Virology 514 (2018) 211–215

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

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

F. Fakri[a,b,*], Z. Bamouh[a], F. Ghzal[a], W. Baha[a], K. Tadlaoui[a], O. Fassi Fihri[b], W. Chen[c], Z. Bu[c], M. Elharrak[a]

[a] Research and Development, MCI Santé Animale, Lot. 157, Z. I., Sud-Ouest (ERAC) B.P: 278, Mohammedia 28810, Morocco
[b] Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco
[c] Key Laboratory of Veterinary Public Health of Ministry of Agriculture and State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences, Harbin 150001, China

ARTICLE INFO

Keywords:
Capripoxvirus vector
Peste des petits ruminants (PPR)
Sheep and goat pox (SGP)
Recombinant vaccine

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

* Correspondence to: MCI Santé Animale, Lot. 157, Z.I., Sud-Ouest (ERAC) B.P: 278, Mohammedia 28810, Morocco.
E-mail addresses: fz.fakri@mci-santeanimale.com (F. Fakri), z.bamouh@mci-santeanimale.com (Z. Bamouh), f.ghzal@mci-santeanimale.com (F. Ghzal), w.baha@mci-santeanimale.com (W. Baha), k.tadlaoui@mci-santeanimale.com (K. Tadlaoui), o.fassifihri@iav.ac.ma (O.F. Fihri), chenweiye1980@yahoo.com.cn (W. Chen), zgb@hvri.ac.cn (Z. Bu), m.elharrak@mci-santeanimale.com (M. Elharrak).

https://doi.org/10.1016/j.virol.2017.11.015
Received 4 August 2017; Received in revised form 10 November 2017; Accepted 18 November 2017

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

carried out with the Invitrogen Superscript III Platinum R one step qRT-PCR system (Cat. no. 11745-100).

2.7. Statistical analysis

Differences among groups regarding hyperthermia, protection index, clinical scoring and serological response were determined using a one-way analysis of variance (ANOVA) followed by Student's *t*-test. A P-value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Vaccine safety

During 14 days following first vaccination and booster, all vaccinated animals remained healthy, without any effect on their appetite and behavior. No abnormal local reactions have been reported, except of a small transitory nodules and a short increase of the temperature not exceeding 1 °C during 2 days in few animals.

3.2. Vaccine dose determination

Sheep vaccinated with GPV-PPR H at $10^{4.5}$ $TCID_{50}$ per dose showed higher PPRV antibody titers comparatively to those who received $10^{3.0}$ $TCID_{50}$ per dose. Anti PPRV antibodies appeared at D21 with a clear booster effect, to reach higher values of VN titer (2.9) on D42. Regarding SGPV response, no significant difference was observed between the 2 groups, titers increase progressively to reach an average of 1.9 at D42. PPRV serological response after vaccination is reported in Fig. 1.

After challenge with virulent SGPV, all unvaccinated control animals exhibited a rise in body temperature between D4 and D11, with a peak at D9 and D10 (Fig. 2). Local reactions at the injection sites were observed from D3, increasing in size the following days. Typical SGP skin secondary nodules not associated with injection sites, appeared between D10 and D11. The obtained virus titre (Table 1) was 6.4 log10 ID50/ml.

Vaccinated sheep showed only slight increase in temperature the first two days pi due to the hypersensitivity reaction at the injection site, in dilutions 10^{-1} and 10^{-2}. There was an important difference between these 2 groups: sheep of high dose showed an average infectious titre of 2.5 log ID50/ml with a protection index of 3.9, sheep of low dose showed an average infectious titre of 4.9 log ID50/ml with a protection index of 1.4. None of the immunized animals showed clinical signs of SGP during the observation period.

