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Original Article
Özgün Araştırma

Immune response in cattle simultaneously vaccinated with foot and mouth disease (FMD) and bovine ephemeral fever vaccines (BEF)

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Abstract: Bovine ephemeral fever (BEF) virus is an arthropod-borne rhabdovirus and causing an acute febrile illness disease in cattle and water buffalo. Foot-and-mouth disease (FMD) is a highly infectious viral disease of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals. FMD and BEF vaccines are widely used in countries where both diseases are seen together. This study was carried out to determine the immunological response of cattle simultaneously vaccinated with BEF and FMD vaccines. For this purpose, the cattle were divided into 4 groups in this study as single FMD vaccination group (Group 1; n=10), single BEF vaccination group (Group 2; n=10), BEF+FMD simultaneously vaccinated group (Group 3; n=10) and unvaccinated control group (Group 4; n=10). After the first vaccinations, booster BEF vaccine was applied to the cattle in groups 2 and 3. Although there was no increase in the neutralizing antibody titers against BEF and FMD virus in the blood serums of unvaccinated cattle on the 30th and 60th days of vaccination, There were significant increases in statistically protective neutralizing antibody levels in the vaccinated cattle. As a result, it was demonstrated with this study that BEF and FMD vaccines can be applied simultaneously to combat both diseases in cattle.

Keywords: BEFV, FMDV, cattle, vaccine, immunity

Şap Hastalığı (FMD) ve üç gün hastalıklarına (BEF) karşı eş zamanlı aşılanmış sığırlarda bağışıklık yanıt

Özet: Sığırların üç hastalığı (BEF) virüsü, artropod kaynaklı bir rabdovirüs olup, sığır ve mandalarda akut ateşli infeksiyona sebep olur. Şap hastalığı (FMD), oldukça bulaşıcı bir viral hastalık olup hassas çift tırnaklı hayvanlarda ciddi ekonomik kayıplara neden olma konusunda büyük bir potansiyele sahiptir. Şap ve BEF aşıları, her iki hastalığın birlikte görüldüğü ülkelerde yaygın olarak kullanılmaktadır. Bu çalışma, BEF ve FMD aşıları ile eş zamanlı olarak aşılanan sığırların immünolojik yanıtını belirlemek amacıyla yapılmıştır. Bu amaçla çalışmada tekli şap aşısı grubu (Grup 1; n=10), tekli BEF aşısı grubu (Grup 2; n=10), BEF+Şap ile eş zamanlı aşılı grup (Grup 3; n=10) ve aşısız kontrol grubu (Grup 4; n=10) olmak üzere 4 sığır grubu kullanıldı. Grup 2 ve 3'teki sığırlara ilk aşılamalardan sonra booster BEF aşısı uygulandı. Çalışma sonucu aşısız sığırların kan serumlarında aşılamanın 30. ve 60. günlerinde BEF ve FMD virüsüne karşı nötralize edici antikor titrelerinde artış olmamasına rağmen, aşılanmış sığırlarda istatistiksel olarak koruyucu nötralize edici antikor seviyelerinde önemli artışlar tespit edilmiştir. Sonuç olarak bu çalışma ile sığırlarda her iki hastalıkla mücadelede BEF ve FMD aşılarının aynı anda uygulanabileceği gösterilmiştir.

Anahtar kelimeler: BEFV, FMDV, sığır, aşı, bağışıklık

Introduction

Bovine ephemeral fever (BEF or 3-day sickness) is an acute febrile illness of cattle and water buffaloes. BEF is caused by an arthropod-borne rhabdovirus, bovine ephemeral fever virus (BEFV) classified as the type species of the genus Ephemerovirus (Chaisiri-rat et al. 2018; Zheng et al. 2012). BEF is geographically distributed from tropical to temperate zones such as parts of Australia, Asia, the Middle East, and Africa (Aziz-Boaron et al. 2015; Karaoğlu et al. 2007; Zeng and Qiu 2012; Yeruham et al. 2010). Infection may be clinically unapparent or result in mild to severe clinical signs including a bi-phasic fever, salivation, ocular and nasal discharge, recumbency, muscle stiffness, lameness and anorexia (Mirzaie et al. 2017). BEF is characterized by rapid onset and rapid recovery, lasting only 1–3 days, but there are reports of prolonged paralysis and ataxia in some

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animals following the acute phase of infection. The most severe cases can result in mortality which may be due to exposure, starvation or pneumonia. Morbidity rates can be very high (approaching 100%) and mortality rates are typically low (<1%). To protect cattle against BEF disease live-attenuated, inactivated and subunit vaccines are being used in the field. Vaccination has been adopted to varying extents in Australia, South Africa, Namibia, Japan, South Korea, Taiwan, Mainland China, the Philippines, Turkey, Israel, Kuwait, Oman, Bahrain, Saudi Arabia and Egypt (Walker and Klement 2015). BEF was first reported in 1985 in Turkey (Girgin et al. 1986). In recent years BEF epidemics have been reported at intervals of 2-4 years (Erganiş et al. 2010) due to global climate changes, the incidence of the disease has increased in recent years. The last two outbreaks of BEF disease have been reported in 2008 and 2012. The outbreaks of BEF disease seen in 2008 were recorded in Turkey's South-East region as a relatively local outbreaks (Erganiş et al. 2010). However, the disease seen in 2012 was detected in some provinces of the Marmara, Aegean and Western Black Sea regions along with the Mediterranean, Central Anatolia, Eastern Anatolia (Alkan et al. 2017; Erol et al. 2015).

