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Original article

COMPARATIVE EFFICACY OF TWO HETEROLOGOUS CAPRIPOX VACCINES TO CONTROL LUMPY SKIN DISEASE IN CATTLE

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Summary

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This longitudinal study was performed for field trial of heterologous vaccine strains of lumpy skin disease (LSD) to provide details on the characteristics of induced immune responses by measurement of specific antibody and target cytokines – critical parameters in immune response that can be related to the durability of protection. The experimental calves were vaccinated with Gorgan-GPV and RM/65-SPV vaccines and humoral and cellular immunity were evaluated weekly. In each vaccinated groups, cross-neutralising antibody titers against LSD virus (LSDV) could be detected, and this rate in GGPV-vaccinated calves (GC) was higher than RSPV-vaccinated calves (RC) in all weeks of experiments. The stimulation index and IFN-γ and IL-4 production in response to homologous virus were higher than to the heterologous virus in all time points. The highest difference between them was observed in RVC, and a significant difference were only shown at 21-day post vaccination (DPV) ($P<0.001$). The results of this study indicated that GGPV-vaccine had a good immunogenic response due to induction of high antibody titre and higher lymphocyte proliferation and IFN-γ and IL-4 production. Therefore, it was considered suitable to control LSD.

Key words: *Capripox* virus, immune response, lumpy skin disease, vaccine

INTRODUCTION

The genus *Capripoxvirus* (CaPV) within the subfamily *Chordopoxvirinae*, family *Poxiviridae* comprises three closely related viruses, namely lumpy skin disease (LSD), sheeppox (SPV) and goatpox (GPV) viruses (Murphy *et al.*, 1999; OIE, 2014). This nomenclature is based on the animal species from which the virus was first isolated, respectively, cattle, sheep and goat. These viruses are the etiological

agents of economically important diseases which collectively constitute the most serious poxvirus diseases of production animals (Buller *et al.*, 2009). Due to the rapid spread and ability to cause irreparable economic losses in livestock industry, capripox viruses are listed in group A diseases of OIE (Carn, 1993; OIE, 2010). Also, these viruses share a major neutralising site, so that animals having been infected with one strain of CaPV family and surviving from it, will be resistant to infection with any other strain. Therefore, the use of vaccine strains of CaPV derived from sheep and goats would be useful to protect cattle against LSD (Davies *et al.*, 1981; Kitching *et al.*, 1987; Kitching, 2003).

Lumpy skin disease is a disease of cattle characterised by pyrexia, generalised skin and internal pox lesions, and generalised lymphadenopathy (Mercer *et al.*, 2007). Vaccination is considered the best suitable way to control LSD, accordingly several CaPV vaccine strains are used for the prevention and control of LSD.

The aim of this study was to evaluate the immunogenicity and efficacy of two live attenuated CaPV vaccines, and how these heterologous vaccines stimulated the immune response against LSD virus. Accordingly, adverse reactions, cell-mediated and humoral immune response of vaccinated calves were monitored throughout 5 weeks.

MATERIALS AND METHODS

Calves

A total of 48 susceptible calves approximately 4–6 months of age were selected from a dairy farm and were divided into: vaccinated calves (2 groups of 20 calves) as treatment groups and 8 unvaccinated calves as a control group.

Types of vaccines and vaccination programme

Live attenuated Romanian sheeppox vaccine (SPV) and Gorgan goatpox vaccine (GPV), were obtained from the Razi vaccine and serum research institute (RVSRI), Iran. One dose of these vaccines for goat and sheep contained $10^{5.2}$ $TCID_{50}$/mL of virus. A ten-fold dose of vaccines was prepared according to the manufacturer's instructions for emergency use against LSD in cattle (Varshovi *et al.*, 2009), Reconstituted vaccines were kept on ice and protected from direct sunlight and used within 1 h. Treated groups were vaccinated subcutaneously with 5 mL of the prepared vaccines, while the control group received only phosphate-buffered saline.

Sampling procedures

All vaccinated calves were daily examined for any increase of rectal temperature, appearance of adverse reaction and delayed-type hypersensitivity (DTH) reaction at the injection site for 5 weeks following vaccination.

Blood samples were collected weekly for antibody, proliferation and cytokine assay. Peripheral blood mononuclear cells (PBMCs) isolation was performed within 2–4 h after bleeding and the sera samples were transferred to cryovials and kept at –20 °C until use. All procedures were carried out under laminar air flow hood in order to avoid contamination.

Virus culture

The LSDV, GPV and SPV viruses were obtained from RVSRI. Virus cultivation was carried out according to the standard protocol of the department of animal viral vaccines at RVSRI following OIE manual (Varshovi *et al.*, 2009; OIE, 2010), and the titre of stock prepared virus was calculated by Reed & Muench (1938) method.

