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Review Article

Special Issue: Molecular Virology and Control of *Peste des Petits Ruminants* Virus

Comprehensive Review on Recent Developments in the Diagnostics and Vaccines against *Peste des Petits Ruminants*

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Abstract | *Peste des petits ruminants* (PPR), a viral disease of goats and sheep caused by a *morbillivirus* of the family *paramyxoviridae* is a major threat to small ruminant farming. Global announcement of PPR eradication by 2030 has opened lot of research gaps for the development of vaccines and diagnostics for differentiating infected and vaccinated animals. With the advent of recombinant DNA technology, recombinant protein based vaccines and/or diagnostics are being tested in various heterologous systems across the globe for development of vaccines and/or diagnostic antigens. The recombinant viral proteins, virus like particle based vaccines, bivalent/multivalent vaccines, recombinant viral vectored vaccines, RNA interference as a therapy, suicidal DNAs, synthetic epitopes and peptides, reverse genetics, anti idiotypic antibody based vaccines and helper cell dependent diagnostics represent the present vaccine/diagnostic development strategies for the effective control and eradication of PPR. This review comprehend the current scenario of recombinant technology based vaccines and diagnostics, virus like particles (VLPs), reverse genetics approach etc., in the development of diagnostics and vaccines against PPR.

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Introduction

Peste des Petits ruminants (PPR), also known as '*Goat plague*', is a viral disease caused by a *morbillivirus* of the family *paramyxoviridae*, related to rinderpest (RP), measles and canine distemper viruses. Goats and sheep are the principal hosts and the disease is characterized by high fever, sores in the mouth, diarrhoea, pneumonia often leading to death. There are serological evidences that cattle and several wild ruminants can also be infected. Disease was first identified in Côte d'Ivoire in 1942, and spread to around 70 countries in Africa, the Middle East and Asia, regions that are home to over 80 per cent of the world's sheep and goats and to more than 330 million of the world's poorest people who depend on them for their

livelihoods. Economic losses caused by PPR are estimated to be USD 1.45 to 2.1 billion each year (OIE and FAO, 2015).

Vaccination using conventional live, cell culture based attenuated strains give adequate protection for three to six years but not robust in terms of thermo tolerance. More over they cannot be used in phase of eradication as they induce a similar antibody profile to that of natural infection making it difficult to differentiate infected animals from the vaccinated ones. Thus development of a vaccine that enables differentiation of infected from vaccinated animals (DIVA) would benefit PPR control and eradication programmes, particularly at the later stages of an eradication campaign. Such developments will not only reduce the time required for eradication but also the economic burden on countries involved thus making an eradication programme a success.

Presently all the diagnostic tests available for PPR are based on a competitive ELISA (cELISA) using a *peste des petits ruminants* virus (PPRV) specific monoclonal antibodies (MAb) directed against the viral nucleocapsid protein (N)/ fusion protein (F)/ haemagglutinin (H) protein (Libeau et al., 1992; Libeau et al., 1994; Singh et al., 2004a; Singh et al., 2004b; Balamurugan et al., 2014a). However, none of these diagnostics are able to meet DIVA requirements.

With the launch of Global PPR Control and Eradication Programme (GCEP) during International conference on the control and eradication of PPR at Abidjan - Cote d'Ivoire, a target year has been set to get rid of sheep and goat plague by 2030. In this context lot of research is needed not only to improve the vaccines but also the diagnostic tools. Thus there is an immediate need for developing a novel marker vaccine which can be either multivalent, thermostable or recombinant technology based one. At the same time development of a recombinant protein based companion diagnostic test to replace the existing live PPRV antigen based test is necessary to ensure safety and efficiency. These tests can serve as a better alternatives for clinical or sero-surveillance of PPR in non-enzootic countries or final eradication in enzootic countries besides serving as a useful tool for investigating the biology of the virus and the pathology of disease, as well as for developing new vaccines and diagnostic methods.

The recent developments in the diagnostics and vaccines for the control of PPR have been reviewed earlier (Diallo et al., 1995; Diallo et al., 2007; Banyard et al., 2010; Sen et al., 2010; Balamurugan et al., 2014a; Parida et al., 2015; Singh and Bandyopadhyay, 2015). The present review comprehend the current scenario of conventional/recombinant bi/multivalent vaccines, recombinant technology based vaccines/diagnostics, development of virus like particles (VLPs), use of reverse genetics etc., in the development of diagnostics and vaccines against PPR.

