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Development and field application of a new combined vaccine against Peste des Petits Ruminants and Sheep Pox

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ABSTRACT

A combined vaccine against Peste des Petits Ruminants (PPR) and Sheep/Goat Pox (SGP) was developed and applied in the field, using a new association of vaccine strains: PPR Nigeria 75 strain with a titre of $10^{4.1}$ TCID50 and Sheep Pox Romania strain with a titre of $10^{4.0}$ TCID50. Safety and efficacy were evaluated on goats and sheep in comparison with monovalent PPR and SGP vaccines. Goats were challenged by PPR virulent strain and sheep by SP virulent strain. The result shows that the combined PPR/SGP vaccine confers a good protection against both PPR and SGP infection with no significant difference with monovalent vaccines. The combined vaccine was used in the field on sheep flocks and good sero-conversion was detected for both diseases as soon as 14 days post vaccination.

1. Introduction

The production of small ruminants is threatened by economically important contagious diseases: the Peste des Petits Ruminants (PPR) and Sheep/Goat Pox (SGP). Both PPR and SGP are transboundary animal diseases listed by the World Organization of Animal Health (OIE).

The control of PPR and SGP is a major goal for a program aimed to poverty alleviation because of the high importance of sheep and goats in endemic regions. The available monovalent vaccines for the control of both PPR and SGP protect after a single injection and the induced immunity covers at least the economic life of the animals, around three 3 years [1–3]. However, the low vaccination coverage due to large space distribution of the animal population and poor infrastructure with difficult access contribute to spread or maintain the infection.

It could be interesting to use an associated bivalent vaccine that protects against the two infections in one shot and this may promote a wider use of vaccination since both diseases are found in the same region. Similar associated vaccines against PPR and SGP infections has been developed in the past and used experimentally with satisfactory results in India [4,5] and in Cameroon [6]. However, no mass vaccination has been conducted with the associated vaccine so far.

The objective of this study was to develop and apply for mass vaccination a combined vaccine that could be used to protect in one-shot small ruminants against both PPR and SGP. The benefits of opting for a single vaccination covering both diseases (PPR and SGP) are numerous: to provide comfort to farmers, reduce stress in animals, especially minimizing vaccination costs for professional farming sector. The combined vaccine was based on highly immunogenic worldwide used strains of PPR (Nigeria 75) and SP (Romania). This strain association was tested for the first time.

2. Material and methods

2.1. Viral strains

The live PPR vaccine strain was Nigeria 75 developed by Diallo et al. [7], for SGP was the Sheep Pox Romania strain [8]. Both strains grown on Monkey African Green kidney (VERO) cells are commonly used for the protection of SGP and PPR.

Local isolated virulent strains: PPR MOR 2008 and SP MOR 1998 were selected for the challenge. Those strains are routinely used in our laboratory for potency testing of monovalent vaccines and characteristic symptoms are observed after experimental infection.

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2.2. Preparation of vaccine

Vaccine strains were grown separately on roller bottles with confluent cells, the inoculation was done using the same multiplicity of infection (M.O.I.) of 0.01. Viral suspensions were harvested when 80% of CPE was observed which happened 3–4 days for PPRV and 4–6 days for SGPV. The viral suspension was then stored at −80 °C before use.

From those two antigens, a monovalent PPR vaccine, a monovalent SGP vaccine and a combined PPR/SGP vaccine, all on lyophilized forms were prepared for this study. Vaccines were formulated in appropriate concentration of PPR and/or SGP for the recommended titre, mixed V/V with a stabilizer of lyophilisation (4% peptone, 8% sucrose and 2% glutamate).

For vaccination, the freeze-dried vaccine vial of 100 doses was reconstituted in 50 ml of the diluent (saline solution) for a vaccine dose of 0.5 ml.

2.3. Vaccination of sheep and goats

Animal experiment was carried out in accordance with guidelines for care and handling of experimental animals, as per the laboratory committee for purpose of control and supervision of experiments on animals. The experiment was conducted on four groups of animals housed in the animal unit of MCI Santé Animale, Mohammedia, Morocco: Group 1 composed by 6 sheep and 6 goats vaccinated with the combined SGP/PPR, Group 2 composed by 4 sheep vaccinated with the monovalent SP, Group 3 composed by 4 goats vaccinated with the monovalent PPR and Group 4 composed by unvaccinated animals (2 goats and 2 sheep). Common local breed sheep and Alpine goats were used in this experiment. All animals were aged 6–8 months, and tested seronegative for PPR and SGP.