Foot and mouth disease (FMD) is an acute and contagious infectious disease of domestic animals including cattle, buffalo, sheep, goats and swine and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals (Hussain et al. 2017). There are seven serotypes of FMD virus (FMDV), namely, O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1 (OIE, 2021). Infection with one serotype does not confer immunity against another. Cattle, pigs, sheep, goats and water buffalo (Bubalus bubalis) are susceptible to FMD and small ruminants can play an important role in the spread of FMDV in different ways (Abubakar et al. 2012; Ahmed et al. 2017). Infection of susceptible animals with FMDV can lead to the appearance of vesicles on the feet, in and around the oral cavity, and on the mammary glands of females. Mortality from a multifocal myocarditis is most commonly seen in young animals and myositis may also occur in other sites. Routine vaccination against FMD is used in many countries or zones recognised as free from foot and mouth disease with vaccination and in countries where the disease is endemic. In contrast, a number of disease-free countries have never vaccinated their livestock but have preferred the use of strict movement controls and culling of infected and contact animals when outbreaks have occurred (OIE 2021).

Materials and Methods

Vaccines

Trivalent FMD vaccine containing serotype A (A Nep-84 (G-VII)), (at least 6 µg / ml), serotype O (at least 8 µg / ml (O TUR 07) and serotype Asia-1 (5 µg / ml-Asia-1 TUR 15) with oil adjuvant (montanide ISA-206 BVG) was obtained from FMD (SAP) Institute Ankara. Attenuated and live BEF vaccine was obtained from Vetal Animal Health Company (Autovaccine-local isolates, BEF-TR2008, BEF AU1978).

Animals

The cattle (n=40, aged 9-18 months) which are not vaccinated with FMD and BEF (Autovaccine-local isolates) and obtained from TIGEM (General Directorate of Agriculture and Forestry, General Directorate of Agricultural Enterprises, in Adana province, Turkey) were used in the study.

Cell cultures

Baby Hamster Kidney (BHK 21-An-30) cells and African green monkey cell culture (Vero) were used for the production of FMD and BEF viruses with Glasgow Modified Essential Medium (G-MEM) containing 10% and %2 fetal calf serum (FCS) (Biochrom Cat no: S-0125) respectively. Both cell cultures were produced as monolayer in 25 cm^2 flasks in a 37°C incubator containing 5% CO_2 (Çokçalışkan et al. 2019; Ammerman et al. 2008).

Non-structural proteins (NSP) ELISA

The antibodies against the NSP proteins of the FMD virus in blood serums of cattle were tested with NSP ELISA kit as recommended by the kit instruction (Priocheck FMDV NS, The Netherlands).

Virus neutralization test (VNT)

The antibody titer levels against FMD and BEF viruses were determined by VNT. Starting from a 1/4 dilution, sera are diluted in a twofold, dilution series across the plate, using at least two rows of wells per serum, and a volume of 50 µl. Previously titrated virus is added; each 50 µl unit volume of virus suspension should contain about 100 $TCID_{50}$ (50% tissue culture infective dose) within an accepted range. Controls include a standard antiserum of known titre, a cell control, a medium control, and a virus titration used to calculate the actual virus titre used in the test. Incubate at 37°C for 1 hour with the plates covered. A cell suspension at 10^6 cells/ml was made up in medium containing 10% FCS (specific antibody negative) for cell growth. A volume of 50

µl of cell suspension was added to each well. Plates were incubated in an atmosphere of 5% carbon dioxide at 37°C for 3 days. Microscope readings was made and the plates were finally fixed and stained routinely on the third day. Fixation was made with 10% formol/saline for 30 minutes. For staining, the plates were immersed in 0.05% methylene blue in 10% formalin for 30 minutes. The plates were rinsed in tap water. Positive wells (where the virus was neutralised and the cells remain intact) were seen to contain blue-stained cells sheets; the negative wells (where virus has not been neutralised) were empty. Titres were expressed as the final dilution of serum present in the serum/virus mixture where 50% of wells were protected (OIE 2021; Tekleghiorghis et al. 2014)

Liquid Phase Blocking ELISA (LPBE)

The immunity level of animals against FMD was determined by LPBE after vaccinations. ELISA plates were coated with rabbit antibody (against anti-FMDV 146S antigens). Meanwhile, test and control sera were added to the carrier microplate at a dilution of 1/16. A working dilution of FMDV type O, type A, and type Asia 1 were added. The carrier and ELISA plates were incubated at 4°C. On the second day of the test, following washing of ELISA plate, a 50 µl mixtures of serum/antigen was transferred from the carrier microplate to the ELISA microplate. Then, the plates were incubated at 37°C with continuous shaking for 1 hour. After washing, 50 µl anti-FMDV type specific guinea pig antibodies were added and incubated in a 37°C for 1 hour. Then 50 µl working dilution (1:2000) of the conjugate was added to the wells and incubated in a 37°C for 1 hour. Chromogen OPD/Substrate (H_2O_2), 50 µl, was added to each well, and then incubated at room temperature for 15 minutes. Finally, 50 µl stop solution (1.25 M sulphuric acid) was added to all the wells. The absorbance was read by the microplate reader (VersaMax, Molecular Devices, USA) at 492 nm. (Basagoudanavar et al. 2013; Sareyyüpoğlu et al. 2019).