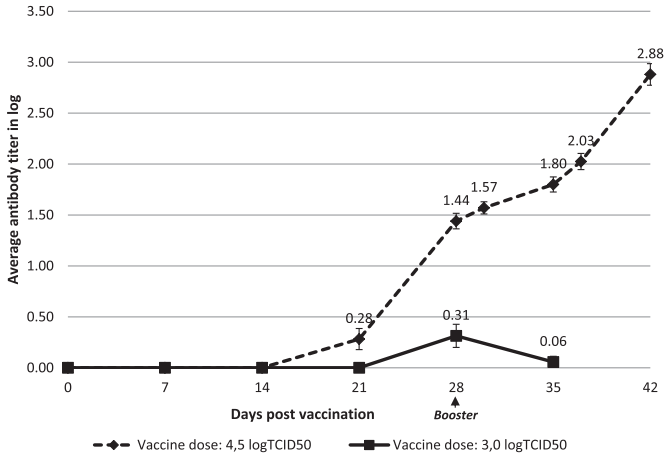

Fig. 1. Neutralizing peste des petits ruminants virus antibody kinetic after vaccination of sheep with the recombinant GPV-PPR H vaccine at 2 doses: $10^{4.5}$ and $10^{3.0}$ TCID50 (mean antibody titer).

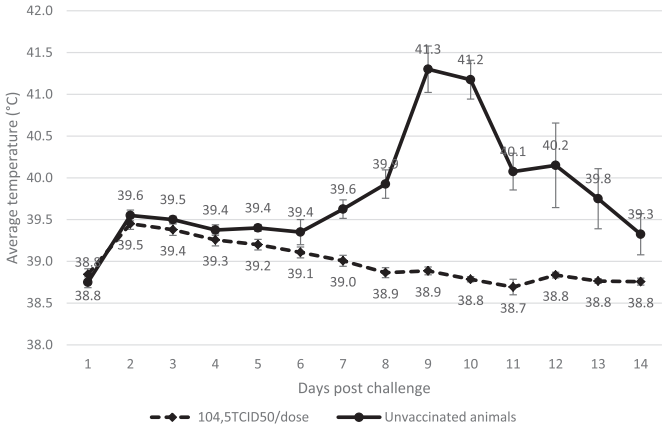

Fig. 2. Obtained average temperature of challenged sheep after vaccination with the recombinant GPV-PPR H at $10^{4.5}$ TCID50/dose.

Table 1
Results of sheep vaccinated with GPV-PPR H at two different doses then challenged with virulent SPV strain.

Group	Number of animals	Vaccination Dose ($logTCID_{50}$/ml)	Challenged animals	Infectious titre (group average) expressed in $logID_{50}$/ml	Protection Index (group average)
G1	14	4.5	8	2,5	3,9
G2	18	3.0	8	4,9	1,4
G3	4	Unvaccinated	4	6,4	0

Two groups of sheep G1 and G2 were vaccinated by respectively dose of $10^{4.5}$ and $10^{3.0}$ TCID50 per ml of GPV-PPR H recombinant vaccine. G3 were kept unvaccinated as control. All groups were challenged after vaccination by virulent SPV strain. The protection index is the difference between the titer observed on controlled animals and the titer observed on vaccinated animals.

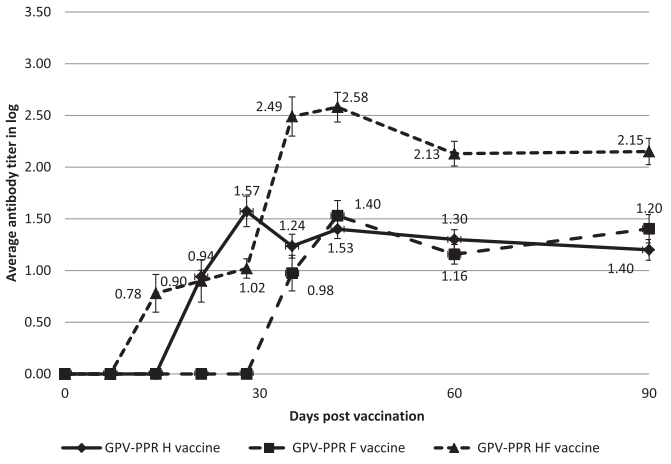

Fig. 3. Neutralizing peste des petits ruminants virus antibody kinetic after vaccination of sheep with three capripoxvirus-vectored peste des petits ruminants vaccines at a dose of $10^{4.5}TCID_{50}$ (mean of antibody titer).