Recombinant Viral Proteins

The PPR virion is enveloped and pleomorphic, enclosing a ~15 Kb single strand negative sense RNA. The RNA is wrapped by the nucleoprotein (N) to form the nucleocapsid, which is associated with two other viral proteins viz. phosphoprotein (P) and the large protein (L) (Crowley et al., 1988; Sidhu et al., 1993; Diallo et al., 1994; Haas et al., 1995). The viral envelope is associated with three viral proteins: the matrix protein (M), the fusion protein (F) and the hemagglutinin (H). By their position and function, both F and H have been attributed to induce a protective immune response against the virus (Barrett et al., 2006).

Nucleocapsid (N) protein is the most abundant and variable immunogenic protein among morbilliviruses (Diallo, 1990; Bodjo et al., 2007). Further, epitopes on the amino-terminal half of the N protein stimulates a humoral immune response more rapidly than that of C-terminal region (Choi et al., 2005). Moreover, it is strongly immunogenic, highly conserved (Francki et al., 1991) and the protein is expressed at high levels in infected cells (Diallo et al., 1994), enabling the identification of varied epitopes of PPRVs into different lineages. Cloning of the N gene of PPRV was done as early as 1994 (Diallo et al., 1994) to deduce its relationship with other morbilliviruses.

Hemagglutinin (H) glycoprotein expressed by PPRV on its outer envelope is crucial for viral attachment to host cells and represents a key antigen for inducing the host immune response. Fusion (F) protein is believed to disrupt the target cell membrane, inducing the virus–cell and cell–cell fusion thereby releasing the viral nucleocapsid into the cell (Choppin and Scheid, 1980). It is also important for the initiation and the progression of the infection (Rima, 1983) and is one of the most conserved genes (Bailey et al., 2005; Dhar et al., 2006), highly immunogenic and confers

protective immunity (Diallo et al., 2007). The Matrix (M) protein is highly conserved and plays a role in the formation of progeny viruses and interaction with the surface glycoprotein in the cell membrane. It also mediates the viral budding process preferentially at specialized regions of the host membrane (Riedl et al., 2002).

Prokaryotic System

The ability to express and purify large quantity of proteins in bacteria has greatly impacted many aspects of biological research. *Escherichia coli* is the most commonly used prokaryotic system for the ease with which the protein can be expressed in bulk without post-translational modifications.

Partial (Yadav et al., 2009; Balamurugan et al., 2014b) and full-length (Yadav et al., 2009) N gene and partial immunodominant ectodomain of F gene (Apsana, 2014) were successfully cloned into pET33b vector and expressed in *E. coli* (BL21). Expressed N/F protein was tested in indirect (Apsana, 2014; Balamurugan et al., 2014b) or cELISA (Yadav et al., 2009) instead of PPRV antigen for serological diagnosis. It reacted well with PPR serum making it an efficient alternative to the live antigen (Yadav et al., 2009). pET30a/F vector also expressed the protein with good immunoreactivity (Wang et al., 2013a). High-level expression of recombinant F protein as well as high titers of rabbit polyclonal antibody against F protein in pCAGGS/F transfected cells offered a valuable tool to further study the pathogenesis of PPRV early infection and the structural and functional characterization of PPRV F protein (Wang et al., 2013a). Alternatively, to obviate the viral genomic cDNA as template, full-length gene encoding the N protein from an outbreak of PPR in Tibet in 2007 was expressed in *E. coli* after synthesizing it in two stages using overlapping PCR. Expressed protein was used in indirect ELISA (iELISA) to detect PPRV antibodies (Zhang et al., 2012). This eliminated the need for virus culture reducing the bio-risk posed by virus dependent manipulation. Low or non-expression of full length M gene in *E. coli* BL21 (DE3) cells was also reported (Liu et al., 2013). But, M protein split into two truncated forms was successfully expressed in *E. coli* at a high level using the pET30a (+) vector (Liu et al., 2013) where both the proteins expressed in an insoluble form. Balb/c mice immunized with the complex of purified proteins under denaturing conditions effectively produced polyclonal antibodies, which reached relatively high titers in ELISA and were specific against both non-denatured and denatured M proteins (Liu et al., 2013).

Eukaryotic System

In theory, prokaryotic hosts can express any gene, but in practice the proteins produced do not always have the desired biological activity or stability. Further, toxic components from the bacteria may contaminate the final product. One must ensure that the recombinant protein must be identical to natural protein in all its properties. As eukaryotic cells share many molecular, genetic and biochemical features, it can be used as an alternative to prokaryotic expression of proteins.

