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# Comparative evaluation of three capripoxvirus-vectored peste des petits ruminants vaccines

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ABSTRACT

Sheep and goat pox (SGP) with peste des petits ruminants (PPR) are transboundary viral diseases of small ruminants that cause huge economic losses. Recombinant vaccines that can protect from both infections have been reported as a promising solution for the future. SGP was used as a vector to express two structural proteins hemagglutinin or the fusion protein of PPRV. We compared immunity conferred by recombinant capripoxvirus vaccines expressing H or F or both HF. Safety and efficacy were evaluated in goats and sheep. Two vaccine doses were tested in sheep, $10^{4.5}$TCDI50 in 1 ml dose was retained for the further experiment. Results showed that the recombinant HF confers an earlier and stronger immunity against both SGP and PPR. This recombinant vaccine protect also against the disease in exposed and unexposed sheep. The potential Differentiating Infected from Vaccinated Animals of recombinant vaccines is of great advantage in any eradication program.

## 1. Background

Capripoxvirus infections (Sheep and goat pox (SGP)) and peste des petits ruminants (PPR) are highly contagious diseases of small ruminants (Albina et al., 2013; Buczkowski et al., 2014; Parida et al., 2016). After the first detection in 1942, PPR distribution expanded from West Africa to the Middle East and South and Central Asia. The disease has recently spread to North Africa and China and has been reported to be expanding southern Africa (OIE, 2015). SGP has almost the same geographical distribution. Despite the existence of highly effective vaccines, SGP and PPR remains one of most important causes of morbidity and mortality in endemic areas (Libeau et al., 2014). The live PPR vaccine based on Nigeria 75 strain has been widely used to control PPR in many countries but the vaccine is heat-sensitive and cannot differentiate infected from vaccinated animals (Diallo et al., 2007; Silva et al., 2011).

Efforts towards developing new generation vaccines against PPR based on Differentiating Infected from Vaccinated Animals (DIVA) concept are of great interest to improve the global eradication strategy (FAO and OIE, 2015). The use of capripoxvirus vaccine vector for inserting PPR virus (PPRV) genes to prepare a bivalent and thermostable vaccine has been reported by Diallo et al. (2002), and Chen et al. (2010). PPRV belongs to the *Morbillivirus* genus in the *Paramyxoviridae* family (Gibbs et al., 1979). PPRV genome encodes two structural glycoprotein which are essential for cell attachment, virus penetration and protective immune response (Barrett and Underwood, 1985): the hemagglutinin (H) and the fusion protein (F). Capripoxvirus recombinant vaccines vectored PPR have been developed using H or F proteins with a controversial opinion on which protein is the most protective (Chen et al., 2010; Diallo, 2003; Diallo et al., 2002).

In this study we compared by challenge the immunity induced by three recombinant capripoxvirus vaccines: one expressing the H protein of PPRV, the second expressing F protein and the third expressing both H and F proteins. We tested the recombinant vaccines on naive and SGP

*Abbreviations:* BSL3, biosafety level 3; DIVA, Differentiating Infected from Vaccinated Animals; D, day; dpi, days post infection; DMEM, Dulbecco's Modified Eagle's Medium; ELISA, enzyme-linked immunosorbent assay; FAO, Food and Agriculture Organization; GPV-PPR, recombinant capripoxvirus vaccines; H, hemagglutinin; F, fusion protein; IN, intra-nasal; IV, intravenous; M.O.I, Multiplicity of Infection; OIE, World Organization for Animal Health; OT, ovine lamb testis; PPR, Peste des petits ruminants; PPRV, Peste des petits ruminants virus; qRT-PCR, quantitative real-time reverse transcriptase-polymerase chain reaction; SC, subcutaneously; SGP, Sheep and goat pox; SPV, sheep pox virus; VN, virus neutralization

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previously exposed goats in the objective to identify a stable DIVA vaccine against both infections.

## 2. Methods

### 2.1. Recombinant vaccines construction

The recombinant viruses, GPV-PPR H, GPV-PPR F and GPV-PPR HF, were generated in the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences. The CPV/AV41 strain, a seed virus of a live attenuated CPV vaccine currently used in Asia, was selected as the recombinant vector. GPV-PPR H and GPV-PPR F were constructed as described by Chen et al. (2010).

The CPV/AV41 strain was firstly use to generate a recombinant vector expressing enhanced green fluorescent protein (eGFP) at TK gene, GPV-eGFP, by homologous recombination with plasmid pTK-gpt-ires-eGFP (Chen et al., 2009) after transfection of infected primary ovine lamb testis (OT) cells with CPV/AV41.

The shuttle plasmid was constructed with the fragments for homologous recombination, TK-Left and TK-Right, identical to those of pTK-gpt-ires-eGFP. The recombinant GPV-PPR HF was generated in OT cells by transfection with pTK-P7.5H/P7.5-F, following infection with GPV-eGFP. The recombinant GPV-PPR HF was screened through viral plaque assay by picking plaques negative for eGFP expression. PPRV H and F genes inserted at TK gene in the recombinant genome were checked by PCR and sequencing (Berhe et al., 2003). The expressions of H and F proteins in GPV-PPR HF were confirmed by Western-blotting as described by Chen et al. (2009, 2010).