3.3. Comparative vaccine protection

As shown in Fig. 3, the obtained PPR antibody titers after vaccination of sheep were clearly higher with the group injected by GPV-PPR HF recombinant vaccine comparatively to those vaccinated by GPV-PPR H and GPV-PPR F. PPR antibodies appeared at D14. The average antibody neutralizing titre seemed to stabilize at 2.1 at D60 to D90. Sheep showed a full protection against SGPV experimental infection; the average protection index on vaccinated sheep for SGPV was 3.9 with no significant difference, comparatively to the unvaccinated

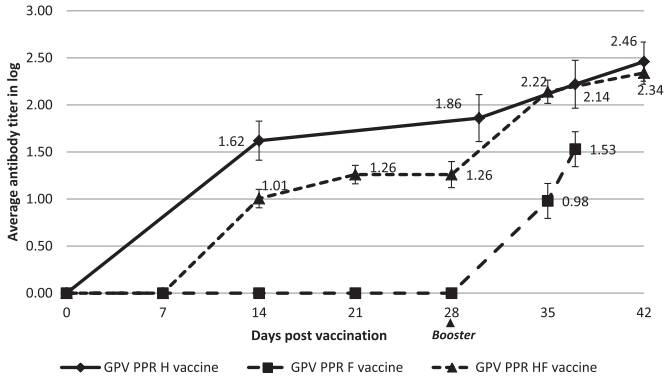

Fig. 4. Neutralizing peste des petits ruminants virus antibody kinetic after vaccination of goats with three capripoxvirus-vectored peste des petits ruminants vaccines at a dose of $10^{4.5}$TCID50 (mean of antibody titer).

animals with a nil protection index.

On goats, the obtained neutralizing PPRV antibody titer after vaccination was low for the group vaccinated by GPV-PPR F, with a maximum value of 1.5 at D37 (Fig. 4). Comparatively groups vaccinated with GPV-PPR H and GPV-PPR HF, showed respectively 2.2 and 2.1 (D37).

After PPRV challenge on goats, unvaccinated animals showed specific clinical signs of PPR infection: dyspnea (3/4), nasal (4/4) and ocular discharges (3/4) followed by a respiratory syndrome with painful polypnea (3/4) and profuse diarrhea (4/4) in the terminal stage. The average clinical scoring was 17 on unvaccinated goats, with no significant difference between animals. For vaccinated animals, no clinical symptom was reported during the 14 days of the observation period, body temperature remained normal and clinical scoring was nil.

3.4. Vaccination in presence of pre-existing SGP immunity

Goats pre-immunized by SGP vaccine with an average antibody neutralizing titre of 1.8 and revaccinated by GPV-PPR H, presented a weak antibody titers against PPR from D14 (0.3), the titers raised to 1.8 at D42 after the booster. At the challenge, no symptom or hyperthermia were observed in vaccinated animals inoculated with the virulent strain of PPRV.

In all previous experiments no antibody to PPR were detected by the IDvet Elisa kit after vaccination.

4. Discussion

The small genome size of some viruses used as vectors facilitates their manipulation but proves to be an important constraint to integrate DNA. The advantages of using poxviruses as vectors are numerous. They tolerate without loss of infectivity insertions of more than 30 kbp which allow multivalent vaccines development (Perkus et al., 1985, 1991; Smith et al., 1984). The intra-cytoplasmic poxviruses replication limits the risk of genetic recombination with the host DNA and allows production of high quantity of antigen. Stability of poxviruses is an advantage in areas where the cold chain is difficult to maintain (Collier, 1955). PPRV is known to be heat sensitive which affects vaccine efficacy in the tropics. For this, development of pox-PPR recombinant represents numerous advantages including the possibility of differentiation between vaccinated and infected animals (DIVA vaccines).

In our study, we compared 3 different capripoxvirus recombinant vaccines expressing H, F or HF proteins in the objective to determine which of those proteins are most efficient to protect animals against PPR infection. We used the CVCC/AV41, a live attenuated goat pox vaccine strain currently used in Asia. This strain offers the advantage to be of goat origin, the most susceptible species to PPR, it protect also against SGPV as reported from the field and tested in our study.

