additional signs include lacrimation with encrustation of the medial canthus, congestion of mucous membranes, mucosal defects of the lips, oral papillae soft and hard palates, nasal discharge with crusts present around the nostrils causing sneezing, deep breathing, profuse salivation, smacking the lips, and often blood-stained, dark, and putrid diarrhoea. Finally, the animals become recumbent and hypothermic, tremble and die within 2–3 days after the first symptoms. Irrespective of the age group, the case fatality rate is high up to 100 %, indicating that gazelles seem to be the most susceptible wild ruminant species for PPR virus (Furley et al. 1987; Hafez et al., 1987; Abu Elzein et al. 2004; Sharawi et al. 2010). A peracute to acute

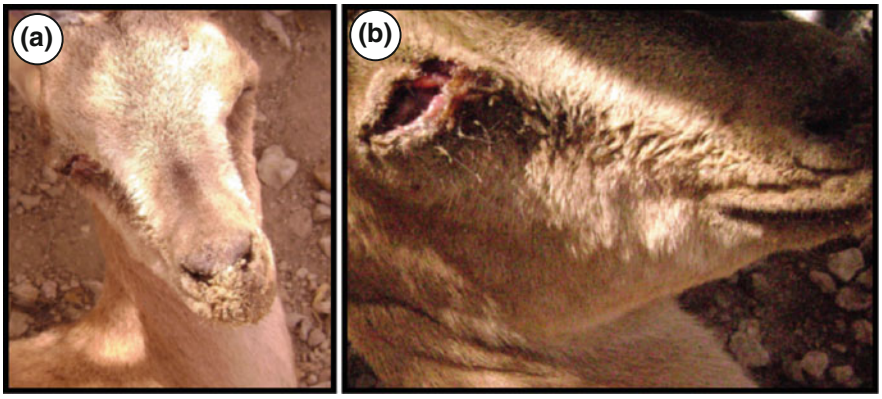

Fig. 6.2 Sindh Ibex (*Capra aegagrus blythi*) in Pakistan with clinical PPR; **a** severe crusting around the mouth and nostrils; **b** severe muco-purulent conjunctivitis with ocular discharge (both figures were kindly provided by Dr. Muhammad Abubakar, Islamabad, Pakistan)

course with diarrhoea is also reported for gemsbok (Furley et al. 1987). Clinico-pathological investigations in gazelles with PPR show haemoconcentration, increased red blood cell count, and lymphopenia (Furley et al. 1987; Abu Elzein et al. 2004).

PPR in Sindh ibex (*Capra aegagrus blythi*) is characterized by fever, anorexia, lassitude, diarrhoea, and emaciation. Additionally, muco-purulent nasal crusting over and around the nostrils and conjunctivitis with ocular discharge are observed (Fig. 6.2a, b). The gums are covered with cheesy necrotic material. Deaths occur in young and adult animals (Abubakar et al. 2011). Bharals (*Pseudois nayaur*) infected with a lineage 4 PPR virus strain show muco-purulent ocular and nasal discharge, diarrhoea, and lameness (Bao et al. 2011). Adult wild goats (*Capra aegagrus*) infected with a PPR virus strain of the same lineage suffer from emaciation, dehydration, weakness, ataxia, muco-purulent nasal discharge, ulcerative keratitis, and conjunctivitis and die in huge numbers (Hoffmann et al. 2011).

Experimental infection of American white-tailed deer (*Odocoilus virgianus*) with PPR virus causes viraemia with a subsequent subclinical course or overt disease with fever, muco-purulent nasal discharge, conjunctivitis, erosive stomatitis, and diarrhoea at a later stage followed by deaths (Hamdy and Dardiri 1976).

6.6 Pathology in Unusual Hosts

6.6.1 Macroscopical Findings

The predominant and most consistently observed morphological finding in PPR virus-infected African and Arabian unusual hosts represents gastroenteritis. In contrast, in wild Asian ungulates, the pulmonary affection predominates. Erosions and ulcerations of the upper digestive tract occur not regularly.

In Indian water buffaloes (*Bubalus bubalis*), gross findings of PPR consist of abomasitis with mural oedema and haemorrhagic gastroenteritis, involving all segments of the intestine (Govindarajan et al. 1997).

Dromedary camels (*Camelus dromedarius*) infected with lineage 4 PPR virus show pulmonary congestion and consolidation mostly of the apical lobes indicative of pneumonia. Lymph nodes are enlarged and inflamed. Additionally, there is gastroenteritis with haemorrhages. The liver is light brown and fragile. Infrequently, swollen lips and haemorrhagic ulcers are observed on the tongue (Khalafalla et al. 2010).

The gross pathology of PPR in Dorcas gazelles (*Gazella dorcas*) has been described in detail according to the stage of the disease (Furley et al. 1987). Animals that died peracutely display severe abomasitis without inflammation of the intestine, but liquid content in the rectum. In more advanced cases, additional inflammation of the intestines and congestion of the ruminal mucosa are observed. Furthermore, linear haemorrhages (so-called zebra or tiger striping) of the rectal and colonic mucosa that represent terminal changes of no diagnostic significance (Roth and King 1982) and erosions of the ruminal mucosa and tongue may occur. These findings were also observed in various wild ruminant species that suffered from PPR in an outbreak in the United Arab Emirates, in which some of the animals showed also suppurative pneumonia, but only few displayed erosive defects of the oral mucosa (Fig. 6.3a; Kinne et al. 2010). Rarely, fibrinous pleuritis and peritonitis, haemorrhages in the spleen, and erosions on the lips and tongue are found. Lesions of the tongue begin as multifocal erosions on the left and right side of the lingual dorsum that progress to ulcerations and extent to affect the whole dorsum. Occasionally, erosions are present on the soft palate. Additional findings in gazelles include thick mucoid deposits on the mucosal surface of the oesophagus, empty rumen with congested papillae, haemorrhagic abomasitis with marked congestion and oedema of the pyloric region, shallow Peyer's patches—interpreted as

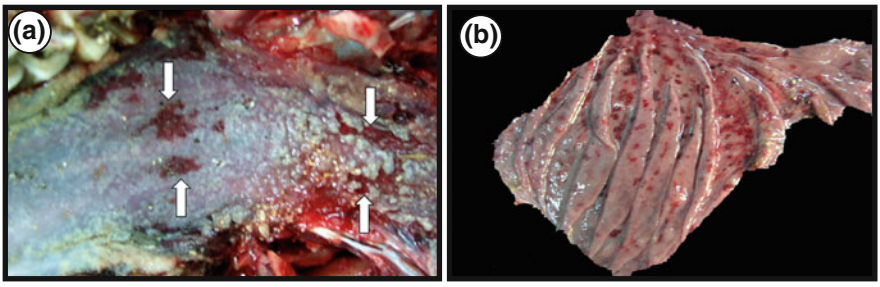

Fig. 6.3 **a** Gazelle (*Gazella subgutturosa subgutturosa*) with erosive to ulcerative lesions in the oropharyngeal mucosa (*arrows*); **b** Laristan sheep (*Ovis gmelini laristanica*) with abomasitis and petechial to ecchymal haemorrhages in the abomasal mucosa (both figures were kindly provided by Dr. J. Kinne, Dubai, UAE)

depletion—with haemorrhagic rims, erosions throughout the gut, congestion of lung, liver, kidney, pancreas and brain, pulmonary oedema, and inactive small lymph nodes and spleen (Abu Elzein et al. 2004).

In gemsbok jaundice, focal fibrinous pleuritis, mottled liver, and a distended gall bladder are described, but the intestine lacks inflammatory changes (Furley et al. 1987).

Laristan sheep (*Ovis gmelini laristanica*) show abomaso-enteritis with pin-point petechial haemorrhages in the abomasal mucosa (Fig. 6.3b) and with zebra striping in the small intestine. The large intestine lacks inflammation; however, it contains liquid content. The ruminal content is also liquid, and focal erosions occur on the lingual dorsum associated with enlarged retropharyngeal lymph nodes (Furley et al. 1987).

In Sindh ibex (*Capra aegagrus blythi*), major changes consist of discoloration and haemorrhagic patches on the lung, splenomegaly, and a friable liver (Abubakar et al. 2011). Bharals (*Pseudois nayaur*; Bao et al. 2011) and wild goats (*Capra aegagrus*; Hoffmann et al. 2011) show pneumonia with caseous necrosis. In addition, hyperaemia of the rectal mucosa and gall bladder enlargement (Bao et al. 2011) as well as striated sub-epithelial haemorrhages in the airways, dilated left cardiac ventricle, enlarged intestinal lymph nodes, and ulcerative kerato-conjunctivitis are recorded (Hoffmann et al. 2011).

Experimental infection of American white-tailed deer (*Odocoilus virgianus*) results in necrotizing stomatitis, abomasal congestion, bronchopneumonia, subendocardial haemorrhages, and catarrhal enteritis (Hamdy and Dardiri 1976).

6.6.2 Histological and Immunohistological Findings

Descriptions on histological lesions of PPR in unusual hosts are very scarce, because of missing sampling due to poor preservation of tissues.

In dromedary camels (*Camelus dromedarius*), histopathology reveals degeneration and denudation of the bronchiolar epithelium and peribronchial mononuclear cell infiltration. The alveolar septa are distended due to congestion and infiltration of mononuclear cells. In addition, pulmonary oedema and emphysema are recorded. Lymph nodes display lymphocytic depletion (Khalafalla et al. 2010).

In wild ungulates with PPR, necrotizing to haemorrhagic enteritis/colitis (Fig. 6.4a), lymphoid necrosis and depletion of the gut-associated lymphoid tissue are observed. Spleen and body lymph nodes display lymphoid depletion and necrosis. In the lung, subacute broncho-interstitial pneumonia with multinucleated syncytial cells, hyperplasia of type II pneumocytes, and bronchiolar epithelial cells is recorded. Occasionally, suppurative to fibrino-purulent bronchopneumonia is found most likely caused by secondary bacterial pathogens. The liver displays multifocal hepatocellular coagulation necroses with infiltration of macrophages. In gastrointestinal epithelial cells, macrophages/reticular cells of lymphoid tissues, bronchial and bronchiolar epithelial cells, syncytial cells, and biliary epithelial cells

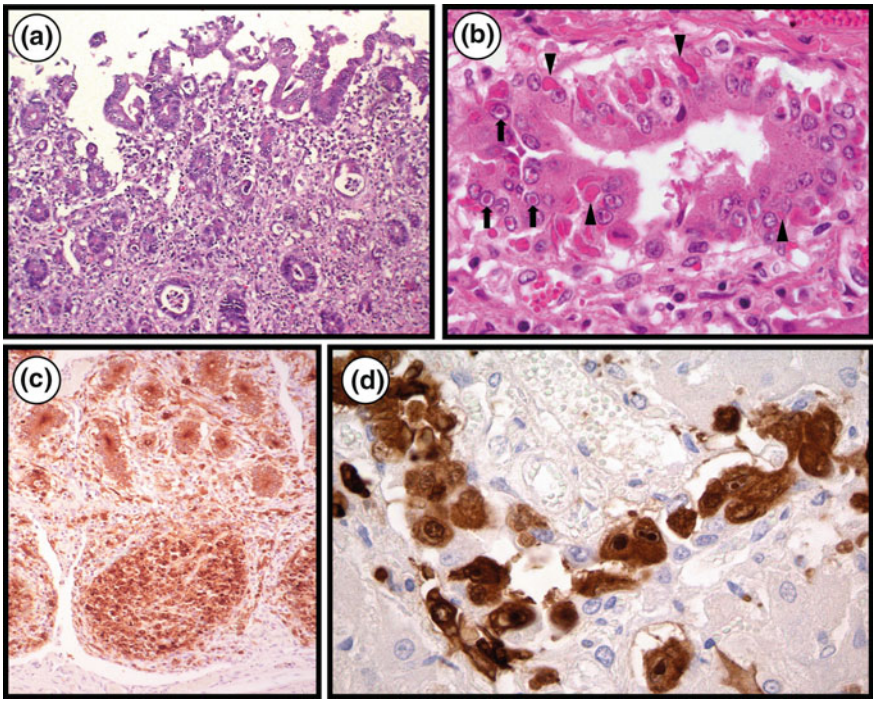

Fig. 6.4 **a** Laristan sheep (*Ovis gmelini laristanica*); intestine with erosive enteritis and dilated crypts; haematoxylin–eosin stain, magnification 200x; **b** Afghan Markhor goat (*Capra falconeri*); liver with numerous nuclear (*arrows*) and cytoplasmic (*arrowheads*) eosinophilic viral inclusion bodies; haematoxylin–eosin stain, magnification 400x; **c** Laristan sheep (*Ovis gmelini laristanica*); immunohistochemical demonstration of morbillivirus antigen in intestinal epithelial cells and cells of gut-associated lymphoid follicles; avidin–biotin–peroxidase complex method, magnification 100x; **d** Afghan Markhor goat (*Capra falconeri*); immunohistochemical demonstration of morbillivirus antigen in the cytoplasm and nucleus of bile duct epithelia; avidin–biotin–peroxidase complex method, magnification 400x

(Fig. 6.4b), eosinophilic cytoplasmic and nuclear Inclusion bodies can be detected. Pneumonia, necrosis, and depletion of lymphatic tissues belong to the spectrum of lesions commonly observed in PPR of small domesticated ruminants (Wohlsein and Saliki 2006).

Indirect immunofluorescence with cross-reacting antibodies to rinderpest virus revealed the presence of morbillivirus antigen in touch smears from spleen and lymph nodes of Indian buffaloes hit by PPR (Govindarajan et al. 1997). In Arabian wild ungulates, morbillivirus antigen is detectable using immunohistochemistry in the cytoplasm and nucleus of intestinal epithelial cells (Fig. 6.4c), bronchial and bronchiolar epithelial cells, syncytial cells, bile duct epithelia (Fig. 6.4d), hepatocytes, and cells of intestinal lymphoid follicles (Kinne et al. 2010) indicating anepithelio- and lymphotropism of PPR virus.

6.6.3 Differential Diagnoses

Rinderpest, which has been eradicated globally now, could cause clinical disease in small ruminants (Anderson et al. 1990; Couacy-Hymann et al. 1995; Rossiter 2001). Therefore, it cannot be excluded that rinderpest in wildlife might have also caused a clinical disease similar to PPR that has been misdiagnosed. In addition, contagious caprine pleuropneumonia (lack of oral lesions), bluetongue (usually lack of diarrhoea), pasteurellosis (lack of oral lesions), contagious ecthyma (lack of pulmonary lesions), foot and mouth disease (lack of pulmonary lesions), sheep and goatpox (cause typical pox-like cutaneous lesions), mycoplasma infections (lack of oral lesions), heartwater (lack of oral lesions), coccidiosis (lack of oral lesions), and mineral poisoning (lack of oral lesions) have to be considered (Rossiter 2004; Office International des Epizooties 2009; Baron et al. 2011). Definitive diagnosis of PPR is possible with the use of laboratory-specific PPR diagnostic kits (Libeau et al. 1994; Singh et al. 2004a).

6.7 Strategy for Serosurveillance of Peste des Petits Ruminants in Wildlife Populations

Serological surveys provide crucial information on the possible presence of any disease in unusual hosts, because information on active disease presence may not be available due to poor accessibility of these animals. However, primary hosts such as sheep and goats are important for routine survey. In addition to the primary hosts, other species of domesticated ruminants such as cattle, buffalos, camels, and wild ruminants and non-ruminant species need to be included in the survey process in the final stage of an eradication programme based on sample availability from different sources, i.e. zoo authorities, samples obtained for screening of other disease from species reported for PPR antigen and antibodies. Given the scope of PPR in wild ungulates that are widespread in many countries, current disease surveillance efforts are inadequate and warrant additional investment (Albina et al. 2013; Munir 2014).

6.7.1 Disease Situation in Peste des Petits Ruminants Endemic Countries

Disease pattern from various Asian and African countries which report outbreaks regularly is available on the Website of World Organization of Animal Health (http://www.oie.int). Analysis of the pattern of PPR disease outbreaks in different countries shows that some of the countries have taken comprehensive steps to control PPR using the vaccines developed from both, lineage 2 and lineage 4

viruses originating from Nigeria (Nigeria75/1 strain) and India (PPRV/Sungri 96), respectively (Diallo et al. 1989; Sreenivasa et al. 2000). In India, after the launch of mass vaccination campaigns in the year 2004, a declining trend of PPR outbreaks was recorded after 2005 (Fig. 6.5). The trend of disease outbreaks from Nepal, Nigeria, Oman, and Iran indicates a more severe pattern up to the year 2001 and subsequently a decline in disease outbreaks probably due to mass immunizations (Singh 2011, 2012). All of this information is available from natural hosts only. The disease pattern and disease situation in unusual hosts are available only as isolated/solitary examples. The PPR virus-susceptible wildlife species are widely spread over all PPR endemic regions, especially in most African countries. However, it is of note that PPR is not reported in most of the countries where the wild susceptible populations are largely concentrated. This is primarily due to either lack of an efficient surveillance system to screen the entire population for virus genome detection or inability of wild ruminants to maintain the virus for a longer time (Munir 2014).

However, a decline in the disease pattern of primary hosts will definitely lead to the disappearance of any clinical case in wild animals due to the poor chance of contacts between infected domesticated small ruminants with the wildlife in any niche/epidemic cycle. It is presumed here that PPR virus is not endemic in any

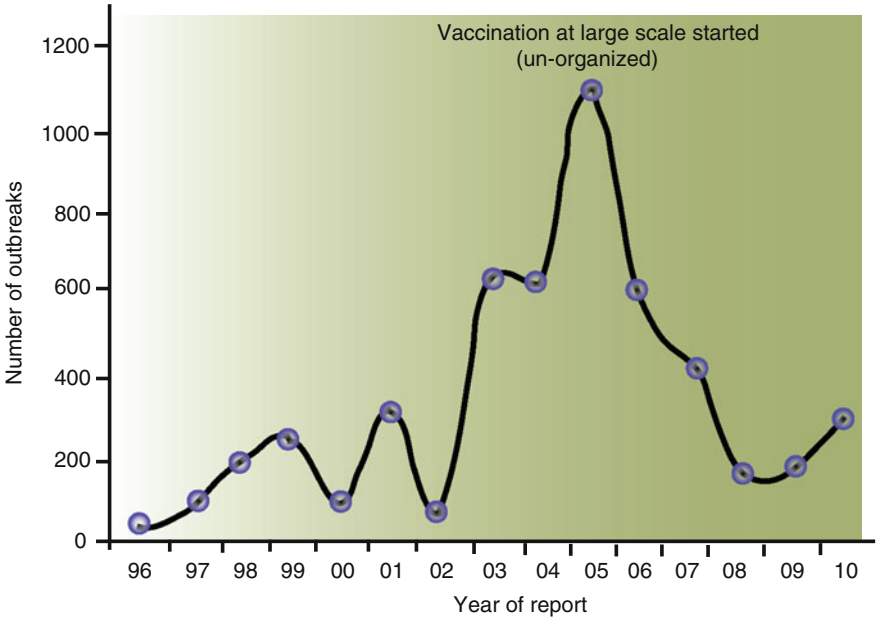

Fig. 6.5 Pattern of PPR virus outbreaks in India between the years 1996 and 2010. Note that number of reported outbreaks increased sharply after the introduction of PPR diagnostic kits between the years 2001/2002 till 2005. Subsequently, number of outbreaks reduced significantly once mass vaccinations were introduced in the year 2004 under a government scheme

wildlife species. Clinical surveillance may be required to establish this fact during the final stage of eradication using a suitable assay (Singh et al. 2004a). A similar effort was made during rinderpest eradication, where a major emphasis was made to vaccinate the primary hosts (cattle and buffalo), followed by a prolonged clinical and serological surveillance of large and small ruminants. These efforts lead to eradication of rinderpest virus, even though wildlife populations were reported to have harboured rinderpest virus with solitary reports (Anonymus 2013).

6.7.2 Sociocultural and Socioeconomic Conditions Influencing Peste des Petits Ruminants Epidemiology

Knowledge of the epidemiology of any contagious disease is essential for the successful implementation of an effective disease control programme. PPR virus is a fragile virus (like rinderpest virus), which requires close contact between animals for disease transmission. Therefore, wild animals, which come in close contact with sheep and goats, will only pick up the infection from the natural host. This is possible from bordering regions of forests, which form a niche between wild and domesticated animals. Important factors influencing disease transmission under these situations are (i) migratory (nomadic) flocks of sheep and goats, which may come in contact with wildlife, (ii) intermixing of domesticated (sheep and goats) animals with semi-domesticated animals which remain in the forest, (iii) grazing grounds in the areas which form a niche for sheep and goats and wild animals, and (iv) any other factor, which increases interaction between domesticated and wild animal populations in any part of the world with high disease intensity.

6.8 Disease Control, Eradication Possibilities, and Role of the Wildlife Population in Peste des Petits Ruminants Disease Control

It seems too early to talk about PPR eradication when there are so frequent outbreaks along with spread of disease in new areas of the world with no lineage restriction, i.e. Asian lineage virus reaching to Africa (Kwiatek et al. 2011). Experiences of global rinderpest eradication over 5 decades with five consecutive large international programmes (Albina et al. 2013) will definitely help to control and eradicate PPR from domesticated and wild animals. Disease control by intensive vaccination using a potent vaccine, which induces long-lasting immunity, will result in a reduced incidence of disease. Repeated vaccinations in combination with clinical surveillance and seromonitoring of vaccinated animals, which was the key to the OIE pathway of rinderpest eradication, should be followed. This must also include clinical surveillance of semi-domesticated ruminant population and

wildlife population to the extent possible following intensive vaccination of sheep and goats. Unlike cattle and buffalos, sheep and goats have high fecundity; therefore, vaccination of new generations of lambs and kids at regular intervals would be required.

The final phase of a PPR eradication programme should focus on detecting any trace of clinical cases of PPR. During this final stage, disease surveillance should be carried out using highly sensitive assays such as PCR-ELISA (Saravanan et al. 2004). During this phase, all the countries have to be vigilant on the situation of their neighbouring countries. This may require creation of immune borders on the national boundaries. Many of these countries may not have prioritized the control and eradication of PPR due to their poor economy and civil unrest. The possible introduction of PPR from neighbouring endemic countries needs to be taken into consideration in any control/eradication campaign, and it should be noted that the global eradication of PPR will only be possible through a massive coordinated campaign in line with the Global Rinderpest Eradication Programme (GREP).

High fecundity of goat and sheep populations and involvement of wildlife populations in the disease epidemiology are the two major hurdles in PPR control (Singh 2011). As per an estimate, about 35–40 % of small ruminants are replaced every year due to high fecundity of these animals and their use as source for meat purposes. Therefore, this population is always available, for vaccinations every year as a susceptible population, unlike the situation in rinderpest, where a very small proportion of cattle and buffalos was replaced due to there longer lifespan and low fecundity and uniparous nature of these animals. The vaccination of free-range domesticated small ruminant populations which come in contact with wild or domesticated animal species is important. Although very potent vaccines are available for disease control, availability of uniform quality of vaccine at livestock owner's door is important. Sometimes, these animals are neither recorded nor have good reach of vaccination teams due to their movement or nomadic nature. In addition to vaccination, effective control of PPR will require quarantine and sanitary measures to be enforced strictly when the animals are transported from one place to another. The effective control and eradication of PPR in a country and maintenance of PPR-free state in any geographical regions will depend on the initiative and interest of neighbouring countries due to porous borders and movement of susceptible wildlife and also domesticated animals across the borders. Sometimes, due to poor national policies and civil unrests, it may not be possible to execute disease control programmes effectively as has been observed with rinderpest in recent past (Singh et al. 2004c; Singh 2011). Under these circumstances, control of PPR may not yield PPR-free state if the neighbouring countries do not show willingness for a similar disease control programme.

Another challenge would be to ensure that there is no PPR in wildlife populations of a country through a stringent surveillance mechanism. Any epidemic cycle or chance of reservoir in such populations may lead to a failure of control and eradication of PPR. In order to culminate the epidemic cycle from all the geographical regions simultaneously, one has to ensure the use of uniform vaccines and diagnostic tests throughout the PPR control programme. Under these circumstances, quality

control of PPR diagnostics for ensuring serosurveillance and seromonitoring is of utmost significance along with the vaccines. Furthermore, it would be advisable to use the vaccine originating from the same lineage (lineage 4 in South Asia and lineages 1, 2, and 3 in African countries), but not based on availability or convenience. Vaccination, seromonitoring, and disease surveillance may require a good logistic system to touch remote areas, especially in the hilly terrains of temperate regions where domesticated small ruminants are in contact with the wildlife population. Given the scope of PPR in wild ungulates that are widespread in many countries, current disease surveillance efforts are inadequate and warrant additional investment.

Furthermore, declaration of a new disease entity in wildlife animals may require the permission of zoo authorities or wildlife authorities sometimes. This may result in under-reporting of these diseases, therefore interfering disease control programmes.

6.9 Concluding Remarks

PPR is a highly infectious morbillivirus infection of domesticated small ruminants causing high morbidity and mortality with possible devastating impact on agricultural communities in developing countries. But it represents also a deadly disease for other artiodactyla including Indian buffaloes, camels, and various species of wildlife ruminants. However, in wild small ruminants, PPR virus infection is not self-sustaining, and most of the epidemics of PPR probably originate from nearby infected domesticated sheep and goats (Munir 2014). In addition, PPR may pose a threat to the survival of endangered species of wild ruminants. However, due to the scarce data on PPR in wild ungulates, the host spectrum of PPR in wild animals still remains to be unfolded. Any additional information on this subject is expected to be of great value regarding the epidemiology of this disease. Outbreaks of PPR in wild animals or in zoological collections could be of considerable significance for virus perpetuation.

With the available vaccines, mass vaccinations can be pursued for disease control and eradication in the primary hosts, i.e. sheep and goats. Development of high flock or herd immunity in primary hosts will automatically curtail the cycle of disease transmission in wild animals, especially small and large ruminants which live in vicinity of domesticated small ruminants. Although mass vaccinations are not possible in wild animal populations, monitoring of these animals for PPR- or PPR-like diseases in captive populations and zoological collections during the final stage of PPR eradication may be necessary to declare a geographical area free from PPR. Similar approaches for rinderpest lead to the disease control and subsequent eradication from a national geographic level to a global level with no much effort to include wild ruminants under the active vaccination and serosurveillance programme. Out of all the species, in which PPR has been reported so far, it is also possible that some of the reports in un-natural hosts especially carnivores and

sometimes buffaloes may be away from the facts, as the laboratories reporting these information are sometimes over-enthusiastic for disease reporting in new species. However, the role of un-natural domesticated and wild hosts can never be ignored during disease control and eradication programme of PPR. It is essential to fully understand the role of wildlife in the spread and potential maintenance of PPR virus in the environment in order to be able to initiate successful control strategies (Baron et al. 2011).

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Chapter 7
Pathology of Peste des Petits Ruminants Virus Infection in Small Ruminants and Concurrent Infections

Oguz Kul, Hasan Tarık Atmaca and Muhammad Munir

Abstract Peste des petits ruminant (PPR) is a systemic viral disease of goats and sheep characterized by gastrointestinal and respiratory system lesions with high rate of mortality. Rhinitis, conjunctivitis, serous-mucopurulent naso-ocular discharge, pneumonia, coughing, dispnea, erosive–ulcerative oral lesions, and diarrhea are the most prominent clinicopathological features of the disease. Histopathologically, pseudomembraneous stomatitis, necrotic tonsillitis, fibrinohemorrhagic enteritis, and proliferative interstitial pneoumonia are seen. Syncytial cells and cytoplasmic and/or nuclear eosinophilic inclusion bodies are considered as pathognomonic. Like the other morbilli viruses, PPR virus can also cause lesions in the kidney, brain, and abomasum. The PPRV tropism can be explained by the mechanism in which PPRV binds to receptors on the cell surface. The PPR in small ruminants often shows co-association with secondary viral, bacterial, and parasitary infections.

7.1 Introduction

Peste des petits ruminants (PPR) is a highly contagious viral disease of goats and sheep that has been widely reported throughout the Middle East, India, and sub-Saharan North Africa during the last 2 decades (Obi et al. 1983; Perl et al. 1994; Alcigir et al. 1996; Amjad et al. 1996; Aruni et al. 1998; Diallo 1988; Ozkul et al. 2002; Taylor et al. 2002; Abu Elzein et al. 2004; Toplu 2004; Yesilbag et al. 2005; Kul et al. 2008). Peste des petits ruminant virus (PPRV) is a member of *Paramyxoviridae* family, *Morbillivirus* genus, and it has a close relationship with rinderpest, measles, canine, and sea mammalian distemper viruses (Plowright 1968; Gibbs et al. 1979;

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Kennedy et al.1989, 1991; Bailey et al. 2005). Rhinitis, conjunctivitis, serous–mucopurulent naso-ocular discharge, pneumonia, coughing, dyspnea, erosive–ulcerative oral lesions, and diarrhea are the most prominent clinical findings of diseased animals (Amjad et al. 1996; Toplu 2004). Erosive–ulcerative stomatitis, catarrhal inflammation and congestion in nasal sinuses and gastrointestinal system, consolidation of antero-ventral lobes of the lungs, and lymph nodes edema are characteristic necropsy findings. Histopathologic changes in PPRV infection includes pseudomembraneous, erosive, and ulcerative stomatitis; necrotic tonsillitis; fibrino-hemorrhagic enteritis; and bronchointerstitial pneumonia (Alcigir et al. 1996; Toplu 2004; Kul et al. 2007; Hammouchi et al. 2012). Syncytial cells in affected oral mucosa and in the lungs, as well as eosinophilic nuclear and cytoplasmic inclusion bodies, especially in the respiratory and/or alimentary tract epithelia, are considered as characteristic histopathology of PPR (Brown et al. 1991; Kumar et al. 2004). Otherwise, recently published reports define non-characteristic pathologic findings of PPR, such as multifocal necrosis in the liver, inclusion bodies in hepatocytes and abomasum epithelia, and unusual immunolocalization of virus in the brain, abomasum, renal pelvis, and heart (Toplu 2004; Kul et al. 2007). PPRV infection may also show urinary and central nervous system involvement resembling to other morbillivirus infections such as canine distemper and distemper of sea mammals (Kennedy et al. 1989, 1991; Taylor et al. 2002; Toplu 2004; Kul et al. 2007). These novel definitions are important, because they are directly related to the clinical course, epidemiology, and pathogenesis of the PPR. Further and detailed field observations are needed to understand the possible changes that take place in the PPR epidemiology and pathogenesis related to its novel urinary and central nervous system involvement.

Peracute PPR may result in high mortality especially among young goats, although the frequency of the disease is higher in older goats (Toplu 2004). Clinic and pathologic findings of PPR has been clearly understood, and there are comprehensive data obtained from both natural and experimental studies (Bundza et al. 1988; Brown et al. 1991; Ugochukwu and Agwu 1991; Alcigir et al. 1996; Amjad et al. 1996; Aruni et al. 1998; Taylor et al. 2002; Abu Elzein et al. 2004; Kumar et al. 2004; Toplu 2004; Gulyaz and Ozkul 2005; Yesilbag et al. 2005; Toplu et al. 2012). But it is not the same for pathogenesis and immunologic studies (Galbraith et al. 2002; Rajak et al. 2005; Emikpe et al. 2010; Jagtap et al. 2012; Chinnakannan et al. 2013). In PPRV infection, virus involvement through central nervous system, kidney, abomasum, skeletal, and heart muscles similar to canine and sea mammalian distemper and human measles have recently been shown (Kennedy et al. 1989, 1991; Galbraith et al. 2002; Kul et al. 2007; Toplu et al. 2012). Tissue tropism of PPRV in different tissues outside of respiratory and alimentary systems can be explained by virus-specific receptors that are expressed on cell membrane (Galbraith et al. 2002).

The other issue that should be clarified is vertical transmission and possible relationship between PPR and abortion. The main transmission route of PPR is horizontal, and widespread PPR outbreaks may occur in a short time. Otherwise, recently published literature indicates that congenital transmission of PPRV is also

possible during PPR outbreaks (Kul et al. 2008). Furthermore, association between PPR and abortion has been increasingly reported (Abu Elzein et al. 2004; Abubakar et al. 2007; Kul et al. 2008). Kulkarni et al. (1996) linked 105 abortions with ongoing PPRV infection in nine different goat flocks. But the results remained inconclusive by the absence of etiologic analyses of aborted fetuses. Similarly, AbuBakar et al. (2007) described 58 abortions in 140 pregnant goats with PPR. In Turkiye, data belonging to congenital transmission of PPRV are reported in concurrent PPRV and pestivirus infections in stillborn twin lambs (Kul et al. 2008). Presence of the both viruses in stillborn lambs and their immunolocalizations around the lesioned liver, lungs, and lymphoid tissues are shown. Thus, PPRV can infect fetuses via vertical route and it can cause abortion. Further works are needed to understand whether congenital infection might occur in PPRV infections (Kul et al. 2012).

7.2 Pathologic Basis of Peste des Petits Ruminants Virus Infection

PPR virus shows lymphotropic and epitheliotropic features like other morbilliviruses. Because of its selective tropism, severe lesions are seen in the organs that are mostly constituted of epithelia and lymphoid tissues such as oral mucosa, tonsils, lungs, intestines, lymph nodes, spleen, and liver (Bundza et al. 1988; Kumar et al. 2004; Toplu 2004; Bailey et al. 2005; Gulyaz and Ozkul 2005; Wohlsein and Saliki 2006; Kul et al. 2007; Atmaca and Kul 2012; Jagtap et al. 2012). PPRV enters the body via respiratory route, and then, its first replication sites are pharyngeal and mandibular lymph nodes and tonsils. Viremia occurs within 2–3 days after this initial virus replication, and then, PPRV disseminates through the spleen, bone marrow, respiratory, and alimentary system (Bundza et al. 1988; Kumar et al. 2004; Rajak et al. 2005; Jagtap et al. 2012).

PPR is considered as the systemic viral disease of sheep and goats. PPR virus has a varying degree of virulence for sheep and goats (Gibbs et al. 1979; Bundza et al. 1988; Gulyaz and Ozkul 2005). In goats, while clinico-pathologic findings show a greater extent and degree, sheep are generally affected mildly (Abu Elzein et al. 2004; Kul et al. 2007; Hammouchi et al. 2012). It is thought that sheep have a genetic resistance to combat clinical and deadly outcome of PPRV infection, but sometimes, field strains of PPRV may cause high incidence of mortality in unvaccinated sheep herds (Obi et al. 1983; Kennedy et al. 1989; Alcigir et al. 1996; Amjad et al. 1996; Aruni et al. 1998; Abu Elzein et al. 2004; Yesilbag et al. 2005; Wohlsein and Saliki 2006; Abubakar et al. 2007). In West Africa where the disease was firstly identified, it is reported that local goat breeds are more susceptible to PPRV infection (Diallo 1988; Wohlsein and Saliki 2006). In an experimental study conducted on alpine goats using Moroccan strain of PPRV, all virus-inoculated goats developed severe pneumonia and lymphadenitis (Hammouchi et al. 2012). It is shown that alpine goats are relatively susceptible to disease and they can be used

as a proper experimental model animal for experimental PPR. Toplu et al. (2004) defined that PPRV infection more frequently affects older animals and morbidity rate is higher than young lambs and kids. Otherwise, they also suggested that PPRV infection in young animals is more likely as non-characteristic because of its deadly per-acute form.

7.2.1 Necropsy Findings of PPR

PPRs are also named as stomatitis–pneumoenteritis complex, pseudorinderpest, erosive enteritis, and stomatitis. In fact, all above-mentioned synonyms of PPR actually reflect its pathomorphologic features (Wohlsein and Saliki 2006; Kul et al. 2007).

There is no obvious macroscopic lesion in the animals that died after peracute PPRV infection. But, in some instances, congestion of oral mucosa and ileocecal valve, and mild erosions in oral mucosa can be noticed (Toplu 2004; Wohlsein and Saliki 2006).

