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REVIEW ARTICLE

# Current perspectives on conventional and novel vaccines against peste des petits ruminants

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**Abstract** Peste des petits ruminants (PPR) is an acute or subacute, highly contagious viral disease of small ruminants, characterized by fever, oculonasal discharges, stomatitis, diarrhoea and pneumonia. This disease is included in the OIE (Office International des Epizooties) list of notifiable terrestrial animal diseases. PPR was first described in the early 1940s in Côte d'Ivoire, and at present, PPR is mainly circulating in Western and Central Africa, the Arabian Peninsula and Southern Asia. Peste des petits ruminants virus (PPRV), the etiological agent of PPR, is classified into the genus *Morbillivirus* in the family *Paramyxoviridae*, as its biological and physicochemical features are closely related to the other morbilliviruses. The first homologous PPR vaccine was developed by an artificially attenuated PPRV, named as Nigeria 75/1, which has been widely used in the production of live attenuated vaccines to protect small ruminants. A new generation of PPR vaccine candidates can be genetically modified to differentiate infected from vaccinated animals (DIVA), which nevertheless is difficult to achieve by conventional vaccines. In this review, we systematically discussed a broad range of vaccines against PPR, including commercially available vaccines and potential vaccine candidates, and further DIVA strategies for immunization with the new generation vaccines.

**Keywords** Peste des petits ruminants · Peste des petits ruminants virus · Vaccine · Immunization · DIVA · Eradication

## Introduction

Peste des petits ruminants (PPR) is an acute or subacute, highly contagious and economically important viral disease of small ruminants, characterized by high fever, oculonasal discharges, pneumonia, stomatitis, and inflammation of gastrointestinal tract (Balamurugan et al. 2010b; Gur and Albayrak 2010; Khan et al. 2008). This disease primarily affects goats and sheep, occasionally infecting wild small ruminants (Kinne et al. 2010), buffalos (Govindarajan et al. 1997), camels (Khalafalla et al. 2010) and even pigs (Nawathe and Taylor 1979), whereas except goats and sheep, the other animals are unlikely to contribute significantly to the epidemiology, since they are unable to excrete viruses as easily as goats and sheep. Outbreaks of PPR are more severe in goats than in sheep; newborn and young animals are more severely affected than adults (Abd El-Rahim et al. 2010). Since morbidity and mortality in PPR-infected animals can be as high as 100 and 90 %, respectively (Luka et al. 2011b), the disease is included in the OIE (Office International des Epizooties) list of notifiable terrestrial animal diseases. PPR was first described in the early 1940s in Côte d'Ivoire (Gargadennec and Lalanne 1942) and at present, is mainly circulating in Western, Central and Eastern Africa (Couacy-Hymann et al. 2005), the Arabian Peninsula (Al-Dubaib 2009) and Southern Asia (Hegde et al. 2009). PPR has remained endemic and given rise to serious socio-economic problems to the rural poor relying on domestic small ruminants as a source of livelihood.

Peste des petits ruminants virus (PPRV), the etiological agent of PPR, is classified into the genus *Morbillivirus* in the family *Paramyxoviridae*, as its biological and

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physicochemical features are closely related to the other morbilliviruses, such as measles virus (MV), canine distemper virus (CDV) and especially rinderpest virus (RPV) (Gibbs et al. 1979). PPRV is a pleomorphic particle (Fig. 1a) with a lipid envelope enclosing a ribonucleoprotein core that contains the genome, a single strand of RNA with negative polarity (Mahapatra et al. 2006), which encodes for six structural (N, P, M, F, H and L) and two nonstructural (V and C) proteins in the order of 3′-N-P (V/C)-M-F-H-L-5′ (Fig. 1b). At the nucleotide level, the full-length genome of PPRV is most similar to that of RPV (Bailey et al. 2005).

Taking advantage of the close relationship between PPRV and RPV, the attenuated tissue culture rinderpest vaccine has been used as a heterologous vaccine for a long time to protect small ruminants from PPR (Diallo 2003). During the past three decades, different PPRV isolates, like Nigeria 75/1 and Sungri/96, were successfully attenuated by serial passages in Vero cells (Saravanan et al. 2010). As demonstrated to be very efficient in the protection of sheep and goats against virulent challenges, these avirulent PPRVs as efficacious immunogens are now widely used in commercially available PPR vaccines. Their thermostability has been dramatically improved by freeze-drying, whereas it is still imperative to maintain a cold-chain for the vaccines shipment and storage, which unfortunately has proven difficult in tropical and subtropical regions.

Unlike conventional vaccines, a new generation of genetically engineered vaccines can avoid cold-chain-associated problems in these regions (Sen et al. 2010). Particularly, immunization with the novel vaccines might provide a way to differentiate between vaccinated and infected or recovered animals. Such a differentiation is difficult to achieve by conventional vaccines. Novel vaccines can be expected to be successfully commercialized for prophylactic immunization in future. In this review, we systematically discussed a broad range of vaccines against PPR, including commercially available vaccines and potential vaccine candidates, and DIVA (differentiating infected from vaccinated animals) strategies for vaccination with the new generation vaccines against PPR.

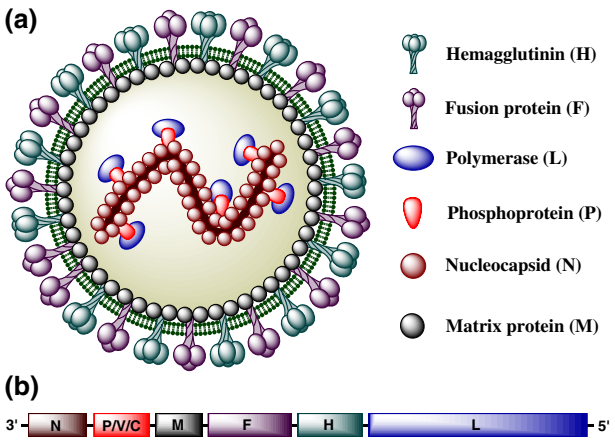


**Fig. 1** Schematic diagram of peste des petits ruminants virus (PPRV) structure. In the virion (**a**), the N, P and L proteins constitute the nucleocapsid that encloses the viral genome, while the H and F proteins as two glycoproteins along with the M protein form the viral envelope. The PPRV genome (**b**) encodes for eight proteins, and each gene encodes for a single protein except the P gene, which is translated into two nonstructural proteins, namely V and C proteins

## Old-fashioned strategies for immunization against PPR

### Immunization with hyperimmune serum

Serum immunization, introduced in the late 19th century (1888–1898) when a severe outbreak of rinderpest occurred in Africa, was involved in the use of serum either alone or in combination with virulent blood to treat animals. Cattles vaccinated with a mixture of rinderpest hyperimmune serum and virulent blood could develop an efficacious immunity. This simultaneous serum-virus vaccination, until the later development of inactivated vaccines, was the most effective way of immunizing cattle against rinderpest and was broadly used in both Africa and India (Brown and Rashid 1965; Mitchell and Le 1946).

Similarly, PPR hyperimmune sera have proven effective in reversing the process of the disease, if administered in animals at the fever stage. Goats inoculated with hyperimmune sera could survive for 10 days before showing evidence of reinfection (Ihemelandu et al. 1985). In comparison with the short-term protection conferred by passive immunity with hyperimmune serum alone, goats inoculated simultaneously both with hyperimmune serum and with virulent PPRVs would even develop a durable immunity against PPRVs. For example, a goat, when inoculated with a mixture of 8 mL of hyperimmune serum and 4 mL of virulent PPRV suspension, could still survive challenge with virulent PPRVs 9 months post-inoculation (Adu and Joannis 1984). However, one difficulty associated with this immunological method was the high cost of PPR hyperimmune sera. An availability of PPRVs for immunization, however, presented another difficulty: they had to be reproduced in vivo, in the absence of related methods to amplify viruses in vitro at that time. In addition, a further consideration was a short shelf-life of virulent bloods, which generally remained for no more than 10 days under the condition of warm weather (Adu and Joannis 1984).

