



# Comparison and efficacy of two different sheep pox vaccines prepared from the Bakırköy strain against lumpy skin disease in cattle

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**Purpose:** Lumpy skin disease (LSD) is a highly contagious and economically important viral infection of cattle, which leads to financial losses in the livestock industry of affected countries. Vaccination is the most effective control measure to prevent the disease. Heterologous sheep pox (SP) vaccine was used against LSD in Turkey. In this research, it was aimed to adapt SP Bakırköy vaccine strain attenuated in lamb kidney cells to Madin-Darby bovine kidney (MDBK) cells to provide better protection than commercial SP vaccine in cattle.

**Materials and Methods:** To evaluate safety and efficacy of vaccines, while animals were immunized with 10 doses ( $10^{4.75}$  50% tissue culture infectious dose [TCID<sub>50</sub>]) and 5 doses of SP vaccine ( $10^4$  TCID<sub>50</sub>) produced in MDBK cells, others were immunized with commercial Penpox-M vaccine ( $10^{3.9}$  TCID<sub>50</sub>). Two cattle were kept as unvaccinated. At day 31 post-vaccination, all animals were challenged with the virulent LSD virus. Blood and swab samples were taken on certain days post-inoculation. Logarithmic differences challenge virus titers between vaccinated and unvaccinated animals were calculated.

**Results:** The clinical sign was not observed in animals immunized with 10 doses of SP vaccine. The differences between the animals immunized with SP vaccine and control group was less than log 2.5 and the viremia occurred in immunized animals. The difference in titer was higher than log 2.5 in animals immunized with the Penpox-M, and viremia did not occur.

**Conclusion:** SP vaccine strain propagated in MDBK cells and can be used for immunization to prevent LSD infections. However, SP vaccine strain propagated in MDBK showed poor protection as compared to Penpox-M.

**Keywords:** Lumpy skin disease, Cattle, Vaccines, MDBK cell, Sheep pox virus

## Introduction

Lumpy skin disease (LSD) is a devastating arthropod-borne viral infection of cattle caused by lumpy skin disease virus (LSDV) of *Poxviridae* family. It is characterized by high fever (40°C to 41.5°C), lymphadenopathy and nodular lesions on skin and mucous membranes [1,2]. Morbidity depends on host immune status and differs greatly from 5% to 100%. Mortality is usually low and varies 5% to 20% during LSD outbreaks and the World Organisation for Animal Health (OIE) categorizes it as notifiable disease and causes huge economic losses to farmers in the form of low production, sterility, abortion, hide damage and death. Besides substantial economic impact, LSD im-

poses international trade restrictions on live animals and their products [3].

Genus capripoxvirus (CaPV) comprises three important viruses of ruminants including LSDV, sheep pox virus (SPPV), and goat pox virus (GTPV) and show 96%–97% nucleotide identity [4,5]. CaPVs are serologically indistinguishable and provide heterologous cross-protection. Sometimes CaPV can be experimentally cross-infected [6]. The LSDV genome consists of approximately 150 kb double stranded DNA. It encodes at least 30 homologous poxviral proteins involved in structural or virion morphogenesis [4].

LSD was first discovered in Zambia in 1929 and endemic in southern, central, eastern, and western Africa [7]. Between 2012 and 2013, the disease appeared in northern Israel and affected both beef and dairy industry [8]. In the same years, 34 outbreaks of LSD were reported in Lebanon, followed by Jordan and eastern and south-eastern European countries (Balkan regions) [9]. In Turkey, the first clinically suspected case of LSD was observed in Kahramanmaraş and Batman Province bordered with Syria in September 2013 and confirmed by laboratory testing to be LSDV. Following first notification, 236 laboratory confirmed LSD cases were reported in 2013 in Southern provinces of Turkey that neighbor with Syria and Iraq. Between 2013 and 2015, LSDV spread throughout Turkey and now has become endemic in the country [10,11]. In Turkey, there were only 18 outbreaks in 2013 and it was reached 784 at the end of 2014. In 2015, the total of LSD outbreaks decreased slightly to 510 because of strict control measures like mass vaccination, increasing the dose of SP vaccine to triple doses and control of animal movements applied in Turkey. In 2016, the measures yielded results and so the number of LSD outbreaks were 221 in 2016 and only 17 outbreaks in 2017 were reported [10,12]. Due to the troubles in the supply of SP vaccine, 169 outbreaks were reported from 2018 to the end of 2019. For 2020, it conducted the vaccination based on homologous LSD strain in the Thrace region and SP Bakırköy strain in Anatolia within the framework of European Union financial aid (Instrument for Pre-Accession Assistance II) and Republic of the Turkey Ministry of Agriculture and Forestry. In the same year, only five outbreak occurred because of the end of vaccination campaign before seasonal vector activities started.