Yeast system

Yeast is an eukaryotic organism and has advantages over *E. coli*. One of the major advantages is that yeast cultures can be grown to very high densities. Fusion gene of Nigeria 75/1 strain (1637 bp) was cloned into pPICZαA, a secretory expression vector of *Pichia pastoris* for the first time in 2007 (Lotfi et al., 2007).

Baculo Virus System

Insect cells are a higher eukaryotic system than yeast and are able to carry out more complex post-translational modifications. They also have the best machinery for the folding of mammalian proteins and, therefore, give the best chance of obtaining soluble protein.

Baculo virus cloned cDNA of the N gene (Nigeria 75/1 strain) was expressed in insect cells and larvae (*Spodoptera frugiperda*) (Ismail et al., 1995). The recombinant protein (58 kDa) was recognized by anti-PPRV antibodies. Crude lysate containing rPPRVN was used in iELISA to detect anti-PPRV antibodies. The recombinant protein was used in an ELISA for the serological diagnosis of PPR. The same antigen was also tried in screening the samples of a suspected outbreak from Cameroon during 1987 (Ismail et al., 1995). The test was simple, rapid and inexpensive compared to serum neutralization test (SNT) or use of monoclonal antibodies.

Nucleoprotein obtained in large quantities from insect cells infected with a nucleoprotein recombinant baculovirus (N-B) was found to react with monoclonal antibody (MAb) in cELISA which had a comparable specificity with the virus neutralisation test (VNT) for detecting specific antibodies to PPRV in sheep and goats (Libeau et al., 1995).

Goats immunized with low doses of purified recombinant extracellular baculovirus carrying a membrane bound form of the HN protein without any adjuvant induced both humoral and cell-mediated immune responses. Antibodies generated could neutralize both PPRV and RPV *in vitro* (Sinnathamby et al., 2001).

Recombinant *Bombyx mori* nucleopolyhedro viruses (BmNPV) displaying the immunodominant ectodomains of F protein of PPRV and the H protein of Rinderpest virus (RPV), on the budded virions as well as the surface of the infected host cells induced immune response in mice against PPRV or RPV (Rahman et al., 2003).

Recombinant baculoviruses, co-expressed M and N proteins in insect cells (Liu et al., 2014a) at a relatively low level. Codon optimized full-length M and H genes with native N gene recombinant baculoviruses also co-expressed M, H and N proteins in insect cells at different levels (Liu et al., 2014b).

Mammalian System

The genes coding for the HN protein of PPRV and H protein of RP virus were cloned in a cytomagalovirus promoter driven expression vector and expressed transiently in mammalian cells. The protein expression was apparent and the expressed proteins were detected at the cell surface. The transiently expressed HN protein was biologically active in possessing hemadsorption and neuraminidase activities (Seth et al., 2001).

Stable Vero cell line constitutively expressing H protein (70kDa) of the vaccine strain (sungri-96) was developed after repeated passages using eukaryotic expression vector (pTarget) and antibiotic selection pressure. The protein reacted specifically with anti-H neutralizing monoclonal and polyclonal antibody in competitive, sandwich and iELISA with high degrees of specificity and sensitivity. H protein can be a sustainable source of safe antigen in countries of non endemicity without the need to handle infectious virus for serodiagnosis (Balamurugan et al., 2006).

Recombinant T7 polymerase produced using highly attenuated MVA strain of vaccinia (MVA-T7) and fowlpox virus (FP-T7) was compared in MDBK, Vero cells and in primary cells of bovine, ovine and caprine origin to check transient expression and to rescue negative strand virus as they are more permissive for the growth of wild type strains of morbilliviruses. Attenuated MVA-T7 was found to be highly cytopathic to primary cells of bovine, ovine and caprine origin, multiplying rapidly and killing the cells within 3-5 days of infection, with very low multiplicities of infection. In contrast, FP-T7 expressed similar amounts of T7 polymerase, but was non-cytopathic in a variety of primary and established cell lines of mammalian origin and was suitable for use in virus rescue experiments. MDBK cells and primary cells, unlike Vero cells, could not be efficiently transfected and so were unsuitable for virus rescue (Das et al., 2000).

Plant System

As a step towards development of a heat stable subunit vaccine, HN protein was expressed in peanut plants (*Arachis hypogea*) in a biologically active form, possessing neuraminidase activity. The protein retained its immunodominant epitopes in their natural conformation. Virus neutralizing antibody responses were elicited upon oral immunization of sheep in the absence of mucosal adjuvant. In addition, HN specific cell-mediated immune responses were also detected in mucosally immunized sheep (Khandelwala et al., 2011).