### Heterologous vaccines against PPR

Since RPV and PPRV share a high homology at the nucleotide level and a high degree of antigenic cross-reactivity (Balamurugan et al. 2010a; Libeau and Lefevre 1990; Raha et al. 2004), in the absence of homologous vaccine, the tissue culture rinderpest vaccine based on the Muguga modification of the Kabete O (RBOK) strain of RPV has been used as a

heterologous vaccine for a long time to protect small ruminants against PPR in Western Africa (Diallo 2003; Lefevre and Diallo 1990). Goats vaccinated with this rinderpest vaccine were protected from PPR for at least 12 months with a high level of neutralizing antibodies against both viruses, and further, vaccinated animals were unable to transmit the challenge virus (Taylor 1979). In addition, the thermostable Vero cell-adapted rinderpest vaccine has been evaluated in terms of immunogenicity as a heterologous vaccine against PPR, suggesting that the thermostable Vero cell-adapted rinderpest vaccine is a suitable candidate for the protection of goats against PPR (Mariner et al. 1993).

Nevertheless, in order to achieve the status of rinderpest-free country or zone following the OIE pathway, the use of rinderpest vaccines in all animal species was discontinued worldwide. Moreover, with the advent of the homologous PPR vaccine in the mid 1990s (Diallo 2006), the practice of PPR control relying on rinderpest vaccines was progressively abolished in every endemic area (Sen et al. 2010). Fortunately, the OIE announced the global eradication of rinderpest on 25 May 2011, further indicating that the homologous PPR vaccine would be the only vaccine permitted for use in sheep and goats against PPRV infections.

## Current prophylaxis against PPR: homologous vaccines

### Vaccine strains

Soon after the first isolation of PPRV in tissue culture in the 1960s, preliminary attempts were made to develop a live attenuated vaccine, but were unsuccessful until the late 1980s (Diallo et al. 2007). The first homologous vaccine was developed by a virulence-attenuated PPRV, named as Nigeria 75/1, and represented a significant milestone in the history of vaccines against PPR, because its introduction bade farewell to the era of excessive reliance on the tissue culture rinderpest vaccines to control PPR. The virus Nigeria 75/1 was initially isolated from a sick Nigerian goat infected with PPR in 1975 (Taylor and Abegunde 1979). In the late 1980s, the isolate was successfully attenuated through consecutive passages in Vero cells (Diallo et al. 1989). The characteristics of progressive attenuation of PPRV virulence by serial passages in Vero cells, meanwhile, was demonstrated by Adu et al. (1990) who found that goats inoculated with the 60th passage suffered from the clinical PPR disease, while others inoculated with the 80th passage did not show any sign of the disease (Adu et al. 1990).

A number of field trials have been conducted on more than 98 000 sheep and goats in the period 1989–1996, demonstrating that the Nigeria 75/1 vaccine could not cause unwanted side effects such as abortion in pregnant animals, and furthermore, vaccinated animals were unable to transmit the challenge virus to others. Anti-PPRV antibodies elicited by the vaccine were highest during 30 to 45 days post-vaccination (Khan et al. 2009) and would last for at least 3 years, an effective economic life of domestic small ruminants (Diallo et al. 2007). Owing to the significant cross-protection between PPRV and RPV (Chandran et al. 1995), the live attenuated Nigeria 75/1 vaccine was able to confer further an additional protection for small ruminants against RPV when rinderpest epidemics occurred in the past. Goats vaccinated with the Nigeria 75/1 vaccine have proven both to be quite capable of resisting challenge with virulent RPVs and to be unable to affect other susceptible animals (Couacy-Hymann et al. 1995).

The Sungri 96, Arasur 87 and Coimbatore 97 strains, all belonging to the lineage IV as per F gene sequence analysis, were isolated in India and have proven experimentally to be potent lineage IV-specific vaccine strains by serial passages of viruses in Vero cells. Out of the three strains, the Sungri 96 was the earliest vaccine strain developed by attenuating the original Sungri isolate up to 60 passages in Vero cells at the Rinderpest Laboratory, Indian Veterinary Research Institute (IVRI). The genome sequence of Sungri 96 showed 96 to 99 % identity with the Asian isolates and 89 to 92 % identity with the African isolates. Phylogenetic analysis of the complete genome sequences revealed that the Sungri 96 vaccine strain clusters with the Asian isolates to a common node away from the African isolates (Siddappa et al. 2014). The Sungri 96 vaccine has been tested extensively in the laboratory and field to demonstrate that it was safe and efficacious in sheep and goats. Further studies were conducted by the IVRI both on the thermostability of the vaccine by comparing various chemical stabilizers (Sarkar et al. 2003) and on the immunosuppressive effects on goats (Rajak et al. 2005), suggesting that the Sungri 96 PPRV vaccine was a potential substitute for the conventional Nigeria 75/1 vaccine for use in domestic small ruminants. In addition, the former can provide stable immunity for at least 6 years and therefore is used throughout India to vaccinate sheep and goats with great efficacy against lineage IV virus (Sen et al. 2010).

The Arasur 87 and Coimbatore 97 were originally isolated from southern part of India and have also been artificially attenuated by serial passages in Vero cells. Although the Arasur 87 is closely related to the Sungri 96 in antigenicity, both can easily be differentiated based on the pattern of cytopathic effect and the degree of neutralization using specific monoclonal antibodies (4B11) (Singh et al. 2010). Potency tests of these vaccines in sheep and goats following the OIE guidelines revealed that all the animals vaccinated with the Arasur 87, Coimbatore 97 or Sungri 96 vaccine withstood the challenge up to 14 days post-challenge, and showed neither rise in rectal temperature nor other PPR-specific signs (Saravanan et al. 2010). These results indicated

that they were potent and could presumably be used for mass vaccination while contemplating a PPR control program. In addition to these four vaccine strains as mentioned above, Egypt 87 was also a local strain of PPRV, which was attenuated by the Veterinary Serum and Vaccine Research Institute, Egypt, and was commercially available at present (Nahed et al. 2004).

Up to now, such live attenuated strains, especially the Nigeria 75/1, have been employed in the commercial production of PPR vaccines in Vero cells on a large-scale basis in roller bottles (Asim et al. 2009; Silva et al. 2008). The commercially available PPR vaccines have been summarized in Table 1. Although results of laboratory and field trials revealed that the vaccines could confer strong protective immunity in sheep and goats, most of them were susceptible to thermodegradation similar to rinderpest vaccines, and thereby should be transported at 2 to 8 °C and be stored at −20 °C.

## PPR and sheep/goat pox combined vaccines

Sheep pox (SP) and goat pox (GP) are contagious diseases of small ruminants caused by sheep pox virus (SPV) and goat pox virus (GPV), respectively, both belonging to the genus *Capripoxvirus* in the family *Poxviridae* (Hosamani et al. 2004). Clinical signs may be mild in indigenous breeds living in endemic areas, but are often fatal in newly introduced animals. Mixed infections of PPR and SP (Ozmen et al. 2009) or GP (Malik et al. 2011; Saravanan et al. 2007) have been increasingly reported in PPR-endemic regions. Therefore, in view of the occurrence of mixed infections caused by a similar geographic distribution of both PPR and GP/SP infection, it is helpful to develop efficacious combined vaccines for prevention of both the mixed infections, particularly in endemic areas. A previous study (Rajak et al. 2005) showed that PPR vaccine virus did not interfere with the immunogenicity to other unrelated antigens in goats, supporting the potential feasibility of PPR and SP/GP combined vaccines.