Vaccination of each group was conducted by subcutaneous route and the monitoring consisted on a clinical observation, temperature, injection site inflammation and serological response.

2.4. Determination of vaccine potency

Determination of the vaccine potency was carried out by challenge on BSL3 containment laboratory.

Goats were challenged at day (D) 28 post vaccination by intravenous (IV) injection and intra-nasal (IN) spray of PPRV virulent strain according to the protocol of Elharrak et al. [9]. The titre of the virulent strains was $10^{5.4}$ ID50/ml and the dose was 1 ml IN and 1 ml IV. Sheep were challenged at D28 by the SP virulent strain with a titre of $10^{5.5}$ ID50/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. 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 [10].

The monitoring consisted on daily observations of specific symptoms, temperature and local inflammation on the site of injection. Clinical scoring and protection index, for each animal and the average for the group was calculated. All surviving animals were euthanized at D14 post infection, autopsied and sampled for further investigations.

2.5. Field trial of combined PPR/SPG vaccine

The combined vaccine was tested in the normal conditions of the field in three farms located in regions nearby Rabat. Three flocks of a minimum of 200 heads of local sheep, between 6 months and 5 years of age, have been used in the trial, observed 3 weeks for the vaccine safety and monitored weekly for serological response. Analyses were carried out on 10% of the vaccinated population.

To monitor vaccination response for both PPR and SGP, serological testing has been done using virus neutralization test as described in the OIE Terrestrial Manual (Chapters 2.7.11 and 2.7.14). ELISA test was also used to detect PPR kinetic of antibodies following vaccination. The kit 'ID Screen® PPR Competition' reference (PPRC-4P ID-VET) was used for that purpose [11].

For the antigen detection on challenged animals, we used real time qPCR as described by Batten et al. [12] for PPR and by Bowden et al. [13] for SGP. DNA extraction was performed using isolate genomic DNA/RNA Mini kit (Bioline® BIO-52066 & BIO-52075) and amplification done with the Kkit superscript Tm III Platinum R one step qRt-PCR system® (Cat. No. 11745-100).

2.6. Statistical analysis

To compare serological responses to monovalent and combined vaccines, data were entered into a database using SPSS 20.0 for Windows (SPSS Inc., Chicago, USA). The independent samples *t* test was used for continuous variables. The difference was considered significant if *p*-value was <0.05.

3. Results and discussion

The PPRV growth with characteristic cytopathogenic effect (CPE) on VERO cells, specific syncytia leading to necrosis was observed after 4–5 days of incubation. The obtained infectious titre was 6.1 ± 0.2 TCID50/ml of the harvested suspension. SPV induced a CPE after 4–5 days of incubation and the titre of the harvested suspension is 5.5 ± 0.2 TCID50/ml.

In this experiment, a monovalent PPR vaccine, a monovalent SGP vaccine and a combined PPR/SGP vaccine, on lyophilized forms were produced. The three vaccines were tested for sterility, purity and identity according international standards. The infectious titre per dose for these vaccines were $10^{4.1}$ TCID50 for PPR, and $10^{4.0}$ TCID50 for SGP.

Safety and efficacy of the vaccination was evaluated on animals comparatively between combined and monovalent vaccines. During the three 3 weeks following vaccination, all vaccinated animals remained healthy, without any effect on their appetite and

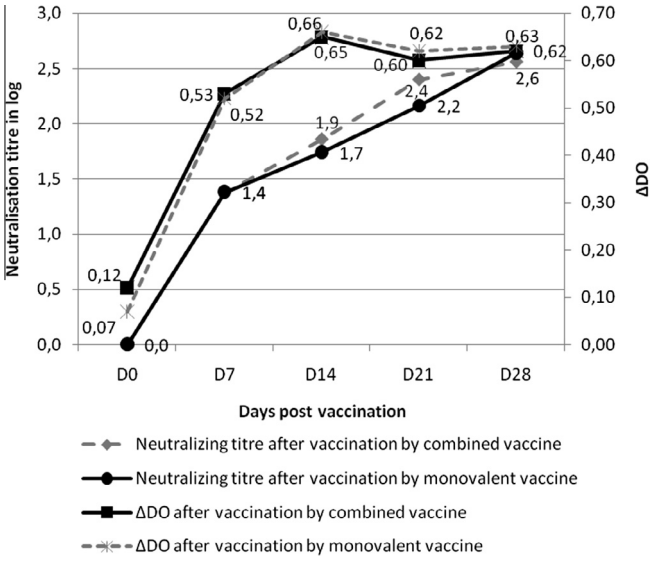

Fig. 1. Neutralizing PPR antibody response and PPR ELISA antibody response after vaccination of goats by combined and monovalent vaccines (group average).