For purification and inactivation of viruses, after the removal cell debris, harvested virus was concentrated by ultracentrifugation in sucrose density gradient (36%), and after the titration, the virus inactivation was carried out according to OIE manual (OIE, 1992; Ryan *et al.*, 2009).

Lymphocyte proliferation assays

PBMCs were prepared as described (Kondo *et al.*, 1996; Norian *et al.*, 2015; Delirezh *et al.*, 2016). Viable and dead cells percent was determined by staining with trypan blue and adjusted to concentration of 2×10^6 cells/mL in RPMI complete medium (Katial *et al.*, 1998; Norian *et al.*, 2015). Proliferation assays were carried out by using MTT assay kit (cell proliferation kit, Roche, Germany). Briefly, 100 µL of PBMCs at a concentration of 2×10^5 cells/well were added to each well of 96-well tissue culture plate (JET BIOFIL, China), and stimulated with 100 µL of inactivated vaccine strains and LSD virus in separate wells for each vaccinated groups, at a multiplicity of infection (MOI) of 0.01 depending on the optimal stimulating capacity of virus (data not shown).

The PBMCs were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 for 4 days (Norian *et al.*, 2015). After the incubation, lymphocyte proliferation assay was carried out according to the kit instructions. The amount of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide formazan produced during the incubation was measured by an ELISA reader (Bio-Tek ELx800) at 550 nm. The results were calculated from the optical density and expressed as stimulation index (SI) (Delirezh *et al.*, 2016).

Cytokine assays

Supernatants from stimulated PBMCs were measured to determine the concentrations of IL-4 and IFN-γ cytokines. Cell-free supernatants were collected on the fourth day of culture and analysed for cytokines concentration (Norian *et al.*, 2015; Delirezh *et al.*, 2016). All samples were stored at −70 ^{o}C until analysis and concentrations of IFN-γ and IL-4 were measured by commercial ELISA kits (USCN Life Science Inc. China). Assays were performed according to the manufacturer's protocol and with reference standards provided by the manufacturers and the mean values used. The limits of detection (LOD) for the individual assays were as follow: IL-4, 6.2 pg/mL and IFN-γ, 12.8 pg/mL.

Antibody titration

Serum samples were collected on days 0 (pre-vaccination), and at DPV 7, 21 and 35. The neutralisation index (NI) was measured according to the standard protocol of RVSRI following the OIE manual (OIE, 2010).

Statistical analysis

Data were analysed by one-way ANOVA using general linear model procedures. A P-value of less than 0.05 was considered significant.

RESULTS

Adverse reactions in vaccinated calves after inoculation

Adverse reactions and rectal temperature of treated groups was recorded daily, and no clinical signs of LSD were detected in any of the calves in all time-point of the experiment.

Table 1. Stimulation (SI) and neutralisation (NI) index of vaccinated calves at days post vaccination. Data are presented as mean ± SEM

Days post vaccination	Gorgan-GPV		RM/65-SPV	
	NI	SI	NI	SI
0	0.20 ± 0.08	1.037 ± 0.058	0.20 ± 0.9	1.021 ± 0.051
7	0.90 ± 0.31	1.599 ± 0.087*	0.60 ± 0.24	1.423 ± 0.079
21	1.80 ± 0.59	1.749 ± 0.093	1.50 ± 0.53	1.592 ± 0.088
35	2.50 ± 0.77	1.356 ± 0.075*	2.00 ± 0.68	1.214 ± 0.064

* $P<0.05$ between GPV and SPV vaccinated calves.

The rectal temperature in the vaccinated groups was first observed 24 h after vaccination, and its duration was 48 to 72 h post vaccination with intermittent low-grade fever, and one week after vaccination the rectal temperature remained within the normal range until the end of the experiment.

The comparison of the measurements of rectal temperature and DTH reactions between the vaccines strains showed that cattle vaccinated with the GPV vaccine had longer fever duration and highest DTH reaction score when compared to the SPV vaccine and unvaccinated calves.

Lymphocyte proliferation response

Lymphocyte proliferation response was calculated as stimulation index (SI) and varied from week to week and calf to calf in each group. PBMCs of vaccinated groups showed higher proliferation than control (non-vaccinated) group. The mean SI of vaccinated groups in response to vaccine strains (homologous virus) increased at 7 DPV and peaked at 21 DPV, and this rate in GPV-vaccinated calves (GC) was higher than SPV-vaccinated calves (SC) in all weeks of experiment, and was significant at days 7 and 35 DPV ($P<0.05$) (Table 1). Also, the results showed that the SI values of homologous virus-stimulated PBMCs were higher than heterologous virus-stimulated PBMCs in all weeks. The highest difference between them was observed in SC and lowest difference were observed in GC at 21 DPV ($P< 0.001$).

