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SHORT COMMUNICATION

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# The effect of Iranian capripoxvirus vaccine strains on neutralizing antibody titer in cattle

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#### ABSTRACT

Lumpy skin disease (LSD) virus, Goat-poxvirus (GPV), and Sheep-poxvirus (SPV) are members of genus capripoxvirus (CaPV) and have close genetic similarity. The use of CaPV-vaccine strains would be useful to protect the cattle against LSD. This study aimed to compare the neutralizing antibody titer of Iranian heterologous sheep pox and goat pox vaccines against LSD in cattle. A total of 100 calves was vaccinated with Gorgan-GPV and Ramyar-SPV vaccines on separate farms. Neutralizing antibody titer and side effects of vaccines were evaluated at days 14, 28, 45, 90, and 180 post-vaccination. The mean of rectal temperature in SPV was higher than GPV and persisted for up to 3 days. Also during the onset time of fever, ocular and nasal discharge were observed, whereas in the GPV and control group no clinical signs were observed. In each vaccinated group, the first detectable antibody titer was after 14 days and rose to peak at 28-45 days post-vaccination, then it decreased in the following days. Although, the mean of the neutralizing index (NI) titer between GPV and SPV was relatively similar and there was no statistically significant difference (p >0.05) at all days of the experiment, but in GPV the titer appeared slightly higher than SPV and reached to protective level (NI  $\geq$  1.5) on day 45 post-vaccination. There was a high antibody titer (Log10<sup>1.07</sup>) in the day 180 post-vaccination. The results showed that GPV vaccine because of the induction of the protective level of antibody titer, and persisting within a long period for up to 180 day post-vaccination, has a good immunogenic response, so is considered a suitable vaccine to control LSD.

#### Keywords

sheep pox virus, goat pox virus, capripox vaccine,

Neutralizing antibody

Abbreviations

*CaPV: Capripoxvirus* DPV: Day's post vaccination GGPV: Gorgan-goat pox virus GPV: Goat pox virus GVC: GGPV-vaccinated calves LSD: Lumpy skin disease

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NI: Neutralizing index OIE: The world organization for animal health RSPV: Ramyar-sheep pox virus RVC: RSPV-vaccinated calves RVSRI: Razi vaccine and serum research institute SNT: Serum neutralization test SPV: Sheep pox virus

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umpy skin disease (LSD) is an infectious disease of cattle caused by a double-stranded DNA virus of the capripoxvirus genus of the Poxviridea family [1, 2]. Lumpy skin disease due to the rapid spread and economic importance in cattle has been considered in "list A" of bovine diseases by OIE [3, 4]. Several capripoxvirus (CaPV) vaccine strains are used for the prevention and control of LSD [5-7]. 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 strains. Therefore, the use of vaccine strains of CaPV derived from sheep and goats would be useful to protect cattle against LSD [5, 8-10]. In Iran, two live attenuated strains of CaPV are used as vaccines for the control of LSD [11]. These are strains of goat and sheep pox virus that are produced by the Razi Vaccine and Serum Research Institute (RVSRI). After a recent outbreak of LSD in Iran in 2014, an emergency vaccination program with heterologous existing vaccines including GPV and SPV vaccines was carried out in the bovine population of the country [10]. Inadequate protective immunity can cause the outbreak of the disease in cattle populations after exposure to the LSD virus [12, 13]. Therefore, evaluation of immune response characteristics of vaccines against LSDV in field trials is very important to assess the status of the existing vaccine strains and to select the best vaccine strain that effectively protects the cattle population against LSDV.

The study aimed to compare the effect of Iranian capripoxvirus vaccine strains on neutralizing antibody titer in cattle. Accordingly, to the evaluation of humoral immunity, the neutralizing antibody levels of vaccinated calves were monitored weekly, to indicate the levels of protection expected.

#### Monitoring of adverse reactions

The individual rectal temperature value of vaccinated calves was recorded daily and rectal temperatures higher than 40 °C were considered febrile. The fever in GPV-vaccinated calves (GVC), and SPV-vaccinated calves (SVC) was first observed 24 h post-vaccination, continued for up to 72 h post-vaccination, then remained within normal range until the end of the experiment. In general, the mean of fever in SVC was higher than GVC, and persisted for up to 3 days. Also, in 32% of SVC (16 calves), ocular and nasal discharge was observed, whereas in GVC and control group any clinical signs were not observed.