Vaccination of cattle

Before vaccination, blood samples of 40 cattle were taken and the presence of antibodies belonging to FMD and BEF viruses in blood serums was investigated. The animals were divided into 6 groups, including 10 cattle in each one. Group-1 (single FMD vaccination), 2 ml trivalent FMD vaccine was injected into 10 cattle subcutanously in the neck area (Çokçalışkan et al. 2019; Sareyyüpoğlu et al. 2019). The cattle in Group-2 (n=10) were vaccinated with the only 1 ml BEF autovaccine at the anterior leg of the chest area. After the first vaccination, booster vaccination of the BEF was applied to all cattle in this group on the 30th day as recommended by the manufacturer. The cattle placed in group 3 (n=10) were vaccinated simultaneously by subcutaneous way with FMD and BEF autovaccine. PBS (2 ml) was injected subcutaneously into the anterior leg of the chest area of cattle in Group-4 (Negative control) (Çokçalışkan et al. 2019; Erganiş et al. 2010; Sareyyüpoğlu et al. 2019) (Table 1). All vaccinated cattle were monitored to record clinical findings of body temperature, local lesions and appetite everyday. Blood samples were taken for the detection of antibody levels against BEF and FMD viruses by using VNT and ELISA (LPBE) the 30 and 60th day after vaccinations.

Table 1. Vaccination study

Groups	Number of animals	Vaccines	
		FMD vaccine (2 ml)	Commercial BEF attenuated autovaccine (1 ml)
1	10	+	Mock injected
2	10	Mock injected	+
3	10	+	+
4	10	Mock injected	Mock injected

Statistical Analysis

In the statistical analysis, Log10 level values of the study data were used. These values were analyzed with Shapiro Wilk and Levene tests and upon determination that parametric assumptions were provided. The Bidirectional Anova test (Repeated Measures Two Way Anova) was applied in repeated measurements in order to determine the time dependent variation of the difference between the groups. Bonferroni multiple comparison test was performed to determine whether the difference between the groups was statistically significant. All data were statistically evaluated at 95% confidence interval using SPSS 22 (Inc. Chicago II, USA) computer program.

Results

In this study; the NSP proteins against FMD virus were negative in all cattle. The antibody titers against FMDV O, A and Asia-1 serotypes were found to be 2.27; 2.63 and 1.22 log10 by VNT and 2.38; 2.34 and 2.27 log10 by ELISA before vaccination of

cattle. Neutralizing antibody titers against BEF virus were determined by VNT as 0.60 and 0.71 log 10 respectively in cattle vaccinated with BEF autovaccine (Group 2) and FMD+BEF (Group 3) before vaccination of cattle.

Antibody responses in cattle vaccinated with single FMD vaccine (Group 1)

The mean antibody titers were found to be 3.30; 3:34; 2.30 on day 30 DPV and 3.24; 3:22; 2.41 log10 against FMDV serotype O, A, Asia-1 respectively by VNT on the 60th day of the vaccination (DPV) (Figure 1, 2, 3).

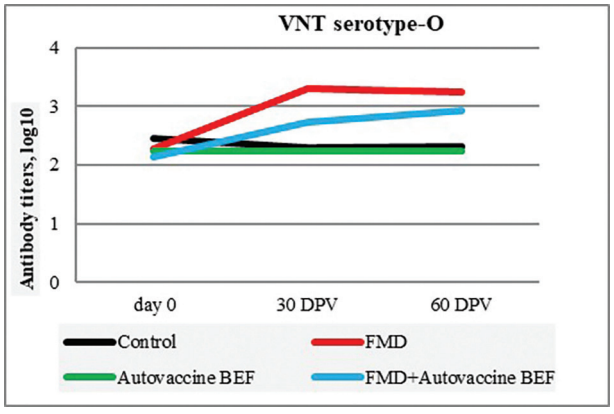

Figure 1. VNT antibody titers against FMDV serotype O

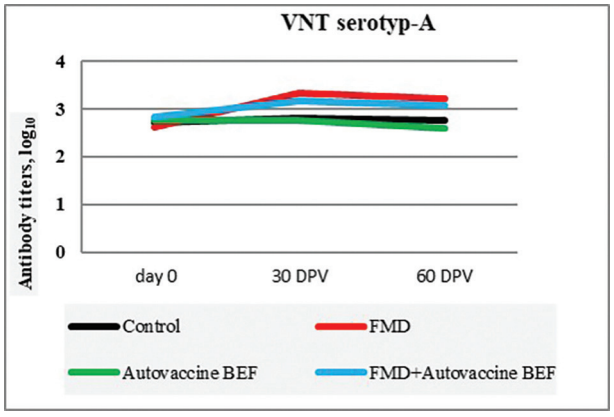

Figure 2. VNT antibody titers against FMDV serotype A

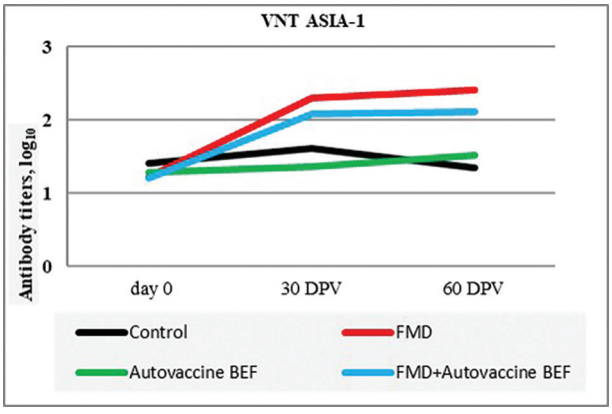

Figure 3. VNT antibody titers against FMDV serotype ASIA-1

According to results of ELISA, the mean antibody titers were found to be 3.24; 3:20; 3.04 log10 on day the 30th and 3.28; 3:19; 3.13 log10 on the 60th DPV against FMDV serotypes O, A, Asia-1 respectively. (Figure 4, 5, 6).