Serology and the challenge results of the first experiment showed that the recommended vaccine dose was $10^{4.5}$ $TCID_{50}$ with GPV-PPR H. Therefore we used this dose to compare the 3 recombinant vaccines. Indeed, sheep vaccinated with $10^{3.0}$ $TCID_{50}$ didn't show high antibody PPRV titers, with only partial protection after challenge by virulent SGPV. The recommended vaccine dose is high if compared to normal pox live vaccine; this could be explained by a lower in vivo replication capacity of the recombinant viruses than the original strain. This dose may have a negative impact on the vaccine cost if no efficient technology process is applied for manufacturing. This funding is not in accordance with what was is reported by Diallo et al. (2002) who claim only 0.1–10 TCID50 for re-ca-FPPR and re-ca-HPPR, to developed recombinant vaccines based on the Kenya KS-1strain, recently found to be a lumpy skin bovine strain (Le Goff et al., 2005; Tulman et al., 2002; Tuppurainen et al., 2014).

The 3 recombinant tested vaccines induced serological response to PPR detected by VN and full protection after challenge by SGPV or PPRV virulent viruses. The commercial ELISA kit ('ID Screen PPR Competition' reference (PPRC-4P ID-VET)) is based on the nucleoprotein then this kit does not recognize vaccinated animals by recombinant H/F vaccine, it can detect only pre-infected animals. No antibody to PPR was detected by the IDvet Elisa kit after vaccination which confirms the DIVA potential of those recombinant vaccines. The nucleoprotein while unable to confer protective immunity, is a strong and early inducer of serum antibodies, and thus may serve as a negative mark in DIVA vaccines as none of vaccinated animals were positive on the Elisa test (Libeau et al., 1995).

Results showed that GPV-PPR HF vaccine conferred a stronger and earlier (D14) antibody response comparatively to GPV-PPR H (D21) and GPV-PPR F (D35), better response was obtained with recombinant H or HF than F alone, which confirm the role of hemagglutinin protein in the immunity process. However the HF vaccine is slightly better in antibody response than H. This result is not in accordance with Diallo et al. (2002) who reported that the fusion protein F is more immunogenic than the H protein.

Goats pre-immunized against SGP, showed a good protection against PPR after challenge. These results give evidence that the recombinant GPV can replicate and confer the protection against PPR on unexposed and exposed SGPV animals. A similar study with a single shot reports a partial protection after challenge in exposed animals to SGP (Caufour et al., 2014). In our study, the adopted vaccination protocol allowed immunity development, thus the vaccine can be used in endemic SGP areas with no loss of protection against PPR. We didn't judge necessary to conduct a study for animal exposed to PPR infection as theoretically no interaction expected between SGP replication and PPR, as demonstrated by Caufour et al. (2014).

The current PPR vaccine based on N75 PPRV strain is known to be heat sensitive but one injection is enough to procure a long lasting immunity. Recombinant vaccines may have disadvantages due to the cost and need of booster, however this study give evidence that they protect efficiently against both infections SGP and PPR, there are much more stable than the current heat sensitive N75 PPR strain. Recombinant tested vaccines showed a potential DIVA property which is a great advantage in any eradication program. The study gives also evidence that the use of 2 proteins H and F confer a better protection comparatively to H or F alone. The vaccine could be used safely in free or endemic regions of SGP and PPR. As PPR is mainly a goat disease GPV-PPR recombinant vaccine would have an advantage on SPV recombinant vector.

Acknowledgements

The authors gratefully acknowledge the support for this study by MCI Santé Animale.

Ethics approval

Animal experiments were carried out in accordance with the international guidelines for care and handling of experimental animals. The study protocol was approved by the Internal Ethic Committee.

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests. MCI Santé Animale authors are members of the Research and Development team and they are claiming that they have no competing interest in the work which is a pure scientific study.

Funding

The study was supported by MCI Santé Animale (RD1R1304-426-427).

Authors' contributions

WC and ZBu performed the construction of recombinant viruses. FF, ZBa and FG performed the experiments. FF and WB performed the serology testing. ME, OF, WC and Zbu designed and supervised the study. FF, Zbu and ME analyzed the data. KT participated in the BSL3 containment research with respect of animal welfare standards. FF, ZBu and MH drafted the manuscript. All authors have read and approved the final manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.virol.2017.11.015.

References

Albina, E., Kwiatek, O., Minet, C., Lancelot, R., Servan de Almeida, R., Libeau, G., 2013. Peste des petits ruminants, the next eradicated animal disease? Vet. Microbiol. 165, 38–44. http://dx.doi.org/10.1016/j.vetmic.2012.12.013.