Chaudhary et al. (2009) developed a PPR and SP combined vaccine, prepared in lyophilized form containing recommended doses of both vaccine viruses, namely the SPV Romanian Fanar and PPRV Sungri 96 (Chaudhary et al. 2009). Sheep immunized subcutaneously with 1 mL of this vaccine resisted challenge with either virulent SPVs or PPRVs on day 30 post-immunization, while control animals developed characteristic signs of disease. Moreover, specific viruses could be detected in the unvaccinated control animals after challenge but not from any of the immunized sheep. Since the component vaccines were compatible with each other, the combination of both the vaccine strains could be used in target population for economic vaccination.

Prior to this study as mentioned above, Hosamani et al. (2006) have even evaluated experimentally the safety and immunogenicity of a combined vaccine comprising attenuated strains of PPRV and GPV in goats (Hosamani et al. 2006). They also found that the combined vaccine was able to induced protective immune response with high safety in goats as evident from seroconversion and challenge studies. More importantly, component vaccines did not interfere with the immunogenicity of each other. Despite the success of both the studies on combined vaccines, further studies should be required to determine the duration of immunity conferred by them.

**Table 1** *Commercially available peste des petits ruminants vaccines worldwide

| Product name | Vaccine type | Vaccine strain | Manufacturer | Licensed country |
|---|---|---|---|---|
| PPR-VAC® | Live | Nigeria 75/1 | Botswana Vaccine Institute | Botswana |
| Freeze Dried PPR Vaccine | Live | Nigeria 75/1 | Central Veterinary Control and Research Institute | Turkey |
| PESTDOLL-S | Live | Nigeria 75/1 | Dollvet | Turkey |
| PPR Vaccine-Sungri 96 strain | Live | Sungri 96 | Hester Biosciences Limited | India |
| PPR Vaccine-Nigerian 75/1 strain | Live | Nigeria 75/1 | Hester Biosciences Limited | India |
| Intervac Pestevac | Live | Nigeria 75/1 | Intervac (PVT) Ltd. | Pakistan |
| PESTEVAC | Live | Nigeria 75/1 | Jordan Bio-Industries Center (JOVAC) | Jordan et al. |
| Peste des Petits Ruminants Vaccine, Live | Live | Nigeria 75/1 | Xinjiang Tecon Co., Ltd | China |
| Peste des Petits Ruminants Vaccine | Live | Nigeria 75/1 | National Veterinary Research Institute | Nigeria |
| Peste des Petits Ruminants Vaccine | Live | Nigeria 75/1 homologous | Nepal Directorate of Animal Health | Nepal |
| Pestvac K™ | Live | Nigeria 75/1 | Vetal Company | Turkey |
| PPR-TC Vaccine Attenuated | Live | Nigeria 75/1 | Veterinary Serum and Vaccine Research Institute | Egypt |
| RAKSHA-PPR | Live | Sungri 96 | Indian Immunologicals Limited | India |

*Source: The homepage of the Center for Food Security&Public Health, the United States (http://www.cfsph.iastate.edu)

## Improvement in thermostability of homologous PPR vaccines

Undoubtedly, the most widely accepted procedure for the long-term preservation of live PPRVs in vaccines is lyophilization (Worrall et al. 2000), which, however, is limited by technological conditions and thus is difficult to enhance thoroughly the thermostability of live attenuated vaccines. Thermostability of a live vaccine can be defined in terms of its shelf-life at ambient temperature simulated with field situation (Sarkar et al. 2003). In tropical and subtropical regions, one of the greatest barriers against vaccination for control of PPR is a loss of potency for the conventional vaccines in the absence of cold-chain infrastructure for shipment and storage. Thus, it is imperative to develop new methods to improve the thermostability of vaccines, due to a lack of cold-chain transport system in many tropical and subtropical countries.

### Chemical stabilizers

The chemical stabilizer is referred to as a substance added to vaccines to prevent unwanted changes in state of immunogens. Different stabilizers, such as lactalbumin hydrolysate-sucrose (LS), Weybridge medium (WBM), lactalbumin hydrolysate-manitol (LM), buffered gelatin-sorbitol (BUGS) and trehalose dihydrate (TD), are mostly used to prepare the lyophilized vaccines (Asim et al. 2008). For example, LS stabilizer can maintain the protective titers of Vero cells-adapted rinderpest vaccine up to 4 h at ambient temperature if reconstituted with 0.85 % NaCl and 1 M $MgSO_4$ (Mariner et al. 1990). The OIE recommended the use of WBM as a chemical stabilizer for PPR lyophilized vaccines, which nevertheless were susceptible to thermal degradation in the absence of a cold-chain system (Silva et al. 2011).

The WBM, LS and LM were broadly used to lyophilize the PPR vaccines. Asim et al. (2008) reported that PPR vaccine lyophilized with the WBM was more stable and maintained the virus titer longer than with two other stabilizers (Asim et al. 2008). In contrast, another study conducted by Sarkar et al. (2003) revealed that the PPR vaccine lyophilized with either LS or TD is more stable than with both WBM and BUGS, having an expiry period of at least 45 days at 4 °C, 15–19 days at 25 °C and 1–2 days at 37 °C. However, at 45 °C, BUGS had a marginal superiority, although lasted for few hours, followed by TD and LS with respect to shelf-life, LS and TD with respect to half-life (Sarkar et al. 2003).

A new extrinsic stabilizer, stabilizer E (trehalose, $CaCl_2$ and $MgCl_2$), was recently introduced into PPR lyophilized vaccines, and was assessed for its stability at different temperatures in lyophilized form as compared with the LS (Riyesh et al. 2011). The results showed that both the stabilizers performed equally well with regard to shelf-life and half-life. The LS was superior at 42 °C with a shelf-life of 44 h, whereas in stabilizer E, a 40 h shelf-life with a comparable half-life was observed. At 45 °C, the half-life in stabilizer E was better than in LS and lasted for 1 day. The vaccine in stabilizer E fared better in 1 mol/L $MgSO_4$ diluent for 30 h at 4 °C and for 24 h at 25 °C as well as at 37 °C. The same vaccine with the LS, 1 mol/L $MgSO_4$ was found suitable for 48 h at 4 °C but at 25 °C and 37 °C, the stability lasted for 24–30 h (Riyesh et al. 2011). Similarly, a report revealed more recently that replacing the formulation from WBM to Tris/Trehalose significantly improved the stability of a PPR vaccine produced at the National Veterinary Institute in Ethiopia (Silva et al. 2014). Based on references (Riyesh et al. 2011; Sarkar et al. 2003), we compared both shelf-life and half-life of three PPR vaccines lyophilized with different stabilizers at various temperatures, and the results were listed in Table 2.

### Thermo-stable vaccine strains

Another method to improve thermostability of PPR vaccines is involved in the use of thermo-stable vaccine strains in terms of their stability at ambient temperature. For the purpose of achieving their thermo-resistance in nature, native PPRVs should generally be cultured consecutively for many passages at a relatively high temperature, which would contribute to obtaining a viral quasispecies characterized by the intrinsic thermo-stability. Such a viral quasispecies can be grown better at a relatively high temperature than conventional vaccine strains, whereas its stability and immunogenicity need to be assessed before considered as a vaccine candidate.