Vaccination, stamping out, animal movement restrictions, and vector control are considered being the main pillars for the successful LSD control. Live attenuated commercial vaccines are derived from sheep pox (SP) and goat pox (GP), and

LSDVs can be used to immunize cattle against LSD because of cross-protection among them [6,13]. Yugoslavian RM65, Romanian SP, and Bakırköy SP vaccine strains were used to protect cattle for LSD [2,14,15]. SPPV and GTPV vaccines can be used in cattle but the safety and efficacy of the vaccines must be proved by experimental challenge studies [3,15]. Live attenuated SP vaccine prepared from Bakırköy strain is used heterologously against LSD in cattle in Turkey. Bakırköy strain was attenuated at primary lamb kidney cell at 65 passages and it is produced as a lyophilized vaccine with virus titer at least  $10^{2.5}$  TCID<sub>50</sub> per vaccine doses and has been used for the prevention of SP, GP, and LSD. Five doses of SP vaccine are currently being administered in cattle in Turkey. Up to date, there is no data on SP vaccine produced in cells of bovine origin. Moreover, there is no study on immunity to heterologous Bakırköy strain in cattle. Therefore, this study was designed to investigate the comparison and efficacy of two different SP vaccines prepared from the Bakırköy strain against LSD in cattle. One of these Penpox-M (Pendik Veterinary Control Institute, İstanbul, Turkey) was produced in primary lamb kidney cells and the other was propagated in Madin-Darby bovine kidney (MDBK) cells. It was expected that live attenuated SP vaccine strain (Bakırköy strain) that was adapted to MDBK cells, multiply more easily in the cattle and cause better immunity.

## Materials and Methods

### Experimental animals

Ten Simmental heifers (age, 6–9 months) imported from the LSD free country (obtained from Germany) were used for the safety and efficacy studies of SP vaccines.

### Propagation of vaccine and challenge virus strains

MDBK cell line (90050801; European Collection of Authenticated Cell Cultures, Salisbury, UK) and ovine testis cells (OA3-Ts, CRL6546; American Type Culture Collection, Manassas, VA, USA) were used to propagate SP vaccine strains and LSDV challenge virus. Both cell lines were grown in Dulbecco's modified Eagle's medium (DMEM, catalog [Cat.] no., F0445; Biochrom, Berlin, Germany) containing NaHCO<sub>3</sub>, supplemented with 10% foetal bovine serum (FBS, S0115; Biochrom), 1% penicillin-streptomycin (Cat. no., P0781; Sigma-Aldrich, St. Louis, MO, USA), and 1% L-glutamine (Cat. no., 609-065EL; Wisent, Saint-Bruno, Canada).

SP Bakırköy strain, passaged 60 times in primary lamb kid-

ney cells, was stocked in Pendik Veterinary Control Institute, Viral Vaccine Production Laboratory. In this research, the newly produced SP vaccine in MDBK cells (SP-MDBK) and commercial SP vaccine (Penpox-M) were used to immunize the cattle to compare the immune response.

A virulent Pendik strain of LSD (accession no., MN995838) at 3 passages in OA3.Ts originated from Pendik Veterinary Control Institute (Istanbul, Turkey) was used for the challenge studies. The viral titer was calculated and adjusted to be  $10^{6.75}$ /mL TCID<sub>50</sub>.

Confluent MDBK cells were prepared in 75 mL flasks by using DMEM containing 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% L-glutamine, and SP virus (Bakırköy strain at 60th passage) was inoculated with titers of  $10^{4.0}$ /mL TCID<sub>50</sub> and allowed to adsorb for 1 hour. After the incubation at 37°C, the inoculum was removed and 10 mL DMEM containing 2% FBS was added in to each flask. Cells were checked daily for vitality and presence of cytopathic effect (CPE). When the 80%–90% CPE was seen, the flask was subjected to 3 freeze/thaw cycles and then taken to the freezer at -20°C. Five serial passages were made in MDBK as adaptation to cells and increasing the virus titer. In order to identify the SP vaccine strain in the cell culture supernatant (bulk vaccine), virus titration test, neutralization test with SP hyperimmune serum, SP-DNA detection by real time polymerase chain reaction (PCR) [16], and observation of intracytoplasmic inclusion bodies were performed.

### Experimental design to immunize cattle

Ten animals were housed in a strictly controlled biosafety level 2 condition and used for immunization studies. They were divided into four groups, and group information, vac-

cine doses, and titers were shown in the Table 1. Animals in group A were utilized for the efficacy and safety study to prove the immune response to 10 doses of SP-MDBK vaccine. The animals in the group B and C were utilized for the efficacy studies, while animals in the group D were kept as negative controls.

Five randomly selected vials of the freeze-dried SP vaccines were reconstituted in 100 mL sterile phosphate-buffered saline (PBS) [3]. Recommended field dose of SP vaccines was prepared according to 5 vials. A total of 2 mL (10 doses,  $10^{4.75}$  TCID<sub>50</sub>) of SP-MDBK was administered subcutaneously into two cattle in group A. On the other hand, 1 mL (5 doses,  $10^{4.4}$  TCID<sub>50</sub>) of Penpox-M and SP-MDBK vaccine (5 doses,  $10^{3.9}$  TCID<sub>50</sub>) produced in this study were administered subcutaneously into animals of group B and group C. After the vaccination, cattle were monitored daily for the development of any clinical sign.

