

Vaccines against peste des petits ruminants virus

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Peste des petits ruminants (PPR) is a highly contagious and economically important viral disease affecting goats, sheep and wild ruminants. The disease is currently circulating in Asian and African countries, creating problems in small ruminant farming. Current control of the disease mainly includes isolation and disinfection of the contaminated environment, and administration of a live-attenuated vaccine, which provides a strong immunity. Maintenance of cold chain for vaccine efficacy has proven difficult in subtropical countries. A thermostable live-attenuated conventional or recombinant vaccine is a way to avoid cold chain-associated problems in tropical and subtropical countries. Mass vaccination of sheep and goats in endemic countries might be a pragmatic approach to control PPR in the first phase of disease eradication. In the future, the development of a marker vaccine with a robust companion test may help in serosurveillance to identify infection in vaccinated animals to control PPR disease. Here, we discuss available potent PPR vaccines and the future possibility of developing new-generation vaccines against PPR.

KEYWORDS: DIVA • live-attenuated vaccine • peste des petits ruminants • PPR marker vaccine • thermostable vaccine

Peste des petits ruminants (PPR), also known as goat plague, is an infectious and fast-spreading viral disease of domestic small ruminants (sheep and goats). Cattle are usually infected asymptotically and are not known to transmit the disease to other animals [1]. Wild ruminants are susceptible but, to date, PPR has been diagnosed only in captive wild ungulates from families of *Gazellinae* (Dorcas gazelle), *Caprinae* (Nubian ibex and Laristan sheep) and *Hippotraginae* (gemsbok) [2]. The disease is characterized by the sudden onset of depression, fever, discharges from the eyes and nose (FIGURE 1), lesions in the mouth and tongue (FIGURE 2 & 3), disturbed breathing and cough, foul-smelling diarrhea leading to necrotizing and erosive stomatitis, pneumonia and enteritis and, ultimately, death [3]. The PPR virus (PPRV) belongs to the *Morbillivirus* genus in the family *Paramyxoviridae* [4]. PPRV is closely related to the rinderpest virus (RPV) that causes disease in cattle, buffalo, sheep, goats and wild ruminants. Owing to the high mortality rate (70–80%), PPR is notifiable to Office International des Epizooties (OIE), the World Organisation for Animal Health. In the new OIE classification, it is categorized in a group of economically important animal diseases, which must be notified to the organization. PPR is a major and serious threat to the small ruminant industry

in endemic countries and has a direct influence on the livelihood of poor farmers and landless laborers, the main owners of sheep and goats [5].

Peste des petits ruminants disease is prevalent in sub-Saharan Africa, the Arabian Peninsula and in the entire Indian subcontinent (FIGURE 4) [6]. The live-attenuated tissue culture rinderpest (TCRP) vaccine has previously been used to protect against PPR but the use of TCRP vaccine was later banned [1], after the launch of the rinderpest eradication program, which stimulated the development of homologous PPR vaccine(s). The first homologous PPR vaccine was developed using the live-attenuated Nigerian strain PPRV Nig 75/1 [7]. Similarly, three other homologous PPR vaccines using Indian isolates of PPRV have been developed and evaluated recently by the Indian Veterinary Research Institute (IVRI; Mukteswar, India) and Tamil Nadu University of Veterinary and Animal Science (TANUVAS; Chennai, India) [8].

Peste des petits ruminants virus

Peste des petits ruminants virus, along with measles, rinderpest, canine distemper and the phocine, dolphin and porpoise distemper viruses, is included in the *Morbillivirus* genus under the *Paramyxoviridae* family [9,10]. Virions of PPRV are pleomorphic, with a lipid



Figure 1. Typical mucopurulent nasal discharge in peste des petits ruminants in a goat.

envelope enclosing a ribonucleoprotein core that contains the single-stranded negative-sense RNA genome of 15,948 nucleotides [5] and is encapsidated by the nucleocapsid (*N*) protein. The genome codes for six structural (*N*, *P*, *M*, *F*, *H* and *L*) and two nonstructural (*C* and *V*) proteins in the order of 3'-*N-P(C/V)-M-F-H-L-5'* [11,12]. The viral envelope is derived from the host cell membrane and is associated with three viral proteins: the matrix protein (*M*), which is located inside the envelope and serves as a link between the nucleocapsid and the two external viral proteins, the fusion protein (*F*) and hemagglutinin (*H*). The virus binds to the host cell receptor through the *H* protein, followed by fusion of the viral envelope with the cell membrane of the host cell. The *F* protein mediates virus fusion to introduce the viral nucleocapsids into the cytoplasm where virus multiplication takes place. The progeny virus is released by budding, during which the virus acquires its envelope. Among the viral proteins, *F* and *H* are considered to be very important for the induction of protective host immune response against the virus, and most of the neutralizing antibodies are directed against *H*. Although *N* is the most abundant and immunogenic viral protein, it does not induce protective immunity against the virus [5].

