Etlik Vet Mikrobiol Derg, 2023; **34 (1):** 16-22
doi: https://doi.org/10.35864/evmd.1272630

Original Article
Özgün Araştırma

Investigation of simultaneous administration of Peste Des Petits Ruminants and Bluetongue vaccines in merino sheep

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Geliş Tarihi / Received: 28.03.2023, **Kabul Tarihi / Accepted:** 08.05.2023

Abstract: Peste des Petits Ruminants (PPR) and Bluetongue (BT), which are among the contagious animal diseases, cause serious economic losses and are included in the notifiable diseases by the World Organization for Animal Health (WOAH). PPR and BT vaccines are widely used in countries where outbreaks occur. In this study, we aimed to examine the antibody response of simultaneously administered PPR and BT serotype 4 vaccines in lambs and sheep. For this purpose, a study was performed under field conditions in a state-owned breeding farm. At first all the experimental animals were controled for PPR virus (PPRV) and BT virus serotype 4 (BTV4) antibodies by virus neutralization test (VNT). Subsequently, the experimental animals were divided into four groups. Group 1 (Group 1/PPR; lamb n=10, sheep n=10) and group 2 (Group 2/BTV4; lamb n=10, sheep n=10) were vaccinated only with the PPR or BTV4 vaccines, respectively. Group 3 was vaccinated simultaneously with PPR and BTV4 (Group 3/PPR-BTV4; lamb n=10, sheep n=10). Group 4 (lamb n=5, sheep n=5) was unvaccinated and served as the control. The vaccinations were carried out in each experimental group only once. At the third level of the study, blood samples were collected at intervals of 1, 3, and 6 months post-vaccination (mpv) to test the animals in terms of neutralised antibodies. A total of 280 post-vaccination blood serum samples were evaluated with virus neutralization test (VNT) for PPRV and BTV4 neutralizing antibodies. When the neutralizing antibody levels of the groups were compared, group 3 did not show any statistically significant difference ($p>0.05$) between group 1 and group 2. Based on the antibody response of simultaneous vaccination, it was shown that the simultaneous vaccination could be administered on the field, and could be labor and cost-effective.

Keywords: BTV, immunity, PPRV, simultaneous administration, vaccine

PPR (koyun keçi vebası) ve mavidil aşısının merinos ırkı koyunlarda eş zamanlı uygulanabilirliğinin araştırılması

Özet: Bulaşıcı hayvan hastalıklar arasında yer alan PPR ve Mavi dil, ciddi ekonomik kayıplara sebep olmalarından dolayı, Dünya Hayvan Sağlığı Örgütü (WOAH) tarafından ihbarı zorunlu hastalıklar içinde yer almaktadır. PPR ve Mavi dil aşıları, hastalık çıkışı olan ülkelerde yaygın olarak kullanılmaktadır. Bu çalışmada, PPR ve Mavi dil aşılarının aynı anda uygulanarak koyunlardaki antikor yanıtı üzerine etkilerinin incelenmesi amaçlanmıştır. Bu amaçla, çalışma devlete ait bir işletmede gerçekleştirilmiştir. Çalışmanın birinci basamağında, seçilen hayvanların aşılama öncesi kanları alınarak her iki hastalığa ait antikorlar yönünden araştırılmıştır. Bunu takiben, dört grup oluşturuldu. Grup 1 (Grup 1/PPR; kuzu n=10, koyun n=10) ve grup 2 (Grup 2/BTV4; kuzu n=10, koyun n=10) sadece PPR veya BTV4 aşısı ile aşılandı. Grup 3 (Group 3/PPR-BTV4; kuzu n=10, koyun n=10) her iki aşı ile eş zamanlı aşılandı. Grup 4 aşısız kontrol grubu (Grup 4; kuzu n=5, koyun n=5) olarak kullanıldı. Tüm aşılama gruplarında tek sefer aşı uygulandı. Çalışmanın üçüncü basamağında, seçilen hayvanlardan nötralize antikorlar yönünden test edilmesi amacıyla 1., 3. ve 6. aylarda serum örnekleri toplanarak toplamda 280 adet serum numunesi PPR ve Mavi dil antikorları virus nötralizasyon testi (VNT) ile nötralizan antikorlar yönünden kontrol edilmiştir. Gruplar arasında nötralizan antikor seviyeleri kıyaslandığında grup 3'ün 1. ve 2. gruplardan istatistiksel olarak anlamlı bir fark ($p >0.05$) göstermediği görülmüştür. Çalışma sonucunda elde edilen antikor yanıta dayanarak, PPR ve Mavidil aşılarının eş zamanlı olarak uygulanabileceği, iş gücü ve ekonomik açıdan tasarruf sağlayabileceği düşünülmektedir.