The animals that died from acute PPRV infection show dehydration and cachexia (Toplu 2004). Perianal skin and muco-cutaneous area may be contaminated with a gray- to green-colored fluidy faeces originating from severe diarrhea. A mucopurulent exudate covers the eyelids, nostrils, and lips (Perl et al. 1994; Aruni et al. 1998; Abu Elzein et al. 2004; Toplu 2004; Kul et al. 2007; Hammouchi et al. 2012). And in severe cases, excessive fibrin-rich exudate adhered to skin around the mouth and nostrils turn to crustous appearance (Obi et al. 1983; Bundza et al. 1988; Abu Elzein et al. 2004; Kumar et al. 2004; Wohlsein and Saliki 2006). Mucopurulent conjunctivitis generally shows co-association with other mucosal lesions. If fibrinopurulent nasal discharge and crusts are removed, underlying hemorrhagic and ulcerative foci can be noticed. In oral mucosa, 1–5 mm in diameter, gray-yellow pseudomembranous and necrotic lesions occur on inner side of the lips, soft and hard

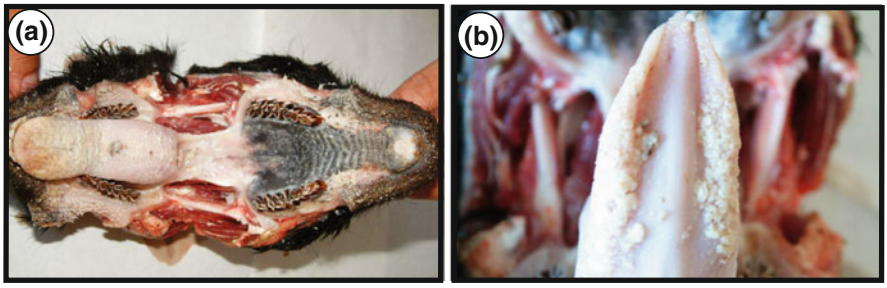

Fig. 7.1 Macroscopic appearance of pseudomembraneous and erosive stomatitis in PPR, goat. *White- to gray-colored foci*; **a** on the soft–hard palate and dorsal surface of the tongue, **b** on the ventral surface of the tongue

palates, and especially ventral and lateral surfaces of the tongue (Fig. 7.1a, b) (Toplu 2004; Wohlsein and Saliki 2006; Kul et al. 2007). Diffuse hyperemia and multifocal erosions take place on nasal conchae mucosa. The oropharyngeal mucosa and adjacent tonsillar surfaces are covered by fibrinous exudates and plaques, those are caseous in severe cases (Toplu 2004; Kul et al. 2007), and severe petechial hemorrhages can be detected in the cut surface of tonsils (Kul et al. 2007).

Through the upper respiratory ways, mostly trachea, there is spongy exudate that is indicative of respiratory distress and lung edema. Nasal conchae, larynx, and trachea mucosae have widespread hemorrhages and fibrinonecrotic lesions (Brown et al. 1991; Kennedy et al. 1991; Aruni et al. 1998; Kul et al. 2007). Retroparyngeal lymph nodes are swollen, congested, and edematous (Aruni et al. 1998).

In the lungs, consolidated and emphysematous areas in the cranial and caudal lobes are seen (Bundza et al. 1988; Brown et al. 1991; Kumar et al. 2004; Wohlsein and Saliki 2006; Kul et al. 2007). Excessive muco-purulent, purulent, and/or necrotic materials may fill bronchial airways, if it is complicated with secondary bacterial infections (Brown et al. 1991). Adhesions between visceral and parietal pleura may occur, and fibrin accumulation all over the lung surfaces gives a cloudy appearance (Toplu 2004).

Spleen is enlarged, congested, edematous, and loose in consistency (Aruni et al. 1998; Toplu 2004).

Rumen, reticulum, and omasum may occasionally contain erosive lesions. If present, linear and regularly distributed erosions, which are covered by blood clot, are typical lesions for omasum mucosa (Brown et al. 1991). Abomasal mucosa is also severely congested and erosive.

Mesenterial lymph nodes enlarge, and they are edematous (Alcigir et al. 1996; Wohlsein and Saliki 2006; Kul et al. 2007).

Mucosa of small intestines is edematous and congested. Its surface is covered by mucous exudate in mild cases. In severe cases, inflammation turns to fibrinohemorrhagic enteritis and intestinal exudate constituted of excessive fibrin and debris of necrotic tissues. Frequently, Peyer's patches and gut-associated lymphoid tissues are necrotic and their mucosal surfaces are covered by pseudomembrans (Obi et al. 1983; Alcigir et al. 1996; Abu Elzein et al. 2004; Kumar et al. 2004; Wohlsein and Saliki 2006; Kul et al. 2007). Duodenum, ileum, cecum, and dorsal colon walls are thickened, and widespread congestion takes place (Toplu 2004). Obviously, the lesions in the small intestines are not so severe and limited to linear hemorrhages and erosions at the beginning part of duodenum and ileum (Bundza et al. 1988). Hemorrhages at ileo-cecal valves and linear congestions alongside the large intestines called as "zebra stripes" are the characteristic pathologic findings of PPR (Alcigir et al. 1996; Wohlsein and Saliki 2006).

Liver is pale, and multifocally gray-white necrotic foci can be observed on its cut surfaces (Alcigir et al. 1996; Toplu 2004).

7.2.2 Microscopic Findings

Histopathological findings of natural PPRV infection include pseudomembraneous, erosive and ulcerative stomatitis, necrotic tonsillitis, fibrinohemorrhagic enteritis, and broncho-interstitial pneumonia (Bundza et al. 1988; Brown et al. 1991; Alcigir et al. 1996; Amjad et al. 1996; Aruni et al. 1998; Abu Elzein et al. 2004; Toplu 2004; Gulyaz and Ozkul 2005; Wohlsein and Saliki 2006; Abubakar et al. 2007; Kul et al. 2007; Atmaca and Kul 2012).

PPR is characterized by pseudomembranous, erosive, and ulcerative lesions in the mucosa of oral cavity, nasal conchae, and trachea (Alcigir et al. 1996; Kul et al. 2007). Oral mucosa epithelia show hydropic and ballooning degeneration, necrosis, and syncytial cell formations (Kulkarni et al. 1996; Wohlsein and Saliki 2006; Kul et al. 2007). Syncytial cells are considered as pathognomonic for PPR, and they contain 2–10 nuclei and intracytoplasmic/intranuclear eosinophilic inclusion bodies (Toplu 2004; Alcigir et al. 1996; Kul et al. 2007). Pseudomembrane formation often shows positive correlation with the degree of virus replication and epithelial necrosis, and the thickness of this layer increases by secondary bacteria contamination (Fig. 7.2a, b). In the latter instance, numerous neutrophil leukocytes are also involved in the nature of the pseudomembrane (Toplu 2004; Wohlsein and Saliki 2006; Kul et al. 2007). In a varying degree of edema, lymphocytes, neutrophil leukocytes, and macrophages infiltrations are also present in submucosa (Toplu 2004).

In the lungs, non-complicated cases show typical interstitial pneumonia appearance. Mostly, mononuclear cells infiltrate through interalveolar septa and around the vessels. These areas are thickened because of excessive edema and inflammatory cell accumulations. Peribronchial cuffing constituted of lymphocytes and epithelial hyperplasia (Fig. 7.2c), and squamous metaplasia on bronchi mucosae is observed (Alcigir et al. 1996; Aruni et al. 1998; Abubakar et al. 2007; Kul et al. 2007; Emikpe et al. 2010). Multinucleated syncytial cells form in pneumocytes. Intracytoplasmic and intranuclear eosinophilic inclusion bodies are seen in these syncitial cells, alveolar macrophages, bronch/iolar epithelia, and in peribronchial glands (Fig. 7.2d) (Kul et al. 2009). Interstitial pneumonia and characteristic histologic findings of PPR in the lungs are shaded by suppurative, fibrinous, and/or necrotic bronchopneumonia lesions, which occur in secondary bacterial complications (Brown et al. 1991; Emikpe et al. 2010). Thus, the demonstration of pathognomonic syncytial cells and eosinophilic inclusion bodies, both cytoplasmic and nuclear, is essential for histopathologic diagnosis. In severe cases, where the histologic details are not available, detection of PPRV-specific proteins and RNA is quite helpful by immunoperoxidase and in situ hybridization techniques (Brown et al. 1991; Alcigir et al. 1996; Toplu 2004; Kul et al. 2007, 2008; Kumar et al. 2009; Toplu et al. 2012).

Widespread lymphoid necrosis is main finding in spleen, lymph nodes, tonsil, and Peyer's patches. Lymphocyte necrosis is characterized by pyknosis and karyorrhexis (Wohlsein and Saliki 2006; Kul et al. 2007). Reticulo-endothelial cells

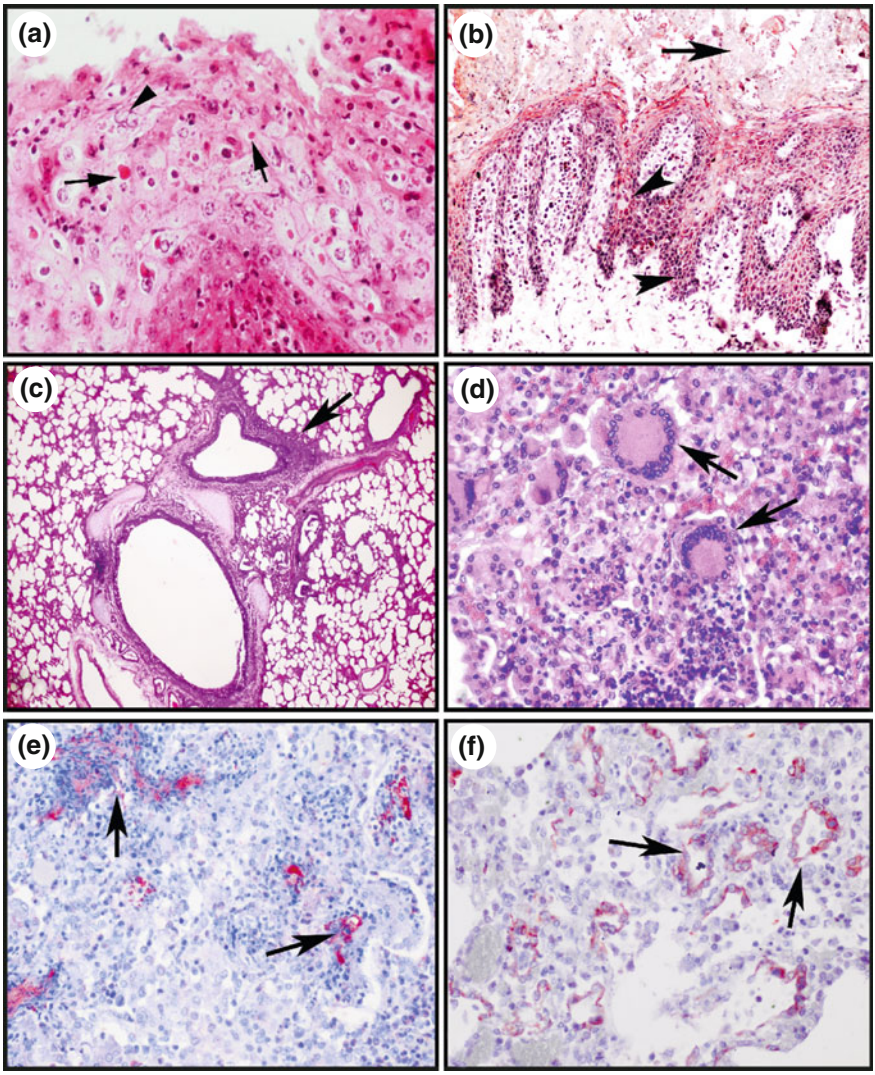

Fig. 7.2 Histopathologic findings of PPR. **a** Intracytoplasmic inclusion bodies (*arrows*) and a syncytial cell (*arrow head*), oral mucosa, hematoxylin eosin (**b**) PPRV immunopositive reactions on Stratum spinosum epithelia (*arrow heads*) and pseudomembran (*arrow*), indirect ABC immunoperoxidase test, anti-PPRV primary antibody, and Mayer's hematoxylin counterstaining (**c**, **d**) Perbronchiolar cuffing and numerous syncytial cells in the lungs, hematoxylin eosin (**e**, **f**) PPRV antigen-positive appearance in necrotic and brochiolar exudate (**e**) and in syncytial cells (**f**), lung, indirect ABC immunoperoxidase test, anti-PPRV primary antibody, and Mayer's hematoxylin counterstaining

become visible after severe lymphocyte depletion, and they seem hyperplastic, and sometimes, syncytial cells originated from adjacent reticular cells resident in reticular network can be observed. Eosinophilic nuclear viral inclusions can also be

associated with reticular cells (Kul et al. 2007). Multifocal and disseminated hemorrhages have been reported in tonsils (Toplu 2004). Otherwise, more characteristically, tonsillar crypt epithelia may have abundant cytoplasmic inclusion bodies in spite of dense cellular necrosis and keratinization of tubular surface of the crypts (Kul et al. 2007). In spleen, there are lymphoid depletion, hyperplastic reticuloendothelial cells in Malpighi corpuscles, and reactive sinusoidal macrophages and plasmacytes in the sinusoidal spaces (Aruni et al. 1998).

Multifocal midzonal and periportal coagulation necrosis in liver have been reported in previous studies (Alcigir et al. 1996; Kul et al. 2007; Toplu et al. 2012). In the lesioned areas; vacuolar changes are seen in the cytoplasm, and pyknosis/karyorrhexis of the nuclei in affected hepatocytes. Numerous neutrophil leukocytes may also accompany with homogenous eosinophilic coagulation necrosis. Kuppfer cells are generally hypertrophic (Aruni et al. 1998; Toplu 2004). Just nearby these coagulation necrosis, syncytial cells are formed by adjacent 2–6 hepatocytes. Hepatocytes, Kuppfer cells, and syncytial cells may contain nuclear viral inclusions. Presence of PPRV in the nuclei of the hepatocytes can be shown by PPRV antigen-specific immunohistochemical tests (Alcigir et al. 1996; Toplu 2004; Wohlsein and Saliki 2006; Kul et al. 2007). In some cases, periportal fibrosis is also a part of liver pathology (Aruni et al. 1998).

Forestomach mucosa rarely invaded by PPRV and vacuolar and hydropic degenerations, cytoplasmic inclusions, and PPRV immunopositivities have been declared in natural cases. Necrosis of principal and parietal cells of secretory glands in Lamina propria of abomasum has been reported (Kul et al. 2007; Toplu et al. 2012).

In small intestines, duodenal gland epithelia are necrotic and intestinal crypts are enlarged by accumulation of degenerative–necrotic cell debris. In lamina propria, there are numerous lymphocytes, macrophages, and rarely eosinophil leukocytes infiltrations (Alcigir et al. 1996; Aruni et al. 1998; Abu Elzein et al. 2004; Wohlsein and Saliki 2006; Kul et al. 2007). Peyer's patches show lymphocytolysis and contain necrotic cell remnants, neutrophil leukocytes, and macrophages (Bundza et al. 1988; Aruni et al. 1998; Rajak et al. 2005).

The renal pelvis transitional epithelia of the goat and sheep that are experienced with end stage of PPRV infection contain vacuolar degeneration and eosinophilic viral inclusion bodies. In the heart, non-purulent interstitial myocarditis and hyalinization of cardiomyocytes have been reported as uncommon pathologic findings of PPR (Kul et al. 2007).

Either in experimental PPRV infection models or in natural PPR cases, PPRV has been demonstrated in epithelial cells of oral mucosa, conjunctiva, trachea, bronch, bronchiol, and ileum, type II pneumocytes, syncytial cells, alveolar macrophages, mesenterial lymph nodes, spleen, and liver (Fig. 7.2e, f) (Alcigir et al. 1996; Toplu 2004; Kul et al. 2007). A recent study's results suggest that PPRV involvement in kidneys, abomasum, heart, and brain shows a great resemblance to the lesions of those other *Morbillivirus* infections, e.g., canine distemper and phocine distemper of sea mammals (Kennedy et al. 1989, 1991; Kul et al. 2007).

7.3 Peste des Petits Ruminants and Concurrent Secondary Diseases

PPRs in small ruminants often show co-association with secondary viral, bacterial, and parasitary infections. The geographic distribution of PPRV infections especially in underdeveloped and developing countries has been increasing the complications with other diseases (Diallo 1988; Abubakar et al. 2007; Malik et al. 2011). Otherwise, improper nutrition, management, and health conditions of the goats, which are known as "the cattle of poor people," increase the frequency and severity of the secondary infections and the outcome of the PPR often becomes more dramatic.

It is not easy to estimate which disease takes place first and then the other worsen the clinical course of the ongoing infection. Thus, it does not seem to possibly differentiate individual contribution of the mixed infections on the prognosis and clinical severity of PPR. The knowledge on the immunopathogenesis of PPRV infections shows that epitheliotropic and lymphotropic features of PPRV have a key role in the development of fatal infections in per-acute and acute cases with or without secondary infections (Rajak et al. 2005; Wohlsein and Saliki 2006; Kul et al. 2007; Jagtap et al. 2012). In other words, during the initial invasion of PPRV to the oral and upper respiratoric mucosa, it should be attached and passed through the epithelial barriers. But this epithelial barrier becomes stronger than a physical wall by the active expression of Th1 cytokines from PPRV-infected oral mucosa and lung epithelia (Atmaca and Kul 2012). IFN gamma and TNF alpha levels are importantly increased in PPRV-infected and damaged epithelia lining the oral mucosa, bronch, and bronchiol in natural infections (Atmaca and Kul 2012). At the beginning of the infection, these Th1 cytokins expressed by epithelial and dendritic cells play a major role in the stimulation of macrophages and lymphocytes which are responsible for the constitution of systemic immune responses. Thus, this is one of the most important reasons why PPR often shows co-association with bacterial infections, which are potentially causing epithelial damage. In a study which is conducted in 40 dwarf goats with clinical PPR, *Staphylococcus* 44.0 %, *Streptococcus* 22.67 %, *Neisseria* 12.00 %, *Pasteurella* 10.67 %, *Pseudomonas* 4.00 %, *Proteus* 4.00 %, and *Corynebacterium* 1.33 % are isolated in nasal swap samples (Ugochukwu and Agwu 1991). The most common bacterial pathogens which are coincidentally diagnosed with PPRV in upper respiratory airways are *Staphylococcus aureus* (30.67 %), *Staphylococcus epidermidis* (13.33 %), and *Streptococcus viridans* (18.67 %) (Ugochukwu and Agwu 1991).

Clinically, PPR is generally complicated with fibrinopurulent bronchopneumonia caused by *Mycoplasma* sp. (contagious caprine pleuropneumonia) and *Manheimia haemolytica* (Brown et al. 1991; Kul et al. 2007; Emikpe et al. 2010). Lung lesions complicated with these bacteria are histologically characterized by oat-shaped leukocytes, coccoid bacteria clusters, widespread necroses, and excessive fibrin accumulation in the interstitial septa and pleura. In an experimentally induced mixed infections using *Mannheimia haemolytica* A2 and Nigerian strain of

PPRV infections in goats, it is reported that syncytial cells, edema, neutrophil leukocyte, and macrophages infiltrations in the alveoli are observed in the third day of intra-tracheal inoculations of the agents (Emikpe et al. 2010). Progressive lung lesions are also necroses of bronch/iol mucosa, BALT hyperplasy, and thickness of inter-alveolar septa. The other important finding of this experimental study is demonstration of *Mannheimia haemolytica* on the vessel walls that are localized in nasal septa, alveolar macrophages, pneumocytes, bronchial, and bronchiolar epithelia (Emikpe et al. 2010). Experimentally induced lung pathology described above the same with the classical broncho-interstitial pneumonia findings that are observed in the lungs of goat and sheep infected with PPRV in the field conditions. It also means that pure interstitial pneumonia containing syncytia and viral inclusions are hardly detected in the most of the routine materials.

PPRV infections may show co-association with other viral diseases such as sheep and goat pox (SGP), contagious echtyma-orf, border disease, and blue tongue (Saravanan et al. 2007; Kul et al. 2008; Malik et al. 2011; Toplu et al. 2012). The occurrence of two different viral agents in a host cell seems divergent to "interference" notion, because "viral interference" basically defines the prevention of successive homologous or heterologous viral infections by the efficacy of type I interferon secreted in virus-infected host cell once (Kul et al. 2008; Chinnakannan et al. 2013). However, it is demonstrated that PPRV v protein inhibits the production of type I interferon from virus-infected cells in in vitro conditions. Not only PPRV but also the other members of *Morbillivirus* genus, e.g., rinderpest virus, canine distemper virus, and measles virus, are also able to block Type 1 IFN responses (Chinnakannan et al. 2013). Interestingly, morbilliviruses may also prevent Type II interferon responses in the host cells in varying degrees.

The most noticeable viral coexistence with PPRV is pestivirus (BDV) infections. The first scientific report belongs to stillborn twin lambs that have had congenital anomalies characteristics of border disease, arthrogryposis, scoliosis, and hydranencephaly, in addition to necrotising bronchitis and syncytial cells in their lungs (Kul et al. 2008). The presence of both PPRV and BDV in these lambs is confirmed by RT-PCR and immunohistochemistry. And this report is also the first evidence of congenital transmission of PPRV from an infected mother to the lambs (Kul et al. 2008). Afterward, co-association between BDV and PPRV in small ruminants is described in a detailed study (Toplu et al. 2012). According to that report, BDV and PPR mixed infection in 26 fetal and neonatal lambs and kids are established. And it is also demonstrated that BDV and PPRV may infect the same cell (Toplu et al. 2012). The higher frequency of BDV and PPRV mixed infections in fetuses and young animals suggests a possible hypothesis on congenital PPRV and BDV transmission which is facilitated by placental damages induced by pestiviruses (Kul et al. 2008; Toplu et al. 2012). Otherwise, Toplu et al. (2012) concluded that brain damage following intrauterine infection with BDV accelerates the transmission of PPRV to the brain and results in infection of neuronal and neuroglial cells by PPRV, as well. There is no case controlled study questioned the placental pathology and whether vertical transmission PPRV alone or mixed infections with BDV. But placental invasion during solely PPRV infections is possible, and as it is recently

shown, PPRV can cause abortion in Kilis goats by the absence of other abortifacient agents such as BDV, Brucella, etc. (Kul et al. 2012).

A report indicates a definite co-association of SGP and PPR in a goat flock in India (Malik et al. 2011). According to clinical history, they defined a severe and fatal outbreak, in which 112 out of 150 goats died, representing clinical findings of PPR, bluetongue (BT), and SGP. Among the serologically examined animals, 8 out of 12 goats are found seropositive against PPR and BT. While SGP is diagnosed based on immunodiffusion test and nodular lesions on the skin, PPR is confirmed by PCR and sequence analyses, but no diagnostic procedure is allowed for BT except serology (Malik et al. 2011).

Another viral sheep and goat disease that is concurrently seen with PPR is contagious echtyma. Saravanan et al. (2007) reported acquired hyperplastic gingival lesions consistent with orf after the entrance of 24 new goats to a goat flock, which had prevalent PPRV infection (Saravanan et al. 2007). While PPR and orf mixed infection prevalence is reported in 11.5 % (20 of 174), 37.35 % (65 of 174) goats died due to secondary ORFV infection and complicated oral lesions.

In conclusion, in fact, not only the immunodeficiency resulting from lymphocytolysis and apparent vulnerability to secondary infections, but also possible dual interactions between coinfections and PPR infection may potentiate the severity and extend the lesion profile of the present PPR infection.

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Chapter 8
Current Advances in Serological Diagnosis of Peste des Petits Ruminants Virus

Geneviève Libeau

Abstract Peste des petits ruminants (PPR) is a contagious disease with potential for high economic impacts. Convalescent and vaccinated small ruminants develop a strong and lifelong immunity and are fully protected against re-infection. Consequently, the presence of antibodies, whether they are against a wild virus or a vaccine, is a marker of the host immune protection. Thus, specific antibodies cannot be missed with classical and validated diagnosis assays, such as conventional enzyme-linked immunosorbent assay (ELISA), an alternative testing method to the conventional virus neutralization test (VNT), allowing to monitor vaccination or locate disease outbreaks. Recent insights into the virions' protein composition, development of expression systems, recombinant vectors to express protective antigens, have contributed greatly to update our current knowledge about diagnostics. In the light of this progress, the chapter focuses on PPRV serological tests with particular attention to diagnostic antigen targets and refinement of assays for accurate serodiagnosis of PPRV.

8.1 Introduction

Peste des petits ruminants (PPR), a highly contagious disease of domestic and wild small ruminants, is classified as a notifiable disease by the World Organization for Animal Health (OIE). Now that rinderpest is eradicated, there has been increasing interest in PPR, the other major morbillivirus disease of livestock that affects mainly sheep and goats. As our knowledge of the epidemiology of PPR has been improved, it appeared that the disease was no more limited to West Africa where it was first described. Its presence has now been recognized in many of the African countries,

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from the Atlantic Ocean to the Red Sea. It is also present from Middle East including Turkey (from where it constitutes a high risk for Europe) to South and West Asia.

PPRV is described to cause an acute disease in small domestic ruminants, although sheep are often less severely affected than goats (Lefèvre and Diallo 1990). In natural (Diop et al. 2005) and experimental infections of goats (Hammouchi et al. 2012; El Harrak et al. 2012), some breeds have shown to be extremely susceptible, displaying severe clinical signs of disease and high levels of mortality. In contrast, in the Sahel zone of Africa for example, PPRV infections are difficult to recognize in breeds of tall sheep and goats and are therefore discovered on the occasion of a serosurvey. Cattle, domestic and wild African buffaloes (*Syncerus caffer*) make a subclinical form of the PPR, and seroconversion can also be observed. Therefore, whatever the severity of the disease, animals that survive infection develop antibodies against PPRV usually from 7 to 10 days post-infection, which persist in the long term. Consequently, detection of specific antibodies against PPRV should perform correctly for the diagnosis of acute, subacute and unapparent forms of the disease. Aside from inducing a lifelong immunity, it is important to note that although four distinct phylogenetic lineages are described up to now, only one serotype is known to exist. One of the issues is to know, however, whether the current diagnostic tools would have at least the same diagnostic potential than virus neutralization test (VNT), the prescribed test in the OIE Terrestrial Manual, in different wildlife populations as well as in camels, an animal recently identified as susceptible to PPRV and exhibiting respiratory distress and mortality (Khalafalla et al. 2010).

Development and use of specific diagnostic tests that can distinguish PPR from diseases with similar signs helped unquestionably to improve our knowledge and understanding in the geographical distribution and spread of the disease in specific areas. Due to the severe economic impact of PPR on small ruminant production, many countries have started to control the disease by using PPR live attenuated vaccines. Although PPRV has been proposed as the next disease to be eradicated after rinderpest, no corresponding international control/eradication programme for PPR is established yet. However, there is currently a high risk of transmission of PPRV to more than one billion of sheep and goats. The consequence of PPR on small ruminant production is so important that many countries are individually implementing serosurveillance for a better understanding of the prevalence and further control of the disease. Other countries are designing plans for better preparedness to emergency situations. This contributes to an increase in more sensitive and specific diagnostic tools and specific immunoreagents for the rapid diagnosis of PPRV. The development of such specific tests is greatly challenged by the antigenic community among the *Morbillivirus* genus. However, although they need to be simplified and improved, differential diagnostic tools able to distinguish PPR from rinderpest were already available in the final stage of rinderpest eradication to limit false positives. Indeed, to recognize countries or regions as free from the disease, the accurate distinction between seropositive and seronegative individuals has become an absolute necessity. More recently, research has focused on simpler, rapid, robust and environment-friendly diagnostic assays which can be adopted as

routine techniques in many laboratories. The availability of modern serological tools will greatly improve and simplify the control of PPR in endemic countries.

For the implementation of targeted and efficient control strategies, it is essential to locate disease outbreaks and to monitor their spread and extent through clinical and serological surveillance. Hence, effective screening of PPR depends on a proper and a rapid diagnosis. In this respect, the present chapter focuses on current knowledge about PPRV serological tests with particular attention to antigenic determinants of proteins with immunological significance on which the development of tests relies. With regard to the progress made in analysis of these determinants, an improvement in reliability and accuracy of serological assays for the diagnosis of PPRV infections may thereby be expected.

8.2 Immunogenicity of Different Viral Proteins

Convalescent sheep and goats and vaccinated animals develop a lifelong immunity and are fully protected against re-infection. A strong humoral immune response has been shown to be a prerequisite for the animal to control the initial phase of the viral replication, to enable virus elimination and ensure host's survival. Neutralizing antibodies elicited by natural infections or by the currently available live attenuated vaccine have been correlated with protection from PPRV-induced disease. A common feature of morbillivirus infections is, however, a transient immunosuppression. Similarly, although current vaccines are highly effective and do not induce clinical signs following their administration, they are also known to induce a short-term immunosuppression. However, to the extent that it can be demonstrated, lifelong immunity associated with transient immunosuppression remains an apparent paradox as virus-induced immunosuppression does not prevent the generation of long-term antiviral immunity (Rajak et al. 2005; Sellin et al. 2009).

Immunogenicity is assessed by measuring the humoral response. While humoral response is important in limiting the spread of virus, the contemporary cell-mediated immunity also helps to eliminate infected cells. During the acute phase of infection with a wild-type virus strain, hyperthermia marks the beginning of the humoral immune response, while after vaccination, antibodies appear around one week and reach a plateau at 3–4 weeks. Neutralizing antibodies are the first defence against infection and have been shown to be important for disease outcome with all morbilliviruses. As with other viral infections, PPRV stimulates the induction of IgM and IgG antibodies, which in the case of IgM disappear soon after infection in favour of IgG that are stable for several years, then declining without disappearing. The protection obtained with vaccination lasts for at least one and probably up to 3 years (Diallo et al. 2007).

Knowledge of the PPRV proteins with immunological significance is relevant for the improvement of serological diagnostic tests. PPRV has a negative-sense, single-stranded RNA genome with a coding scheme synthetizing eight known proteins of which six are structural proteins: the core nucleocapsid proteins, the nucleoprotein

(N), phosphoprotein (P), matrix protein (M), large protein (L) and two surface proteins, hemagglutinin (H) and fusion proteins (F) (Bailey et al. 2005). Essentially, three viral proteins N, H and F of the six structural proteins of the virus appear to induce immunity. The nucleoprotein N induces the most abundant immune response, but neutralizing and protective antibodies are directed against two surface glycoproteins, H and F. Correlation between neutralizing antibody titres and protection has long been recognized for canine and phocine distemper virus infections (Osterhaus and Vedder 1988) and for measles (Chen et al. 1990). The same occurs for PPR, which F and H proteins, located within the viral envelope, have been targeted for use in recombinant vaccines to confer protection in goats. Both recombinant proteins are found to confer immunity when expressed either together or separately. Examples are given by heat-stable capripoxvirus vectors expressing individual F (Berhe et al. 2003) or H (Diallo et al. 2002; Diallo 2003), or both proteins (Chen et al. 2010), and recombinant canine adenovirus expressing the H of PPRV (Qin et al. 2012). In the case of the recombinant capripox, neutralizing antibodies are observed after a single injection of the vaccine. Neutralizing antibody titres are maintained at a significant level up to at least 6 months and are increasing rapidly following challenge. The protective efficacy of antibodies to PPRV is also illustrated by protection conferred to young animals to virulent challenge from passively acquired maternal antibodies (Balamurugan et al. 2012). However, the passive protection of kids born to vaccinated or exposed mothers is relatively short compared to other species, showing a declining trend from 60–90 days onwards and a complete loss at 120 days (Libeau et al. 1995). Consequently, the presence of antibodies, whether they are neutralizing or not, can serve as surrogate markers of protection when detected with serological tests, despite the fact that cellular immunity is implicated in the control of PPRV replication (Mitra-Kaushik et al. 2001).

8.3 Antigenic Relationships of Morbilliviruses

8.3.1 Serological Cross-Reaction

Antigen/antibody cross-reaction among morbilliviruses is one of the main constraints for achieving a reliable diagnosis. Among the genus, the problem of differential diagnosis of PPRV arose only with RPV, two viruses used to give rise to a very similar clinical picture in ruminants. The task was not easy, particularly during rinderpest seromonitoring activities in the endemic areas where both viruses had overlapping geographical distribution and host range. Nevertheless, RP has been eradicated from the world. Despite this notable progress, lessening the cross-reactions with rinderpest and other morbilliviruses in the development of tests designated for the differential serological diagnosis of PPRV is highly desirable. The resulting tools are expected to be able to define the exact distribution of the PPRV to help containing any extension and facilitate its distinction from other similar diseases.

The strong immunological relationship is due to sequence similarities at both the nucleotide and amino acid sequences among morbilliviruses. Analysis of the protein sequences now suggests that these similarities are likely to represent structurally and functionally equivalent domains among the genus (Chard et al. 2008; Bailey et al. 2007). Cross-reactivity was first highlighted by the earliest serological assays developed for disease diagnosis, which are immunofluorescence, complement fixation and agar gel immunodiffusion (Orvell and Norrby 1974). These reactions involve mainly the core nucleocapsid proteins, N, P, M, L and one of the two surface proteins, the F protein. Carried out empirically, at least at the beginning, the cross-protection experiments reveal associations in pairs such as MV/CDV (Norrby and Appel 1980; Appel et al. 1984), MV/RPV (Plowright 1962; Provost et al. 1968, 1971), RPV/PPRV (Bourdin et al. 1970, Gibbs et al. 1979, Taylor 1979) and CDV/PDV (Osterhaus and Vedder 1988). The matching was confirmed subsequently by phylogenetic studies, except for the RPV/PPRV pair. Indeed RP/MV and CDV/PDV form phylogenetic clusters within the *Morbillivirus* genus, PPRV being related to the RPV/MV group (Diallo et al. 1994; Kwiatek et al. 2007; Minet et al. 2009).

8.3.2 PPRV Serodiagnostic Protein Targets

A clear understanding of the PPRV proteins involved in the serological immune response is a crucial step that should allow to identify the most immunogenic as well as specific epitopes. This in-depth understanding is highly relevant for the improvement of serological diagnosis tests. It has been shown that among the surface proteins, only H, an important component required for attachment of the virus to its cellular receptors, induces little cross-reactivity among viruses of the genus. Host-specificity of morbilliviruses may be responsible for part of this variability. H protein is also an important antigenic component. The residues known to be involved in cell tropism and binding the host-specific signalling lymphocytic activation molecule (SLAM) receptor, also known as CD150, are exposed to the host immune system and therefore induce a specific neutralizing immune response (Vongpunsawad et al. 2004). In measles, another neutralizing epitope was demonstrated to interfere with the H–F interaction (Tahara et al. 2013). Differential neutralization effect of homologous and heterologous antibodies against RPV and PPRV was used to clarify the antigenic relationship of these viruses and classify them as two distinct viruses in the *Morbillivirus* genus (Gibbs et al. 1979; Taylor 1979; Saliki et al. 1993). H PPRV protein also possesses hemagglutination activity as it is seen for MV (Wosu 1985; Seth and Shaila 2001; Osman et al. 2008) as well as it exhibits neuraminidase activity (Devireddy et al. 1998; Seth and Shaila 2001). Major neutralizing epitopes were defined using monoclonal antibodies (mAbs) able to inhibit neuraminidase and hemagglutination activity and were topographically mapped on this protein using overlapping truncated proteins. Two discontinuous regions of amino acid sequence 263–368 and 538–609 are required for efficient

binding of mAbs to the H protein, those targeting the latter domain being able for distinguishing PPRV from RPV. This comprehensive map of functional domains shows that mAbs may recognize epitopes with conformational sequences (Renukaradhya et al. 2002). The HPPRV-mAb-based tests were used for serological differential diagnosis (Anderson and McKay 1994).

