Tropical Biomedicine 39(1): 47-54 (2022)
https://doi.org/10.47665/tb.39.1.002

RESEARCH ARTICLE

Antibody response of sheep to simultaneous vaccination of foot-and-mouth disease, peste des petits ruminants, sheep pox and goat pox, and bluetongue vaccines

Sareyyüpoğlu, B.[1*], Gülyaz, G.[2], Saraç, F.[3], Uzar, S.[3], Kabaklı, Ö.[4], Çokçalışkan, C.[1], Gündüzalp, C.[1], Uzun, E.A.[1], Camli, O[5]

[1]Republic of Turkey, Ministry of Agriculture and Forestry, Institute of Foot and Mouth Disease (SAP), Ankara, Turkey
[2]Harran University, Faculty of Veterinary Medicine, Virology Department, Şanlıurfa, Turkey
[3]Republic of Turkey, Ministry of Agriculture and Forestry, Pendik Veterinary Control Institute, Viral Vaccine Production Laboratory, İstanbul, Turkey
[4]Republic of Turkey, Ministry of Agriculture and Forestry, Etlik Central Veterinary Research Institute, Viral Vaccine Production Laboratory, Ankara, Turkey
[5]Middle East Technical University, Department of Statistics, Ankara, Turkey
*Corresponding author: beyhan.sar@gmail.com

ARTICLE HISTORY

Received: 23 November 2021
Revised: 28 December 2021
Accepted: 28 December 2021
Published: 31 March 2022

ABSTRACT

There are many infectious animal diseases in Turkey and generally, vaccination is the primarly control strategy to combat them. However, it is difficult to apply all vaccines in a definite period in the field due to limitations of the labor and finance. Rapid vaccination and effective use of labor can be possible with the help of simultaneous vaccine administrations. The study aims to show the effects of simultaneous foot-and-mouth disease (FMD), peste des petits ruminants (PPR), sheep pox and goat pox (SGP), and bluetongue (BT) vaccine administration on the antibody response of sheep. For this aim, 30 sheep were divided into one experiment and 5 control groups. Blood samples were collected in each group at 0, 30 and 60 days post-vaccination (DPV). Immune response was measured with virus neutralization test (VNT) and, liquid phase blocking ELISA (LPBE) for FMDV; VNT for BTV and PPR. A live virus challenge study was performed to determine the immune response of SGP vaccine. As a result, antibody titers for each vaccine agent decreased on 60 DPV with the simultaneous vaccination except FMD. The difference between means of antibody titer decrease with single and simultaneous vaccinations is significant especially for BTV and PPR vaccines at 60DPV ($p<0.05$). Briefly, this decreasing immune response of three live vaccines can be explained with the development of the interference, administration of these vaccines from the same injection site, the effect of cytokines, especially IL-10 effect of SGP vaccine. It was concluded that four vaccines can not be used simultaneously in sheep.

Keywords: Antibody response; viruses; simultaneous; sheep; vaccination.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease caused by Picornavirus. The disease affects cattle, pigs, sheep, goats and other cloven-hooved ruminants. It decreases the livestock production and causes an enormous economic impact. Sheep pox and goat pox disease (SGP), which is a generalized viral infection in sheep and goats, cause severe clinical disease in either sheep or goats. SGP virus is classified in the *Poxviridae* family (Kitching, 1986). The disease causes lesions in the skin and internal organs besides abortion, mastitis, and deaths in lambs and kids. Peste Des Petits Ruminants (PPR) is an acute viral infection caused by the morbillivirus (OIE, 2020). PPR is widespread in Africa, Arabian Peninsula, Middle East, and Turkey (Özkul *et al*., 2002). Blue Tongue (BT) is a viral disease of ruminants and it is transmitted by insects. The virus is classified in the genus Orbivirus of the *Reoviridae* family. The disease is characterized by congestion, oedema, and haemorrhage in the oral and nasal tissues, inflammation of the coronary band, and lameness besides decreasing the fertility rate causing significant economic losses in the industry (Maclachlan, 2011).

Vaccination is the primary control strategy against FMD, SGP, BT, and PPR in Turkey and other endemic countries (Özkul *et al*., 2002; Perry & Rich, 2007; Saegerman *et al*., 2008; Khorasani *et al*., 2016). The animals are injected simultaneously at different sites of the body during the simultaneous vaccine implementations. Since many factors can affect the immunity of animals, the effect of simultaneous vaccine administrations

on immune response should be examined in detail (Trotta *et al*., 2015). The main considering factor should be the interference. In this case, one viral vaccine agent suppresses the replication of another which cause inadequate immunity in the animal. The interference between different components of vaccines can result in very different immunological effects such as antigenic competition. It means that a vaccine antigen may lead to a decrease in immune response in the presence of the other antigen (Boikos *et al*., 2017; Tizard, 2017). Another factor is the epitope sharing between the vaccine viruses, in that an increased or a decreased immune response may be observed in the organism (Dagan *et al*., 1998). Interactions of adjuvants among the vaccines can be possible another factor to consider during the vaccine co-administration (Fox *et al*., 2013). A live vaccine must replicate in an organism to show it's efficacy. In other words, it almost gives the same immune response as the natural immunity of its agent. The main mechanism is the stimulation of macrophage cells by the live antigen following the presentation of antigen to the T-lymphocyte cells. Then, the cellular and humoral immunity develop by the secretion of interleukins and other immune elements (Tizard, 1990; Tizard, 2017).

Rabies, swine fever, vesicular stomatitis, anthrax, Brucellosis, rinderpest, hemorrhagic septicemia, and parvovirus vaccines are used simultaneously with the FMD vaccine in the world (Castenada *et al*., 1976; Joseph & Hedger, 1984; Hedger *et al*., 1986; De Clercq *et al*., 1989a; Srinavasan *et al*., 2001; Trotta *et al*., 2015; Hancı, 2016; Çokçalışkan *et al*., 2019; Gülyaz *et al*., 2019). In a study (Hedger *et al*., 1986), the adequate immune response were provided against both agents during the simultaneous rinderpest and FMD vaccine administrations. In another study (De Clercq *et al*., 1989b), the interference was not observed with the simultaneous administration of IBR, adenovirus, and parainfluenza-3 vaccines in pigs. The result was favorable with the simultaneous vaccination of three modified live virus vaccines in pigs (Kristensen *et al*., 2018). However, in contrast to these studies, in a research (Casteneda *et al*., 1976) the lack or the low antibody response were obtained with the simultaneous application of vesicular stomatitis and FMD vaccines, and it was reported that interference was responsible for the inadequate antibody response.