### Challenge studies

All cattle were challenged with the virulent LSDV on the 31 days post-vaccination (dpv). Log10 dilutions of the Pendik-LSD challenge virus ( $10^{6.75}$ /mL TCID<sub>50</sub>) were prepared in sterile PBS and seven dilutions were inoculated intradermally (0.1 mL per inoculum) on chest and flank region of experimental cattle. After the challenge, all cattle were monitored for 25 days in terms of rectal temperature, general condition, and specific signs of LSD. The hypersensitivity reaction at sites of inoculation within 24 hours was ignored which was quickly subsided. The titer of the challenge virus was daily calculated according to Spearman-Kärber for the vaccinated and unvaccinated control animals; a difference in titer  $\geq \log_{10} 2.5$  was taken as evidence of protection [3]. In order to

**Table 1.** Experimental design, vaccines, and titers of administered doses

Group	Cattle no.	Study name	Vaccine	Dose	Titer of administered dose (TCID <sub>50</sub> )
Group A	1	Efficacy and safety	SP-MDBK	10	$10^{4.75}$
	2	Efficacy and safety	SP-MDBK	10	$10^{4.75}$
Group B	1	Efficacy	SP-MDBK	5	$10^{4.4}$
	2	Efficacy	SP-MDBK	5	$10^{4.4}$
	3	Efficacy	SP-MDBK	5	$10^{4.4}$
Group C	1	Efficacy	Penpox-M	5	$10^{3.9}$
	2	Efficacy	Penpox-M	5	$10^{3.9}$
	3	Efficacy	Penpox-M	5	$10^{3.9}$
Group D	1	Control group	Unvaccinated	-	-
	2	Control group	Unvaccinated	-	-

TCID<sub>50</sub>, 50% tissue culture infectious dose; SP-MDBK, sheep pox Madin-Darby bovine kidney.

decide that the challenge virus is working properly, scab sample was taken from the inoculation area and inoculate in to the Oa3.Ts cell line [17] and DNA sample was tested with gel-based PCR [18].

**Detection of CaPV DNA and antibody to CaPV**

Blood samples were collected on 12, 20, 27, and 31 dpv from all cattle and serum was harvested. After vaccination, blood samples were analyzed by real time PCR for the detection of possible viremia due to vaccine virus [16]. Serum samples were tested for the presence of vaccine-induced antibodies by enzyme-linked immunosorbent assay (ELISA) (IDvet ID Screen Capripox Double Antigen Kit; IDvet, Grabels, France) and virus neutralization assay (constant-virus/varying-serum) [19].

Swabs (ocular and nasal), blood, and serum samples were collected on 9, 11, 15, 21, and 25 days of post-challenge (dpc) from all cattle. Swabs and blood samples were analyzed by real time PCR for detection of possible viremia due to LSDV challenge virus [16]. Serum samples were tested for the presence of antibodies by ELISA and virus neutralization assay [19].

**Necropsy**

On 25 dpc, all animals were euthanized and necropsied. Skin and oedema swabs, hair follicle, muscle tissue from the injection site, gluteal muscle tissue, lung, heart, kidney, liver, and rumen samples were taken. Sample selection was made by taking into consideration the findings from the previous experimental infection study with Pendik-LSD challenge virus (unpublished data). All samples were analyzed by real-time PCR for LSDV-DNA after the DNA extraction by using a commercial DNA extraction kit (Roche High Pure Viral Nucleic

Acid Kit, cat. no., 11858874001; Roche Diagnostics, Basel, Switzerland).

**Ethical approval**

This study was carried out in accordance with the animal rights statement. The permission was obtained from the ethics committee of experimental animals of Istanbul University (no., 2015/109).

**Results**

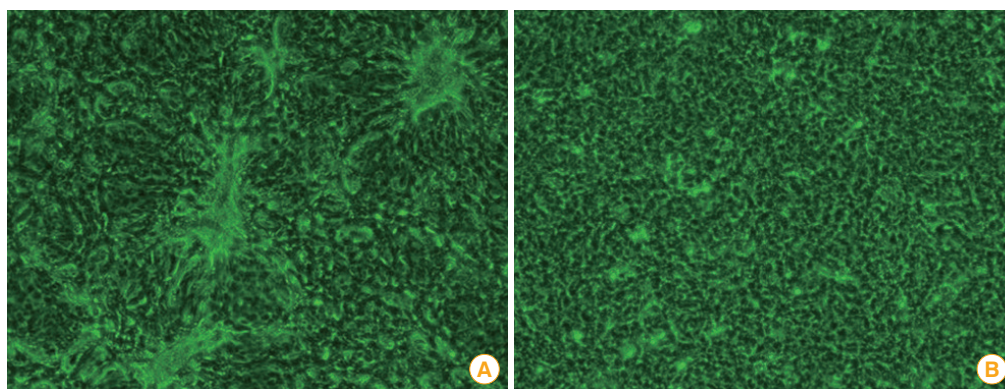
**Preparation of vaccine in confluent MDBK cells**

Following inoculation of SP vaccine strain into confluent MDBK cells, CPE formation was observed on day 10 post-inoculation (Fig. 1A). No CPE was seen in control cells (Fig. 1B). The titer of the SP-MDBK vaccine was calculated as  $10^{5.75}$ /mL TCID<sub>50</sub>. After one-hour incubation of SP vaccine strain with SP hyperimmune serum, CPE formation was not observed in MDBK cells showing the inhibition of virus. When checked by real-time PCR, cycle threshold value was determined as 24.32. The SP vaccine strain that propagated in MDBK cells was identified as the SP virus.

