RESEARCH ARTICLE

# Antibody responses against foot-and-mouth disease vaccine differ between the sexes in cattle

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Received: 27.04.2013, Accepted: 15.06.2013
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## Özet

**Şevik M.** Sığırlarda şap hastalığına karşı aşılamada cinsiyetler arasında antikor yanıtı farklılığı. **Eurasian J Vet Sci, 2013, 29, 4, 205-210**

**Amaç:** Sığırlarda şap virusuna karşı aşılamaya bağlı gelişen humoral immun yanıt üzerine cinsiyetin etkisinin araştırılması amaçlanmıştır.

**Gereç ve Yöntem:** Sığırlar (n=252) yaşlarına (0-11 ay, 12-35 ay ve >35 ay) ve cinsiyetlerine (erkek-dişi) göre 6 gruba ayrıldı. Her bir gruptaki hayvanlar yağ adjuvantlı bivalent ($O_1$ Manisa, $A_{22}$ Irak suşları) aşı ile aşılandı. Aşılanan sığırlardaki antikor yanıtı solid faz kompetitif ELISA ile belirlendi.

**Bulgular:** Yüz yirmi altı erkek serumunun, 86 (%68.2)'sında serotip O, 90 (%71.4)'ında serotip A'ya karşı antikor tespit edildi. Dişi hayvanlarda ise 126 serumun 106 (%84.1)'sında serotip O, 112 (%88.8)'sinde serotip A'ya karşı oluşan antikor tespit edildi. Dişi serumlarının 89 (%70.6)'unda serotip O, 98 (%77.7)'sinde serotip A'ya karşı koruyucu düzeyde antikor yanıtı belirlendi. Erkek sığır serumlarının ise 67 (%53.1)'sinde serotip O, 81 (%64.2)'inde serotip A'ya karşı koruyucu düzeyde antikor varlığı tespit edildi. Dişi ve erkek hayvanlar arasında hem serotip O (P=0.0063) hem de serotip A (P=0.0259)'ya karşı koruyucu düzey antikor yanıtları arasındaki farklılık istatiksel olarak önemli bulundu.

**Öneri:** Sonuçlar yağ adjuvantlı bivalent ($O_1$ Manisa, $A_{22}$ Irak suşları) aşı ile aşılanan dişi hayvanların, erkek hayvanlardan daha yüksek antikor yanıtlarına sahip olduğunu göstermektedir. Dişi ve erkek hayvanlar arasında şap aşılamasına bağlı gelişen immun yanıt farklılığının aydınlatılması için daha fazla çalışmaya ihtiyaç vardır.

**Anahtar kelimeler:** Şap hastalığı, sığır, aşılama, cinsiyet, antikor yanıtı

## Abstract

**Sevik M.** Antibody responses against foot-and-mouth disease vaccine differ between the sexes in cattle. **Eurasian J Vet Sci, 2013, 29, 4, 205-210**

**Aim:** The aim of this study was to investigate the effects of sex on the humoral immune response induced in cattle by vaccination against foot-and-mouth disease virus (FMVD).

**Materials and Methods:** Cattle (n=252) were classified into six groups according to the age (0-11 months, 12-35 months, and >35 months) and sex (male-female). Animals in each group were vaccinated with oil-adjuvanted bivalent vaccine ($O_1$ Manisa, $A_{22}$ Iraq FMDV strains). Solid-phase competitive ELISA was used to measure antibodies produced in vaccinated cattle.

**Results:** Serotype O antibody was detected in 86 (68.2%) and serotype A antibody in 90 (71.4%) of 126 male sera. In female animals, serotype O antibody was detected in 106 (84.1%) and serotype A antibody in 112 (88.8%) of 126 sera. Protective level of antibody against serotype O was detected in 89 (70.6%) and serotype A in 98 (77.7%) of 126 female sera. Protective level of antibody against serotype O antibody was detected in 67 (53.1%) and serotype A in 81 (64.2%) of 126 male sera. The differences between the level of protective antibody against both serotype O (P=0.0063) and serotype A (P=0.0259) in female and male animals were statistically significant.

**Conclusions:** Results showed that female animals vaccinated with oil-adjuvanted bivalent vaccine (containing $O_1$ Manisa, $A_{22}$ Iraq FMDV strains) had higher antibody responses than male animals. In order to elucidate difference between immune response of male and female animals to FMD vaccination more studies are needed.

