

## Original Article

# Evaluation of Humoral and Cell-mediated Immunity of Two Capripoxvirus Vaccine Strains against Lumpy Skin Disease Virus

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## Abstract

**Background and Aims:** Prevention of Lumpy skin disease (LSD) in cattle is heavily dependent on vaccination. Since the genetic structure of LSD virus has the close relatedness with other Capri-pox virus (CaPV). Therefore, the use of vaccine strains of CaPV derived from sheep and goat would be useful to protect cattle against LSD.

**Materials and Methods:** The susceptible calves of dairy farms were vaccinated with two available LSD vaccines. These vaccines were live attenuated sheep pox and goat pox vaccine strains. To evaluate vaccine-induced immune responses, whole blood and serum samples were collected up to 5 weeks post vaccination from both vaccinated and control groups.

**Results:** The findings showed that, lymphocyte proliferation index in response to recall antigen in goat pox vaccine was higher than sheep pox vaccine in all time-point of experiments, and this difference was significant in weeks 1 and 5 post vaccination ( $p < 0.05$ ). Although the levels of antibody production in both vaccinated groups was almost the similar, and there was no statistically significant difference, but in goat pox vaccine slightly higher than sheep pox vaccine. Also, the interferon gamma and IL-4 production in goat pox vaccine were higher than sheep pox vaccine in all-time point and statistically significant at week 3 post vaccination ( $p < 0.05$ ).

**Conclusions:** From this study we found that live attenuated goat pox vaccine induced high level of lymphocyte proliferation and interferon gamma and IL-4, so it considered good vaccine to control of Lumpy skin disease.

**Keywords:** LSD, Capri Pox Virus, Vaccine.

## Introduction

Lumpy skin disease (LSD) is a viral disease of cattle caused by Capri poxvirus genus of the Poxviridea family (1, 2). Lumpy skin disease is an infectious, eruptive, occasionally fatal disease of cattle and due to the rapid spread and ability to cause irreparable economic losses in livestock industry has been considered in "list A" of

disease by OIE (3, 4). The disease was first reported in 1929 in south of Africa in cattle and then spread in most of the central and northern of African countries (4-6), and recently through import of live cattle that carry the LSDV from endemic countries has been aggressively spreading in the Europe, India, south-west of Middle East and other countries (7). Several Capripoxvirus (CaPV) vaccine strains are used for the prevention and control of LSD. These vaccines are live attenuated CaPV strains including Neethling strain of LSDV, Kenyan sheep and goat pox virus (KSGPV), Yugoslavian strain of sheep pox virus (YSPV), Romanian strain of sheep pox virus (RSPV) and Gorgan strain of goat pox virus (GGPV) (8-10). According to many studies, it has been proven that CaPV strains

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share a major neutralizing site, So that animals are infected with one strain of CaPV family and survived from it, will be resistant to infection with any other strain. Therefore, the use of vaccine strains of CaPV derived from sheep and goat would be useful to protect cattle against LSD (8, 11, 12).

Recently, live attenuated goat pox virus and sheep pox virus strains are used as vaccines for the control of LSD (13). The live attenuated vaccines stimulate mainly the cell-mediated immunity and therefore a previously described hypersensitivity test was slightly modified and used to investigate the immunogenicity of the vaccines. In endemic regions vaccine failure is a severe impediment for the effective control of LSD. Although LSD vaccines are widely used in the face of outbreaks in countries, vaccine breakdown and re-infection of vaccinated animals have been reported (14, 15). Therefore, evaluation of immune response of the vaccines against LSDV in field trial is very important to assessment the status of the existing vaccine strains and to select the best vaccine strain that effectively protects cattle population against LSDV. Consequently, the immune response of calves was evaluated following emergency administration of live attenuated GPV and SPV vaccine formulated with Razi vaccine serum research institute of Iran.

The aim of this study was to evaluate the immunogenicity and efficacy of two live attenuated CaPV vaccines, and to what extent these vaccines stimulated the immune response against LSD virus. Evaluation of cell-mediated immune response was carried out by using lymphocyte proliferation assay and Interferon Gamma Bioassay (IFN- $\gamma$ ), while humoral immunity was evaluated by using antibody titration. Result was provided that goat pox vaccine gave good, lifelong protection which is dependent on stimulating humoral and cellular immunity.