In Turkey, there are only three reports about simultaneous vaccine administration in veterinary field (Hancı, 2016; Çokçalışkan *et al*., 2019; Gülyaz *et al*., 2019). The first of these studies (Hancı, 2016) is related to the simultaneous use of brucella and FMD vaccines in calves and lambs, as a result it was not determined any decrease in FMD vaccine antibody titers while an overall increase in brucella antibody titers was detected. The researchers reported that it was the positive effect of the oil adjuvant in the FMD vaccine on the cellular immunity of animals through cytokines. Since the adjuvants are used to stimulate the immune system more by creating a depot effect in vaccines. Thus, they increase the quantity of immunoglobulin obtained by stimulating macrophages. The second study (Çokçalışkan *et al*., 2019) is related to the simultaneous use of anthrax and FMD vaccines and it was detected higher antibody response to FMD virus in the simultaneous group. The authors declared that the higher FMD response was related to the cytokine increase induced by the live anthrax vaccine and this was supported the explanation based on cellular immunity. Briefly, the vaccine agents of these two studies are composed of live bacterial agents (anthrax and brucella) and the stimulation of the cellular immune response by live bacterial cells was one of the most reliable explanations. Third study (Gülyaz *et al*., 2019) is related to the simultaneous and combined use of ecthyma and FMD vaccines in sheep and it was reported antibody decrease in simultaneous group and especially this decrease was significant in combined group. Thus, it was reported that ecthyma virus could have stimulated the release of immunomodulatory cytokines and this could lead a decrease in the cellular immune response. Therefore, in that study, immunostimulant effect of ecthyma vaccine could not be determined on the antibody response of sheep. Altogether, it is difficult to compare these three studies to this study since the vaccine agents, the number of vaccine agents in vaccine among the simultaneous vaccine administration groups used with FMD vaccine are different. The vaccines used in the present study are the commercial vaccines routinely used in the vaccination campaign in Turkey. The simultaneous application of these vaccines will reduce the number of visits to farms and significantly decrease the labor force and cost used in the vaccinations. In general, the immune response for each vaccine agent with simultaneous vaccine administration is affected positively or at least it was not changed in previous studies. For this purpose, this study aimed to determine the effect on the immune response of sheep against each of four vaccine agents when the SGP, BT, PPR, and FMD vaccines were used simultaneously.

MATERIALS AND METHODS

Vaccines

A commercial (TURVAC oil) 6PD50, bivalent, inactive double oil emulsion FMD vaccine containing O/TUR07 and A/NEP84 strains and Montanide ISA 206 adjuvant (Seppic, France) produced by the SAP Institute in Ankara. Live attenuated sheep goat pox (SGP) vaccine (PENPOX-M) including at least $10^{2.5}$DKID50/ml-strain of Bakırköy is produced at Pendik Veterinary Research Institute in İstanbul. Live attenuated PPR vaccine (PEST-S ETVAC) containing at least the $10^{2.5}$DKID50/ml-strain of Nigeria 75/1 and live attenuated BTV vaccine (BLU T4 ETVAC) containing at least the 10^{2}DKID50 /ml-strain of SA/BTV-4 were produced by the Etlik Central Veterinary Research Institute in Ankara.

Animals and immunisation route

A total of 30, sixth-months-old male FMD, BT, SGP, and PPR viruses antibody seronegative Merino sheep obtained from a state farm were used in the study. The animals were randomly divided into 6 groups (Table 1). To determine their seronegativity against FMDV, BTV, PPR and SGP; blood samples were collected on day 0 and analyzed via enzyme linked immunoassay (ELISA) and virus neutralization test (VNT) for FMDV and virus neutralization test (VNT) for BTV, PPR, and SGPV. Then, the animals were vaccinated subcutaneously against the four different agents at the same time at Pendik Veterinary Research Institute. A total of 1 ml FMD vaccine was administrated to the right pre-axillar region of animals and 1 ml of each live vaccines (BT, PPR and SGP) were administrated with separate injection sites on the left side of the pre-axillar region. Blood sera were collected 30 days post-vaccination (DPV) and then, sheep pox and goat pox virus challenge study was initiated. Afterthat, blood samples were collected at 60DPV. Serum samples were stored at -20°C until analyzed. All vaccinated sheep were monitored daily for the body temperature, SGP lesions, and appetite during the study. The study was conducted according to EU directive 2010/63EU for animal experiments and under the authorization of the local animal ethics committee (23.01.17/03-1).

Table 1. Group of animals

Groups		Number of animals (n)
1	FMD, SGP, PPR, BTV simultaneous vaccination	10
2	FMD single vaccination (control)	4
3	SGP single vaccination (control)	4
4	PPR single vaccination (control)	4
5	BTV single vaccination (control)	4
6	Unvaccinated (negative control)	4

Serological assays and live virus challenge study

Foot and Mouth Disease

FMDV virus circulation of animals was determined via antibody detection against FMDV non- structural protein (NSP) as recommended by the kit with the day 0 sera (PRIOCHECK FMDV NS ELISA, The Netherlands) (Sørensen *et al*., 1998). To determine total antibody response of animals, LPBE was performed (Hamblin *et al*., 1986; OIE, 2021a). Briefly, ELISA plates were coated with rabbit antibody (against anti-FMDV 146S antigens). Meanwhile, test and control sera were added to the carrier microplate. Then working dilution of FMDV type O, type A were added. The carrier and the ELISA plates were incubated at 4°C. On the second day of the test, following washing of ELISA plate, the mixture of serum/antigen was transferred from the carrier microplate to the ELISA microplate. Then, the plates were incubated at 37°C for 1 hour. After washing, 50 µl anti-FMDV type specific guinea pig antibodies (DAKO, P0141, Denmark) were added and incubated at 37°C for 1 hour. Then 50 µl working dilution of the conjugate was added to the wells and incubated in at 37°C for 1 hour. Then, chromogen OPD/Substrate (H_2O_2) was added to each well, and incubated at room temperature for 15 minutes. Finally, 50 µl 1.25 M sulphuric acid was added to the wells. The absorbance was read by the microplate reader (Versamax, Molecular Devices, USA) at 492 nm. The protective cut-off titer of LPBE was accepted as 1/96.

