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Effect of simultaneous administration of foot-and-mouth disease (FMD) and anthrax vaccines on antibody response to FMD in sheep

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Purpose: Foot-and-mouth disease (FMD) and anthrax are important diseases in sheep. Vaccination is a favorable strategy against both infections. Simultaneous administration of vaccines does generally not impede the immune responses of each other, although there are some exceptions, and it may help reduce the labor and costs of vaccination as well as distress on animals. Although oil adjuvant FMD vaccine has been tried with live anthrax vaccine in cattle, there are no reports on the simultaneous use of both vaccines in sheep.
Materials and Methods: In this study, FMD seronegative sheep were used to investigate the impact of the simultaneous vaccination of FMD and anthrax on FMD antibody titers of sheep. Virus neutralization test and liquid phase blocking enzyme-linked immunosorbent assay were used to determine the antibody response to the FMD vaccine.
Results: The results demonstrated that both vaccines can be used simultaneously without any interference with the FMD response. Moreover, the simultaneous administration with anthrax vaccine had a stimulating effect on the early (day 7 post-vaccination) virus neutralization antibody response to the FMD vaccine.
Conclusion: The simultaneous use of the FMD and anthrax vaccines did not hinder the response to the FMD vaccine in sheep.

Keywords: Foot-and-mouth disease, Anthrax, Simultaneous vaccination, Sheep, Virus neutralization

Introduction

Foot-and-mouth disease (FMD) is one of the most important viral diseases in cloven-hoofed animals. The cost of each new FMD incursion into Turkey was estimated to be nearly 230 million dollars according to the 2015-outbreak data [1]. Vaccines are used in countries where the disease is endemic or where there is no infection [2]. Despite emerging new technologies, conventional vaccines are still being used, and significant success has been achieved [3]. Vaccines against many pathogens are generally administered simultaneously for epidemiological reasons. Moreover, the simultaneous use of different vaccines has benefits for animal welfare and the economy. According to the manual of the European Medicine Agency, it must be demonstrated that the vaccines for combined or simultaneous administration do not affect each other's safety and efficacy. These requirements are described in guideline part 5.3 [4]. In livestock

vaccination, cost-benefit aspects are also important. The primary purpose is to maintain the production of animals.

The simultaneous use of active and inactive agents for vaccination is generally safe and efficacious. However, when two live agents are used, a 4-week lapse is generally preferred between two administrations to avoid immunodominance of one agent over the other [5].

There are many reports of the successful simultaneous use of FMD vaccines with other vaccines, such as vaccines against classical swine fever, rinderpest, rabies, hemorrhagic septicemia, brucellosis, parvovirus, and anthrax [6-12]. On the other hand, negative results have been obtained with the simultaneous use of FMD vaccines with *Vesicular stomatitis* live vaccine [13]. The antibody response to both vaccines was reduced with simultaneous use. Sharing the common epitopes of different antigens in multi-disease vaccinations can lead to an increased or decreased response to antigens [14]. Particularly in combined vaccines with *Haemophilus influenzae* type b polysaccharide conjugates and diphtheria-tetanus-pertussis in children, the immune response was lower than expected [14].

Another important disease in farm animals is anthrax, which is a zoonotic disease caused by the resistant, Gram-positive bacterium *Bacillus anthracis*. The disease is endemic in many parts of the world [10]. Due to the higher economic importance of cattle, the spread of the disease among sheep may be underestimated, which could lead to an increased risk of sheep-to-human transmission of the disease [15].