Following the identification, the vaccine virus bulk was lyophilized by mixing with stabilizers (5% lactalbumin and 10% sucrose). The titer of the lyophilized vaccine was determined to be  $10^{5.75}$ /mL TCID<sub>50</sub> (The required titer for the 100-dose viral SP vaccine was a minimum  $10^{4.5}$ /mL TCID<sub>50</sub>). Standardized vaccine controls were made, and the vaccines were found to meet the OIE requirements and appropriate for usage [3].

**Clinical observation of vaccinated cattle**

No clinical symptoms and a raise of rectal temperature were monitored in experimental cattle before the injections. No



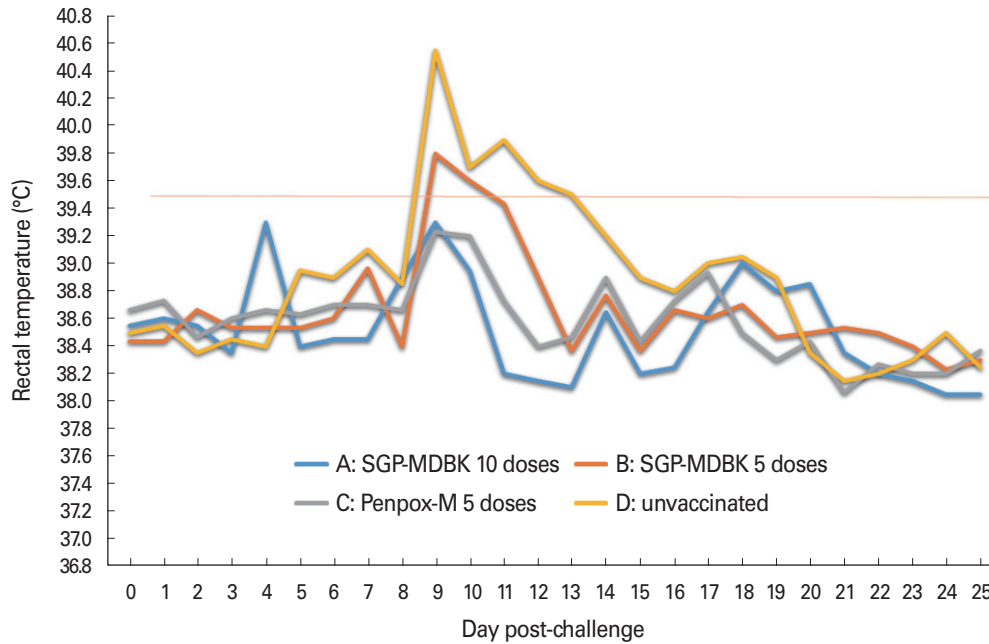
**Fig. 1.** Appearance of confluent Madin-Darby bovine kidney (MDBK) cells. (A) MDBK cells inoculated with  $10^4$ /mL TCID<sub>50</sub> (50% tissue culture infectious dose) at day 10 post-inoculation. (B) Negative control.



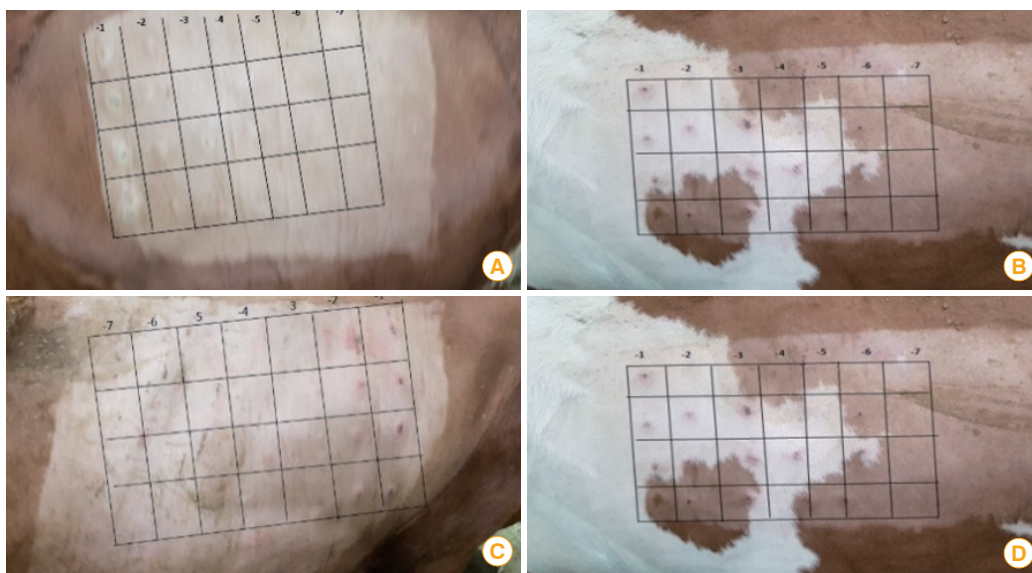
adverse reactions were noted in group A and group B cattle, immunized with 5 and 10 doses of SP-MDBK vaccine and also in group C immunized with 5 doses of Penpox-M. In these cattle, rectal temperatures were in the normal range (38.0°C to 39.4°C) and no swelling or lesions were observed at the injection sites.