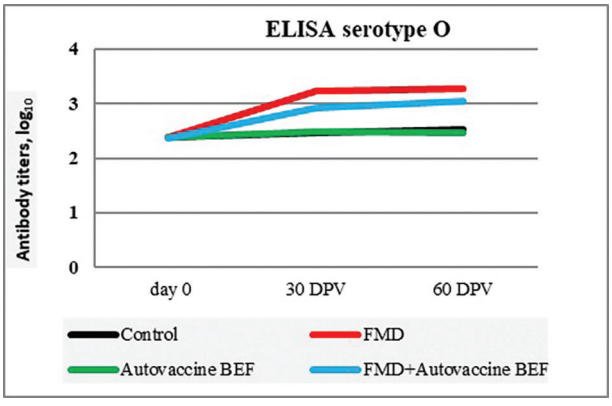

Figure 4. ELISA antibody titers against FMDV serotype O

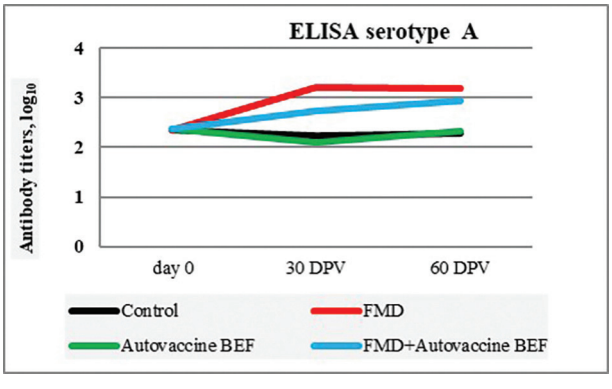

Figure 5. ELISA antibody titers against FMDV serotype A

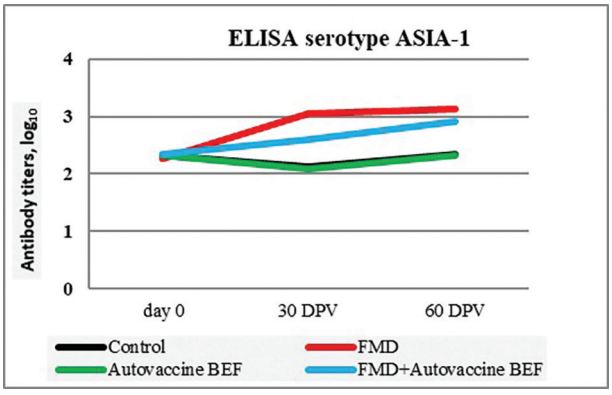

Figure 6. ELISA antibody titers against FMDV serotype ASIA-1

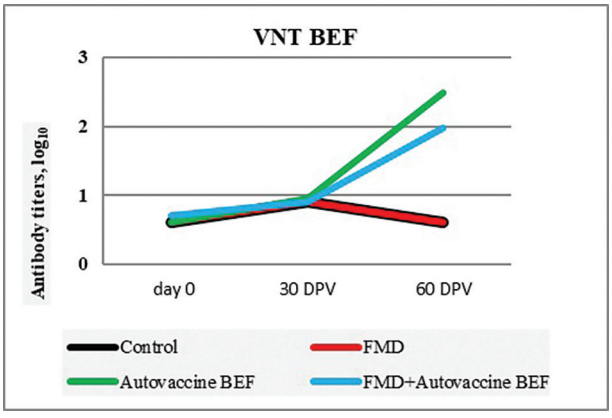

Figure 7. VNT antibody titers against BEF virus.

Antibody responses in cattle vaccinated with single BEF (Group 2)

The antibody titres against BEF virus were determined as 0.96 on the 30th and 2.48 log10 on the 60th DPV by VNT (Figure 7).

Antibody responses in simultune vaccination grup (FMD+BEF) (Group 3)

Antibody titers were found to be 2.77; 3.18; 2.08 on 30 DPV and 2.93; 3.07; 2.12 log10 on 60 DPV against serotype O, A and Asia-1 of FMDV by VNT (Figure 1, 2, 3). ELISA titers were obtainted at 2.93; 2.74 and 2.61 on 30 DPV and 3.04; 2.93; 2.92 log10 on 60 DPV (Figure 4, 5, 6). The antibody levels against BEF virus were found to be 0.90 on the 30 DPV and 1.98 on the 60 DPV (Figure 7).

The antibody titers of cattle placed in control group against FMDV serotype O, A and Asia-1 were found to be 2.29; 2.81; 1.62 log10 on 30th day and 2.32; 2.77; 1.35 log10 on 60 DPV by VNT. ELISA antibody titers were obtainted as 2.46; 2.23 and 2.12 on 30 DPV and 2.53; 2.29 and 2.35 log10 on 60 DPV. Meanwhile, antibody levels against BEF virus were determined as 0.90 on 30th day and 0.60 log10 on 60th day. In cattle vaccinated with single FMD (Group 1), the differences of neutralizing antibody titers between before (on the 0th day) and after (on the 30th day) vaccination against the serotypes of FMD virus O, A and Asaia-1 were detected as 1.03; 0.71 and 1.08 respectively. According to the results of the ELISA, these differences were found to be 0.85, 0.86 and 0.77 log 10, respectively. The differences of the mean antibody titer between before (on the 0th day) and after vaccination (on the 30th day) in the cattle vaccinated with BEF+FMD (Group 2) were seen to be 0.59; 0.35; 0.86 with VNT, and 0.57; 0.37; 0.26 with ELISA against O. A and Asia-1 serotypes, respectively.

