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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.

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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

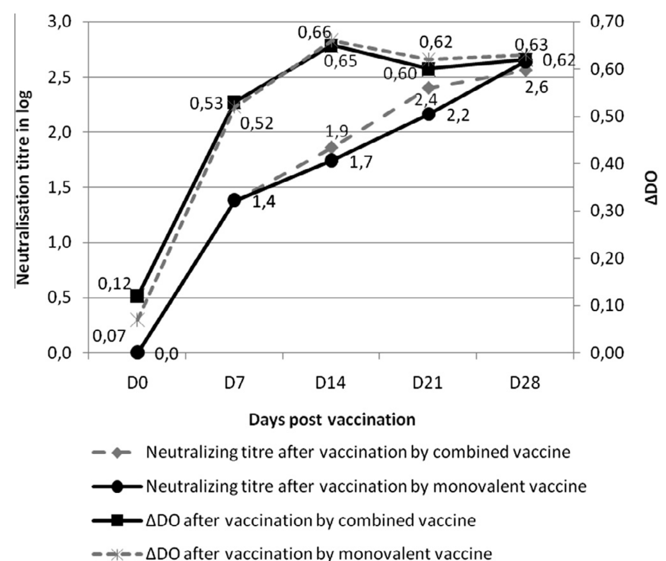

Fig. 1. Neutralizing PPR antibody response and PPR ELISA antibody response after vaccination of goats by combined and monovalent vaccines (group average).

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