Cytokine production of stimulated PBMCs

The production of IL-4 and IFN-γ cytokines in response to virus stimulated-PBMCs were significantly increased when compared to non-stimulated cultures (control group) at all-time points of experiment (data not shown). The mean values of cytokine production of each vaccinated groups demonstrated a wide range of values. The IFN-γ and IL-4 production of vaccinated groups increased at 7 DPV, peaked at 21 DPV and decreased thereafter. The highest difference of IFN-γ and IL-4 between the vaccinated groups was detected only at 21 DPV ($P<0.05$) (Fig. 1). Also, the production level of these cytokines in response to vaccine strains (homologous virus) were higher than to LSD virus (heterologous virus) in all time points. The highest difference between them was observed in SC group at 21 DPV ($P<0.001$).

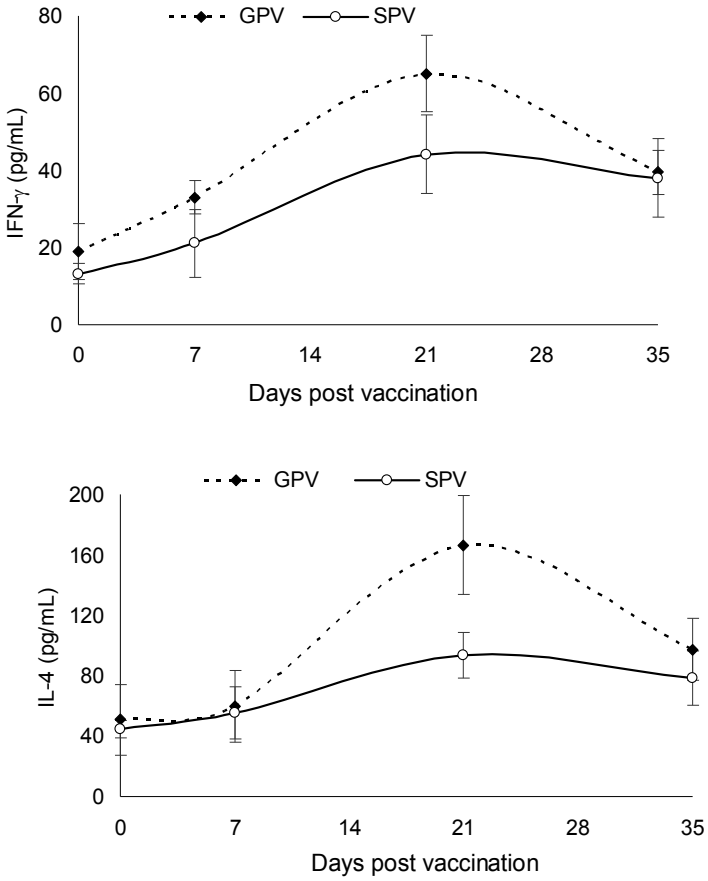

Fig. 1. Cytokine concentration in the supernatants of stimulated PBMCs in response to inactivated vaccine strains at days 0 (before vaccination) and days 7, 21 and 35 post vaccination.

Neutralising antibody titres following vaccination

The results of each treated groups showed that the neutralising antibody titre was detectable after 7 DPV and rose to peak at 21–35 DPV ($P<0.05$). According to the results presented on Fig. 2, although the mean neutralising antibody titre between vaccinated groups at all weeks of experiment was relatively similar without statistically significant difference, in GC it appeared slightly higher (Table 1). The serum neutralisation index of vaccinated groups in response to homologous virus was higher than to the heterologous virus in all time points, with significant difference between DPV 21 and 35 ($P<0.05$). This difference was higher in SC than in GC.

