RESEARCH ARTICLE

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Evaluation of an Inactivated Lumpy Skin Disease Vaccine for Cattle

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In the study, the efficacy of paxdoll and ndoll vaccines in Lumph Skin Disease (LSD) was investigated.

Blood samples were collected from healthy, 35 calves at 6-8 months of age in Şanlıurfa province. Eight sero-negative calves were selected and sent to Ankara-Sap institute, following all the official procedures regarding internal animal's movement. At the first stage, animals were kept at the non-contaminated facility. Animals were divided into 3 groups: First group of 3 animals were vaccinated with Poxdoll, and second group of 3 animals were vaccinated with LSD-Ndoll. 2 animals of the third group were served as a control and didn't receive any vaccine. Blood Samples were taken on the first, 14 and 28 days of vaccination. The animals were observed for body temperature, general condition and any lesions at the vaccination site. Second stage were provided at the high containment facility where the animals at the 28th day after vaccination were moved. Each virus dilution was inoculated in 4 points at the left side and 4 at the right side.

On the 14th day, the infectious doses of virus in control and vaccinated animals were calculated. Protective power of vaccines was determined by calculation of the difference between viral titer in control and vaccinated animals.

As a result, sheep and goat pox vaccine of Bakrky strain at the concentration of 10 sheep doses as well Neethling vaccine provided protection against LSD, and both strains were shown to be safe for administration among cattle. In addition, no adverse reactions were observed.

Key Words: *Lumpy skin disease, cattle, vaccines, poxdoll, LSD-ndoll*

Sığırlar için İnaktive Edilmiş Lumpy Skin Disease Aşısının Değerlendirilmesi

Bu çalışmada pax doll ve ndoll aşılarının Lumpy Skin Disease (LSD) etkinliği araştırıldı.

Şanlıurfa ilinde 6-8 aylık sağlıklı yaklaşık 35 buzağıdan kan örnekleri alındı. Sekiz sero negatif buzağı seçildi ve tüm resmi prosedürler takip edilerek Ankara-Şap enstitüsüne gönderildi. İlk aşamada hayvanlar kontamine olmayan tesiste tutuldu. Hayvanlar 3 gruba ayrıldı: birinci grupta bulunan 3 hayvan Poxdoll ile, ikinci gruptaki 3 hayvan ise LSD-Ndoll ile aşılandı. Üçüncü gruptan 2 hayvan kontrol olarak seçildi ve herhangi bir aşı yapılmadı. Aşılanan hayvanlarda aşılamanın 1, 14 ve 28. günlerinde kan örnekleri alındı. Hayvanlar vücut ısısı, genel durum ve aşılama yerinde herhangi bir lezyon açısından gözlemlendi. İkinci aşama, ilk aşılamadan sonraki 28. günde hayvanların taşındığı yüksek muhafaza tesisinde sağlandı. Her virüs dilüsyonu sol tarafta 4 ve sağ tarafta 4 noktaya uygulandı.

Kontrol ve aşılanmış hayvanlardaki Enfeksiyöz virüs dozları 14. günde hesaplandı. Aşıların koruyucu gücü, kontrol ve aşılanmış hayvanlardaki viral titre arasındaki fark hesaplanarak belirlendi.

Çalışmada koyun ve keçi çiçeği aşısı Bakrkoy suşunun 10 koyun dozunda ve Neethling aşısının LSD'ye karşı koruma amaçlı kullanımını desteklediği, her iki suşun da sığırlar arasında uygulanmasının güvenli olduğu gösterilmiş, herhangi bir yan etki gözlenmemiştir.

Anahtar Kelimeler: *Lumpy skin disease, sığır, aşı, poxdoll, LSD-ndoll*

Introduction

Lumpy Skin Disease (LSD), is an acute, sub-acute or in apparent viral disease of cattle characterized by pyrexia, generalized skin lesions, and generalized lymphadenopathy (1). Disease is caused by a virus in the genus Capripoxvirus of the family Poxviridae. The virus is believed to be transmitted mechanically by blood feeding flying insects such as *Stomoxys* sp. and *Aedes* sp. and potentially by ticks (2-4)

In Türkiye0, LSD was first reported in August 2013. In 2015, LSD was spread all over the country resulting in 510 outbreaks. In 2016, the number of outbreaks and cases were decreased after the control measures taken by the Ministry of Food Agriculture and Livestock of Turkish Republic. In addition to the control of vectors and quarantine measures, vaccination of animals is the main disease control measure (5, 6).

Local sheep and goat pox vaccine strain (Bakrkoy) is used for vaccination. This vaccine contains at least 102,5 TCID50 viruses per dose and it is still currently in use against LSD in Türkiye (7).