The cattle vaccinated with single BEF autovaccine (Group 2), the differences the average antibody titer between on the 30 (0.96 log 10) and 60th day (2.48 log10) was determined as $TCID_{50}$ 1.52 log10 after booster vaccination.

In the control group (Group 4), the average neutralizing antibody titers that existed prior to vaccination against FMD virus O, A and Asaia-1 serotypes and BEF virus did not increase on the 30th and 60th days.

Statistical Analysis

Antibody levels for A NEP-84 and O TUR-07 showed a statistically significant increase on the 30th day after vaccination ($p<0,05$) by VNT depending on the days. Although a decrease in antibody level was observed for these two serotypes on the 60th day, a statistically significant increase was observed compared to the 0th day (Table 2). The highest titer for the serotype Asia-1-Tur-15 was detected on the 60th day. According to the results obtained with ELISA, a statistically significant increase were detected in antibody titers from day 0 to day 60 in all three serotypes ($p<0,05$) (Table 3).

There was a statistically significant increase in the antibody levels of the cattle vaccinated with BEF vaccine from day 0 to day 60 ($p<0.05$) according to the cattle without BEF vaccine. Higher antibody titers were observed numerically only in the cattle vaccinated with FMD (Group 1). However, simultaneous administration of FMD+BEF vaccines did not show any statistically significant effects on both FMD and BEF antibody titers in animals (Group 3) (Table 2).

Table 2. Comparision of VNT antibody titers of groups on 0 day-30th and 60th days (Log10)

The comparison of antibody titers after vaccinations		Antibody Titers											
		Mean Values (X)											
Vaccinations		FMD ANEP-84 Serotype			FMD O TUR-07 Serotype			FMD Asia-1-Tur-15 Serotype			BEF		
		Days											
Groups		0	30	60	0	30	60	0	30	60	0	30	60
Control		2,63	2,81	2,77	2,27	2,28	2,32	1,41	1,62	1,35	0,60	0,60	0,60
FMD		2,71	3,34	3,22	2,45	3,29	3,23	1,22	2,30	2,41	0,60	0,90	0,60
BEF		2,75	2,75	2,60	2,23	2,24	2,24	1,28	1,37	1,51	0,60	0,96	2,63
FMD+ BEF		2,83	3,17	3,07	2,14	2,72	2,93	1,21	2,08	2,12	0,70	0,90	2,48
BEF	Control	2,67	3,07	2,99	2,36	2,78	2,77	1,31	1,96	1,88	0,6	0,75	0,6
	BEF	2,75	2,75	2,6	2,23	2,24	2,24	1,28	1,37	1,51	0,6	0,96	2,63
FMD	Control	2,69	$2,78^{c}$	$2,68^{b}$	2,25	$2,28^{c}$	$2,32^{c}$	1,34	$1,49^{b}$	$1,43^{c}$	0,6	$0,78^{b}$	$1,61^{b}$
	FMD	2,71	$3,34^{a}$	$3,22^{a}$	2,45	$3,29^{a}$	$3,23^{a}$	1,22	$2,30^{a}$	$2,41^{a}$	0,6	$0,9^{a}$	$0,6^{c}$
	FMD+ BEF	2,83	$3,24^{b}$	$3,11^{a}$	2,20	$2,90^{b}$	$2,93^{b}$	1,36	$2,18^{a}$	$2,11^{b}$	0,6	$0,90^{a}$	$2,56^{a}$
Days	0		$2,73^{C}$			$2,27^{B}$			$1,28^{B}$			$0,62^{C}$	
	30		$3,02^{A}$			$2,63^{A}$			$1,84^{A}$			$0,84^{B}$	
	60		$2,91^{B}$			$2,68^{A}$			$1,85^{A}$			$1,58^{A}$	
SEM			0,047			0,049			0,39			0,12	
						P							
Days			<0,00			<0,00			<0,00			<0,00	
BEF			<0,00			0,01			0,01			0,01	
FMD			<0,00			0,10			0,03			0,02	
FMD*BEF			0,1			0,1			0,1			0,1	

Discussion and Conclusion

Bovine ephemeral fever (or 3-day sickness) is an acute febrile illness of cattle and water buffaloes caused by an arthropod-borne rhabdovirus, bovine ephemeral fever virus (BEFV) (El-habbaa and Radwan 2019). The disease occurs seasonally over a vast expanse of the globe encompassing much of Africa, the Middle East, Asia and Australia (Aziz-Boaron et al. 2013; Walker and Klement. 2015). There are also significant impacts on trade to regions in which the disease does not occur, including the Americas and most of Europe. In recent years, unusually severe outbreaks of BEF have been reported from several regions in Asia, the Middle East and Turkey with mortality rates through disease or culling in excess of 10–20% (Abaylı et al. 2017; Tonbak et al. 2013; Oğuzoğlu et al. 2015; Walker and Klement 2015).