Recently, a study [10] on the simultaneous vaccination with FMD and anthrax vaccines has been conducted in cattle. The immune response to the FMD vaccine was measured using liquid phase blocking enzyme-linked immunosorbent assay (ELISA) (LPBE) for the total antibody response and isotype and single dilution avidity ELISAs for the isotype antibody response and avidity. The results showed that the simultaneous administration of both vaccines did not affect the response against the FMD vaccine. The researchers concluded that both vaccines could be used simultaneously. A total of 1,498,008 cattle have been simultaneously vaccinated with FMD and anthrax vaccines in Argentina since 2004. Up to now, no human or animal case has been reported in the country since the campaign started [16]. Although they are vulnerable to both infections and have an irrefutable role in FMD epidemiology, sheep are generally not included in vaccination programs [17]. Perhaps for this reason, there are no simultaneous vaccination studies in sheep with both agents, except for one study with an aluminum gel FMD vaccine 40 years ago [18].

In this study, the simultaneous administration of an inactive oil adjuvant FMD vaccine and a 34F2 live anthrax vaccine has been evaluated for FMD neutralizing and total antibody responses in sheep by virus neutralization test (VNT) and LPBE. Hence, by the simultaneous use of both vaccines in areas under development, where the farms are scattered and both diseases are prevalent, repeated visits could be prevented, thereby reducing costs and labor as well as vaccination stress on animals.

Materials and Methods

Vaccines

TURVAC 17/25 manufactured by the FMD Institute in Ankara/Turkey, a commercial trivalent inactive double oil emulsion FMD vaccine containing O/TUR07, A/NEP84, AS/TUR15 vaccine strains, and Montanide ISA 206 adjuvant (Seppic, France), was used together with ANT-ETVAC, a live spore *Bacillus anthracis* 34F2 strain vaccine manufactured in the Central Veterinary Control Institute in Ankara/Turkey.

Animals and immunization route

Seven-month-old male merino sheep obtained from a state farm were used. The animals were randomly divided into four groups (Table 1). One milliliter FMD vaccine was administered intramuscularly to the hind legs of the animals, and 0.5 mL anthrax vaccine was injected subcutaneously in the back of the front leg. The animal experiments were conducted according to the recommendations in the International Harmonization of Animal Care and Use guidelines. The study was approved by the ethics committee of the FMD Institute with protocol number 17/03-2.

Table 1. Number of sheep in the groups

Group	No. of animals
Experiment groups	
FMD vaccine alone	16
Simultaneous FMD and anthrax vaccine	16
Control groups	
Anthrax vaccine alone	5
Non-vaccinated group	5

FMD, foot-and-mouth disease.

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Virus neutralization test

The virus neutralization test was performed according to the World Organisation for Animal Health manual [19]. Briefly, serum samples were heat-inactivated for 30 minutes at 56°C. Two-fold serial dilutions of serum samples from 1:4 to 1:512 were prepared by an automated platform (Integra Viaflow, Chur, Switzerland). The diluted sera were then incubated with 100 $TCID_{50}$ homolog viruses for 1 hour at 37°C in a 5% CO_2 chamber. After 1 hour of incubation, BHK-21 cell suspension (600,000 cells/mL) was added to all the wells. The cells were stained with crystal violet dye at the end of the 72-hour incubation time. The endpoint titers were determined by observing the cytopathic effect formation.

Liquid phase blocking ELISA

The assay was performed according to Hamblin et al. [20]. On the first day of the test, ELISA plates were coated with trapping rabbit antibody (anti-FMD virus [FMDV] 146S antigens of serotypes O, A, Asia-1). Meanwhile, test and control sera were added to the wells of the U-bottom 96 well carrier microplates at a dilution of 1/16. Working dilution of FMDV antigens 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 three complete wash cycles of the ELISA plate, a 50 μL mixture of serum/antigen was transferred from the carrier microplate to the ELISA microplates. After sealing, 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 were incubated on an orbital shaker housed in a 37°C incubator with continuous shaking for 1 hour. Then 50 μL working dilution (1:2,000) of the conjugate (polyclonal rabbit anti-guinea pig IgG) was added to the wells and incubated in a 37°C incubator with continuous shaking for 1 hour. Chromogen (OPD, Sigma, St. Louis, MO, USA)/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 immediately added to all the wells. The absorbance was read by the microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA) at 492 nm [20].