Anahtar kelimeler: Aşı, bağışıklık, BTV, eş zamanlı uygulama, PPRV

Introduction

Animal diseases, particularly viral epidemics influence production and trade ending with severe economic consequences. For this reason, it is necessary to determine useful and feasible control strategies against diseases (Diallo 1995). Successful vaccination regimens are major tools, particularly for prevention and sustainable control programs. Achieving these goals requires the use of high-quality and efficacious vaccines (Peta 2021). *Peste des petits*

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ruminants (PPR) and Bluetongue (BT) viruses are included as highly contagious pathogens that require strict control measures (Baron 2016).

PPR is a severe contagious viral disease of small ruminants. In naïve populations, it may reach high mortality rates. Although PPR is not considered a pathogen for cattle it is shown in case of exposure to *Peste des petits ruminants virus (*PPRV) cattle may seroconvert (Baron 2016; Selvaraj 2021).

As a member of Morbillivirus, PPRV is closely related to the Rinderpest virus (RPV). PPRV is assumed to spread widely following the strict control and eradication of RPV. Currently, for the eradication of PPR, WOAH, and FAO developed and endorsed the PPR Global Control and Eradication Strategy (PPR GCES) (Baron 2016; Eloiflin 2022).

There is a wide range of clinical outcomes including high fever, coughing, purulent nasal discharge, ulceration at oral commissure and tongue, pneumonia, diarrhea, anorexia and abortions in pregnant animals, and death. Host susceptibility varies considerably depending on immune status, host species, breeds, and environmental factors. Generally, goats are more susceptible than sheep (Eloiflin 2022).

PPRV is an enveloped virus with monosegment and negative sense ribonucleic acid (RNA). PPRV belongs to genus *Morbillivirus, in the* family Paramyxoviridae (Bamouh 2019; Baron 2016). As PPRV is not resistant to environmental conditions, direct contact is the most effective way of the infection between infected and healthy animals (Baron 2016).

For the control and prevention of PPR in endemic areas, vaccination is the most effective tool. Currently, PPRV/Nigeria 75/1 (lineage II) and PPRV/Sungri/96 (lineage IV) are the most common, safe, and efficacious vaccine strains and validated by WOAH for mass vaccination campaigns (Balamurugan 2014; Baron 2016; Bitew 2019).

In recent years there is in an uptrend in arboviral infections. As the effect of climate change is accelerated, an increase in arboviral infections may be expected depending on the changes in competent vector distribution as well as other factors (Belbis 2017; Chambaro 2020). *Bluetongue virus* (BTV) is of these arboviruses which belongs to the genus of Orbivirus within the family Reoviridae, and transmitted between its hosts by midges, especially *Culicoides* spp. (Ries 2021). BT is a major viral infectious disease of ruminants and has variable clinical symptoms. Camelidae show no clinical sign but turn seropositive with the infection and serve as reservoirs. Particularly in sheep the outcome of the clinical symptoms are severe. These cases are characterized by hemorrhagic fever, respiratory distress, loss of productivity, and death (Caporale 2014; Chambaro 2020; Mohd 2014).

The surface protein VP2 enables receptor binding, and hemagglutination and induces serotype-specific neutralizing antibodies. By this way VP2 plays the most important role in serotype affiliation. In recent years, in addition to 24 notifiable serotypes (based on virus neutralization test), the number of BTV serotypes has increased to a total number of 36 (Ries 2021). Both cellular and humoral immune responses are effective for preventing either BT infection or disease. The immune status of the infected animal has a direct effect on the course of BT. Since there are lots of challenges for vector control this makes the BT vaccination a more effective tool for disease control and prevention (Caporale 2014; Sánchez-Cordón 2015).