VNT was performed for neutralizing antibody response (OIE, 2021a). Glasgow-MEM containing 50 µl of Fetal Calf Serum (FBS-BIOWEST, France) were placed in all wells of a 96-well plate. 50 µl of the each serum was placed as duplicated in the first wells of the plate and, 50 µl solution from the first wells were transferred to the lower wells to make a two-fold dilutions of the serum. FMD virus strains in 50 µl of 100 $DKID_{50}$ was added to the two-fold dilution of serum samples in the wells and left to neutralize for one hour at 37°C. At the end of the incubation period, 50 µl of BHK21 cell culture was added to all wells and incubated in 5% CO_2 medium for 72 hours at 37°C. Cells were checked daily for CPE and evaluated by staining with crystal violet (SIGMA-ALDRICH, USA). The protective cut-off titer of VNT was accepted as 1/22.

Sheep Pox and Goat Pox

To determine the antibody response of animals at day 0, VNT was performed. Every step almost similar to the FMDV VNT assay, but the main difference was at the end of the incubation period, 50 µl of Vero cell culture was added to all wells and incubated in 5% CO_2 medium for 10 days at 37°C. Cells were checked daily for CPE. Since the immunity to SGP is predominantly cell-mediated and infected or unprotected animals after vaccination may produce undetectable low levels of neutralising antibodies, the immune response of SGP vaccination was evaluated according to live virus challenge study (OIE, 2021d).

To perform the live virus challenge study, $10^{6,25}$/ml $TCID_{50}$ titer of SP(İ)LK5 pathogen field strain was diluted tenfold and 0.1 ml of each dilution was injected via intradermal route at four different sites with 5 cm interval among the injection sites at the abdomen of sheep. The dilutions of 10^{-1}, 10^{-2} and 10^{-3} were applied to the left side. The dilution of the 10^{-4} and 10^{-5} were applied to the right side (Group 1 and 3). Sheep were monitored daily for 14 days immediately after the live virus challenge study for body temperature, SGP lesions, changes in the inoculation sites. The immune response of SGP vaccination was evaluated by observation of clinically protected animal numbers in the population (n=10). The challenge titer of virus was calculated at 8-10. day of challenge. The titre of the challenge virus between the vaccinated and control animals must be $\geq \log10^{2,5}$ and a difference of log10 titre$\geq$2.5 is accepted as evidence of protection (OIE, 2021d).

Peste Des Petits Ruminants

To determine the immune response of animals, VNT was performed (OIE, 2021c). Each serum was worked in double-well. A total of 80 µl EMEM (GIBCO, USA) were placed in the first row of a 96-well plate. Then 50 µl EMEM was added the other wells. 1/5 dilution was prepared in the first row when the 20 µl serum samples were added to the first row. After that, 50 µl solution from the first wells was transferred to the lower wells to make a two-fold dilutions of the serum. PPR virus of Nigeria 75/1 strains in 50 µl of 1000 DKID50 was added to the two-fold dilution of serum samples in the wells and let to neutralize for one hour at 37°C. At the end of the incubation period, 50 µl of Vero cell culture was added to all wells and incubated in 5% CO2 medium for 12 days at 37°C. Cells were checked daily for CPE. The protective cut-off titer of VNT was accepted as 1/10.

Bluetongue

To determine the immune response of animals, VNT was performed (OIE, 2021b). Each serum was worked in double-well. A total of 90 µl EMEM (GIBCO, USA) were placed in the first row of a 96-well plate. Then, 50 µl EMEM was added the other wells. 1/10 dilution was prepared when the 10 µl serum samples were added in the first row. After that, 50 µl solution from the the first wells were transferred to the lower wells to make a two-fold dilutions of the serum. BT virus of SA/BT-4 incubation period, 50 µl of Vero cell culture was added to all wells and incubated in 5% CO2 medium strains in 50 µl of 100-300 DKID50 was added to the two-fold dilution of serum samples in the wells and let to neutralize for one hour at 37°C. At the end of the for 5-7 days at 37°C. Cells were checked daily for CPE. The protective cut-off titer of VNT was accepted as 1/10.

Statistical analysis

Parametric and non-parametric statistical methods were used for independent samples with single and simultaneous vaccinations at 30DPV and 60DPV. The Shapiro-Wilk test, two sample t-test, and Wilcoxon rank sum test were performed to test the normality assumption for all samples. The proportion of protected animals with the single and simultaneous vaccination were compared to investigate the effect of SGP vaccine at 60 DPV since the results of live virus challenge study are not quantitative. Z-test was used for the comparison of the proportions. R language and environment (R Core Team, 2020) was used to carry out all analyses in this study (Carey, 2015).

RESULTS

It was not determined any seropositive animal against FMD, BT, PPR, and SGP viruses at day 0 (data not shown). The mean antibody titers for FMD, BT, and PPR on day 60 DPVs with the simultaneous and single vaccination groups were shown in Figure 1. The mean antibody titers, standard errors and p-values for the significance of differences in mean antibody titers between the single and simultaneous vaccinations at 30 and 60DPVs are shown in Tables 2 and 3, respectively. It can be concluded that the difference between means of antibody titer decrease with single and simultaneous vaccinations are significant for BT and PPR vaccines at 60 DPV ($p \leq 0.05$). However, since the p-values are less than the significance level of 0.05, the decision is to reject the null hypothesis. According to statistical results, a decrease of antibody titers was found statistically significant between the 30DPV and 60DPV especially for BTV and PPR vaccine agents ($p<0.05$), (Tables 2 and 3).