**Outcome of challenge studies**

No clinical symptoms specific to LSD were noted in the immunized animals. After the inoculation of the challenge virus, the rectal temperatures of A-2, B-1, B-3 cattle, and control group D-1 and D-2 cattle were measured above 39.5°C, while the other immunized animals showed normal temperature range (Fig. 2). A decrease in feed intake with cachexia and enlargement of subscapular lymph nodes were found in



**Fig. 2.** Average rectal temperatures of each group after challenge with virulent Pendik lumpy skin disease virus strain. MDBK, Madin-Darby bovine kidney.



**Fig. 3.** Skin reactions in cattle challenged with lumpy skin disease virus. (A) Vaccinated with 10 doses of sheep pox Madin-Darby bovine kidney (SP-MDBK) vaccine. (B) Vaccinated with 5 doses of SP-MDBK vaccine. (C) Vaccinated with 5 doses of Penpox-M. (D) Unvaccinated control animal.

D-1 (control animal) at 7 days after the challenge. No clinical signs were seen in D-2 (control animal).

Hypersensitivity reactions were noted at the inoculation site for 3 days after injection of the LSD challenge virus. After they subsided at 4 dpc, swelling and lesions appeared at 1/10 dilution site (Fig. 3).

The difference in challenge titers between immunized and control group were calculated with the average log 5.63 in only D-1 cattle, because of the absence of lesion development in the number D-2 cattle (Table 2). There were no lesions developed in A-1 and B-1 cattle and logarithmic differences were calculated as log 5.63.

The logarithmic differences were 3 in A-2, 3.26 in C-1, 4.76 in C-2, and 3.01 in C-3 and the minimum protective difference was greater than log<sub>10</sub> 2.5. The logarithmic differences were 1.76 in B-2 and 0.75 in B-3 and minimum protective dif-

ferences were found to be less than log<sub>10</sub> 2.5 (Table 2).

The challenge virus was re-isolated in Oa3.Ts cells at day 4 post-inoculation (Fig. 4) and confirmed as LSDV by gel-based species specific PCR (Fig. 5).

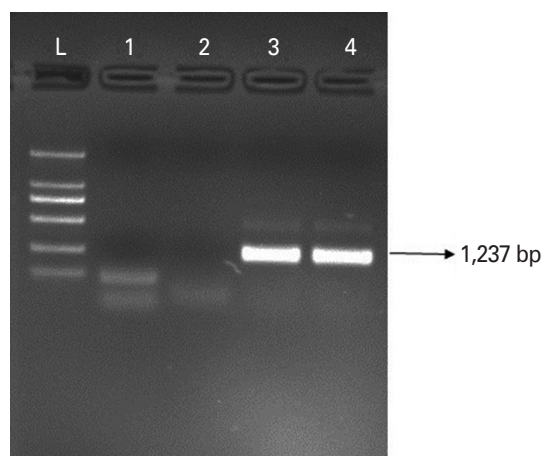
**Detection of CaPV-DNA**

Even though SP vaccine strain DNA was not found in the immunized cattle indicating the absence of viremia, after injection of LSD challenge virus in group A, B, and D cattle, viremia (CaPV-DNA) was detected on 11 dpc by real-time PCR and also in B-3 on 15 dpc. Besides, no viremia was detected in cattle group C immunized with Penpox-M (Table 3). Challenge virus DNA (LSD-DNA) was not detected in ocular and nasal swab samples in groups A, B, and C. But it was detected in nasal and ocular swab samples in D-1 and nasal swab in D-2 on 15 dpc (Table 4).

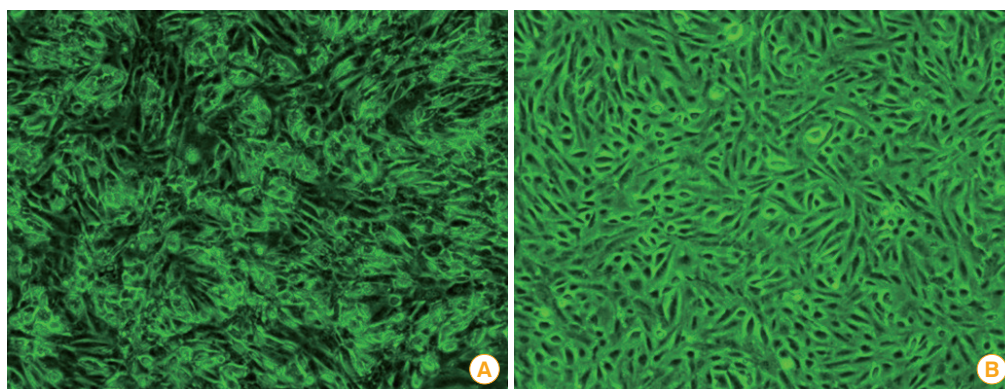
**Table 2.** Logarithmic differences between vaccinated and unvaccinated cattle

Cattle no.	Vaccine and dose	Average challenge titer (log)	Logarithmic difference
A-1	SP-MDBK/10 dose	0	5.6
A-2	SP-MDBK/10 dose	2.6	3
B-1	SP-MDBK/5 dose	0	5.6
B-2	SP-MDBK/5 dose	3.8	1.7
B-3	SP-MDBK/5 dose	4.8	0.7
C-1	Penpox-M/5 dose	2.3	3.2
C-2	Penpox-M/5 dose	0.8	4.7
C-3	Penpox-M/5 dose	2.6	3
D-1	Unvaccinated	5.6	-
D-2	Unvaccinated	0	-

SP-MDBK, sheep pox Madin-Darby bovine kidney.