More recently, one group initially reported the successful development of thermo-stable and virulence-attenuated PPRVs derived from a virulent isolate (Jhansi 2003), by their up to 50 serial passages in thermo-adapted Vero cells grown at 40 °C. As a result, such viruses were found sterile, innocuous in mice and guinea pigs, and safe in seronegative goats and sheep. More importantly, the thermo-stable strains did not induce any adverse reaction at high dose ($10^5$ $TCID_{50}$) in goats and sheep, and provided complete protection even at low dose ($10^2$ $TCID_{50}$) in goats when challenged with virulent viruses. Additionally, there was no shedding and horizontal transmission of the attenuated viruses to in-contact animals (Balamurugan et al. 2014). The results indicated that the developed thermo-stable strains were innocuous, safe, immunogenic and potent, and could be used as efficacious vaccine candidate alternative to the existing PPR vaccines.

**Table 2** #Comparison of both shelf-life and half-life of three PPR vaccines lyophilized with different stabilizers at various temperatures

| SL or HL (d/h) | Stablizers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | LS* | WBM* | BUGS* | 2.5 % TD* | 5 % TD* | LS** | LS*** | E** | E*** |
| SL[a] | ND | 123 d | 239 d | 2051 d | ND | ND | ND | ND | ND |
| HL[a] | ND | 30 d | 42.25 d | 500 d | ND | ND | ND | ND | ND |
| SL[b] | 15 d | 5 d | 12 d | 16 d | 19 d | 23.29 d | 22.28 d | 25.64 d | 22.56 d |
| HL[b] | 4.76 d | 1.83 d | 2.17 d | 4.67 d | 4 d | 4.68 d | 4.9 d | 4.62 d | 4.81 d |
| SL[c] | 1.58 d | ND | 1.55 d | 1.05 d | 1.96 d | 7.62 d | 6.82 d | 6.95 d | 5.51 d |
| HL[c] | 17.8 h | 10 h | 7.79 h | 8.57 h | 14.07 h | 1.76 d | 2 d | 1.94 d | 1.8 d |
| SL[d] | ND | ND | ND | ND | ND | 3.68 d | 2.61 d | 3.48 d | 2.29 d |
| HL[d] | ND | ND | ND | ND | ND | 0.66 d | 0.59 d | 0.72 d | 0.67 d |
| SL[e] | ND | ND | ND | ND | ND | 43.18 h | 23.8 h | 39.25 h | 40.5 h |
| HL[e] | ND | ND | ND | ND | ND | 10.6 h | 7.12 h | 11.1 h | 9.68 h |
| SL[f] | 5.72 h | 0.56 h | 10.8 h | 7 h | 8.11 h | 22.87 h | 9.52 h | 24.67 h | 26.95 h |
| HL[f] | 2.29 h | 1.33 h | 2.4 h | 1.3 h | 1.96 h | 6.21 h | 4.14 h | 8.4 h | 12.87 h |

#Based on references (Riyesh et al. 2011; Sarkar et al. 2003); *, ** and *** corresponding to Sungri/96, Jhansi/2003 and Revati/2006; the superscript letters, a, b, c, d, e and f, refer to at 4, 25, 37, 40, 42 and 45 °C; d: days; h: hours; *SL* shelf-life; *HL* half-life; *LS* lactalbumin hydrolysate-sucrose; *WBM* Weybridge medium; *BUGS* buffered gelatin-sorbitol; *TD* trehalose dehydrate; *E* trehalose, $CaCl_2$ and $MgCl_2$; *ND* not done

## Production and use of homologous PPR vaccines

### Production of lyophilized vaccines

Live attenuated vaccines against PPR have been commercially produced using different vaccine strains on a large scale basis by many manufacturers. Hegde et al. (2008) described the standardization of large-scale production of PPR vaccines in detail (Hegde et al. 2008) and an outline of production has been properly summarized by us to show in Fig. 2. There are various considerations, such as quality, safety, efficacy and GMP (good manufacturing practice), involved in a process of producing PPR vaccines. Technologies and production methods underlying vaccines must operate in compliance with the principles of current GMP, for detailed review see (Heldens et al. 2008).

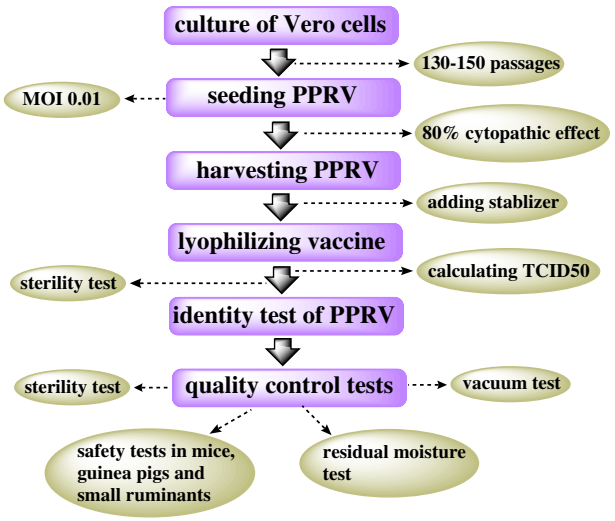


**Fig. 2** Flow chart of commercial production of peste des petits ruminants lyophilized vaccine. *MOI* multiplicity of infection, *TCID* tissue culture infective dose, *PPRV* peste des petits ruminants virus

Nowadays, PPR vaccines are produced in Vero cells using a roller bottle-based conventional method, nevertheless involving high efforts concerning consumables, having limited scalability and increasing bioprocess costs. Therefore, there is a need for better production processes of vaccines controlling future PPR outbreaks (Silva et al. 2008). Microcarriers enable most anchorage-dependent animal cells to grow in suspension cultures, and can be used to increase the surface area of traditional monolayer cultures. Microcarrier (Cytodex-1®) beads, if used to culture Vero cells for infection and amplification of PPR vaccine viruses, have proven to be effective in enhancing the viral yield: peak infectivity titer of PPRVs in microcarrier culture was found to be 9.81 $\log_{10}TCID_{50}$/0.1 mL at 72 h post-infection, being two log higher amount of virus than the stationary culture (Mohan et al. 2009). This microcarrier-based culture system may be used as a basis for further development of a large-scale process for PPR vaccine production.

### Clinical immunization with lyophilized vaccines

Control of PPR may be attained using measures including immunization for sheep and goats, slaughter of infected animals, removal of potential fomites and a restriction on the importation of animals from endemic areas (Abubakar et al.

2011a). Control of PPR outbreaks may also rely on movement control (quarantine) combined with the use of focused "ring" vaccination and prophylactic immunization in high-risk populations (Abubakar et al. 2011b).

Compared with large ruminants, smaller ruminants have a high turnover, which can be estimated that every 3 years all animals of a flock are susceptible to PPR in absence of pre-immunization, and thereby require frequent immunization for themselves (Diallo 2006). However, most of the developing countries affected by PPR cannot afford drastic sanitary measures, e.g., a strict stamping out policy. Moreover, it seems that in most cases, there has been no actual program for effective control of PPR in certain endemic areas as yet and therefore, vaccination would be also implemented mainly as a precaution in face of PPR outbreaks to avoid possible transmission in future. In particular, the only effective means to control PPR in PPR-endemic countries is mass immunization with commercially available vaccines. To date, PPR vaccines for domestic small ruminants have played a significant role in prophylaxis of this disease worldwide. The conventional Nigeria 75/1 vaccine has been mostly used with high efficacy for sheep and goats, conferring immune protection at 1 month post-vaccination with a single dose (Khan et al. 2009). This protection can usually remain for at least 1 (Rashid et al. 2010) and probably 3 years without side reactions normally noticed, regardless of the lineage type circulating in particular countries or regions.

