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Immune response characteristics of Capri pox virus vaccines following emergency vaccination of cattle against lumpy skin disease virus

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Keywords

Goat Pox Virus, Sheep Pox Virus, Lumpy skin disease, IL-4, IFN-γ

Abstract

In this research immune response characteristics of two available heterologous vaccines including Gorgan goat pox virus (GPV) and Romanian sheep pox virus (SPV) vaccines against lumpy skin disease have been examined, by using the monitoring of humoral and cell-mediated immune responses in vaccinated calves in the field. The evaluation of humoral immune response showed that the neutralizing antibody titers in both vaccinated groups started at day 7 post-vaccination, then reached to the protective level at day 21 post-vaccination and persisted till 35 day post-vaccination. The neutralizing antibody titers in GPV-vaccinated calves (GVC) the ratio was higher than SPV-vaccinated calves (RVC), and on days 21 and 35 post-vaccination were significantly different (p <0.05). Also, in vitro evaluation of cellular immune responses showed that the lymphocyte proliferation index and IFN-γ and IL-4 production levels in both vaccinated groups began to increase at day 7 post-vaccination until reached to its peak at day 21 post-vaccination and decreased in the period thereafter. So that, in GVC this ratio was higher than that in RVC and was significant at day 21 post-vaccination ($p < 0.05$). The findings show that the live attenuated GPV vaccine due to induction of high level of antibody titer and higher lymphocyte proliferation and IFN-γ and IL-4 production has a good immunogenic response, so it is considered a suitable vaccine to control lumpy skin disease.

Abbreviations

CaPV: Capri pox Virus
DPV: Day Post Vaccination
GGPV: Gorgan-Goat Pox Virus
GPV: Goat Pox Virus
GVC: GGPV-vaccinated calves
LSD: Lumpy Skin Disease
LSDV: Lumpy Skin Disease virus
RSPV: RM/65-Sheep Pox Virus
RVC: RSPV-vaccinated calves
SI: Stimulation Index
SNT: Serum Neutralization Test
SPV: Sheep Pox Virus
TCID50: 50% Tissue Culture Infective Dose

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Introduction

Goat pox virus (GPV), Sheep pox virus (SPV) and Lumpy skin disease virus (LSDV), are the members of Capri poxvirus genus of the Poxviridea family [1, 2], which can cause significant economic losses in goat, sheep and cattle, separately. They are the most important Capri- poxviruses of animals, listed in group A diseases of The World Organization for Animal Health (OIE) [3, 4]. Lumpy skin disease (LSD) is an endemic disease in Africa in cattle [4-6], and recently through import of live cattle that carries the LSDV from endemic countries has been aggressively spreading in the Europe, India, south-west of Middle East and other countries [7]. Several Capri poxvirus (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 (SPV) and Gorgan strain of goat pox virus (GPV) [8-10]. According to many studies, it has been proven that CaPV strains 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]. In Iran, two live attenuated strains of CaPV are used as vaccines for the control of LSD [13]. These are strains of GPV (Gorgan strain) and SPV (RM/65 strain) that are produced by Razi Vaccine and Serum Research Institute (RVSRI). So, after a recent outbreak of the LSD in west and northwest of Iran in 2014, an emergency vaccination program with heterologous existing vaccines including GPV and SPV was carried out in bovine population of the country. In many scientific studies have been reported, that inadequate protective immunity induced by LSD vaccines can cause the failure of vaccination and outbreak of disease in cattle population after exposure to LSD virus [14, 15]. Therefore, evaluation of immune response characteristics of vaccines against LSDV in field trial is very important to assess 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 by RVSRI.

The aim of this study was to evaluate the immunogenicity of two Iranian live attenuated CaPV vaccines, and how these heterologous vaccines stimulated the immune response against LSD virus. Accordingly, to evaluate humoral immunity, the antibody levels of vaccinated calves were monitored weekly to give an indication of the levels of protection expected. Also, to assess the cell-mediated immune response, the peripheral blood mononuclear cells (PBMCs) of vaccinated calves were re-stimulated in vitro by inactivated virus to identify lymphocyte proliferation and Th1-type (IFN-γ) and Th2-type (IL-4) cytokine response, to determine the relationship between cytokine levels and antibody response.

Results

Adverse reactions monitoring

Clinical examination of both RVC and GVC was daily performed, and no clinical signs of LSDV were detected in any of the calves in all time-point of the experiment. In GVC, mild local reactions in the form of redness and mild swelling was appeared, as in previous study has been shown [3, 16-18], but in RVC local reaction at the vaccination site was much lower than GVC. Rectal temperature value of all vaccinated calves was recorded daily and temperatures greater than 40°C were considered fever. The fever in the GVC was first observed in 24 h post-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 normal range until the end of the experiment.