## Methods

**Animal experiments.** Random sampling method was used to select the study calves from the dairy farms to vaccinate them with the vaccine strains. Forty-eight Holstein breed male calves of approximately 4-6 months of age were selected from two dairy farms (each farm 24 calves). All of the calves were daily examined by a veterinarian and were in good health during the study period.

**Vaccines.** Live attenuated capri pox vaccine strains were obtained from the veterinary organization. These vaccines include sheep pox virus (SPV) romanian strain and goat pox virus (GPV) gorgan strain produced by the Razi vaccine serum research institute (RVSRI) of Iran. According to the manufacturer's instructions 10-fold dose of the vaccines was prepared for vaccination program in two dairy farms (13). Reconstituted vaccines were kept on ice and protected from direct sunlight and used within 1 h until injection.

**Vaccination and experimental design.** The calves in each dairy farm divided into groups; treated group A were vaccinated with goat pox vaccine and treated group B were vaccinated with sheep pox vaccine according to the manufacturer's instructions, and the control groups received phosphate bufer saline (PBS) alone.

**Sampling.** Blood samples were collected weekly up to 5 week post vaccination from the tail vein in sterile conditions for antibody, proliferation and cytokine assay.

**Virus preparation.** Virus cultivation was carried out according to the standard protocol of the department of animal viral vaccines of RVSRI following OIE manual (4, 13), and the titer of stock prepared virus was calculated by Reed & munch method (16). For purification and inactivation of virus, after the cell debris removal, harvested virus was concentrated by ultrafiltration, and after titration, the virus inactivation was carried out by conventional method (17).

**Antibody titration.** Serum samples were collected on weekly for up to 5 week post vaccination, and the antibody titer was

measured according to the standard protocol of RVSRI institute following OIE manual (4).

**PBMCs Isolation.** Peripheral blood mononuclear cells (PBMCs) isolation was carried out from whole blood by density gradient centrifugation according to the standard protocol (20, 21). Briefly, the whole blood was mixed with an equal volume of PBS and overlaid onto 15 ml Histopaque (Sigma). The gradients were centrifuged for 30 min, 20-25°C at 1300 x g. Cells at the interface were aspirated and washed in PBS by centrifugation for 10 min at 670 x g. If after washing, red blood cell (RBC) contamination was evident; cells were incubated with 0.83% (w/v) ammonium chloride buffer for 5 minute before three further washes as described above. PBMCs were cultured in RPMI-1640, containing, non-essential amino acids, l-glutamine, sodium bicarbonate 2 g/L, HEPES 10  $\mu$ M and gentamycin 100  $\mu$ g/ml, supplemented with 10% fetal calve serum (FCS), in 96-well U-bottomed microtitre plates (Nunc, Napierville, IL). Viable and dead cells percentage was determined by staining with trypan blue and adjusted to concentration of  $2 \times 10^6$  cells / ml in RPMI complete medium(22, 23).

**Lymphocyte proliferation assays.** In vitro lymphocyte proliferation assay was performed in 96-well bottomed microtitre plates (jet biofil, china). PBMCs were plated at a concentration of  $2 \times 10^5$  cells/well in RPMI medium as described above, and stimulated with inactivated vaccine strain in separately wells for each vaccinated groups, at a MOI of 0.1 depended on the optimal stimulating capacity of virus (data not shown), and The cultured were incubated for 4 days at 37°C in a humified atmosphere containing 5% CO<sub>2</sub>. After the incubation time, lymphocyte proliferation assay was carried out according to the instructions Kit (cell proliferation kit, Roche, Germany).

MTT (Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved at 5 mg/ml in PBS and used essentially as previously described (20, 24). Briefly, 20  $\mu$ l of MTT solution were added to each well, and the microplates were further incubated at 37°C for 4 h. Supernatants