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In the current study, it is aimed to determine the protection efficacy of sheep and goat pox vaccine, Poxdoll vaccine and LSD-Ndoll vaccine against LSD, and whether both strains are safe for cattle.

Material and Methods

Research and Publication Ethics: The animals included in the study were obtained within the framework of the Ethics Committee Report (2022/04) given by Dollvet Inc. Animal Experiments Local Ethics Committee according to these principles.

Study Preparation: Blood samples were collected from healthy, Sheep pox vaccine non vaccinated, about 35 calves 6-8 months old in Şanlıurfa province. Before sampling animal owners were asked about the vaccination and sickness history of animals.

Serum was separated and all the sera samples were tested by Neutralization test against LSD vaccine strain Neethling. Eight seronegative calves were selected and sent to Ankara-Şap institute, following all the official procedures regarding internal animal's movement.

Vaccines and Viruses: In this study two vaccines were used:

1. Poxdoll vaccine (Bakrkoy strain, Dollvet Biyoteknoloji AŞ, Şanlıurfa, TÜRKİYE), 100 sheep doses vials were dissolved in 25 mL vaccine diluent and administrated subcutaneously in 2.5 mL for calf (10 sheep doses for calf).

2. LSD-Ndoll vaccine (Neethling strain, Dollvet Biyoteknoloji AŞ, Şanlıurfa, TÜRKİYE), 50 doses vials were dissolved in 100 mL dilution buffer and administrated subcutaneously in a dose 2 mL for calf. LSD-Ndoll safety in cattle has been already approved during the vaccine quality control trails.

Virulent LSD strain was obtained from Pendik institute. 7 tenfold dilutions were prepared (10-1 -10-7) in PBS.

Study Design: The study was provided in Şap institute at the animal facility under the supervision and control of competent institute officers. At the first stage, animals were kept at the non-contaminated facility. Animal were divided into 3 groups: First group of 3 animals were vaccinated with Poxdoll, and second group of 3 animals were vaccinated with LSD-Ndoll according the above mentioned protocol. 2 animals of the third group were served as a control and didn't receive any vaccine. Blood Samples were taken at the first, 14 and 28 day of vaccination. Animals were observed for body temperature, general condition and any lesions at the vaccination site.

Second stage were provided at the high containment facility, where animals at the 28th day after vaccination were moved. Dilutions of the virulent virus were administrated for animals all groups. Each virus dilution was inoculated in 4 points at the left side and 4 at the right side.

All animals were observed for favor, general condition and the lesions at inoculation sits. At the 14th day the Infectious doses of virus was calculated on control animals as well the virus infectious dose on vaccinated animals. Protective power of vaccines was calculated by determined the deference between viral titer in control and vaccinated animals.

Results

Vaccinated animal haven't developed any clinical signs, raisin temperature or lessons at vaccination site. Group animals vaccinated with LSD-Ndoll developed a small swell at the injection site for 3 days which were recovered later.

After challenge the nodules at inoculation site of the first dilution was developed at the 3-4th days in vaccinated and control animals. Animals of vaccinated groups were fully recovered at the 9th day of challenge while nodules in animal control group continued developing and generalized on all skin to the end of experiment. Results of progressive viral effect on animals reported in Table 1.

The maximum infective titer of the challenging virus in both groups of vaccinated animals reached in the 6th day of challenge with an average 1.7 log in groups animals vaccinated with Poxdoll and 1.68 log in group animals vaccinated with LSD-Ndoll (Figure 1).

The variation in viral infective values among animals of same group were minimal and within the acceptable standards Figure 2.

At the same regards, it is clear the difference in viral infectious titer in control and vaccinated animals is almost more than 2.5 log at all stages of disease developing (Figure 3).

Tablo 1. Virulent LSD virus titration results on animals (titer virus = value log_{10})

ID / Day	Control		Poxdoll			LSD-Ndoll		
	7756	7759	7757	7760	7761	7755	7758	7762
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	1.5	0	0	0	0	0	0
4	1.25	2.375	1.125	1.75	1.5	1.5	1.5	1.5
5	1.875	3.750	1.375	1.875	1.5	1.5	1.75	1.75
6	3.625	5	1.625	1.875	1.5	1.5	1.75	1.75
7	4	6.25	1.5	1.625	1.5	1.25	1.375	1.5
8	4	6.25	1	1	1.125	0.75	0.75	1
9	4	6.5	0.75	0.5	0.5	0.5	0.5	0.5
10	4	6.5	0	0	0	0	0.25	0
11	4	6.5	0	0	0	0	0	0
12	4	6.5	0	0	0	0	0	0
13	4	6.5	0	0	0	0	0	0