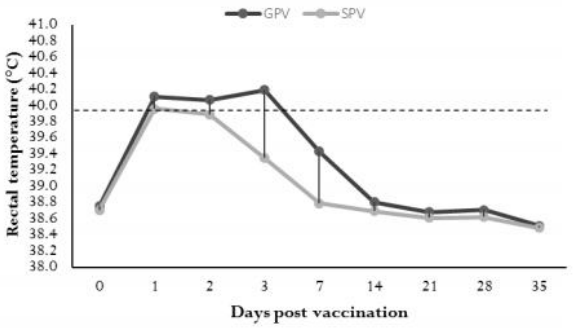

Figure 1
Daily rectal temperatures of vaccinated calves with live attenuated GPV and SPV vaccine. Mean temperatures for all calves during the 35 days post vaccination period are shown. The upper limit of the normal temperature range (40°C) is indicated by dashed lines..

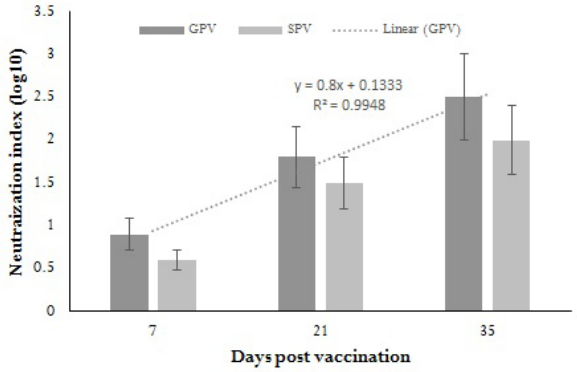

Figure 2
Comparative evaluation of serum neutralization antibody titers (mean ± SD) of GPV and SPV vaccinated calves on each day of the follow up period (up to day 35) in response to inactivated virus (vaccine strain).

Also, in RVC slight increase in fever was observed in 24 to 48 post-vaccination and was remained within normal range until the end of the experiment. In General, the fever in GVC was higher than RVC, and persisted for up to 3 days. In a few calves of GVC during the onset time of fever, ocular and nasal discharge was observed, whereas in RVC no clinical signs were observed (Figure 1).

Neutralizing antibody titers following vaccination

Neutralizing antibody titre of all calves were negative before vaccination, but after the vaccination, the neutralizing antibodies were detected in the serum of all cases. In each vaccinated group, the first detectable neutralizing antibody titer was after 7 days and rose to peak at 21-35 days post vaccination, and in comparison to day 0 a significant difference was observed at days 7, 21 and 35 ($p < 0.05$) (Figure 2). Although, the mean of the neutralizing antibody titer between GVC and RVC at days 7, 14 and 35 post vaccination was relatively similar and there was no statistically significant difference ($p > 0.05$), though in GVC appeared slightly higher.

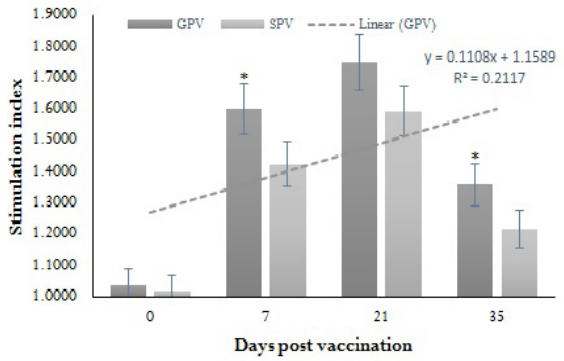

Figure 3
Lymphocyte proliferation index of vaccinated calves. PBMCs in response to inactivated GPV and SPV vaccines at days 0 (before vaccination), 7, 21 and 35 post vaccination. The calves vaccinated-PBMCs were cultured with inactivated virus for 4 days to determine the intensity and longevity of a detectable circulating memory T cell response. The x-axis shows the days after the vaccination, when the PBMCs were sampled and prepared.