Due to their high economic impact, both PPR and BT are included in World Organisation for Animal Health (WOAH) listed diseases (Belbis 2017; Bréard 2011). In Türkiye, control and prevention of these infections are carried out by PPR and BT4 vaccination. In this study, it was aimed to investigate simultaneous administration of PPR and BT vaccines in lambs and sheep in order to evaluate the level of protective immunity.

Materials and Methods

Animals, vaccines, and experimental design

Merino sheep were preferred in field studies as they are one of the most susceptible breeds against BT. In the study, 35 merino lambs between 4-8 months old, and 35 PPR seropositive merino sheep over 1 year old were used. The study was performed at a state farm in Polatlı province.

Live attenuated PPR (Nigeria 75/1 strain) and Bluetongue (SA BT-4 strain) lyophilized vaccines were obtained from the Viral Vaccine Production Laboratory of the Veterinary Control Central Research Institute.

In the first phase of the study, serum samples were collected pre-vaccination and investigated in terms of neutralizing antibody for the presence of both diseases.

Following serological controls the sheep were randomly selected into four groups and vaccinated as follows:

- Group 1/PPR: 10 lambs and 10 sheep received subcutaneous injections in the loose skin of the axillary region, of 1ml containing $10^{3,7}$ tissue culture infective dose 50% ($TCID_{50}$)/ml of PPR vaccine.

- Group 2/BT: 10 lambs and 10 sheep received subcutaneous injections in the loose skin of the axillary region, of 1ml containing $10^{3,3}TCID_{50}$/ml of BT vaccine.

- Group 3/PPR-BT: 10 lambs and 10 sheep received 2 subcutaneous injections in the loose skin of both axillary regions, of 1ml containing $10^{4,2}TCID_{50}$/ml of PPR and $10^{3,6}$ $TCID_{50}$/ml of BT vaccine.

-Control group: 5 lambs and 5 sheep were left unvaccineted.

All the groups were observed daily for clinical signs for a period of 21 days post-vaccination.

Serum samples collection

Serum samples were collected from all animals prior to vaccination (day 0) and at 1, 3, and 6 months post-vaccination (mpv).

Sera collected were examined for antibodies to PPR and BTV by neutralization test.

Virus neutralization test

The virus neutralization (VN) tests were performed as per WOAH Terrestrial Manuels (WOAH 2021b, 2021a). Briefly, for PPR twofold serial dilutions of inactivated serum samples were prepared to start from 1/5 dilution, and mixed with 100µl of virus at 1000 $TCID_{50}$/ml PPR vaccine strain. Following incubation at 37°C, 5% CO2 for 1 hour, Vero cell suspension 600.000/ml was added to 96-well tissue culture plate, and incubated at 37°C, 5% CO_2 till the cytopathic effect (CPE) in virus control wells for virus was evident (7–12 days).

For BT, 50 µl of twofold serial inactivated serum dilutions, starting from 1/10 mixed with an equal volume of BTV4 vaccine strain and incubated at 37°C, 5% CO2 for 1 hour. After incubation, 10^4 Vero cell was added to each well in a volume of 100µl. Plates were incubated at 37°C, 5% CO2 for up to 7 days.

Both tests were accepted valid if serial dilutions of the test virus gave the following results: 100% CPE at 100 and 10 $TCID_{50}$/well; %50 CPE at 1 $TCID_{50}$/well; %0 CPE at 0.1 $TCID_{50}$/well.

For both infections a neutralizing titer of greater than >1/10 (1 $\log_{10}$) was considered as positive (protective titer) (Saravanan 2010; WOAH 2021b, 2021a).

Statistical analysis

Antibody titers were log-transformed to log 10 for all statistical processes and are presented as arithmetic mean titers. VN test results were used to compare titers between vaccination groups for the duration of the immunity study by conducting T-test at each sampling point post-vaccination. Since the number of samples in each group was less than 30, T-test was used to calculate the results and perform statistical analyses. A one-tailed T-test for 2 independent means was used to calculate the main results. A p value of <.05 was accepted as a significant result for all parameters.

Results

In this study, the protective immunity of simultaneous administration of PPR and BT4 vaccines was evaluated according to WOAH recommendations (WOAH 2021b, 2021a).

