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Protective efficacy of a single immunization with capripoxvirus-vectored recombinant peste des petits ruminants vaccines in presence of pre-existing immunity

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ABSTRACT

Sheeppox, goatpox and peste des petits ruminants (PPR) are highly contagious ruminant diseases widely distributed in Africa, the Middle East and Asia. Capripoxvirus (CPV)-vectored recombinant PPR vaccines (rCPV-PPR vaccines), which have been developed and shown to protect against both Capripox (CP) and PPR, would be critical tools in the control of these important diseases. In most parts of the world, these disease distributions overlap each other leaving concerns about the potential impact that pre-existing immunity against either disease may have on the protective efficacy of these bivalent rCPV-PPR vaccines. Currently, this question has not been indisputably addressed. Therefore, we undertook this study, under experimental conditions designed for the context of mass vaccination campaigns of small ruminants, using the two CPV recombinants (Kenya sheep-1 (KS-1) strain-based constructs) developed previously in our laboratory. Pre-existing immunity was first induced by immunization either with an attenuated CPV vaccine strain (KS-1) or the attenuated PPRV vaccine strain (Nigeria 75/1) and animals were thereafter inoculated once subcutaneously with a mixture of CPV recombinants expressing either the hemagglutinin (H) or the fusion (F) protein gene of PPRV (10^3 $TCID_{50}$/animal of each). Finally, these animals were challenged with a virulent CPV strain followed by a virulent PPRV strain 3 weeks later. Our study demonstrated full protection against CP for vaccinated animals with prior exposure to PPRV and a partial protection against PPR for vaccinated animals with prior exposure to CPV. The latter animals exhibited a mild clinical form of PPR and did not show any post-challenge anamnestic neutralizing antibody response against PPRV. The implications of these results are discussed herein and suggestions made for future research regarding the development of CPV-vectored vaccines.

1. Introduction

Peste des petits ruminants (PPR), an infectious and highly contagious disease of domestic and wild small ruminants, is caused by peste des petits ruminants virus (PPRV), which is classified in the genus *Morbillivirus*, family *Paramyxoviridae* [1]. PRRV shares structural and clinico-pathogenic characteristics with other members of the genus, among which include measles virus, canine distemper virus and rinderpest (RP) virus [1,2].

Fever, conjunctivitis, gastroenteritis, pneumonia and high mortality characterize the acute form of PPR, also known as stomatitis pneumoenteritis complex [3]. PPR may however manifest itself in

Abbreviations: CPE, cytopathic effects; CP, capripox; CPV, capripoxvirus Conventional; RT-PCR, cnRT-PCR; DIVA, differentiating infected from vaccinated animals; GTPV, goat poxvirus; H, hemagglutinin protein; IFAT, immunofluorescent antibody test; F, fusion protein; OIE, World Organization for Animal Health; rCPV-PPR vaccines, capripoxvirus-vectored recombinant PPR vaccines; $rCPVH_{RPV}$, CPV recombinant containing the RPV H gene; $rCPVH_{PPR}$, CPV recombinant containing the PPRV H gene; $rCPVH+F_{PPR}$, mixture of $rCPVH_{PPR}$ and $rCPVF_{PPR}$; $rCPVF_{RPV}$, CPV recombinant containing the RPV F gene; $rCPVF_{PPR}$, CPV recombinant containing the PPRV F gene; RP, Rinderpest; RPV, rinderspest virus; PPR, Peste des petits ruminants; PPRV, Peste des petits ruminants virus; VNA, virus neutralizing antibody; VACV, vaccinia virus.

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different clinical forms ranging from mild to severe, a situation bearing similarities to RP [4]. PPR is a notifiable disease for the World Organization for Animal Health (OIE). It is widespread and emerging in Africa, the Middle-East and Asia [5]. An effective, live-attenuated vaccine is currently in use in many countries, providing life-long immunity after a single injection [6]. However, this vaccine is thermolabile, a characteristic that might be a constraint in hot and remote areas with limited cold chain facilities. Also, it is not a marker vaccine that would allow for differentiating infected from vaccinated animals (DIVA)[6]. Therefore, a thermostable DIVA vaccine for the control of PPR would be valuable especially with the growing interest in PPR eradication after the successful eradication of RP in 2011.

Also widespread in Africa, Asia and the Middle East, sheeppox and goatpox are two infectious and contagious diseases, notifiable to OIE, showing an expanding distribution with large overlapping geographical areas with PPR [7]. Sheeppox virus (SPPV), goatpox virus (GTPV) and lumpy skin disease virus (LSDV) of cattle belong to the genus *Capripoxvirus*, family *Poxviridae* [7]. Several attenuated CPV vaccine strains have been used to control CP in many countries [7,8], including LSDV KS-1 strain which has been largely used [9]. Their thermostability, large transgene insert capacity and genetic stability made these attenuated CPV strains attractive vectors for the development of multivalent vaccines to control ruminant diseases.

Their relevance as vectors of choice has been confirmed through the outstanding protective efficacy of KS-1-vectored recombinant RP/PPR vaccines administered in a single subcutaneous (SC) dose in naïve animals [10–15]. With respect to the bivalent rCPV-PPR vaccines and their areas of potential use in countries where both PPR and CP co-exist, one issue deserving attention is the potential effect of pre-existing immunity against PPRV or against CPV on the vaccine protective efficacy against CP or PPR, respectively. Should this effect be significant, it would represent a hurdle for CPV-vectored vaccine development. This question has been addressed for other viral vectors and strategies have been developed accordingly to circumvent the interference of pre-existing immunity [16–18]. In contrast, these aspects have been scarcely addressed for CPV-vectored vaccines; few data are available today and no definitive conclusion can be drawn [12,13,19].