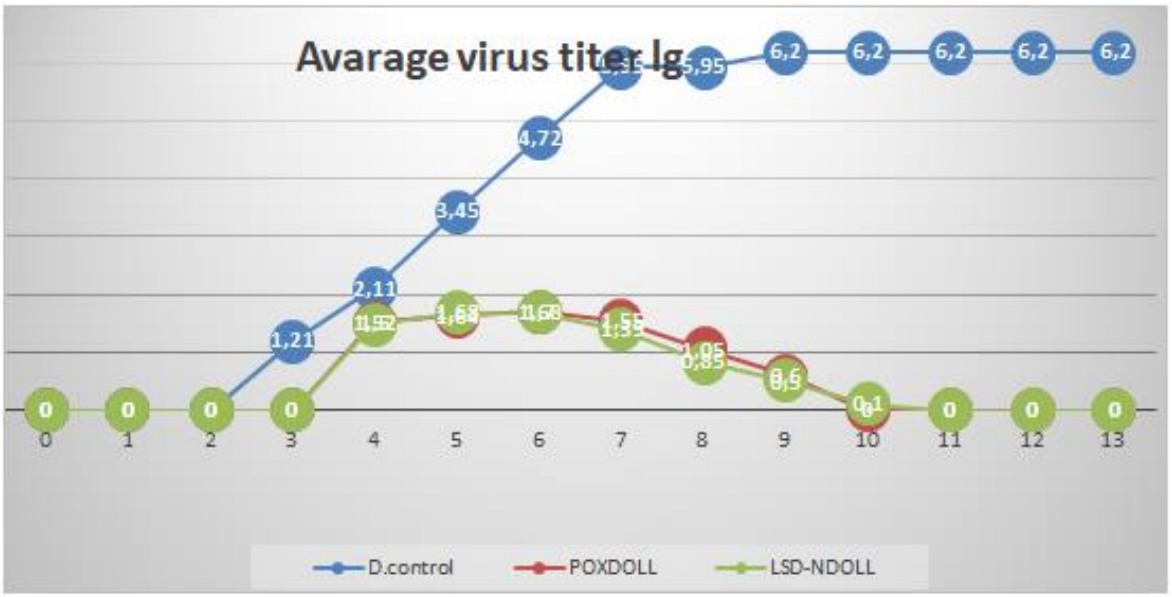

Figure 1. Virus infective titer average in control and vaccinated croups in log_{10}