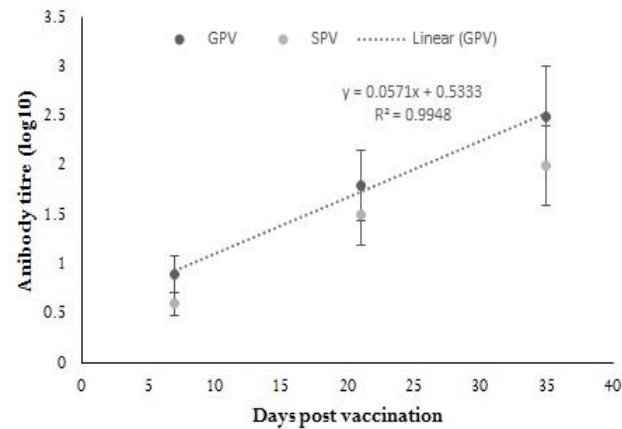
were then discarded and 200  $\mu$ l of acidified isopropanol (0.04 N HCl in isopropanol) were added to the cultures and mixed thoroughly to dissolve the dark blue crystals of formazan. Formazan quantification was performed using ELISA reader (Bio-Tek ELx800) at a test wavelength of 550 nm. The results were calculated on the optical density and expressed as a stimulation index (SI)(20, 24).

**Cytokine assays.** Concentrations of Th1-like cytokine: IFN- $\gamma$  and Th2- like cytokine IL-4 were determined in supernatant of cultured PBMCs. Cell-free supernatants of PBMCs cultured with virus or control suspension were collected 4<sup>th</sup> day after the start of the culture. My pervious experiments had shown that IFN- $\gamma$  and IL-4 concentrations in the supernatant were optimal at this time point (20, 22). All supernatant samples were stored at -70 °C until analysis and concentrations of IFN- $\gamma$  and IL-4 cytokines were measured using commercially available ELISA-kits (USCN Life Science Inc. China). Assays were performed according to the manufacturer's protocol and the optical dencity was measured by ELISA reader (Bio-Tek ELx800) at a test wavelength of 450 nm.

**Statistical analysis.** In this study, data were analyzed by the analysis of variance (one-way ANOVA) using general linear model procedures and descriptive statistics was used to quantify levels of antibody titres across each sampling days. A P-value of less than 0.05 was considered significant.

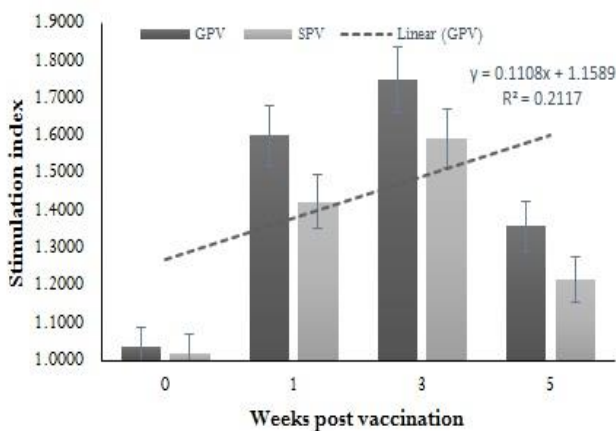
## Results

**Antibody titers.** Anti-LSD antibodies were detectable in serum of vaccinated groups. This was first detectable after 1 week post vaccination and the titre rose to peak at 3-5 weeks post vaccination, after which they were maintained for the duration of the experiment. The mean titre of vaccinated calves showed that the mean of the antibody titer between vaccinated groups at all weeks post vaccination was relatively similar and there was no statistically significant difference ( $p > 0.05$ ), though in goat pox group appeared slightly higher than sheep pox group (fig. 1).



**Fig. 1.** Antibody titer of vaccinated calves at days' post vaccination.

**Lymphocyte proliferation response.** PBMCs from animals receiving whole virus vaccine will respond to virus antigen in an in vitro re-stimulation assay, although it was found necessary to boost the vaccinated animals to aid detection of the antigen-specific proliferation (27, 28). PBMCs of vaccinated groups (A&B) did show higher proliferation response than control group (non-vaccinated). Lymphocyte proliferation response of vaccinated calves began to increase till reach to its peak at 3<sup>th</sup> week, then decreased until the end of the experiment. Stimulation index in goat pox group was higher than sheep pox group during all weeks of experiment, and this difference was significant at weeks 1 and 5 post vaccination ( $p < 0.05$ ) (fig. 2).