### 2.2. Recombinant vaccine manufacturing

OT cells were used for the propagation and titration of the recombinant strains. The three viruses were propagated on cells and maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 1% irradiated fetal calf serum. Viral inoculation was carried out using a Multiplicity of Infection (M.O.I) of 0.01. The live vaccine was prepared with the virus suspension by addition of a stabilizer (4% peptone, 8% sucrose and 2% glutamate) followed by lyophilization. Final products were tested for sterility, identity, purity and the infectious titre before use according to the OIE Terrestrial Manual (OIE, 2017).

### 2.3. Vaccination protocol

In this experiment, we used sheep of local known sensitive breed (Fakri et al., 2017) and Alpine goats between 6 and 9 months of age from our own experiment farm. Experiments was performed under biosafety level 3 (BSL3) in accordance with the guidelines described for the care and handling of experimental animals by the Laboratory Committee for Control and Supervision of Animal Experimentation.

#### 2.3.1. Determination of the recombinant GPV-PPR vaccine dose

Three groups composed respectively of 14, 18 and 4 sheep, were constituted. The first 2 groups were vaccinated subcutaneously (SC) at day (D) 0 and boosted at D28, by GPV-PPR H respectively at $10^{4.5}$ and $10^{3.0}$$TCID_{50}$ per dose of 1 ml. Sheep of group 3 were kept unvaccinated. Eight animals of each vaccinated group and 4 unvaccinated controls were challenged with the virulent strain of SGPV to evaluate potency.

#### 2.3.2. Comparative protection study

GPV-PPR H, GPV-PPR F and GPV-PPR HF recombinant vaccine were compared by vaccination of sheep and goats by each vaccine: 8 sheep per group for vaccinated animals and 4 sheep unvaccinated, for goats, 4 animals per group. Vaccination was carried out SC at the dose of $10^{4.5}$ $TCID_{50}$, with GPV-PPR H, GPV-PPR F and GPV-PPR HF vaccines at D0 with a booster at D28. Sheep and goats were then respectively challenged with virulent strains of SGPV and PPRV at D42 to assess proffered immunity.

#### 2.3.3. Vaccination in presence of pre-existing SGP immunity

Four goats were vaccinated SC by a SGP vaccine (Romania strain), at the manufacturer recommended dose of $10^{2.5}$ $TCID_{50}$. Three months later, the animals were injected by the recombinant vaccine GPV-PPR H at the dose of $10^{4.5}$ $TCID_{50}$. The animals were challenged at D42 for potency against PPRV.

### 2.4. Vaccination monitoring

Vaccination response was monitored by virus neutralization antibody titration (VN) for SGPV and PPRV and enzyme-linked immunosorbent assay (ELISA) for PPRV, performed on weekly collected serum. VN was performed in 96-microwell plates. The test is based on a serial ¼ dilutions of heat inactivated sera mixed with infectious virus (100 TCID50). The neutralizing antibody titer was calculated in accordance to Reed and Muench method (OIE, 2016, 2013).

ELISA kit ('ID Screen PPR Competition' reference(PPRC-4P ID-VET)) was used to detect kinetic of PPR antibodies (Libeau et al., 1995).

### 2.5. Challenge for vaccine potency

Local virulent strains isolates on cell culture of PPR (2008) and SP (1998) were used for challenge. Those strains are used routinely for challenge and known to induce characteristic symptoms.

#### 2.5.1. PPR

Alpine goats were challenged by intravenous (IV) injection and intra-nasal (IN) spray of PPRV virulent strain according to the protocol of Elharrak et al. (2012). The titre of the virulent strains was $10^{5.4}$ TCID50/ml. Monitoring was based on a daily observation of hyperthermia and clinical signs from D1 to D14 days post infection (dpi) according to Elharrak et al. (2012) and Hammouchi et al. (2012). Clinical scores were used to evaluate the severity of clinical signs and to allow comparison between animals and groups. A clinical scoring system was followed with a ranking from 0 to 4 based on the severity of: general clinical appearance, hyperthermia, alimentation, behavior, diarrhea, nasal discharge, salivation, respiratory symptoms including dyspnea, coughing and sneezing. A total cumulative score of the assessed signs per animal per day were then calculated. The animals that showed clinical symptoms of the disease were euthanized when the clinical score reach between 15 and 18 according severity of the symptoms (dyspnea, diarrhea). All surviving animals were euthanized at the end of the study. Specific samples after autopsy were taken from lung, mesenteric nodes, pulmonary nodes, trachea and liver for virus or RNA detection.

#### 2.5.2. SGP

Sheep were challenged with the virulent strain of SGPV at a titre of $10^{5.5}$ TCID50/ml, using the protection index protocol that consisted on a virus titration by intra-dermal injection of serial dilutions on the flank of each animal. Sheep were monitored daily for clinical signs, rectal temperature and the development of inflammation in each of the injection site. The presence of any inflammation was considered positive for the virus titration. The obtained titre for each group was compared with the titre of the unvaccinated control animals and the difference between the two titres expressed in log represent the Protection Index (Fassi-Fehri et al., 1984).

### 2.6. qPCR screening

PPRV genome detection was performed using quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) as described by Batten et al. (2011). RNA extraction was accomplished using a RNA kit (Bioline BIO-52075, isolate II RNA Mini kit). Amplification was