Epidemiology

Peste des petits ruminants was first reported in the Ivory Coast, West Africa during 1942 [13] and was later found in Senegal [14], Central Africa [15], Sudan [16], India [17], East Africa [18], Arabia [19], Dakar [20], The

Middle East [21], United Arab Emirates [22], Iraq [23], Ethiopia [24], Southern Nigerian states [25–27], Bangladesh, Pakistan, Nepal, Israel and Saudi Arabia [28–39]. The chronological identification of PPR disease in different countries between 1942 and 1996 is provided in TABLE 1. The disease is now enzootic in several African and Asian countries, including India [40]. This disease accounts for significant economic losses in small ruminants and affects the marginal and landless farmers who depend on small ruminant rearing as a means of livelihood. Goats are more susceptible to PPRV infection than sheep [41]. There were descriptions of a PPR outbreak in a zoological collection in Al Ain in the Arabian Gulf and showed clinical infection in *Gazellinae*, *Caprinae* and *Hippotraginae* and subclinical infection in Nilgai (*Tragelophinae*) [2]. Cattle are susceptible to subclinical infection of PPRV [1]. Generally, the morbidity of PPR infection in sheep and goats ranges from 10 to 83% [42], with occasional reports of mortality up to 100% [24]. The mortality rate of PPR disease in small ruminants ranges from 10 to 90% [43]. The case–fatality rates are higher in goats (71.6%) compared with sheep (55.4%) [44].

Molecular epidemiology of PPRV based on the *F* gene sequence from all over the world has defined the existence of four different lineages of virus (I–IV), of which lineage IV is prevalent in the Indian subcontinent and the other lineages are prevalent in African countries [45]. Analysis of partial sequence data of the *F* gene has pointed out small variations between PPRV strains and has allowed their grouping into four lineages, which better reflects their geographical origins than the variations of genes of the external glycoprotein *H* [5,45]. Lineage I circulates in West Africa, lineage II in Nigeria and Cameroon, lineage III in East Africa and lineage IV in Asia. However, recently there was an incursion of lineage IV virus into Africa; this was identified following a large epizootic in Morocco [201]. Based on the partial



Figure 2. Mouth and tongue lesions in peste des petits ruminants.



Figure 3. Typical lesions of peste des petits ruminants in goats infected with the disease.

From gene sequencing, there is a solitary report of lineage III PPRV occurrence in Southern India [30]. However, since then there has been no further report of this lineage in India. The importance of lineages is that it helps in monitoring of the virus and tracing the source of outbreaks, and is also used for devising control strategies. The prevalence of a particular lineage would help in choosing a candidate vaccine prototype that is homologous to ensure adequate immunization of a susceptible flock. Vaccination with a heterologous lineage would risk the introduction of a lineage hitherto not present. Thus, lineage identification is an important prerequisite of PPR seroepidemiology and control.

Attenuation of virulent PPRV for vaccine preparation

Gilbert and Monnier were the first to adapt PPRV to grow in cells *in vitro*, and reported that the cytopathic effect (CPE) of the virus was manifested by the appearance of large syncytia in sheep liver cells (primary cell lines) [14]. Subsequently, Laurent investigated the CPE upon different cell systems wherein the CPE manifested mainly in the form of refringent and rounded cells, which gradually detached themselves [46]. Upon staining with hematoxylin and eosin, there was a visualization of syncytia, including mini-syncytia. These types of polykaryons were also described earlier for CPE of RPV in calf liver cells [47]. Microsyncytia are mainly

found in the initial stages of PPR, followed by large syncytia. They showed that the infection of young bovine kidney cells with RPV resulted in the rapid appearance of CPE and also better production of virus [47,48]. Diallo *et al.* [7], while adapting the Nigeria 75/1 isolate, reported that at the start of the serial passages, the CPE was not perceptible till 4–7 days postinfection (dpi) for the initial ten passages and the virus could only be harvested by 8–13 dpi. However, after the PPRV was adapted for 50 passages or more in the tissue culture *in vitro*, this period was practically reduced to 2 days, which was evident by observation of refringent cells from the second day onwards. Cell rounding was maximum at 3–6 dpi, which coincided with peaks in virus titer, and the entire monolayer was infected between 4–6 dpi. Sabin and others [49] had shown that serial passages of poliovirus in very high doses resulted in the development of mutants. Work proceeded likewise for obtaining the TCRP virus, which was later widely utilized as TCRP vaccine, leading to successful eradication of rinderpest from various parts of the world [48,50,51]. This work demonstrated that RPV exhibited a minor surge in its pathogenicity during the initial stages of multiplication, which later waned off, leading to the development of an avirulent strain at the 40th passage [48,50,51].