In contrast, the F protein seems to be one of the most conserved proteins. MV F protein is mentioned as primarily responsible for the cross-protection of dogs against CDV protein after immunization with MV (Appel et al. 1984). F is confirmed in the role of the major cross-protecting antigen, as both N- and C-termini are conserved, as described for PPRV (Meyer and Diallo 1995; Chard et al. 2008). An anti-F1 monoclonal antibody produced against PPRV also defines a unique epitope on all RPV and PPRV strains but is absent from CDV and MV (Libeau et al. 1997). It should also be noted that, due to its high homology, the F gene is now currently used for molecular epidemiological studies of PPRV (Banyard et al. 2010; Munir et al. 2012).

The N protein is antigenically well conserved among morbilliviruses and as it is expressed to a very high level compared to the other viral proteins, is highly immunogenic while being located internally to the virus. The amino acid sequence of the PPRV N proteins is known to present three main domains of differing similarity (Diallo et al. 1994): the amino-terminal region of medium similarity, the highly conserved central region and the poorly conserved carboxy-terminal domain covering the last 105 aa. The identification of serological immunodeterminants of the N protein was achieved by mapping the B-cell epitopes using pepscan of overlapping peptides complemented by analysis of truncated N proteins. This could delimit the sequence of the immunodominant peptidic residues required for efficient binding of mouse mAbs to the N protein of both RPV and PPRV. Two regions of amino acid sequence 1–262 (Choi et al. 2005; Bodjo et al. 2007) and 448–521 (Choi et al. 2005) are involved in the immune response. The mapping of epitopes in both the amino-terminal and carboxy-terminal ends of N protein, the least conserved amino acid sequences, has been previously reported for other morbilliviruses, including measles virus (Buckland et al. 1989; Longhi et al. 2003) and rinderpest virus (Choi et al. 2004; Bodjo et al. 2007). In addition to being detected by mouse mAbs, PPRV epitopes were confirmed as immunodominant in ruminants as demonstrated by their reactivity with sera from immunized or convalescent goats and cattle (Bodjo et al. 2007). It appears that the most interesting anti-N mAbs property is their ability to distinguish between PPRV and RPV strains. The marked differential capacity was first demonstrated by McCullough et al. (1991). In addition, the characterized mAbs were used in the development of ELISAs for serological differential diagnosis (Libeau et al. 1992, 1994, 1995, 1997).

In summary, information on viral proteins has thereby provided a good foundation for the selection of mAbs to be included in ELISAs. A range of mAbs obtained against PPRV but also against RPV have identified important common or specific antigenic sites on these two viruses. A selection of them was later on employed in diagnostic procedures, particularly for routine serodiagnosis.

8.4 Serological Assay Formats

Confirmatory diagnosis of PPR is established when serological tests are implemented in combination with clinical observations while relying whenever possible on virus detection and epidemiological data. PPR can be easily confused with other diseases such as pasteurellosis or contagious caprine pleuropneumonia (CCPP), or even rinderpest since this virus also infects small ruminants. In the absence of specific manifestation of the disease, as it happens in the Sahel zone of Africa where PPRV infections are difficult to recognize in sheep and goats, diagnosis depends on reliable serology tests. Therefore, for the purpose of differential diagnosis, considerable efforts have been made to develop more reliable and accurate serological for PPRV infections. The most commonly used methods for the detection of PPRV antibodies include virus neutralization test and the enzyme-linked immunoassays (ELISAs). Other tests such as hemagglutination inhibition (HI) and indirect immunofluorescence assay (IFA) have been described for PPRV serology, but in the recent years, they were progressively substituted by modernized serological tools in order to improve and simplify the control of PPR in endemic countries. An overview of serological assays for antibody detection of PPRV infection is detailed in the following chapters and a summary of which is given in Table 8.1.

8.4.1 Virus Neutralization Test

One of the earliest serological assays used for determination of protective immunity to PPR virus was the VNT. This is the prescribed test for international trade in the OIE Terrestrial Manual (OIE 2013). The principle of the method is to titrate serum antibodies by evaluation of their neutralization effect on virus infectivity on cells. To this end, serum dilutions are incubated with the viral suspension and distributed over a cell culture in tubes or plates. After one to two weeks incubation, neutralizing antibodies will inhibit visible cytopathic effects (CPE) comparatively to the virus alone. The serum titre is the last dilution of the test serum for which no CPE is observed. This reaction requires culture stocks of sensitive cells and of vaccine virus. The method was developed first for rinderpest (Plowright and Ferris 1961; Rioche et al. 1969); however, before VNT was established for PPR (Taylor 1979; Taylor and Abegunde 1979), both immune responses could not be distinguished. The PPR test was refined later by Rossiter et al. (1985) for use in 96-well microplate format and soon established as a screening test. The positive threshold was settled at a titre of 10, the minimum value for considering an animal as immune. The PPR VNT used as a differential test played therefore a critical role in rinderpest serosurveillance and eradication programs (Kock et al. 2006), in domestic as well as in wild population, the latter considered as a sentinel population during vaccination. Serum samples titrated in parallel by the VNT against PPRV and RPV, ensured distinction of the homologous from the heterologous immune response (Obi et al. 1984).

Table 8.1 Overview of serology assays for antibody detection of PPRV infection

Technique used	Antigen	Detected antibodies	Specificity	References
VNT	Titrated live virus	Anti-H, anti-F	High specificity (most specific serological tool for PPRV diagnosis)	OIE (2013), Taylor (1979), Taylor and Abegunde (1979)
HI	Chicken RBC	Total Ig	Low specificity	Dhinakar et al. (2000)
IFA	Infected monolayer	Total Ig	Low specificity, improved specificity with optimally infected cell monolayer	Libeau and Lefevre (1990), Wang et al. (2013)
Indirect ELISA	Full rPPRV N, insect cell-baculovirus expression systems	Anti-N	High cross-reactivity within the Morbillivirus genus	Ismail et al. (1995)
Indirect ELISA	Full rPPRV N, E. Coli expression systems	Anti-N	"	Zhang et al. (2012)
Indirect ELISA	Full rPPRV H, transgenic cells	Anti-H	"	Balamurugan et al. (2006)
b-ELISA	Whole PPRV, vaccine	Anti-H	High specificity, low cross-reaction with RP antibody due to steric hindrance	Saliki et al. (1993)
c-ELISA	Whole PPRV, vaccine	Anti-H	"	Anderson and McKay (1994), Singh et al. (2004)
c-ELISA	Full rPPRV N, insect cell-baculovirus expression systems	Anti-N	"	Libeau et al. (1995), Choi et al. (2005)
c-ELISA	Partial and full rPPRV N, E. coli expression systems	Anti-N	"	Yadav et al. (2009)
Peptide-based ELISA	N synthetic peptide	Anti-N	High specificity, low cross-reaction with RP antibody	Dechamma et al. (2006)
Epitope-based c-ELISA	Epitope-based N synthetic peptide	Anti-N	High specificity	Zhang et al. (2013)

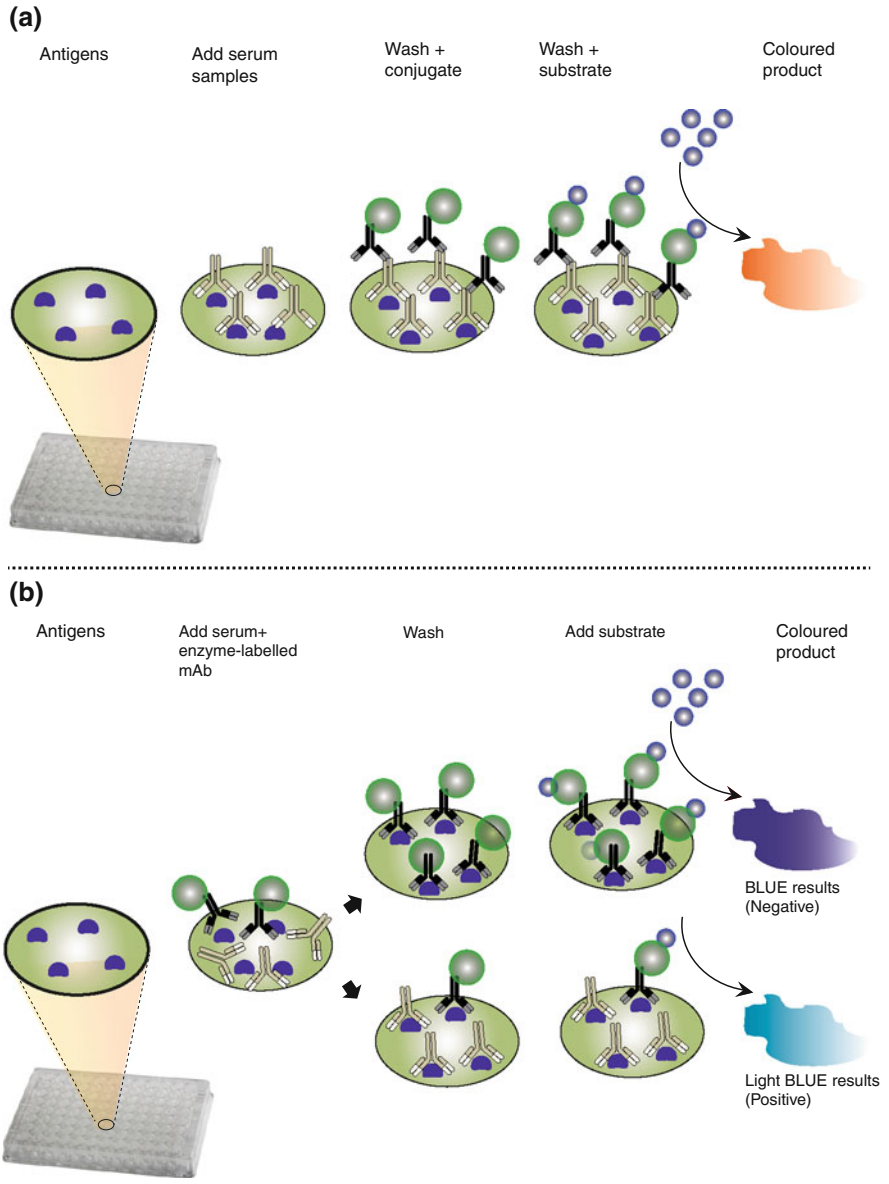
When possible, the global pattern in the same herd was considered to give confidence in the given status.

Although regarded as the gold standard, the method is, however, quite laborious and not readily adaptable to large-scale surveys. In addition, the test necessitates 10–12 days for completion since the virus and cell growth take some time. Moreover, it can only be performed with standardized stocks of PPR vaccine and Vero cell cultures and also specific laboratory equipment and procedures, which are unaffordable for most laboratories in regions where PPR is endemic. Consequently, now that rinderpest is eradicated, the utility of such a test is much less primordial and only implemented in suitable facilities or reference laboratories as a diagnostic discriminator in validation studies. In this regard, VNT is still needed to validate new tests to evaluate their diagnostic potential in unexplored populations, camel and different wild species, shown to be susceptible to PPRV. On these same goals, the classical VNT assay has been recently improved by substituting the conventional vaccine strain, Nigeria 75-1, by a recombinant of the same virus expressing the green fluorescent protein, GFP. The test thus amended allows an observation of the fluorescent cells as early as day 4 and is completed at day 6–8, four days earlier than the conventional test. The new method was validated using sera from vaccinated animals. Neutralizing titres resulting from the assays using either the standard or the GFP-tagged virus did not show statistical difference (Hu et al. 2012).

8.4.2 Various ELISA Formats Based on Whole PPRV

From the early 1990s, different ELISA formats, Indirect ELISA as well as mAb-based ELISAs were developed for the detection of PPR antibodies in the serums of sheep and goats. The conventional antigen used in these different formats is prepared from the whole PPRV extracted from infected Vero cells. The indirect ELISA format developed by Balamurugan et al. (2007), in which the serum is incubated with a solid-phase PPRV antigen, induces colour development when antiserum enzyme conjugate and substrate chromogen are added. The test is not in principle discriminating between morbillivirus cross-reacting antibodies and is therefore recommended to be used as screening tests, followed by a confirmatory test in case of positive results. In the blocking format (b-ELISA, Saliki et al. 1993), the serum is pre-incubated with a solid-phase PPRV antigen, and then, a specific mAb is added. The competitive format (c-ELISA, Anderson and McKay 1994; Singh et al. 2004) is a variant of the previous format where the two reagents are added simultaneously. Both formats are developed using the whole virus as an antigen and the competing mAb targets the H protein. These assays work on the principle that sera containing antibodies to PPRV either block or compete with the binding of the anti-H mAb and result in the reduction of the expected colour when the enzyme-labelled anti-mAb antibody and substrate chromogen are added (Fig. 8.1).

The assays based on H-specific mAbs use purified or semi-purified PPRV vaccine as antigens, strains being either the PPRV Sungri (Singh et al. 2004) or the


(a)
Antigens
Add serum samples
Wash + conjugate
Wash + substrate
Coloured product
(b)
Antigens
Add serum+ enzyme-labelled mAb
Wash
Add substrate
Coloured product
BLUE results (Negative)
Light BLUE results (Positive)

◀ **Fig. 8.1** Configuration of indirect and competitive ELISA. For both indirect and competitive ELISA, either whole-virus antigen or recombinant antigen is used to coat the surface of microwells. **a** In the indirect format, serum is added to wells, and PPRV-specific antibodies, if present, bind to the antigen. All unbound materials are washed away. Antibody–antigen complex is incubated with enzyme conjugate. Excess enzyme conjugate is washed off, and chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the colour generated is proportional to the amount of specific antibodies in the sample. **b** In the competitive format, sample serum PPRV antibodies inhibit binding of enzyme-labelled specific mAb to PPR viral antigen coated on the microwells. Binding of the enzyme-labelled mAb is detected by the addition of chromogenic enzyme substrate which colour product development is subsequently quantified after a fixed incubation period. Strong colour development indicates little or no competition with enzyme-labelled mAb binding and therefore the absence of PPRV antibody in sample sera. Weak colour development due to competition with mAb binding to the antigen indicates the presence of PPRV antibodies in sample sera. For both indirect and competitive formats, results are read by an ELISA reader and compared in parallel with calibrator and controls

Nigeria 75-1 (Anderson and McKay 1994; Saliki et al. 1993). Crude antigen from infected cell cultures were harvested when showing cytopathic effect is prepared by cell disruption/sonication and clarification. Concentration is obtained by high-speed centrifugation and pelleting on sucrose gradient or precipitation using PEG. The resulting concentrated virus is used as the ELISA antigen. Optimized validation conditions relied on sera for which the status for PPR and rinderpest was already established by the VNT. The b-ELISA format appeared to have an increased sensitivity and specificity compared to the c-ELISA format. Using a threshold settled at 45 % of mAb inhibition, as defined by the average inhibiting % +2 standard deviations from a negative population (n = 277), this test presented, compared to the VNT, a sensitivity and specificity of 90.4 and 98.9, respectively. On field samples, the overall agreement between the two tests (n = 253) was 0.91 (Saliki et al. 1993). The H-based C-ELISA developed by Anderson and McKay (1994) utilizes a mAb, designated C77. This ELISA has subsequently been used throughout Africa, Middle East and Asia on several species of small ruminants and has proved to be extremely reliable and robust and is currently commercialized by BDSL. However, some studies showed that, in contrast to the highly specific rinderpest c-ELISA, the PPR c-ELISA does detect some antibodies against RPV. The specificity of the test drops significantly when used with rinderpest-vaccinated animals (Anderson et al. 1991; Couacy-Hymann et al. 2007).

8.4.3 Hemagglutination Inhibition (HI)

HI has also been described for routine serology. With the aim to be implemented as a simple test, an HI assay employing 0.5 % chicken RBC has been described to correlate well with the VNT (Dhinakar et al. 2000). The method measures the whole-serum immunoglobulins and as for the VNT and c-ELISA is able to test sera from any species, making it useful for the surveillance studies. The other value is that expensive and specialized equipment are not required. However, the drawback

is that strong cross-reactivity occurs among morbilliviruses. Moreover, quality control and standardization of reagent may be difficult to implement, while these parameters are of paramount importance for confident testing.

8.4.4 Indirect Immunofluorescence Assay (IFA)

IFA may be used as a serology test although not adaptable to large-scale surveys. Mainly implemented in laboratories with suitable facilities, the test is based on the use of formalin- or acetone-fixed infected cell monolayers incubated in successive steps with sera and antispecies fluorescent conjugates. Reactions are viewed under indirect fluorescence microscopy. With IFA, PPRV immune responses generate a characteristic fluorescence pattern in susceptible infected cells. Although this assay warrants practice and skill, it has advantages compared to indirect ELISA to add specificity since characteristic positive reactions can be discriminated from background by well-delineated intracellular fluorescence, which corresponds to antigen localized in the cell. To improve the specificity of the test, cells may be optimally infected to obtain 20–40 % fluorescence of the monolayer, hence, facilitating the detection of PPRV-specific fluorescent foci (Libeau and Lefevre 1990).

Implementation of IFA in research can also be exemplified by the recent publication of Wang et al. (2013), showing the production of polyclonal antibody against the recombinant F protein which specificity was confirmed by IFA in transfected cells. Authors of the publication intend to use polyclonal antibody against F protein produced at high-level titres as a tool for further study of the pathogenesis of PPRV early infection and the structural and functional characterization of PPRV F protein.

8.5 Recombinant Protein- and Peptide-Based ELISAs

New generation antigens produced by cloning-specific genes in different expression systems were developed. The insect cell-baculovirus and *Escherichia coli* expression systems appear to be a suitable alternative for producing immunogenic antigens for use in diagnostics. The corresponding delivered proteins were used for their introduction into PPR serology tests. Advantages of recombinants over the whole virus issued from infected cells are multiple. Recombinant proteins overcome the cumbersome and costly production of semi-purified viral particles. These antigens meet the requirement of homogeneous and standardized antigens for optimal sensitivity and specificity and can be expressed in large quantities (approximately 20 mg/litre of culture), thus constituting good candidate antigens in diagnostic tests. Finally, the recombinant proteins comply with the demand for safe and reliable tests for serological surveys of PPRV whether the country is infected or not.

Of the six structural proteins, the N protein is known as the major viral protein. As such, this protein that accumulates in larger amount during viral infection compared to the other viral proteins is also highly immunogenic and thus constitutes a good candidate to be used as an antigen in standardized diagnostic tests. Taken together with the possible expression of the protein in large amounts, ELISA formats based on PPRV recombinant N protein (rPPRV N) allow for standardized and industrialization process, hence for high throughput screening of sera. Although antibodies against N are not neutralizing, several N-based ELISAs have been described assuming that in natural infection or vaccination, immune response against N is parallel to that of the protective immunity. To this end, full-length or truncated rPPRV N have been generated by recombinant baculoviruses (Ismail et al. 1995; Libeau et al. 1995; Choi et al. 2005) or *E. coli* expression systems (Yadav et al. 2009; Zhang et al. 2012). These rPPRV N were in a first step characterized by Western blot analysis, the full length was shown to have a molecular weight of 58 kDa.

8.5.1 Indirect ELISA Based on rPPRV N or Sonicated Transgenic Cells

To detect the immune response against PPR, an indirect ELISA based on rPPRV N may be used (Ismail et al. 1995; Zhang et al. 2012). Using rPPRV N instead of whole virus circumvents antigen contamination with cell constituents from the latter, which is the main factor responsible for false-positive reactions in indirect ELISA. This ELISA format although based on a recombinant protein is not in principle specific and is recommended to be used as screening tests for the reason that nucleoprotein is highly conserved among the genus. However, now that the rinderpest is eradicated, the indirect ELISA based on rPPRV N may not provide any evidence that other ruminant morbilliviruses could obscure the reliable serodiagnosis of PPRV. In addition, it is worth stressing that the format favours easy implementation in diagnostic laboratories. If indirect format is to be widely adopted, it is, however, important to draw attention to a phenomenon linked to this format: the negative population of an endemic country will behave differently than that of a free country, exhibiting a higher background level. Therefore, extensive validation should be needed, especially the cut-off settlement.

An indirect ELISA based on the use of recombinant rPPRV N was first described by Ismail et al. (1995). The test was evaluated for routine diagnosis based on goat sera collected during a suspected outbreak of PPR in Cameroun. All field samples ($n = 18$) gave ELISA titres of 8 to ≥1,024, while neutralizing antibody titres ranged from <4 to 4,096. No VNT-negative sera reacted positively with indirect ELISA. More recently, Zhang et al. (2012) used the full length of the N gene from a Tibet strain of PPRV for expression in *E. coli*. Initial validation of the rPPRV N issued from the Tibet strain was based on the comparison in the c-ELISA format with the rPPRV N issued from Nigeria 75-1 strain using the protocol and mAb developed

earlier (Libeau et al. 1995). Differences in the percent inhibition values among both tests were comprised between 5 and 15 %. Based on sera from healthy goats raised in an isolated area (n = 198), cut-off value of the indirect format based on rPPRV N Tibet was settled at a positive/negative optical density value of 2.18, and a preliminary study for assessing the suitability of the assay for serosurveillance was implemented using serum samples with known status (n = 697). All sera were shown to be reassessed in indirect format as previously defined by the competitive format.

Other serological tests have been explored for use in diagnosis of PPRV infections. Balamurugan et al. (2006) have cloned PPRV H gene under a CMV promoter-DNA construct into the Vero cell genome. H protein, displayed as a stable Vero cell line constitutively expressing this PPRV protein, was assessed for its potential use as a diagnostic antigen for ELISA and validated extensively for serodiagnosis use on field goat serum samples. To be used as an antigen in the ELISA format, the cell suspension expressing the H protein was sonicated on ice. When compared to tests based on whole virus, a high degree of relative specificity and sensitivity was found.

8.5.2 C-ELISA Based on rPPRV N

C-ELISA tests based on PPRV recombinant antigens were validated through extensive studies comparatively to methods previously developed, particularly against the VNT and proved to have at least the same diagnostic accuracy in small ruminant populations from various geographical regions. In addition, these antigens helped increasing the improvement of standardized results among laboratories. Test conditions were optimized and the method revealed to be highly accurate and robust.

Expression and purification efficiencies of the recombinant protein obtained by the Baculovirus expression system were evaluated. Crude lysate or affinity-purified rPPRV N was further used as the coating antigen for PPR antibody detection in competitive ELISAs. The first c-ELISA assay based on a recombinant protein described for PPRV was validated with experimentally infected or vaccinated animals and then implemented for sero-surveys. The method originally described in the publication of Libeau et al. (1995) was one of the OIE-approved PPR diagnosis techniques. It was subsequently reproduced by Choi et al. (2005). The mAb used in the ELISA of Libeau and colleagues was chosen for its high affinity for PPRV strains. The epitope recognized on the native N is also present on the rPPR N protein expressed by baculovirus in insect cells, thus allowing the rPPR N protein to behave as the whole virus in the c-ELISA format. The relative sensitivity and specificity of this c-ELISA were 94.5 and 99.4 %, respectively, compared to the VNT reference method, as determined with sera from experimentally infected animals (n = 148). The correlation between the two tests (r = 0.94) was established on the serum titres generated by both tests (n = 683). C-ELISA also showed to be

more friendly and easy in use than the VNT, especially for large-scale seroprevalence surveys in tropical conditions. The test was also reliable in the monitoring of maternal antibody decrease in the kids of vaccinated mothers in Mauritania, after colostrum ingestion. The good performances of this test were further evidenced with African dwarf goats and Sahelian goats field sera during an outbreak of PPR in Senegal (Diop et al. 2005). This ELISA has subsequently been used on several species of small ruminants in different geographical areas (Kwiatek et al. 2007; Ayari-Fakhfakh et al. 2011). It is worth stressing that the test is now commercially available (ID Screen® PPR Competition kit from IDVet).

E. coli expression system has been alternatively explored to express partial and full-length PPRV N gene. The histidine-tagged proteins were purified using a Ni-NTA resin chromatography matrix and eluted. Microtitre wells coated with the purified antigen were tested for reactivity with PPRV immunized rabbits and a specific mAbs for further use in c-ELISA serological diagnosis of PPR infection (Yadav et al. 2009). N expressed with the *E. coli* system behaves as the *whole* virus in the c-ELISA system (Singh et al. 2004) as demonstrated by the specificity of the test with sera from goats having no history of either vaccination or natural exposure to PPRV (n = 70). A preliminary study for assessing the suitability of the assay for serosurveillance was implemented using sheep and goats serum samples collected randomly from an area where the disease was present (n = 120). However, comparatively to the PPRV antigen-based test, 73 samples out of 93 samples were found positive by recombinant protein-based c-ELISA. Comparison with the VNT was not undertaken due to the limited number of samples analysed in this preliminary study.

8.5.3 Peptide- or Epitope-Based ELISA

Despite the fact that c-ELISAs developed for PPR diagnosis show satisfactory performance in PPR enzootic areas, a cross-reactivity with rinderpest antisera has often been shown, very likely linked to steric hindrance (Couacy-Hymann et al. 2007; Anderson and McKay 1994). Promising methodology for increasing PPR serodiagnosis specificity relies on short synthetic peptides representing a single epitope as alternative antigens to recombinant proteins or to the whole microorganism. Used in an indirect ELISA format, they afford the advantage of eliminating non-specific reactions or steric hindrance and are therefore considered more specific than the whole antigen-based assays. In addition the method does not require virus culture or the production of mAbs. Among the structural proteins, the N protein as the most abundant viral protein correlated with a prompt anti-N antibody response was selected for the synthesis of amino acid sequences representing specific antigenic epitopes of PPRV. Preliminary to peptide-based ELISA development, immunogenic peptides on the variable domains of the N PPRV protein were defined by in silico sequence—structure analysis to delineate high antigenic index propensity. Then peptides tested for reactivity with PPR antiserum from infected goat in

comparison with rinderpest antiserum raised in rabbits against the vaccine strain, lead to a reliable and rapid identification of epitopes specific to PPR. Dechamma et al. (2006) showed that peptide ^{132}STEGPSSGSKKRIN^{144} from the N-terminal region, and peptides ^{433}ATREEVKAAIP^{443} and ^{454}RSGKPRGETPGQLL-PEIMQ^{472} from the C-terminal region of the N PPR protein of PPRV showed high specific reactions with PPRV antibodies from infected goat, while although reduced, a reaction with rinderpest antiserum rose in rabbits against the vaccine strain. Identification of immunodominant but PPRV-specific epitopes and domains will provide the foundation in designing an N-based diagnostic immunoassay for PPRV. However, performance of peptide-based ELISA still remains to be compared with conventional VNT and c-ELISA for routine purposes. For RPV, peptide ^{479}PEA-DTDPL^{486} (Choi et al. 2004) or the whole variable domain from 421 to 490 of the C-terminal of the N protein expressed in *E. coli* (Parida et al. 2007) has been used as antigen in a peptide-based ELISA. Although encouraging, one can fear that specificity improvements with peptide-based ELISAs may in contrast lead to the erosion of sensitivity performances.

To further improve the specificity of the PPRV c-ELISA tests, researchers have recently based on the knowledge of defined immunogenic and specific N PPRV epitopes to produce polyclonal anti-sera in order to replace mAbs usually included in this assay format. The development of epitope-based c-ELISA is sensitive and specific, quickly developed and overcoming production of mAbs usually time-consuming, and finally allows the replacement of the virus by a synthetic peptide (Zhang et al. 2013). Validation of this epitope-based c-ELISA based on 1,039 serum samples defined the relative sensitivity and specificity values of 96.18 and 91.29 %, respectively, comparatively to a commercially available c-ELISA. The added and welcomed value is the use of a focused method for obtaining polyclonal antibodies suitable in c-ELISA, an assay usually mAb-based. The drawback, comparatively to a stable antibody-producing hybridoma, is the difficulty to standardize such a production to avoid affecting antibody affinity and avidity and as a consequence sensitivity and specificity of the assay.

8.6 Companion Diagnostic Tests for DIVA Vaccine

At present, it is not possible to discriminate previously PPRV-infected from vaccinated animals. Small ruminants vaccinated on a large scale with PPR vaccines will still act as serological reactors for the presence of wild-type virus strains. Thus, for predicting the impact of vaccination strategies on the control of the diseases, DIVA vaccines and companion tests would be of great help. Ideally, serological surveillance using adapted tools should be able to identify and differentiate serum antibodies from animals infected by field virus only, from vaccinated animals, and from vaccinated and co-infected animals. All these situations would occur in enzootic areas where disease control by vaccination is implemented. It is worth mentioning that an abortive replication of the challenge virus might occur in

vaccinated animals as suggested for RPV (Walsh et al. 2000). This limited replication might be sufficient to induce new antibody production in the vaccinated animals. To address the issue of a marker vaccine and its accompanying tests that would allow the identification of all three categories of animals, an important prerequisite is the knowledge of immunodominant regions on the PPRV proteins. DIVA vaccines will be thus obtained by deletion or incorporation of mutated epitopes into rescued recombinant PPRV using reverse genetics techniques. A proof of concept based on the RPV backbone has demonstrated the possibility to mutate residues critical for recognition of the viral H protein by mAb C1, a mAb that was used in the c-ELISA for RPV serosurveillance (Buczkowski et al. 2012). These findings are a good foundation for the development of marked vaccines for PPRV. DIVA vaccines and companion diagnostic tests would be ideal tools for emergency vaccination in case of the occurrence of an outbreak in a PPR-free country or zone and would be the most realistic and cheapest measure than a stamping-out policy leading to the destruction of large populations of animals. In addition, marker vaccines will reduce surveillance efforts and speed the steps leading to disease control and eradication.

8.7 Conclusions

PPR is a devastating disease having high impact on economy and negative effects in terms of food security and incomes. In addition to these constraints, infected countries are excluded from international trade. The virus is now present in many countries of Africa, Middle East and Asia and given its high spreading capability, it is a threat for the other non-affected countries of Europe, Africa and Asia. Differential diagnosis is also masked by the different clinical features of the disease, as well as similarity in respiratory signs with CCPP or with pneumonic pasteurellosis, cited as major causes of small ruminant mortality. A rapid and accurate serological diagnosis is therefore a critical first step in any outbreak. On the other hand, PPR vaccine has shown its effectiveness in establishing a durable antibody response protecting animals at least three years. This will be further evaluated with the measurement of the presence of serum antibodies with standardized serologic diagnostic methods.

In this context, c-ELISA is considered as highly accurate, standardized and robust test, able to measure the immune response either due to infection or vaccination. This ELISA format has the potential to replace the conventional VNT method which presents technical challenges for its implementation in some laboratories. Based on the use of mAbs targeting to a single epitope, the impact of c-ELISAs proved to significantly increase diagnostic specificity. Indeed, PPR ELISA under the competitive format, whether it relies on whole or on recombinant virus as antigen, has already proved its utility by indirectly facilitating programmes previously implemented to eradicate rinderpest such as the Pan-African Programme for the Control of Epizootics (PACE Programme) when both diseases were present in

the same areas. Given their performances, these assays are suitable for testing small ruminant samples in all endemic countries as well as in countries still free of the disease. Although wildlife is not considered as reservoir but certainly infected through possible spillover from domestic animals, the serosurveillance of the disease in wild species is highly relevant for the management of outbreaks and determining the exposure status to PPRV. In addition to the role of camel in the epidemiology of the disease, it is still ignored.

PPRV has become an important subject of interest particularly now that rinderpest is eradicated. Public international institutions grasped the full significance of the disease, particularly in the recent years and realized the importance of its devastating effects. They have reached the level of now organizing a global control, foreseeing, as for rinderpest, a possible eradication. In the final stage of vaccination campaigns, serological tools that can accurately discriminate between seropositive and seronegative individuals will become an unconditional necessity for countries wishing to be recognized as free from the disease. Therefore, improvement of sensitive and specific tests is required to reliably detect true seropositive animals. Based on the relevant performance characteristic of improved tests, help will be given to determining testing strategies and planning disease control measures. In this perspective, it is pretty safe to say that serodiagnostic tools and specific immunoreagents for the rapid diagnosis of PPR will be given central stage for this battle.

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Chapter 9
Current Advances in Genome Detection of Peste des Petits Ruminants Virus

Emmanuel Couacy-Hymann

Abstract Molecular techniques have given various opportunities to detect the genome of peste des petits ruminants virus (PPRV) at high resolutions, and these powerful methods are very sensitive and specific. In the past, radioisotope-based techniques have been used for diagnostic purposes. However, because of associated hazards these may cause to human and environment, the radioelement hybridization techniques are no more in use. Alternative techniques such as hybridization with the digoxigenin/anti-digoxigenin system have been developed and are being practised. Moreover, genome amplification with different polymerase chain reaction (PCR) chemistries (conventional PCR, real-time PCR, multiplex real-time PCR, LAMP-PCR) has been developed to easily detect genome of PPRV, independent of lineage variations. Prior to these, adequate samples should be taken from sick animals and should be well conserved and transported rapidly to the laboratory for analysis. Despite convincing performance of these new diagnostic methods, currently, it is not possible to directly differentiate lineages of PPRV strains, which are now prevalent without distinct geographical demarcation. Currently, the amplified PCR products are sequenced to determine genetic classification of PPRV lineages and to establish epidemiological links.

9.1 Introduction

Various molecular tools are available to be used for the specific and sensitive detection of the genome of peste des petits ruminants virus (PPRV). These genome detection techniques range from the cDNA probe in a hybridization procedure up to diverse polymerase chain reaction (PCR) techniques. There is also a wide range of equipments needed to perform these methods. However, current methods are mainly based on PCR techniques combined often with the hybridization methods to

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specifically detect the PPRV genome and to increase the sensitivity of the diagnostic assays.

PPR has currently emerged as one of the major transboundary emerging diseases (TAD) in many countries of Africa and Asia. Therefore, timely and efficient diagnosis of PPRV is indispensible to reduce its negative impact on rural populations and to save by this way their livelihood since small ruminants remain an important source of revenue for these populations. Before the eradication of rinderpest virus (RPV) from the world, a very closely related virus to PPRV (Gibbs et al. 1979) has emerged, and thus, it was a strong need to make a clear differentiation between RPV and PPRV since both viruses replicate in large and small ruminants, while PPRV causes only subclinical disease in large ruminants and the infecting virus does not spread to contact animals (Diallo et al. 1989; Couacy-Hymann et al. 1995). In this effort, several diagnostic techniques have been developed with different chemistries and sensitivities.

The ultimate objective of any techniques designed to specifically detect PPRV in the field samples should have compliance with the following criteria:

- Sensitive and specific,
- Simple to be implemented on a routine basis,
- Less time-consuming,
- To be applied to a large number of samples at a time, high throughput of diagnostic test.

The molecular techniques offer above-mentioned advantages, in comparison with conventional techniques such as virus isolation or antigen detection. However, the results of the analysis significantly rely upon the quality of field samples. Therefore, it is advisable that laboratories and mainly field staff should have sufficient training on the collection, storage and transportation of samples. Once clinical samples are safely reached to the laboratory, the extracted nucleic acid (DNA or RNA) will then be subjected to different diagnostic methodologies. Besides detection, a stretch of sequence of the F gene (Forsyth and Barrett 1995) or the N gene (Couacy-Hymann et al. 1993, 2002) is required to genetically classify PPRV into 4 lineages. However, it has been demonstrated recently that the N gene is more divergent and therefore more suitable for phylogenetic distinction between closely related PPRVs (Kwiatek et al. 2007; Kerur et al. 2008; Banyard et al. 2010).

9.2 Genome Structure of PPRV

As a member of the *Morbillivirus* genus of the *Paramyxoviridae* family, the genome of PPRV is a non-segmented, single- and negative-strand RNA, which wrapped in a ribonucleoprotein (RNP). The genome length is around 16,000 nucleotides. This genome encodes for six structural proteins, which remain contiguous and non-overlapping proteins. The gene order (N-P/C/V-M-F-H-L) is typical as in other morbilliviruses from 3′ to 5′ ends. There are two more non-structural proteins, C and

V, produced during the infection phase. An intergenic region of variable sequence lengths separates each gene without yet known functionalities (Diallo et al. 1989). PPRV is morphologically pleiomorphic and enveloped particle with two external glycoproteins, F and H (see Chap. 2 for detail).

Regarding PPR molecular diagnosis, mainly the F and N proteins, genes are targeted. Moreover, nucleoprotein from the Np gene is the first among the structural proteins to be produced and in abundance and therefore well indicated for the development of sensitive and specific test.