Statistical analysis

Levene's test was utilized to demonstrate the differences between groups. Pearson's correlation analysis was used to show the concordance of the ELISA and VNT results.

Results

Virus neutralization test

Following the vaccination, an increase was detected in both groups containing FMD vaccine. On day 7 post-vaccination (pv), the arithmetic means of the virus neutralization (VN) antibody titers were detected as 1:56, 1:20, and 1:18 for OTUR/07, ANEP/84, and ASTUR/15, respectively, in the FMD-alone group. In the simultaneous group, the arithmetic means of VN antibody titers were determined as 1:118, 1:40, and 1:35 for OTUR/07, ANEP/84, and ASTUR/15, respectively, on day 7 pv (Fig. 1A-C). The highest level of VN antibodies was detected in both groups for type O on day 7 pv; however, a slight decrease was observed later (Fig. 1A). For both groups, the increase in VN antibody continued up to day 60 pv for type A (Fig. 1B). For ASIA-1 type, the peak values were obtained on day 14 pv (Fig. 1C). At day 7 pv, the increase in VN antibody titers was significantly higher in the simultaneous group than in the FMD-alone group. The differences between both groups

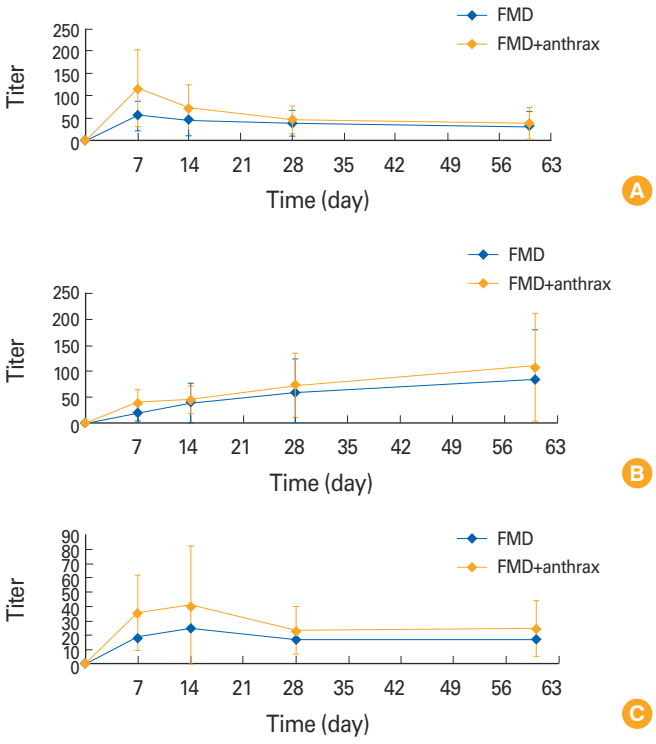

Fig. 1. (A) The post-vaccinal arithmetic means of virus-neutralizing antibody titers against OTUR/07 for the experiment groups. (B) The post-vaccinal arithmetic means of virus-neutralizing antibody titers against ANEP/84 for the experiment groups. (C) The post-vaccinal arithmetic means of virus-neutralizing antibody titers against ASTUR/15 for the experiment groups. FMD, foot-and-mouth disease.