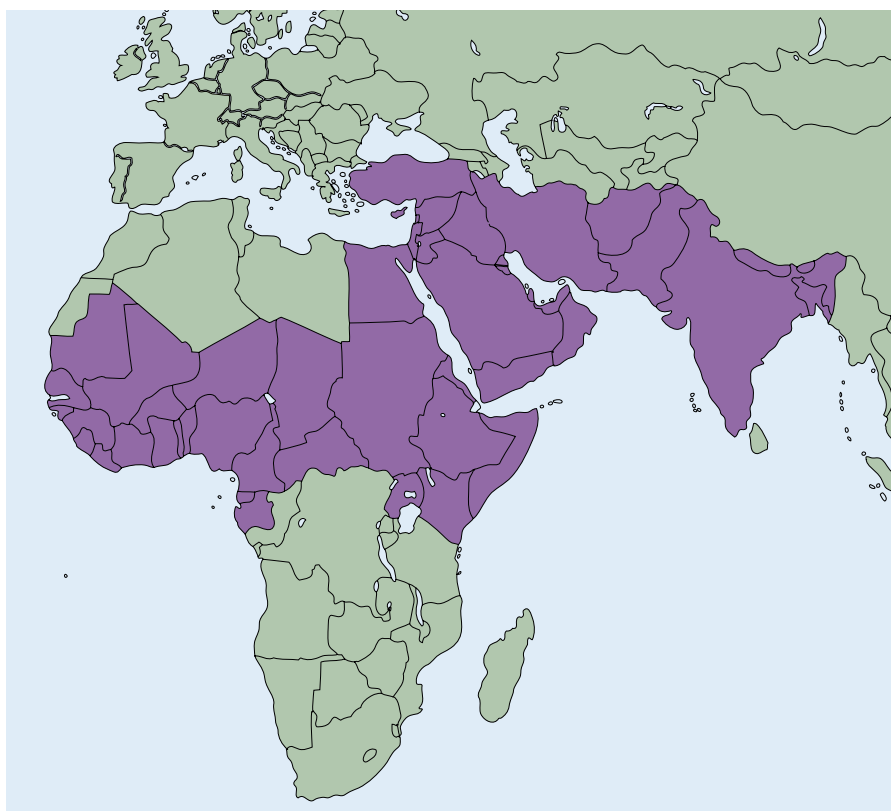


Figure 4. Prevalence of peste des petits ruminants worldwide. The areas shaded purple indicate an endemic zone for peste des petits ruminants disease. It covers Arabia, the Middle East, Southeastern Asia, the Indian subcontinent, and East and Central Africa. Time of occurrence of peste des petits ruminants and respective country: 1962: Senegal; 1981: endemic in West and Central Africa; 1984: Sudan; 1987: Southern India; 1990s: reports from East African countries (Kenya, Uganda and Ethiopia); 1993–1995: epidemic in Arabia, Middle East, Southeastern Asia and the Indian subcontinent.

Table 1. First reports of peste des petits ruminants from different countries.

Serial number	Location/country	Ref.
1	Ivory Coast	[13]
2	Senegal	[102]
3	Chad	[103]
4	Togo	[52]
5	Benin	[57]
6	Ghana	[59]
7	Nigeria	[104]
8	Oman	[105]
9	Sudan	[106]
10	Saudi Arabia	[2]
11	India	[17]
12	Jordan	[107]
13	Israel	[108]
14	Ethiopia	[24]
15	Kenya and Uganda	[109]
16	Pakistan	[28]

The PPR Nigeria 75/1 virus adapted to cell culture also lost its virulence quickly as it resulted in slight hyperthermia at the 20th passage, while it was totally avirulent after the 55th passage in Vero cells as the virus lost its pathogenicity and could even protect the animals against a virulent virus infection [7]. This vaccine virus (at the 63rd passage) when used for immunization elicited good immunity and resulted in appearance of neutralizing antibodies from day 7 postimmunization [7].

Gilbert and Monnier, in an effort to attenuate a PPRV strain, observed that the virus was highly pathogenic at the 6th passage and it elicited only a slight thermal reaction at the 55th passage [14]. However, their studies, followed by other work performed by Benazet [52], did not result in a totally avirulent virus, despite 65 passages in sheep liver tissues. The failures in attenuation could be due to either: the inherent nature of the strain; the tissue culture system employed (sheep renal cells); or the use of very low virus concentrations that did not facilitate the generation of mutants.

The PPRV Sungri isolate (lineage IV) was isolated from Sungri in Himachal Pradesh, India, in 1994 from a goat [53]. This virus was initially adapted in B95a (Marmoset lymphoblastoid) cells for ten passages and the subsequent passages were given in Vero cells, for up to 59 passages [53]. Vero cells were chosen as they are deficient in interferon production [54] and have been used extensively for developing viral vaccines of human and veterinary significance. PPR (Sungri) vaccine virus became avirulent after 56 passages directly in Vero cells [55].