A previous study carried out in Uganda suggested that vaccinated 1- to 2-year-old animals had a better sero-positivity to PPR than any other age groups (Luka et al. 2011a). This finding was in agreement with other reports (Abd El-Rahim et al. 2010; Rashid et al. 2008), which revealed that goats were more susceptible to PPRVs than sheep, and kids and lambs were at higher risk than adults. Therefore, the suitable immunization schedule recommended by manufacturers for small ruminants is that lamb and kids can be vaccinated between 4–6 months old. This could be a suitable period to avoid window of susceptibility to PPRVs and the effort to eliminate PPR infection from susceptible populations (Balamurugan et al. 2012). Additionally, a minimum dosage required for vaccination is regarded as 100-fold the lowest dose of vaccine virus able to induce a 50% immunizing response. For instance, a 102.5 TCID50/dose is recommended immunizing response. For instance, a 102.5 TCID50/dose is recommended by the OIE as the required minimum titer for Nigeria 75/1 vaccine.

## DIVA strategies for development of novel PPR vaccine candidates

One major objective of current efforts on the development of veterinary vaccines is to obtain a formulation facilitating the generation and maintenance of specific pathogen-free herds, as this is one of the best means concerning long-term serological surveillance for epizootics. Conventional vaccines remain inactivated or attenuated forms of the targeted viruses, resulting in an impossibility to differentiate which subjects are infected or only vaccinated by laboratory tests (Peeters et al. 2001). In comparison with the conventional ones, new generation vaccines to distinguish infected from vaccinated animals are very desirable for the viral infection in livestock production, and can make vaccination a much more valuable tool in approving programs for the eradication and monitoring of animal diseases (Goldenberg et al. 2011; McElroy et al. 2009).

### Design of DIVA vaccines

The vaccine characterized by the differentiation of infected from vaccinated animals is defined as "DIVA vaccine", previously known as "marker vaccine", which not only confers effective protection but also contributes to epidemiological survey on animal diseases accompanied with proper diagnostic tests (Meeusen et al. 2007). In general, there are two feasible methods for the development of PPR DIVA vaccines: (1) "positive marker", by containing at least one heterologous protein or epitope in a potent vaccine, and (2) "negative marker", by the absence of at least one homogenous protein or epitope compared with a corresponding wild-type PPRV. Several studies were involved in the development of both the PPR DIVA vaccines (Table 3), such as viral vector vaccine, chimeric virus vaccine and subunit vaccine, most of which were designed based on the "negative marker" strategy but all of which have not been commercialized as yet.

PPR DIVA vaccine candidates should meet minimum demands, such as no side effects to vaccinated animals, easy to produce under industrial conditions, low cost and long-lasting immunity. In addition to meeting these requirements for immunization, they also should enable easy but accurate serological DIVA accompanied with proper diagnostic tests. Unlike other types of vaccines, DIVA vaccines must work together with their accompanying serological diagnostic tests. Thus, if a candidate vaccine were to be designed for DIVA strategies, it would be considered how to develop and to assess an effective diagnostic test (Dong and Chen 2007).

### Selection of markers for accompanying diagnostics

DIVA diagnostics for PPRVs, such as enzyme-linked immunosorbent assay (ELISA), is exclusively based on the detection of antibodies induced by either a positive or negative marker, whereas somewhat has a limitation for the reason that detection of differentiating antibodies in animals should be carried out a few weeks post-viral infection. Therefore, if many markers designed for the purpose of DIVA vaccination were weakly immunogenic, the titer of DIVA-specific antibodies induced by the markers would keep a relatively low level in such a short time, and accordingly would be difficult

**Table 3** New generation vaccine candidates against PPR

| Type of vaccine candidate | Characteristics | Immunized animal | Challenge | | | Ref. |
|---|---|---|---|---|---|---|
| | | | virus | p.v. | Result | |
| Viral vector vaccine | | | | | | |
| rVV vaccine | rVV simultaneously expressing F and H proteins of RPV | Goats | PPRV | 35 d | No deaths | (Jones et al. 1993) |
| rCPV vaccine | rCPV expressing either F or H protein of RPV | Goats | PPRV | 28 d | No deaths | (Romero et al. 1995) |
| rCPV vaccine | rCPV expressing H protein of PPRV | Goats | PPRV | 21 d | No deaths | (Diallo et al. 2002) |
| rCPV vaccine | rCPV expressing F protein of PPRV | Goats | PPRV | 14 d | No deaths | (Berhe et al. 2003) |
| rCPV vaccine | rCPV expressing either H or F protein of PPRV | Goats and sheep | CPV | 21 d | No deaths | (Chen et al. 2010) |
| rCAV-2 vaccine | rCAV-2 expressing H protein of PPRV | Goats | ND | ND | ND | (Qin et al. 2012) |
| rHAV-5 vaccine | rHAV-5 expressing either F or H protein of PPRV | Sheep | PPRV | 42 d | No deaths | (Rojas et al. 2014) |
| rAD vaccine | rAD expressing H and F proteins of PPRV | Goats | ND | ND | ND | (Wang et al. 2013a) |
| rAD vaccine | rAD expressing H protein of PPRV | Goats | PPRV | 15 w | No deaths | (Herbert et al. 2014) |
| Chimeric virus vaccine | | | | | | |
| Chimeric BV vaccine | Chimeric BV carrying a membrane bound form of H protein of PPRV | Goats | ND | ND | ND | (Sinnathamby et al. 2001b) |
| Chimeric BV vaccine | Chimeric BV carrying immunodominant ectodomains of F protein of PPRV | Mice | ND | ND | ND | (Rahman et al. 2003) |
| Vaccine using reverse genetics | | | | | | |
| rPPRV vaccine | rPPRV expressing GFP | ND | ND | ND | ND | (Hu et al. 2012) |
| rPPRV vaccine | rPPRV expressing FMDV VP1 | Goats | FMDV | 40 d | No deaths | (Yin et al. 2014) |
| Subunit vaccine | | | | | | |
| H protein subunit vaccine | H protein of PPRV expressed in transgenic peanut plants | ND | ND | ND | ND | (Khandelwal et al. 2011) |
| VLP-based vaccine | VLP composed of PPRV M and H (or F) proteins | Mice and Goats | ND | ND | ND | (Li et al. 2014) |
| Nucleic acid vaccine | | | | | | |
| Suicidal DNA vaccine | Recombinant pSCA1 plasmid expressing H protein of PPRV | Mice | ND | ND | ND | (Wang et al. 2013b) |

*AD* adenovirus, *BV* baculovirus, *FMDV* foot-and-mouth disease virus, *GFP* green fluorescent protein, *ND* not done, *p.v.* post vaccination, *PPR* peste des petits ruminants, *PPRV* peste des petits ruminants virus, *rVV* recombinant vaccinia virus, *rCPV* recombinant capripoxvirus, *rCAV-2* recombinant canine adenovirus type-2, *rHAV-5* recombinant human adenovirus type-5, *RPV* rinderpest virus, *rPPRV* recombinant peste des petits ruminants virus, *VLP* virus-like particle

to be detected with conventional diagnostic methods. Thus, how to choose one or more specific markers is a hard problem to develop accompanying diagnostics for PPR DIVA immunization. It is very important for the unbiased evaluation of diagnostic methods for PPR. Poor performance in the sensitivity and specificity can detract from the success of DIVA approaches, allowing undetected marker-specific antibodies in animals.