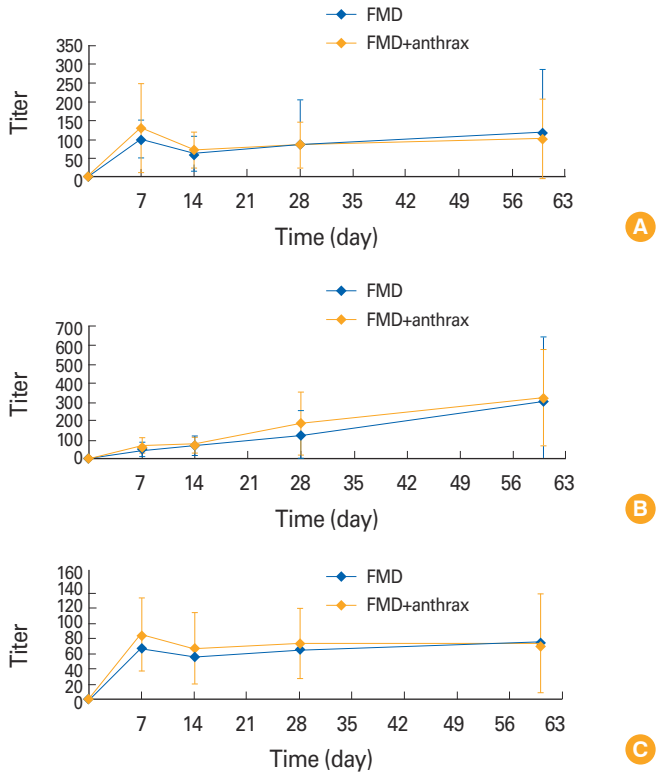

Fig. 2. (A) The post-vaccinal arithmetic means of the liquid phase blocking enzyme-linked immunosorbent assay (LPBE) antibody titers against OTUR07 for the experiment groups. (B) The post-vaccinal arithmetic means of the LPBE antibody titers against ANEP/84 for the experiment groups. (C) The post-vaccinal arithmetic means of the LPBE antibody titers against ASTUR/15 for the experiment groups. FMD, foot-and-mouth disease.

were not significant on the days thereafter. On all days, no VN antibody against FMD virus was detected in the sera of the control groups.

Liquid phase blocking ELISA

The total antibody response curves that were obtained by LPBEs were similar to the antibody response curves obtained in the VN assay. On day 7 pv, the arithmetic means of the LPBE antibody titers of the FMD-only vaccinated group were detected as 1:101, 1:43, and 1:68 for OTUR/07, ANEP/84, and ASTUR/15, respectively. In the simultaneous group, the arithmetic means of the LPBE antibody titers were found to be 1:130, 1:70, and 1:86 for OTUR/07, ANEP/84, and ASTUR/15, respectively (Fig. 2A-C). The highest values were obtained for type O and type Asia-1 on day 7 pv (Fig. 2A, C) and for type A on day 60 pv (Fig. 2B). No significant difference was detected between the FMD-alone group and the simultaneous group for the whole period of the study. No liquid phase blocking antibody was detected in the sera of the control groups on all days. All ELISA and VNT results were statistically concordant.

Discussion

Vaccination is almost the only weapon in the fight against viral animal diseases because wide-spectrum antivirals are not available and sanitary measures are generally ineffective in the field. To combat wide range viral agents, many inactivated or attenuated conventional vaccines have routinely been used in immunization campaigns [3].

The FMD vaccine is one of the earliest animal vaccines. Although many studies have been carried out to develop new FMD vaccines, inactivated whole virus vaccines are being used throughout the world today [2]. Disadvantages of the vaccines are the inability to induce a cytotoxic T-cell response [3] and provide short-term immunity [21]. The protective response needs repeated administration every 6 months [2]. Even repeated vaccinations cannot produce sterile immunity, which means that vaccinated animals can also become infected [21].

The live spore Sterne vaccine (34F2) is most commonly used in animals against anthrax. $CD4^+$ T cells, which secrete specific interferon γ (IFN-γ) cytokines against anthrax toxins such as LF, play an essential role against infection. Similarly, following exposure to LT and ET, rodent lymphocytes secrete cytokines such as interleukin (IL)-3, IL-4, IL-5, IL-6, IL-10, IL-17, tumor necrosis factor α, IFN-γ, and granulocyte-macrophage colony-stimulating factor [22,23].

