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 Capripoxvirus genus of the Poxviridea family (1, 2). Lumpy skin disease is an infectious, eruptive, accationally 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 extend
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-γ), 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 (RVRSR)
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
RVRSRI 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 RVSR1 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 µM and gentamycin 100 µg/ml, supplemented with 10% fetal calf 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 × 10^6 cells / ml in RPMI complete medium.

**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 × 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% CO2. 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 µl 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 µl 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-γ 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 4th day after the start of the culture. My pervious experiments had shown that IFN-γ 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-γ 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).
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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 3th 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).

Cytokine response. We found IFN-γ and IL-4 in variable levels in the supernatant of stimulated PBMCs in both vaccinated groups. The mean production of IL-4 and IFN-γ 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-γ and IL-4 production of each vaccinated groups are shown in fig. 3 A&B, and demonstrated a wide range of values.

The IFN-γ production of vaccinated groups began to increase in first day post vaccination till reach to its peak at 3th week, then decreased until the end of the experiment at 5th 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 3th 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 3th week, then decreased until the end of the experiment at 5th 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 3th 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-γ and IL-4 cytokines production between them was observed in goat pox group. Also, a significant difference was showed at 3th week post vaccination (p< 0.001).
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 defenses, 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 vaccinated 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 3th day post vaccination and reached its peak on the 10th day then decreased till the 30th 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 1th week and increased up to 5th week. These finding 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 vaccines strains, the production of IL-4 and IFN-γ cytokines were observed at 1th week post vaccination, peaked at 3th week post vaccination and decreased in the weeks thereafter, and a significant difference between the groups was detected at 3th week post vaccination (p<0.05). The level of IL-4 and IFN-γ 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-γ and IL-4 production were only showed at 3th week post vaccination (p<0.001). That result was found with Heba and Charles, who detected that experimentally infected calves produced serum IFN-γ, IL-12 and other pro inflammatory cytokines, but not IFNα. Despite the lack of IFN-α, innate immunity via the IL-12 to IFN-γ 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.