The PPRV H and F proteins are two glycoproteins protruding from lipid membrane, and both induce neutralizing antibodies against PPRVs, which are integral elements in immune protection. Unlike both the proteins, the N protein has an inability to confer protective immunity against PPRVs, and in other words, its deletion has almost no effect on immunoprotection. More importantly, N protein-specific antibodies, as induced early in infection, can be detected easily using a proper diagnosis. Thus, the N protein serving as a negative marker plays a potential role in the development of DIVA vaccines, and further a number of studies are increasingly reported to establish successfully serological diagnostics concerning the N protein (Choi et al. 2005; Libeau et al. 1995; Zhang et al. 2013). Indeed, most of the PPR vaccine candidates with properties of DIVA were reasonably designed both in the absence of the N protein and in the presence of the H and (or) F proteins, and parts of them were discussed in the next section in detail.

## Different types of DIVA-specific PPR vaccine candidates

### Poxvirus vector vaccine

Capripoxviruses (CPV), including SPV and GPV, have been attenuated and further used as replicating viral vectors for the studies on vaccines against a variety of viruses, such as human immunodeficiency virus (Shen et al. 2011), RPV (Romero et al. 1994) and bluetongue virus (Perrin et al. 2007). Due to a host range specific to sheep and goats, the CPV is an excellent vector for the development of recombinant multivalent vaccines to enable delivery of immunogenic genes from the host-specific PPRV sharing the same geographical distribution as the CPV.

Due to the significant cross-protection between PPRV and RPV, goats have proven to be protected against a lethal challenge of PPRVs following vaccination with recombinant CPVs expressing either the F or H glycoprotein of RPV (Romero et al. 1995). As early as 1993, such an ideal efficiency of heterologous recombinant vaccine against PPR has also been demonstrated through a vaccinia virus double recombinant expressing both the F and H glycoproteins of RPV, conferring complete protection for goats against challenge with virulent PPRVs (Jones et al. 1993). Although animals inoculated with viral vector vaccines expressing the glycoproteins of RPV could survive challenge with virulent PPRVs, the vaccines allowed the replication of challenge PPRVs for some time prior to their final elimination.

To date, the attenuated CPVs have been employed as vectors to express either the H (Diallo et al. 2002) or the F (Berhe et al. 2003) glycoprotein of PPRV, both conferring dual protection against CPV and PPRV. Particularly, a dose of the recombinant expressing the F gene as low as 0.1 PFU could protect goats against challenge with a virulent PPRV strain. More works were required to establish further the duration of immunity provided by both the vaccines (Berhe et al. 2003; Diallo et al. 2002) and to test their efficacy in presence of antibodies against PPRVs. Unfortunately, there was no follow-up report available so far.

The potencies of both the vectored vaccines based on expression of either the H or the F protein have not been directly compared with each other in goats and sheep. To address this problem, Chen et al. (2010) generated two recombinant CPVs, namely rCPV-PPRVH and rCPV-PPRVF, which expressed the H and F proteins of PPRV, respectively (Chen et al. 2010). Vaccination studies with different dosages of recombinants showed that the rCPV-PPRVH was a more potent inducer of neutralization antibodies against PPRVs than the rCPV-PPRVF. One dose of the rCPV-PPRVH was enough to seroconvert 80 % of immunized sheep, and booster vaccination with the same dose significantly induced a higher titer of neutralization antibodies against PPRVs. In comparison with the rCPV-PPRVH, the rCPV-PPRVF may be a more potent inducer eliciting cell-mediated immune response, contributing to the protection against PPRVs. Due to the expression of either the H or the F glycoprotein alone in host cells, vaccination with either of the vaccines could elicit a glycoprotein-specific antibody response, which was different from another response induced by the natural infection with wild-type PPRVs. This difference indicates that either of the recombinant CPVs as mentioned above, in conjunction with a proper serological test, is a promising DIVA vaccine candidate.

### Adenovirus vector vaccine

Over the past few decades, adenoviruses have emerged as a promising vehicle to deliver foreign antigens for vaccine design. They are double-stranded DNA viruses with high genetic stability, exhibiting no mutations after multiple rounds of replication in vitro. To determine whether the PPRV H protein can be exploited to generate an effective vaccine, a replication-competent recombinant canine adenovirus type-2 (CAV-2) expressing the H gene of PPRV (China/Tibet strain) was generated in transfected MDCK cells and used to immunize goats. All vaccinated animals 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 at least 7 months. This recombinant adenovirus could not be isolated from the urine or feces of vaccinated goats, up to the end of the monitoring period on day 35 post-vaccination, further indicating that it could not contaminate the environment during the period (Qin et al. 2012). Nonetheless, there was no more data to reveal whether the recombinant virus would shed from the vaccinated animals during a longer period.

More recently, two groups (Herbert et al. 2014; Wang et al. 2013a) independently reported that replication-defective recombinant adenoviruses expressing PPRV glycoproteins could induce both high levels of neutralizing antibodies and strong cell-mediated immune responses in goats, whereas co-expression of F and H proteins induced higher titer of neutralizing antibodies than expression of either F or H alone did. Furthermore, in order to assess the potential of adenovirus expressing the H protein as a DIVA vaccine, either of the groups analyzed the development of antibodies against the PPRV N protein in vaccinated goats before and after challenge with PPRV. As expected, none of the goats secreted N-specific antibodies before challenge, thereby offering the possibility of an effective DIVA vaccine (Herbert et al. 2014).

### Recombinant PPRV-based vaccine

Since the establishment of reverse genetics systems for RPV (Baron and Barrett 1997) and CDV (Gassen et al. 2000) more

than a decade ago, many groups have attempted to develop reverse genetics systems of other paramyxoviruses. Using reverse genetics, it will be possible to transform a full-length genome of parental paramyxovirus into a new stable infectious clone, which can be used as a new modified virus conferring as optimal protection as the parental virus. Furthermore, the reverse genetics technology contributes to the rational design of DIVA vaccines by either inserting a marker gene or deleting an unnecessary parental gene at the genomic level. Nowadays, various chimeric RPVs have been rescued by the replacement of the RPV H (Das et al. 2000), F (Das et al. 2000), M (Mahapatra et al. 2006) and N (Parida et al. 2007) genes with corresponding those of the PPRV by means of reverse genetics. Although these chimeric viruses carrying the PPRV glycoproteins might be useful to control PPR, field trials have never been conducted in the post rinderpest eradication era. Even in the era of rinderpest, monoclonal antibodies-based both the RPV and the PPRV N proteins competitive ELISAs had no efficiency to differentiate between both the infections due to cross-reaction each other, thus limiting the application of this type of chimeric DIVA vaccines.