Virus Like Particle-based (VLP) Vaccines

For many viral diseases, vaccination with VLPs has shown considerable promise as a prophylactic approach; however, the processes of assembly and release of PPRV VLPs are not well characterized and their immunogenicity in the host is unknown.

Recombinant baculoviruses with M and N proteins constructed by Liu et al. (2014a) though resulted in low level expression of these proteins the interaction between them caused the formation of VLPs which morphologically resembled authentic PPRVs but lacked spikes protruding from the particulate surfaces. Interestingly, the diameter of VLPs ranged from 100 to 150 nm, far less than the mean diameter (400-500 nm) of parental virions. VLPs were also generated in a baculovirus system through simultaneous expression of M and F proteins. The released VLPs showed morphology similar to that of the native virus particles. Subcutaneous injection of these VLPs (PPRV-H, PPRV-F) into mice and goats elicited specific IgG production, increased the levels of virus neutralizing antibodies and promoted lymphocyte proliferation. Without adjuvants, the immune response induced

by the PPRV-H VLPs was equivalent to amounts of PPRV vaccine. VLPs containing M and H or F protein are potential vaccine candidates for DIVA in the surveillance and eradication of PPR (Li et al., 2014). Further, recombinant baculoviruses consisting of codon optimized full-length open reading frame (ORF) corresponding to the M and H along with native N ORF lead to budding of VLPs from cell membrane based on self-assembly of the three proteins. The VLPs, morphologically resembled authentic PPRVs but were smaller in diameter (Liu et al., 2014b). Mice immunized with purified VLPs (sucrose density gradient centrifugation) induced both types of antibodies, indicating a potential of VLP-based vaccine candidate against PPR (Liu et al., 2015).

Bivalent / Multivalent vaccines

Sheeppox (SP), goatpox (GP), foot and mouth disease, lumpy skin disease (LSD), Rift Valley fever (RVF) are some of the highly contagious ruminant diseases along with PPR in Africa, the Middle East and Asia imposing a major burden on economic activity and public health. While commercial vaccines against these viruses are available, the cost of implementing regular vaccination regimens against multiple diseases is prohibitive for most farmers. A single, affordable multivalent vaccine that simultaneously protects against many diseases would be of significant benefit to the livestock sector. It could also serve as a platform for the development of new vaccines of significance to other developing countries around the world. Combined vaccine comprising of attenuated strains of PPRV and goat poxvirus (GTPV) (Hosamani et al., 2006) or sheeppox virus (Chaudhary et al., 2009) are safe and immunogenic. Goats immunized with vaccine consisting of PPRV and GTPV generated specific antibodies directed against both GTPV and PPRV by iELISA and cELISA, respectively following immunization (Hosamani et al., 2006). Sheep immunized with live attenuated combined sheep pox (Romanian Fanar (RF) strain) and PPR vaccine (PPRV-Sungri/96 strain) in lyophilized form developed specific antibodies to sheep pox virus (SPPV) by iELISA and SNT and to PPR virus by cELISA and SNT. All the immunized animals resisted challenge with virulent SPPV or PPRV on day 30 post immunization. Component vaccines did not interfere each other and can be used in target population for economic vaccination strategies (Hosamani et al., 2006; Chaudhary et al., 2009).

Recombinant Viral Vectored Vaccines

Capripox Virus (CPV)

Recombinant CPV (rCPV) is a promising candidate for DIVA vaccine against PPR. CPV vectored recombinant PPR vaccines (rCPV-PPR), have been developed and shown to protect both Capripox (CP) and PPR, would be critical tool in the control of these important diseases. In order for rCPV to be successfully used in the field, there should exist dependable indicators for quality control of vaccine products, surveillance and vaccine evaluation. Viral neutralization antibody (VNA) is correlated to protection against PPR and is a technically feasible indicator for this purpose. The immunogenicity of this vectored vaccine in goats and sheep, however, has not been fully evaluated.

Goats were protected against a lethal challenge of PPRV following vaccination with rCPV containing either the F or H gene of RP virus (Romero et al., 1995). A rCPV vaccine containing a cDNA of F gene was used as quick and efficient method to select a highly purified recombinant virus clone. A dose as low as 0.1 PFU protected goats against challenge with a virulent PPRV strain (Berhe et al., 2003).