**Fig. 2.** Lymphocyte proliferation index of vaccinated calves at weeks post vaccination.

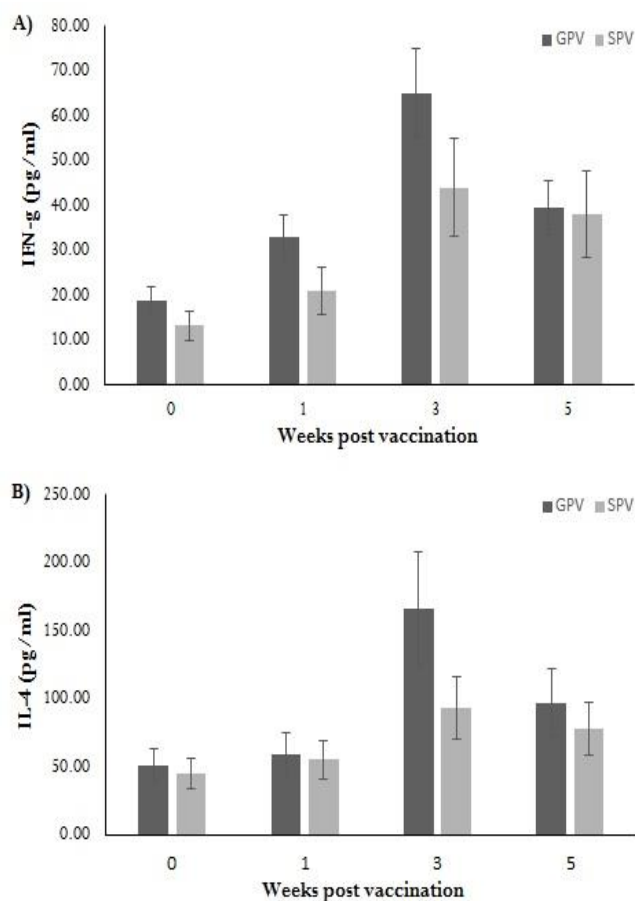
**Cytokine response.** We found IFN- $\gamma$  and IL-4 in variable levels in the supernatant of stimulated PBMCs in both vaccinated groups. The mean production of IL-4 and IFN- $\gamma$  cytokines in response to virus stimulated-PBMCs were significantly increased when compared with the control group at all-time points (data not shown). The mean values of IFN- $\gamma$  and IL-4 production of each vaccinated groups are shown in fig. 3 A&B, and demonstrated a wide range of values.

The IFN- $\gamma$  production of vaccinated groups began to increase in first day post vaccination till reach to its peak at 3<sup>th</sup> week, then decreased until the end of the experiment at 5<sup>th</sup> week post vaccination. In the both vaccinated groups a significant difference was showed between day 0 and weeks 1, 3 and 5 post vaccination ( $p < 0.05$ ), and also a significant difference was observed at 3<sup>th</sup> week post vaccination ( $p > 0.05$ ) (fig. 3A).

Also, the IL-4 production of vaccinated groups began to increase in first week post vaccination till reach to its peak at 3<sup>th</sup> week, then decreased until the end of the experiment at 5<sup>th</sup> week post vaccination. For both vaccinated groups, a significant difference of cytokine production was found between day 0 and week 3 post vaccination ( $p < 0.01$ ), and also a significant difference between the groups was only detected at 3<sup>th</sup> week post vaccination ( $p < 0.05$ ) (fig. 3B).

Between individual calves the most of cytokine production was varied and were seen at weeks 3-5 post vaccination, and occasionally a low-responder was detected. Fortunately, in each vaccinated groups non-responder was not observed.

According to the results presented in fig. 3, the production level of these cytokines in response to goat pox virus were higher than sheep pox virus in all time-point, and the highest difference in the level of IFN- $\gamma$  and IL-4 cytokines production between them was observed in goat pox group. Also, a significant difference was showed at 3<sup>th</sup> week post vaccination ( $p < 0.001$ ).



**Fig. 3.** Cytokines concentration in the supernatants of stimulated-PBMCs at weeks post vaccination.

## Discussion

Vaccination is widely prescribed as an effective control measure for LSD (4, 8, 9). However, little is known on the immunological response and immune dynamics, vaccine-cytokines response and how they contribute to a protective response against lumpy skin disease is available against this disease. To prevention and control of lumpy skin disease, several Capri poxvirus-vaccine strains are currently used, and despite of regular LSD vaccination of cattle, the cases of vaccine failure and re-occurrence of the disease have been reported (29). In the present study, the commercially available goat pox vaccine elicited a stronger immune response in the cattle than the sheep pox vaccine.