#### Neutralizing antibody titers

The serum neutralizing antibody titer of all treated calves were negative before vaccination, but after the vaccination, the neutralizing antibodies were detected. In each vaccinated group, the first detectable neutralizing antibody titer was after 14 days and rose to peak at 28-45 days post-vaccination (dpv). Then it decreased in the following days, while in the control group there was no antibody response. Although, the mean of the neutralizing antibody titer between vaccinated groups at days 14, 28, 45, 90, and 180 post-vaccination were relatively similar, but in GVC was slightly higher than SVC, and in the 90 and 180 dpv it had a higher antibody titer (Log10<sup>1.07</sup>) than SVC. As shown in Fig. 1, the mean neutralization index was higher for SVC on days 14 and 28 post-vaccination and for GVC on days 45, 90, and 180 post-vaccination.

# Comparison of the seroconversion status of vaccinated calves



#### Figure 1.

Comparison of the neutralizing antibody titer of vaccinated calves in response to sheep pox virus (SPV) and goat pox virus (GPV) vaccines on different days post-vaccination.

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#### Table 1.

Generalized estimating equations test results in evaluating the effect of time and vaccine type and their interactions on altering the antibody titer

Variables	Parameter	В	95% confidence interval for B	p-value
Vaccine	SPV	-0.26	-056 to 0.04	0.085
	GPV (reference)		•	
Time	Day 180	0.04	-0.21 to 0.29	0.75
	Day 90	0.06	-0.12 to 0.24	0.51
	Day 45	0.52	0.29 to 0.75	<0.001
	Day 28	0.30	0.11 to 0.49	0.002
	Day 14 (reference)			
Vaccine*Time	SPV*Day 180	-0.56	-0.89 to -0.24	0.001
	SPV*Day 90	-0.50	-0.82 to -0.18	0.002
	SPV*Day 45	-0.40	-0.72 to -0.08	0.015
	SPV*Day 28	-0.18	-0.44 to 0.08	0.17
	SPV*Day 14	•		

According to the obtained results, the effect of "vaccines" (two Iranian capripoxvirus vaccines) in immune response stimulation and antibody production was not significantly different and the mean of antibody titer in each vaccinated group was similar (p = 0.59, DF = 1, Wald's *Chi*-Square = 0.29). But, the effect of "time" in antibody production was significant, so that the mean antibody titer in each vaccinated group were statistically significant different in five measurement periods (p < 0.001, DF = 4, Wald's *Chi*-Square = 65.16).

Also, the interactive effects of "time" and "vaccines" type on immune response were significant, and the mean antibody titer between the two types of the vaccines did not remain constant over time (p = 0.018, DF = 4, Wald's *Chi*-Square = 11.94). Table 1 shows the details of the generalized estimating equations statistical parameters. In this table, the B value shows the difference of mean antibody titer of each vaccinated group relative to the reference group.

In evaluating the effect of the vaccine type on antibody production, the B = -0.26 value was obtained for SPV, and indicating that the mean antibody titer in SPV was about 0.26 units less than GPV, and this difference was not significant. In interpreting the effect of time on antibody production, the 14th-day post-vaccination was considered as reference day, and the mean antibody titer of the other days was compared to the 14th day. The mean antibody titer on days 180 (p = 0.75, B = 0.04) and 90 (p = 0.51, B = 0.06) in comparison to day 14 (reference day) were not significantly different, but on day 45 (p < 0.001, B = 0.52), and 28 (p = 0.002, B = 0.30) this difference was significant. In evaluating the interaction between the two variables; time and vaccine type, the mean antibody titer in SPV was 0.56 units less than of GPV on day 180 (compared to day 14), and this difference was significant (B = -0.56, p < 0.001). Also, on days 90 and 45, these differences were -0.50 and -0.40 significant. But, on day 28, the mean antibody titer of the two vaccines was not significantly different (B = -0.18, p = 0.17).

Because of reasonable prices, vaccination has been considered as a practical and effective method to control LSD [4-6]. To prevent and control lumpy skin disease, several capripoxvirus vaccine strains are currently used [14], and despite regular LSD vaccination of cattle, the cases of vaccine failure and re-occurrence of the disease have been reported [15]. However, little information about the immune dynamics and vaccine-cytokines response to LSD is available. In this study, Gorgan-GPV and Ramyar-SPV vaccine-induced immune responses were measured by neutralization antibody titer, because it is a critical parameter in immune response and can be related to the durability of protection.