Lymphocyte proliferation response

Analysis of the recall immune response is found necessary to boost the vaccinated calves to aid detection of the virus-specific proliferation [19, 20]. Therefore, a memory response of immune cells was provable when the PBMCs of vaccinated calves were re-stimulated in vitro with inactivated virus [21, 22]. Blood samples had been taken from the calves before (0 day) and after (up to 5 weeks) vaccination, and lymphocyte proliferation assay were performed. Stimulation index (SI) was calculated and varied from week to week and calve to calve in each group. PBMCs of vaccinated groups did show

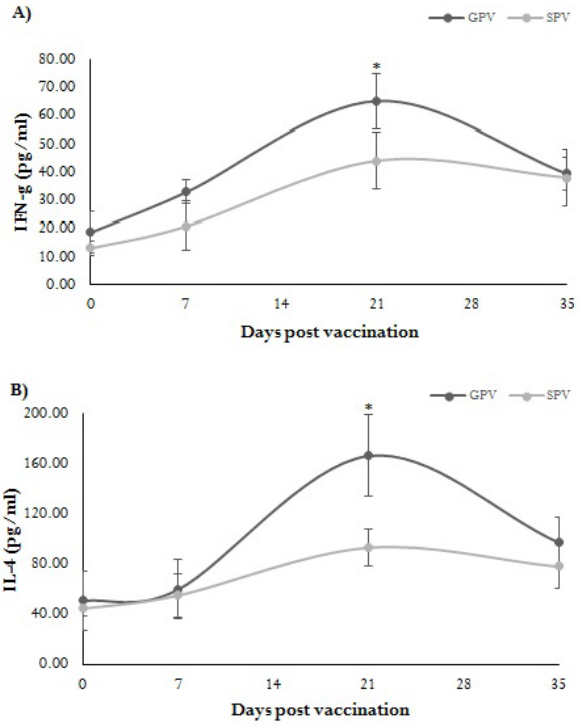

Figure 4
Cytokines concentration in the supernatants of stimulated PBMCs with inactivated vaccine strain at days 0 (before vaccination), 7, 21 and 35 post vaccination. Supernatants were taken from the PBMC cultures at day 4 of culture after treatment with inactivated virus. These were tested by ELISA for the presence of IFN-γ and IL-4 to determine the level of cytokine present (pg/ml). Again, the x-axis shows the time in days after the vaccination.

higher proliferation than control (non-vaccinated) group (data not shown). The mean SI of vaccinated groups in response to vaccine strains increased at 7 dpv and peaked at 21 dpv and decreased thereafter. A significant difference was found in both vaccinated groups between day 0 and days 7, 21 and 35 dpv ($p < 0.05$). Also, SI in GVC was higher than RVC in all weeks of experiment, and this difference was significant at days 7 and 35 ($p < 0.05$) (Figure 3).

Cytokine production of stimulated PBMCs

Supernatants from the stimulated PBMCs were analyzed for the presence of cytokine proteins by ELISA technique. The Th1-like cytokine, IFN-γ, and the Th2-like cytokine, IL-4, was found in variable levels in the supernatant of virus-stimulated PBMCs in both GVC and RVC, and were significantly increased when compared with the unstimulated cultures (control group) at all-time points (data not shown).

The median values of IFN-γ and IL-4 production of each vaccinated groups demonstrated a wide range of values, and increased at 7 dpv, peaked at 21 dpv and decreased in the period thereafter. In the GVC a significant difference was found between day 0 (19 ± 7.29 pg/ml) and days 7 (33.02 ± 4.24 pg/ml), 21 (65.06 ± 9.81 pg/ml) and 35 (39.50 ± 5.75 pg/ml) dpv for IFN-γ ($p < 0.05$), and between day 0 (50.77 ± 23.59 pg/ml) and days 21 (166.52 ± 32.58 pg/ml) dpv for IL-4 ($p < 0.01$) (Figure 4A).

Also, in RVC this difference only was observed between day 0 (13.23 ± 2.63 pg/ml) and days 21 (44.07 ± 10.15 pg/ml) and 35 (38.03 ± 10.22 pg/ml) dpv for IFN-γ ($p <0.05$), and between day 0 (44.78 ± 5.87 pg/ml) and days 21 (93.29 ± 15.04 pg/ml) dpv for IL-4 ($p < 0.01$) (Figure 4B).

In general, the IFN-γ and IL-4 production in GVC was higher than RVC at all time points of the experiment, and the significant difference between the groups was only detected at 21 dpv ($p < 0.05$).

Discussion

In this research we sought to provide detail on the immune response characteristics of the GGPV and RSPV vaccines by measurement of specific antibody and target cytokines, because they are critical parameters in immune response and can be related to the durability of protection.