**Fig. 5.** Amplification of re-isolated challenge virus DNA. L, ladder marker 2 kbp; 1, Bakirköy sheep pox vaccine strain DNA; 2, negative control; 3, scab sample DNA; 4, positive control.



**Fig. 4.** Appearance of confluent Oa3.Ts (ovine testis cell line) cells. (A) Cytopathic effect formation of lumpy skin disease virus at 4-day post-inoculation. (B) Negative control.

**Table 3.** Real time polymerase chain reaction results of post-challenge blood samples

Cattle no.	Vaccine/dose	Blood samples dpc				
		9 dpc	11 dpc	15 dpc	21 dpc	25 dpc
A-1	SP-MDBK/10 dose	-	32.46	-	-	-
A-2	SP-MDBK/10 dose	-	23.63	-	-	-
B-1	SP-MDBK/5 dose	-	25.46	-	-	-
B-2	SP-MDBK/5 dose	-	26.87	-	-	-
B-3	SP-MDBK/5 dose	-	32.58	32.65	-	-
C-1	Penpox-M/5 dose	-	-	-	-	-
C-2	Penpox-M/5 dose	-	-	-	-	-
C-3	Penpox-M/5 dose	-	-	-	-	-
D-1	Unvaccinated	-	25.52	-	-	-
D-2	Unvaccinated	-	26.6	-	-	-

Dpc, day post-challenge; SP-MDBK, sheep pox Madin-Darby bovine kidney.

**Table 4.** Real time polymerase chain reaction results of post-challenge swab samples

Cattle no.	Vaccine/dose	Swab samples dpc									
		9 dpc		11 dpc		15 dpc		21 dpc		25 dpc	
		N	O	N	O	N	O	N	O	N	O
A-1	SP-MDBK/10 dose	-	-	-	-	-	-	-	-	-	-
A-2	SP-MDBK/10 dose	-	-	-	-	-	-	-	-	-	-
B-1	SP-MDBK/5 dose	-	-	-	-	-	-	-	-	-	-
B-2	SP-MDBK/5 dose	-	-	-	-	-	-	-	-	-	-
B-3	SP-MDBK/5 dose	-	-	-	-	-	-	-	-	-	-
C-1	Penpox-M/5 dose	-	-	-	-	-	-	-	-	-	-
C-2	Penpox-M/5 dose	-	-	-	-	-	-	-	-	-	-
C-3	Penpox-M/5 dose	-	-	-	-	-	-	-	-	-	-
D-1	Unvaccinated	-	-	-	-	29.42	36.5	-	-	-	-
D-2	Unvaccinated	-	-	-	-	35.36	-	-	-	-	-

Dpc, day post-challenge; N, nasal; O, ocular; SP-MDBK, sheep pox Madin-Darby bovine kidney.

**Table 5.** Real time polymerase chain reaction results of necropsy samples

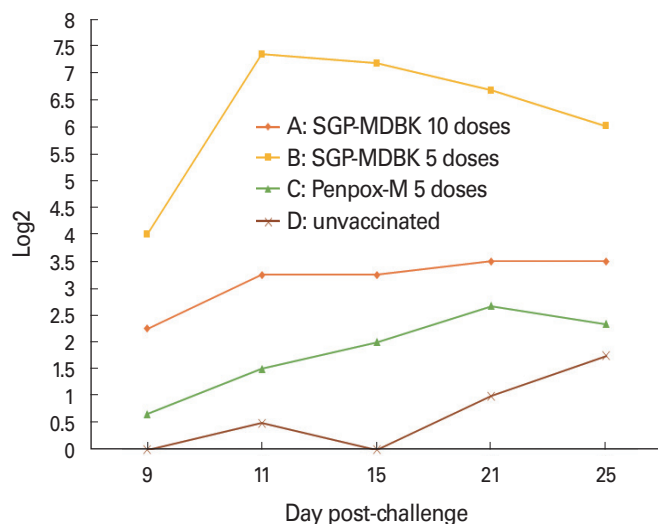
Cattle no.	Vaccine/dose	Skin swab	Hair follicles	Muscle injection site	Musculus gluteus	Lung	Heart	Kidney	Liver	Rumen	Oedema Swab
A-1	SP-MDBK/10 dose	-	-	-	-	-	-	-	-	-	-
A-2	SP-MDBK/10 dose	-	-	-	-	-	-	-	-	-	-
B-1	SP-MDBK/5 dose	-	-	-	-	-	-	-	-	-	-
B-2	SP-MDBK/5 dose	-	-	-	-	-	-	-	-	-	-
B-3	SP-MDBK/5 dose	-	-	-	-	-	-	-	-	-	-
C-1	Penpox-M/5 dose	-	-	-	-	-	-	-	-	-	-
C-2	Penpox-M/5 dose	-	-	-	-	-	-	-	-	-	-
C-3	Penpox-M/5 dose	-	-	-	-	-	-	-	-	-	-
D-1	Unvaccinated	35.2	22.1	-	-	-	-	-	-	-	-
D-2	Unvaccinated	-	-	-	-	-	-	-	-	-	-

SP-MDBK, sheep pox Madin-Darby bovine kidney.