Although a PPRV mini-genome rescue system was described in 2007 (Bailey et al. 2007), the rescue of recombinant PPRV from a full-length cDNA clone of the virus genome was not reported until 2012, when one group successfully rescued a recombinant PPRV by using a RNA polymerase II promoter to drive transcription of the full-length virus antigenome. The rescued PPRV contained a green fluorescent protein (GFP), which was not known or expected to bind to any cell surface receptors, and which could be employed as a genetic marker to allow serological differentiation between vaccinated and infected animals (Hu et al. 2012). Rescue efficiencies of such recombinant PPRVs have proven to be acceptable but not high, and thus should be further improved to develop a novel vaccine candidate. More recently, a follow-up study was completed by this group, who used reverse genetics to construct successfully a recombinant PPRV expressing the foot-and-mouth disease virus (FMDV) VP1 (rPPRV/VP1), which did not greatly affect replication of the recombinant PPRV in vitro. Moreover, vaccination with one dose of rPPRV/VP1 induced FMDV neutralizing antibody in goats and protected them from challenge with virulent FMDV (Yin et al. 2014). Owing to its introduction into the genome of recombinant PPRV, the foreign VP1 gene could serve as a positive marker used for the DIVA diagnosis for PPR.

Although the PPRV N protein is widely used to establish diagnostics (Choi et al. 2005; Libeau et al. 1995; Zhang et al. 2013), its C-terminal domain appears to be a relatively less conserved area among related viruses (Diallo et al. 1994). This domain remains a suitable candidate for developing a test that can differentiate PPRV from other morbilliviruses (Dechamma et al. 2006). Therefore, it can be speculated that if the C-terminus of PPRV N protein were to be replaced with those of other morbilliviruses, a resulting recombinant PPRV would be a potential negative DIVA vaccine candidate. A similar substitution has proven to be effective in the rescue of a chimeric RPV with the N protein derived from PPRV (Parida et al. 2007). Fig. 3a and c schematically showed positive and negative DIVA strategies for immunization of goats with recombinant PPRVs, respectively, as compared with Fig. 3b illustrating a natural infection with wild-type PPRV.

## Chimeric baculovirus vaccine

The baculovirus surface display system is a specialized baculovirus expression system, in which target proteins are incorporated into the envelope of recombinant baculovirus and are displayed on its surface without affecting its infectivity (Feng et al. 2006). As early as 2001, Sinnathamby et al. investigated the immune responses in goats immunized with low doses of purified recombinant extracellular baculovirus carrying a membrane bound form of the PPRV H protein without any adjuvant. They indicated that the immunized goats developed both humoral and cellular immune responses and antibodies in the immunized animals could neutralize both PPRVs and RPVs in vitro (Sinnathamby et al. 2001b). Subsequently, Rahman et al. (2003) constructed recombinant baculoviruses that displayed immunodominant ectodomains of the PPRV F protein and the RPV H protein. Following infection of the insect larvae or the host-derived BmN cells with recombinant baculoviruses, the expressed GP64 fusion proteins were displayed on the host cell surface and the budded virions. Antigenic epitopes of the recombinant proteins were properly displayed and furthermore the recombinant viruses induced immune responses in mice against PPRVs or RPVs (Rahman et al. 2003). This specific immune response is presumably attributed to posttranslational modifications of the heterologous protein in the insect cells, thus augmenting the immunoreactivity of the displayed antigens.

## Edible subunit vaccine

The surface glycoproteins of morbillivirus mediate virus attachment and penetration to host cells and play a vital role in induction of protective immunity. Although the F protein can be the target of neutralizing antibodies, most of the protective immune responses to morbilliviruses are directed against the H protein (Sugiyama et al. 2002). Recombinant H protein of RPV expressed in insect cells can elicit not only humoral but also cell-mediated immune responses in cattle (Sinnathamby et al. 2001a). Furthermore, the transiently expressed H protein of PPRV was found to be biologically active in possessing hemadsorption and neuraminidase activities (Seth and Shaila 2001). Therefore, the PPRV H protein is a better candidate to be incorporated in a vaccine than the F protein.

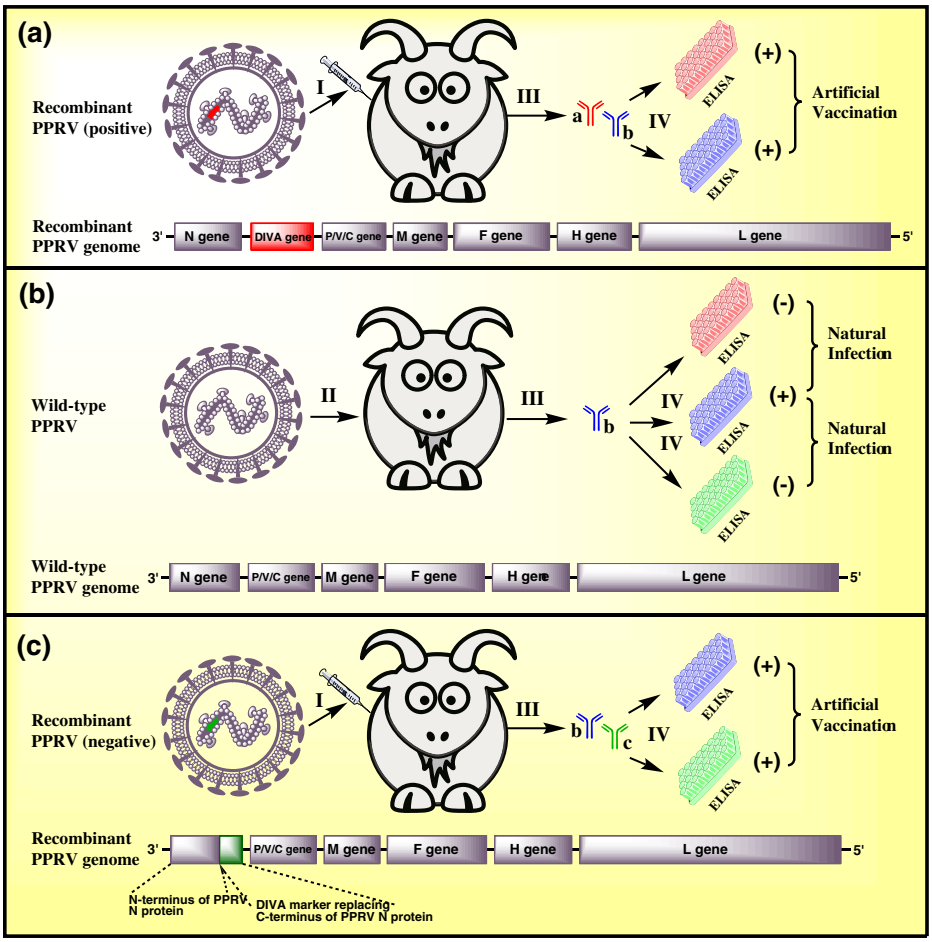


**Fig. 3** Schematic representation of "differentiating infected from vaccinated animals (DIVA)" strategy for immunization with positive or negative DIVA vaccine against peste des petits ruminants virus (PPRV). The positive DIVA vaccine is composed of the positive recombinant *PPRV* in which a foreign DIVA gene is selectively inserted between the N and P genes in the viral genome (**a**). In contrast, in the negative recombinant *PPRV* the C-terminal domain of the N protein is replaced with that of another morbillivirus to form a new "N protein" in the viral genome (**c**). However, neither of the DIVA-specific markers is enclosed in the wild-type PPRV (**b**). Both the recombinant PPRVs were used to vaccinate goats, respectively, in which different types of antibodies **a**: antibody against positive DIVA marker; **b** PPRV-specific antibody; **c** antibody against negative DIVA marker) can be identified by proper serological tests many weeks post-immunization. Antibodies (**a** and **b**, or **b** and **c**) induced by either the positive or negative DIVA vaccine are different from the only one (**b**) induced by natural infection. Such a difference can be recognized by combinedly using two corresponding ELISA assays to achieve finally the DIVA objective. I, II, III and IV: vaccination with recombinant PPRVs, natural infection with wild-type PPRV, secretion of antibodies and ELISA assays. (+) and (-): positive and negative results in ELISA assays

The use of plants for production and delivery of vaccine proteins has shown promise in research conduced over the past two decades (Rice et al. 2005). Transgenic plants as a production system for subunit vaccines have been considered safe and economical, in comparison with traditional cell culture-based methods. Genes encoding bacterial and viral antigens are faithfully expressed, processed, and assembled in plant cells to form immunogenic proteins (Mason et al. 2002). Moreover, compared with parenteral antigen delivery, oral vaccine delivery is a convenient way of immunization and offers effective protection against pathogens interacting with host mucosal surfaces by the induction of mucosal immunity (Abubakar et al. 2011a).