Barrett, T., Underwood, B., 1985. Comparison of messenger RNAs inducedin cells infected with each member of the morbillivirus group. Virology 145, 195–199.

Batten, C.A., Banyard, A.C., King, D.P., Henstock, M.R., Edwards, L., Sanders, A., Buczkowski, H., Oura, C.C.L., Barrett, T., 2011. A real time RT-PCR assay for the specific detection of Peste des petits ruminants virus. J. Virol. Methods 171, 401–404. http://dx.doi.org/10.1016/j.jviromet.2010.11.022.

Berhe, G., Minet, C., Le Goff, C., Barrett, T., Ngangnou, A., Grillet, C., Libeau, G., Fleming, M., Black, D.N., Diallo, A., 2003. Development of a dual recombinant vaccine to protect small ruminants against peste-des-petits-ruminants virus and capripoxvirus infections. J. Virol. 77, 1571–1577. http://dx.doi.org/10.1128/JVI.77.2.1571-1577.2003.

Buczkowski, H., Muniraju, M., Parida, S., Banyard, A.C., 2014. Morbillivirus vaccines: recent successes and future hopes. Vaccine 32, 3155–3161. http://dx.doi.org/10.1016/j.vaccine.2014.03.053.

Caufour, P., Rufael, T., Lamien, C.E., Lancelot, R., Kidane, M., Awel, D., Sertse, T., Kwiatek, O., Libeau, G., Sahle, M., Diallo, A., Albina, E., 2014. Protective efficacy of a single immunization with capripoxvirus-vectored recombinant peste des petits ruminants vaccines in presence of pre-existing immunity. Vaccine 32, 3772–3779. http://dx.doi.org/10.1016/j.vaccine.2014.05.025.

Chen, W., Hu, S., Qu, L., Hu, Q., Zhang, Q., Zhi, H., Huang, K., Bu, Z., 2010. A goat poxvirus-vectored peste-des-petits-ruminants vaccine induces long-lasting neutralization antibody to high levels in goats and sheep. Vaccine 28, 4742–4750. http://dx.doi.org/10.1016/j.vaccine.2010.04.102.

Chen, W., Qu, L., Hu, S., Hu, Q., Zhang, Q., Zhi, H., Huang, K., Bu, Z., 2009. Recombinant goat pox virus expressing PPRV H protein. Chin. J. Biotechnol. 25, 496–502.

Collier, L.H., 1955. The development of a stable smallpox vaccine*. J. Hyg. 53, 76. http://dx.doi.org/10.1017/S002217240000053X.

Diallo, A., 2003. Control of peste des petits ruminants: classical and new generation vaccines. Dev. Biol. 114, 113–119.

Diallo, A., Minet, C., Berhe, G., Le Goff, C., Black, D.N., Fleming, M., Barrett, T., Grillet, C., Libeau, G., 2002. Goat immune response to capripox vaccine expressing the hemagglutinin protein of peste des petits ruminants. Ann. N.Y. Acad. Sci. 969, 88–91.

Diallo, A., Minet, C., Le Goff, C., Berhe, G., Albina, E., Libeau, G., Barrett, T., 2007. The threat of peste des petits ruminants: progress in vaccine development for disease control. Vaccine 25, 5591–5597. http://dx.doi.org/10.1016/j.vaccine.2007.02.013.

Elharrak, M., Touil, N., Loutfi, C., Hammouchi, M., Parida, S., Sebbar, G., Chaffai, N., Harif, B., Messoudi, N., Batten, C., Oura, C.A.L., 2012. A reliable and reproducible experimental challenge model for peste des petits ruminants virus. J. Clin. Microbiol. 50, 3738–3740. http://dx.doi.org/10.1128/JCM.01785-12.

Fakri, F.Z., Elhajjam, A., Bamouh, Z., Jazouli, M., Boumart, Z., Tadlaoui, K., Fassi-Fihri, O., Elharrak, M., 2017. Susceptibility of Moroccan sheep and goat breeds to peste des petits ruminants virus. Acta Vet. Scand. 59. http://dx.doi.org/10.1186/s13028-017-0323-y.

FAO, OIE, 2015. Global strategy for the control and eradication of PPR. In: Proceedings of the International Conference for the Control and Eradication of Peste des Petits Ruminants (PPR). Abidjan, Ivory Coast.