Recombinant Goatpox virus (GPV) containing H gene (rGPV-PPRV-H) could express H protein in lamb testis cells. Immunized goats produced very good neutralization antibodies of GPV and PPRV establishing a foundation for the industrialization of the PPRV recombinant GPV vaccine (Chen et al., 2009). rCPV-PPRVH is a potent inducer of VNAs than rCPV-PPRVF. One dose (rCPV-PPRVH) was enough to seroconvert 80 per cent of immunized sheep. A second dose induced significantly higher VNA titers which were detected for over six months in 80 per cent of both goats and sheep. Booster vaccination at 6-month intervals induced significant re-boost efficacy of VNAs. Moreover, two doses could completely overcome the interference caused by pre-existing immunity to the CPV vaccine backbone in animals. Vaccination also protected goats from virulent CPV challenge. It could be a practical and useful candidate DIVA vaccine in countries where PPR newly emerges or where stamp-out plans are yet to be implemented (Chen et al., 2010).

Consortium of Canadian and South African researchers have initiated an approach to develop single, multivalent capripoxvirus-vectored vaccine that

would protect against five diseases (LSD,SP,GP,RVF and PPR) of livestock (Boshra et al., 2013).

Animals immunized with mixture of CPV recombinants (KS-1) expressing either H or F gene (Nigeria 75/1) when challenged with a virulent CPV strain followed by a virulent PPRV strain three weeks later demonstrated full protection against CP for vaccinated animals with prior exposure to PPRV and a partial protection against PPR for vaccinated animals with prior exposure to CPV. The latter animals exhibited a mild clinical form of PPR and did not show any post-challenge anamnestic neutralizing antibody response against PPRV (Caufour et al., 2014).

PPR Virus

Immunization with recombinant PPRV expressing the FMDV VP1 gene (rPPRV/VP1) induced FMDV and PPRV neutralizing antibodies in goats and protected them from challenge with virulent FMDV and served as a potential dual live vectored vaccine against PPR and FMD (Yin et al., 2014).

Fowlpox Virus

Immunogenicity of recombinant fowlpox (FP) expressing F and H proteins, in goats was evaluated to enable DIVA (Herbert et al 2014).

Adenoviruses

Goats immunized with replication-competent recombinant canine adenovirus type-2 (CAV-2) expressing the H gene (China/Tibet strain) with human cytomegalovirus (hCMV) promoter/enhancer and the BGH early mRNA polyadenylation signal, transfected into MDCK cells produced antibodies upon primary injection that were effective in neutralizing PPRV *in vitro*. Higher antibody titer was obtained following booster inoculation, and the antibody was detectable in goats for seven months. No serious recombinant virus-related adverse effect was observed and no adenovirus could be isolated from the urine or feces of vaccinated animals. Recombinant virus was safe and could stimulate a long-lasting immune response in goats. This strategy has not only provided an effective PPR vaccine candidate for goats but also a valuable DIVA tool (Qin et al., 2012).

Goats inoculated with replication-defective recombinant adenoviruses (rAds) expressing F (rAd-F), H (rAd-H), and F-H (rAd-F-H) developed PPRV-specific VNAs by three weeks post immunization. Moreover, the seroconversions were maintained for approximately 21 weeks after primary immunization. Stronger lymphocyte proliferation responses were induced in goats immunized with three rAds. Goats inoculated with rAd-F-H developed significantly higher VNA titers and stronger cell-mediated immune responses than goats inoculated with rAd-F or rAd-H alone. rAds served as attractive candidate differentiating infected from vaccinated animals (DIVA) vaccines for preventing PPRV infection (Wang et al., 2013b).

Replication-defective recombinant human adenovirus 5 (Ad), expressing F and H proteins induced higher levels of virus-specific and neutralising antibodies in goats and primed greater numbers of CD8+ T cells. Importantly, a single dose of Ad-H, with or without the addition of Ad expressing ovine granulocyte macrophage colony-stimulating factor and/or ovine interleukin-2, not only induced strong antibody and cell-mediated immunity but also completely protected goats against challenge with virulent PPRV. Replication-defective Ad-H therefore offers the possibility of an effective DIVA vaccine (Herbert et al., 2014).