DISCUSSION

Vaccination is considered the suitable way to control lumpy skin disease (Carn *et al.*, 1995; Kitching, 2003; OIE, 2010). Vari-

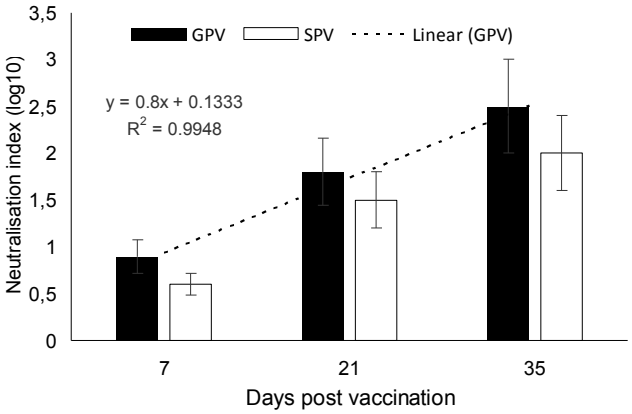

Fig. 2. Comparative evaluation of antibody titres of GPV and SPV-vaccinated calves in response to vaccine strain on follow-up days 7, 21 and 35.

ous types of *Capripoxvirus* vaccine strains are currently used in vaccination programmes, and despite regular LSD vaccination of cattle, vaccine failure and re-occurrence of the disease have been reported (Gari *et al.*, 2011). The aim of this study was to compare the *in vitro* immunological response and efficacy of two heterologous *Capripoxvirus* vaccine strains against LSD virus in dairy cattle population.

In the GC group, mild local reactions in the form of redness and mild swelling have appeared (OIE, 1992; Carn, 1993; Coetzer, 2004; Diallo *et al.*, 2007), but in SC group local reaction at the vaccination site was much attenuated as compared to GC group. In cases where the local reactions at the vaccination site were very slight or not observed, it may also indicate that the vaccine virus was over-attenuated and therefore failed to produce an effective cell-mediated immune response. Also, highest DTH reaction responses in vaccinated calves were observed in the GC group, which indicated higher immunogenicity of the vaccine, and the lowest DTH reaction responses reaction observed with the SC group, might be related to the poor immunogenicity of the vaccines, resulting from lower antigenic similarity to LSD virus or over-attenuation resulting in genetic alteration leading to a failure in the generation of protective immunity (Tuppurainen *et al.*, 2014; Gari *et al.*, 2015).

Humoral and cellular immune response are considered as the most important factors in the protective immune response against *Capripoxvirus* (Abdelwahab *et al.*, 2016). For this reason, lymphocyte proliferation of cultured PBMCs of vaccinate calves were analysed after re-stimulation with inactivated vaccine strains and as well as their cross-proliferation with inactivated LSDV. Cross-reactive lymphocyte proliferation in stimulated PBMCs is probably caused by recognition of conserved epitopes within or even between serotypes which have genetical relationship (Saiz *et al.*, 1992; Eble *et al.*, 2006). Stimulation index in response to homologous and heterologous virus in GC group was higher than in SC group in all time points, but the highest SI difference between homologous vs heterologous virus-stimulated PBMCs, was observed in RSPV group at 21 DPV

($P<0.05$). The close genetic similarity of the GPV virus to LSD virus might be the reason why the GPV vaccine showed better levels of stimulation than SPV vaccines against LSDV (Tulman *et al.*, 2002). In many previous studies, immune responses of capripoxviruses have been investigated, but the functional role of induced cytokines by vaccination and how they contribute to protective responses have not been clearly identified (Abdelwahab *et al.*, 2016; Khafagy *et al.*, 2016). Since cytokines are generally produced locally and at low levels, they might be difficult to be systemically detected; hence *in vitro* stimulation of cultured PBMCs with the virus can be helpful to investigate virus-induced cytokine production. The level difference of IL-4 and IFN-γ production between homologous virus-stimulated PBMCs and the heterologous virus-stimulated PBMCs in SC group was higher than in GC group at the same time. Also, a significant difference for IFN-γ and IL-4 production were shown only in SC vaccinated group at 21 DPV ($P<0.001$).

According to the results of this study, all calves in both vaccinated groups were able to produce antibodies in response to vaccine strains, and the neutralisation antibody titres of vaccinated calves were increased at each day of follow-up after DPV 7 and increased up to DPV 35. These findings are consistent with results obtained in other studies demonstrating that vaccinated calves produce neutralising antibodies before day 7 after vaccination (Barman *et al.*, 2010; Khafagy *et al.*, 2016). Vaccinated calves reached protective level (1.5) at DPV 21 that increased gradually to DPV 35. NI ≥1.5 is considered a protective level of neutralising antibody against *Capripox* viruses (OIE, 2010; Abdelwahab *et al.*, 2016; Khafagy *et al.*, 2016).

CONCLUSION

The results allowed deducing that GGPV vaccine gave good immunogenic potential, inducing a higher level of antibody titre and IL-4 and IFN-γ cytokines production in response to vaccine strain and LSDV, than the RSPV vaccine. On the other hand, the GGPV vaccine antigens due to the stronger stimulation of both Th1-like and a Th2-like cells, induced better humoral and cell mediated immune response than RSPV vaccine, so it was considered a suitable vaccine to control the disease in the field.

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