Fassi-Fehri, M., Eharrak, M., Johnson, D., Abbadi, M., EL-idrissi, A.H., 1984. Etude expérimentale de l'immunité anticlaveleuse post-vaccinale. Ann. Rech. Vétérinaires 15, 59–64.

Gibbs, E.P., Taylor, W.P., Lawman, M.J., Bryant, J., 1979. Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. Intervirology 11, 268–274.

Hammouchi, M., Loutfi, C., Sebbar, G., Touil, N., Chaffai, N., Batten, C., 2012. Experimental infection of alpine goats with a Moroccan strain of peste des petits ruminants virus (PPRV). Vet Microbiol. 160, 240–244.

Le Goff, C., Fakhfakh, E., Chadeyras, A., Aba-Adulugba, E., Libeau, G., Hammami, S., Diallo, A., Albina, E., 2005. Host-range phylogenetic grouping of capripoxviruses: genetic typing of CaPVs. Appl. Gene-based Technol. Improv. Anim. Prod. Heal. Dev. Ctries 727–733.

Libeau, G., Diallo, A., Parida, S., 2014. Evolutionary genetics underlying the spread of peste des petits ruminants virus. Anim. Front. 4, 14–20. http://dx.doi.org/10.2527/af.2014-0003.

Libeau, G., Préhaud, C., Lancelot, R., Colas, F., Guerre, L., Bishop, D.H., Diallo, A., 1995. Development of a competitive ELISA for detecting antibodies to the Peste des Petits Ruminants virus using a recombinant nucleoprotein. Res. Vet. Sci. 58, 50–55. http://dx.doi.org/10.1016/0034-5288(95)90088-8.

OIE, 2017. Tests for sterility and freedom from contamination of biological materials intented for veterinary use. In: OIE Terrestrial Manual, pp. 1–15.

OIE, 2016. Sheep pox and Goat pox. In: OIE Terrestrial Manual, pp. 1–13.

OIE, 2015. Peste des petits ruminants. Bulletin 1–124.

OIE, 2013. Peste des petits ruminants. In: OIE Terrestrial Manual. pp. 1–14.

Parida, S., Muniraju, M., Altan, E., Baazizi, R., Raj, G.D., Mahapatra, M., 2016. Emergence of PPR and its threat to Europe. Small Rumin. Res. 142, 16–21. http://dx.doi.org/10.1016/j.smallrumres.2016.02.018.

Perkus, M., Piccini, A., Lipinskas, B., Paoletti, E., 1985. Recombinant vaccinia virus: immunization against multiple pathogens. Science 229, 981–984.

Perkus, M.E., Goebel, S.J., Davis, S.W., Johnson, G.P., Norton, E.K., Paoletti, E., 1991. Deletion of 55 open reading frames from the termini of vaccinia virus. Virology 180, 406–410. http://dx.doi.org/10.1016/0042-6822(91)90047-F.

Silva, A.C., Carrondo, M.J.T., Alves, P.M., 2011. Strategies for improved stability of Peste des Petits Ruminants Vaccine. Vaccine 29, 4983–4991. http://dx.doi.org/10.1016/j.vaccine.2011.04.102.

Smith, G.L., Mackett, M., Moss, B., 1984. Recombinant vaccinia viruses as new live vaccines. Biotechnol. Genet. Eng. Rev. 2, 383–407. http://dx.doi.org/10.1080/02648725.1984.10647806.

Tulman, E.R., Afonso, C.L., Lu, Z., Zsak, L., Sur, J.-H., Sandybaev, N.T., Kerembekova, U.Z., Zaitsev, V.L., Kutish, G.F., Rock, D.L., 2002. The genomes of sheeppox and goatpox viruses. J. Virol. 76, 6054–6061. http://dx.doi.org/10.1128/JVI.76.12.6054-6061.2002.

Tuppurainen, E.S.M., Pearson, C.R., Bachanek-Bankowska, K., Knowles, N.J., Amareen, S., Frost, L., Henstock, M.R., Lamien, C.E., Diallo, A., Mertens, P.P.C., 2014. Characterization of sheep pox virus vaccine for cattle against lumpy skin disease virus. Antivir. Res. 109, 1–6. http://dx.doi.org/10.1016/j.antiviral.2014.06.009.