9.3 Sample Collection

Good-quality samples should be taken from sick animals at the onset of hyperthermia (viremia phase) after it has been slaughtered or on fresh dead animal. Samples that can be used for diagnostic purposes may consist of: lachrymal, nasal and oral swabs, blood for serum and whole blood in EDTA, on live animal and with tissue collected on autopsy: lung, lymph nodes (bronchial, mesenteric), tonsils, spleen along with intestine on slaughtered or freshly dead animals. These collected samples should be rapidly sent to the laboratory on the same day, if possible, stored on ice or liquid nitrogen. In the field conditions, samples can be kept in a solution to prevent RNA degradation such as guanidium thiocyanate (4 M) or Trizol (GIBCO BRL). This procedure reduces the cold chain maintenance during transportation up to the laboratory.

Filter papers for rapid sample collection have been developed as another mean of sample preservation up to the laboratory that is suitable under tropical conditions. This method allows molecular detection and genotyping of viruses when stored over long periods at elevated temperatures. Filter papers with dried blood containing viruses can be cut in small pieces and added directly to the tube for conventional PCR (Michaud et al. 2007). Alternatively, the nucleic acid can be eluted using elusion buffer and used for amplification purposes.

9.4 Laboratory Set-Up for the Molecular Detection

The lack of expertise in performing molecular reactions may lead to generate false-positive results. It is required to use several working rooms or working areas dedicated to these reactions. For example, to perform the PCR technique, a minimum number of working rooms required is three (four rooms are better). Each working room shall be supplied with its specific materials. Tubes containing PCR products must not be opened in previous working rooms to avoid contamination of the atmosphere with DNA molecules, aerosols. Controls (positive and negative) shall always be included in the PCR. Micropipettes should be sterilized using a cross-linker oven for DNA degradation by UV light, and benches should be cleaned with 10 % bleach.

9.5 Molecular Diagnosis of PPR

9.5.1 Hybridization Techniques

The method is a powerful tool for the detection of specific genes and can be applied to the diagnosis of viral, bacterial and parasitic infections (Fig. 9.1). For this method, the nucleic acids need to be extracted from biological samples.

9.5.2 Radioactive Probes

Nucleic acids labelled with radioactive isotopes as probes in clinical laboratories can be used as diagnostic tools for the identification of disease-causing agents. The methodologies coupled with autoradiography provide high degree of sensitivity and resolution (Manak 1993). However, high precautions have to be in place in the

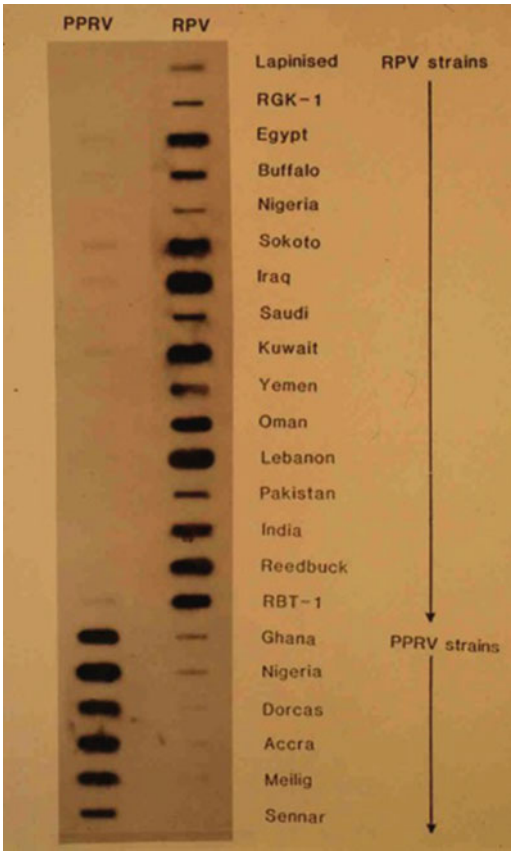

Fig. 9.1 Hybridization of PPRV and RPV cDNAs with RNA from cell infected with a range of PPRV and RPV isolates (Diallo et al. 1989)

laboratory where these radioisotopes are handled to prevent any contaminations. Laboratory workers should wear gloves, working rooms marked with a radioactive sign, bench tops covered with absorbent plastic-backed paper, etc. Special care for radiochemicals and wastes should be taken.

Applied to PPRV, cDNA probe derived from mRNAs for the nucleocapsid protein of PPRV and labelled with [^{32}P] dATP was used to specifically detect PPRV genome in sample. After RNA extraction from infected tissues, the hybridization with the cDNA probe is carried out to confirm the presence of PPR virus by the detection of PPRV genome (Diallo et al. 1989). Oligonucleotides obtained from PPRV genome sequence targeting specific genes can be also labelled with radioactive isotopes as probes for PPRV diagnosis.

Despite real advantages encountered, this technique with radioactive isotopes limits deeply its wide and routine use mainly in developing countries due to the health hazards linked to the radioactive labels as well as cost and disposal of radioactive waste product along with the lack of suitable equipments. Consequently, the radioactive probe techniques are not currently in practice for the diagnosis of PPRV (Couacy-Hymann et al. 2002).

9.5.3 Non-radioactive Probes (Synthetic Oligonucleotide Probes with Non-radioactive Isotopes)

For safety reasons, radioactively labelled DNA probes have been replaced by an alternative non-radioactive DNA labelling and detection versions. The standard procedure can be divided into three parts:

Labelling step: incorporation of a nucleotide analogue (digoxigenin-11-dUTP) into DNA by the random primed labelling technique.

Hybridization step: the digoxigenin-labelled probe is bound to the immobilized DNA.

Detection step: the digoxigenin-labelled probe is detected with an antibody–enzyme conjugate, anti-digoxigenin–alkaline phosphatase. The location of the antibody–antigen conjugate is visualized by an enzyme-linked colour reaction.

Synthetic oligonucleotide, complementary to PPRV-specific genes labelling with the systems digoxigenin-dUTP or biotin-dUTP, biotin-dATP, are used as non-radioactive probes to detect PPRV genes. After hybridization, these digoxigenin-labelled or biotin-labelled probes are detected using an anti-digoxigenin–enzyme or avidin/streptavidin–enzyme conjugates. These systems, including digoxigenin/anti-digoxigenin–enzyme and biotin/avidin or streptavidin–enzyme, are powerful tools for PPR diagnosis. Mainly, these probes are used to detect PCR products in the post-PCRs to increase the sensitivity of the PCR technique (Couacy-Hymann et al. 2002). This hybridization method with a non-radioisotopic element is commonly used in the laboratory for the diagnosis of animal diseases such as PPRV, mainly after RT-PCR, on the amplicons. These amplicons are transferred onto a membrane through

Southern blot or dot-blot techniques and detected by the digoxigenin-labelled probe. The technique is safe, sensitive and specific enough to be preferred to the radioisotope-labelled probe.

9.5.4 Polymerase Chain Reaction (PCR) Technique Applied to PPRV

Currently, different PCR techniques have been developed for the genome detection of PPRV, which range from conventional PCR to reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) PCR with variable sensitivities and specificities (Table 9.1).

9.5.5 Conventional RT-PCR

The PCR has become established as a widely used technique for the diagnosis of animal diseases. It is a technique for in vitro amplification of specific DNA sequences by the simultaneous primer extension of complementary strands of DNA. This technique was applied to the detection of PPRV genome in 1990s. As a RNA virus, it was necessary to carry out the reverse transcription reaction (RT) for the synthesis of cDNA, which is needed in the PCR (Figs. 9.2, 9.3).

Mainly, two conventional PCRs have been developed to amplify viral genes for both detection and characterization purposes. First is based on the amplification of the N gene, which is an internal protein and is the most abundant viral protein among all proteins (Couacy-Hymann et al. 1993, 2002). Second is based on the F protein, which is a surface glycoprotein (Forsyth and Barrett 1995). For either of the assay, extraction of the genomic material is required. Both techniques are similar and can be used for the detection of PPRV genome. Regarding N gene, the design of the primers for use in the RT-PCR assay has been made after the alignment of the second half of the N gene of ten PPRV (wild-type strain plus the vaccine strain). Indeed, RNA viruses are known to high nucleotide substitution error frequencies (Steinhaurer et al. 1989). Therefore, for routine diagnosis of the virus, it is advisable to take into consideration the sequences from several strains from different origins. This strategy has guided the design of the primers NP3/NP4 for PPR diagnosis by RT-PCR for an amplified product of 351 bp. An internal oligonucleotide to the amplified fragment, SP3, was designed to be used as a probe after labelling with the digoxigenin-dUTP.

There is also an alternative method where the genomic nucleic acid is not extracted to perform the reaction, the direct PCR (Michaud et al. 2007). The PCR product is relatively large and can provide a template for sequencing and subsequent phylogenetic analysis. The conventional PCR technique is nowadays widely used for the diagnosis of PPR in countries where it is endemic. It is a specific and sensitive technique, mainly when it is coupled with the hybridization with a specific probe.

Table 9.1 Sequences and hybridization positions of primers and probes for conventional and rRT-PCR currently being used for the diagnosis of PPRV

Primer identification	Locations	Sequence (5′–3′)	References
Conventional RT-PCR (N gene)			
NP3	1,232–1,255	TCTCGGAAATCGCCTCACAGACTG	Couacy-Hymann et al. (2002)
NP4	1,530–1,506	TCAGCCGATCTTTGAGCCTCACGAG	
Probe SP3	1,292–1,316	CAGGCGCAGGTCTCCTTCCTCCAGC	
Conventional RT-PCR (F gene)			
F1 (F)	777–801	ATCACAGTGTTAAAGCCTGTAGAGG	Forsyth and Barrett (1995)
F2 (R)	1,124–1,148	GAGACTGAGTTTGTGACCTACAAGC	
Real-time RT-PCR (N gene)			
PPR_Np_F298	405–428	CGCCTTGTTGAGGTAGTTCAAAGT	Polci et al. (2013)
PPR_Np_R366	473–455	ATCAGCACCACGTGATGCA	
Probe	438–453	6FAM-CAGTCCGGGTTGACCT-MGBNFQ	
NPPRf	1,438–1,461	GAGTCTAGTCAAAACCCTCGTGAG	Kwiatek et al. (2010)
NPPRr	1,516–1,534	TCTCCCTCCTCCTGGTCCTC	
Probe NPPRp	1,472–1,495	FAM-CGGCTGAGGCACTCTTCAGGCTGC-BHQ1	
LAMP-PCR			
N gene position			
F3 forward outer	191–209	19-mer ACATCAACGGGTCAAAGCT	Li et al. (2010)
B3 reverse outer	398–417	20-mer ACTCGAGGGTCCTTCAGTTG	
FIP forward inner	(F1C + TTTT + F2)		
F2,	213–231	44-mer; F1C, 21-mer; F2, 19-mer CCGCTGTATCAATTGCCCGGG-TTTT-CGGCGTGATGATCAGCATG	
BIP	Reverse inner (B1C + TTTT + B2) B1C, 296–317; B2, 358–377	46-mer; B1C, 22-mer; B2, 20-mer GCATCCGCCTTGTTGAGGTAGT-TTTT-TTGTCCAAATCAGCACCACG	

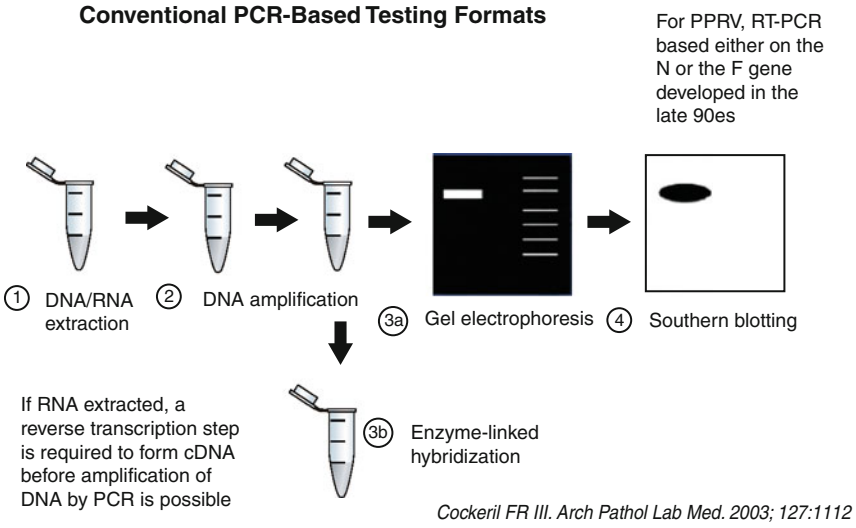

Fig. 9.2 Principle of conventional RT-PCR

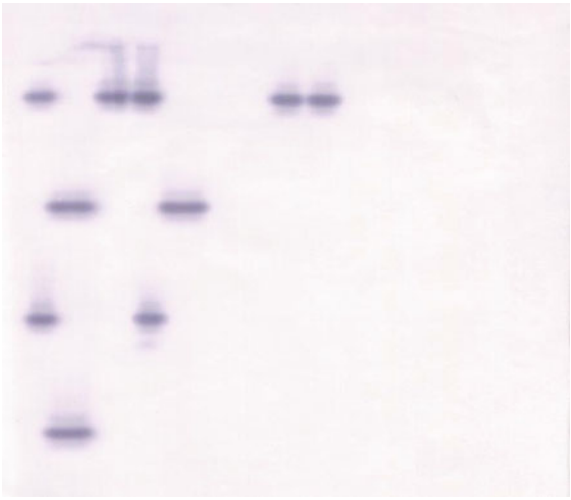

Fig. 9.3 Conventional PCR products were transferred from the gel onto nylon membrane. By Southern blot and probed with a digoxigenin-labelled internal oligonucleotide (Couacy-Hymann et al. 2005)

9.5.6 Real-Time RT-PCR

A wide range of real-time PCR assays have been developed and applied for the diagnosis of animal diseases including PPR. The use of labelled primers facilitates the specificity, compared to conventional agarose-gel-based PCR assays (Buston et al. 2005). Detection of target sequence occurs by monitoring the fluorescence generated by intercalating dyes, fluorophore-labelled primers or sequence-specific

probes (Fig. 9.4). Quantification of target sequences is typically achieved by determining the number of amplification cycles required to generate PCR product and corresponding fluorescence at the beginning of the exponential phase of the PCR, in order to cross a threshold fluorescence line (threshold cycle, Ct). The threshold cycle is the number of cycles required to reach the threshold. In real-time assays, Ct values correlate closely with the original quantity of target sequence. In contrast, conventional PCR endpoint detection occurs in the plateau phase. The combined properties of high sensitivity and specificity, low contamination risk and speed have made real-time PCR technology a highly attractive to tissue culture, or immunoassay-based methods for diagnosing many infectious diseases (Hoffmann et al. 2009). Various real-time detection formats exist; however, the fluorophore-labelled oligonucleotide probes are most widely used for the specific detection of desired sequences, mainly in the hydrolysis probe (5'-exonuclease assay) system with the TaqMan probes (Figs. 9.4, 9.5). Particularly, for the detection of PPRV genome, the TaqMan technology approach has been used by various authors (Bao et al. 2008; Wang et al. 2009; Kwiatek et al. 2010; Batten et al. 2011; Polci et al. 2013).

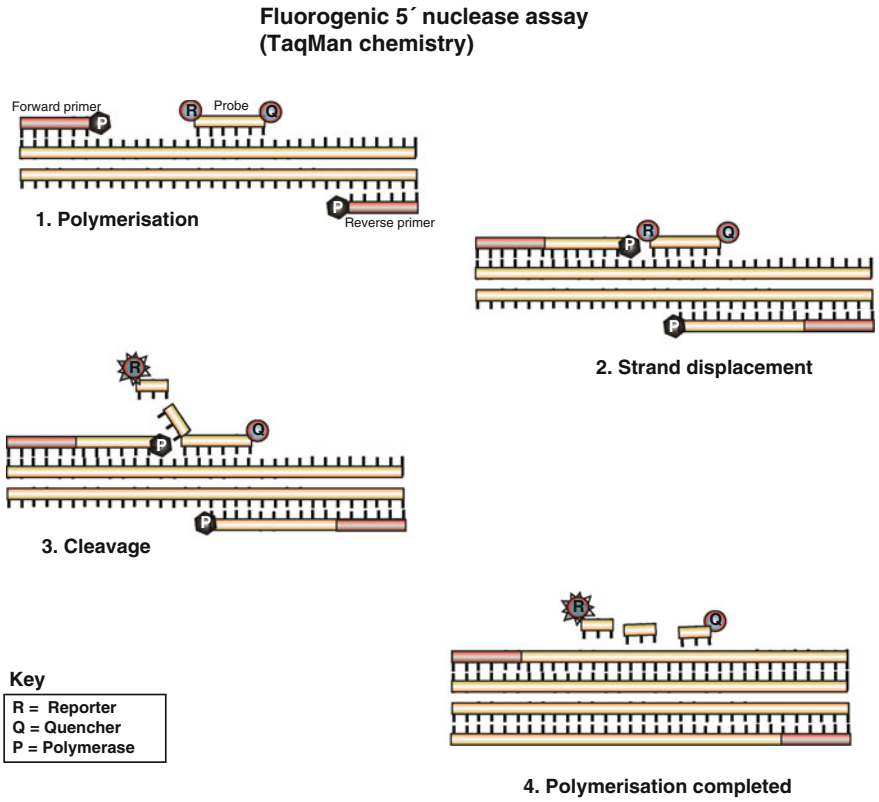

Fig. 9.4 Principle of real-time PCR using TaqMan chemistry (Libeau, Cirad–France)

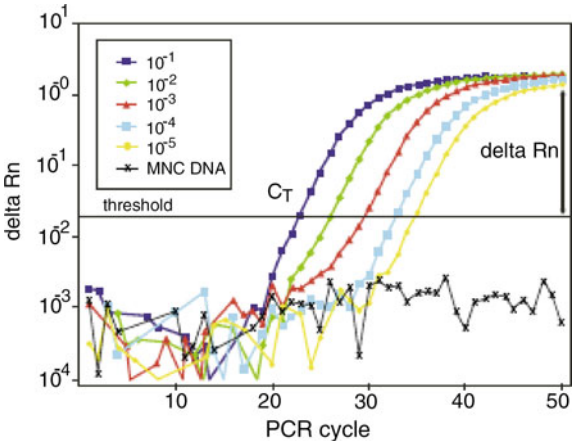

Fig. 9.5 Result and interpretation of curves above the threshold

The real-time RT-PCR is more sensitive to detect low viral RNA load compared to conventional PCR method. In addition, the technique allows the quantification of the targeted viral genome. This is important in viral diagnosis and research studies.

These PCR techniques appeared to be a powerful and helpful tool to conduct the surveillance of PPR in regions where the disease is present after full validation on field samples since virus isolation remains tricky and most laboratories in developing countries do not have cell culture facilities. Both PCR techniques are able to specifically detect four known lineages of PPRV. However, real-time PCR is not common in developing countries such as in African countries, mainly due to the cost of the equipment and the reagents, especially the TaqMan probe.

9.5.7 Internal Control System

The use of internal control (IC) is an important aspect of quality control of RT-PCR assays. The presence of IC in the assay serves to ensure the adequate efficiency of RNA extraction and to confirm the absence of PCR inhibitors in sample what contributes to avoid false-negative results. Co-amplification of an IC increases the reliability of the results and is used to validate negative results.

9.5.8 Various IC Systems Exist

Detection of endogenous gene: it concerns genes that occur naturally in the test sample. These genes should have a constant, basal cell cycle-independent level of transcription that is not influenced by the cellular pathology associated with the disease targeted the PCR assays. Genes with these criteria are named housekeeping

genes including glyceraldehydes-3-phosphate (GADPH), beta-actin, 18S ribosomal RNA, glutamate decarboxylase (GAD), and B2-microglobulin.

Detection of exogenous gene: this type of IC can be designed to contain heterologous target sequence or a complete heterologous viral genome, which is not related to the sequence to be detected. Exogenous IC can be added to each test sample before either the extraction phase or prior to co-amplification in the PCR assay. Depending on the particular design of these ICs, amplification of the control sequence may require additional set of primers to be included in the reaction. Alternatively, mimic ICs (in vitro transcripts, plasmids or chimeric viruses) can be engineered, containing homologous target sequences for the same primer pair as the diagnostic test. Moreover, these mimic ICs can contain internal sequences not present in the target amplicon to yield an amplification product with a different length, which can be differentiated, from wild-type amplicon by a second IC-specific probe. Since these controls are amplified using the assay target-specific primers, a benefit of this type of control is that it directly monitors the performance of diagnostic primer sets, ensuring that the correct PCR components have been added. However, in contrast to the previously described exogenous ICs, these target-specific control systems cannot be transferred to other assays, although a cassette approach can be designed to incorporate primer sequence of multiple diagnostic test (Hoffmann et al. 2009; Polci et al. 2013).

Both internal control systems have been used to perform PCR technique for the specific detection of PPRV genome (Polci et al. 2013).

9.5.9 Multiplex RT-PCR

The multiplex PCR methods are based on the use of multiple primers for the amplification of multiple templates within a single reaction in a single PCR tube. This method is used to diagnose all possible pathogens suspected to be responsible for the disease complex. Even though conventional PCR assays used this multiplex method, real-time PCR is more suitable for multiplexing using individual probes labelled with different fluorophores with a specific colour for each set of primers. It is a cost-effective technique since it gives answers to several diagnostic questions in a single reaction. However, the design of these primers is more complex taking into consideration the possible competition, which could appear among them (Hoffmann et al. 2009; Pestana et al. 2010).

Different authors developed various multiplex systems for the detection of PPRV genome such as a duplex real-time PCR (Polci et al. 2013) and multiplex RT-PCR (George et al. 2006; Yeh et al. 2011). Mainly, N, M and F gene-based multiplex PCR is in use in the laboratory for the specific diagnosis of PPR. However, this multiplex method is not implemented in many laboratories for the diagnosis of PPR or mucosal disease including PPR. In general, simplex real-time PCR is performed for this issue.

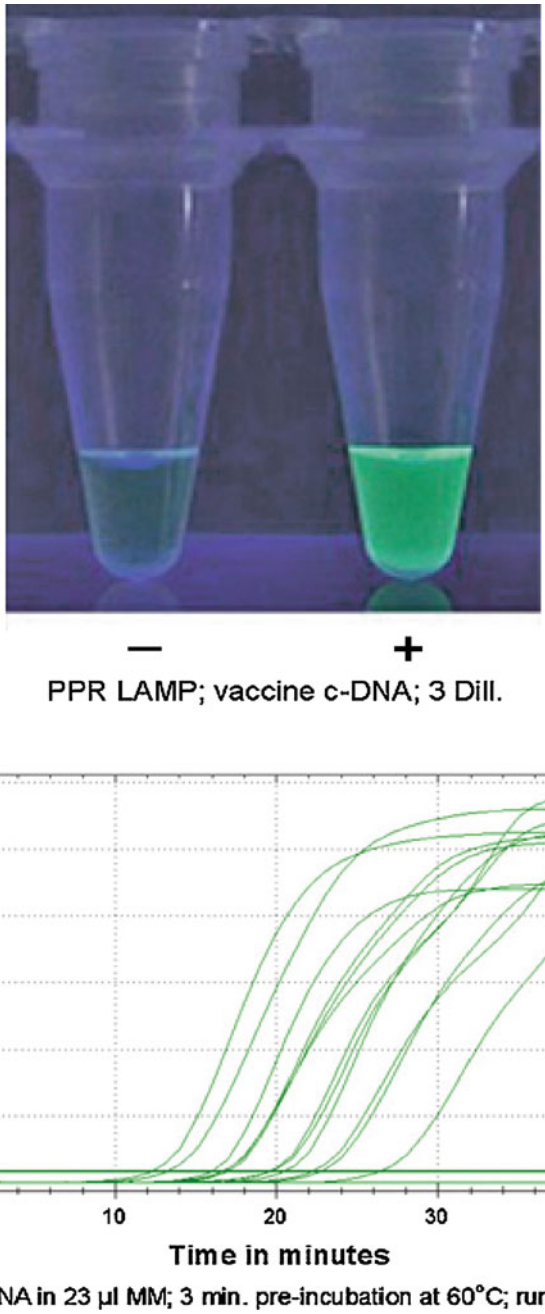

Fig. 9.6 LAMP-PCR results. A: Analysis of RT-LAMP products by visual inspection within tubes. Positive reaction turned green and negative remains orange when fluorescent detection reagent is added to the reaction tube (Li et al. 2010). B: Lamp real-time curves

9.5.10 *Reverse Transcriptase Loop-Mediated Isothermal Amplification Assay (RT-LAMP-PCR)*

LAMP-PCR is a novel PCR technique that can amplify a few copies of DNA in less than an hour under isothermal conditions. The test is simple and easy to perform, as it requires four specific primers, Bst DNA polymerase and a regular laboratory heat block or water bath (Notomi et al. 2000). RNA molecules can be amplified simply by adding reverse transcriptase to the reaction mixture; reverse transcription (RT) and DNA amplification can then be accomplished at a constant temperature in one step (Li et al. 2009, 2010).

The LAMP-PCR technique is a more cost-effective alternative to thermal DNA amplification. The presence or absence of the isothermally amplified DNA product can be detected visually (i.e. a change in colour) (Fig. 9.6). Another advantage of the LAMP-PCR platform is that it can be developed for use on-site or on farm as a pen-side rapid diagnostic test. This new PCR technique has been applied to the diagnosis of PPR targeting the matrix gene (Li et al. 2010) or the NP gene (Wei, unpublished data) of PPRV. However, the LAMP-PCR has not been fully validated on a large number of field samples. The LAMP products can be analysed by naked eye (Fig. 9.6). This on going process is planned for the effective implementation of this technique throughout developing countries. Its low cost and the possibility to use it in the field as a pen-side test make it a useful technique. It needs to be fully validated and implemented in countries for several diseases. These powerful tools used for the diagnosis allow the detection of any lineages of PPRV. The sequencing method is used to differentiate these lineages.

9.6 Conclusions

The efficient control of PPR is ensured by a rapid diagnosis of the disease in order to stop its diffusion and to put in place adequate control measures. Current genome detection technologies offer the possibility to achieve this main objective. For this purpose, it is necessary to present a good quality of samples to make them applicable.

Since 1990s, improved technologies to diagnose animal diseases exist, besides the conventional ones, to better ensure food security and poverty alleviation. These new technologies are more sensitive, specific and cost-effective diagnostic tools. Most of these novel techniques have been applied to the diagnosis of PPR. Assays involving PCR yield specific DNA products that can be investigated and analysed by several means such as conventional sequencing or deep sequencing. The data generated from these analyses help to set up a phylogenetic study for the molecular epidemiology purposes.

These assays allow detecting any strains of PPRV, regardless of lineages. Moreover, technique such as LAMP-PCR needs less sophisticated equipment with

a reduced cost and able to be applied directly in the field conditions. Multiplex PCR and microarray techniques applied to the diagnosis of animal diseases including PPR open a new way of disease investigation, giving the opportunity to target several causative agents in a single reaction, decreasing time consumption and cross-contamination and improving early response to control diseases.

Recently, the existence of more than one lineage has been reported in single country. Moreover, due to the fact that disease is emerging and one lineage may have superior character to overwhelm or dry out other lineage(s), it is advantageous to wisely design diagnostic assays that can detect and differentiate all four lineages simultaneously. Efforts for the development of these techniques have been started; however, currently, no such technique has been published.

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Chapter 10
Host Immune Responses Against Peste des Petits Ruminants Virus

Gourapura J. Renukaradhya and Melkote S. Shaila

Abstract The immune responses to morbilliviruses generated in the respective hosts are contributed by the hosts' innate immune system as well as the adaptive immune system. While the innate immune responses against peste des petits ruminants' virus (PPRV) have not been investigated till date to any extent, a limited number of reports exist providing information on the adaptive immune responses elicited to either PPRV infection or to vaccination. For other members of this genus, specifically measles virus, cellular and molecular level investigations have thrown light on the functioning of the molecules of the innate immune system as well as details of humoral and cellular immune mechanisms. This chapter highlights the limited knowledge on the humoral and cell-mediated immune responses in sheep/goats upon infection by PPRV or vaccination. A small section is devoted to provide information on what is known about immune suppressive effects of morbillivirus infections in general and PPRV infection wherever known.

10.1 Introduction

Peste des petits ruminants (PPR), also known as "goat plague," is an acute, highly contagious viral disease of goat and sheep, caused by peste des petits ruminants virus (PPRV), which is a member of the genus *Morbillivirus* under the family *Paramyxoviridae* (Rowland et al. 1969, 1971). PPR disease is prevalent in West

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and Central Africa, the Middle East, and Southern Asia (Nanda et al. 1996; Shaila et al. 1996). It is also spread to central Asia and China (Kwiatek et al. 2007; Wang et al. 2009). Our understanding of the mechanisms used by morbilliviruses to induce a protective immune response in their hosts or their abilities to interfere with the host immune responses is limited to measles virus to a large extent and to canine distemper virus (CDV) as well as rinderpest virus (RPV) to a certain extent. A limited number of investigations have been reported that throw light on the immune responses elicited by HN protein of PPRV, and there is even lesser information on the F protein-induced immune responses. This chapter reviews PPRV-induced immune responses in relation to what is known with other morbilliviruses, during natural infection and after vaccination with different types of vaccines. Further, immunosuppression induced by morbillivirus infection of the host or cells in vitro is reviewed.

10.2 Immune Responses During Natural Infection

Morbillivirus infections cause significant mortality and morbidity in both humans and animals, and the viruses belong to the family paramyxoviridae. The important members of this genus include measles virus (MV) of humans, RPV of large ruminants, PPRV of small ruminants, CDV of dogs, and a couple of other viruses which cause disease in marine mammals (Barrett 1999). The viral components of the host-derived envelope consists of two surface glycoproteins, the hemagglutinin (H) and the fusion (F) proteins, are responsible for viral attachment to and fusion with the host cell membrane, respectively. The other structural proteins of these viruses are nucleocapsid (N) protein which encapsidates the viral RNA and matrix (M) protein which lies beneath the envelope. The RNA polymerase proteins L and P are associated with the nucleocapsid protein to form the viral nucleocapsids (see Chap. 2 for detail). There are limited number of studies, which have examined in detail and the immune responses during natural infection by most morbilliviruses in general and PPRV in particular.

10.2.1 Role of Humoral Immunity

Humoral immune responses have been shown to be important for infection outcome with CDV and phocine distemper virus (Cosby et al. 1983; Rima et al. 1990). For RPV, Lund's group has shown that animals infected with a virulent strain of RPV failed to develop an antibody response, which is suggestive of severity of the disease (Lund et al. 2000). In the case of MV, it has been shown that after passive antibody transfers to Cotton rats, MV vaccine-induced immunity and protection from challenge is inhibited (Schlereth et al. 2003). Acute encephalitis caused by CDV infection in hamsters has been shown to be reduced in the presence of

maternal antibody (Cosby et al. 1983). Low levels of maternal antibody have been detected in the sera of cattle, sheep, and goats born to RPV- and PPRV-infected mothers (Ata et al. 1989; Libeau et al. 1992).

10.2.2 Role of Cell-Mediated Immunity

Although there is no direct evidence for the role of cell-mediated immunity during natural infection of cattle with RPV aiding the recovery process, it is important for protection by vaccination against challenge infection. Yamanouchi and his group has shown that whole body irradiation with X-ray aggravated the clinical course of RPV infection, leading to an increased fatality rate, suggesting an important role for bone marrow cells in recovery from RPV infection (Yamanouchi et al. 1974). While the relative importance of cell-mediated and humoral immunity has not been deciphered in studies with PPRV and CDV, the contribution of each has been examined in animal models with MV. The contribution of the cytotoxic T lymphocyte (CTL) response for protection against MV infection in rhesus monkeys has been shown to be much higher than the B cell response (Permar et al. 2004).

10.2.3 Protective Antigens of Morbilliviruses

The surface glycoproteins of CDV, MV, RPV, and PPRV have been shown to possess protective antigenic properties. The H and F proteins of CDV expressed in vaccinia or canarypox viruses have been tested successfully for their immunogenicity in dogs (Pardo et al. 1997). All vaccinates seroconverted to CDV and serum antibody titers were comparable to modified live virus-vaccinated animals. In a challenge exposure study, neither morbidity nor mortality was observed in vaccinates; while in control dogs, 100 % morbidity and 86 % mortality were observed. Similarly, vaccinated ferrets survived the CDV challenge, and none of the ferrets developed viremia. However, ferrets which received the modified live virus vaccine lost weight became lymphocytopenic and developed the erythematous rash typical of CDV (Stephensen et al. 1997). These data showed that ferrets are an excellent model for evaluating the ability of CDV vaccines against symptomatic infection, because the pathogenesis and clinical course of CDV infection of ferrets are quite similar to that of other morbillivirus infections, including measles. It has been shown for MV that the F protein contributes to protective immunity (Malvoisin and Wild 1990). Employing a vaccinia-measles virus F recombinant, anti F monoclonal antibodies have been generated which neutralize virus infectivity, as well as passively protect mice when challenged intracerebrally with virulent MV. A double recombinant vaccinia virus expressing the F and H genes of RPV protects cattle against RP (Giavedoni et al. 1991). Cattle vaccinated with the recombinant virus were 100 % protected from challenge inoculation with greater than 1,000 times the

lethal dose of RPV. Further, no transmission of recombinant vaccinia virus from vaccinated animals to contact animals was observed.

The cross-protective nature of protective antigens of morbilliviruses has been reported, for example, recombinant RPV H and F candidate vaccine cross-protects against PPRV (Romero et al. 1995). Both MV and RPV recombinant vaccines cross-protect ferrets and dogs from virulent CDV infection in short-term trials (Jones et al. 1997; Taylor et al. 1991). Recombinant capripoxvirus expressing RPV H (Romero et al. 1994a) and RPV F (Romero et al. 1994b) elicits protection in cattle against RPV challenge; also protect goats against PPRV challenge by eliciting adequate B and T cell responses (Romero et al. 1995). While the H recombinant capripox virus produced high titers of neutralizing antibody to RPV in the vaccinated goats, and the F recombinant capripox virus failed to stimulate detectable levels of neutralizing antibody (Romero et al. 1995). The recombinant capripox virus induces significant levels of neutralizing antibodies in vaccinated cattle and protects them from clinical RP in challenged cattle with a lethal dose of a highly virulent heterologous strain of the virus. Protection was achieved using vaccine doses lower than those used with a similar recombinant expressing the fusion protein gene of RP (Romero et al. 1994a).

10.2.4 Immune Responses After Vaccination

10.2.4.1 Live Attenuated Vaccine

Bivalent PPRV and goat poxvirus vaccine were found to be safe and induce protective immune response in goats as evident from seroconversion as well as results of challenge studies (Hosamani et al. 2006). Similarly, a combined sheep pox and PPRV vaccine also protects against both the viral challenges (Chaudhary et al. 2009).

10.2.4.2 Replicating Recombinant Vaccine

Capripox virus is a highly host-specific virus, with the host range restricted to cattle and small ruminants. The recombinant capripox virus (rCPV) that expresses PPR F protein has been shown to protect goats against both PPR and Capri pox challenge at a dose as low as 0.1 pfu (Berhe et al. 2003). The immunogenicity studies using a single dose of rCPV expressing PPRV H and F glycoproteins have indicated that only rCPV-PPRV HN induces VN antibody response (but not rCPV-PPRVF) in both goats and sheep (Chen et al. 2010). Goats vaccinated with RPV H and F double recombinant vaccine were protected against PPRV challenge, but the vaccine failed to elicit virus-neutralizing antibody response against PPRV, suggesting the important role played by cell-mediated immunity in PPRV clearance (Jones et al. 1993). It is likely that the F protein may contribute more to cell-mediated immunity.