As a step towards the development of thermo-stable subunit vaccines against PPR, Khandelwal et al. (2011) have expressed the PPRV H protein in peanut plants (*Arachis hypogea*) in a biologically active form. The expressed H protein possessed neuraminidase activity and retained its immunodominant epitopes in natural conformation. Neutralizing antibody responses to viruses were elicited upon oral immunization of sheep in the absence of any mucosal adjuvant. Additionally, anti-H protein-specific cell-mediated immune responses were also detected in mucosally immunized sheep (Khandelwal et al. 2011). In fact, Khandelwal et al. (2003) prior to this report had demonstrated that the RPV H protein expressed by transgenic plant was antigenically authentic as revealed by reactivity with H protein-specific

antibodies and convalescent sera. High titers of antibodies were induced in mice immunized with plant-derived H protein of RPV and have proven to be characterized by H protein-specificity (Khandelwal et al. 2003).

*Virus-like particle-based vaccine*

Virus-like particles (VLPs), composed of one or more structural proteins but no genomes of native viruses, mimic the organization and conformation of authentic virions but have no capability of self-replication in cells. Most viruses comprise multiple proteins, out of which, one or more immunogenic proteins are removed probably still resulting in induction of neutralizing antibodies but lack of the other antibodies against removed proteins. Based on this principle, VLP-based vaccine candidates offer a promising strategy for DIVA, as VLPs lacking either monovalent or multivalent antigen can be constructed on the need for serological surveillance, systematically highlighted in our recent review (Liu et al. 2013).

We have constructed previously two types of recombinant baculoviruses, which co-expressed either the PPRV M, H and N proteins (Liu et al. 2014b) or the PPRV M and N proteins (Liu et al. 2014a) in insect cells, and furthermore, have confirmed the generation of two types of PPR VLPs (VLP-MHN and VLP-MN) by viewing of a transmission electron microscope. More importantly, the results in immunization of mice with VLP-MHNs showed that purified PPR VLPs induced virus neutralizing antibodies at a relatively high level, indicating a given potential of VLP-based vaccine candidate against PPR (data not shown). A further experiment should be performed to determine whether ELISA tests on the PPRV F, P or L protein-specific antibodies can serve as DIVA diagnostics for VLP-MHN-based immunization. More recently, Li et al. (2014) used a baculovirus expression system to construct other types of PPR VLPs, namely VLP-MH and VLP-MF, both of which have subsequently proven to elicit PPRV-specific neutralizing antibodies in mice and goats (Li et al. 2014). Due to the absence of the PPRV N protein inside them, both the VLPs, if accompanied with a diagnostic test on the N protein, would be a promising DIVA vaccine candidate for the surveillance of PPR.

*Nucleic acid vaccine*

Naked DNA vaccines represent an attractive approach for generating antigen-specific immunity, owing to their stability and simplicity of delivery in organisms. Wang et al. (2013) recently developed a suicidal DNA vaccine based on the Semliki Forest virus replicon and further tested its ability to induce immunogenicity in mice. The results showed that such a vaccine could express the PPRV H protein in BHK-21 cells. Specific antibodies, neutralizing antibodies and lymphocyte proliferation responses were all induced in mice (Wang et al. 2013b), but unfortunately there was no another similar test on small ruminants. Due to the expression of the H protein alone, this type of nucleic acid vaccine, if accompanied with a specific serological test, represents a promising approach for the development of PPR DIVA vaccines.

## DIVA strategies for global PPR eradication

1924 and 2011 represent two milestones related to the global campaign against rinderpest: the first one is the creation of the OIE following a disastrous rinderpest incursion in Europe and consequent spread to other continents; the second one is an official declaration of rinderpest eradication worldwide. The global eradication of rinderpest mainly results from the following factors: the reliable vaccines covering all strains, the sensitive and specific diagnostics available, a short incubation period for rinderpest, viral transmission only by close contact, a narrow range of hosts and restricted geographic distribution. Considering the current situation of PPR around the world, it is clear that many requirements for the eradication of PPR are as well established as those for rinderpest. For example, there is only one serotype of PPRV; effective diagnostics and potent vaccines are available in endemic areas; the range of hosts is mainly restricted to small ruminants; PPR is endemic to tropical and subtropical countries.

As described previously, prophylaxis against PPR would be essentially improved by the development of both versatile DIVA vaccines and companion diagnostics, combinedly used for serosurveillance of PPR. The purpose of vaccination with DIVA vaccines is not only to prevent the disease, but also more importantly, to be the implementation of a serological surveillance system. Unfortunately, the DIVA vaccines against PPRV have not entered the veterinary marketplace as yet. Regardless of their commercialization, the launching of an eradication campaign against PPR worldwide appears technically feasible and practically attainable without considering the economic incentive (Baron et al. 2011; Singh et al. 2009). Fortunately, a resolution on a global strategy to control and finally to eradicate PPR was recently adopted by the OIE at its 82nd general session in Paris in 2014. A global PPR control strategy is being developed within the Global Framework for the Progressive Control of Transboundary Animal Diseases, a joint initiative between the OIE and Food and Agriculture Organization (FAO). The strategy will serve as a model for PPR eradication programs worldwide, and there are good reasons to believe that the eradication of PPR is an achievable goal like that of rinderpest.

## Conclusions

At present, mass vaccination in endemic areas has been the best measure to protect sheep and goats from PPR. Taking advantage of the close antigenic relationship between RPV and PPRV, tissue culture rinderpest vaccines had been used for a long time to protect small ruminants against PPR until the PPRV isolate Nigeria 75/1 was successfully attenuated by multiple passages in Vero cells. The Nigeria 75/1 and other subsequently licensed vaccines appear to provide a life-long immunity against PPR and are extensively used for immunization. However, the relatively low thermostability of conventional vaccines is still a major concern, especially in tropical and subtropical regions where the maintenance of a cold-chain during storage, transport and distribution of vaccines is difficult. Both genetically engineered vaccines characterized by strainseither intrinsic thermostability or acquired thermotolerance can avoid cold-chain-associated problems under high temperature conditions.

Although live attenuated vaccines are able to induce both humoral and cell-mediated immune responses and to keep long-term neutralizing antibodies against PPRVs at a high level, a potential possibility in the reversion of vaccine strains to virulence, albeit unreported so far, should not be neglected. Moreover, antibody responses they induce in animals cannot be distinguished from those following a natural infection. Thus, it becomes more necessary to increase studies on the new generation vaccines with properties of high safety, stability and especially DIVA capability for control of PPR. With the advent of DNA recombinant technology, efforts are being made to develop effective PPR vaccines to enable the differentiation of infected from vaccinated animals, allowing countries to implement both vaccination and disease surveillance programs at the same time. It can be expected that the availability of effective DIVA vaccines along with their companion serological tests will greatly assist in designing efficient control strategies for PPR in the near future.

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