The immunological status of the vaccinated animals was monitored during a follow-up of study of 6 months. While all the animals were seronegative for BT pre-vaccination, initial serum samples of sheep (>1 year old) and 9 lambs showed seropositivity (log10 titer=1-1.3) for PPR.

All the animals in the control group remained seronegative for BTV throughout the study. For PPR, the unvaccinated lamb control group remained seronegative. No difference was detected in the endpoint titer of seropositive sheep (>1 year old) during the sampling period.

When the control group and the vaccinated groups were compared, no difference was observed in terms of clinical and local findings.

VN antibody response and duration of immunity

Post-vaccination mean VN antibody titers at each sampling interval over 6 months for the three groups were presented in Table 1.

Group 1 (lambs) and group 3 (lambs) were comparison is shown in Figure 1. At the end of the 1 mpv, all the animals in each group demonstrated >1 log10 VN antibody titer for PPR. There were 3 animals in each group which was seropositive pre-vaccination, most likely originating from maternal antibodies. Following the PPR vaccination, the evolation of endpoint titer by VN antibody response gives a four-fold increase. According to the titers at the end of 6 months, group 1 (lambs) *(M= 1.93, ±SD=0.2)* compared to group 3 (lambs) *(M=*

1.89, ±SD=0.21) demonstrated that the result was not significant (p > .05)*; t= 0.08, p= .47* (Figure 1).

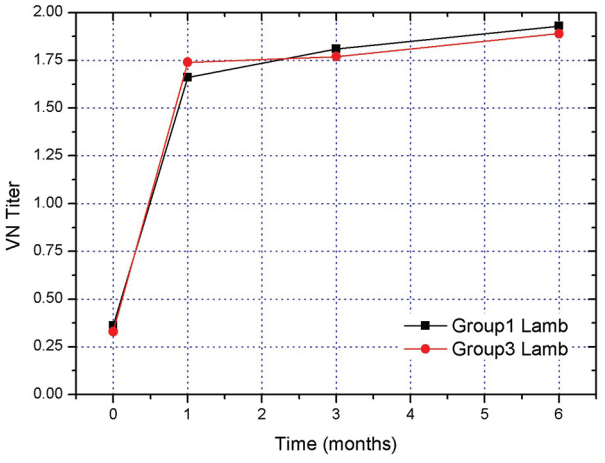

Figure 1. Group 1 (lamb) and group 3 (lamb) mean (±SD) antibody titers over 6 months

Group 1 (sheep) and group 3 (sheep) were both seropositive for PPR, so pre-vaccination antibody titers were considered as the baseline, and differences in endpoint titer obtained from experimental PPR vaccination were evaluated (Table 1). When the results of antibody titers of sheep in group 1 and group 3 were compared with each other for all sampling intervals (1 mpv, 3 mpv, and 6 mpv) they showed no significant difference (p>.05) *t=0.009, p= .50; t=0.137, p= .44; and t=0.08, p= .47,* respectively (Figure 2).

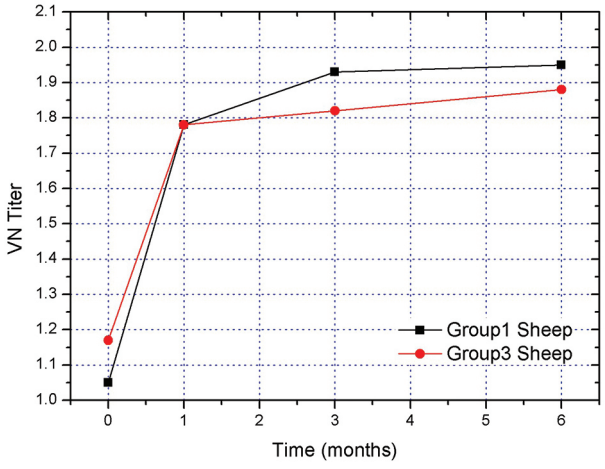

Figure 2. Group 1 (sheep) and group 3 (sheep) mean (±SD) antibody titers over 6 months

Comparing antibody titers of the 1 mpv, 3 mpv, and 6 mpv serums for both PPRV and BTV respectively revealed that the sheep in each group was higher than lambs, but the difference was not significant (p>.05) between the age groups (Table 1).