HEK293A cells infected with recombinant replication-defective human adenovirus serotype 5 (Ad5) containing the F and H genes elicited PPRV-specific B- and T-cell (CD4+ and CD8+) responses in mice (Rojas et al., 2014a). Sheep vaccinated with Ad5 expressing either the F or H elicited PPRV-specific B- and T-cell responses. In addition, a significant antigen specific T-cell response in vaccinated sheep against two different PPRV strains, indicated that the vaccine induced heterologous T cell responses. Importantly, no clinical signs and undetectable virus shedding were observed after virulent PPRV challenge in vaccinated sheep. These vaccines also overcame the T cell immunosuppression induced by PPRV. Adenovirus constructs could be a promising alternative to current vaccine strategies for the development of PPRV DIVA vaccines (Rojas et al., 2014b).

Vaccinia Virus

Although, goats vaccinated with a vaccinia virus double recombinant expressing the H and F genes of RPV, developed antibodies (neutralizing and ELISA) to RPV and not to PPRV, they were completely protected when challenged with virulent PPRV. This would indicate that protection is most probably due

to cell-mediated immunity (Jones et al., 1993).

Goats vaccinated with attenuated Modified Vaccinia virus Ankara virus (MVA) (MVA-F and MVA-H) expressing the full length PPRV F and H glycoproteins, were completely protected from the clinical disease. Mass vaccination of small ruminants with either of the above or both recombinant inexpensive virus vaccines could help in possible eradication of PPRV from endemic countries like India and subsequent seromonitoring of the disease for DIVA strategy (Chandran et al., 2010).

RNA Interference as Therapy against PPR

Morbilliviruses are important pathogens of humans, ruminants, carnivores and marine mammals. Although good vaccines inducing long-term immunity are available, recurrent outbreaks of measles, canine distemper and PPR are reported. Moreover, vaccines cannot offer protection to the animals before 14 days post-vaccination. Thus, in control strategies, antivirals could be useful to confine virus spread and application of interfering RNAs is a promising approach, provided they can be delivered efficiently into the host cells. Two regions in the nucleocapsid genes of PPRV can be targeted efficiently by synthetic short interfering RNAs (siRNAs), for reducing the virus replication more than 80 per cent. Thus siRNA molecules can be developed as therapeutic agents for the treatment of PPRV infections (Servan d Almeida et al., 2007). Silencing of the N gene mRNA efficiently shuts down the production of N transcripts, the expression of N protein, and the indirect inhibition of M protein resulting in the inhibition of PPRV progeny by 10,000-fold (Keita et al., 2008). Chemically synthesized siRNAs transfected into B95a cell line were potent inhibitors of SLAM expression both at m-RNA and protein levels. They also caused the maximum reduction of virus replication and virus titre. This further confirmed that SLAM is one of the (co) receptors for PPRV. However, the presence of other putative virus receptor(s) is/are not ruled out (Pawar et al., 2008).

Recombinant adenovirus and baculovirus vectors expressing short hairpin RNAs (shRNAs) against the PPRV inhibited generation of infectious progeny and the nucleoprotein expression. Baculoviral shRNA-expressing vectors have the potential for therapeutic use against morbillivirus infections (Nizamani et al., 2011).

Suicidal DNA as a Vaccine

pSCA1, an Semliki Forest virus (SFV) replicon vector with H (Wang et al., 2013c) and F (Wang et al., 2015) gene generated specific antibodies, neutralizing antibodies and lymphocyte proliferation responses in mice. Increase in IL-2, IL-10, IFN-γ and TNF-α was also recorded. This could be considered as a novel strategy for vaccine development against PPR.

Synthetic Epitopes / Peptides

Through the years, various chemical modifications of antigen have been attempted to enhance efficacy and safety. Recent approaches with modified epitopes, peptides, adjuvants, recombinant antigens, T-cell tolerizing constructs, and improved oral approaches have been demonstrated in various clinical studies to provide measurable benefit in treatment of various diseases. Recent developments in structural biology have paved the way for better understanding of biological behaviour of the peptides and protein. Peptide science can provide novel methodologies for the diagnostics, therapeutics and prophylactic measures. Further, the superior synthetic peptide designs can improve the practical applications of these methodologies. The peptides have wider biomedical applications in diverse areas like drug design, targeted drug delivery, chemotherapy, serodiagnosis, oncology and vaccinology (Briand et al., 1992; Bais et al., 2008). Computer based prediction algorithms was applied to identify antigenic determinants on the N protein of PPRV. Specificity and antigenicity of each peptide was evaluated by solid phase ELISA. Six specific peptide sequences were evaluated in multiple antigenic peptide (MAP) form and immune response was evaluated by supplementing universal T-helper epitope human IL-1beta peptide (VQGEESNDK, amino acids 163-171). Out of the six peptides 19mer sequence corresponding to 454-472 region of N protein of PPRV was found to be highly immunogenic and specific to PPRV. Evaluation of overlapping peptides differing in length for this 452-472 region, showed minimum length of 14 amino acid residues were required for the stable affinity binding of antigen-antibody. The results of immunization and indirect ELISA indicated the presence of T-helper epitope at the N-terminal end and Linear B epitope at the C-terminal region of 454-472 19mer of nucleocapsid peptide of PPRV-nucleocapsid protein. The antipeptide antibodies developed against this region showed specificity to PPRV antigen differentiating it from RPV when used in in-