An infectious recombinant canine adenovirus type 2 (rCAV-2), which expresses the HN protein of PPRV has been generated (Qin et al. 2012). The rCAV-2-PPRV H vaccine was administered to goats through the intramuscular route (but not by either oral or intranasal route) elicits enhanced levels of virus-neutralizing antibody response. Virus-neutralizing antibody was detected in goats for at least 7 months; also, this vaccine induced cell-mediated immune responses. A chimeric RPV vaccine with F and H proteins of PPRV elicited protective immune response in the challenge study, but the rescued virus grew very slowly in the tissue culture (Das et al. 2000). Further, the growth characteristic of the chimeric virus is rescued by incorporating the homologous M, along with the F and H proteins of PPRV into the RPV vaccine strain (Mahapatra et al. 2006).

10.2.4.3 Non-replicating Subunit Vaccine

Immunoaffinity purified HN and F proteins of PPRV, purified from virus-infected Vero cells have been shown to elicit virus-neutralizing antibodies in immunized rabbits. These immune rabbits were protected against challenge with lapinized RPV. PPRV HN protein was shown to confer better protection than F protein (Devireddy et al. 1998). The HN protein of PPRV has been expressed in transgenic peanut (*Arachis hypogeal L)* plants in a biologically active form. The immunogenicity of the peanut plant-derived PPRV HN protein vaccine administered to sheep by oral route elicited increased virus-neutralizing antibody response, as well as cell-mediated immune responses in the absence of any mucosal adjuvant. The virus-neutralizing antibodies were present at significant levels by 5-week post-oral immunization. Further, the animals exhibited enhanced cell-mediated memory response upon injection of subimmunogenic dose of the live attenuated vaccine (Khandelwal et al. 2011). These results are promising and indicate that recombinant subunit oral vaccines could be tested as DIVA vaccine for PPR eradication. However, the ability of the plant expressed HN to protect target animals from challenge infection with virulent virus has not been investigated.

An immunodominant antigenic region of MV F (p32; amino acids 388–402) represents an amino acid sequence within the highly conserved cysteine-rich domain of the F protein of paramyxoviruses. Epitope mapping of p32 peptide indicated that the complete 15 amino acid sequence is necessary for high-affinity interaction with anti-MV antibodies. Immunization of mice with the p32 peptide induces antipeptide antibodies, which neutralize the MV infectivity in vitro. Further, passive transfer of antipeptide antibodies confers significant protection against fatal rodent-adapted MV-induced encephalitis in susceptible mice. These results indicate that the p32 peptide of MV F represents a candidate for inclusion in a future peptide vaccine against MV (Atabani et al. 1997). Presence of such a functional region on other morbilliviruses including PPRV cannot be ruled out, but it needs further investigation.

10.3 Identification of B and T Cell Epitopes on Structural Proteins of PPRV

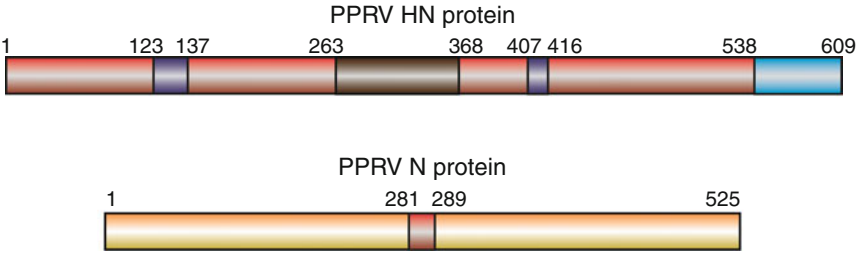
The HN protein of PPRV binds to its cognate receptor on the host cell during the first step of viral infection process, and it acts as a major antigen that stimulates protective immune response in the host (Berhe et al. 2003; Das et al. 2000; Sinnathamby et al. 2001). Unlike other morbilliviruses, the H protein of PPRV apart from having the hemagglutinin activity (Ramachandran et al. 1995) has a detectable neuraminidase activity, and thus it is termed as hemagglutinin-neuraminidase (HN). Transiently expressed PPRV HN protein has been shown to be biologically active in possessing hemadsorption and neuraminidase activities (Seth and Shaila 2001). The presence of neuraminidase activity in PPRV HN protein is unusual among members of the morbillivirus genus.

10.3.1 B Cell Epitopes on PPRV HN Protein

B cell epitopic regions having PPRV neutralizing antibody epitopes on the HN protein have been mapped employing four different monoclonal antibodies generated against recombinant baculovirus particles expressing the HN protein on the surface. The epitopic regions were mapped using deletion mutants of PPRV HN and RPV H proteins expressed in *E. coli* as well as PPRV-HN deletion proteins expressed transiently in mammalian cells (Renukaradhya et al. 2002). Two discontinuous regions of HN comprising of amino acids 263–368 and 538–609 react with four monoclonal antibodies, indicating that the epitopes are conformational. The binding regions for three mAbs were shown to be immunodominant by employing competitive ELISA using serum samples of PPRV-vaccinated sheep. All the four mAbs have been shown to neutralize the virus infectivity in vitro, while two of the mAbs are able to inhibit neuraminidase activity.

10.3.2 T Cell Epitopes on PPRV HN Protein and N Proteins

In goats immunized with low doses of purified recombinant extracellular baculovirus carrying a membrane bound form of PPRV HN protein, both humoral and cell-mediated immune responses are elicited. Antibodies generated in the immunized goats neutralized both PPRV and RPV in vitro. Using a combination of *E. coli* expressed deletion mutants of PPRV-HN and RPV H proteins, and synthetic peptides corresponding to the highly conserved N-terminal sequences of MV H protein, N-terminal T cell determinant (possibly Th epitope) has been mapped—which corresponds to the N-terminal region between amino acids 123–137 (Sinnathamby et al. 2001). Further, in the same study, a C-terminal domain (amino acids 242–609)



Functional T and B cell epitopes on PPRV HN and N proteins

Virus Protiens	Epitope	Amino Acid region	References
PPRV HN	Th cell	123-137	Sinnathamby et al. 2001
PPRV HN (also in RPV H)	CTL	407-416	Sinnathamby et al. 2001
PPRV HN	B cell 4 epitopes	263-368 & 538-609	Renukaradhya et al. 2002
PPRV N (also in RPV N)	CTL	281-289	Mitra-Kaushik et al. 2001

Fig. 10.1 Functional T and B cell epitopes on PPRV HN and N proteins. A table showing the amino acid regions of identified functional Th cell, CTL, and B cell epitopes of PRRV HN and N proteins. Both the CTL epitopes overlap with the respective RPV H and N proteins. A near-to-scale schematic diagram of location of indicated epitopes of PRRV HN and N proteins is also shown

harboring potential T cell determinant(s) in goats has also been revealed. CTL-specific for a conserved region on HN of PPRV and H of RPV have also been demonstrated. Using lymphocytes of cattle immunized with a recombinant extracellular baculovirus (rECV) expressing RPV H and autologous skin fibroblasts transiently expressing truncations of both H and HN proteins in a BoLA class I restricted lymphoproliferation assay, a conserved epitope (407–416 amino acids) on these two proteins has been shown to function as a potential CTL epitope (Sinnathamby et al. 2004) (Fig. 10.1).

The N protein of MV and CDV has been shown to induce strong cell-mediated immune response, which aid in protective immunity (Cherpillod et al. 2000; Hickman et al. 1997). The most frequently recognized T cell epitopes in both naturally infected and vaccinated donors were located in the genetically heterogeneous C-terminal half of the N protein. Analysis of patterns of peptide reactivity among vaccinated and naturally infected subjects has identified the peptides, 221–240 and 237–256, in 100 % of naturally infected donors and in 37.5 % of vaccinated donors. Thus, MV N protein-specific T cells constitute a major fraction of the virus-specific memory cells (Hickman et al. 1997). Employing assembled recombinant nucleocapsids of N protein expressed in *E.coli*, a highly conserved MV-specific CTL epitope has been mapped to the N proteins of PPRV and RPV in the region 281-289 amino acids in the mouse model (Mitra-Kaushik et al. 2001). The murine CTL epitope functions in the bovine system as a CTL epitope. Since this sequence is conserved in the N proteins of morbilliviruses, it conforms well to

the predicted algorithm for some of the most common BoLA CTL antigenic peptides. The relative contribution of this CTL epitope in protection against PPRV infection remains to be elucidated.

10.3.3 Conserved Epitopes on F Protein Is Shown to Be Protective in MV

An immunodominant antigenic region from the MV F protein (amino acids 388–402) was found to represent a highly conserved cysteine-rich domain of the F protein of paramyxoviruses. Epitope mapping of this peptide sequence indicates that the complete 15-amino acid sequence is necessary for high-affinity interaction with anti-MV antibodies. Immunization of mice with the peptide (amino acids 388–402) induces antipeptide antibodies, which cross-react with and neutralize MV infectivity. Passive transfer of antipeptide antibodies conferred significant protection against fatal rodent-adapted MV-induced encephalitis in susceptible mice. These results indicate that epitopes region (amino acids 388–402) represents a candidate for inclusion in the future peptide vaccine for measles (Atabani et al. 1997).

In sera from naturally MV immune donors, the majority of VN antibodies are specific to MV H protein, but up to 10 % of neutralizing antibodies are specific for MV F protein (de Swart et al. 2009). A chimeric recombinant protein having parts of the ectodomains of HN and F proteins of PPRV surface-displayed on a baculovirus has been tested for its immunogenicity in the mouse model. This was done by inserting the F and H protein sequences having the epitopes in-frame within the amino-terminal region of Bombyx mori nuclear polyhedrosis virus envelope glycoprotein GP64 expressed under the strong viral polyhedrin (polh) promoter. The high levels of virus-neutralizing antibody generated in mice have shown the usefulness of properly displayed epitopic regions of both HN and F proteins to elicit virus-neutralizing antibodies (Rahman et al. 2003).

10.4 Immunosuppression by Morbilliviruses

It has been known that morbillivirus infections lead to the development of an acute immunosuppression in their natural hosts (Griffin and Bellini 1996). The immunosuppression caused by MV modulates the cell-mediated immune responses (Borrow and Oldstone 1995). The immunosuppression caused by MV is not restricted to natural infection, but can also occur after vaccination with live attenuated measles vaccine (Hussey et al. 1996). CDV infection also results in short- and long-term immunosuppression, leading to secondary infections (Krakowka et al. 1980). RPV infection is also followed by lymphodepletion in different lymphoid tissues (Wohlsein et al. 1995), and vaccination of cattle with live attenuated vaccine decreases proliferative responses of leukocytes upon stimulation with a mitogen

(Lund et al. 2000). PPRV infection has been shown to induce lung infection which is often followed by secondary bacterial infection (Lefevre and Diallo 1990). Both virulent and attenuated PPR viruses have been shown to cause marked immunosuppression in goats as seen by leucopenia and lymphopenia, reduced antibody responses to both specific and nonspecific antigens (Rajak et al. 2005). The magnitude of immunosuppression is higher during the acute phase of the disease, while the vaccine virus-induced immunosuppression is transient and immune response to nonspecific antigen is not significantly affected.

Morbillivirus proteins have been shown to inhibit the immune response in the absence of viral replication both in vivo (Marie et al. 2001) and in vitro (Heaney et al. 2002). It has been shown that the N protein of MV, CDV, and PPRV brought about the development of both delayed-type hypersensitivity response and contact hypersensitivity response in C57/BL/6 mice (Kerdiles et al. 2006). Further, the work of Kerdiles's group has shown that N protein of morbilliviruses including PPRV N protein interacts with the FcγRII receptor (Kerdiles et al. 2006). This work has suggested that since FcR plays an important immunoregulatory role, its engagement by N protein during MV infection may be responsible for some of the immunosuppressive effects during infection. In an in vitro system, Schlender's group have shown that proliferation of naïve peripheral blood lymphocytes from patients shows significantly reduced capacity to proliferate in response to mitogens, allogens, or recall antigens (Schlender et al. 1996). Further, these authors have identified that a complex of both F and H proteins is critically involved in triggering MV-induced suppression of in vitro proliferation of lymphocytes.

10.5 Conclusions

In conclusion, the host immune responses against either PPRV infection or vaccination employing a variety of replicating and non-replicating vaccines including isolated or recombinant protective antigens have been reviewed. The relative roles of humoral and cell-mediated immunity in protection have been discussed. From the survey of published works, it is evident that there is a need to intensify research on the generation of an effective candidate vaccine which possesses the immunodominant epitopes of both the protective antigens, covering B cell and T cell epitopes, either as surface-displayed virus-like particles or as a non-replicating mucosal vaccine which could be employed as a DIVA vaccine.

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Chapter 11
Vaccines Against Peste des Petits Ruminants Virus

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Abstract *Peste des petits ruminants* (PPR) is an acute and highly contagious viral disease of sheep, goats and wild ruminants. The disease is controlled by the use of live-attenuated vaccines that give a lifelong immunity. Currently, several PPR vaccines are available for use in the field that are based on the tissue culture passage attenuation of wild-type PPR isolates. Four such preparations, viz., PPRV/Nigeria/75, PPRV/Sungri/96, PPRV/Arasur/87 and PPRV/Coimbatore/97 are licensed for use. The PPR vaccines comprising of PPRV/Nigeria/75 and PPRV/Sungri/96 are commercially available. While highly efficacious, a drawback to these vaccines is the requirement of a cold chain to preserve vaccine titre in the field. Thermostable live-attenuated vaccines have recently been developed in an attempt to circumvent the problems associated with the maintenance of a cold chain in tropical and subtropical countries. Despite this issue, targeted vaccination programmes for PPR in high-risk populations of sheep and goats constitute an effective control strategy. Lessons learnt from the rinderpest eradication programme suggest that the availability of a DIVA vaccine, to enable the differentiation between infected and vaccinated animals (DIVA), and a companion diagnostic test would greatly aid both the control and the surveillance for PPRV although it is recognised that eradication could be achieved in the absence of these tools. In this chapter, we discuss both the current and potential future vaccine strategies for PPRV and highlight elements of vaccines that are highly desirable in newer vaccine preparations.

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11.1 Introduction

Historically, the use of vaccines to protect small ruminants against *peste des petits ruminants* virus (PPRV) started with the recognition that heterologous rinderpest vaccine (Tissue Culture Rinderpest Vaccine—TCRP) could be successfully used to protect sheep and goats (Gibbs et al. 1979). Following the characterisation of PPRV as a distinct viral entity within the *Morbillivirus* genus (Mornet et al. 1956; Gibbs et al. 1979), the knowledge that rinderpest virus (RPV) and PPRV were antigenically closely related enabled use of the TCRP vaccine to protect small ruminants (Bourdin et al. 1970; Dardiri et al. 1976; Plowright and Ferris 1962). Interestingly, immunisation of goats with TCRP vaccine was found to elicit neutralising antibodies against RPV but not against PPRV. However, all TCRP-vaccinated animals survived challenge with PPRV and rising levels of PPRV-neutralising antibody activity was reported (Taylor 1979; Taylor and Abegunde 1979). This feature of vaccination suggested that the attenuated vaccine was able to replicate in animals, albeit transiently, and resulted in the TCRP vaccine being successfully used to protect small ruminants against PPR disease. Initial studies reported that the resultant immunity lasted for at least one year (Taylor 1979) although later studies suggested the duration of immunity to be significantly longer (Diallo et al. 2007). The cross-protection afforded by TCRP vaccine was probably following the induction of neutralising antibodies to the F protein which was highly conserved between the two viruses (Diallo et al. 2007). As the rinderpest eradication programme gathered momentum, it was recognised that the use of the TCRP vaccine in small ruminants interfered with the sero-surveillance programme and use of this heterologous vaccine to protect against PPR was prohibited (Anderson and McKay 1994). Following this action, there was an urgency to develop a homologous PPR vaccine to combat the disease. In the process of making a homologous PPR vaccine, the first attenuated vaccine developed against PPR was the Nigerian PPR vaccine incorporating the lineage I African isolate, PPRV/Nigeria 75/1 (Diallo et al. 1989). Subsequently, three PPRV vaccines were developed in India using lineage IV PPRV isolates (PPRV/Sungri/96, PPRV/Arasur/87, PPRV/Coimbatore/97 isolates) (Sreenivasa et al. 2000; Palaniswamy 2005) and one PPR vaccine was developed in Pakistan, again using the Nigerian PPRV isolate (PPRV/Nigeria/75/1) (Asim et al. 2009). As for RPV, the presence of only a single serotype of the virus has meant that each vaccine has been of great utility in several areas as the vaccines induce sterile immunity regardless of the lineage of PPR circulating. This feature of these viruses ultimately make them relatively viable targets for eradication and was a factor of great importance during the RPV eradication campaign where for the majority of areas only a single vaccine was required to protect against all three circulating lineages of RPV (Roeder 2011).

11.2 Attenuation of PPRV in Cell Culture

The history of the development of morbillivirus vaccines starts with attempts to attenuate rinderpest virus. Numerous different approaches were taken to generate a truly attenuated virus for vaccination purposes and it was not until adaptation to tissue culture passage was successful that a viable attenuated vaccine was produced (Plowright and Ferris 1959a, b). Following the adoption of this practice to both rinderpest and measles viruses, attempts were made to make a homologous live-attenuated vaccine for PPRV. The practice of tissue culture cell attenuation of PPRV was first attempted in vitro in sheep liver cells by Gilbert and Monnier (1962). In these cells, the infection was seen to cause a cytopathic effect that took the form of large fusions of cells within a monolayer termed syncytia. Subsequently, Laurent (1968) investigated this cytopathic effect in different cell systems and reported that PPRV-specific effects are manifested mainly by the formation of refringent and rounded cells which gradually detach themselves. Staining with hematoxylin and eosin (H&E) of the PPRV-infected cell monolayer led to visualisation of syncytia and mini-syncytia. These types of polykaryons had previously been described by Plowright and Ferris (1959a) for RPV upon infection of calf liver cells. For PPRV infection of tissue culture cells, polykaryons were reported to be mainly found during the initial stages of infection. During attempts to attenuate the virus, Gilbert and Mornier (1962) noticed that the virus remained highly pathogenic following 6 passages, but by 12 passages, it only caused mild pyrexia in vivo. Despite initial studies that showed promise, these attempts to develop a completely attenuated PPR vaccine were unsuccessful (Benazet 1973).

11.3 Conventional Live-Attenuated Vaccines

11.3.1 The African Nigeria 75/1 Vaccine

Diallo et al. (1989) finally succeeded in developing a homologous live-attenuated PPR vaccine by serial passage of the Nigeria/75/1 isolate in Vero cells. The virus strain used by Diallo et al. (1989) was isolated in sheep liver cell culture from a goat that had died from PPRV infection (Taylor and Abegunde 1979). Passage attenuation in tissue culture showed that the mutations that led to true attenuation could vary at subsequent passage levels. Indeed, following initial passage, syncytia formation was not observed until 4–6 days following infection; however, further passage of the virus in cell culture appeared to enhance the replicative efficiency of the virus with a reduction in the time to the observation of syncytia to almost 2 days. By passage 20, the in vivo virulence was reduced greatly, while by 55 passages, the virus only caused a mild hyperthermia in the animals and was finally reported as being completely attenuated by the 63rd passage. Importantly, at this passage level, the vaccine was able to induce a neutralising antibody response from

7 days post-vaccination (Diallo et al. 1989). Numerous field trials were conducted using this vaccine with more than 98,000 sheep and goats being vaccinated with this preparation between 1989 and 1996 (Couacy-Hymann et al. 1995). The vaccine was found to be safe even in pregnant animals, produced solid immunity following a single vaccination that lasted for a minimum of 3 years and was also able to protect ruminants against RPV in a manner similar to that seen through the application of the TCRP to protect small ruminants from PPRV (Couacy-Hymann et al. 1995).

11.3.2 The First Indian Vaccine (IVRI)

In India, the Indian Veterinary Research institute (IVRI) developed a live-attenuated PPR vaccine using an indigenous isolate of PPRV (PPRV/Sungri/96) isolated from a goat in 1996 from Sungri village in Himachal Pradesh. This virus was initially adapted to in vitro growth with nine passages in a marmoset lymphoblastoidal cell line (B95a) before subsequent passage in Vero cells for a further 50 passages after which the virus was found to be suitably attenuated in vivo (Sreenivasa et al. 2000). The Sungri/96 vaccine has been tested extensively in both experimental and field trials and is safe and highly effective in small ruminants. Using this vaccine, studies have been undertaken with respect to pathogenicity and immunogenicity at various in vitro passages (Sreenivasa et al. 2000) as well as on the thermostability of the preparation (Sarkar et al. 2003). The vaccine virus at passage 59 proved to be completely safe and did not revert back to virulence following five in vivo passages in small ruminants (Sarkar et al. 2003). Studies on the immunomodulatory effects of this vaccine virus revealed that the vaccine caused a transient lymphopenia, which was not considered to cause a biologically significant immunosuppression (Rajak et al. 2005). Furthermore, the vaccine was found to be safe in pregnant animals and conferred solid immunity to PPRV challenge virus (Sreenivasa et al. 2000). The duration of protective immunity following vaccination has been reported as being more than 6 years, long past the economic life span generally considered for small ruminants (Saravanan et al. 2010a). As such, the PPR vaccine of Indian origin (PPRV/Sungri/96) is safe for mass vaccination campaigns under field conditions and has the potential to be used effectively in endemic areas where lineage IV virus is circulating.

11.3.3 Further Indian Vaccines (TANUVAS)

Two more vaccines based on Indian strains of PPRV have been developed at the Tamil Nadu University of Veterinary and Animal Science (TANUVAS), India. These vaccines, PPRV/Arasur/87 (from a virulent sheep isolate) and PPRV/Coimbatore/97 (developed from an isolate from an infected goat), were attenuated following 75

passages in Vero cells. Both laboratory and field trials confirmed their complete attenuation (Palaniswamy 2005), and despite different virological properties and backgrounds (Singh et al. 2010), both are effective (Saravanan et al. 2010b). The two vaccines have found utility in the southern states of India to protect the sheep and goat population from PPRV.

11.4 The Impact of Mass Vaccination Using the Sungri/96 Vaccine in India

Outbreaks of PPR in India continue to be reported, but some outbreaks can go unreported due to geographical remoteness, lack of diagnostic tests and inadequate reporting systems. However, the availability of diagnostic tests and reagents from 2002 onwards (Singh et al. 2004a, b) and efforts of the government towards improving the reporting systems has led to a considerable increase in the number of outbreaks reported since 2002. Similarly, a decline in the number of PPR outbreaks after 2005 coincides with the application of mass vaccination efforts initiated in 2004 under the Assistance to States for Control of Animal Diseases (ASCAD) programme, funded by the Indian government (Singh 2011). Alongside this, the production and supply of vaccines by government bodies like the Institute of Animal Health and Veterinary Biologicals (IAHVBs) in Bangaluru and Hyderabad and private companies (e.g. Indian Immunologicals Limited, and Intervet India Pvt Ltd.) have aided the situation in India.

11.5 Worldwide Availability of Conventional PPR Vaccine

The utilisation of different vaccine preparations within different regions is illustrated by Sen et al. (2010). From this, it is clear that only the Nigerian (PPRV/75/1) vaccine and the Indian (PPRV/Sungri/96) vaccine have been used extensively. The PPR Sungri/96 vaccine has been used mainly in India, although there are recent plans to supply this vaccine outside of India by Indian Immunologicals and MSD, while the Nigerian PPRV/75/1 vaccine has been used across the majority of remaining areas where the virus is endemic. Importantly, there appears to be no restriction to the use of vaccines from distinct genetic lineages in different areas as vaccines have been shown in outbreak situations across Africa and Asia to protect against other lineages of PPRV.

11.6 The Development of Thermostable PPRV Vaccines

The acknowledgement of the need for thermostable morbillivirus vaccines is not new. During the rinderpest eradication campaign, it was quickly realised that the viability of vaccine in tropical areas was reduced (Plowright et al. 1970, 1971). The reduction in titre of live vaccine virus was shown to be particularly reduced once lyophilised vaccine was reconstituted for inoculation. This hurdle remains partially problematic for PPRV vaccines. The studies performed with the TCRP vaccine were directly applicable to the PPRV vaccine, and as such, some of the early trials with RPV (Mariner et al. 1990) streamlined the process for developing thermostable PPRV vaccines. Certainly, the application of freeze-drying technologies enabled vaccine preparations to be stable for relatively long periods of time prior to reconstitution. It is at this stage that current studies with PPRV are attempting to further prolong PPR vaccine viability.

To address the problem of vaccine thermolability, trehalose has been used as a stabilising agent for the Nigeria/75/1 PPR vaccine. Freeze-drying of this vaccine—in an excipient containing trehalose—results in an increased vaccine stability being able to resist temperatures of up to 45 °C for a period of 14 days with minimal loss of potency (Worrall et al. 2000). A further method of creating a thermostable vaccine preparation has been applied to Indian PPRV vaccines. Here, the PPRV/Ind/Revati/2006 (a vaccine of sheep origin) and PPRV/Ind/Jhansi/2003 (a vaccine of goat origin) isolates were passage attenuated in Vero cells at a higher temperature (40 °C) for 50 passages in a media containing high concentration of serum. The thermostability of both vaccines was assessed at both 37 °C and 40 °C, and it was established that they had a shelf life of 7.62 and 3.68 days, respectively, compared to 1.58 days at 37 °C for the commercially available Sungri/96 vaccine. Post-reconstitution of PPRV/Jhansi/2003 vaccine was found to be thermostable up to 48 h at 4–25 °C when used with stabiliser E (trehalose, calcium chloride and magnesium chloride). At 37 °C, the PPRV/Jhansi/2003 vaccine maintained a protective titre for up to 42 h when reconstituted with diluents containing NaCl and $MgSO_4$. Similarly, the PPRV/Revati/2006 vaccine showed a good stability at 4 °C for about 36 h and a protective titre was maintained at 25–37 °C for up to 24 h (Riyesh et al. 2011). These vaccines have undergone successful in-house trials in both sheep and goats, and both provide complete protection against virulent virus challenge.

11.7 The Use of Heavy Water to Enhance Thermostability

The application of deuterium to enhance the thermostability of PPR vaccines has also been evaluated using heavy water to reconstitute lyophilised vaccine. For the development of a deuterated vaccine, the culture medium included 20 % deuterated D_2O (also known as heavy water). Furthermore, heavy water has been used as a

diluent (87 %) with 1 M $MgCl_2$. When a solution of deuterated water (D_2O) and $MgCl_2$ was used as the reconstituting diluent, the deuterated vaccine maintained titres of greater than $10^{2.5}$ $TCID_{50}$/ml for a period of 28 days at both 37 °C and 40 °C. In contrast, the conventional vaccine only maintained a useable titre for 14 days when stored in a reconstituted form in the standard diluent at 37 °C and 40 °C. Interestingly, the heavy water–$MgCl_2$ combination served as a better diluent for reconstitution than heavy water alone regardless of the vaccine used. In conclusion, higher titres were maintained when vaccine preparations were deuterated and reconstituted in heavy water-based diluents when compared with vaccines reconstituted in the conventional way (Sen et al. 2010).

11.8 Combined Vaccines

Historically, the development of combined/combination vaccines has been attempted to generate protection against multiple pathogens while reducing the costs associated with multiple inoculations. Combination vaccines have found great utility in human medicine, particularly for paediatric vaccines (e.g. DTaP/MMR). Combination vaccines for rinderpest were experimentally assessed by numerous researchers combining RPV with foot and mouth disease virus vaccine preparations (Kathuria et al. 1976; Hedger et al. 1986; Guillemin et al. 1987), anthrax/black-quarter vaccine (Macadam 1964) and most successfully contagious bovine pleuropneumonia (CBPP) (Provost et al. 1969). In recent years, the number of combination vaccine products has grown considerably not only within the medical sector but also in the field of veterinary medicines. Here, we describe attempts to develop combination vaccines for PPRV.

11.8.1 Combined PPR and Capripox Vaccines

Both PPR and capripox (including both sheep and goat pox) are OIE (World Organisation for Animal Health)-notifiable diseases. In endemic regions, infections with capripox viruses and PPRV are important factors that prevent the development and sustainability of small ruminant agriculture (Perry et al. 2002; Bhanuprakash et al. 2006). Experimental studies with dual vaccines against PPRV and goatpox have been reported (Martrencher et al. 1997; Berhe et al. 2003), and similarities in the epidemiological distribution of the viruses suggest that combination vaccines represent an appropriate preparation. Recently, a Vero cell-based live-attenuated goat pox and PPR combined vaccine has been developed (Hosamani et al. 2006). This vaccine is a combination of the Uttarkashi isolate of goat pox at passage level 60 (P-60) and PPR Sungri/96 at passage level 60 (P-60) in Vero cells. The vaccine was found to be safe even when administered at a higher dose. Further, protection against challenge virus was observed following administration of a low dose of the

vaccine (100 $TCID_{50}$) (Hosamani et al. 2006). However, this combination vaccine does have contraindications with administration of goat pox vaccines being problematic through the potential for teratogeny. To overcome this problem, a next-generation combination vaccine that contains a further attenuated preparation of goat pox in combination with a thermostable PPRV vaccine has been developed (Anon 2008). This novel preparation has been shown to be safe in pregnant animals in experimental trials and further trials under field conditions are underway.

Alongside the development of a combination vaccine for PPRV and goat pox, other researchers have developed a similar preparation for PPRV and sheep pox. This PPRV/sheep pox combined vaccine is a combination of the Rumanian Fanar (RF) strain of sheep pox virus and the Sungri/96 strain of PPRV. Each vial of vaccine contains each of the constituent attenuated viruses at a concentration of 10^3 $TCID_{50}$/ml. The vaccine has been tested for its safety and immunogenicity in sheep (Chaudhary et al. 2009) and was found to elicit a strong neutralising response in vaccinated animals that protected against challenge with virulent virus. This indicates that the component vaccines did not interfere with each other and can be used in target populations for economic vaccination strategies (Chaudhary et al. 2009). Currently, the duration of immunity conferred by this combined vaccine is being evaluated in large-scale field trials to ensure that the response to vaccination is adequate for the economic life span of the animal.

11.9 Selection of PPR Vaccine(s) for Worldwide or Regional Use in Mass Vaccination Programmes

While the genetic typing of PPRV isolates in endemic areas remains challenging, the available data suggests that isolates that group within lineages I and IV are the most prevalent. However, with no restriction to the ability of individual vaccines to protect against all lineages of PPRV, the applicability of these vaccines to different regions is not in question. Indeed, the Nigerian vaccine has been used extensively across many countries (Sen et al. 2010) having been the first commercially licensed vaccine available (Diallo et al. 1989). This vaccine is now marketed by many commercial vaccine companies and is supplied to vast areas where all lineages of wild-type PPRV circulate. Alongside this, the Indian Sungri/96 PPR vaccine has been used extensively across India since 2004. These two vaccines have significantly reduced outbreaks of PPRV in endemic regions as well as being used recently to prevent the potential spread of PPRV from Tanzania to the completely naïve populations of small ruminants present across the South African territories, in essence averting a humanitarian crisis (FAO 2010). The Sungri/96 vaccine was initially only manufactured and sold by the IVRI alongside a few IAHVBs across India in states including Karnataka, West Bengal, Maharashtra, Haryana and Andhra Pradesh. The vaccine was manufactured and sold on a "No-Profit-No-Loss" basis to the state Animal Husbandry departments in India. M/S Indian Immunologicals Limited,

Hyderabad, has been producing and selling PPR vaccine (https://www.indimmune.com/livestock-vaccines.pdf, retrieved October 26, 2013) for the last 4–5 years. More recently, MSD Animal Health (known as Merck Animal Health in the USA and Canada) has announced the launch of a new vaccine "OVILIS® PPR", against *Peste des Petits Ruminants* (PPR) which was made commercially available in August 2012 (http://www.merck-animal-health.com/news/2012-08-08-ovilis-ppr-launch.aspx, retrieved October 26, 2013). The communication also stated that studies are ongoing to generate data to enable export to countries where the disease is endemic.

Many more companies have acquired the PPR vaccine technology from IVRI and are likely to be producing PPR vaccines in the near future. The launch of a mass vaccination programme against PPR in India has led to increased commercial interest in vaccine manufacture, and as such, it is likely that more private companies will develop further vaccines. The other two vaccines, viz., Arasur/87 and Coimbatore/97 have not yet entered into commercial production.

A future vaccine requirement for PPRV control in India was extrapolated while assessing the technological capacity of the country in launching a PPR mass vaccination and eradication programme coined as the National Programme on PPR Eradication (NPPPRE)—based on the similar initiative developed for Rinderpest Eradication (NPRE) in India which included the RP-Zero programme that was operational until 2006 (Singh et al. 2009). Singh et al. (2009) have extrapolated all the requirements in terms of vaccine requirement, sero-monitoring and sero-survey in an anticipation of launch of NPPPRE.

Availability of vaccine and importantly the infrastructure in endemic areas to define the need, procurement and distribution of vaccine in different areas is key to any attempts to vaccinate populations of small ruminants. Suggestions have been made as to the necessity to vaccinate populations with a vaccine derived from a lineage homologous to PPRV strains circulating in any one region although the existence of a single serotype of the virus makes this idea redundant.

11.10 Existing Preparations and Future Vaccines for PPR

The conventional PPR vaccines have proved effective in inducing protective immunity in sheep and goats. However, the issues surrounding thermostability of these preparations remain yet unsolved. To combat this, novel preparations are being developed that may serve to overcome this problem in future. Alongside this, the potential utility of both multivalent vaccines and DIVA vaccines highlight future hopes for PPRV control by mass vaccination that will enable differentiating infection from vaccination. Development of PPR vaccines (Diallo et al. 1989; Sreenivasa et al. 2000), alongside a battery of diagnostic assays including PPR sELISA (Singh et al. 2004b), monoclonal antibody-based PPR competitive ELISA tests (PPR cELISA) (Singh et al. 2004a), RT-PCRs (Forsyth and Barrett 1995; Couacy-Hymann et al. 2002, Balamurugan et al. 2006); PCR-ELISA (Sarvanan et al. 2004), indirect ELISA (Balamurugan et al. 2007), and a recombinant PPRV truncated N protein antigen-based ELISA (Yadav et al. 2009)

for diagnosis, sero-monitoring, and serosurveillance of PPR have taken a long way in giving confidence to India, which now led to the launch of national PPR Eradication programme by Government of India in XI plan period which has also been extended in XII Plan period. Research and development of user-friendly pen-side tests warrants the immediate attention of the PPRV research community, while capacity enhancement for augmented production of PPRV vaccine and related biologicals should engage immediate attention of the global community where PPRV circulates.

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Chapter 12
Why Is Small Ruminant Health Important—Peste des Petits Ruminants and Its Impact on Poverty and Economics?

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Abstract The impact of *peste des petits ruminants* can be devastating for smallholders and small ruminant owners; however, the degree and extend will depend on a variety of factors linked to the role small ruminants plays within different production systems. Unpacking both the tangible (i.e., meat and milk) and intangible (i.e. insurance) impacts of PPR can be complicated and requires inputs from a diverse set of experts. Failure to do so will result in underestimating the impacts and result in underinvestment and thereby a continued spread of the PPR disease. It is argued that capturing all dimensions of impacts is especially important for PPR as the majority of small ruminants are kept in smallholder subsistence systems, which characteristically requires a multidimensional approach in controlling the disease. This needs to be supported by political will at the international and national levels for financing progressive disease control interventions.

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12.1 Introduction

Peste des petits ruminants (PPR) is a contagious viral disease which mainly affects small ruminants. It is characterized by morbidity and mortality rates which can range from 10 to 100 %. If it enters a naïve population of sheep or goats, it is known to devastate the flock. Yet to fully understand the impact of PPR on livelihoods of small ruminant keepers and national economies across the world, it is necessary also to: (re-) examine the role and importance of goats and sheep within agriculture, understand the multiple uses and services it provides, and the place small ruminants have within different farming systems. It also requires a thorough understanding and application of the socio-economic approaches to impact assessment of animal diseases and the possibilities and option for interventions. Failure to do so may result in underestimating the impacts and resultant underinvestment or inappropriate actions. It can be argued that capturing all dimensions of impacts is especially important for PPR since the majority of small ruminants are kept in smallholder subsistence systems in areas that extend from Mauritania in West Africa, through the plateaus of Central Asia, and also requiring a more multidimensional approach in controlling it. In the era of fast intercontinental trade, areas in Central America or the Far East are also at risk.