As for SGP vaccine, 5 sheep were clinically protected in the population (n=10) and proportion of protected animals is not significantly different between the single and simultaneous vaccination groups with a p-value = 0.1712, which is greater than the significance level α = 0.05. This result may be partially explained by the small sample size in the group. The rectal temperatures of sheep were depicted in Table 4. It was seen constant high rectal temperatures of non-protected sheep (Sheep code: 28) but it was determined only a short period increase after the first five days of challenge in protected animal rectal temperature (Table 4). Titer differences of protected and non-protected animal in the simultaneous vaccination group were shown in Table 5.

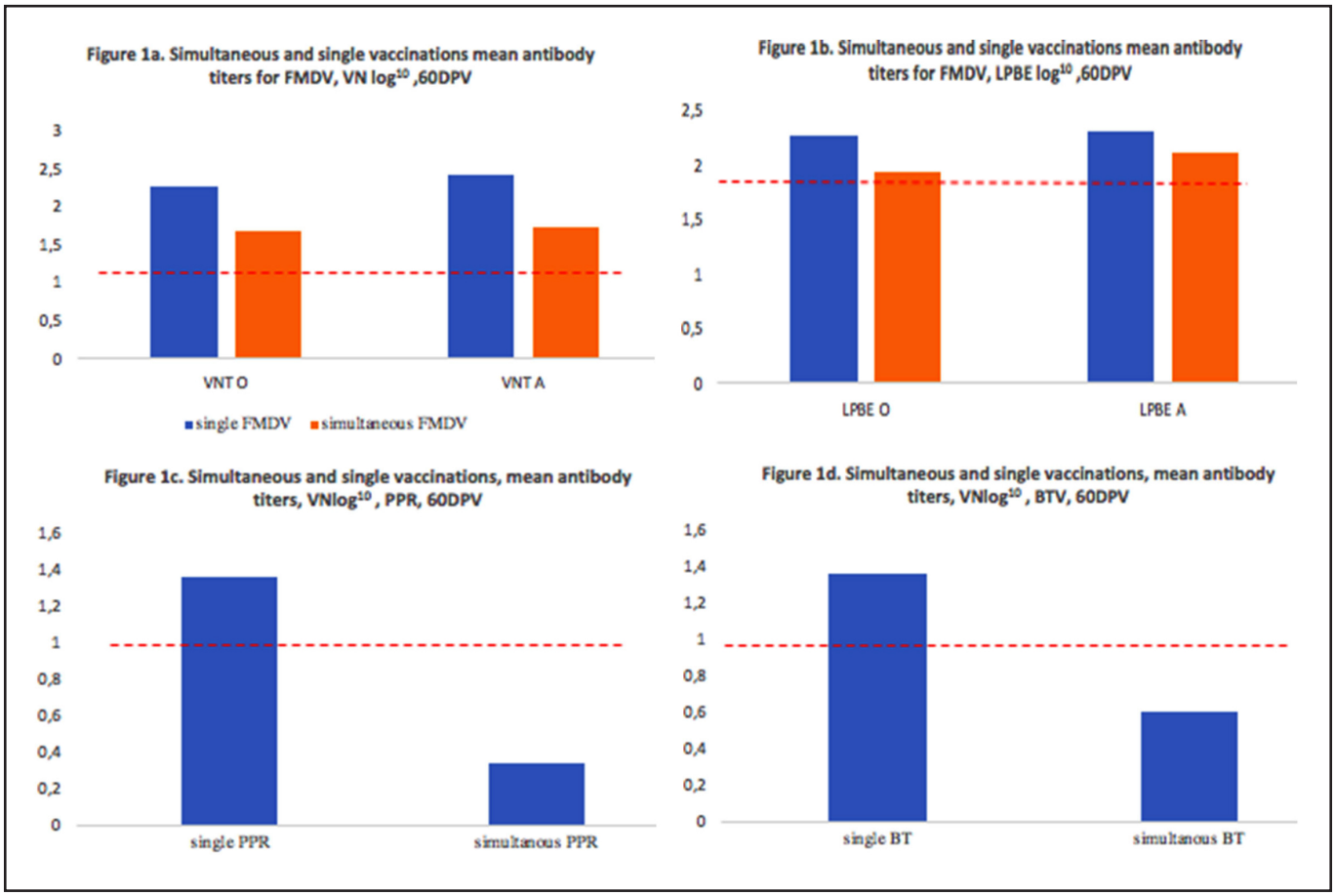

Figure 1. Comparison of mean antibody titers (log^{10}) in simultaneous and single vaccination groups of each vaccine, 60 days post vaccination (60DPV). FMDV VNT antibody titers for O TUR O7 and ANEP 84, FMDV ELISA antibody titers for O TUR O7 and A NEP 84, PPR VNT antibody titers, BTV VNT antibody titers. Red lines show the protective cut off titers (log^{10}) for each vaccine agents.

Table 2. Mean and standard errors of antibody titers and p-values at 30 DPV

Vaccine agent/test method	Single vaccination	Simultaneous vaccination	p-value
FMDV serotype O/ VNT	1.73 ± 0.47	1.64 ± 0.29	>0,05
FMDV serotype A/ VNT	1.73 ± 0.47	1.41 ± 0.24	>0,05
FMDV serotype O/ LPBE	2.02 ± 0.14	1.82 ± 0.13	>0,05
FMDV serotype A/ LPBE	2.04 ± 0.17	2.06 ± 0.14	>0,05
PPR/ VNT	1.06 ± 0.15	0.71 ± 0.11	>0,05
BTV /VNT	1.06 ± 0.15	0.83 ± 0.17	>0,05

All p-values are compared to significance level, α=0.05.