### Necropsy

After completion of the efficacy study, all cattle were necropsied. A macroscopic examination revealed oedema in the

subcutaneous tissue at the injection site of the challenge virus. No macroscopic and pathological findings were noted in the internal organs. CaPV-DNA was detected in the swab and



**Fig. 6.** Serum neutralization test average results of experimental animals after challenge with virulent Pendik lumpy skin disease virus strain (log2). SP-MDBK, sheep pox Madin-Darby bovine kidney.

hair follicles taken from the skin area of the cattle D-1 in the control group (D) and was not detected in all immunized cattle (Table 5).

**Sero-conversion of immunized cattle**

Serum neutralization test (SNT) and ELISA did not detect antibodies to CaPV in all groups on 12, 20, 27, and 31 dpv against CaPV indicating that seroconversion to post-vaccination did not occur. The results of post-challenge seroconversion studies in ELISA and SNT were relatively similar except for the D-10 serum sample on 25 dpc, it was negative by ELISA while it is log2 1 by SNT. Both ELISA and SNT did not detect antibodies in C-1 cattle but there was a significant increase in cattle A-2, B-2, and B-3 which had more lesions after inoculation of the challenge virus. A lower titer was detected in cattle A-1, B-1, C-2, and C-3 with the occurrence of few or no lesions. In the control group cattle D-1 and D-2, the late antibody response was determined. These data were consistent with average ELISA and SNT results in challenged groups (Fig. 6, Table 6).

**Discussion**

LSD was first seen in Turkish cattle in 2013. It was then later spread all over Turkey and neighboring countries and leading to economic losses. It is widely agreed that vaccination is the only effective way to control the spread of LSDV in endemic countries [20]. Our experience to mitigate LSD in Tur-

**Table 6.** Average enzyme-linked immunosorbent assay optical density results of experimental animals challenged with virulent Pendik lumpy skin disease virus strain

Days	A: SP-MDBK 10 doses	B: SP-MDBK 5 doses	C: Penpox-M 5 doses	D: unvaccinated
Dpc 9	0.7	1.511	0.388	0.15
Dpc 11	0.87	1.82	0.52	0.26
Dpc 15	0.87	1.57	0.661	0.16
Dpc 21	0.8515	1.65	0.6	0.33
Dpc 25	0.904	1.567	0.59	0.58

Dpc, day post-challenge; SP-MDBK, sheep pox Madin-Darby bovine kidney.

key has also shown that vaccination is an important way to control the disease. Therefore, this study was planned to investigate the comparison and efficacy of SP vaccines in cattle. At present, live attenuated commercial vaccines derived from CaPV’s can be used to immunize cattle against LSD because of cross-protection among them [13,21]. Because of the emergence of the LSD control which suddenly appeared in Turkey in 2013, the SP vaccine (Bakırköy strain-PenPox-M) was used in the field [10,15].

CaPV can propagate in tissue culture of bovine, ovine, or caprine origin. Primary or secondary cell cultures of kidney and testis cells are the most susceptible to both virus isolation and vaccine production [3]. In this study, Bakırköy SP vaccine strain was easily adapted to MDBK cell line. The titer of the vaccine strain was calculated as  $10^{5.75}$ /mL TCID<sub>50</sub> and it was determined that SP vaccine production did not cause any effect on the virus titer in MDBK cells. Similar studies have been performed on the propagation of SP and GP virus in MDBK cells [22,23].

In this study, no seropositivity was found as a result of ELISA and SNT in blood samples taken on 12, 20, 27, and 31 dpv. Antibody response may not develop in cattle immunized with sheep goat pox vaccine as the immunity against CaPV is predominantly cell-mediated after vaccination [15,24]. Cell-mediated immunity handles long-term protection in case of inability to detect antibodies after vaccination meaning that cattle devoid of antibody confers to protection [25].

The efficacy of CaPV vaccines against LSD should be confirmed by challenge studies with sero-negative LSDV in young dairy cattle [3]. In this study, to determine the efficacy of both SP vaccines, 10 Simmental heifers (6 to 9 months old) were challenged with virulent LSDV. In group A (immunized with 10 doses of SP-MDBK), although the logarithmic difference was found to be higher than 2.5 and giving to results of



swab samples did not spread the challenge virus, the LSDV-DNA was detected on the 11 dpc in the blood sample and the rectal temperature was measured high in A-2. Ten doses of SP-MDBK vaccine did not stop the viremia and did not provide complete protection against LSD. In group B (immunized with 5 doses of SP-MDBK), although agreeing to results of swab samples did not spread the challenge virus, the logarithmic difference was found to be lower than 2.5 in B-2 and B-3, the LSDV-DNA was detected in the blood sample and the rectal temperature was measured high in B-1 and B-3. Five doses of SP-MDBK vaccine did not stop the viremia and did not protect against LSD. In group C (immunized with 5 doses of Penpox-M), the logarithmic difference was found to be higher than 2.5, agreeing to results of swab samples did not spread the challenge virus and the rectal temperature was measured at normal range. Penpox-M stopped the viremia and provided complete protection against LSD. In group D (unvaccinated control group), the titer of challenge virus was confirmed ( $10^{5.75}/100 \mu\text{L TCID}_{50}$ ) at D-1 cattle. LSDV-DNA was detected in blood samples on 11 dpc, in ocular and nasal swabs on 15 dpc. Rectal temperatures were measured as high, and it was confirmed that the challenge virus was virulent in unvaccinated cattle.