The third and fourth attenuated strains of PPRV (lineage IV) were Asasur 87 (sheep origin) and Coimbatore 97 (goat origin), respectively, which were developed by TANUVAS [56]. The attenuation of these viruses was achieved in Vero cells after 75 serial passages [8].

Vaccines used against PPRV

Vaccination against PPR is an effective means of controlling the disease. Heterologous rinderpest vaccine was used for many years to control PPR disease. However, after the eradication of rinderpest, it was necessary to restrict the use of this vaccine. The first attenuated vaccine developed against PPR involved using the lineage I African isolate, Nigeria 75/1 [7]. Subsequently, three more attenuated vaccines were developed in India by using the lineage IV Indian isolates.

Heterologous vaccines against PPRV

The attenuated Plowright's TCRP vaccine was initially used as a heterologous vaccine to protect against PPR, since PPRV and RPV are closely related morbilliviruses [57–61]. TCRP vaccine provided protection for at least 1 year and probably for the economic life of the vaccinated animals [62]. Use of this vaccine was necessitated by a series of failures in the past in developing a homologous PPR vaccine [2,14,52]. However, the use of rinderpest vaccine in all animal species was discontinued worldwide so as to achieve the status of rinderpest-free country or zone following the OIE pathway [61]. Hence the practice of heterologous PPR control was abolished in most countries.

Homologous vaccines against PPRV

The availability of a homologous vaccine for PPR is fortunate since the use of the rinderpest vaccine in all animal species has now been discontinued worldwide. The reasons for this have been previously mentioned. The attenuated homologous PPRV vaccine is now the only vaccine permitted for use in sheep and goats against PPRV infections [5].

PPRV Nigeria 75/1 vaccine

A very efficient PPR homologous live-attenuated vaccine was developed using the PPR Nigeria 75/1 isolate by continuous passages in Vero cells [7]. During 1975, this virus was isolated in sheep liver cell culture from a goat that had died from PPRV infection [58]. Several vaccine trials were conducted during 1989–1996 which demonstrated the efficacy of this vaccine in 98,000 sheep and goats in the field. During those trials, no unwanted side effects, such as abortion in pregnant animals, were recorded. The animals vaccinated with this attenuated PPRV were unable to transmit the challenge virus to animals they were in contact with. Anti-PPRV antibodies generated against this vaccine last for at least 3 years, which is the effective economic life of sheep and goats. This vaccine provides long-term immunity and protects goats against virulent RPV [63]. Therefore, the attenuated PPR 75/1 virus was initially used to protect small ruminants against different lineages of PPRV in the different PPR-endemic zones. Recently, it has been demonstrated that freeze-drying of this vaccine in an excipient-containing trehalose makes it very thermostable and it resists temperatures up to 45°C for a period of 14 days, with minimal loss of potency [64].

PPRV Sungri/96 vaccine

Since lineage IV is mostly restricted to Asian countries, the use of Nigeria 75/1 vaccine in Asian countries may increase the likelihood of mixing up of lineages and the development of mutants

with high virulence. Thus, it is imperative to consider using the lineage-specific vaccine available for use in Asian countries. To this effect, a live-attenuated Vero cell-adapted vaccine against PPRV, Sungri/96 vaccine, was developed by the IVRI [53]. The Sungri isolate was adapted in Vero cells and was found to be attenuated fully at 59 passages (P-59). This vaccine has been tested extensively both experimentally as well as in the field and has been found to be safe and potent in small ruminants. A recent experimental trial was conducted for comparative evaluation of PPR vaccines manufactured in India. The results are summarized in TABLE 2 [8]. Studies on thermostability, pathogenicity and immunogenicity of this vaccine have also been undertaken [55]. The PPR vaccine at P-59 was found to be safe and efficacious in pregnant animals and also has no record of biologically significant immunosuppression [53,65]. The vaccine provides sterile immunity for more than 6 years [BANDOPADHYAY SK, SINGH RK, SREENIVASA BP, UNPUBLISHED DATA]. Thus, the Sungri 96 PPR vaccine is safe for mass vaccination campaigns under field conditions. Some of the salient features of PPRV Sungri/96 (IVRI) vaccine are listed in Box 1. This vaccine is used throughout India to vaccinate sheep and goats with great efficacy and has the potential to be used effectively against lineage IV virus circulating in the Indian subcontinent and in other Asian countries.

PPRV Arasur/87 vaccine & PPRV Coimbatore/97 vaccine

The third and fourth conventional live-attenuated vaccines were PPRV Arasur 87 (sheep origin) and Coimbatore 97 (goat origin), respectively, which were developed by TANUVAS [56]. These vaccines are being used in southern states of India. The CPE pattern (Box 1) of this virus differs from that of PPR Sungri /96 [66]. However,

these vaccines equally provide protection (TABLE 2) against PPRV Izatnagar/94 [8]. They are also as equally safe and protective as Sugri 96 in sheep and goats and have potential for commercial vaccine production.