Table 3. Mean and standard errors of antibody titers and p-values at 60 DPV

Vaccine agent/test method	Single vaccination	Simultaneous vaccination	p-value
FMDV serotype O/ VNT	2.28 ± 0.09	1.68 ± 0.25	>0,05
FMDV serotype A/ VNT	2.43 ± 0.08	1.74 ± 0.26	>0,05
FMDV serotype O/ LPBE	2.28 ± 0.26	1.93 ± 0.16	>0,05
FMDV serotype A/ LPBE	2.32 ± 0.25	2.12 ± 0.15	>0,05
PPR/ VNT	1.36 ± 0.15	0.56 ± 0.04	<0,05
BTV/ VNT	1.36 ± 0.15	0.60 ± 0.08	<0,05

All p-values are compared to signifiance level, α=0.05.

Table 4. The rectal temparature of each sheep after days post challange (DPC) in the simultaneous vaccination group

Animal code	DPC 1	DPC 2	DPC 3	DPC 4	DPC 5	DPC 6	DPC 7	DPC 8	DPC 9	DPC 10	DPC 11	DPC 12	DPC 13	DPC 14
6	40,3	40,2	40,9	41,3	41,5	40,3	39,8	39,6	39,7	39,6	39,3	39,4	39,5	39,2
23	39,5	39,5	39,7	40,3	40,6	39,7	39,5	39,6	39,6	39,2	39,3	39,5	39,1	39
1*	40	40,4	40,9	41	41	39,8	39,8	39,4	39,3	39,5	39,6	39,3	39,2	39,3
3	39,2	39,5	39,9	41,1	41,8	41	40,5	40,7	40,5	40,3	39,9	39,7	39,9	39,9
28*	40	40,1	41,2	40,7	41,6	40,6	40,3	40,3	40,5	40,4	40,1	39,9	39,8	40
17	39,6	39,6	39,9	39,8	39,7	39,6	39,9	39,6	39,4	39,2	39,3	39,5	39,4	39,3
4	39,6	39,7	41,1	41,4	41,5	40	39,8	39,7	39,8	39,7	39,6	39,6	39,5	39,4
18	39,5	39,4	40,9	41,2	40,6	40,3	39,9	39,9	39,7	39,6	39,8	39,9	40,1	39,9
29	40,2	39,8	40,5	41,3	41,9	41	40,5	40,2	40,2	39,9	40,1	39,7	39,5	39,4
21	40,6	40,5	40,7	40,9	41	40,8	40,2	40,5	40,6	41,1	40,5	39,9	40,1	39,9

*The rectal temperatures were signed in 1 and 28 codes of sheep in the Table to show an example for the protected and nonproted sheep temperatures.

Table 5. Titer difference of protected and non-protected animal in simultaneous vaccination group

Animal code	Difference of log10 titre	Result
6	2,5	Protected
23	2,5	Protected
1*	3,0	Protected
3	2,5	Protected
28*	0	Non-protected
17	5,5	Protected
4	0	Non-protected
18	1,0	Non-protected
29	0	Non-protected
21	1,0	Non-protected

*The lesions in 1 and 28 codes of sheep were shown in Figures 2a and 2b to express an example for the protected and non-proted sheep.

The comparison of skin reactions of protected (Sheep code:1) and non-protected animals (Sheep code:28) in the simultaneous vaccination group was shown in Figure 2.

When we look at the FMD vaccine results, it was not determined any statistical importance between the single and simultaneous vaccination groups for FMD vaccine.

DISCUSSION

Vaccination is the main control strategy of FMD, BT, PPR, SGP diseases in Turkey. Simultaneous vaccinations help to increase work-speed, reduce labor and vaccination stress for the animals. In the present study, it was aimed to determine the effect on the immune response of sheep after the simultaneous SGP, BT, PPR, and, FMD vaccine administration. It was determined that simultaneous administration of above-mentioned vaccines elicited adequate neutralizing and total antibody titers against the FMD vaccine, however, it was not provided sufficient immune response to the other three live attenuated vaccines (BT, PPR and SGP), (Figures 1a, b, c, and d). Here, all of three live vaccines (BT, PPR, and SGP) were administrated in the left side of the pre-axillar region but FMD vaccine was on the right side. This probably had a benefit in favor of response to FMD antigen since there will be no immune competition with other agents on the right side. Poxviruses are known to activate a wide variety of cellular proteins such as viral interleukins (IL-2,4,10) to escape host immune defence These cytokines suppress the cellular responses in various ways. Especially the IL-10 is the most important anti-inflammatory cytokine which suppresses cellular responses in various ways (Fleming *et al*., 1997; Friebe *et al*., 2004; Gülyaz *et al*., 2019). The local interleukin-10 response probably suppressed the immune response of the other three vaccine agents except FMD vaccine on the left side. These might be the reasons why FMD immune response was not affected negatively with simultaneous administration.

Similar to the result of this study, inadequate immune responses were obtained when the vaccines were simultaneously administrated in a few studies. Casteneda *et al*. (1976) has shown that low antibody titers were determined with the simultaneous vesicular stomatitis and FMD vaccinations. In another study (Ruben *et al*., 1973), measles seroconversion rates were decreased during the simultaneous administration of measles, smallpox, yellow fever vaccines In these studies, researchers reported that interference was responsible for the low antibody titers.