direct ELISA and western blot analysis (Dechamma et al., 2006). A cELISA for detecting serum antibody against PPRV was developed by chemically synthesising epitopic peptides of the N protein of Tibetan PPRV for monitoring PPRV infections with good sensitivity and specificity values. This method eliminated the requirement for virus culture and MAb preparation, reduced the biorisk posed by virus-dependent manipulations, and the performance of the resultant cELISA compared favorably with a commercially available cELISA kit (Zhang et al., 2013).

Reverse Genetics Approaches

Negative sense, non-segmented RNA genomes, host range and pathogenic determinants of all *paramyxoviruses* have been extensively studied using reverse genetics. This technology enables a more rational approach to be taken with respect to vaccine design. It is possible to engineer PPR marker vaccines which can allow DIVA. The F and H proteins, have been introduced into the genome of a capripox vaccine strain. The generated viruses have proved to be effective as a dual vaccine candidates offering to protection against PPR and CP infections (Diallo, 2003).

PPRV minigenome was constructed and expressed in transfected cells. Minimum requirements for minigenome rescue were shown to be the cis-acting elements of the genome (GP) and antigenome (AGP) promoters as well as the three trans-acting helper proteins N, P (phosphoprotein) and L (large polymerase). Homologous PPRV helper proteins were compared to their heterologous analogues from the closely related RPV and heterologous minigenome rescue was found to be a much less efficient process. By engineering two GP/AGP chimerics, differences between the two viruses were identified in the specific interactions between the promoters and the transcriptase / replicase complexes. The PPRV minigenome was also shown not to strictly comply with the "rule of six" *in vitro* (Bailey et al., 2007).

The large (L) polymerase gene and the 5'-terminal UTR of the genome (vaccine strain Nigeria 75/1) expressed in eukaryotic cells and its polymerase activity was quantitatively measured in a PPR reverse genetics assay using a reporter minigenome. Comparative sequence analysis of functional L gene with corresponding genes of other morbilliviruses showed a degree of conservation exceeding 70 per cent. The multiple sequence alignment and the phylogenetic study of L gene discriminated the morbilliviruses in six clusters, which are more closely related to Tupaia and Henipa viruses than to other paramyxoviruses. Important protein domains and functional motifs of the L polymerase of the PPRV Nigeria 75/1 vaccine were also identified by using different bioinformatics tools (Minet et al., 2009).

Recombinant PPRV from a full-length cDNA clone of the virus genome was successfully rescued using a RNA polymerase II promoter to drive transcription of the full-length virus anti genome. A virus expressing a tracer protein (green fluorescent protein, GFP) was constructed and the recombinant virus replicated well and could stably express GFP. The newly established reverse genetics system for PPRV provides a novel method for constructing a vaccine using PPRV as a vector, and will also prove valuable for fundamental research on the biology of the virus. Recombinant virus allowed more rapid and higher throughput assessment of PPRV neutralization antibody titers via the virus neutralization test (VNT) compared with the traditional method (Hu et al., 2012).

Mutated residues critical for C1 mAb binding were identified by phage display and assessed in an in vitro fusion assay. Mutated epitopes were incorporated into a full length clone and recombinant RPV was rescued using reverse genetics techniques. Here we describe a novel mechanism of marking morbillivirus vaccines, using RPV as a proof of concept, and discuss the applicability of this method to the development of marked vaccines for PPR (Buczkowski et al., 2012).

Anti idiotypic antibody based vaccine has also been attempted.Immunization of sheep with DNA coding for the variable region of anti-idiotypic antibody generated humoral and cell mediated immune responses specific for PPRV (Apsana et al., 2015).