Thermostable PPR vaccines

The new methods for freeze-drying conventional rinderpest and PPR vaccines have added a new dimension in increasing the thermostability of these vaccines [60,64]. Mariner *et al.* developed a method for freeze-drying wherein the moisture content of vaccine was greatly reduced, thereby increasing the thermostability of the TCRP vaccine [67]. Similarly, Worral *et al.* reported an ultra-rapid method (Xerovac) for the dehydration and preservation of live-attenuated rinderpest and PPR vaccines [64]. However, such vaccines have only marginal advantages in terms of thermostability. As such, there is a need to develop an intrinsically thermoresistant PPRV vaccine that will have better thermostability. However, the thermostability of even such a thermoresistant virus vaccine can further be enhanced by using a better combination of stabilizers and heavy water. Heavy water has recently been reported to increase thermostability for polio [68] and yellow fever vaccines [69]. We have developed two thermostable vaccines (PPR/Revati and PPR/Jhansi) at the IVRI and tested their thermal degradation profile. At 37 and 40°C, these thermostable vaccines had a shelf life of 7.62 and 3.68 days, respectively, when compared with 1.58 days at 37°C for native Sungri/96 vaccine. The novel thermostable vaccines developed were also tested after reconstitution. The Jhansi PPR vaccine was stabilized at 4–25°C using stabilizer E (trehalose, calcium chloride and magnesium chloride) and could maintain the protective

Table 2. Peste des petits ruminants vaccines available worldwide.

Product name	Vaccine type	Strain	Manufacturer	Country
PESTEVAC	Modified live	Nigeria 75/1	Jordan Bio-Industries Center (Amman, Jordan)	Afghanistan, Albania, Bahrain, Ethiopia, Iraq, Jordan, Kuwait, Lebanon, Libya, Yemen, United Arab Emirates, Syria, Pakistan and Oman
PPR-VAC	Live	PPRV 75/1	Botswana Vaccine Institute (Gaborone, Botswana)	Botswana
Not available	Live	Egypt 87	Veterinary Serum and Vaccine Research Institute (Cairo, Egypt)	Egypt
Not available	Live	PPRV 75/1 homologous	Biological Products Division (Kathmandu, Nepal)	Nepal
Not available	Live	PPRV 75/1	National Veterinary Research Institute (Vom, Nigeria)	Nigeria
PESTDOLL-S	Live	PPRV Nigeria 75/1	Dollvet (Sanliurfa, Turkey); Vetall Company (Adiyaman, Turkey); Veterinary Control and Research Institute (Ankara, Turkey)	Turkey
PPR vaccine	Live	PPRV Sungri 96	IVRI (Bareilly, India)/National Research Development Corporation (New Delhi, India)	India
PPR vaccine	Live	PPRV Arasur/87	TANUVAS (Chennai, India)	India
PPR vaccine	Live	PPRV Coimbatore/97	TANUVAS	India

IVRI: Indian Veterinary Research Institute; PPR: Peste des petits ruminants; PPRV: Peste des petits ruminants virus; TANUVAS: Tamil Nadu University of Veterinary and Animal Science.

Box 1. Salient features of peste des petits ruminants vaccine (Sungri/96).**Nature of vaccine:**

- Vero cell-based live-attenuated peste des petits ruminants vaccine

Target species:

- Goats and sheep

Phylogeny:

- Lineage 4 (Asian lineage)

Vaccine presentation:

- 50/100 doses per vial

Diluents:

- 1M MgSO₄/0.85% normal saline solution

Stability of freeze-dried vaccine:

- Shelf life is more than 1 year at 4°C

Stability of the diluted vaccine:

- At least 8 h

Cold chain requirement:

- Required

Dose:

- 1.0 ml of reconstituted vaccine

Route of administration:

- Subcutaneous in neck region

Duration of immunity:

- More than 6 years tested so far

titer even up to 48 h. At 37°C, it maintained the protective titer for up to 42 h when reconstituted with diluents containing NaCl and MgSO₄. Similarly, the Revati vaccine showed a good stability at 4°C for about 36 h and it could maintain the protective titer at 25–37°C for up to 24 h [70].

The application of deuterium for enhancing the thermostability of PPR vaccines has been evaluated using heavy water as reconstituting diluent. For the development of deuterated virus, 20% heavy water was incorporated in the culture medium, and for use as a diluent, a combination of 87% heavy water and 1 M MgCl₂ was tested. Furthermore, the ability of heavy water–MgCl₂ and conventional saline diluents was tested for increasing the thermostability of the live-attenuated PPR vaccine. It was observed that when heavy water–MgCl₂ were used as the reconstituting diluents, then deuterated vaccine maintained titers greater than 10^{2.5} TCID₅₀/ml until 28 days on exposure at 37 and 40°C, whereas the conventional PPR vaccine maintained titer until 14 days when exposed at 37 and 40°C. The heavy water–MgCl₂ combination was a better reconstituting diluent than heavy water alone for both the deuterated and conventional PPR vaccine. There was a marked superiority of the deuterated virus over the conventional virus in terms of residual titers obtained at 37°C after 28 days of exposure. In general, higher titers were seen for deuterated virus reconstituted in heavy water-based diluents when compared with the conventional virus [71–74].