PPR was first reported and described in 1942 in the Ivory Coast (though its origins would likely be found in Central Asia) and has been causing impacts on livelihoods for many more centuries needs to change to years. However, only recently has this disease gained more attention, and two reasons are identified for this new interest. Globally, the Millennium Development Goals have focused attention on reducing poverty of the poorest in the world, and part of this work has involved examining livelihood portfolios and assets. Among the poor, goats and poultry are often the mainstay as livestock assets. For poor livestock keepers, goats and sheep play an important role in wealth accumulation. Therefore, there is an increased need to protect this asset, and preventing PPR and other diseases fits within this agenda.

The second reason can be found from within the international and national veterinary services and the recent success in eradicating rinderpest that affected large ruminants such as cattle, buffalo, and yaks and several species of wildlife. The eradication of rinderpest, as declared by the World Organization for Animal Health and the Food and Agriculture Organization of the United Nations in 2012, has led to a renewed conviction of the ability to rid the world of animal diseases. Due to the similarities between rinderpest and PPR, the latter is seen as a good candidate for eradication. There are many lessons from rinderpest that can be applied to PPR. These include the role communities can play in disease control, the importance of understanding the epidemiology of the disease, having a highly effective vaccine and ensuring its delivery, and the collaboration between countries to ensure eradication. Yet, there are also very clear differences between PPR and rinderpest that will need to be taken into account in an eventual control and eradication strategy.

The heightened interest in eradicating PPR among veterinarians and others has led to an increased demand in social and economic impact assessments of the disease. Yet to date, limited research, data, and approaches are available to examine

and understand the social and economic impact of PPR and importance of the small ruminant production. This chapter will examine the state of knowledge on PPR socio-economic impact assessment, identify the major issues for impact assessment within small ruminant systems, and provide an initial framework to capture the pertinent issues of socio-economic impact of PPR.

12.2 Small Ruminant Systems and the Various Dimensions of Potential Impact

Though both rinderpest and PPR are caused by viruses belonging to the same genus, *Morbilivirus*, and though many of the lessons learned from the rinderpest eradication campaign can be applied to PPR, there are differences between these diseases that need to be highlighted and examined. The most obvious difference is the susceptible hosts and therefore the farming system they affect; rinderpest mainly affects cattle and PPR mainly affects small ruminants. Small ruminants play an important role in subsistence farming, are found mainly on poor agricultural land and in resource-poor areas; in general, they are seen as the cattle of the poor, and they have an important gender dimension. Similarly, small ruminants straddle a range of production systems globally. Their size makes them very mobile, easy to buy, and invest in, but also relatively easy to sell, and they require minimal investment and management, thereby making them a very versatile animal within many different farming systems and livelihood strategies. Additionally, their interaction with agroecological and sociocultural settings means that they may fulfill different roles in different environments.

This background implies that the people with small ruminants have a weak or nonexistent political voice, which in turn limits the access of these people to public resources to support small ruminant development. These are important differences in comparison to cattle or buffalo rearing or the dairy sector and need to be considered if impact assessments and policy advice on PPR are to be useful and effective.

12.3 Geographic Distribution and PPR Risk Areas

Globally, there are about 1.84 billion small ruminants (FAOSTAT 2010), of which 1 billion are sheep and about 900 million are goats. Asia accounts for about half of the global population of small ruminants. Unlike cattle, the distribution of small ruminants is more localized and less widespread (FAO 2007). For sheep, major populations can be found in the Near East, throughout Australasia, in the UK and in southern Brazil and Uruguay. There is a band of high-density sheep populations going from Spain through northwest Africa to northwest India. Other pockets of

Table 12.1 Small ruminant populations (*source* FAOSTAT (2012)

	Small ruminant population	Proportion (%)
Africa	497,178,619.30	27
Americas	132,055,115.80	7
Asia	885,715,551.80	48
Oceania	153,190,195.20	8
Europe	172,484,255.60	9
Total	1,840,623,737.70	100

small ruminants are found in the African Sahel, South Africa, southern India, north central China, and Mongolia. Goats are even more locally situated, even within countries and states, such as southern Texas, northeast Brazil, around eastern Turkmenistan, western Tajikistan and Kyrgyzstan, and southern Kazakhstan. In Africa, they are more pervasive and are overall more common than sheep. India and Pakistan also have a high population of goats (Table 12.1).

12.4 Small Ruminant Products and Services: Foundations of Food Security and Poverty Alleviation

Unpacking and understanding the different functions, uses, and outputs of small ruminants at farm level will give a better understanding of the impact of how PPR will impact small ruminant production and livelihoods. The products and uses of outputs of small ruminant production can be grouped into diverse categories of tangible products and by-products, intangible benefits, and services. A product is hereby defined as primary output for which small ruminants are kept, while by-product is derived from the primary production. Evaluating the impact of the disease, as magnitude and value, of each becomes more difficult as one goes down the list (Table 12.2).

Table 12.2 Products and services from small ruminants

Tangible		Intangible
Products	By-products	Benefits
Meat	Manure and Fertilizer	Bank
Milk		Smooth out cash flows
Skins and hides	Fuel and biogas	Risk reduction and diversification
Fiber and wool		Pathway out of poverty
		Shock buffer and resilience
	Horns	Food security
	Weed control	

12.5 Products and By-products as Cornerstones of Food Security

Physical products, meat and milk, are the most common outputs of small ruminant production and relatively easy to tag with a monetary value. This includes a variety of products; for meat, this includes raw, cooked, blood and soup, and milk can take a variety of forms as well, including fresh, sour, yoghurt, butter, ghee, and cheese. Yet the diversity of outputs can make quantification and monetary estimations challenging when doing an impact study. Meat estimated from live animals sold for commercial slaughter as well as those slaughtered at household level to provide meat for household complicate matters further. Similar with milk production, a certain amount of milk might be sold on the market, and the rest can be dedicated to home consumption, while another portion is suckled by kids and lambs. For milk production, it is difficult to obtain precise data on production and to appreciate the differences between goat and sheep milk production, since it is often culturally and geographically defined. Valuing the primary products—milk and meat—is also complicated as the farm gate and primary market prices are determined by markets factors such as infrastructure and level of integration. One would also need to determine which market level price to use, as well as the challenge of getting average prices as this would require temporal, spatial, and age-group/species considerations. Ørskov (2011) reports goat milk usually receives a premium compared to cow's milk. For instance, in Malaysia, goat milk is six times more expensive than cow's milk, in Vietnam three times, and in Kenya it is believed it is even better than cow's milk for HIV/AIDS patients. Both goat and sheep's milk are seen as very nutritious, goats milk has a similar profile to human milk, 'containing 4.5 % fat, 4.0 % lactose, and 3.0–4.0 % protein depending on the goat's nutrition, breed, and stage of lactation' (Peacock 1996). While it is recognized that a proportion of this price differential relates to the relative quantities of cow and goat milk in the market, goat's milk does have some very important characteristics. Goats' milk can also be easily digested and therefore is good for lactose-intolerant people. An important aspect of small ruminant milk is that lactating females can be regularly milked for small quantities and might still produce this highly nutritious foodstuff in harsher environments when cows do not. In the Horn of Africa and during drought periods, transhumance pastoralists leave behind women and children and a portion of the small ruminant flock as they move to search for pasture, and these household members rely on goats and sheep for milk and blood. Quantifying milk and meat output from live animals also requires good understanding of the proportion of young, immature, and adult animals disaggregated by sex as well as the proportion of adult females that are lactating.

In addition to direct consumption of milk and meat products and besides direct sale at market, live animals are used in bartering for grains in areas where markets and cash economy are poorly developed. Livestock income, from sale of products, accounts for 4–100 % of the household income depending on the production systems, the wealth group owning them, and geographical location. Among the better-off households in a pastoral setting of Turkana, Kenya, 61 % of the household

income (all from livestock) is spent on food which implies that livestock plays an important role in financial access pillar food security.

Other products such as hides and sheepskin are used for shoes, containers, clothing, beddings, and tents. Fiber and wool are important products within certain farming systems, such as cashmere production. In Gansu Province in western China, goat keepers are able to earn their main cash income from cashmere. In Kyrgyzstan, cashmere is still a secondary income source after selling live animal because of the lack of quality wool (LPP 2010). Important for each of these systems, is that the small ruminants can be used for a series of function from own consumption within the household, to traded or sold at the market for an income.

By-products of small ruminant production are numerous. An important one is the ability to recycle nutrient in the soil through manure, and this by-product can also be used as fuel and for biogas production and in some aquaculture systems as feed for fish. These linkages to pasture and crop production are an important aspect of small ruminant production. Another by-product of small ruminant production within smallholder systems is role goats and sheep can play in weed management. In Sri Lanka, Malaysia, Indonesia, and the Philippines, goats and sheep are used to control weeds and vegetation under coconut trees and oil palms (Devendra 1991). They have been known to control bush encroachment. However, this is often a contested role since goats have also been seen as the cause of environmental degradation through tree-felling and overgrazing and sheep with pulling out grass roots when feeding on grassy soils (Peacock 1996). Yet it can be argued that it is not the animal itself, and instead, it is the management of these animals. Goats are known to be browsers, and often small and light, and can complement rather than compete with cattle for feed (Lebbie 2004). Further, goats are also known within some societies to act as herding animals for sheep (Peacock 1996).

Like other animal diseases, PPR impacts on the function performed by small ruminants and their products and services. Understanding the population, their location, farming systems, and therefore those at risk gives some clarity of the potential impact and socio-economics of small ruminants and PPR at the broad global and national level. Yet unpacking the different functions, uses, and outputs of small ruminants at farm level will give a better understanding of how PPR will impact small ruminant production and livelihoods. The relative importance of the roles played by the small ruminants varies with each production systems. It is important to appreciate that 98 % of small ruminant production happens within smallholder household level.

12.6 Services and Benefits of Small Ruminants for Poverty Alleviation and Food Security

The services and intangible benefits are more difficult to quantify and estimate since they are often context specific, and thereby, the context will determine the importance. However, there are some very particular service and benefits that can

be attributed to small ruminants that are crucial to understanding their role within livelihoods and why smallholders keep them.

Often mentioned is small ruminants' role as a bank or a savings account. If the majority of goats and sheep are meant for meat production and sale, it is clear that they are essentially a form of banking or wealth accumulation. Anecdotal evidence shows that farmers are more likely to sell their small ruminants when they are in need of money for uniforms or medicine and not only when the animals are a perfect weight or condition for the market. As with banking, small ruminants are also seen as the account with least risk. By investing into several small ruminants as compared to cattle, when one goat dies, there are still several left to continue production with. This is why Ørskov (2011) refers to goats as being the current account and cattle as the savings account.

Important in this is that small ruminants provide a buffer from shocks and an opportunity for building resilience within their systems. In other words, small ruminants provide for a steady income into the bank account, since, due to their size, the hurdle for selling small ruminants is often lower and will have less impact on the overall portfolio. In addition, small ruminants can be sold off relatively easily and quickly; the small ruminant value chain is reasonably accessible compared to cattle, with fewer inherent barriers such as standards and transportation, and the market is also more flexible. This ease of selling also means it can smooth out cash flows within households, a vital function in fragile environments. Small ruminants also allow for the generation and potential accumulation of capital. Regeneration of own stocks is also an important aspect of goat herds and sheep flocks, since it means they can continue reproducing their livelihood systems without external inputs.

Goats and sheep provide an important component of risk reduction and risk spread both within pastoralists and agro-pastoral systems with mixed herds and within mixed farming systems. Pastoral and agro-pastoral systems keep a variety of livestock including cattle, camels, donkeys, and small ruminants, and the latter are the most numerous and kept nearly by all households. Within these systems, diversification is an important survival strategy (Ellis 2000), and small ruminants provide this. If a disease or other adverse incident such as drought affects the crops, the small ruminants might still be able to keep the household producing and free from food insecurity. It is also an integral part of these systems, since small ruminants might also be sold to buy seeds for planting or to pay for labor during harvest, and their manure will enhance productivity of the land. Therefore, in these systems, small ruminants are often seen as holding the system together.

The role of small ruminants in food security is an important one to highlight. The four pillars of food security can be directly supported by small ruminants. The four pillars include food availability (refers to food supply), food access (refers to the ability of people to physically and economically obtain food), stability of both availability and access to ensure adequate food at all times, and utilization that incorporates food safety and nutritional well-being. Small ruminants contribute to food availability through regular (and constant) production of milk and milk products and occasionally meat at household level, as well as sale of surplus

through formal and informal markets and as food for other households. In pastoral systems in the horn of Africa, livestock products produced and consumed at household level annually account for 2–63 % of the annual kilocalories based on a 2,100 kcal daily requirement (Save the Children 2007; Levine and Crosskey 2006).

Within the intangible category, the importance of goats and sheep in poverty alleviation or as a pathway out of poverty needs to be considered. Livestock plays a crucial role in keeping people out of poverty (Kristjanson et al. 2004; Peacock 2005; Randolph et al. 2007; World Bank 2007), and this merits that it could be further promoted by having farmers invest in raising a small number of smaller animals leading the way to build up to bigger flocks or species and more sustainable farming systems and assuring their livelihoods. The concept of a ladder out of poverty implies that with an initial investment of several poultry, for instance, could be possible to gain enough profit to move onto the next level of small ruminants and then from there to larger ruminants or camels. Small ruminants also play a role in accumulation of wealth where they may be sold for other income activities including purchasing donkeys for transport, oxen for ploughing, and replacement heifers for milk production.

Sickness within the household and funerals are two of the main shocks that drain resources from smallholders in rural areas (Kristjanson et al. 2004). Yet by selling small stock, such as goats and sheep, to cover these expenses, smallholders will be able to allay costs without dire consequences, thereby avoiding people from reaching poverty lines. Therefore, in such systems, maintaining the small ruminant assets becomes vital and can be seen as an investment in resilience.

Sociocultural issues are often seen as intangible and therefore largely ignored in considering the importance of small ruminant production for their owners or the community. Ejlertsen et al. (2012) described the use of goats and sheep as gifts, for religious rites, as dowries, and for funerals, as important. In recent research on the function of sheep and goats in smallholder systems in the Gambia, ceremonies and dowries ranked second or third in breeding objectives of goats and sheep, along with savings, insurance, and income, depending on the wealth ranking (Ejlertsen et al. 2012). Prestige in ownership of small ruminants is also often mentioned as important (Devendra and Thomas 2002; Peacock 2000).

In understanding ways to alleviate poverty, understanding small ruminants' ownership is also important. Goats and sheep are convenient for women and children to take care of and to own. Small ruminants require limited resources and can often be kept within home compounds. Children are often charged with herding small ruminants and can do this as a chare before or after school. Women often take care of sheep and goats because they can combine it with other duties near the house, and because when the animals are stall-fed, it enters the woman domain as taking care of the homestead (Ampaire and Rothschild 2011). Women are indeed more likely to own small stock than large animals, since the barrier to investment is smaller. Yet there seem to be limits to the production parameters or herd size for women since women will have limited time for such activities (Devendra 2007). Since they interact daily with their herds, women are often involved with their health care. Two aspects that cannot be underestimated in relation to women and small ruminants, the ability to empower women, and provide a sense of security.

Empowering them because they are often given control over goats and to a lesser degree sheep, it allows them to decide when to sell and how to use the income. And sense of security, as goats are often an asset women can take with them in case of divorce or migration, and therefore, they provide a sense of security.

In other words, livestock and by extension small ruminants, if managed well, are vital for food security and provide important building blocks to poverty alleviation. Yet more work needs to be done to understand the linkages and to provide modeling and quantification on the contribution of each livestock species to household food security and poverty alleviation.

12.7 Small Ruminant Systems: Magnitude of Impact Related to Production Function

Though it is clear that goats and sheep fulfill a diversity of functions and usages, understanding the degree and importance of the impact of a loss, such as PPR, will only be possible if small ruminant production is placed in the broader context of the farming systems, the function they play, and the value chain. In other words, whether the farmer has one or many goats will not adequately predict the impact it will have on their livelihood.

12.8 Market-Oriented or Value-Driven Small Ruminant Producers

Before understanding the production system within which PPR will infect the flock, it is just as important to understand the goal driving ruminant systems and the aspiration of the small ruminant keeper. For goat and sheep production, it ranges from the highly commercial profit-oriented production to the household with one goat being used for a variety of purposes. One can characterize these two extremes as market-oriented systems and the social value-driven systems (Ørskov 2011).

The motivation behind why producers keep small ruminants will determine the impact of the disease and degree of loss for the owners, it will also determine how much they will invest in preventing the disease.

12.9 Small Ruminant Production Systems

Most livestock production systems are based on a classification of farming systems linked with agro-ecology, and this allows for resource endowment to be factored in and thus also an understanding of the potential of the system (Otte and Chilonda

Table 12.3 Different small ruminant production systems (adapted from Ørskov 2011)

	Market-oriented systems	Social value oriented
Overall goals	Profit maximization	Risk minimization
	Cash generation	Family support
	Productivity	Stability and sustainability
		Income smoothing
Targets	Increased production	Multifunctional animal
	Single-purpose animal	Improved viability of animals
	Genetic homogeneity	Biological vigor
Risk of PPR	Smaller	High
Potential impact	Small	Variable—high
Disease approach	Invest in protecting	Reduction of impact
	Input driven	Limited inputs

2002). Taking this principle, but highlighting the actual dependence in the systems of small ruminants, provides the following classification adapted from Otte and Chilonda (2002), Peacock (2005), Devendra (2010).

12.9.1 Rangeland and Pastoralist Base

Pastoralist systems are dependent on livestock for their livelihoods and can be found in Central Asia, South Asia, the Maghreb, and sub-Saharan Africa and in pockets across the world. In Kenya, Table 12.3 shows small ruminant herd sizes of between 2 and 35 for the very poor and poor and 24–100 for middle and better pastoral households. Goats and sheep usually make up part of a mixed flock and can be a main asset of the producer. Flocks might be divided among different family groups in order to spread the risk of disease. In these systems, the quantity of animals usually gets preference to quality of animals. Often, children are engaged in herding, and women play an important role in safeguarding the small stock. Nomadic livestock keeping can travel extensively, for instance, in Rajasthan in India, goats and sheep may travel up to 1,800 km per year in herds of 2–3,000 animals, along the way they have been known to provide manuring services in exchange for access to pasture lands (Devendra and Thomas 2002). Peacock (2005) points out that there is a trend among pastoralists in East Africa, specifically the Maasai in Kenya and the Afar in Ethiopia to move toward keeping more small ruminants in proportion to cattle, and this is because they are cheaper, reproduce quicker, and provide a quicker recovery of the herd, keeping it more viable in times of calamity.

For these systems, the impact of a disease such as PPR can be devastating and felt directly in the reduction of income or savings (Table 12.4).

Table 12.4 Number of livestock asset owned by wealth category and pastoral livelihoods zones in Kenya Save the Children (2007) and Levine and Crosskey (2006)

Wealth Group					
Livelihood zone	number of livestock assets	Very poor	Poor	Middle	B/ off
Mandera and Garissa Peri-urban	Proportion of human population	21	27	29	23
	Camel and cattle	–	–	–	–
	Sheep and goats n	2	8	24	40
Garissa urban drop off pastoralists	Proportion of human population	24	22	34	20
	Camel and cattle	0	0	0	13
	Sheep and goats	0	0	0	0
Wajir South grassland livelihood zone	Proportion of human population	25	30	30	15
	Camel and cattle	2	6	25	50
	Sheep and goats	8	18	32	75
Garissa Riverine	Proportion of human population	34	29	23	14
	Camel and cattle	–	7	14	35
	Sheep and goats	14	25	44	100
Turkana Central	Proportion of human population	55		25	20
	Camel and cattle	0	0	7	40
	Sheep and goats	20	35	60	100

12.9.2 Mixed Farming

In contrast to pastoralists and rangeland systems, mixed farming will usually see more modest herds, yet they are known to contribute 46 % of the meat globally and 88 % of the milk (Smith et al. 2013). Ejlertsen et al. (2012) describe goat herd sizes between 1–30 head and sheep of 1–80 head for smallholder found across Africa and Asia as mixed farming systems. Yet, the management practices differ across mixed farming systems; they may be free grazing during the day on communally allocated lands to return home during the evening, or they might be completely zero-grazed and stall-fed. Integration between crop–livestock is important in these systems, where the small ruminants can add value to the household through eating stover and fodder resources from crop production and in return provide much needed manure. Diversification in these systems is also important; therefore, if the market for a specific crop collapses, farmers will still be able to benefit from their small ruminants (i.e., risk spread in investment terms). The impact of PPR will vary in these systems, since the numbers and degree of dependency differ per household.

12.9.3 Commercial Systems

Globally, there are limited commercial systems of goat and sheep production. Some can be found in South Africa, Australia, and along the Mediterranean Basin. Both the

wool and meat can mean the need for a more commercialized approach to production. For goats, milk can also be an important commercial function as it can be produced at a larger scale and there is a vibrant market for goat cheese. Should PPR enter these commercial operations, the impact would indeed be very devastating. However, in most cases, they would be most likely to have the necessary precautions to prevent the disease to enter their systems (biosecurity, including trained contract workers).

12.9.4 Urban Systems

Population numbers for urban and peri-urban goat and sheep keeping are very difficult to come by because of the ad hoc nature of these systems. However, the sight of goats scavenging through many urban areas in developing countries is very common. It can also be speculated that the transmission of PPR within these systems is limited since the actual connection point between infected animals is minimal. More information would be needed to understand the actual impact of PPR upon such as system.

12.9.5 Beyond Producers and the Value Chains

Though in general the value chains for small ruminants can be considered as relatively short, it does support the livelihoods of various categories of traders, processors, wholesalers, and retailers involved in local, national, regional, and international trade of live animals, sheep and goat meat, and goat milk. The main goal of the postproduction value chain actors is income generation. The number of the actors, volumes, and value of trade depend on the geographical location, meat consumption preferences, production volumes and marketing infrastructure and linkages between production and consumption clusters.

12.10 The Future of Small Ruminant Production and PPR

It is forecasted that the demand for mutton (both goat and sheep meat) will increase (Delgado et al. 1999b). A growing middle class within certain developing countries will mean more disposable income to spend on protein. Predictions show sub-Saharan consumption of mutton will increase by 137 % from 2000 to 2030, and for low-income countries, mutton has a predicted increase of 177 %, second to poultry (Robinson and Pozzi 2011). This demand will need to be met. In Africa, it will most likely be met through increased regional trade, which will have implications for disease spread, as well as future livelihoods as more people start getting dependent on small ruminant production for income and business.

12.11 Impact Assessment and PPR

12.11.1 The Use of PPR Impact Assessments

Differential impacts are expected when PPR infects flocks and herds in the different systems characterized and described above. The approach for assessing the impacts depends on the anticipated use of the information generated. Two main uses can be identified; it can help understand the magnitude of the problem, and therefore, justify potential funding for its prevention and livelihoods support. It can also be used to inform the development of control strategies catered to the prevailing circumstances, by defining the biological and social dynamics in which the disease and production systems exist. When providing evidence for funding, a demonstration of impact is required by the donor.

However, when the issue of limited available resources especially in developing countries and considering the competing problems across multiple sectors, there is need to provide further information for decision making. Important information is, for instance, the potential benefits of control and specifically the control approaches yielding the highest benefit or return to investment. At this point, intangible losses or benefits, ordinarily left out, should also be incorporated. Smallholders dropping out of small ruminant production can become a long-term strain for government's social support systems. There is also a need to gather socio-economic data on aspects that may affect the adoption and implementation of the control strategies. These include prevailing producer circumstances that range from motivation for engagement in small ruminant production, perception toward PPR and its impacts, importance of small ruminant production, appropriateness and feasibility of prevention and control options, response capacity of the private and public sector service providers, and the overall economic development of the farming systems.

All types of assessment will depend on the targeted user of the assessment or who is interested in investing in mitigation measures. When the user of information is the national government, macro-level impact on national income and regional and international trade as well as returns to investment are often the main focus. The national veterinary service focuses on macro-level impacts and returns to invest analysis for alternate control options to identify the best bet approach. In pastoral systems, where poverty and food insecurity are common, non-governmental organizations (NGOs) working on humanitarian assistance and livelihood programs are interested in impacts on poverty, food security, and gender, especially for the vulnerable households. The latter often concentrates on the community and household level. In the recent past and since the Horn of Africa crisis of 2011, resilience building of livelihoods is the major focus for support, and therefore, a PPR assessment for NGOs and governments would look at PPR within a lens to improve resilience of the communities.

Generally speaking, all socio-economic assessment of PPR impacts needs to capture a degree of the complexity related to the multiple uses, services, and farming systems of small ruminants. Failure to do so may result in underestimating

the impacts and resultant underinvestment. It can be argued that capturing all dimensions of impacts is especially important for PPR since the majority of small ruminants are kept in smallholder and/or subsistence systems, requiring a more multidimensional approach for its control.

A clear understanding of the information required subsequently determines the data requirements and possible approaches that can be used. The nature and magnitude of the socio-economic impacts of PPR on livelihoods depends on several independent or correlated factors that include the following: morbidity and mortality rates, size of small ruminant population at risk, flock immunity level and contact rates, frequency and magnitude of outbreaks; roles and importance of small ruminants in livelihoods, response capacity of the small ruminants value chain actors; response measures and levels of implementation, interactions between small ruminants and other livelihood strategies and farming systems (including cross-border ecosystem linkages).

12.11.2 Identifying and Calculating the Elements of PPR Impact

Any impact analysis of PPR on small ruminants will therefore have to address the various aspects of the function, use, and context within which PPR is spreading. It will also have to look at the complex roles, both tangible (can be described measured and valued) and intangible (difficult to describe, measure, or place values on), of small ruminants in livelihoods, communities, and economies. In addition, there are a variety of levels at which impacts happen and can be estimated, and they include animal, herd, household, community, livestock subsector, agricultural sector, national economy, and global economies. The range of tangible and intangible losses goes from easily quantifiable economic costs of the disease at production levels of the small ruminant value chains to more complex approaches that capture impacts beyond the producer, as well as those that measure PPR dimensions of poverty and food security. Besides capturing the tangible and intangible, the need to identify the direct and indirect impacts of the disease can add an extra level of needed complexity. Direct tangible impacts include production losses from sick and dead animals, while the indirect are, for instance, additional costs for treatment, market restrictions, and denied access to better markets (Fig. 12.1).

At the producer household level, the tangible direct and indirect losses are related to productivity losses (e.g., production loses, cost of prevention and treatment, forgone income from market disturbances and changes in flock value as a result of abortions). For others on the value chain, the direct impacts mainly revolve around income loss from reduced sales of milk and meat (from supply shocks associated with death of animals and market restrictions) and forgone profits and incomes from live animal trading, marketing, processing, and retailing of products

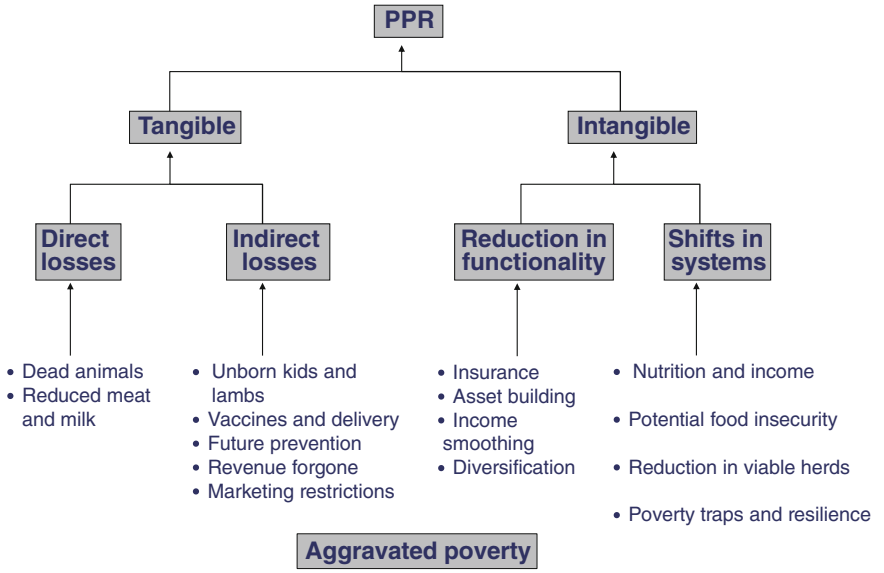

Fig. 12.1 The impacts of PPR

and by-products. The governments too are impacted through the mobilization of its personnel and expenditures for prevention and control costs. The intangible impacts are mainly considered at household and community levels and as mentioned above the most difficult to compute. The consequent impacts of production and income losses on livelihoods can be measured in terms of food, income, and expenditure shifts. Lastly, for some diseases, suboptimal use of production potential (small ruminant species, genetics, and production practices) reduces the potential contribution of the animals involved to the roles played in the value chain. Impacts on small ruminant value chain livelihoods beyond producers' results from ripple effects while spillover of effects results in impacts to sectors with linkages to the livestock sector (i.e., feed and fodder, transport, slaughterhouses, and employment).

Ideally, a disease impact assessment also considers with disease and without disease scenarios. The latter requires good understanding in terms of the roles played by livestock along the value chain livelihoods and specifically the dimensions, quantities, and values in case of monetary benefits. A with disease scenario also requires an understanding on how morbidity and mortality, market disturbances affects the roles played and quantifications of the reductions. Consequently, therefore, available data to support impact assessment are key consideration in determining the analytical approaches to use and therefore the range of impacts information produced.

12.11.2.1 Tangible and Quantifiable Impacts of PPR

The most tangible and direct impact of PPR for a small ruminant owner is the death or mortality of an animal, resulting in the depletion of assets and the subsequent forgone production in terms of milk, live animal off take, and unborn kids and lambs. Other tangible impacts are the morbidity losses as sick animals produce less milk both for household consumption and sale and for the feeding of kids and lambs. Less milk for kids/lambs may also reduce survival rates and therefore affect herd growth and value. Within commercial systems, PPR-associated weight loss results in increasing feeding costs or keeping animals in the system longer to reach the desired weight. Production and productivity losses directly impact on key livelihood dimensions of food sources and quantities, size and value of the household livestock production assets. The livelihoods of other value chain actors are impacted mainly as a result of supply constraints of live animals and their products. In case of market disturbances, increased costs of alternate sourcing of small ruminant and products are likely outcome. In severe outbreaks, consumer may be affected by supply shock driven increased consumer prices of products.

At the livestock sector level, the impacts include changes in the national flock structure and value; reduced gross national livestock production and income; and prevention and control costs by the national governments. Agricultural sector and national economy impacts result from reduced livestock production and income from forgone revenue.

Mortality and Morbidity

PPR case mortality rate differs. In naive populations, rates as high as 75 % (Kenya 2006 outbreaks) and 72 % (Tanzania 2009–2011 outbreaks) have been reported (FAO 2009). Lower mortality rates of 20 % and morbidity rates of 75 % were reported in Kenya in 2012 following fresh outbreaks in Marakwet District (unpublished investigation report), which implies that the disease could be moving toward stability since introduction in 2006.

Morbidity broadly refers to the extent of disease in a defined population normally measured as point prevalence rate (total number of cases of disease present at a particular time in a specific population) or period prevalence (percentage of the population with disease during a defined range of time). Morbidity rates and therefore consequent impacts have been shown to differ between endemic and new infected countries/regions. In naïve population, such as was the case of the 2006–2011 outbreaks in Kenya, Tanzania, Uganda, and DR Congo, PPR morbidity as high as 73 % has been reported (FAO 2009). In endemic countries, lower morbidity rates are reported. PPR morbidity also seems to differ among small ruminant species (more in goats than in sheep) and age categories (Merck Veterinary Manual 2013). In Ethiopia, an endemic country, animals older than 3 years were more likely to be serologically positive for PPR compared to younger animals (Waret-Szkuta et al. 2008). The reverse seems to be true in newly infected

countries/naïve populations. In the first outbreaks reported in Kenya 2007, sheep and goat and all age categories were equally affected (FAO 2009).

Farming systems and animal movement patterns (of infected and healthy small ruminants) can affect morbidity rates by influencing the transmission and spread of PPR. It is important to note that each of these influences is the result of human behavior and the demands it places on the animal and the production system. In endemic countries such as Ethiopia, the proportions of seropositive animals significantly differed across farming systems and regions with a range between 0 and 52.5 % (Waret-Szkuta et al. 2008). Low-altitude areas reported higher prevalence than highlands, which was attributed to different production systems with exchanges and movements in areas in the lowlands being more frequent and involving larger numbers of animals. Seasons associated with heavy animal traffic during the months of March through June have been associated with PPR outbreaks in the country. In the same country, differential morbidity rates have been reported in different farming systems where the prevalence rates of 86, 43, and 33 % were reported among small ruminants from nomadic, sedentary, and mixed farms, respectively (EMPRES 1998). Movement of small ruminants for trade, pasture, water, and internal or cross-border conflicts, for instance in sub-Saharan Africa, often results into the gathering and interaction of animals of different origins. An assessment of PPR introduction into Tandahimba District of Tanzania in 2009 (FAO 2009) indicated that the goat/sheep meat traders played a role in the dissemination of the disease. Traders mixed animals purchased in other districts markets with their own flocks, yet as a consequence, the traders were among those who lost most of their goats to PPR.

The practice of gathering sheep and goats of different ages together and the failure of separating sick from healthy animals influence PPR transmission and spread as well. Similarly, introduction of new animals from the market, the return of village animals to the flock after not being sold in market, and practices related to sick animal carcass disposal are management practices influencing the transmission and spread of PPR (EMPRES 2008).

Other aspects that will influence the PPR morbidity and mortality are the size of small ruminant population at risk and the population density. Assuming similar immunity levels, and for a contagious disease such as PPR, an area with a higher population density experiences higher transmission rates and more infected and dead animals compared to areas of low population density. Hedge et al. (2009) report the density of PPR-susceptible populations in different Indian districts played a major role in disease incidence. In India, it was also observed that the impact of PPR was influenced by environmental factors and that outbreaks increased gradually during the late monsoon and pre-winter periods and the greatest number of outbreaks occurred during winter (Hedge et al. 2009).

Morbidity- and mortality-associated production losses are estimated as the sum of the product of quantities of reduced production (meat, milk, fiber/wool, manure, hides, and skins) and their market prices. Milk losses from dead animals are taken to be 100 %, while infected and recovered animals milk loss depends on the proportion of milk production loss as result of PPR. In some cases, dead animals are

valued at their market price which is assumed to represent loss of products, and in such a case, milk and other product loss from dead animals is not estimated.

Estimation of forgone revenue or income for households is estimated by considering the number of months that market quarantines are implemented and average monthly off take rate to get the number that animals that could have been sold but were not. In some cases, animals could be sold through informal markets, and such dimensions and associated price effects of market disturbances need to be considered. Care should be taken to interpret forgone revenue as un-marketed animals remain in the flocks. It is more important to highlight the consequences of the forgone revenue on household expenditures and income shifts. PPR has been associated with abortions, and the losses can be estimated as milk losses from prolonged lambing and kidding interval, and overall changes in herd composition and size as results of fetal losses.

Beyond producers, other value chain activities such as small ruminant live animal trading, transportation, live animal markets, and meat processing represent another group of livelihoods which get disrupted in PPR outbreaks. The impacts can result in reduced supply of small ruminant products as a result of death and morbidity or from market bans. The magnitude of the impacts depends on the degree to which the value chains are developed and linked to other national and global value chains. The number of actors, volumes handled before and during PPR outbreaks, transactions costs, and gross margins are all important considerations. These value addition losses are estimated from quantities of reduced sales volume and profit margins as well as idling costs associated with business premises and labor. Live animals traders could incur extra costs while sourcing animals from alternative sources.