Rectal temperature and local reactions in the form of redness and mild swelling at the vaccination site in GVC was higher than RVC, however, there was no statistically significant difference ($p > 0.05$) [3, 16-18]. The recorded clinical signs were also in agreement with Diallo and Viljoen (2007), who stated that the clinical signs caused by different Capri pox viruses are very variable, depending not on individual host susceptibility, but also on the virus strain. In cases where the local reactions at the vaccination site were very low or not observed, may also indicate that the vaccine virus was over-attenuated and therefore failed to produce an effective cell-mediated immune response [10].

The result of this study showed that all calves in both vaccinated groups were able to produce antibodies in response to vaccine strains, indicating that the specific immune defenses had been efficiently induced [23, 24]. The neutralization antibody titers of vaccinated calves were increased at each day of follow up period after day 7 and increased up to day 35. These finding are consistent with results obtained in other studies, indicating that vaccinated calves produce neutralizing antibodies before day 7 after vaccination [25, 26]. Protective level (1.5) at day 21 post vaccination increased gradually to day 35. Protective level of neutralizing antibody against CaPV were considered NI ≥1.5 [4, 26, 27].

Based on previous studies, it has been proven that cell mediated immune response plays an important role against CaPV beside humoral immunity [28, 29]. Accordingly, cell-mediated immune responses were demonstrated using the lymphocyte MTT proliferation assay in which responses are probably mainly attributable to T-helper cells [30, 31], and caused by recognition of conserved epitopes within or even between serotypes having genetic relationship [21, 30]. Stimulation index of calves vaccinated-PBMCs in GVC was higher than that in RVC in all time points of the experiment, indicating high stimulatory effects of the GPV vaccine. These results were in agreement with other studies, who reported the increase of lymphocyte activity at day 3 post vaccination and reached its peak on day 10 dpv, then decreased till day 30 post vaccination [32, 33].

In many previous studies, immune responses of CaPV have been investigated, but the functional role of induced cytokines by vaccination and how they contribute to protective responses have not been clearly identified [26, 27]. Since cytokines are

generally produced locally at low levels, they might be difficult to detect systemically; hence in vitro stimulation of cultured PBMCs with virus can be helpful to investigate virus-induced cytokine production. IFN-γ and IL-4 are the most important cytokines in the host defense against infection by viral and microbial pathogens and their activities can directly inhibit the viruses.

In this study after re-stimulation of calves vaccinated-PBMCs with vaccine strains, the production of IL-4 and IFN-γ cytokines were observed and a significant difference between the groups was detected, so that, this increase in GVC was higher than RVC and was significant at 21 dpv ($p < 0.05$). From the above results it can be deduced that GGPV vaccine provides good immunogenicity, inducing a higher level of IL-4 and IFN-γ than RSPV vaccine. These results were in agreement with other studies, who detected that experimentally infected calves produced serum IFN-γ, IL-4, IL-12 and other pro inflammatory cytokines [34].

Conclusion

In this study experimental challenge post vaccination was not performed to evaluate the efficacy of the induced cellular and humoral response in protecting the calves against LSDV invasion. However, there is an argument that protective level of neutralizing antibody against LSDV challenge is NI ≥1.5. Based on this study we conclude that GGPV and RSPV heterologous vaccines against LSDV induced humoral and cell mediated immune response, with the induction of both a Th1-like and a Th2-like activity. GGPV vaccine due to inducing high level of antibody titer and IL-4 production level, and also higher lymphocyte proliferation and IFN-γ production level against LSD virus is considered a suitable vaccine to control the disease in the field.

Materials and methods

Animal experiments

We used 48 Holstein breed male calves with approximately 4-6 months of age from two dairy farms (each farm 24 calves). Random sampling method (RSM) was used to select the study calves from each farm and to vaccinate with the vaccine strains. The calves on each farm were divided into two groups; vaccinated calves (20 calves) as a treated group and unvaccinated calves (4 calves) as a vaccine control group.

Types of Vaccine and Vaccination study

Gorgan-goat pox virus (GGPV) and Romanian-sheep pox virus (RSPV) which produced by the Razi vaccine serum research institute (RVSRI) of Iran were used. These vaccines were live attenuated, lyophilized and one dose of each vaccine for goat and sheep contained 105.2 TCID50/ml of virus. Ten-fold dose of vaccines was prepared according to the manufacturer's instructions for emergency use against LSD in cattle [13]. In the vaccination study, in order to confirm that the calves were free from maternal antibody against lumpy skin disease virus, serum neutralization test (SNT) were used. Treated groups in each farm were vaccinated with live attenuated GGPV-and RSPV-vaccines, respectively, and the control groups received PBS alone. These calves were received 5 ml volume of 10-fold dose of vaccine subcutaneously (SC) according to the manufacturer's instructions. All immunized calves were daily examined for any increase of rectal temperature and appearance of adverse reactions for 35 days post vaccination.