PPR & goatpox combined vaccine

Similar to PPR, goatpox (GP) affects sheep and goats and is recognized as a notifiable disease by the OIE. GP virus belongs to the genus *Capripoxvirus* in the family *Poxviridae*. Both PPR and

GP infections inflict substantial losses on sheep and goat production in endemic regions. These diseases share a more or less similar pattern of geographic distribution, with high seroprevalence in the target species [40]. A number of mixed viral infections, particularly involving PPR and Orf (type spp. of *Parapoxvirus*, *Poxviridae*) or PPR and GP, have been previously recorded [75–78]. Live-attenuated vaccines are currently in use for controlling GP as PPR. Thus, considering a similar geographic distribution of both PPR and GP virus infection, as well as the occurrence of mixed infections, there is potential for using a combined vaccine for control of these two infections, particularly in endemic areas. The choice of the target population to be immunized and the concurrent occurrence of infection from the point of view of both geographical and seasonal influences need to be thoroughly assessed before devising a combined approach for immunization. Compatibility of vaccines also needs careful assessment. If feasible, this approach would be economic and assist mass immunization programs to a great extent.

A number of multivalent live viral vaccines have been successfully used or are being tested in humans. The common ones include the trivalent/tetavalent measles, mumps, rubella and varicella vaccine in children [79,80]. However, only a few multivalent veterinary vaccines are available, such as those used against canine, poultry or bovine viral infections. In canines, commercial multivalent preparations containing modified live canine distemper virus, canine adenovirus type 2, canine parvovirus type 2b and canine parainfluenza virus have been found to elicit protective immunity [81]. A bivalent poultry vaccine comprising live turkey herpes virus and infectious bursal disease vaccines has also been successfully used in poultry [82]. Similarly, BOVI-SHIELD® 4 is a freeze-dried preparation of modified live virus strains of infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza-3 and bovine respiratory syncytia viruses marketed by Pfizer. Bisec vaccine, which is a combination of rinderpest and contagious bovine pleuropneumonia vaccine, has been widely used in the Global Rinderpest Eradication Programme (GREP) [61,202]. Vero cell-attenuated homologous GP and PPR vaccines have been developed at IVRI using Indian isolates. The PPR vaccine has been extensively tested in field conditions, with over 6 million doses being used across the country. Recent experimental and limited field trials using GP vaccine have shown that the vaccine is safe, potent and immunogenic in goats. However, while the PPR vaccine is seen as being totally safe in pregnant animals, the GP vaccine used to induce abortion in 0.01% of vaccinated pregnant animals [SINGH RK, SINGH SK, HOSAMANI M, SEN A, UNPUBLISHED DATA].

In order to protect goats from PPR and GP, a combined live-attenuated PPR and capripoxvirus vaccine of sheep origin has been used in Cameroon [76]. However, goats could not withstand challenge with a wild strain of GP virus in this experiment, and the failure of the vaccine to protect against virulent virus challenge was ascribed to a lack of protection between sheep poxvirus (SPV) strain RM65 and the wild strain of GP virus used in the experiment. Furthermore, a partial cross-protection among SPV and GP virus vaccine has been suggested; hence homologous vaccines are recommended for full protection [83]. We have re-evaluated

the efficacy of the combined vaccine (PPR and GP) in goats and showed that the bivalent vaccine induced a protective immune response against homologous challenge in goats. Seroconversion, as well as protection upon homologous challenge in goats, indicates that combined PPR and GP vaccines do not interfere with each other's immunogenicity [65]. Thus, both PPR and GP vaccine viruses are compatible with each other for making a live bivalent vaccine that can control PPR and GP infections in the same geographic distribution [84].

PPR & sheep pox combined vaccine

The combined sheep pox and PPR vaccine was prepared in lyophilized form containing recommended doses of both vaccine viruses. The safety and immunogenicity of this combined vaccine were evaluated in sheep [85]. Sheep immunized subcutaneously with 1 ml of live-attenuated vaccine consisting of 10^3 TCID₅₀ each of Romanian Fanar strain SPV and Sungri/96 strain of PPRV were monitored for clinical and serological responses for a period of 30 days postimmunization and then for a further 2 weeks postchallenge. Specific antibodies directed to SPV could be demonstrated by indirect ELISA and serum neutralization test. Competitive ELISA and serum neutralization test were used for demonstration of antibodies to PPRV. All of the immunized animals resisted challenge with virulent SPV or PPRV on day 30 postimmunization, while the control animals developed characteristic signs of disease. Challenge viruses could only be detected in the unvaccinated control animals but not from any of the immunized sheep. This indicates that the component vaccines did not interfere with each other and can be used in target populations for economic vaccination strategies [85].