Helper Cell Dependent Diagnostics

Helper cell dependent form of PPRV was created by removing the entire RNA polymerase gene and complementing it with polymerase made constitutively in a cell line. The resultant L-deleted virus grows efficiently in the L-expressing cell line but not in other cells. Virus made with this system is indistinguishable from normal virus when used in diagnostic assays, and can be grown in normal facilities without the need for high level biocontainment. The L-deleted virus will thus make a positive contribution to the control and

study of this important disease (Baron and Baron, 2015). The available new diagnostics/vaccines/therapeutics for PPR is summarized in Table 1.

Future Perspectives

PPR is an important animal viral disease of sheep and goats, which now threatens the billion-strong small ruminant population in Africa, the Middle and Near East, South-West and Central Asia. PPR is the one of the priority animal diseases whose control is considered important for poverty alleviation. (Balamurugan et al., 2014). More research is needed on the host virus interaction through cellular receptor, immunological events including protective mechanisms, development of marker vaccine to differentiate between virulent and vaccine virus antibodies and also on development of thermostable vaccine. With the launch of Global PPR Control and Eradication Programme (GCEP), lot of research is needed not only to improve the vaccines but also the diagnostic tools. A great deal of more research into this aspect of the disease is need of an hour. Development of a vaccine that enables differentiation of infected from vaccinated animals (DIVA) would benefit PPR control and eradication programmes, particularly at the later stages of an eradication campaign. Thus there is an immediate need for developing a novel marker vaccine which can be either multivalent, thermostable or recombinant technology based one. The availability of an effective marker vaccine along with its companion serological tests will greatly assist in designing effective control programmes in future. The new diagnostics/vaccines/therapeutics described above either improve existing

Table 1: *Recent developments in diagnostics/vaccines/therapeutics against PPR*

System	Gene of interest	Reference
Prokaryotic system	N, F, M	Yadav et al., 2009; Zhang et al., 2012; Wang et al 2013; Liu et al., 2013; Balamurugan et al., 2014; Apsana, 2014
Eukaryotic syste, Yeast system:	F	Lotfi et al., 2007
Baculo virus system	N, F, M, H, HN	Ismail et al., 1995; Libeau et al., 1995; Sinnathamby et al., 2001; Rahman et al., 2003; Liu et al., 2014a, 2014b.
Mammalian system	H, HN	Seth et al., 2001; Balamurugan et al., 2006; Das *et al.,* 2000
Plant system	HN	Khandelwala et al., 2010
Virus like particle-based (VLP) vaccines	M, N, F and H	Liu et al., 2014; Li et al., 2014a; Liu et al., 2014b; Liu et al, 2015
Bivalent/Multivalent vaccines	Live attenuated combined vaccines	Hosamani et al., 2006; Chaudhary et al., 2009
Recombinant viral vectored vaccines Capripox virus (CPV)	H and F	Romero et al., 1995; Chen et al., 2010; Berhe et al., 2003; Caufour et al., 2014
Goatpox virus	H	Che et al., 2009
PPR virus	FMDV VP1	Yin et al., 2014
Fowl pox virus	F and H	Herbert et al 2014
Adenoviruses	F, H, F-H	Wang et al 2013
Canine adenovirus type-2	H	Qin et al., 2012
Human adenovirus 5	F and H	Herbert et al., 2014; Rojas et al., 2014
Vaccinia virus	H and F (RPV)	Jones *et al.,* 1993
Vaccinia virus Ankara virus	F and H	Chandran et al., 2010
RNA interference as therapy against PPR	N	Servan d Almeida et al., 2007; Keita et al., 2008
Suicidal DNA as a vaccine	H, F	Wang et al., 2013; Wang et al., 2015
Synthetic epitopes/peptides	N	Dechamma et al., 2006; Zhang et al., 2013
Reverse genetics	F, H, N, P, L	Diallo, 2003; Bailey et al., 2007; Minet et al., 2009; Hu et al., 2012; Buczkowski et al., 2012
Anti idiotypic antibody based vaccines	-	Apsana et al., 2015
Helper cell dependent diagnostics	-	Baron and Baron, 2015
Chemically synthesized siRNAs	-	Pawar et al., 2008
shRNAs	-	Nizamani et al., 2011

techniques or develop new approaches to old questions in order to control and eradicate the disease in a more efficient way.

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Conflict of Interest

No conflicts of interests are declared by authors for the contents in this manuscript.

Authors' Contribution

A.R. Gomes planned and wrote the draft of the manuscript. B.M. Veeregowda and S.M. Byregowda gave the technical guidance and support. V. Balamurugan provided inputs, guidance, support and edited manuscript.

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