Virus preparation

The viruses (vaccine strains) was obtained from RVSRI and was used to make a master stock of the virus, and also, to eliminate a potential source of variability, a single batch of virus was used. SPV and GPV virus cultivation was carried out according to the standard protocol of the Department of Animal Viral Vaccines of RVSRI following OIE manual [4, 13]. Briefly, the monolayer cell lines (LK) were prepared in test tube by using DMEM and Hanks media that contained 5-10% fetal calf serum (FCS). Ten-fold dilution of viruses (10^{-1}- 10^{-5}) was prepared in Hanks solution. Then, 0.1 ml of each dilution was inoculated into prepared cultures tubes. The tubes were incubated at 37°C and examined for the presence of CPE till 10 days post inoculation and the titer of stock prepared virus, calculated by Reed & munch method [35]. 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 [22].

Antibody titration

The serum neutralization test is the gold standard to detect antibodies against CaPV [4, 36, 37]. Serum Samples were collected on days 0 (pre-vaccination), 7, 21 and 35 post-vaccination (dpv) and the neutralizing index (NI) was measured in a micro-neutralization assay, according to the standard protocol of RVSRI institute following OIE manual [4].

PBMCs Isolation

Whole blood was taken weekly from the jugular vein of calves and were collected in sterile heparin tubes. Peripheral blood mononuclear cells (PBMCs) were isolated within 4-8 h by density gradient centrifugation through Ficoll-Hypaque solution (Histopaque-1077), according to standard protocol [31, 38]. Viable and dead cells percent was determined by staining with trypan blue (Sigma-Aldrich, Germany) and adjusted to concentration of 2×10^6 viable cells /ml in RPMI complete medium [39, 40].

Lymphocyte proliferation assays

To evaluate the extent of the cell-mediated immune response, vaccine strains-and LSDV-induced in vitro lymphocyte proliferation response for each vaccinated and control

groups up to 5 weeks after vaccination was performed by using MTT assay. Briefly, 100 µl of PBMCs at a concentration of 2×10^5 cells/well in triplicate were added to each well of 96-well tissue culture plate (Jet Biofil, China), and stimulated with 100 µl of inactivated virus in separate wells for each vaccinated groups, at a MOI of 0.1 depended on the optimal stimulating capacity of virus (data not shown). One well of PBMC from each subject was stimulated with 2.5 µg/mL Phytohemagglutinin mitogen (PHA) (Sigma, St Louis, MO) as a positive control. The PBMCs were cultured at 37°C in a humidified atmosphere containing 5% CO2 for 4 days (96 h). After the incubation time, lymphocyte proliferation assay was carried out according to the instructions of the Kit (cell proliferation kit, Roche, Germany). The amount of MTT formazan produced during the incubation was measured by an ELISA reader (Bio-Tek ELx800) at a test wavelength of 550 nm and a reference wavelength of 630 nm. The results were calculated on the optical density and expressed as a stimulation index (SI)[31, 41], which was calculated as follows:

SI= (mean OD550 of stimulated PBMCs) -(mean OD550 of blank)/ (mean OD550 of unstimulated PBMCs).

Cytokine assays

Functional analysis of the effector cells generated upon activation of PBMCs with vaccine strains should contribute to the elucidation of the basic mechanism responsible for the stimulation of the immune system [21, 29]. For this purpose, cytokine production analyses focused on the secretion of Th1-like cytokine, IFN-γ, and Th2- like cytokine, IL-4, because they play an important role in humoral and cellular immune response against infectious agents. Concentrations of IL-4 and IFN-γ cytokines in supernatants of vaccine strain-stimulated PBMCs were measured for each vaccinated group. Cell-free supernatants were collected on the fourth day of culture and analyzed for cytokines concentration. My pervious experiments had shown that IFN-γ and IL-4 concentrations in the supernatant were optimal at this time point [31, 39]. All samples were stored at -70°C until analysis and concentrations of IFN-γ and IL-4 were measured by using commercially available 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. In each assay, a control recombinant cytokine was diluted over the recommended detection range from which a standard curve was generated, and cytokine concentrations in each sample were calculated using this standard curve.

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 SNT antibody titres across each sampling day. A P-value of less than 0.05 was considered significant.

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Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Conceived and designed the experiments: N.A.A., H.R.V. Performed the experiments: R.N., H.R.V. Analyzed the data: R.N., H.R.V. Research space and equipment: R.N., H.R.V. Contributed reagents/materials/analysis tools: R.N., H.R.V.

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