Recombinant marker vaccines

For effective control and declaration of freedom from disease, post-outbreak serosurveillance is an important tool. To facilitate post-outbreak serosurveillance, a marker vaccine and a companion test that can detect infection in vaccinated animals (differentiation of infected and vaccinated animals [DIVA]) are the key. Therefore, although the current live-attenuated vaccine is efficacious against clinical disease, to facilitate the serosurveillance and seromonitoring, a recombinant marker vaccine (either positive or negative marker) approach may be beneficial.

Heterologous recombinant PPR vaccines

The development of a number of vaccinia virus recombinant vaccines for rinderpest has been reported in the past. When vaccinated with a vaccinia virus (VACV) double recombinant expressing the *H* and *F* genes of RPV, goats were completely protected against challenge with virulent PPRV, although they only developed antibodies (neutralizing and ELISA) to RPV and not to PPRV [86–89]. Goats were also protected against a lethal challenge of PPRV following vaccination with a recombinant capripoxvirus containing either the *F* gene or the *H* gene of RPV. The *H* gene recombinant produced high titers of neutralizing antibody to RPV in the vaccinated goats, whereas the *F* gene recombinant failed to stimulate detectable levels of neutralizing antibody [90]. A similar response to the

two recombinant vaccines has previously been reported for cattle. Neither recombinants produced detectable levels of specific antibodies to PPRV, even though they afforded protection against PPRV. In another study, recombinant VACV and capripoxvirus expressing the *H* and *F* genes of RPV were reported to be able to protect goats against PPRV challenge [89,90]. Although animals vaccinated with all these heterologous vaccines are protected from disease, they allow to some extent the replication of the PPR challenge virus for some time prior to its final elimination [90].

Homologous recombinant PPR vaccines

A recombinant capripoxvirus expressing the PPRV *F* gene provided protection in vaccinated goats [91]. The vaccine, even when used at a dose as low as 0.1 pfu, protected animals against virulent PPRV (Guinee Bissau/89 isolate at 10^4 as subcutaneous injection) and virulent capripox (Yemen isolate). Such a dual recombinant vaccine could be exploited to provide protection against two diseases (PPR and capripox) that are of great economic importance in many developing countries. However, the duration of immunity provided and its efficacy in the presence of antibodies against PPR or capripox need to be established.

The genes coding for the surface glycoproteins HN of PPRV and H of RPV were cloned in a cytomegalovirus promoter-driven baculovirus expression vector and expressed transiently in mammalian CV-1 cells [92]. The transiently expressed PPRV HN protein was found to be biologically active in possessing hemadsorption and neuraminidase activities, while the RPV H protein exhibited neuraminidase activity but was deficient in hemadsorption activity. Furthermore, it was found that the transiently expressed PPRV F protein could bring about both fusion and hemifusion, whereas the RPV F protein could only bring about hemifusion, and fusion required the presence of an attachment protein (HN) [92].

Sinnathamby *et al.* produced recombinant HN glycoprotein of PPRV in a baculovirus system. Goats that were immunized with these recombinant proteins produced an immune response against PPRV and antibodies generated in the immunized animals could neutralize both PPRV and RPV *in vitro* [93].

Recombinant *Bombyx mori* nucleopolyhedroviruses (BmNPV) displaying the immunodominant ectodomains of F glycoprotein of PPRV and the H protein of RPV, on the budded virions as well as on the surface of the infected host cells, have been constructed. The F and H protein sequences were inserted in-frame within the amino-terminal region of BmNPV envelope glycoprotein GP64 expressing under the strong viral polyhedrin (polh) promoter. The recombinant virus selection in BmNPV was improved by incorporating the green fluorescent protein (*GFP*) gene as a selection marker under a separate promoter within the transfer cassette harboring the desired genes. Following infection of the insect larvae or the host-derived BmN cells with these recombinant BmNPVs, the expressed GP64 fusion proteins were displayed on the host cell surface and the budded virions. The antigenic epitopes of the recombinant proteins were properly displayed and the recombinant virus particles induced an immune response in mice against PPRV or RPV [94].

A study was conducted to determine the antigenicity and immunogenicity of silk worm larvae-expressed recombinant PPRV F protein. The silk worm larvae containing PPRV F antigen were found to be negative in polyclonal antibody-based sandwich ELISA, while the cell culture virus and known positive tissue materials tested positive. Goats orally fed with silk worm larvae and goats injected with larval extract failed to produce a humoral immune response and tested negative by PPR competitive and PPR indirect ELISA, and serum neutralization test up to 56 days postimmunization [SINGH RK, SEN A, SARAVANAN P, UNPUBLISHED DATA]. This could be due to the fact that the F protein of PPRV mostly elicits a cell-mediated immune response [5], which was not tested in this study.