FMD disease is an acute and infectious disease of domestic animals, including cattle, buffalo, sheep, goats and swine having communicable potential. There are seven serotypes of FMD virus which are antigenically and immunologically different and each serotype has a vast range of distinct subtypes (OIE 2021; Tekleghiorghis et al. 2014). The difficulty regarding the control of FMD disease is due to its wide host range and geographical distribution along with poor cross immunity, antigenic diversity and establishment of a carrier state.

FMD disease continues to occur sporadically in Turkey (Çokçalışkan et al. 2019; Sareyyüpoğlu et al. 2019). In order to combat the FMD disease, campaign vaccinations have been used to control the disease. The policy of which consists of two doses of vaccinations of adult cattle in 6 months intervals and booster application in calves which receive the

Table 3. Comparision of ELISA antibody titers of groups on 0 day-30th and 60th days (Log10)

The comparison of antibody titers after vaccinations		Antibody Titers								
		Mean Values (X)								
Vaccinations		FMD (A-NEP-84) Serotype			FMD (O-TUR-07) Serotype			FMD (Asia-1-Tur-15) Serotype		
		Days								
Groups		0	30	60	0	30	60	0	30	60
Control		2,36	2,23	2,29	2,38	2,46	2,53	2,31	2,12	2,35
FMD		2,34	3,20	3,19	2,40	3,24	3,28	2,27	3,04	3,13
BEF		2,33	2,10	2,33	2,38	2,49	2,48	2,33	2,08	2,33
FMD+BEF		2,37	2,74	2,93	2,37	2,93	3,04	2,33	2,60	2,92
FMD	Control	2,34	2,16	2,31	2,38	2,47	2,50	2,32	2,10^{c}	2,34^{c}
	FMD	2,35	2,97	3,06	2,38	3,08	3,16	2,3	2,82^{a}	3,02^{a}
	FMD+BEF	2,37	2,74	2,93	2,37	2,93	3,04	2,33	2,60^{b}	2,92^{b}
BEF	Control	2,35	2,71	2,74	2,39	2,85	2,90	2,29	2,58	2,74
	BEF	2,35	2,42	2,63	2,37	2,71	2,76	2,33	2,34	2,62
Days	0		2,35^{C}			2,38^{C}			2,31^{C}	
	30		2,57^{B}			2,78^{B}			2,46^{B}	
	60		2,68^{A}			2,83^{A}			2,68^{A}	
SEM			0,03			0,03			0,025	
		P								
Days			<0,00			0,01			0,01	
BEF			0,1			0,1			0,1	
FMD			0,15			0,98			0,04	
FMD*BEF			0,18			0,82			0,13	

first dose of the vaccine at 2 months of age if they consumed adequate colostrum and their mothers were vaccinated regularly (Çokçalışkan et al. 2019; Sareyyüpoğlu et al. 2019). As the potency of FMD vaccines produced as of 2014 was increased, the disease focuses related to FMD decreased in the following years, and in 2018 and 2019, the only FMD serotype O was seen.

As a part of the fight against FMD and BEF diseases in Turkey, especially in provinces with risk of occurrence of the BEF, the cattle are vaccinated against both diseases separately during the spring every year. Additionally, the vaccines against LSD and Anthrax diseases should be administered during spring with 21-day intervals. As a result, difficulties are faced with regards to vaccination costs, task force and time spent when administering FMD, BEF, Anthrax and LSD vaccinations during the spring. Simultaneous administration of some vaccines in compulsory vaccination programs provides an advantage to combat diseases. For this purpose, many studies have been conducted on simultaneous administration of vaccines in countries where it is obligatory to carry out more than one vaccination (Çokçalışkan et al. 2019; Srinivasan et al. 2001; Elbagoury et al. 2014; Kasem et al. 2017; Trotta et al. 2015; Yang et al. 2015). Successful results have been obtained in studies on simultaneous administration of vaccines against FMD, Anthrax, LSD, Brucella diseases included in seasonal vaccination program in Turkey, and simultaneous vaccinations have started in the field (Çokçalışkan et al. 2019).

This study was conducted for simultaneous administration of FMD and BEF vaccine in especially southern and western parts of Turkey. In this study, it was observed that antibody titers against FMDV in-

creased at 30th and 60th days, and this increase was statistically significant in cattle, which received only FMD and BEF + FMD vaccine. On the other hand, the fact that the antibody titers did not increase on day 30 and day 60 in the control group cattle. These results showed that increase in antibody titers against FMDV in cattle that received only FMD vaccine and BEF + FMD vaccine was completely associated with the vaccination. Based on these data, the cattle that were applied simultaneous BEF+FMD vaccines exhibited statistically significant increases in the antibody titers at the protective level against all 3 serotypes of the FMDV virus. These antibody titers are very compliant with the antibody titers of the cattle that were given only FMD vaccine.

Numerous studies have been conducted for development of live attenuated and inactive vaccines to use against BEF disease, and successful results have been obtained (Ibrahim et al. 2016; Aziz-Boaran et al. 2014; Erganiş et al. 2010; Tzipori et al. 1978; Yang et al. 2015). In order to combat BEF disease, studies have been carried out in other countries to apply BEF vaccine with Akabane and FMD vaccines as simultaneous and combined (El-Bagoury et al. 2014; Yang et al. 2015).