The impacts of PPR on trade, local and international, are only likely to be significant in newly infected countries, as quarantines can be used to minimize rapid spread, though often with limited success. Diallo (2006) points out that although PPR is a contagious transboundary disease, it usually does not impact international trade. The argument presented is that most countries where the disease is present, it is endemic and therefore trade bans have not much use. Similarly, trade of small ruminants is often informal in nature and would therefore not be affected by a formal trade ban. Therefore, the major impact of PPR will only be at national level and mostly felt at household level (Diallo 2006), although even this will depend on effectiveness of the bans. If bans are widespread and disrupt trade, then livelihoods of others along the marketing and processing segments of the value chains are also affected.

Prevention and Control

A very tangible, but indirect, cost of an animal disease is the prevention and control costs associated with the disease. This means the associated monetary costs of implementing control measures, including the externality and negative side effects of the measures and cost of these measures. Vaccination is one of the most direct

ways of preventing and controlling PPR. For this, the major costs are vaccine procurement and delivery. The delivery costs include the necessary equipment, allowances, salaries, and transport. To date, most PPR vaccination is done through official campaigns, with a limited number through private initiatives. Yet at household level, livestock keepers do make substantial investments in drugs to treat PPR clinical cases, which are often not reflected in impact studies. Vaccination minimizes PPR impacts by reducing the size of susceptible population and preventing further spread by creating an immunized buffer zone. In infected countries, PPR vaccinations continue with varying coverage levels which in turn affect the susceptible host population. Other than costs of vaccine procurement and delivery, a major concern around vaccine is the failure to maintain a cold chain leading to ineffective doses being administered (Nawathe 1984) and thereby low effective vaccine coverage, as observed in East Africa by the authors. Where vaccination coverage is low, reduction of disease morbidity is limited and the associated prevention costs can be seen as sunk costs. Finally, possible side effects of PPR vaccinations, such as abortions and mild signs of PPR associated with the vaccine, are further prevention and control costs or impacts. Beyond vaccination, where quarantines are implemented, their implementation costs are also relevant, and the consequent cost due to loss of incomes for businesses, as well as revenues to the local governments and the veterinary services.

Key to reducing magnitudes of disease outbreaks through prevention and control is early detection and response. It is important to look at the timelines and time lags from introduction to confirmation of PPR and response. In newly introduced countries, the response capacity of the communities and the national veterinary services can determine the magnitude of impacts at household, community, and national level. A delay in detection and response results in wider outbreaks, higher morbidity, and mortality outcomes resulting in higher prevention and control costs and disease impacts on livelihoods and economies. PPR's differential diagnosis with diseases such as CCPP, Rift Valley fever, sheep and goat pox, and orf, which can occur in the same geographical areas, can delay diagnosis and increasing the likelihood of spreading.

12.11.2.2 Intangible Losses and Non-monetary Impacts

Though the intangible losses and non-monetary negative impacts of PPR are more difficult to identify and quantify, they compound the effects of the disease and can be detrimental for households and communities. Intangible outcomes can range from a change in management to aggravated poverty.

Shifts in Management and Viable Herds

An important shift will be in the flock structure, with PPR impacting the ability of the herd to reproduce, or be viable. A viable herd has been described as the

minimum number of animals capable of recovery and reconstituting itself (Sidhamed 1998). The number of animals required for a flock to be viable will vary depending on the environment and system. PPR can infect a herd and reduce the numbers of animals in the herd, making it unviable. If this occurs, external inputs will be required to maintain production, which can be lacking in the often fragile setting where small ruminants are usually found. Dropout is now a common feature of pastoralist systems in the Horn of Africa following frequent shocks such as drought and animal disease outbreaks. In Turkana, in north western Kenya, FAO (2009) showed that PPR resulted in dropout mostly affecting the poor and very poor households whose only assets were the sheep and goats.

Shifts in Nutrition, Income, and Expenditure

Shifts in nutrition and household expenditure can also be a result of PPR, by reducing the amount of milk and meat produced, and by limiting the income, household can acquire from small ruminants to purchase food from markets. At household level, an outbreak will mean reduction in accessible meat and milk, which will mean a reduction in food security and availability of nutritious foodstuffs. Within the pastoral systems of Kenya, FAO (2009) showed that the poor and very poor categories, whose main livestock were small ruminants, turn to wild foods, food aid, and other social support systems to access food in the wake of the PPR outbreaks of 2007. To compensate for loss of livestock income, there were considerable efforts to look for other incomes sources such as casual labor and wood products harvesting (charcoal and firewood) to improve access to money, as they had become gradually more dependent on markets for their food. The degree of shift between household nutrition and income and its implications will depend on the production system. In pastoral systems, where small ruminants directly contribute to food availability and household income, the impacts will revolve around shifts in income and food compared to commercial systems where reduced income will have the bigger impact and might mean the need to reduce investment in the system. It is important to explore measuring the malnutrition indicators in children when PPR outbreaks occur.

Poverty Traps and Reduced Resilience

The majority of small ruminants are found in smallholder systems, where livelihoods are made up of a diverse agricultural and non-agricultural portfolio. The entry of PPR will reduce the options and resilience of these households. By taking out the small ruminants, there will be one less insurance against any emergency such as crop failure or a drought.

Within these systems, where producers mainly rely on a few small ruminants, it is still possible to gradually build the small ruminant asset base to escape poverty since a herd can be self-replicating. But similarly, if a household does not have a

viable herd, it can fall into a poverty trap. This can happen when households do not have large enough flocks to help cover important costs, such as medical bills and funerals meaning they get trapped in poverty (Kristjanson et al. 2004, van Campenhout and Dercon 2012). In Kenya, a recent assessment (FAO 2009) showed that the proportion of poor and very poor increased by 10 %, while the livestock asset thresholds for other categories were redefined to reflect depletion of livestock assets as a result of PPR, and in other words, household that had been considered wealthy was now considered as part of the middle-income group.

Having animals die from a disease like PPR can also impact the standing and position of someone in the community. Often people within a community will judge a person by their ability to take care of their animals and will use it as a means to judge where they are to be trusted or not. Though not often analyzed, issues such as this will have an impact on how any control strategy is developed and how people will participate in such a strategy.

12.12 The Current State of Knowledge—Impact and Socio-Economics of PPR

Despite the interest in PPR, there is limited evidence or data available to provide useful information on impact for strategy and policy development. Much of what has been written about PPR and its impact is very case and time specific, and yet, in many cases has been extrapolated to make sweeping statements on the importance of PPR and its economic impact. In line with this, there are very few actual studies looking specifically at the economic impact of PPR and none that look at the social or intangible impact of the disease or other socio-economic issues revolving around its prevention and control. Apart from specific studies on this, two literatures that provide insights are the PPR virus technical publications which dedicate a paragraph or two to this subject, and the livestock health and development literature. The latter provides insights into PPR as contrast to other livestock diseases but also places sheep and goats and their role within livelihood strategies and resilience building. Though both provide information, they do not help in understanding the dynamics of the disease on people's livelihoods and their possible reactions to the disease and therefore have limited usefulness for strategy or policy development.

Another area of concern within the literature is the restricted geographic reach of studies when and where they are conducted. Several studies have examined the situation in West Africa and in India, and only recently has work started to come out of East Africa, yet globally there are areas and farming systems where little or nothing is known about the actual socio-economic impact of this disease and its impact on small holders and livelihoods. A review of the limited studies also brings out the issues of diversity of entry points and approaches used for these studies. They range from making specific economic analysis of PPR (Stem 1993), to understanding the cost-effectiveness of PPR vaccines (Awa 2000), to national

populations multiplied by prevalence calculations (Hamdy et al. 1976). Yet, studies in impact and cost of foot-and-mouth disease (Rushton and Knight-Jones 2012) and rinderpest (Roeder and Rich 2009) have also had similar limitations in data, evidence, and approaches available.

12.12.1 Past Studies

The most widely cited and comprehensive study on the socio-economics of PPR is an article produced by Stem (1993) on the economic analysis of the prevention of PPR in goats in Niger. Stem examined the potential effectiveness of the Nigerien government's decision to nationally vaccinate goats in 1986. He undertook this using data from 1981 to 1987 and by building a 5-year dynamic model based on 30 herds used in a PPR prevalence study. He concludes that the investment of US$ 2 million for PPR vaccines would generate a US$ 25 million in return for a 5-year vaccine campaign. This study also provides two interesting insights not often highlighted, that herders questioned generally seemed willing to pay the $0.73 per head for the vaccine, suggesting that they found the disease important enough to invest in, and that understanding the externalities of increased off-take as related to increased demand for labor and increased pressure on already very fragile ecosystems was very important.

Other often-cited articles on socio-economics of PPR are Awa et al. (2000), Tillard (1991), and Hamdy et al. (1976). Hamdy et al. (1976) looked at the cause of stomatitis pneumo-enteritis among Nigerian dwarf goats and determined the impact of about USD$ 1.5 million per year. However, upon further examination, the mortality rate used was that of pneumonia 80–90% and not death or case fatality rates of PPR. The article does not explain how it arrived at the figure, unless 100 % of the pneumonia cases were fatal. One could speculate that the mortality rate was applied to the national population and the value obtained by further applying an average price of live animals.

Tillard (1991), Thys and Vercruysse (1990), and Awa et al. (2000) broaden the discussion by researching the cost benefit analysis of curative versus preventive approaches to PPR. In the Tillard et al. 6,000 sheep and goats were monitored in Senegal for a 5-year period using different prophylaxis and concluded that vaccinating small ruminants would be cost-effective for the producers. However, they also point out that the unpredictability of the vaccines makes it more difficult to calculate exactly the benefit side of the equation. Another point made by the authors is that it would only be cost-effective if they did it simultaneously with the rinderpest vaccine and that the cost-effectiveness would be higher for sheep since they had a higher commercial value. They make the crucial point that in these decisions, understanding the prevalence of the disease becomes very important in each specific region before making a decision to invest. Thys and Vercruysse (1990) take up this discussion in their review and also agree that linking different prophylaxis might not be most cost-effective for small ruminants. Awa et al. (2000) puts this to

the test in northern Cameroon and experiments with 18,400 sheep and goats. This study showed that yearly vaccinations against PPR together with a biannual anthelmintic treatment had cost/benefit ratio of 2.26–3.27 for goats and 3.01–4.23 for sheep.

In a specific area of India, where the morbidity rates were 52 and 53 % for sheep and goats, respectively, and where mortality rates were 13.5 in sheep and 8.5 % in goats, the losses were at Rs 918 (approximately 18 dollars) for sheep and Rs 945 (approx. 19 dollars) for goats (Thombare and Sinha 2009). The disaggregated losses include production losses (based on weight reduction and meat price), reduction in price due to abortion, reduction in market value, and reduction in wool/fiber quality. The additional unit cost of medicines to treat and manage sick cases was estimated to be Rs. 162 and Rs. 155 in sheep and goats, respectively. At a farm level, financial losses due to mortalities were estimated to be Rs. 21,368 and Rs. 1,673 for sheep and goat farms, respectively. Though this study gives a good insight as to the impacts, it is does have elements of double accounting that need to be taken into consideration.

The other entry point to examining socio-economics of PPR has come from some of the broader studies on animal health and the ranking of disease by impact and several other factors. Perry et al. (2002) identified priority animal diseases of the poor for investment. The study divides the impacts at household/herd/productivity level and national level. Out of 13 specific diseases, three syndromes (i.e., neonatal mortality) and four general disease categories (i.e., gastro-intestinal parasitism), PPR appears in the top 10 for South Asia and in the top 20, number 17, for West Africa for impacting the poor. Interestingly, it appears in the top 20 diseases as ranked according to their impact on the poor for each production system, 18th for pastoralist systems, 15th for mixed systems, and 16th in peri-urban production.

The World Livestock Disease Atlas (World Bank and TAFS forum 2011) analyzes livestock units that are lost due to disease based on official OIE reported data for the years 2006–2009. Of a list of 68 diseases, PPR figures at 25th in total amount of lost livestock units (LSUs). This is quite high considering that a goat or sheep is calculated at 0.1 LSU, while a cow is 0.9 LSUs. The authors also calculated that a total of 2,565 LSU were lost from PPR 2006–2009. In the same publication, it figured as the third most important disease of sheep and goat diseases. This type of data is important since it provides an insight to the relative impact of a disease in relation to others, but is skewed in that the data analyzed represent official declarations and likely underreporting of incidence or prevalence.

Recently, a range of studies have been undertaken which also start providing insight into the different dynamics, the intangible losses, and multiple impacts of PPR. A report on the PPR outbreak of 2007 in East Africa (FAO 2009) demonstrates the livelihood impacts of PPR due to recent outbreaks in Kenya and Tanzania. The studies show how PPR outbreaks have resulted in better-off households slipping into poverty, while the poor and very poor become impoverished. The estimated livestock asset loss due to 2 years of PPR circulation ranged from 52 to 68 % depending on the wealth categories. The disease caused among other things shifts in food consumption, food availability, and income sources. The livestock-derived income

dropped by 99 % for poor and very poor households and by 55 % for the middle wealth groups and 42 % among better-off households. This study illustrated that in these production systems, most households are unable to maintain a sustainable flock size and might therefore leave their pastoralist livelihoods in an environment that supports very little else in terms of livelihoods. In some cases, this means an increased long-term dependency on food aid and a drain on the national resources.

12.12.2 Analytical Frameworks for PPR Impact Assessments

As illustrated above, there have been limited studies looking very specifically at the socio-economic or tangible and intangible impacts of PPR at different levels. Yet, for the more tangible and economic analysis, there are a range of works that can guide any analysis, and for the intangible aspects, approaches are much needed and are still in need of development. The purpose of these different approaches is an oversight of what is available.

The approaches to economic assessments of animal diseases have been described by Rushton et al. (1999), Morris (1999), Ramsay et al. (1999), Marsh (1999), Rich et al. (2005a,b). Approaches to social assessments for intangible aspects within animal health have only recently been starting to gain ground; however, they borrow heavily from the sustainable livelihood literature (DFID and Carney 1991) and others such as Kristjanson et al. (2004), Bush (2006).

The conceptual and analytical framework that is to be adopted for the assessment depends on:

(i) The question or information required. Will the information be to elicit funding? then an economic impact study might be enough or is it to understand the social and economic dynamics and impacts surrounding the disease to help inform and develop control programs. Even if only disease impacts are considered, the extent of impact inclusion (tangible and intangible losses) needs to be further determined. The depth and type of socio-economic questions are mostly determined by target group requesting the information (i.e., is it for policy makers, livestock keepers, disease control implementers, or veterinarians, each will require a different type of information) and the uses to which the information will be put.

(ii) The level of analysis (animal, herd, household, community, livestock subsector, agricultural sector, national economy, or global economies). Household-/herd-level analytical methods include partial/enterprise budget, gross margins, breakeven analysis, household modeling, sustainable livelihoods analysis (SLA), and household economy approach (HEA). Sector-, national-, and international-level methods include cost–benefit analysis (CBA), linear and dynamic models, simulation modeling, cost-effectiveness analysis (CEA), decision analysis, policy analysis matrix (PAM), partial and

computable general equilibrium analysis, input–output (I–O) models, and social accounting matrices (SAMs).

(iii) The prevalence and occurrence of PPR in endemic, sporadic, and epizootics situations. Endemic situations are best analyzed through partial budgeting and simple CBA and more complex livelihoods analysis while sporadic outbreak situations require consideration for both impact and the probability through decision analysis or more complex CBA. Epidemics are best approached through decision analysis. Quantification and the parameters of the disease add another level of biological complexity mainly resolved through epidemiological inputs and considerations.

Combining the scale of analysis and socio-economic questions narrows the analytical approach options, but rarely points to a single dominant approach. According to Rich et al. (2005), none of the economic models or approaches is universally appropriate. Since PPR occurs as endemic in most countries and with potential for small- to medium-scale epizootics in newly introduced countries and considering that most small ruminants are key livelihood assets, its analysis is of interest to implementers of development policies and decision makers in the quest to eradicate poverty, improve, and safeguard livelihoods, as well as the household. The household-level analysis brings out relationship between the disease impacts on poverty, gender, and food security.

12.12.3 Nationally—Aggregation and Spread Sheets

At the national level, conventional economic CBA frameworks would be best suited to understand the impact of PPR. Spreadsheet-based frameworks with detailed animal population, production indices, and prices levels are used to quantify particular costs of PPR outbreaks. With this data, production losses and the value of mortalities can be calculated. To include disease prevention and control costs, detailed activity/equipment/allowances budgets and costs would need to be included. If control program analysis is included, details would require capturing variables representing PPR with and without control scenarios. If an epidemiological model for PPR transmission is available, it can be linked to economic CBA framework to simulate alternative disease mitigation strategies and determine changes in income under different scenarios. Assumption is made on the proportion by which each control option reduces PPR prevalence based on expert opinion or scientific data. Where the focus is on impacts or both impacts and control options, it is important to consider the need for longitudinal statistics with a starting baseline (and updates on an annual basis and then for several years) for meaningful interpretation. Some studies have only estimated impacts over a one-year period which does not allow interpreting cumulative impacts. When analysis considers future and past years, discounting projected future (on previous years) revenues and costs at an appropriate rate introduces dynamism in CBA (Perry et al. 2003; Randolph et al.

2002). However, CBA or disease impact frameworks would fail to capture post-producer losses such as price effects, spill over to other market commodities, long-term dynamic effects, and impacts at a broader scale (Rich et al. 2005a, b; Otte et al. 2004). It would also fail to capture the numerous intangible impacts mentioned above. CBA short comings on post-producer losses could be overcome by (i) partial equilibrium models to capture producer and consumer surplus, aggregate impacts, and distributional aspects of a disease shock such as price changes, linkages, or welfare changes or (ii) input–output models and SAM that offer the ability to capture linkages between economic sectors. Unlike partial equilibrium models, input–output models and SAM do not allow for price changes and are unable to consider dynamic changes. Though price changes are critical in the agriculture sector, they are less attractive than partial equilibrium models when looking at medium- and long-term effects. Also I–O framework assumes that any changes in the economy are due to shifts in the demand curve rather than supply constraints. However, the CGE, I–O, and SAM models may not exit or could be outdated in many countries limiting their application to PPR or other diseases.

12.12.4 Partial Budgets

Partial budget analysis looks at the effects of relatively small changes (net increase or decrease) in the existing system, and it is based on expected values. The four basic costs and benefit elements of a partial budget include new costs and revenues forgone, and costs saved and new revenues accrued. It can therefore be used to analyze changes that would be associated with diseases and their control programs in a system. When applied in evaluating disease control programs, the focus of analysis would be increased costs of control compared to incremental benefits. In evaluating the impacts of PPR control, prevention and control costs would be the main new costs, while cost saved would be production losses avoided additional income and herd value.

12.12.5 Herd Level or Upstream Businesses with Gross Margin

Gross margin methods are basically output minus variable costs and are used to evaluate the economic viability of an enterprise, ignoring the fixed costs. These are normally expressed as output per standard unit such as hectares, acres, or livestock units. Gross margin analysis of small ruminant enterprise should include live animals and milk off takes (home consumption and sold), skins from home slaughter (only the usable ones), wool, in addition to change in the value of the flock over time. The variable costs include all purchases. This approach is considered

appropriate mostly for production systems with a commercial enterprise orientation. In a majority of the pastoral systems and agro-pastoral system households, live animal or milk off take for sale is not a major production objective, and the milk and meat products for household consumption and barter trade could be considered to be more important. In the systems, it is difficult for variable costs to meet the required criteria of being specific to the small ruminant production and vary approximately in proportion to the size of the enterprise. In low-input mixed livestock systems, it might be complicated to identify precisely the expenditure outlays for small ruminants.

Gross margin analysis can also be undertaken for small ruminant live animals' traders and other business actors along the small ruminant value chains. The gross margin analysis for herd or business level can be upgraded to an enterprise budget to provide profitability information by including fixed costs which are ignored in the gross analysis. Fixed costs do not vary with the enterprise and include land, labor, and capital. Gross margin analysis and enterprise budgets have limited applicability in smallholder systems. Their usefulness to PPR assessments would revolve around providing baseline productivity data which can be used to assess potential or actual impacts of disease. In absence of actual enterprise data, models developed using spreadsheets or commercial packages can be used and supported by data collected through rapid appraisals.

12.12.6 Household and Livelihood Frameworks

Impacts at household level can be very diverse and sometimes far reaching; the livelihoods approach provides a framework whereby it is possible to capture some of the inter-linkages. The livelihoods framework looks at the capabilities, assets, and activities that people have to make up their economic survival and their social wellbeing, i.e., their living. The framework looks at the various capitals that people have, including social, financial, human, natural, and physical. For instance, for small ruminants, they can be seen as a physical capital; however, they also require natural capital to be able to access grassland for fodder and social capital to access information on where and when to vaccinate them. Similarly, when a small ruminant gets sold, the returns are financial capital, which can be used for schooling of a family member which will mean the livelihood portfolio is increasing in its human capital. The framework therefore provides us with a tool to improve the understanding of people's resources and the way they use them, particularly the livelihoods of the poor, since these are often not monetized.

The livelihoods approach can be adapted to animal health, production, and disease threats in explaining how disease shocks could or do affect the livelihood strategies of livestock owners. Because of the often intangible impacts of PPR, the livelihoods approach can easily be adapted to PPR. In other words, it allows for impact such as the loss of small ruminants and the consequent impact on

availability of schooling fees to become apparent. The sustainable livelihoods approach (SLA), as developed by the UK Department for International Development (DFID), has become the most prominent framework currently applied for understanding livelihoods and effective poverty reduction. It also allows for a better understanding of the link between animal disease and how these aggravate poverty, and thereby allow policy makers and project implementers to understand where to intervene to increase resilience for communities. Gathering this data can be done through a series of in-depth interviews and participatory approaches such as focus group discussions scoring and ranking.

Another supplement to the livelihood framework is the HEA (Bush 2006; Levine and Crosskey 2006) used to examine household operations and how households across the wealth spectrum source food and income, their expenditure patterns, social relationships, and how they cope with hazards. HEA data are normally collected to support interventions on poverty alleviation, food security, and livelihood sustainability. Baseline data are used to simulate the impacts of shocks such as drought or high food prices. A main aspect of the HEA is that it requires this comprehensive baseline, ideally one from before there is a disease outbreak, such as PPR. The HEA analysis process itself involves four analytical steps: (i) livelihood zoning, (ii) wealth group clustering, (iii) an understanding of the livelihood strategies by wealth group and livelihood zones, and (iv) the consequent development of scenarios. For PPR, these scenarios will be developed through a problem specification, where analysts can gauge the immediate effect of PPR and how it may affect access to food and income for all wealth groups. The last step is analysis of coping strategies to highlight how producers are responding to the impacts of the shocks on livelihoods to identify consequent impacts.

In the case of a PPR outbreak in Kenya in 2007 (FAO 2009), a household baseline data set had been collected in 2006 for Turkana households. To model the impacts of PPR on livelihood dimensions, the baseline data were used to develop a spreadsheet excel framework of the following tables disaggregated by wealth group and livelihood: (i) herd dynamics for all livestock species, for each livestock species, number of livestock owned at the beginning of the year, year inflows and outflows and balance at the end of the year; (ii) annual food sources as a percentage of the recommended kilocalories; (iii) annual household incomes by sources in absolute figures; and (iv) annual household expenditure lines in absolute figures. To determine the magnitude of the PPR problem, rapid appraisal data were collected to determine PPR morbidity and mortality, overall livestock losses, wealth group categorization and basis, PPR impacts and other concurrent shocks on herd dynamics changes, incomes, food sources, and expenditures. To tease out the actual contribution of PPR to the livelihood changes or losses, PPR mortality, which was high, at about 60 %, was applied to shock the baseline scenario. The assumptions made on the model incorporated field data observations especially on food and income shifts. The output from the model included impacts of PPR on wealth ranking in relation to poverty, extent of depletion of livestock assets, herd dynamics changes, income sources, values and shifts, food sources expenditures lines shifts and reductions. The main disadvantage of the framework is that it is data intensive

and can only apply when baseline information prior to PPR introduction is available. It can be argued that it is very useful in endemic situations. Nevertheless, household economy data in PPR endemic areas are important in informing strategies based on the general livelihood data.

12.13 Conclusion

The impact of PPR can be devastating for small ruminant owners. Yet the magnitude and value can depend on a variety of issues linked to the role small ruminant production plays within different production systems. But it is becoming more and clearer that the links between PPR, food security, and poverty alleviation will require an increased attention from the international community to really comprehend the extent of the long-term impact of PPR. This attention needs to be translated into political will at the international and national levels for financing progressive disease control interventions. Unpacking both the tangible and intangible impacts of PPR can be complicated and requires inputs from a diverse set of experts; however, failure to do so will result in underestimating the impacts and resultant underinvestment and consequently a continued spread of PPR. It can be argued that capturing all dimensions of impacts is especially important for PPR as the majority of small ruminant is kept in smallholder subsistence systems and also requiring a multidimensional approach to controlling the disease.

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Chapter 13
Strategies and Future of Global Eradication of Peste des Petits Ruminants Virus

G. Dhinakar Raj, A. Thangavelu and Muhammad Munir

Abstract Peste des petits ruminants virus (PPRV) is a highly contagious and infectious virus of small ruminants and camel and is endemic in several African, Middle Eastern and Asian countries. Recently, intensive efforts have been made to develop and produce potent vaccines and efficient diagnostic kits, and to understand the molecular epidemiology in endemic countries. These efforts, along with success story of rinderpest, have established baseline for the control of PPRV. Efforts for effective control and subsequent eradication include focused vaccinations in high-risk small ruminants followed by carpet vaccination, understanding socio-economic and culture situation of the small ruminants holder, established infrastructure to cope emergence of disease, and co-operation in countries where disease is endemic with essential involvement of international organizations. In this chapter, all these requirements and deliverables are comprehensively discussed.

13.1 Introduction

The word "eradicate" is defined as "to pull up by the roots" or "to remove totally" or "to get rid off". Disease eradication has been defined as permanent reduction of the worldwide incidence of a disease to zero as a result of deliberate efforts, obviating the need for implementation of further control measures. Such practices have led to the eradication of large ruminant disease, rinderpest, around the globe.

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In contrast, disease elimination is the permanent reduction to zero of the incidence of a specified disease in a defined geographical area. However, continued intervention measures are required. A classical example of disease elimination was the outbreak of foot and mouth disease during 2001 in United Kingdom and rabies in many parts of the world.

13.2 Infection and Disease

The difference between infection and disease is that an infection implies growth of pathogenic microorganisms in the host. As a result, body functions may or may not be affected. The disease is a change from a state of health with impaired body functions. The disease state is associated with the manifestation of symptoms such as fever and pain.

13.3 Incidence and Prevalence

Incidence is the rate of new or newly diagnosed cases of the disease. It is generally reported as the number of new cases occurring within a period of time (e.g., per month, per year). The prevalence is the measure of the total number of cases of disease in a population rather than the rate of occurrence of new cases. Thus, incidence relates to the risk of contracting the disease, whereas prevalence indicates how widespread the disease is.

13.4 Motivations for the Eradication of a Disease

Disease eradication requires complete removal of the disease burden in susceptible host population in given geographical locations. Therefore, the disease eradication programme can be extremely expensive and may require global co-operation and collaborations. The amount of resources' input is rewarded, once the disease is permanently eradicated, in terms of vaccine utilization, and social and welfare impact. There are several biological indicators that could favour the disease eradication programme:

1. Diseases caused by viruses that are genetically stable and antigenically homologous
2. No animal reservoir hosts
3. Diseases that are not very contagious
4. Availability of a potent vaccine eliciting long-lasting immunity
5. A high proportion of population already being vaccinated
6. Availability of sensitive and specific diagnostic tools
7. A global consensus and collaboration

Table 13.1 How did these indicators favour small pox and rinderpest eradication?

Biological indicators	Smallpox	Rinderpest
Reservoir and host	Man	Cattle
Transmissibility	Very low	High
Vaccine efficacy	>98 %	Lifelong immunity
Carrier state/subclinical infections	Never or rare	No carrier state
Incubation period	12 days	3–15 days (4–5 days)
Genetically stable	Yes	Yes
Seasonality	Striking	Not seasonal
Public concern/economic importance	High	Very devastating
Diagnostics	Easy	Sensitive assays available

Several human diseases are considered as potential candidates for disease eradication, such as dracunculiasis (guinea worm disease), poliomyelitis, lymphatic filariasis, leprosy, measles and malaria. World Health Organization (WHO) adopted elimination of dracunculiasis in 1986 and polio in 1988. However, although both diseases appear close to eradication, they are still present even long after the deadlines for global eradication. This highlights the daunting nature of disease eradication programmes. Two diseases, smallpox and rinderpest, have been eradicated from humans and animals, respectively. A parallel comparison of some of biological indicators is shown in Table 13.1. These factors could probably be assessed along with the nature of eradicated viruses for any successful efforts to eradicate PPRV from the globe.

13.5 Factors Supporting PPRV Eradication Campaign

Some indicators are crucial on PPR eradicability and are discussed briefly.

13.5.1 Economic Concerns

Although all diseases are theoretically candidates for disease eradication, the major driver is the cost/benefit ratio between eradication and alternative use of resources. In this context, the decision-making is not easy and is largely determined by the economic status of the country and the perceived advantages. There would be no second opinion that rabies has to be eradicated because of its zoonotic nature and is a hundred per cent fatal disease, which is totally preventable. In contrast, if goat pox needs to be eradicated, its economic importance may preclude it from immediate implementation of disease eradication. Therefore, it is a better candidate for effective control rather than eradication.

A comparison between control of a disease and eradication of a disease is crucial factor not only for motivation but also to build commitments for any organization in initiating alliance on disease eradication. The main challenge is to convince financial administrators to mobilize resources for such programmes. They need convincing based on economical gain accrued following eradication; many of the health effects following absence of disease may not be very tangible. The offshoot benefits of eradication programmes are establishment of laboratories for disease surveillance and develop well-trained and highly motivated para-veterinary staff and a sense of togetherness across disciplines and departments. Some of these benchmarks apply to PPRV since economic importance has been realized owing to its disease impact, global alliance has been initiated and due to the presence of a good eradication model, rinderpest.

13.5.2 Social and Political Concerns

Political support is another success-determining factor in any disease eradication programmes. For this to occur, the disease chosen for eradication should be need-based, of international relevance, technically feasible and should be considered as a worthy goal if achieved. There should be a strong commitment at different levels primarily in the field staff, which should be clearly awarded of the goals and the modalities and are motivated with incentives. The other essential social requirement in disease eradication programmes is perseverance. Currently, both social importance and political importance of PPR have been realized and could further be exploited to favour the PPR eradication programme at an international level.

13.6 Why Peste des Petits Ruminants Virus (PPRV) Can Be Considered as an Ideal Candidate for Eradication of the Next Animal Disease?

The moral or logical standpoint in countries where rinderpest and PPR may have coexisted is that PPR was indirectly under check due to the cross-protection effects of rinderpest vaccines, which was used, even in small ruminants in Southern India. Thus, when rinderpest vaccinations were stopped, there was an upsurge of PPR in small ruminants. Albeit difference between PPRV and rinderpest virus (RPV), eradication of RP left an excellent model for antigenically and genentically related virus, PPRV. There are several points that require discussion before any final conclusions can be made.

13.6.1 Is PPR a Global Disease?

The disease is only prevalent in countries of Asia, Africa and Middle East with inclusion of China, Turkey and Morocco. However, the disease has not been reported in Europe, America and Australia, but there is always threat especially in the context of increasing trend in the spread of the disease in recent times due to globalization and trade.

13.6.2 Is PPR an Economically Important Disease?

The disease imparts both direct and indirect economic losses in small ruminants. Although in adults, it is self-limiting, the kids and lambs succumb to infections. Morbidity is almost 100 %, while mortality ranges from 50 to 80 %. The mortality and morbidity rates varied depending on the species/breeds affected and the strain of virus involved.

There have been reports of virus outbreaks affecting predominantly sheep or goats or both species. When different strains of PPRV were infected into West African dwarf goats experimentally (Couacy-Hymann et al. 2007a, b), some strains of PPRV produced peracute disease; some produced acute disease; and some produced acute to mild disease, while some others caused a mild disease with recovery of infected animals. In another study, when PPRV replication in peripheral blood mononuclear cells was compared across 4 breeds of goats in India, it was seen that virus titres were higher in Barbari and Tellicherry breeds as compared to Kanni and Salem Black (unpublished data). Severe outbreaks of PPR have been reported in newly introduced Barbari goats with mortality rates of 16.67–65.0 % (Rita et al. 2008; Amjad et al. 1996). Recently, a severe outbreak of PPR was reported in Tellicherry breed of goats with 100 % mortality among young and 87.5 % mortality among adults (Parimal Roy et al. 2010). Infection rate was 52.99 % in sheep and 51.47 % in goats. The mortality rate was 13.50 % in sheep and 8.53 % in goats (Thombare and Sinha 2009). Collectively, these studies indicate that mortality and morbidity rates depend on both virus and host factors including age of the host, its population density, nutrition status and innate immunity.

Due to the confusing clinical presentation of the disease, it is not differentially diagnosed with other infections, and thereby, the economic impacts of PPR are under estimated. However, it has been realized well in time that PPR is one of the major constraints of small ruminant farming in the tropics (Taylor 1984).

The complete and comprehensive economic impact of PPR on the small ruminants is currently not known; however, few recent studies have estimated economic losses. Some of these are summarized below:

- PPR causes annual economic losses of about US$342.15 million (Hussain et al. 2008)
- Direct economic losses of US$3.6 million with the rate 5 % mortality

- PPR causes economic losses of 1,800 million Indian rupees (Rs.) (US$39 million) every year in India
- Direct economic losses of US$13 millions with the rate 17 % mortality in sheep and 29 % mortality in goats in India (Singh et al. 2009)
- The total losses are estimated as Rs. 918 in sheep to Rs. 945 in goats. Reduction in the market value of animals leads to a loss of Rs. 404 (44 %) in sheep and Rs. 408 (43 %) in goats. Indirect losses include production and yield reduction, expenditure on medicine, veterinary and labour services. Major loss is also due to infertility (Thombare and Sinha 2009).
- The average annual economic loss due to seven diseases in goats over a 15-year period (1991–2005) was Rs. 264.8 lakh (26.48 million). The average annual economic loss was highest due to PPR (Rs. 91.4 lakh or 9.14 million) (Singh and Shivprasad 2008).
- Opasina and Putt (1985) estimated an annual sum ranging from 2.47£ per goat at high loss and 0.36£ per goat at lowest.
- The losses due to PPR in Nigeria were estimated to be 1.5 million dollars annually (Hamdy et al. 1976).
- The economic benefits of vaccination against PPR in Niger showed that the anticipated net present value return in 5 years of 24 millions USD following an investment of two millions USD.

In most of these cost-benefit analyses, indirect losses have not been considered which occur in the form of biosecurity measures, trade restrictions, and curtailing animal movements and consequent local trading.

13.6.3 What Is the Nature of Spread of PPR Disease?

There is no vector-mediated transmission in PPRV, and the spread is only by close contact or through fomites. However, the disease is highly contagious. In Morocco, the first outbreak of PPR was confirmed on 18 July 2008 although the first case is believed to be detected on 12 June 2008. More than 92 outbreaks were identified by 4 August 2008 in which more than 2833 animals were identified with 50 % case fatalities (Defra 2008).

Infected animals shed the virus in secretions and excretions (from the mouth, eye and nose, faeces, semen and urine) for 10 days after the onset of fever. Sneezing and coughing by the infected animal can spread infection through droplets. Transmission of virus can occur through close contact of infected and non-infected animals, inhalation (over a distance of 10 m), ingestion and conjunctival penetration. It is unlikely to occur through fomites due to its rapid inactivation in external dry conditions. The estimated half-life of PPRV is 2.2 min at 560 °C and 3.3 h at 370 °C (Rossiter and Taylor 1994).

The recovered animals show strong immunity. Although there is no carrier state, infection is likely to be spread during the incubation period before the onset of clinical signs (Couacy-Hymann et al. 2007a, b). Ezeibe et al. (2008a, b) have shown that goats infected with PPRV can shed viral haemagglutinin antigens in faeces for 11 weeks after recovery.