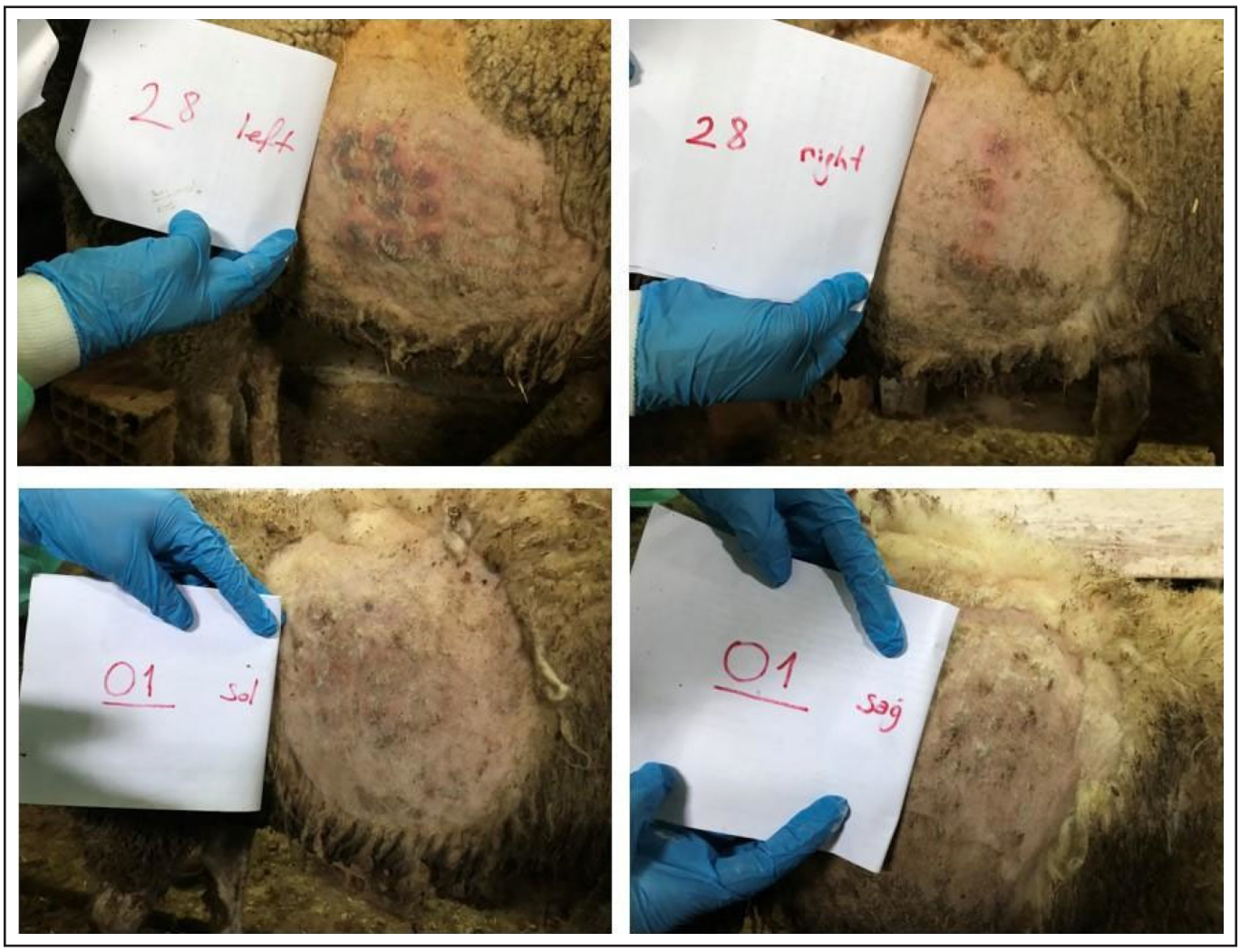

Figure 2. Comparison of skin reactions of protected and non protected animals in the simultaneous vaccination group. Skin reactions of non protected animal after simultaneous vaccination (28: animal code) at 8. Days Post Challenge (DPC) and skin reactions of protected animal after simultaneous vaccination (1: animal code) at 8. DPC (left and right sides of body).

When we look at the studies that gained successful results, in a study (Srinivasan, 2001); FMD, rabies, Pasteurella, and Clostridium vaccine agents were used in the combined formulation. Formulating the many vaccines in a one syringe can help the workload of field vaccination, but, some drawbacks are possible. For example, negative interference may decrease the protective immune response to one or more component of the candidate vaccine, combined vaccines are more prone to shortage because of its complex nature, the risk is increased if one batch of combined vaccines can not pass one of the vaccine control tests. For this reason, simultaneous vaccine administration is more preferable than combined vaccine administration (Fletcher *et al*., 2004). Therfore, here simultaneous vaccination was preferred. In other two different studies (Trotta *et al*., 2015; Çokçalışkan *et al*., 2019), FMD and anthrax vaccines were used simultaneously without any interference. Live attenuated Brucella and FMD vaccines in cattle were used simultaneously and researchers declared that the antibody titers against Brucella were higher during the simultaneous administration (Hancı, 2016). The vaccine agents of these three studies are composed of live bacterial agents (anthrax and brucella) and it was the positive effects on the cellular immune response through cytokines. This is probably the main reason why succesful results were gained in these studies.

The result of the present study explained in general terms via the interference, depressed or exhausted immunity (Ruben *et al*., 1973; Casteneda *et al*., 1976; Berger *et al*., 1988; Vidor, 2007; Yi *et al*., 2010; Kenney *et al*., 2015). A live vaccine can not replicate in the same organism when the other live vaccine agent and/or agents have already started the replication (Boikos *et al*., 2017). Another factor mentioned by a few researchers is the cross-reactive epitopes between the vaccine antigens during the vaccine co-administration (Dagan *et al*., 1998; Kenney *et al*., 2015). However, in our study the viral antigens of three live vaccines (BTV, PPR, and, SGP) do not belong to the same virus family and thus, there are no cross-reactive epitopes shared between these three viruses.

Simultaneous vaccine administration is very similar to a coinfection due to the exposure of different antigens at the same time (Kenney *et al*., 2015). Kenney *et al.* (2015) evaluated T cell memory response during a simultaneous coinfection in laboratory mice. The researchers showed that some of the coinfected mice have sufficiently altered memory T cell responses, this change was related to decreasing the protection and overwhelming infection. They reported that a better understanding of the human T cell response to vaccination is required to optimize immunization strategies in general means. As suggested in Kenney's study, detailed future cellular immunity researches are necessary with the

simultaneous vaccine practice, especially in live-live vaccine combinations for animal diseases.

In conclusion, the simultaneous administration of FMD, SGP, PPR and, BTV vaccines elicited adequate neutralizing and total antibody against FMD vaccine. However, The simultaneous administration does not provide sufficient immune response against the other three diseases. Therefore, it was reported that these four vaccines can not be administrated simultaneously in sheep. Future studies are required to detail out the immune response studies with more animal numbers and different species. The simultaneous administration of FMD-SGP-PPR or FMD-SGP-BT vaccines in different regions of the body might be studied at first. The combined formulation of some vaccine agents can be tried secondly. Lastly, it would be better to design new vector vaccines for this purpose.