Combined or simultaneous vaccinations are needed because of the existence of multiple pathogens in animals. Many successful co-applications of different agents consist of bacterial and viral antigens, such as rinderpest, contagious bovine pleuropneumonia [24], Pest Des Petits Ruminants, clostridial agents [25], *Brucella* [26], pneumo-3 virus, and sheeppox virus [27]. There are limited numbers of studies on FMD immunization together with other vaccines [10,12,28]. On the other hand, in practice, many vaccines, including FMD, are routinely applied almost at the same time or within short periods of time, and interactions between these vaccines are not known. Co-administration of different vaccines makes vaccination more practical, economic, and timesaving, especially when the animals are scattered in the field. Another advantage can be the reduction in vaccination stress for the animals [29].

Srinivasan et al. [11] reported that a combined vaccine

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containing FMD, rabies, *Pasteurella*, and *Clostridium* agents could be successfully utilized in countries where the diseases are endemic. Another combined vaccine experiment used FMD and ephemeral fever agents with Montanide ISA 206 oil adjuvant. No negative effect has been observed on the immune response to both agents in calves [29]. Another study experimented with Rift Valley fever and FMD vaccines in pregnant sheep [30]. According to the results of this study, the lambs born to dams vaccinated with combined vaccine have antibody titers of a protective level. Recently, vaccine manufacturers have combined hemorrhagic septicemia (*Pasteurella multocida*) and FMD vaccine and obtained a prolonged immune response to both antigens in buffaloes [31]. Although combining the vaccines in one syringe can help to minimize the labor of vaccination campaigns, for manufacturers it is a complicated procedure, and each antigen ingredient still has to be produced separately [32]. Besides, some manufacturers produce only a single type of agents. Therefore, some studies have focused on the simultaneous administration of different vaccines.

Hanci et al. [12] investigated the simultaneous application of live attenuated *Brucella* and FMD vaccines in cattle. The authors found that the antibody titers against *Brucella* were higher in the simultaneous administration than in the *Brucella*-alone group. However, the FMD titers were the same in both groups. Elham and Abeer [33] found that the simultaneous administration of FMD and polyvalent *Pasteurella* vaccines did not hamper the cellular or humoral responses of each other. The only negative result obtained in the simultaneous application of FMD with other vaccines was with *Vesicular stomatitis* vaccines. The FMD antibody titers were found to be lower than normal when the two vaccines were used at the same time [13].

Trotta et al. [10] simultaneously administered tetravalent FMD and anthrax vaccines in seropositive cattle and gathered the results by ELISA. The results showed that after a booster administration, no significant difference was detected for FMD antibodies except for the type O response. Only the total antibody response to the O_1 Campos strain was detected to be higher in the simultaneous group. The authors explained that the higher response was caused by a cytokine increase induced by the live anthrax vaccine. The results confirmed that anthrax live vaccine prepared by the Sterne strain could be used together with an oil adjuvant FMD vaccine [10].

In our study, in naïve sheep, the FMD virus-neutralizing antibody response in the simultaneous vaccination group was found to be significantly higher than that in the FMD-alone group ($p<0.05$) on day 7 pv. On the other hand, on the other days of the study, the higher mean antibody titers in the simultaneous vaccination group were found not to be statistically significant. This finding indicates that simultaneous administration with anthrax vaccine has a stimulating effect on the early VN antibody response to FMD vaccine. Although we did not utilize a test which evaluate cell-mediated immunity, earlier reports showed that anthrax vaccine induces a cytokine response and Th1-type immunity [14]. As discussed by Trotta et al. [10], cytokines might have played a role in the transient high FMD antibody response, which did not continue beyond day 7 pv in our study. Our ELISA results supported the VN test findings.

In conclusion, the simultaneous use of the FMD and anthrax vaccines did not hinder the response to the FMD vaccine in sheep. Although it is highly unlikely that it would be suppressed by the presence of a weak inactivated antigen (FMDV), the immune response to anthrax should be investigated when simultaneously applied with the FMD vaccine. Thus, both vaccines can simultaneously be used in sheep, and labor and costs can be reduced, particularly in the case of emergency vaccinations.

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