In the present study, using the KS-1-based recombinants expressing either the H or F gene of PPRV, we addressed successively the influence of PPRV pre-immunity on the protective efficacy against CP and the influence of CPV pre-immunity on the protective efficacy against PPR. These questions were addressed under experimental conditions meeting the requirements necessary for the implementation of vaccination campaigns on small ruminants in developing countries, and especially in the low-input smallholder husbandry systems.

2. Materials and methods

2.1. Cells and viruses

The ovine lamb testis cell line OA3.Ts (ATCC CRL-6546) and Vero cells (ATCC CCL-81) were used to propagate CPV and PPRV respectively. Madin Darby bovine kidney (MDBK) cells (ATCC CCL-22) were used in the immunofluorescent CPV antibody test (IFAT).

The virulent field strain goatpox virus (GTPV) Oman 84 was a kind gift from Dr E.S.M. Tuppurainen (Institute for Animal Health, Pirbright laboratory, United Kingdom).

All other viruses were obtained from the virus bank of CIRAD, Montpellier, France: attenuated CPV vaccine strain KS-1 (Kenyan 0240 strain) [9], attenuated PPRV vaccine strain Nigeria 75/1 (PPRV Nig.75/1) [6], CPV recombinants (KS-1-based) containing either the PPRV H gene ($rCPVH_{PPR}$) or PPRV F gene ($rCPVF_{PPR}$) [10,11] and the virulent field strain PPRV India 94.

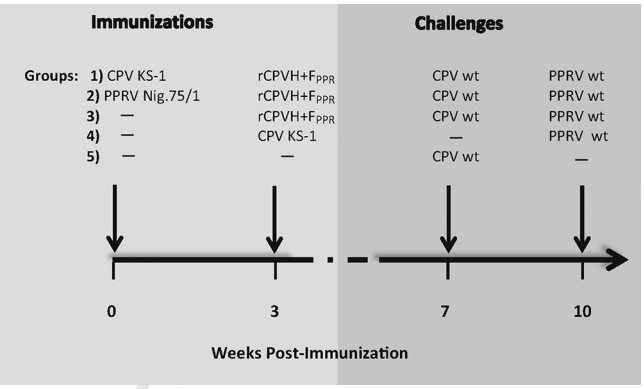

Fig. 1. Experimental design of immunizations and challenges. Groups 1 and 2 were first immunized SC with KS-1 (10^3 $TCID_{50}$/animal) or PPRV Nig.75/1 (10^3 $TCID_{50}$/animal) respectively. Three weeks later, groups 1–3 were inoculated SC with a mixture of $rCPVH_{PPR}$ and $rCPVF_{PPR}$ ($rCPVH+F_{PPR}$), 10^3 $TCID_{50}$/animal of each. At this time, group 4 (PPR control group) was inoculated with KS-1 (10^3 $TCID_{50}$/animal). Four weeks later, groups 1, 2, 3 and 5 (CP control group) were challenged SC with virulent field strain GTPV Oman 84 (CPV wt), 10^5 $TCID_{50}$/animal. Finally, 3 weeks later, groups 1–4 (PPR control group) were challenged SC with virulent field strain PPRV India 94 (PPRV wt), $10^{3.8}$ $TCID_{50}$/animal. The timing of immunizations and challenges are indicated by arrows.

2.2. Immunization and experimental challenge of goats

We selected 24 healthy male goats (Ethiopian breed, Western highlands), 12–18 months old, which tested negative for antibodies to PPRV by competitive ELISA (ID Screen PPR Competition; IDVET, France) and to CPV by indirect fluorescent antibody test (IFAT). Goats were ear-tagged, randomly split into five groups, each comprising either 5 animals (groups 1–4) or 4 animals (group 5). These groups were housed in separated rooms at the secure animal facilities of National Animal Health Diagnosis and Investigation Center (NAHDIC), Sebeta, Ethiopia. This research laboratory did not have an animal ethics committee at the time of the experimental work. Nevertheless, it had received the necessary authorization to conduct the study from the Ministry of Agriculture and Rural development of Ethiopia. In addition, all experiments were carried out according to the guidelines in the Guide to the Care and Use of Experimental Animals provided by the French Ministry of Agriculture.

The animals were immunized as shown in Fig. 1:

- A first round of immunization with groups 1 (G1) and 2 (G2), inoculated subcutaneously (SC) with KS-1 (10^3 50% tissue culture infective dose ($TCID_{50}$)/animal) or PPRV Nig.75/1 (10^3 $TCID_{50}$/animal) respectively.
- Three weeks later, a second round of immunization with groups 1–3 (G3) inoculated SC with a mixture of $rCPVH_{PPR}$ and $rCPVF_{PPR}$ ($rCPVH+F_{PPR}$), 10^3 $TCID_{50}$/animal of each. At this time, group 4 (PPR control group) was inoculated with KS-1 (10^3 $TCID_{50}$/animal) concomitantly with these immunizations.

Two consecutive challenges were thereafter performed (Fig. 1): groups 1, 2, 3 and 5 (G5, CP control group) were first inoculated SC with virulent field strain GTPV Oman 84 (10^5 $TCID_{50}$/animal). Then, 3 weeks later, groups 1–4 (G4, PPR control group) were inoculated SC with virulent field strain PPRV India 94 ($10^{3.8}$ $TCID_{50}$/animal).

Animals were examined daily for clinical signs of CP or PPR and the rectal temperatures were recorded. Blood, collected either for

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