ACKNOWLEDGMENTS

All animal experiments were conducted in Pendik Veterinary Research Institute, İstanbul, Turkey. The authors wish to thank Burak Güngör, Hakan Enül, Cumhur Adiay, Sedat Kılıç, Neşe Kalya, Ayşe Parmaksız for their help. This study was financially supported by Foot-and-Mouth Disease Institute and Pendik Veterinary Research Institutes, The Republic of Turkey, Ministry of Agriculture and Forestry.

Conflict of interest

The author declares that they have no conflict of interests.

REFERENCES

Berger, R., Just, M. & Gluck, R. (1988). Interference between strains in live virus vaccines I: Combined vaccination with measles, mumps and rubella vaccine. *Journal of Biological Standardization* **16**: 269-273. https://doi: 10.1016/0092-1157(88)90014-5

Boikos, C., Papenburg, J., Martineau, C., Joseph, L., Scheifele, D., Chilvers, M., Lands, L.C., Serres, G.D. & Quach, C. (2017). Viral interference and the live-attenuated intranasal influenza vaccine: Results from a pediatric cohort with cystic fibrosis. *Human Vaccine & Immunotherapeutics* **13**: 1254-1260. https://doi.org/10.1080/21645515.2017.1287641

Carey, V.J. (2015). Generalized Estimation Equation Solver. In: R Package Version 4. UK: rdrr.io

Castañeda, J., Espinoza, M., Bernel, C., Jiménez, J. & Aguirre L. (1976). Simultaneous vaccination of cattle with against foot and mouth disease and stomatitis live virus vaccines. *Developments in Biological Standardization* **35:** 429-436.

Çokçalışkan, C., Göktuna, P.T., Türkoğlu, T., Uzunlu, E., Gündüzalp, C., Aras Uzun, E., Sareyyüpoğlu, B., Kürkçü, A. & Gülyaz, V. (2019). Effect of simultaneous administration of foot-and-mouth disease (FMD) and anthrax vaccines on antibody response to FMD in sheep. *Clinical and Experimental Vaccine Research* **8**: 103-109. https://doi.org/10.7774/cevr.2019.8.2.103

Dagan, R., Eskola, J., Leclerc, C. & Leroy, O. (1998). Reduced response to multiple vaccines sharing common protein epitopes that are administered simultaneously to infants. *Infection and Immunity* **66**: 2093-2098. https://doi.org/10.1128/IAI.66.5.2093-2098.1998

De Clercq, K., Koenen, F., Strobbe, R. & Debecq, J. (1989a). Simultaneous vaccination of piglets against foot-and-mouth disease and classical swine fever. *Veterinary Microbiology* **20**: 215-221. https://doi.org/10.1016/0378-1135(89)90045-x

De Clercq, K., Strobbe, R., Van Opdenbosch, E., Wellemans, G., Theys, H. & Debecq, J. (1989b). The serological response to foot-and-mouth disease vaccination is not affected by simultaneous infectious bovine rhinotracheitis/adenovirus/parainfluenza 3 vaccination. *Archiv für Experimentale Veterinaermedizin* **43**: 409-413.

Fox, C.B., Kramer, R.M., Banes, V.L., Dowling, Q.M. & Vedvick, T.S. (2013). Working together: interactions between vaccine antigens and adjuvants. *Therapeutic Advances in Vaccines* and Immunotherapy **1**: 7-20. https://doi.org/10.1177/2051013613480144

Fleming, S.B., Mccaughan, C.A., Andrews, A.E., Nash, A.D. & Mercer, A.A. (1997). A homolog of interleukin-10 is encoded by the poxvirus orf virus. *Journal of Virology* **71**: 4857-4861. https://doi.org/10.1128/jvi.71.6.4857-4861.1997

Fletcher, M.A., Fabre, P., Debois, H. & Saliou, P. (2004). Vaccines administered simultaneously: directions for new combination vaccines based on an historical review of the literature. *International Journal of Infectious Diseases* **8**: 328-338. https://doi.org/10.1016/j.ijid.2004.03.006

Friebe, A., Siegling, A., Friederichs, S., Volk, H.D. & Weber, O. (2004). Immunomodulatory effects of inactivated parapoxvirus ovis (ORF virus) on human peripheral immune cells: induction of cytokine secretion in monocytes and Th1-like cells. *Journal of Virology* **78**: 9400-9411. https://doi.org/10.1128/JVI.78.17.9400-9411.2004

Gülyaz, V., Saraç, F., Çokçalışkan, C., Satır, E., Sareyyüpoğlu, B., Arslan, A., Uzar, S., Karakaya, M., Kara, O. & Öztap, G. (2019). Determination of the effect of ecthyma vaccine virus on immunity against the foot and mouth disease vaccine in sheep. *Journal of Animal Health and Production* **8**: 19-26. http://doi.org/10.17582/journal.jahp/2020/8.1.19.26

Hamblin, C., Barnett, I.T.R. & Hedger, R.S. (1986). A new enzyme-linked immunoassay (ELISA) for the detection of antibodies against foot-and-mouth disease virus I. Development and method of ELISA. *Journal of Immunological Methods* **93**: 115-121. https://doi.org/10.1016/0022-1759(86)90441-2

Hancı, İ., Gürcan, S. & Sönmez, A. (2016). The effect of simultaneous application of Brucella and FMD vaccines on antibody level in cattle and sheep. *Journal of Etlik Veterinary Microbiology* **27**: 27-37. https://doi.org/10.35864/evmd.514164

Hedger, R.S., Taylor, W.P., Barnett, I.T.R., Reik R. & Harpham, D. (1986). Simultaneous vaccination of cattle against foot-and-mouth disease and rinderpest. *Tropical Animal Health and Production* **18**: 21-25. https://doi.org/10.1007/BF02359652