**References**

1. OIE. Manual of Diagnostic tests and vaccines for terrestrial animals. paris,World Organization for Animal Health, 2004; pp: 1-17.
2. Murphy F, Gibbs E, Horzinek M, Studdert M. Veterinary Virology. 3rd edition. USA, Academic Press, 1999; pp: 277-291.
3. Carn VM. Control of capripoxvirus infections. Vaccine. 1993;11(13):1275-1279.
4. OIE. Lumpy skin disease; Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. paris, World Organization for Animal Health, 2010; pp: 1-13.
5. Kitching RP. Vaccines for lumpy skin disease, sheep pox and goat pox. Dev Biol (Basel). 2003;114:161-167.
6. Carn VM, Kitching RP. The clinical response of cattle experimentally infected with lumpy skin disease (Neethling) virus. Arch Virol. 1995;140(3):503-513.
7. Gari G, Abie G, Gizaw D, Wubete A, Kidane M, Asgedom H, et al. Evaluation of the safety, immunogenicity and efficacy of three capripoxvirus vaccine strains against lumpy skin disease virus. Vaccine. 2015;33(28):3256-3261.
8. Kitching RP, Hammond JM, Taylor WP. A single vaccine for the control of capripox infection in sheep and goats. Res Vet Sci. 1987;42(1):53-60.
9. Davies FG, Otema C. Relationships of capripox viruses found in Kenya with two Middle Eastern...
strains and some orthopox viruses. Res Vet Sci. 1981;31(2):253-255.
10. Varshovi HR, Keyvanfar H, Aghaiypour K, Pourbakshh SA, Shoosharti AH, Aghaebrahirmin M. Capripoxvirus identification by PCR based on P32 gene. Archives of Razi Institute. 2009;64(No.1):19-25.
11. Tuppurainen ES, Oura CA. Review: lumpy skin disease: an emerging threat to Europe, the Middle East and Asia. Transbound Emerg Dis. 2012;59(1):40-48.
12. Brenner J, Bellaiche M, Gross E, Elad D, Oved Z, Haimovitz M, et al. Appearance of skin lesions in cattle populations vaccinated against lumpy skin disease: statutory challenge. Vaccine. 2009;27(10):1500-1503.
13. Reed LJ, Muench H. A Simple Method Of Estimating Fifty Per Cent Endpoints. American Journal of Epidemiology. 1938;27(3):493-497.
14. Ryan JE, Dhiman N, Osvyannikova IG, Vierkant RA, Pankratz VS, Poland GA. Response surface methodology to determine optimal cytokine responses in human peripheral blood mononuclear cells after smallpox vaccination. J Immunol Methods. 2009;341(1-2):97-105.
15. Babiuk S, Bowden TR, Boyle DB, Wallace DB, Kitching RP. Capripoxviruses: An Emerging Worldwide Threat to Sheep, Goats and Cattle. Transbound Emerg Dis. 2008;55(7):263-272.
16. Gari G, Biteau-Coroller F, LeGoff C, Caufour P, Roger F. Evaluation of indirect fluorescent antibody test (IFAT) for the diagnosis and screening of lumpy skin disease using Bayesian method. Vet Microbiol. 2008;129(3-4):269-280.
17. Delirezh N, Norian R, Azadmehr A. Changes in some pro-and anti-inflammatory cytokines produced by bovine peripheral blood mononuclear cells following foot and mouth disease vaccination. Archives of Razi Institute. 2016;71(3):199-207.
18. Kondo T, Sugiu T, Kamada M, Imagawa H. Colorimetric Assay of Equine Peripheral Lymphocyte Blastogenesis Using MTT. Journal of Equine Science. 1996;7(3):63-66.
19. Norian R, Delirezh N, Azadmehr A. Evaluation of proliferation and cytokines production by mitogen-stimulated bovine peripheral blood mononuclear cells. Veterinary Research Forum. 2015;6(4):265-271.
20. Katial RK, Sachanandani D, Pinney C, Lieberman MM. Cytokine production in cell culture by peripheral blood mononuclear cells from immunocompetent hosts. Clin Diagn Lab Immunol. 1998;5(1):78-81.
21. Norian R, Delirezh N, Azadmehr A. Evaluation of proliferation and cytokines production by mitogen-stimulated bovine peripheral blood mononuclear cells. Vet Res Forum. 2015;6(4):265-271.
22. Barnard AL, Arriens A, Cox S, Barnett P, Kristensen B, Summerfield A, et al. Immune response characteristics following emergency vaccination of pigs against foot-and-mouth disease. Vaccine. 2005;23(8):1037-1047.
23. Eble PL, de Bruin MG, Bouma A, van Hemert-Kluitenberg F, Dekker A. Comparison of immune responses after intra-typic heterologous and homologous vaccination against foot-and-mouth disease virus infection in pigs. Vaccine. 2006;24(9):1274-1281.
24. Gari G, Bonnet P, Roger F, Waret-Szkuta A. Epidemiological aspects and financial impact of lumpy skin disease in Ethiopia. Prev Vet Med. 2011;102(4):274-283.
25. Coetzer JAW. Lumpy skin disease; Infectious Diseases of Livestock. Cape Town, Oxford University Press Southern Africa,2004; 2:1268-1276.
26. OIE. Manual of recommended diagnostic techniques and requirements for biological products. Rue de Prony, World Organization for Animal Health,1992; pp.1-5.
27. Diallo A, Viljoen GI. Genus capripoxvirus. In: Andrew A, Mercer, Axel Schmidt, Olaf Weber: Poxviruses. 1th edition. Springer. 2007; pp.167-181.
28. Tulman ER, Afonso CL, Lu Z, Zsak L, Sur JH, Sandybaev NT, et al. The Genomes of Sheeppox and Goaptox Viruses. J Virol. 2002;76(12):6054-6061.
29. Saiz JC, Rodriguez A, Gonzalez M, Alonso F, Sobrino F. Heterotypic lymphoproliferative response in pigs vaccinated with foot-and-mouth disease virus. Involvement of isolated capsid proteins. J Gen Virol. 1992;73 (Pt 10):2601-2607.
30. Heba A, Khafagy MGA, Abdelmoneim M, Mustafa, Mohamed A, Saad, AA. Preparation and field evaluation of live attenuated sheep pox vaccine for protection of calves against lumpy skin disease. Benha Veterinary Medical Journal. 2016;31(2):1-7.
31. Mohamed G, Abdelwahab HAK, Abdelmoneim M, Moustafa, Mohamed A, Saad. Evaluation of Humoral and Cell-mediated Immunity of Lumpy Skin Disease Vaccine Prepared from Local strainin calves and Its Related to Maternal Immunity. Journal of American Science. 2016;21(10).