In this study, the increases of antibody titers detected against BEFV on the 30th (1.98 log 10) and 60th (2.48 log 10) days after booster application of BEF vaccine in cattle vaccinated with only BEF and BEF+FMD vaccines was close to the values reported by other researchers and significant increases in antibody titer was observed statistically, which are $TCID_{50}$ 1.83 log10 (Ibrahim et al. 2016), 2.3 log10 (El-Bagoury et al. 2014), at least 1/64-1/28 (Aziz-Boaron et al. 2014), 1/32 1/512 (Yang et al. 2015), at least 1/362 (Vanselow et al. 1995) and 1/128-256 (Tzipori et al. 1975).

As a result, it was found that simultaneous administration of BEF and FMD vaccines to cattle provide sufficient levels of neutralizing antibodies against both diseases, and no side effects occured in the animals following administration of both vaccines. This result suggests that these vaccines can be simultaneously used in fighting programs against FMD and BEF diseases, and this will provide significant convenience for breeders and vaccinating staff.

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Compliance with ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Animal Rights Statement: This study was conducted under the supervision of the General Directorate of Agricultural Research and Policies of the Ministry of Agriculture and Forestry. In addition, permission (Decision no: HDYEK-604-1315/2014) was obtained from the ethics committee of experimental animals of FMD (SAP) Institute.

References

Abayli H, Tonbak S, Azkur AK, Bulut H. (2017) Complete genome analysis of highly pathogenic bovine ephemeral fever virus isolated in Turkey in 2012. Arch Virol. 162, 3233-3238. https://doi.org/10.1007/s00705-017-3470-6

Abubakar M, Arshed MJ, Ali Q, Hussain M. (2012) Spatial trend of foot and mouth disease virus (FMDV) serotypes in cattle and buffaloes, Pakistan. Virol Sin. 27, 320-323. https://doi.org/10.1007/s12250-012-3271-8

Ahmed H I, Farooq U, Zahur A B, Naeem K, Latif A, Irhsad H. (2017) Evidence of foot-and-mouth disease virus excretion in the milk of apparently healthy vaccinated buffaloes in Islamabad, Pakistan. Turk J Vet Anim Sci. 41, 431-434. https://doi.org/ 10.3906/vet-1512-96

Alkan F, Albayrak H, Timurkan MO, Ozan E, Coskun N. (2017) Assessment of the molecular epidemiology of bovine ephemeral fever in Turkey. Vet Arhiv. 87 (6), 665-675. https://doi.org/10.24099/vet.arhiv.160711

Ammerman NC, Sexton MB and Azad AF. (2008) Growth and maintenance of vero cell lines. Curr Protoc Microbiol. available in PMC 2019. https://doi.org/ 10.1002/9780471729259.mca04es1.

Aziz-Boaron O, Gleser D, Yadin H, Gelman B, Kedmi M, Galon N, Klement E. (2014) The protective effectiveness of an inactivated bovine ephemeral fever virus vaccine. Vet Microbiol. 173, 1-8. https://doi.org/10.1016/j.vetmic.2014.06.021.

Aziz-Boaron O, Leibovitz K, Gelman B, Kedmi M, Klemen E. (2013) Safety, immunogenicity and duration of immunity elicited by an ınactivated bovine ephemeral fever vaccine. Plos one. Vol. 8 (12)| e82217. https://doi.org/10.1371/journal.pone.0082217.

Aziz-Boaron O, Brettschneider S, King R. (2015). Seroprevalence of Bovine Ephemeral Fever Virus in Domesticated and Wildlife Species during Epidemic and Inter-epidemic Periods (2000–2009) in Israel. Transbound Emerg Dis. 62, 183-187. https://doi.org/10.1111/tbed.12104.

Basagoudanavar S H, Hosamani M, Tamil Selvan R P, Sreenivasa BP, Saravanan P, Chandrasekhar Sagar BK, Venkataramanan R. (2013) Development of a liquid-phase blocking ELISA based on foot-and-mouth disease virus empty capsid antigen for seromonitoring vaccinated animals. Arch Virol. 158, 993-1001. https://doi.org/ 10.1007/s00705-012-1567-5.

Chaisiririrat T, Sangthong P, Arunvipas P, Petcharat N, Thangthamniyom N, Chumsing W, Lekcharoensuk P. (2018) Molecular Characterization of Bovine Ephemeral Fever Virus in Thailand between 2013 and 2017. Vet Microbiol. 227, 1-7. https://doi.org/ 10.1016/j.vetmic.2018.10.013

Çokçalışkan C, Göktuna PT, 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. Clin Exp Vaccine Res. 8(2), 103-109. https://doi.org/10.7774/cevr.2019.8.2.103

El-Habbaa AS, Radwan ME. (2019) Detection and genetic analysis of bovine ephemeral fever virus G gene in buffy coat samples from cattle at Qualyubia, Egypt 2017. J Virol Sci. 5, 55-65.

El-Bagoury GF, El-Habbaa AS, Gamil MA, Fawzy H.G. (2014) Evaluatıon of an ınactivated combined oil vaccıne prepared for foot and mouth disease and bovine ephemeral fever viruses. BVMJ. 27(1), 221-231.

Erganiş O, Yavru S, Sayin Z, et al., (2010) The development of vaccine for bovine ephemeral fever infection. In: 9th National Veterinary Microbiology Congress, Lefkose, Republic of North Cyrpus.

Erol N, Koç BT, Gür S, Çağlav VO, Tan MT. (2015) Aydın ve Muğla İlleri'nde Bovine Ephemeral Fever Virus Enfeksiyonu'nun Serolojik Olarak Araştırılması. Kocatepe Vet J. 8(2), 45-49. https://doi.org/ 02175570010156

Girgin H, Yonguc AD, Akcora A, (1986) First outbreak of bovine ephemeral fever in Turkey (Türkiye'de ilk bovine ephemeral fever salgını). Etlik Vet Mikrobiyol Enst Derg. 5, 5-12. https://dergipark.org.tr/tr/pub/evmd/issue/61195/734436.