In this study, there was no lesion development in A-1, B-1, and D-2 but viremia occurred in these cattle on 11 dpc because of subclinical infection due to challenge virus. In one study, only 40%–50% of inoculated animals have shown clinical signs following the results [26]. In unvaccinated D-2 animal, LSDV-DNA was detected in nasal swab. In A-1 and B-1, the absence of LSDV-DNA in the ocular and nasal swabs of the animals shows that the vaccination did not provide complete protection in these cattle, but prevented the spread of the disease.

In the comparison of the SP-MDBK vaccine efficacy 5 doses to 10 doses, viremia occurred in both groups. Although the lesions in the 5 doses' administered cattle (group B) were larger and the logarithmic difference was below the protective value, the lesions that developed at 10 doses' administered cattle (group A) were smaller and the logarithmic difference was above the protective value. Because of the viremia, 10 doses of the SP-MDBK vaccine did not provide complete protection. These findings show that the SP-MDBK vaccine may provide immunity when administered higher than 10 doses.

Live and attenuated vaccines can be used in the control of LSD but the safety of 10 doses should be determined in target

animals [3]. Although homologous, live attenuated LSD vaccines are more effective, it causes mild to severe clinical symptoms after vaccination [8,27]. In a field study, the commercially used homologous LSD vaccine caused local reactions at a rate of 12% after vaccination [27,28]. Another vaccine used against LSD is the SP virus derived from Yugoslavia RM65 strain [29]. There was no clinical finding in cattle administered 1 and 5 doses of SP vaccine (RM65) but clinical side effects in animals that belong only to the group received 10 doses of the SP vaccine ( $1 \times 10^{4.5} \text{TCID}_{50}$ ). The side effects observed were fever, a decrease in feed intake and milk production, as well as skin lesions [9]. The findings of the present study show that vaccination of cattle with 10 doses of SP-MDBK did not cause swelling, fever, viremia, and any clinical symptoms, and the vaccine was safe in cattle.

In the present research, there was no seropositivity in immunized animals, but after the challenge, antibodies were found on 9, 11, 15, 21, and 25 days by ELISA and SNT. In immunized cattle, serum antibody titers were higher where the lesions were visible and larger due to the challenge virus. As seen in Fig. 4 and Table 6, the antibody response after the challenge was remarkably high in animals that the vaccine did not fully protect. Serum antibody titers increased by 21 dpc in unvaccinated challenged animals (group D). According to OIE Terrestrial Manual, antibodies against CaPV can be detected from day 2 after the onset of clinical signs, but a significant increase in antibody titer usually occurs between days 21 and 42 [3]. In a similar investigation on antibody response, some cattle do not have local reactions and there was no measurable antibody level after LSD vaccination but these animals are immune when challenged [30,31]. In a study, skin lesions were occurred after administration of vaccines derived from virulent LSD field isolates which were extracted from interleukin-10-like and interferon-gamma receptor-like genes and high neutralizing antibodies were detected. In the same study, neutralizing antibody development was not detected in cattle after LSD Neethling strain vaccination [32]. In the present study, 90 serum samples of all experimental cattle were examined serologically. According to the results of SNT and ELISA, 89 were similar whereas 1 sample was negative in the ELISA test but was found to be positive in SNT. These results agree with other studies [30,31]. In a previous study, ELISA gave similar results with SNT but more specific method than ELISA [33]. It was mentioned that the immune response of the virulent LSDV inoculated cattle is variable in individual animal, and large sampling sizes needed [32].

After necropsy, there was no LSDV-DNA was in tissue and organ samples were taken from all immunized cattle and both vaccines prevent the spread of the challenge virus in the body. In unvaccinated challenged cattle D-1, LSDV-DNA was detected in hair follicles and skin swab samples that were collected from the inoculation site. This may show LSDV replication in hair follicle epithelium [34,35], and also it shows the LSD challenge virus infected experimental animals accurately.

In this study, live attenuated SP vaccine strain was adapted to bovine origin MDBK cells to multiply more easily in the cattle. However, there was a decrease in immunogenicity while propagating in MDBK cells and did not provide the desired immunity in cattle. In contrast, commercial Bakırköy SP vaccine produced in primary lamb kidney cells, protected against LSD in cattle. Live viral vaccines against LSD are attenuated by performing multiple passages in the cell culture or embryo egg [7,36]. The cell lines used in viral vaccine production have a significant effect on both immunogenicity and attenuations. Determining the changes in the viral genome after the attenuation of the vaccine virus in cell culture passages will contribute to vaccine studies. Considering the outbreak numbers from 2013 to 2020 in Turkey and based on the results of this study, SP Bakırköy vaccine was effective in spread and controlling LSD. Although it is a heterologous vaccine, it appears to protect with a high rate of seasonably mass vaccination. SP vaccines used against LSD may be a good alternative in countries with high cattle populations such as Turkey or where preventive vaccination programs apply in LSD-free countries.

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