Clinical examination of both SVC and GVC was performed daily, and in SVC, mild local reactions

and mild swelling were appeared in the site of vaccine injection, as in the previous studies has been shown [3, 10, 16-18]. While in GVC the local reaction at the vaccination site was lower than SVC [7, 10, 19, 20]. According to the incubation period of the disease, as well as reports of disease in this area, it is likely that SVC was in the incubation period and then showed symptoms following the vaccine injection [7, 21]. In many studies, the effective and protective immune system against LSD and other capripoxviruses is the cellular immune response, although both cellular and humoral immunity play a role in regulating of immune responses [22]. For example, Norian et al [10] and Barman et al [23] showed that not only the cellular immunity but the humoral immunity actively protect the cattle against the capripoxvirus disease.

In our study, the neutralizing antibody titer was detected in both groups of vaccinated calves. The vaccinated calves were able to produce antibodies before day 14 post-vaccination. Similarly, in many research studies, it has been indicated that the vaccinated cattle produce neutralizing antibodies before day 7 post-vaccination [20, 21].

The mean of NI antibody titer of two vaccine strains was increased on each day of follow-up and reached the high value at day 45 and decreased until the follow-up ends at day 180. There is accumulated evidence that the capripoxviruses (LSD, goat, and sheep pox viruses) are genetically related as well as their induced antibodies cross-react one another [4, 24]. Accordingly, though our viral strain vaccines were derived from sheep pox (Ramyar strain) and goat pox (Gorgan strain), a significant difference was not observed in antibody induction among the two viral strains used for our experiment across the study time. Between the two vaccinated groups, the seroconversion rates were relatively higher in GPV compare to SPV. The probable and known variables which influence the immune response to capripoxvirus vaccines include differences in virus strain, the dosage of the vaccine, or a difference in the percentages of exotic germplasm composition. The result of this study showed that all calves were able to produce antibodies in response to vaccine strains and reached to protective level at 28 and 45 day post-vaccination [4, 10, 24-26].

#### **Executive Limitations**

There were two executive limitations in this study: First, due to the inability to provide the necessary biosecurity, as well as the lack of funding, we were unable to perform the challenge of LSD virus in the vaccinated cattle by the capripoxvirus. While for approval of the protective effect of CaPV vaccines against LSD virus, we need to expose the calves with

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LSD virus. But since the LSD virus was on the list A of OIE protocol, this exposure was impossible. Second, the stimulation of immune systems and increases of neutralizing antibody titer may be due to vaccine antigens or the presence of other antigens in the animal's environment. So, the experiment was not performed in the antigen-free area.

Based on the above study we concluded that the Gorgan-GPV and Ramyar-SPV heterologous vaccines stimulated the humoral immune response against LSDV, and induced comparable SNT antibody response after vaccination. Both vaccines induced neutralization antibodies before 14 days, increased regularly thereafter, reached the peak at day 45, and then decreased with a gentle slope until the follow-up ends at day 180 post-vaccination. It seems that the GPV vaccine due to inducing a high level of neutralizing antibody titer against LSD virus, and longer shelf life, can be a suitable vaccine to control the disease in the field.

#### Study Area and Study Population

This study was conducted in the Zanjan province of Iran. 100 Holstein breed calves with approximately 5-6 months of age were selected from several dairy farms. The calves on each farm were divided into two groups; vaccinated calves as a treated group and unvaccinated calves as a vaccine control group.

#### **Types of Vaccine**

The two types of vaccine strains used were Ramyar-Sheep pox virus (SPV) and Gorgan-goat pox virus (GPV), produced by RVSRI of Iran. These vaccines were live attenuated, lyophilized and one dose of each vaccine for goat and sheep contained 10<sup>3.2</sup> TCID50/ml of the virus. A ten-fold dose of vaccines was prepared according to the manufacturer's instructions for emergency use against LSD in cattle [10, 27].

#### Vaccination

In the vaccination trials, treated groups in each farm were vaccinated with GPV or SPV vaccines separately, and the control groups received PBS alone. The calves were received 5 ml volume of vaccine through subcutaneous, according to the manufacturer's instructions. All immunized calves were daily examined to the appearance of adverse reactions and clinical signs for 180 days following vaccination.

#### Sample collection

Blood samples were collected regularly at days 0 (before vaccinations), 14, 28, 45, 90, and 180 post-vaccinations. The collected samples were transported to the animal viral vaccine department of RVSRI institute and allowed to clot for 16-24 hrs at room temperature and then the sera sam-

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ples were transferred to cryovials under a laminar airflow hood in ordered to avoid contamination and the serum was kept at -20°C temperature until processed.

#### Virus preparation

The GPV- and SPV-vaccine strains and LSDV were obtained from RVSRI and were used to make a master stock of the virus, and also, to eliminate a potential source of variability, a single batch of the virus was used. Virus cultivation was carried out according to the standard protocol of the department of animal viral vaccines of RVSRI following OIE manual [4, 27], and the titer of stock prepared virus, calculated by Reed & munch method [28].