A decline was observed in 3 mpv for each vaccine and age group. Thereafter, the seronegativity percentage was raised up 80 %, and the VN antibody titer at the remaining animals was close to the threshold *(M= 1-1.75, ±SD=0-0.1)*. For this reason, the BT VN antibody titers were evaluated over 3 mvp. At 1 mpv, mean BT antibody titers were 1.84 log10 *(±SD=0.20)*, and 1.76 log10 *(±SD=0.21)* for group 2 (lamb) and group 3 (lamb), respectively (Figue 3). T-test calculations were t=0.172, p = .432 and the result was not significant (p > .05), and 3mvp mean antibody titers were seen to decline to 1.48 log10 *(±SD=0.30)*, and 1.54 log10 *(±SD=0.29)* for group 2 (lamb) and group 3 (lamb), respectively with a t-test result t= 0.147, p= .441. This result was also not significant (p >.05). (Table 1).

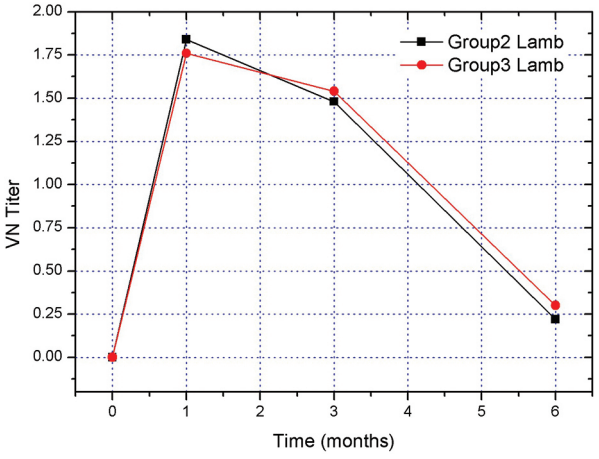

Figure 3. Group 2 (lamb) and group 3 (lamb) mean (±SD) antibody titers over 6 months

Similar results were observed in group 2 and group 3 sheep (Table 1). Group 2 which received BT alone was compared at the end of 1 mpv *(M=1.87, SD=0.30)* and 3 mpv *(M=1.57, SD=0.31)* to group 3 *(M=2.08, SD=0.20)* and *(M=1.88, SD=0.21)* respectively. The statistical evaluation of pairwise mean antibody levels recorded in both sampling periods showed that the differences were not significant (*p >.05)* between the two groups (Figure 4).

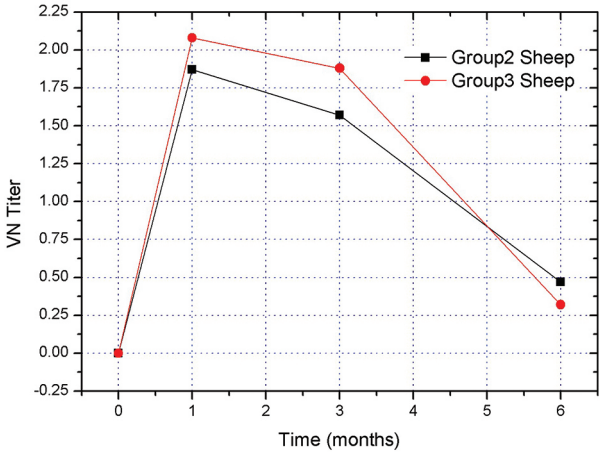

Figure 4. Group 2 (sheep) and group 3 (sheep) mean (±SD) antibody titers over 6 months

Table 1. Comparison of mean antibody titers between the 3 groups at each post-vaccination sampling time