Due to the fact that such emergency vaccines are designed to provide protective immunity the present work sought to provide detail on

the characteristics of the induced immune response (22). Specific antibody can be related to the durability of protection, for which reason its induction is a critical parameter. An additional important event for the efficacy of emergency vaccination is the rapid induction of innate defences, as reported in terms of cytokine production and stimulation of leukocyte (23, 24).

Vaccination efficacy was also monitored by determining leukocyte responsiveness to in vitro re-stimulation (22, 23). Both lymphocyte proliferation and inducible cytokine activity were observed, with a clearly higher proficiency of PBMCs from the vaccinates compared to controls.

Cell-mediated immune response plays an important role against capripox virus beside humoral Immune response. Accordingly, cell-mediated responses of vaccinated calves were demonstrated after vaccination using the lymphocyte MTT proliferation assay in which responses are probably mainly attributable to T-helper cells (20, 33). Lymphocyte proliferation of cultured PBMCs of vaccinate calves were analyzed after re-stimulation with inactivated virus. Lymphocyte proliferation in stimulated PBMCs is probably caused by recognition of conserved epitopes within or even between serotypes which have genetically relationship (26, 33).

Lymphocyte MTT proliferation assay was chosen for estimation of cell mediated immune Response (7, 30). Cell mediated immune response in vaccinated calves in goat pox-vaccinated calves was higher than sheep pox-vaccinated calves in all time point of experiment. The result recorded previously by previous reports who reported the increase of lymphocyte activity by the 3<sup>th</sup> day post vaccination and reached its peak on the 10<sup>th</sup> day then decreased till the 30<sup>th</sup> day post vaccination (30).

Humoral Immune response depending mainly on the antibody titers in sera of vaccinated calves. The result of this study showed that all calves in both vaccinated groups were able to produce antibodies in response to vaccine strains, and the antibody titers of vaccinated calves were increased at each week of followed

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up after 1<sup>th</sup> week and increased up to 5<sup>th</sup> week. These findings are consistent with results obtained in other studies, they have shown that vaccinated calves produce neutralizing antibodies before first week after vaccination (36, 37).

In many previous studies, immune responses of Capri pox viruses have been investigated, but the functional role of induced cytokines by vaccination and how they contribute to protective responses have not been clearly identified. (37, 38). Since cytokines are generally produced locally and at low levels, they might be difficult to detect systematically; hence in vitro stimulation of cultured PBMCs with virus can be helpful to investigate virus-induced cytokine production. In this study after re-stimulation of calves vaccinated-PBMCs with vaccine strains, the production of IL-4 and IFN- $\gamma$  cytokines were observed at 1<sup>th</sup> week post vaccination, peaked at 3<sup>th</sup> week post vaccination and decreased in the weeks thereafter, and a significant difference between the groups was detected at 3<sup>th</sup> week post vaccination ( $p < 0.05$ ). The level of IL-4 and IFN- $\gamma$  production between goat pox virus-stimulated PBMC in compare to the sheep pox virus-stimulated PBMCs, in goat pox virus was higher than sheep pox virus at the same time. Also, a significant difference for IFN- $\gamma$  and IL-4 production were only showed at 3<sup>th</sup> week post vaccination ( $p < 0.001$ ). That result was found with Heba and Charles, who detected that experimentally infected calves produced serum IFN- $\gamma$ , IL-12 and other pro inflammatory cytokines, but not IFN $\alpha$ . Despite the lack of IFN- $\alpha$ , innate immunity via the IL-12 to IFN- $\gamma$  circuit possibly contributed to early protection against LSD, since neutralizing antibodies were detected after viremia had cleared. The present work demonstrates that the capripox virus vaccines for emergency vaccination against LSDV efficiently stimulates a humoral and cellular immune response.

## Conclusion

We concluded that goat pox vaccine was highly lymphocyte proliferation and interferon-

gamma and IL-4 cytokine level, highly immunogenic, inducing a higher level of antibody titer with prolongation of the duration of immunity, so it considered the best choice of vaccine to control the LSD disease in the field.

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## Conflict of interest

The authors declare they have no conflict of interest.

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