#### Serum Neutralizing Test

In OIE, the serum neutralizing test (SNT) is the gold standard method to detect neutralizing antibodies against capripoxviruses [4, 29, 30]. Therefore the evaluation of the humoral immune responses of all samples was done by this method. The level of neutralizing antibodies index was measured in a micro-neutralization assay in duplicate, according to the standard protocol of RVSRI institute following the OIE manual [4]. Results were read on day seven for the neutralization index. The neutralization titer was recorded as the reciprocal of the initial dilution of serum which caused suppression of cytopathic effect. The neutralization index (NI) was calculated following the method of Reed and Munch [20].

#### Statistical analysis

For evaluation of the effects of vaccine type and time (as independent variables) on antibody titer changes (as dependent variable), and because of abnormal distribution of antibody titer and time interval differences, the generalized estimating equations method (GEE) was used. A *p*-value of less than 0.05 was considered significant.

# **Authors' Contributions**

Conceived and designed the experiments: MS and HRV. Performed the experiments: HI, HRV, and MS. Analyzed the data: HI and HRV. Research space and equipment, reagents/materials/analysis tools: HI and HRV.

# Acknowledgments

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# **Competing Interests**

The authors declare no conflict of interest.

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Abstracts (in Persian)

# تاثیر سویه های ایرانی واکسن های کاپری پاکس ویروس بروی تیتر آنتی بادی خنثی کننده در گاو

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### **یکید**ہ

ویروس بیماری لمپی اسکین، آبله بزی و آبله گوسفندی از اعضاء جنس کاپریپاکس ویروسها است. استفاده از واکسن های کاپریپاکس ویروس می تواند در محافظت گاوها در برابر بیماری لمپی اسکین (LSD) کمک کند. هدف از این مطالعه مقایسه تیتر آنتی بادی واکسنهای هترولوگ آبله بزی و گوسفندی در برابر بیماری LSD در گاو است. ۲۰۰ گوساله توسط واکسن های آبله بزی(GPV) و آبله گوسفندی (SPV) در فارم های جداگانه واکسینه شدند. تیتر آنتی بادی واکسن در روزهای ۲۰، ۲۸، ۲۵، ۴۰ و ۱۸۰ بعد از پاکسیناسیون بررسی شد. میانگین دمای مقعد گوساله های واکسینه شدند. تیتر آنتی بادی واکسن در روزهای ۲۴، ۲۸، ۲۵، ۴۰ و ۱۸۰ بعد از پالاتر بود بلکه ترشحات چشمی و آبریزش از بینی در زمان تب نیز مشاهده شد در حالیکه در گوساله های واکسینه شده با GPV هیچ علایم بالاتر بود بلکه ترشحات چشمی و آبریزش از بینی در زمان تب نیز مشاهده شد در حالیکه در گوساله های واکسینه شده با GPV هیچ بالاتر بود بلکه ترشحات پیشمی و آبریزش از بینی در زمان تب نیز مشاهده شد در حالیکه در گوساله های واکسینه شده با GPV هیچ در روزهای بعد از واکسیناسیون به بالاترین میزان خود رسید و سپس در روزهای بعد کاهش یافت. با وجودیکه میانگین تیتر آنتی بادی در هر یک از گروه های واکسیناسیون شده با GPV این میزان خود رسید و سپس در روزهای بعد کاهش یافت. با وجودیکه میانگین تیتر آنتی بادی در هر یک از گروه های واکسینا شده با GPV این میزان خود رسید و سپس در روزهای بعد کاهش یافت. با وجودیکه میانگین تیتر آنتی بادی در هر یک از گروه های واکسیناسیون شده با GPV این میزان در روز ۴۸ به سطح محافظت کنندگی خود رسیده بود و در روز ۱۸۰ نیز دارای تیتر بالاتر از SPV داشت. نتایچ شده با GPV این میزان در روز ۴۸ به سطح محافظت کنندگی خود رسیده بود و در روز ۱۸۰ نیز دارای تیتر بالاتر از SPV داشت. نتایچ شده با GPV بهری برخوردار بوده و می توان به نون و اندگاری بیشتر آنتی بادی در ماه های متوالی بعد از واکسیناسیون، از خاصیت ایمنی زادی بهتری برخوردار بوده و می توان بعنوان واکسن مناسب در برابر LSD بهره برد.

<u>واژ</u>گان کلیدی

آبله بزی، آبله گوسفندی، کاپری پاکس واکسن، آنتی بادی خنثی کننده