Sampling intervals (mpv)	Group 1		Group 2		Group 3			
	Mean VNT titer (log10)/ (±SD)		Mean VNT titer (log10)/(±SD)		Mean VNT titer (log10)/(±SD)			
	Lamb	Sheep	Lamb	Sheep	Lamb		Sheep	
					PPR	BT4	PPR	BT4
0	1.20 (0.14)	0,16 (0.20)	-	-	1.1 (0.14)	-	1,3 (0.24)	-
1	1,66 (0,25)	1,78 (0.27)	1,84 (0.18)	1,87 (0.29)	1,74 (0.30)	1,76 (0.21)	1,78 (0.20)	2,08 (0.20)
3	1,81 (0.23)	1,93 (0.14)	1,48 (0.30)	1,57 (0.31)	1,77 (0.29)	1,54 (0.30)	1,82 (0.30)	1,88 (0.21)
6	1,93 (0.19)	1,95 (0.16)	0.22 (0.41)	0,47 (0.58)	1,89 (0.21)	0,30 (0.48)	1,88 (0.21)	0,32 (0.48)

Discussion

Vaccine-induced immunity is not only related to vaccine quality, also proper vaccination is another important point. Our study was aimed to compare the protective immunity of PPR and BT vaccines in combination vs. individual administration, to support labor and cost-effective vaccination strategies, and to contribute to animal welfare by reducing vaccination stress in animals.

The field trial carried out with 70 animals and none of the vaccinated animals experienced any adverse effect. The results demonstrated there was no significant difference between elicted immune response with both admistration methods.

For both PPR and BT in addition to cellular immunity, VN antibodies have an essential role in the immune response. Since, modified- live-attenuated virus (MLV) vaccines elicit a strong immune response, vaccine induced VN antibodies are considered as an indicator of protective immunity (Huismans 1987; Jeggo, Wardley, and Brownlie 1984; Tatar and Kabaklı 2006).

Pre-vaccination seropositivity for PPR in lambs and sheep was an expected result because of the mass PPR vaccination. Lambs born from PPR vaccinated sheeps retain passive immunity between 4-5 months (Tatar and Kabaklı 2006). Following the vaccination of group 1 and 3 with PPRV seropositive lambs (n=9) showed a two- to four-fold increase in VN antibody levels. Maternal PPR VN antibodies present at the first vaccination are associated with reduced titers following vaccination (Tatar and Kabaklı 2006). In a study it was stated that Measles maternal antibodies lost avidity at a faster rate than antibodies induced by natural disease (Collins 2020). PPR antibodies increase following the vaccination of lambs can be explained by low affinity antibodies.

In a doctoral thesis completed in 2019, the avidity level developed after PPR vaccination was examined and it was stated that the avidity in the 12th month following the vaccination was higher than the antibody avidity due to natural infections ($p<0.001$) (Ali and Özkul 2019).

The duration of antibody mediated immunity was compared between groups. In all three groups, the duration of PPR immunity lasted for 6 months. This result was consistent with previous studies (Baron 2016; Tatar and Kabaklı 2006).

Since the ongoing global PPR eredication campaigns (PPR GCES) there is a very high percentage PPR seropositivity. All the animals in group1 and group 3 showed a VN antibody titer increase following both single (PPR) and combined vaccination (PPR-BT), because of the existing antibodies, probably we obtained a partial antibody increase following the vaccination. A long term study should be conducted for the follow up results.

It was determined that the antibody response following the BT vaccination in all groups decreased below the acceptable titer value of 1 log10 in the period up to the 6th month. However, there are studies showing that the cellular immune response maintains its protective effect despite this decrease in neutralizing antibodies. In these studies, it has been reported that both neutralizing antibodies and cytotoxic T lymphocytes play a major role in the protective immune response to BT, and the cellular response plays a vital role even in the absence of neutralizing antibodies (Jeggo1984; Rojas 2011).

According to the comparison results, it was determined that there was no statistical difference between the simultaneous and individual administration of both vaccines. Both administration methods created sufficient protective antibody levels. As a result, it was concluded that live attenuated PPR and Bluetongue vaccines can be administered simultaneously.

Information: 3-5 November 2022 – The oral presentation was performed at the 4th International Vaccine Science Congress.

Acknowledgments: We are grateful to Mustafa GÖÇTÜ veterinary officer in the Ministry of Agriculture and Forestry

Deney hayvanları kullanımı etik kurulu ve diğer etik kurul kararları ve izinler: Gıda ve Kontrol Genel Müdürlüğü 229.12.2014 tarihli ve 55016929-325-01.04/47612 sayılı yazısına istinaden 18.02.2015 tarih ve 2015/03 karar no ile deney hayvanları kullanımı etik kurul raporu alınmıştır.

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