Epidemiological studies have indicated that the outbreaks of PPR are normally associated with the following:

1. Recent introduction of new animals
2. Housing of animals from different origins and ages
3. Sharing of food, water sources or shelters with "foreign" animals or returning animals that were not sold in the market
4. Stress related to changes in diet, habitat, rains, climate change, intensification and most predominantly transport.

13.6.4 Is There Any Carrier Status of the Disease?

PPR is an acute contagious disease with no persistent infections reported. Carrier status of the disease has also not been reported. Although cattle get infected and show seroconversion, they do not excrete the virus and may not show clinical manifestations. In fact, the major difference between rinderpest and PPR was the host specificity. Initial observations of the disease in Ivory Coast in 1942 indicated that the disease was not transmissible from small ruminants to cattle. This led them to believe that the identified virus was different from RPV (Gargadennec and Lalanne 1942).

13.6.5 What Is the Role of Wildlife in the Spread of PPR?

PPR has been reported from a number of wild animals such as camels, gazelle, water buck, Impala, Afghan goats, Bubal hartebeests and several others. Mortality has also been observed in some outbreaks in wild animals. Although these species are susceptible to virus infection, whether they shed the virus that can in turn infect small ruminants needs more extensive studies. Whether they serve as dead-end hosts or act as a focus of infection for small ruminants needs to be more systematically explored.

Peste des petits ruminants infection has been reported in many species of wildlife such as Bharal, Dorcas gazelle, Black tailed gazelle, Thomson's gazelle, Rheem gazelle, Lanstan sheep, Nubian Ibex, African buffalo, Water buck, Spring buck, Kob, Bushbuck, Impala and white tailed deer (Munir 2013) either through serology, RT-PCR or clinical assessments. However, virus isolations have been done in

Table 13.2 The pros and cons of wild life vaccination for PPR

Advantages of wildlife vaccination against PPR	Disadvantages of wildlife vaccination against PPR
Reduce transmission rates of PPRV to small ruminants	Wildlife acts as sentinel and would indicate the presence of infection in sheep and goats. In the face of vaccination of wild animals, this advantage would be lost
Deter virus spread across countries trading and exchanging wildlife	Very difficult for vaccine coverage and monitoring
Wildlife living in herds facilitate virus spread between them, and vaccination would reduce it	Need for differentiating vaccine and virulent PPRV for diagnostic relevance
Prevent crossing species barriers for virus survival and maintenance	Serology of wild animals, which is the easiest method, would be of no diagnostic relevance

few cases only. In many cases, it appears that PPR disease outbreaks in small ruminants resulted in virus spread to wild animals and not vice versa (Abubakar et al. 2011a, b).

Another interesting feature is that all PPRV in wild animals belong to lineage IV only and no other lineage has been identified so far. Whether this is a reflection of the massive spread of this lineage virus recently or whether other lineages are not capable of infecting wild animals also needs detailed future analysis.

The advantages of wildlife in PPRV epidemiology is that they can serve as an indicator for the presence of infection in small ruminants since, in the absence of wildlife vaccination, all the antibodies would be from infection. A comparison of the advantages and disadvantages has comprehensively been shown in Table 13.2.

13.6.6 Genotypes of PPRV—Relevance to Disease Control?

The PPRV is genetically grouped into four distinct lineages (I, II, III and IV) based on the sequences of a 372-bp product covering position 777–1148 nucleotides of the fusion protein (F) gene (Forsyth and Barrett 1995). Lineage I includes isolates from Western Africa, lineage II includes isolates from West African countries, the Ivory Coast, Guinea and Burkina Faso, lineage III represents strains from Eastern Africa, the Sudan, Yemen and Oman, and lineage IV includes isolates from the Arabian Peninsula, the Middle East and South Asia and recently from the Africa (Dhar et al. 2002; Shaila et al. 1996).

Classification of PPRV is also based on the sequence analysis of N gene. It is also suggested that N gene being more divergent is more appropriate for molecular characterization of closely related isolates. Despite four distinct lineages, only a single serotype of PPRV is reported.

The classification of PPRV into lineages has broadened our understanding of the molecular epidemiology and spread of PPR viruses across the world. For example,

phylogenetic analysis of the PPRV strain involved in the Aboumi outbreak belonged to lineage IV (Maganga et al. 2013) similar to the Cameroon strain. It has been predicted that the Asian lineage IV was introduced in Cameroon and later spread to Gabon through importation of living animals. However it's spread from the Republic of Congo could have also occurred. The way lineage IV PPRV is spreading it may encompass all PPRV endemic countries in near future.

The relevance of lineages in disease eradication is unclear. It may have a role in diagnosis of PPR if nucleic acid-based detection methods such as PCR or real-time PCR are be used. While designing primers and probes, it has to be ensured that the sequences are conserved across all of the 4 lineages of PPRV to ensure detection of at least all known lineages of PPRV. A one-step real-time RT-PCR assay for PPRV has been developed to detect all four lineages of PPRV by targeting the nucleoprotein (N) gene of the virus (Kwiatek et al. 2010). The sensitivity of this assay for detection of lineage II PPRV was higher than the earlier method developed by Bao et al. (2008).

It is supposed that cross-protection should occur across PPRV lineages since PPRV belongs to a single serotype. In fact, we even propose a "debatable" hypothesis that if a vaccine belonging to a lineage other than one occurring in a particular country is used, then it becomes easy to differentiate vaccine from field viruses at least till a time when other lineage viruses are not reported in that particular country. Even DIVA may be possible if some epitopes in the sequentially different regions of F or N gene are identified and peptide ELISA could be developed and validated.

13.6.7 Genotypes Versus Serotypes Versus Protectotypes

Although genotypic studies have no relevance in assessing the protection in vivo, it has become a easy and effective tool for virus epidemiology studies. The serotypic classification is based on in vitro virus neutralization tests using homologous and heterologous serum. If two viruses are serotypically different, then neutralization by heterologous serum would be very low or non-existent. Even when two viruses are serotypically different, it is possible that they may show some degree of cross-protection in vivo wherein stimulation of cross-reactive immune responses may trigger it (cross-protection). In this case, they are said to belong to the same protectotype, even if they are serotypically different.

In the case of PPR, there is only one serotype of virus known. PPRV and RPV are serotypically different in terms of neutralization titres although there exists cross-protection between PPRV and RPV, probably triggered by the existence of conserved proteins between the two viruses. Three genetically distinct lineages (1–3) of RPV have been recognized. However, a single vaccine was used throughout the world, the RBOK strain (rinderpest bovine Kabete "O" strain), as that of PPRV. Some of these characteristics are summarized in Table 13.3.

Table 13.3 Comparison of genotypes, serotypes and protectotypes

Feature	Genotype	Serotype	Protectotype
What it means?	Grouping based on genetic characterization	Grouping based on reaction between the virus- and serotype-specific antibodies by VNT in vitro	Grouping based on immunologic relationships in vivo by challenge experiments
Requirement	Thermal cycler, sequencer	Monospecific serum, assay system	Vaccination and challenge
Advantages	Characterize isolate even in mixed infections	Assessment of antigenic nature of virus	Pragmatic as immunologic relationship is assessed
	Virus isolation not required		
	Sequence data available in public domain		
Disadvantages	Does not reflect nature of virus in vivo as only a part of gene is considered	Panel of monospecific sera needed	Genotypic or antigenic nature not known
Application	Molecular epidemiology	Antigenic relatedness	For assessing protection by existing vaccines

13.7 How Is PPRV Eradication Different from RPV?

Despite having fundamental antigenic and immunological similarity, there are differences in these related viruses that require consideration in the context of any PPR controlling strategies (Table 13.4).

Table 13.4 Factors that differ between PPR and RP

Factors	RP	PPR
Duration of immunity	Lifelong	3 years
Sterile immunity	Yes	Yes
Reproduction rate	Slow	High
Movement	Restricted	More extensive
Population stability	More stable	High turnover
Seroconversion	High	Low
Hosts	Cattle	Small ruminants, wildlife?
Thermostable vaccine	1995?	No thermostable vaccine

13.8 Diagnosis of PPR

Diagnosis of a viral disease is conventionally based on the following:

- Direct examination of the virus by electron microscopy
- Virus isolation and identification
- Detection of antigen
- Detection and/or quantification of the genome
- Rise in specific antibody titres

Each assay has its own features of specificity, sensitivity, cost, availability of reagents, quality and cross-reactivity of the reagents, varying interpretation of results based on the context in which it is applied, etc. For disease diagnosis in general, a tier system of diagnosis is proposed. For example, in the case of human immunodeficiency virus (HIV) infections, the first tier of mass screening would be based on strip tests, a second tier based on ELISA and the confirmation based on Western blotting assays.

The choice of the assays is governed by the ease of performance *vis-a-vis* the specificity of the results generated. Although ELISA is amenable to mass screening, the chances for false positive is present that needs to be ruled out by Western blotting which is cumbersome to perform and time consuming, although confirmatory. For any endemic disease diagnosis, a test with higher specificity could be preferred under conventional conditions of diagnosis. For any emerging infections, a test with higher sensitivity should be the test of choice, so that any focus of infections is not missed out. During a disease eradication strategy, the same would be applicable. When vaccinations have been stopped, a test with greater sensitivity should be used. During mass vaccinations, a test with higher specificity may be preferred!

During the phase when RP and PPR coexisted, the context of diagnosis should always be linked to differential diagnosis of these diseases. But since RP has been declared eradicated, now PPR diagnosis could be a "stand-alone" diagnostic situation!

The following section lists the tests that have been applied for PPR diagnosis in the literature. The antigen detection tests used in PPR diagnosis include agar gel immunodiffusion (AGID), counterimmunoelectrophoresis (CIEP), haemagglutination (HA) test and immunocapture ELISA (ICE).

The AGID test is the most commonly used, simple, cheap technique, which gives result in 1 day and useful as an initial test. However, AGID is not sensitive enough to detect low quantities of excreted virus, as may be the case with milder forms of PPR. Counterimmunoelectrophoresis is a more rapid test for detecting PPR viral antigen giving results in an hour. CIEP has been shown to be more sensitive than AGID (80.3 % vs. 42.6 %) (Obi and Patrick 1984).

Unlike rinderpest virus, PPRV have been shown to possess HA property (Wosu 1985). In addition to HA, neuraminidase activity has also been demonstrated (Seth and Shaila 2001). The HA property of PPRV has been used by different authors for

the detection of PPRV antigen and antibody detection (Dhinakar Raj et al. 2000; Manoharan et al. 2005). However, HA is not detected in all the PPRV isolates. Indian PPRV vaccine strains Arasur 87 and Sungri 97 show HA property, while the Sungri 96 vaccine strain lacks HA property (Hegde et al. 2009). Hence, this aspect has to be taken into account in applying HA and HI tests in PPR diagnosis.

The immunocapture (sandwich) ELISA is suitable for routine diagnosis of RPV and PPRV in field samples such as ocular and nasal swabs. The limit of virus detection using this test is $10^{0.6}TCID_{50}$/well for PPRV, but only $10^{2.2}TCID_{50}$/well for RPV. The main advantages of this assay are rapidity (it can be performed in precoated plate in less than 2 h) and specificity (Libeau et al. 1994).

Detection of viral nucleic acid is an alternative approach used in the diagnosis of viral disease. In the case of PPR, first method used for nucleic acid detection was DNA hybridization. This technique was used as early as 1989 to differentiate PPR and RPV infections using cDNA clones corresponding to their respective N genes as probes (Diallo et al. 1989). The technique is not suitable for routine diagnosis due to the short half-life of the ^{32}P isotope, health hazard due to radioactivity and the need for special equipment to protect the users.

Polymerase chain reaction is the most popular and highly sensitive tool available so far for diagnosis of PPR. Since the genome of PPRV consists of a single strand of RNA, it must be first copied into DNA using reverse transcriptase, in a two-step or a single-step reaction known as reverse transcription–polymerase chain reaction. Several reverse RT-PCR methods have been developed for the rapid and specific detection of PPRV genome (Table 13.5).

However, these conventional RT-PCR assays are labour intensive, as they require gel analysis for the detection of PCR products with a consequent high risk of contamination, and they are not suitable for high-throughput testing. To overcome these drawbacks, real-time RT-PCR, which completes amplification and analysis in a closed system, has been recently developed by few researchers.

The real-time PCR assays (qRT-PCR) developed for PPR has been shown to be 10–100 times more sensitive than conventional PCR assays.

Another improvement over the regular PCR assay is the loop-mediated isothermal amplification (LAMP) assay. In this assay, the target amplification occurs at single temperature and the developed product can be detected by naked eye. The sensitivity of the assay was similar to that of qRT-PCR and tenfold higher than that of conventional RT-PCR (Li et al. 2010)

One of the common problems of using RT-PCR, qRT-PCR or LAMP assays in a routine diagnostic laboratory is its potential to generate aerosol contamination leading to false-positive results in the absence of good laboratory practices (GLP) adherence. Various methods of detecting those using appropriate controls such as no-template controls to rule out reagent contamination and controls using RNA in place of cDNA to rule out genomic DNA controls have been recommended. Of the assays, qRT-PCR may be the test of choice especially using TaqMan probe chemistry since it avoids potential aerosol contamination using the UNG enzyme, reads result in real time without the need for opening the assay tubes and use of probes avoids non-specific amplification. Although the quantification of genome of

Table 13.5 Tests developed for PPR diagnosis

S. No.	Diagnostic test	Target detected	Features	References
1.	Agar gel immuno-diffusion (AGID)	Precipitinogens	Simple to perform, low sensitivity	Obi and Patrick (1984), OIE (2008), Banyard et al. (2010)
2.	Counter immuno electrophoresis (CIEP)	Precipitinogens	Simple to perform, low sensitivity, rapid	Obi and Patrick (1984), OIE (2008)
3.	Haemagglutination	H protein	Simple test, to be confirmed by haemagglutination inhibition test	Wosu (1985), Dhinakar Raj et al. (2000), Osman et al. (2008)
4.	Dot ELISA	Virus antigen	Simple test	Obi and Ojeh (1989), Saravanan et al. (2006)
5.	Latex agglutination test (LAT)	Virus antigen	Simple test, rapid, suitable as field test	Meena et al. (2009)
6.	Immunocapture ELISA	N protein	High sensitivity and specificity	Libeau et al. (1994)
7.	Sandwich ELISA	N protein	High sensitivity and specificity	Saravanan et al. (2008)
8.	Immunofiltration	N protein	Simple test, rapid, suitable as a field test	Dhinakar Raj et al. (2008)
9.	RT-PCR	Viral genes	High sensitivity, sequence data can be obtained	Forsyth and Barrett (1995), Brindha et al. (2001)
10.	qRT-PCR	N or M gene	High sensitivity and specificity, rapid	Bao et al. (2008), Balamurugan et al. (2010)
11.	RT-PCR ELISA	Viral genes	High sensitivity and specificity	Saravanan et al. (2004), Senthil Kumar et al. (2007)
12.	Loop-mediated isothermal amplification (LAMP)	Viral gene	High sensitivity, prone to contamination	Wei et al. (2009), Li et al. (2010)
13.	Virus isolation	Infective virus	Gold standard, virus properties can be studied, time consuming, and requires cell culture facilities	Anderson et al. (2006), Brindha et al. (2001), Sreenivasa et al. (2006)
14.	Competitive ELISA	H or N protein-specific antibody	Sensitive and rapid, correlates with in vivo protection, suitable for large-scale seromonitoring/surveillance	Libeau et al. (1995), Singh et al. (2004a, b)
15.	Neutralization test	Virus neutralizing antibody	Gold standard, correlates with in vivo protection time consuming, and requires cell culture facilities	Diallo et al. (1995), OIE (2008), Abubakar et al. (2011a)

PPRV is not essential in a diagnostic set-up, the above advantages drive towards the use of this assay for PPR diagnosis at least during the later stages of disease eradication.

Virus isolation is the most confirmatory technique in diagnosis. PPR virus can be isolated during the acute stage of the disease when clinical signs are still apparent. Virus is present for approximately 10 days after the onset of fever. Swabs of the eye (conjunctival sac), nasal secretions, mouth and rectal linings and whole blood (with EDTA anticoagulant), may be used for isolation. At post-mortem fresh samples of spleen, lymph nodes and affected sections of alimentary tract mucosa may be collected for virus isolation. The most widely used cell culture systems are primary lamb kidney, Vero cells, B95a, MDBK and BHK-21. However, it should be noted that virus isolation can only be attempted in well-equipped laboratories and on freshly collected samples.

When undertaking PPR eradication programme, highly sensitive and rapid tests are required. Rapid field-level tests would be preferable as the diagnosis can be made at the site itself facilitating immediate control measures at the locality. Laboratory assays such as ICE ELISA and qRT–PCR will be useful as confirmatory tests. Sensitive and rapid antibody detection assays suitable for large-scale sample processing are required to assess vaccination efficacy. Validated competitive ELISA techniques having good correlation with neutralization test will be useful. The tests developed for PPRV detection and antibody assessment are tabulated below.

13.9 Vaccines Against PPR

During the time when RP and PPR coexisted, there existed a cross-protective effect of RPV vaccination against PPRV especially in places where RP vaccines were used in sheep and goats. This was also evident from the fact that PPR infections increased dramatically when the RP vaccination totally ceased. Not to forget that PPR diagnosis and differential diagnosis also evolved during this period with the advent of molecular techniques.

The first homologous PPR vaccine was developed by attenuation of PPRV Nigeria 75/1 in Vero cells (Diallo et al. 1989). It is used widely in many countries. In India, three live attenuated vaccines, namely Arasur 87, Coimbatore 97 and Sungri 97, were developed from local isolates by attenuation in Vero cells. The Arasur 87 (sheep isolate) and Coimbatore 97 (goat isolate) vaccine viruses were developed by attenuation of the viruses by 75 serial passages in Vero cells. The Sungri 97 (goat) vaccine strain was developed by attenuation of the virus by 60 serial passages in Vero cells. All the three vaccines have been shown to be potent and suitable for commercial production and use (Saravanan et al. 2010) (Table 13.6).

In the eradication context, attenuated live vaccines are available for PPRV and production strategies need to be geared up to meet the demand of vaccines. Developing vaccines in fermenters using either Vero cells in microcarrier systems

Table 13.6 Details of available PPRV vaccine strains

S. No.	Vaccine strain	Lineage	Isolated from species	Property
1	Nigeria 75/1	I	Goat	Haemagglutinating
2	Arasur 87 (AR 87)	IV	Sheep	Haemagglutinating, fast multiplication in cell culture (Hegde et al. 2009; Singh et al. 2010)
3	Coimbatore 97 (CBE 97)	IV	Goat	Haemagglutinating, fast multiplication in cell culture
4	Sungri 96	IV	Goat	No haemagglutination, slow replication (Hegde et al. 2009)

or in suspension cultures such as BHK_{21} cells may also be considered if sufficient doses may not be produced in stationery cultures or roller bottles.

13.9.1 Quality Assurance

When large doses of vaccines are required for such disease eradication programmes, the quality of vaccines produced needs to be strictly monitored. One way to facilitate strict compliance is to have an independent evaluation and certification of vaccine quality assurance. The quality control of vaccines also needs to be ensured, if possible by a "third-party" evaluator distinct from the vaccine manufacturers. Similar international standards for the laboratory diagnosis of PPR with harmonisation of test protocols and availability of reference reagents to be used in these tests are also a prerequisite for effective diagnostic surveillance.

13.9.2 New Generation Vaccines

Different strategies are used for the development of new generation PPR vaccines. These studies aim at developing thermostable vaccines, which can also act as marker vaccines so that it is possible to differentiate infected from vaccinated animals (DIVA) (Table 13.7).

Capripox virus has been used as vector for the development of dual vaccines to protect against PPRV and capripox. PPRV F protein expressed in capripox virus was effective at a low dose of 0.1 PFU (Berhe et al. 2003). Recombinant capripox virus expressing PPRV H or F proteins elicited long-lasting immunity against PPR (Chen et al. 2010).

Vaccinia virus has also been used as vector for developing recombinant PPRV vaccines. Recombinant attenuated modified vaccinia virus Ankara viruses (MVA)

Tabel 13.7 New generation vaccines under development for PPR

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

expressing the PPRV fusion (F) and hemagglutinin (H) glycoproteins, induced protective immune response in goats. The vaccinated goats were completely protected against virulent virus challenge 4 months post-vaccination (Chandran et al. 2010).

PPRV expressing green fluorescent protein (GFP) was generated by reverse genetics approach. This virus was found suitable for high-throughput assessment of neutralization test (Hu et al. 2012).

Buczkowski et al. (2012) have developed a new strategy by incorporating mutation in viral protein by reverse genetics method for the production of morbillivirus DIVA vaccines. This is an alternative to expression of PPRV proteins in other viruses. In this method, the epitope in the vaccine virus is altered so that the vaccine immune response lacks antibodies against the epitope.

Recombinant canine adenonovirus type 2 (CAV-2) expressing PPRV H protein was generated and shown to induce neutralizing antibody response in goats (Qin et al. 2012). Recombinant adenovirus expressing PPRV H, F or H–F fusion proteins was shown to induce neutralizing antibodies, which lasted for 21 weeks post-vaccination (Wang et al. 2013).

Production of edible vaccines by expression of viral antigens in plants is a cost-effective method for vaccine manufacture. The hemagglutinin (H) protein of PPRV was expressed in peanut plants (*Arachis hypogea*). The expressed protein was in sheep by oral immunization. Virus neutralizing antibodies and cell-mediated immune responses were detected in the immunized sheep (Khandelwal et al. 2011).

All these new generation vaccines are in various stages of development and validation. The live attenuated conventional vaccines available for PPR can be used to initiate disease eradication programmes. However, the DIVA vaccines may have a role in later stages of disease eradication.

13.9.3 Thermostable Vaccines for PPR

The conventional live attenuated PPR vaccines require maintenance of proper cold chain as PPRV is thermolabile. Hence, thermostabilization of existing vaccine strains or evolving thermostable vaccines would be very useful for PPR eradication, as the cost of cold chain maintenance will be saved.

PPRV freeze-dried in the trehalose containing stabilizer has been shown to become more thermotolerant. This vaccine remained stable for 14 days at 45 °C with minimal loss of potency (Worrall et al. 2001).

PPRV freeze-dried with lactalbumin hydrolysate–sucrose and trehalose stabilizers were more stable than Weybridge medium and buffered gelatine–sorbital stabilizers. The vaccine diluent 1 M magnesium sulphate was shown to maintain required vaccine titre for a longer time when compared to water and 85 % saline (Sarkar et al. 2003). Deuterated vaccine preparation reconstituted with heavy water maintained higher titres when compared with conventional vaccine (Sen et al. 2009).

Stabilizer containing Tris with trehalose was found to stabilize PPRV vaccine virus better when compared to Weybridge medium. The freeze-dried vaccine prepared with Tris trehalose stabilizer maintained the viral titre above 104 $TCID_{50}$/ml for 21 months at 4 °C and 144 h at 37 °C (Silva et al. 2011).

PPRV AR 87 was exposed to higher temperatures in succession to evolve a thermo-adapted vaccine virus. The titre and potency of the thermo-adapted vaccine remained constant at $10^{5.5}$ $TCID_{50}$/100 µl level up to 1 month at room temperature (Palaniswami et al. 2005). Sen et al. (2010) developed two thermostable PPR vaccines. The shelf life of these vaccines was 7.62 and 3.68 days at 37 and 40 °C, respectively.

13.10 Strategies for Control of PPR

Disease eradication has to be undertaken in two phases:

- Present no susceptible hosts to virus and abolish transmission cycle
- Prove the above process/attempt is successful

For any eradication strategy, there is a need for an integrated approach combining the following:

- Vaccination
- Biosecurity
- Epidemiological understanding of the disease

When deciding to vaccinate, decision should be taken on either "targeted vaccination" or "mass vaccination". For rinderpest eradication, while India initially followed the mass vaccination model, the South Asia Rinderpest Eradication Campaign used vaccination only in identified places where virus transmission was occurring.

The disadvantages of mass vaccination are as follows:

- Very expensive
- Vaccinating animals that are not perceived to be at risk
- Non-compliance since risk not perceived

Challenges of mass vaccinations include:

- Limited human resources
- Limited stakeholder involvement
- Financial constraints
- Inadequate cold chain
- Civil unrest
- Poor infrastructure
- Poor communication
- Uncontrolled movement across borders

The only country, which reduced its rinderpest incidence to zero, without recourse to a mass vaccination campaign, was Pakistan where the last detected outbreaks occurred in Karachi in 2001. Although mass vaccinations led to decrease in rinderpest disease incidence, it was no way near eradication. Hence, a targeted pulsed, 2-year-long intensive vaccination strategy in the enzootically infected states of southern was adopted that was highly successful and led to the eradication of rinderpest.

13.11 Disease Surveillance

Before disease surveillance is embarked upon, the country should publicly cease vaccination in a declaration of provisional freedom from disease.

This freedom should be proven through the following:

- Clinical disease surveillance
- Serosurveillance and
- Wildlife surveillance

Disease surveillance needs to be undertaken, through veterinary searches within the community that had previously experienced disease/infection and provide negative incidence reports. The required number of searches should be based on statistical methods.

Then, unvaccinated animals must be sampled for PPRV antibodies using an OIE validated assay to assess the trend of antibodies in its population. Statistically significant numbers of samples need to be collected to obtain evidence of freedom from disease as well as freedom from infection status. With PPRV antibodies present in several wildlife species, both clinical surveillance and serological surveillance in wildlife populations are a prerequisite of global eradication.

Now, to embark on a vaccination strategy for PPR, the epidemiology of the disease in each country needs to be ascertained and a decision made.

PPR is widely endemic in India (unlike rinderpest which was more common in Southern India). However, it appears that goats are more severely affected in Northern India and sheep in Southern India. There is unrestricted movement of sheep and goats across several states. Small ruminants are more prone to mixing during purchase fairs, grazing, etc.

Keeping the above factors, it may be needed to use a mass vaccination strategy initially for 3–5 years, vaccinating the newly born kids and lambs every year. Although costly and a huge challenge in terms of personnel and infrastructure costs, the disease epidemiology (unlike large ruminant disease, rinderpest) probably requires this approach.

The second stage could be a more focussed targeted vaccination where still disease/infection has been reported or for high-risk groups of animals. This needs to be coupled with disease diagnosis strategies with the development of infrastructure, assays, training, reporting, documentation, etc. Once freedom from infection is achieved, then the OIE pathway for disease/infection surveillance may be followed, following stoppage of vaccination.

Briefly, PPR eradication should not only rely on the use of vaccines but also on concomitant biosecurity measures. First, small ruminants should be immunized in sufficient "depth" that fresh transmission chains are not be established and, secondly, to "stamp out" the infection in enzootic areas by high-intensity, pulsed vaccination.

During devising the strategy for PPR eradication, the following distinct epidemiological aspects should also be borne in mind (that may be different from rinderpest):

PPR clinical picture is more "subdued" especially in adults where the disease may be self-limiting. Thus, it may be more easily misdiagnosed or undiagnosed leading to persistence of the focus of infection. This could also lead to slaughter of infected animals again favouring disease spread. There seems to be a wide variation in the genetic susceptibility of the breeds and species.

The movement and intermixing of small ruminants are higher in the form of migration of flocks and local markets, fairs and common grazing. It is well documented and observed that PPRV infection occurs especially when new animals are

introduced into a flock or when animals that were not sold were brought back from the local animal fairs.

Sheep and goats are higher in population density than cattle/buffaloes. Higher density would increase disease transmission rates providing effective contact between infected and susceptible animals.

Providing vaccine coverage among small ruminant animals may be more difficult. Even if 100 % animals are vaccinated, only 80–85 % seroconversion is seen due to practical immunization methods, time taken for animal restraint, environmental temperature, cold storage and transport facilities.

The birth of young ones is more in small ruminants, and thereby, the availability of naive susceptible animals that could serve as a focus of new infections is higher.

Disease spread could be much higher due to large distances of movement among small ruminants. Further social requirements could also be a deterrent for eradication. In Tamil Nadu, India, there are no camels, but during festive seasons, camels are transported from states such as Rajasthan that can facilitate disease transmission through distances of more than 2,000 km. Disease introduction from across borders may be easier through straying of small ruminant animals.

13.12 What Is the Role of Cattle or Buffaloes in Disease Control?

Though PPRV multiplication and seroconversion occur in cattle and buffaloes, they do not suffer the disease. In cattle and buffaloes, an overall PPRV antibody prevalence of 4.58 % has been reported in a study conducted in India (Balamurugan et al. 2012). This indicates that under field conditions, natural PPRV infection occurs in cattle and buffaloes. These species could be used as additional indicators of infection foci during later stages of PPR eradication.

13.13 Pulse PPRV Vaccination Strategy?

Pulse vaccination is the repeated application of a vaccine over a defined age range. This method has been applied successfully as Pulse Polio campaigns in India. Unlike constant vaccination, where high vaccine coverage is essential (more than 95 %), pulse vaccination requires only low vaccination coverage to prevent epidemic outbreaks. The “inter-pulse” intervals should be decided based on the population dynamics.

However, a mixed strategy may be considered for PPR. Initially, the constant vaccination may be applied to reduce the number of susceptible animals by means of a high percentage of vaccination coverage, and the second (pulse) vaccination

with relatively low coverage with very long inter-pulse intervals, for kids and lambs between 4 and 12 months that would create a "infection-free" condition for preventing the PPRV focus of infection.

13.14 Strategies for Disease Control in Borders

When PPR occurs in a particular country and eradication efforts are on, the neighbouring countries should also place the same emphasis. This requires political will and a "non-political" medium for communication between countries. Across borders, governments should favour joint rather than unilateral action. Regional bodies can play a pivotal role through coordinating and ensuring that governments act together.

The disease preparedness should also be high at these borders. This may be complemented with diagnostic methods, which can provide a result in minutes at the field level. Although several assays such as lateral flow devices are available, the sensitivity of these methods is questionable. The more recent technique called recombinase polymerase amplification (RPA) assay appears to have a great potential since results are obtained in 4–10 min and also can be done without the need for many equipment at the field. This assay has been tested for many biological warfare agents (Wang et al. 2013).

13.15 Differentiation of Infected from Vaccinated Animals (DIVA)

The term "differentiation of infected from vaccinated animals (DIVA)" was coined in 1999 by Jan T van Oirschot. Marker vaccines were deletion mutants of wild-type pathogens, used along with a companion diagnostic test (CDT). The underlying principle is based on a DIVA vaccine producing an antibody response that is different from the response produced by the wild-type pathogen.

13.15.1 DIVA Strategy

When undertaking disease control by vaccination, it will be useful to identify infected animals from vaccinated animals. This is possible in certain diseases such as FMD where inactivated vaccine is used. Hence, vaccination antibodies are mainly against structural proteins, while post-infection, there is antibody response against non-structural proteins also. However, it may not be possible to identify and differentiate infection induced antibodies from vaccination antibodies when conventional live or

inactivated viral vaccines are used. If the vaccine organism lacks an antigen or contains an additional antigen (marker vaccine), it is possible to differentiate infected animals from vaccinated animals by using suitable companion diagnostic tests.

The advantages of application of the DIVA strategy are as follows:

- Facilitation of identification of residual focus of infections
- Identification of remerging infections in "disease-free" or "vaccination-ceased" zones

This can be used to initiate "stamping out" of infected animals to remove these foci and hasten eradication

The disadvantages of DIVA vaccine are as follows:

- If DIVA is made through reverse genetics approaches, regulatory compliance in all countries may not be easy
- The developed vaccine need to be tested all over again in multitudes of animals for its safety and potency
- The CDT also need to be validated
- Lag time before it is available across the world
- Technology transfer/licensing issues

13.15.2 Is DIVA Needed for PPR Control?

In the case of PPR, presently, vaccines are live attenuated vaccines which induce immune response against all viral proteins not distinguishable from the immune responsible induced by natural infection. Hence, it is not possible to conduct sero-epidemiosurveillance of the disease in areas where vaccination is carried out.

Rinderpest was eradicated without the availability of DIVA vaccine. Scientifically, DIVA would be needed during the later phases of PPR eradication and not initially.

13.16 Progressive Control Programme (PCP) as Envisaged for FMD—Lessons for PPR

The PCP-FMD is a tool that has been developed jointly by FAO and OIE to assist endemic countries to progressively control the disease and reduce its impact on rural livelihoods.

It is a set of 5 FMD control activity stages:

Stage 1: To understand the epidemiology of FMD and develop a comprehensive approach to reduce its impact

Stage 2: To implement control measures such that the impact of FMD is reduced in one or more livestock sectors and/or in one or more zones

Stage 3: Progressive reduction in disease incidence, followed by elimination of FMD virus circulation in domestic animals in at least one zone of the country

Stage 4: Eventual freedom with vaccination

Stage 5: Freedom without vaccination—disease eradication

Whether a PCP as envisaged for FMD would also apply to PPR is debatable.

Implementation of disease control strategies in one zone of the country may be fraught with danger especially for sheep and goats since they are highly migratory and controlling their migration is a bigger task. Further, this approach may be valuable for diseases such as avian influenza where in slaughter of infected poultry is practised, but not in other cases where slaughter is not possible.

13.17 Risk Analysis

A risk-based approach of controlling diseases tends to be more effective. Hence, during an eradication programme, the efforts can be concentrated on critical points of the disease transmission cycle.

With respect to PPR, the following predisposing factors have been shown to be associated with disease onset or progression:

- Susceptible population of sheep and goats and susceptible breeds
- New animal introduction and animal movement
- Poor biosecurity measures
- Trade and migratory routes driven by seasons
- Livestock markets
- Cultural practices and production systems, water sharing or grazing land sharing
- Geographical and environmental factors—high temperature and low humidity reduce virus survival and decrease risk
- Presence and interaction of domestic/wildlife
- Quality of veterinary services—access to quick diagnosis and preventing spread
- Quality of ante-mortem examinations
- Disease information from other parts of the country and world
- Porous borders
- Poor roads and inaccessible terrains

These factors may vary from place to place and needs to be determined. The identification of risks enables for contingency planning and surveillance.

13.17.1 Risk Management

New outbreaks of PPR can be prevented using enhanced surveillance systems and early detection methods. When the reports of unusual mortalities in sheep/goat, especially kids, are received, immediate diagnosis and relevant activities to manage the disease should be initiated. The disease reporting system should be incentivized and rewarded. Surveillance will be strengthened in areas, which have had infection using participatory disease search methods.

13.18 Whether Vaccine Can Be Given During Outbreaks?

PPR occurs throughout the year. In some countries such as Nigeria, it peaks in April increasing from December. Hence, a vaccination around November is suggested. In countries such as India where temperatures are high during summer (March–June), it is also recommended to vaccinate during the October–November months due to lower possibility of inactivation of vaccine viruses.

Abubakar et al. (2012) have reported excretion of PPRV in faeces. During a clinical outbreak of PPR in goats in Pakistan, some infected animals were vaccinated in the face of outbreak and some were left unvaccinated. They report that animals that were vaccinated excreted antigen in faecal matter for 1 month following vaccination, while unvaccinated animals continued to shed virus antigen for 2 months. The virus excretion in faeces adds another dimension to PPR epidemiology and needs a thorough examination.

13.19 Conclusions

To sum the detailed discussion, following conclusions can be drawn:

- The nature of the PPR disease, the availability of tools and its economic importance lead us to believe that this could be the next animal disease to be targeted for eradication.
- Some of the epidemiological features of the disease such as role of wildlife and excretion in faeces need to be studied more thoroughly
- Approaches to thermostabilize the available PPRV vaccines may be strengthened as also the field-based diagnosis of PPR
- DIVA vaccines may be developed and validated for use in later phases of PPR eradication
- Lessons learnt from rinderpest and FMD control or eradication programmes must be utilized, and a unique PPRV eradication programme must be evolved that could vary based on geographical regions

- All the countries must join hands in this fight to protect the small ruminants—the 'poor man's cows' for the ultimate goal of poverty alleviation and economic inclusion.

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