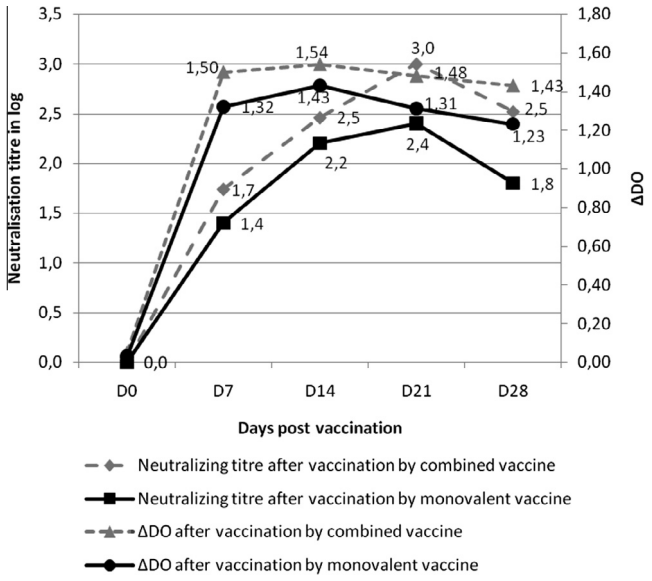

Fig. 2. Neutralizing PPR antibody response and PPR ELISA antibody response after vaccination of sheep by combined and monovalent vaccines (group average).

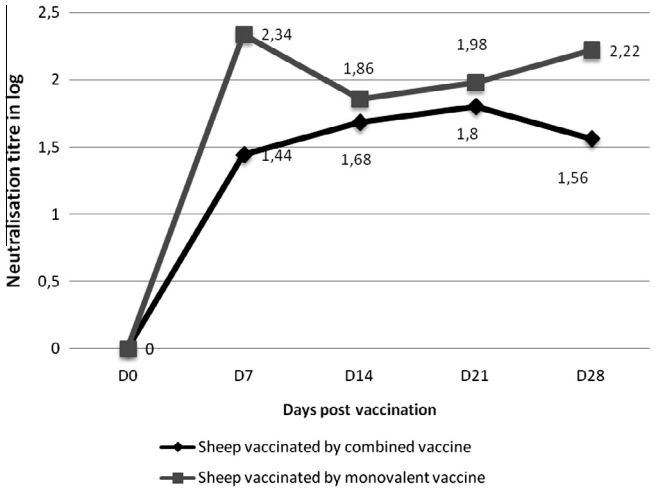

Fig. 3. Neutralising SPV antibody response after vaccination of sheep by combined and monovalent vaccines (group average).

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 sheep of Group 1 and Group 2. This reaction is routinely observed with Sheep Pox live attenuated vaccine based on Romania strain [8].

Serological response after vaccination is reported in Figs. 1–3. Good SGP antibody response obtained as soon as 7 days post vaccination. The neutralisation titre seems to stabilize between 1.8 and 2 log (Fig. 3).

Regarding PPR, the antibody titre increased progressively in goats until D28 with no significative difference ($p > 0.05$) between combined or monovalent vaccine as tested by SN and confirmed by ELISA (Fig. 1). For sheep a similar response was obtained with PPR antibodies after vaccination (Fig. 2). No significant difference in serological response between monovalent and combined vaccine or between sheep and goats regarding PPR valence. A similar observation was reported by Ayalet et al. [14] and by Chaudhary et al. [4].

Regarding SGP, serological response tested in sheep after vaccination show no significant difference between monovalent and combined vaccine. This valence has not been tested on goats because it has been demonstrated previously that Romania strain provides good protection against Goat Pox infection: vaccinated goats challenged with a virulent strain of Goat Pox virus appears to be fully protected (unpublished data). This cross protection between genus of Capripoxviruses have been reported by several authors [15,16].

Both vaccinated and unvaccinated control animals were challenged 28 days after vaccination by the corresponding virulent strain.