The RPV H protein was expressed on transgenic tobacco and peanut plants. The authenticity of the antigen was verified by using anti-H monoclonal antibody and convalescent antisera. A high antibody titer was observed in mice inoculated intraperitoneally with the leaf extracts. Mice and cattle that were fed these leaf extracts had a very good immune response in terms of IgG and IgA titers against RPV and also a lymphoproliferative response [95]. Similarly, the two surface glycoproteins of PPRV hemagglutinin-neuraminidase and F protein confer protective immunity. The successful generation of transgenic pigeon pea (*Cajanus cajan* [L.] Millsp.) plants and expression of HN protein with biological activity was reported. A 2-kb fragment containing the coding region of the HN gene from an Indian isolate was cloned into the binary vector pBI121 and mobilized into *Agrobacterium tumefaciens* strain GV3 101. Cotyledonary nodes from germinated seeds of pigeon pea were used for transformation. The presence of transgenes, NPTII and HN in the plants was confirmed by PCR. The expression of HN protein in the transgenic lines was further confirmed by western blot analysis using polyclonal monospecific antibody to HN and, more importantly, plant-derived HN protein was shown to be biologically active as demonstrated by neuraminidase activity. However, a clinical trial of this edible vaccine needs to be performed that can demonstrate the efficacy of this vaccine. Similarly, the subunit vaccine that contains PPR-specific expressed protein may be a useful vaccine candidate [96].

Another approach for producing recombinant vaccines is by replacing gene/s from related viruses and rescuing chimeric viruses. Following this approach, a rinderpest–PPR FH recombinant virus was produced where the F and H gene of RPV were swapped with PPRV F and H genes [97]. Later, the M, F and H genes of RPV were replaced by those of PPRV using the RPV vaccine as the backbone. Goats were protected against virulent PPR challenge with the resulting chimeric PPR marker vaccine [98]. These investigators also showed that the monoclonal antibody tests based on the response to the H(1) and N [99,100] proteins of RPV and PPRV could be used to distinguish between vaccinated and naturally recovered animals and also vaccinated animals that subsequently become infected [98]. Unfortunately, the monoclonal antibody-based RPV and PPRV N c-ELISA cross-react with each other, which has limited the use of this genetically marked vaccine. However, this problem will soon be overcome with the development of a non-cross-reacting test using the carboxy-terminal variable region of the rinderpest

N protein [101]. The widespread use of such marker vaccines in the future, along with the diagnostic tests to identify their serological signature, would greatly improve the surveillance capabilities for disease preparedness and emergency prevention procedures.

Expert commentary

The PPR live-attenuated vaccine is efficacious and can be used in PPR control programs. However, the low thermolability of this live-attenuated vaccine is a major concern, especially in subtropical countries where the maintenance of a cold chain during vaccine transport is difficult. Thus, new vaccine formulations with improved stability are the hallmark of efficacy of current PPR live-attenuated vaccines. After the eradication of rinderpest, it would be pertinent to consider the possibility of eradicating other livestock diseases. A substantial amount of funding has gone in the development of infrastructure to ensure the success of rinderpest eradication. Considering the economic losses that arise from PPR and the state of preparedness in terms of the vaccines and diagnostic tests available, it would be worthwhile to consider an eradication program for PPR.

Combined vaccines like PPR and GP or PPR and sheep pox are economic and may be useful for controlling mixed infections in small ruminants. Recombinant marker vaccines associated with DIVA tests would make an ideal substitution for the conventional live-attenuated vaccines and would facilitate serosurveillance and seromonitoring for the control and eradication of PPR.

Five-year view

Rescue of recombinant PPRV employing reverse genetics has the potential for opening up new areas of study especially in the field of marker vaccines and in the area of virus–host interactions. This approach will facilitate manipulation of the genes of the recombinant PPRV to insert a positive marker that may help to develop a companion test. Similarly, deleting an unnecessary portion of the gene or mutating nucleotides in a particular gene or producing a chimeric virus may help to develop a negative marker vaccine and a DIVA test. Research in this area is ongoing in quite a few laboratories throughout the world and success is predicted. More fundamental immunological research is required for PPR to find out the immune correlates of protection. Therefore, work on cell-mediated immunity is an area of importance for future research.

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Key issues

- Availability of a sufficient vaccine to cover almost all endemic areas in order to increase the herd immunity is one of the major key issues in the first phase of a peste des petits ruminants control program.
- Strengthening the infrastructure (i.e., validated diagnostic tests for serosurveillance and seromonitoring) is a key issue in endemic countries.
- Development of thermostable vaccines with an improved shelf-life is an important point for controlling disease in tropical and subtropical peste des petits ruminants-endemic countries.
- For easy eradication of disease, an efficient marker vaccine and a robust differentiation of infected and vaccinated animals test are required.

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