Joseph, P.G. & Hedger, R.S. (1984). Serological response of cattle to simultaneous vaccinations against foot-and-mouth disease and haemorrhagic septicaemia. *The Veterinary Record* **114**: 494-496. https://doi.org/10.1136/vr.114.20.494

Kenney, L.L., Cornberg, M., Chen, A.T., Emonet, S., De la Torre, J.C. & Selin, L.K. (2015). Increased immune response variability during simultaneous viral coinfection leads to unpredictability in CD8 T cell immunity and pathogenesis. *Journal of Virology* **89**: 10786 -10801. https://doi.org/10.1128/JVI.01432-15

Khorasani, A., Madadgar, O., Soleimanjahi, H., Keyvanfar, H. & Mahravani, H. (2016). Evaluation of the efficacy of a new oil-based adjuvant ISA 61 VG FMD vaccine as a potential vaccine for cattle. *Iran Journal Veterinary Research* **17**: 8-12. https://doi.org/10.22099/IJVR.2016.3596

Kitching, R.P. (1986). The control of sheep and goatpox. *Revue Scientifique et Technique (International Office of Epizootics)* **5**: 503-511. https://doi.org/10.20506/rst.5.2.241

Kristensen, C.S., Kvisgaard, L.K., Pawlowski, M., Carlsen, S.H,. Hjulsager, C.K., Heegaard, P.M.H,. Bøtner, A., Stadejek, T., Haugegaard, S. & Larsen, L.E. (2018). Efficacy and safety of simultaneous vaccination with two modified live virus vaccines against porcine reproductive and respiratory syndrome virus types 1 and 2 in pigs. *Vaccine* **36**: 227-236. https://doi.org/10.1016/j.vaccine.2017.11.059

Maclachlan, N.J. (2011). Bluetongue: History, global epidemiology, and pathogenesis. *Preventive Veterinary Medicine* **102**: 107-111. https://doi.org/10.1016/j.prevetmed.2011.04.005

Özkul, A., Akça, Y., Alkan, F., Barrett, T., Karaoğlu, T., Dağalp, S.B., Anderson, J., Yeşilbağ, K., Çokçalışkan, C., Gençay, A. *et al*. (2002). Prevalence, distribution and host range of pste des petits ruminants virus, Turkey. *Emerging Infectious Disease* **8**: 709-712. https://doi.org/10.3201/eid0807.010471

Perry, B.D. & Rich, K.M. (2007). Poverty impacts of foot-and-mouth disease and the poverty reduction implications of its control. *Veterinary Record* **160**: 238-241. https://doi.org/10.1136/vr.160.7.238

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Ruben, F.L., Smith, E.A., Foster, S.O., Casey, H.L., Pifer, J.M. Wallace, R.B., Atta, A.I., Jones, W.L., Arnold, R.B., Teller, B.E. *et al.* (1973). Simultaneous administration of smallpox, measles, yellow fever, and diphtheria-pertussis-tetanus antigens to Nigerian children. *Bulletin of the World Health Organization* **48**: 175-181.

Saegerman, C., Berkvens, D. & Mellor, P.S. (2008). Bluetongue epidemiology in the European Union. *Emerging Infectious Disease* **14**: 539-544. https://doi.org/10.3201/eid1404.071441

Sørensen, K.J., Madsen, K.G., Madsen, E.S., Salt, J.S., Njindi, J. & Mackay, D.K.J. (1998). Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. *Archives of Virology* **143**: 1461-1476. https://doi.org/10.1007/s007050050390

Srinivasan, V.A., Reddy, G.S., Rao, K.A. & Kihm, U. (2001). Serological response of bovines to combined vaccine containing foot and mouth disease virus, rabies virus, *Pasteurella multocida* and *Clostridium chauvoei* antigens. *Veterinarski Arhiv* **71**: 37-45.

Tizard, I. (1990). Risks associated with use of live vaccines. *Journal of the American Veterinary Medical Association* **196**: 1851-1858.

Tizard, I. (2017). Immunology in viral diseases. In: Veterinary Immunology, Tizard, I. (Editor) 8th edition. USA: Saunders, pp. 89-297.

Trotta, M., Lahore, J., Cardoso, N., Melucci, O., Catena, M., Pérez-Filgueira, M., Fernández, F. & Capozzo, A.V. (2015). Simultaneous immunization of cattle with foot-and-mouth disease (FMD) and live anthrax vaccines do not interfere with FMD booster responses. *Trials in Vaccinology* **4**: 38-42. https://doi.org/10.1016/j.trivac.2015.04.001

Vidor, E. (2007). The nature and consequences of intra- and inter-vaccine interference. *Journal Comparative Pathology* **137**: S62-S66. http://doi.org/10.1016/j.jcpa.2007.04.014

World Organisation for Animal Health (OIE) (2020). Peste Des Petýts Rumýnants. In: OIE Terrestial Manual 2020. https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/PESTE_DES_PETITS_RUMINANTS.pdf. Accessed 15 October 2021.

World Organisation for Animal Health (OIE) (2021a). Chapter 3.1.8 Foot-and-mouth disease (Infection with foot and mouth disease virus. In: OIE Terrestial Manual 2021. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.08_FMD.pdf_Accessed 15 October 2021.

World Organisation for Animal Health (OIE) (2021b). Chapter 3.1.3 Bluetongue virus (Infection with bluetongue virus). In: OIE Terrestial Manual 2021. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.03_BLUETONGUE.pdf Accessed 15 October 2021.

World Organisation for Animal Health (OIE) (2021c). Chapter 14.7 Infection with peste des petits ruminants Virus. In: OIE Terrestial Animal Health Code. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_ppr.pdf Accessed 15 October 2021.

World Organisation for Animal Health (OIE) (2021d). Chapter 2.7.13 Sheep pox and goat pox. In: OIE Terrestial Manual 2021. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.13_S_POX_G_POX.pdf. Accessed 15 October 2021.

Yi, J.S., Cox, M.A. & Zajac, A. (2010). T-cell exhaustion: characteristics, causes, conversion. *Immunology* **129**: 474-481. https://doi.org/10.1111/j.1365-2567.2010.03255.x