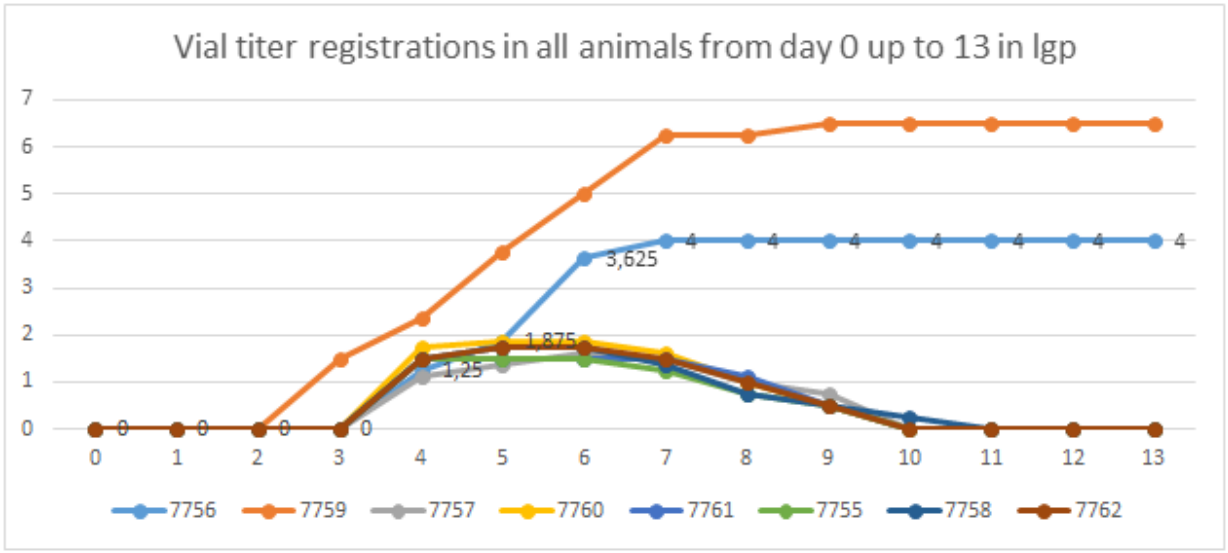

Figure 2. Virus infective titer in all animals from day 0 up to 13 log_{10}

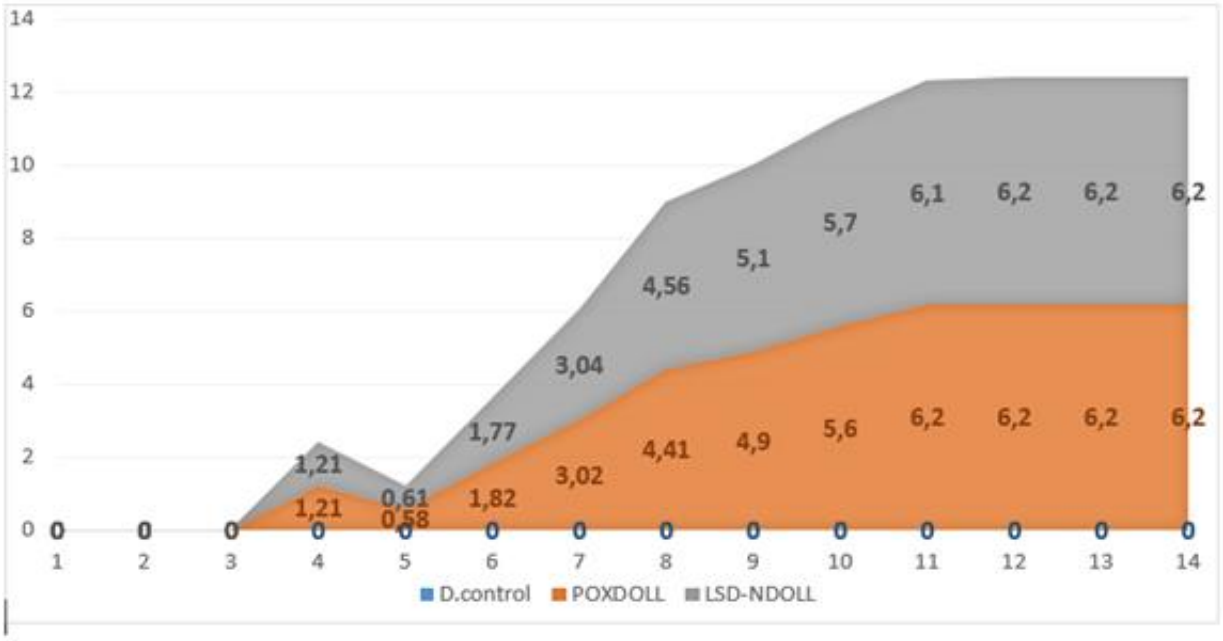

Figure 3. Difference between vaccinated and control animals during the experiment period log_{10}

Discussion

In Türkiye vaccination of animals against Lump Skin Disease started in 2014, any way vaccination was carried out with the heterologous vaccine based on sheep pox strain (Bakrkoy strain). According many authors, the heterologous vaccine based on sheep pox strain is less effective than the homologous strain vaccine (8). The yearly vaccination coverage achieved was around 10% in 2014, 46% in 2015 and 66% in 2016, and, although showing a marked decreasing trend since the first year of epidemics, LSD outbreaks are reported even in 2016 that is the fourth year of LSD epidemics, which started in Türkiye in August 2013, and the third year of vaccination (9). In the other hand, according EFSA report, Balkan countries started vaccination campaign with the homologous vaccines strain and it was reported where higher quicker vaccination coverage is achieved, the lower the number of monthly outbreaks

recorded and the quicker the outbreaks fade out (9). According the results of our provided experiment, no protection deference has been registered in animals vaccinated with LSD Neethling strain and those vaccinated with 10 sheep and goat doses of vaccine strain. Application 10 sheep doses of sheep pox Bakrkoy strain for cattle vaccination, was decided on the background of serological studies of vaccinated calves with 5 sheep doses, where the antibody titre was very low against LSD virus in neutralization test.

On the other hand, Ribonucleic analysis of Bakrkoy sheep and pox vaccine strain genome, reported identity with Neethling strain in 99,9%. Felid studies are very essential for drawing full image regarding the behaviour of Bakrkoy strain against the field infection with LSD.

The results of the present study support the use of sheep and goat pox vaccine, Poxdoll (Bakrkoy strain) and LSD-Ndoll (Neethling strain) vaccine for protection against LSD. However, both strains were observed to be safe for administration among cattle and without any adverse effects.

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