PPR challenge was conducted on goats and the obtained results are described in Table 1. Unvaccinated animals showed specific clinical signs of PPR infection: dyspnoea, nasal and ocular discharge followed by a respiratory syndrome with painful polypnea and profuse diarrhea in the terminal stage. The body temperature exceeded 40.8 °C. The two unvaccinated control goats died at D8 and D10 post infection with a clinical scoring of 19 and 17 respectively. Analysis by qPCR showed a high level of viral multiplication with excretion detected in ocular and rectal swabs (Ct 14.7). At post mortem examination, specific lesions of pneumonia and inflammatory nodules were observed in lung and digestive tractus with high viral concentration as detected by qPCR (Ct 17.9 in mesenteric nodes and 19.4 in lungs).

The obtained results complain with other studies on Alpine goats reported by Hammouchi et al. [17] and Elharrak et al. [9] whose defined a challenge model for PPR. However other experimental infection carried by several authors shows irregular or non-conclusive results depending on the animal species and breeds, the challenge strain and inoculated doses [18]. For vaccinated animals with monovalent PPR or combined vaccine, no clinical symptom was reported during the 14 days of observation period, the body temperature remained normal and the clinical scoring estimated between 0 and 2. PCR testing of ocular and rectal swabs of vaccinated and challenged animals confirmed the absence of virus excretion. These give evidence that combined and monovalent vaccines provide complete protection against the disease and the infection with PPRV.

Table 1
Results of vaccinated and unvaccinated goats challenged by PPR virulent strain.

	Animal	Clinical scoring	Mortality	Maximum viral charge in ocular swab (Ct value)	Maximum viral charge in rectal swab (Ct value)	Maximum viral charge in post-mortem organs(Ct value)
Combined SPV-PPR	134	0	–	37.1	29.2	–
	146	0	–	>34.6	28.2	–
	172	0	–	34	35.6	–
	182	0	–	>30.1	28.3	–
Monovalent	132	0	–	>34.2	31,2	–
	166	2	–	37.1	35.4	–
Unvaccinated	164	19	D8	23.9	22.7	17.9
	150	17	D10	19.6	14.7	19.4

Table 2
Results of vaccinated and unvaccinated sheep challenged by SP virulent strain.

	Identification	Serology (log neutralisation doses 50%)	Infectious titre (ID50/0.2 ml)	Protection value
Unvaccinated sheep	958	0	5.5	0.4
	957	0	5.9	0
Sheep vaccinated by combined vaccine	951	1.98	0	5.7
	952	1.26	0	5.7
	953	1.26	0	5.7
	954	1.74	0	5.7
Sheep vaccinated by SPV Ro monovalent vaccine	955	2.46	0	5.7
	956	1.98	0	5.7

Maximum viral charge (Ct value) for unvaccinated sheep

Primary lesions	Secondary lesions	Nodes		Organs	
Inoculation site	Skin papules	Mesenteric	Pulmonary	Lung	Spleen
11.3	12.9	9.72	17.2	27.2	Undetermined

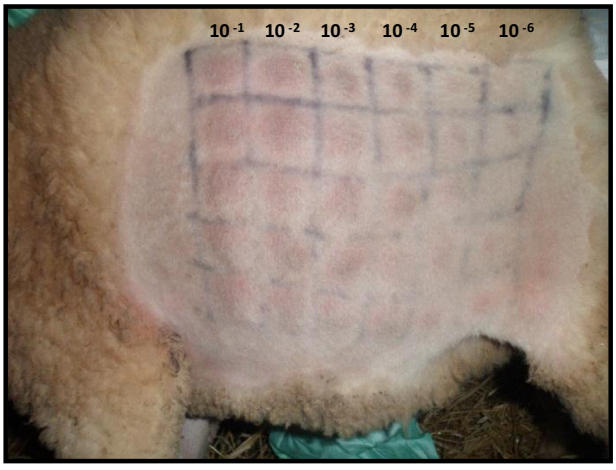

Fig. 4. Figure of a challenged control sheep showing local inflammation on site of inoculation (flank) with 10^{-1} to 10^{-6} dilutions (left to right) of virulent SGPV in five replica.

SGP challenge was conducted on sheep and the obtained results are described in Table 2. The used challenge model allows quantification of the conferred immunity and then comparison between different vaccines.

After challenge, unvaccinated sheep showed local inflammation in the site of injection (Fig. 4) starting at D3 pi at the lowest dilution with the maximum reaction observed between D7 and D9. The two control animals presented hyperthermia with a peak value up to 41.4 °C at D7 pi, and secondary lesions from D8. The obtained titre was 5.5 and 5.9 ID50/0.2 ml in the two controls respectively. After euthanasia at D14 pi, qPCR analysis showed a maximum viral charge with a Ct 11.3 in primary lesions (inoculation site), Ct 12.9 in secondary lesions (skin papules); an important viral charge was also observed in some nodes (Ct 9.72 in mesenteric nodes, to 17.2 in pulmonary node) and a low charge in lung (Ct 27.2) and negative in spleen.