Hussain A, Abubakar M, Shah H, Arshed MJ, Hussain M, Afzal M. (2017) Socioeconomic impact of foot and mouth disease vaccination in Pakistan. Pak J Life Soc Sci. 15(3), 183-191.

Ibrahim M.M, El Zieny A, Christine A. (2016) Further evaluation of locally prepared live attenuated bovine ephemeral fever vaccine in cattle. VSVRI. 23(2),174- 183.

Kasem S, Abdel Fatah S, Khodier M Desouky A. (2017) Evaluation of the Efficacy of Simultaneous Vaccination of Cattle Against Rabies and Foot and Mouth Disease Viruses. Global Veterinaria. 18 (3), 226-233. https://doi.org/10.5829/idosi.gv.2017.226.233.

Karaoğlu T, Ozgünlük I, Demir B, Ozkul A, Burgu I. (2007) Seroprevalence of culicoides-borne disease in cattle in European Turkey. Ankara Univ Vet Fak Derg. 54,121-125. https://doi.org/10.1501/Vetfak_0000000266.

Mirzaie K, Bahonar A, Mehrabadi M.F, Hajilu G, Yaghoubi M. (2017) Determinants of bovine ephemeral fever outbreak during 2013, in Qazvin Province, Iran. Asian Pac J Trop Dis. 7(12), 744-747. https://doi.org./10.12980/apjtd.7.2017D7-22.

Oğuzoğlu TC, Ertürk A, Cizmeci SG, Koç BT, Akça Y. (2015) A report on bovine ephemeral fever virus in Turkey: Antigenic variations of different strains of EFV in the 1985 and 2012 outbreaks using partial glycoprotein gene sequences. Transboundary and Emerging Diseases. 62. https://doi.org/10.1111/tbed.12187.

OIE, (2021). OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Terrestrial manual: foot and mouth disease: OIE terrestrial manual, Chapter 3.1.8. https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access.

Sareyyüpoğlu B, Gülyaz V, Cokçalışkan C, Ünal Y, Çökülgen T, Uzunlu E, Gürcan S, İlk O. (2019) Effect of FMD vaccination schedule of dams on the level and duration of maternally derived antibody. Veterinary Immunology and Immunopathology Vol: 217. https://doi.org/10.1016/j.vetimm.2019.109881.

Srinivasan VA, Reddy GS, Rao KA, Kihm U. (2001) Serological response of bovines to combined vaccine containing foot and mouth disease virus, rabies virus, Pasteurella multocida and Clostridium chauvoei antigens. Vet Arhıv. 71(1), 37-45.

Tekleghiorghis T, Weerdmeester K, Hemert-Kluitenberg FV, Moormann RJM, Dekker A. (2014) Comparison of test methodologies for foot-and-mouth disease virus serotype A vaccine matching. Clinical and Vaccine Immunology. 21(5), 674-683. https://doi.org/ 10.1128/CVI.00034-14.

Tonbak S, Berber E, Yoruk MD, Azkur AK, Pestil Z, Bulut H. (2013) A large-scale outbreak of bovine ephemeral fever in Turkey, 2012. J. Vet Med Sci. 75(11), 1511-1514. https://doi.org/10.1292/jvms.13-0085.

Trotta M, Lahore J, Cardoso N, Melucci O, Catena M, Perez-Filgueira M, Fernandez F, Capozzo AV. (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. Tzıporı S, Spradbrow PB and Doyle VT, (1975) Laboratory and fıeld studıes wıth a bovine ephemeral fever vaccine. Aust Vet J. Vol. 51. https://doi.org/10.1111/j.1751-0813.1975.tb06924.x

Tzıporı S, Spradbrow PB. (1978) A cell culture vaccıne agaınst bovıne ephemeral fever. Aust Vet J. Vol. 54. https://doi.org/10.1111/j.1751-0813.1978.tb02480.x.

Walker PJ, Klement E, (2015) Epidemiology and control of bovine ephemeral fever. Vet Res. 46, 124. https://doi.org/10.1186/s13567-015-0262-4.

Vanselow BA, Walthall JC, Abetz I, (1995) Field trials of ephemeral fever vaccines. Vet Microbiol. 46 (1-3), 117-30. https://doi.org/10.1016/0378-1135(95)00077-n.

Yang D-K, Kim H-H, Jo H-Y, Choi SS, Cho In-S. (2015) Development of inactivated Akabane and bovine ephemeral fever vaccine for cattle. Korean J Vet Res. 55(4), 227-232. https://doi.org/ 10.14405/kjvr.2015.55.4.227.

Yeruham I, Van Ham M, Stram Y, Friedgut O, Yadin H, Mumcuoglu KY, Braverman Y. (2010) Epidemiological Investigation of Bovine Ephemeral Fever Outbreaks in Israel. Vet Med Int. Article ID 290541. https://doi.org/10.4061/2010/290541.

Zheng F,Qiu C. (2012) Phylogenetic relationships of the glycoprotein gene of bovine ephemeral fever virus isolated from mainland China, Taiwan, Japan, Turkey, Israel and Australia. Virol J. 9, 268. https://doi.org/10.1186/1743-422X-9-268.