Regarding vaccinated sheep, no clinical sign of the disease was observed and no secondary lesion reported for both monovalent and combined vaccine. After the challenge virus injection in the animal flank, a hypersensibility reaction was observed after the first and the second day pi which disappear completely the following days. No inflammatory reaction reported in the injection sites even in the lowest dilution giving evidence of the complete neutralisation of the virulent virus by the conferred immunity. Similar results have been also reported by Precausta et al. [8], whose authors demonstrated that Romania strain is very effective for protection against Sheep Pox infection. The protection index was evaluated to 5.7 ID50/0.2 ml in vaccinated sheep which mean a solid and durable immunity. In support of the present

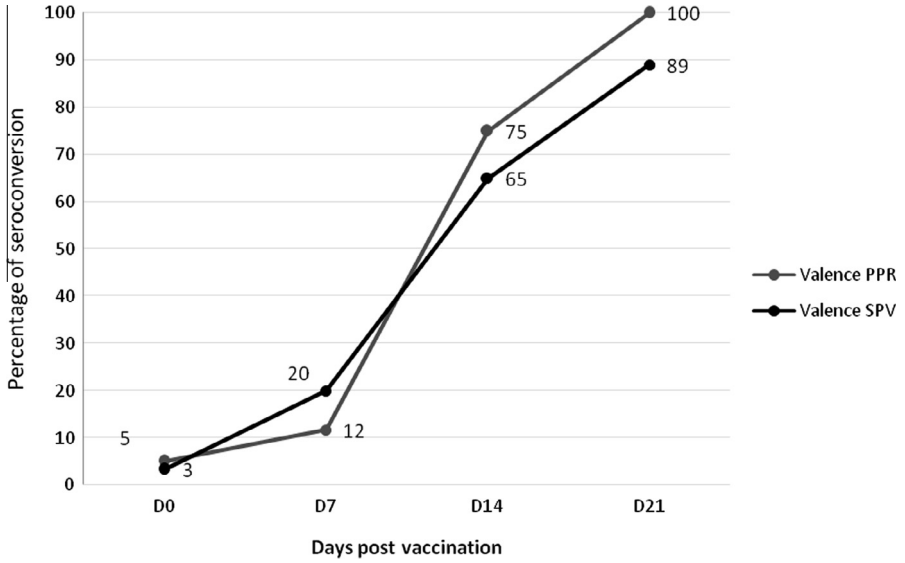

Fig. 5. Percentage of seroconversion for PPR and SPV of vaccinated sheep by the combined PPR/SGP vaccine in three farms.

observations, previous studies conducted by Ayalet et al. [14] and by Chaudhary et al. [4] showed that all the immunized animals resisted challenge with virulent SPV or PPRV, while control animals developed characteristic signs of disease.

Consequently, the combined vaccine was tested at large scale under normal field conditions on three flocks of sheep in the central region of Morocco as described in material and methods. Results were expressed by percentage of seropositive animals as tested by SN for PPR and SGP (Fig. 5).

In two farms, few animals were found positive for PPR due to a previous vaccination. The global serological response obtained by ELISA reaches almost 100% at D14 after vaccination with some differences between farms. When tested by SN, 75% of the animals were protected at D14 and 100% at D21 against PPR (Fig. 5). This difference between the two technics could be explained by high sensitivity of ELISA compared to SN. For SGP 65% of animals were protected at D14 to around 90% at D21 (Fig. 5). Consistent results were published by Hosamani et al. [5] using combined PPR and Goat Pox vaccine and by Sreenivasa et al. [2] using PPR vaccine only.

4. Conclusion

In conclusion the combined PPR/SGP vaccine confers a good protection against both PPR and Sheep Pox infections and diseases. At laboratory level solid protection was obtained with no viral multiplication in vaccinated and sssschallenged animals, results confirmed by serology monitoring after large scale vaccination under normal field conditions. The combined vaccine could be used for vaccination campaigns to protect small ruminants in one shot against two economically and medically important diseases. The combined vaccine could be an efficient tool in endemic countries with low infrastructure and extensive breeding management of small ruminant population. In addition, it could be interesting to use this combined vaccine to reduce vaccination costs which might be highly attractive for farmers in these countries.

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