**Keywords:** Foot and mouth disease, cattle, vaccination, sex, antibody response

## Introduction

Foot-and-mouth disease (FMD) is a major livestock disease, causing economical losses due to loss in milk and meat production, mortality of young animals, and restrictions on trade (Kitching et al 2007). Lameness, vesicles on the tongue, nose, teats and feet are the conspicuous clinical signs of the disease (Alexandersen and Mowat 2005). It has been eradicated from many regions of the world, but continues to exit in Asia, Africa, South America and Anatolian Region of Turkey (WRLFMD 2013).

Foot-and-mouth disease virus (FMDV) is a non enveloped virus with icosahedral symmetry that is classified as the member of the *Aphthovirus* genus in the *Picornaviridae* family (Van Regenmortel et al 2000). Its genome is single stranded positive RNA. The FMDV virion is made up of 60 copies of the four structural proteins, VP1 to 4 (Grubman and Baxt 2004). The G-H loop of VP1 has been identified as the major antigenic site (Borrego et al 1995). There are seven distinct serotypes of FMD, and there is no cross-protection across serotypes and subtypes. FMDV may cause asymptomatic but persistent infection in vaccinated or naturally immune ruminants (Kitching et al 2007). FMDV has very high mutation rates during replication (Domingo et al 2003). One of the most troubling consequences of high mutation rates is antigenic diversity. The antigenic variation makes FMD very difficult to control (Grubman and Baxt 2004). Vaccination is one of the most powerful tools to protect animals against FMD, although vaccines do not induce lifelong protection (Cloete et al 2008, Rodriguez and Grubman 2009). Three species (cattle, sheep and pigs) are the main target of FMD vaccines. Conventional vaccines make use of inactivated viral strains of FMDV. To protect an animal against all the prevailing FMDVs would require a vaccine combining multiple, representative strains. All currently available FMD vaccines are chemically inactivated and blended with suitable adjuvants, based on cell culture derived preparations of whole virus (Jamal et al 2008, Rodriguez and Grubman 2009). Typically, FMD vaccines formulated with the adjuvant of aluminium hydroxide gel-saponin (AS) or oil, and can be monovalent, bivalent and multivalent, including viruses of different strains and/or serotypes (Jamal et al 2008). Oil adjuvant FMD vaccines have been shown to induce higher antibody titres than AS vaccines (Cloete et al 2008). Systematic vaccination programs with conventional vaccines have successfully reduced the prevalence of disease in enzootic areas (Smitsaart et al 1998). FMD vaccines may initiate protection against disease within 4 to 5 days of vaccination (Kitching et al 2007). Protection against FMD correlates with levels of neutralizing antibodies induced by immunization. Neutralizing antibody production is associated mainly with the infective 146S virus particles (Wang et al 2011). Vaccinated animals produce antibodies to structural (SP) proteins only but infected animals produce antibodies to both SP and non-SP (Kitching et al 2007). Immunity level of the vaccinated cattle population is readily measured by detecting antibodies to the capsid or structural proteins of the virus (Smitsaart et al 1998). It has been shown that there is a good correlation between antibody response and degree of protection (Jamal et al 2008).

In this study, I investigated the effects of sex on antibody serotype responses induced in cattle by oil-adjuvanted bivalent vaccine (a commercial vaccine, containing $O_1$ Manisa, $A_{22}$ Iraq FMDV strains).

## Material and Methods

*Animals and sampling*

A total of 252 cattle (at the 95% confidence level with an allowable error of 4.4%) consisting of 126 females (Brown Swiss hybrid) and 126 males (Brown Swiss hybrid) were investigated. Cattle were kept under similar care and feeding conditions. Cattle were classified into six groups according to the age (0-11 months, 12-35 months, and older than 35 months) and sex (male-female) to determine sex related differences in antibody responses. FMD vaccination statuses of subgroups are presented in Table 1. Animals in each group were vaccinated with oil-adjuvanted bivalent vaccine ($O_1$ Manisa, $A_{22}$ Iraq FMDV strains, payload of the antigens 6 µg and 4 µg, respectively) formulated in a double oil emulsion adjuvant. Same batches of a commercial vaccine were used. Vaccination was performed by injection of 2 mL volumes subcutaneously in the dewlap in the region of the brisket. Serum samples were collected 28 days after vaccination. No other vaccinations were administered to these animals. Pregnant animals were not used in this study.

*Test reagents*

Anti-FMDV O and A serotype specific strong positive antiserum (C++), weak positive antiserum (C+) and negative serum (C-), serotype specific (O and A) rabbit anti-FMDV sera (trapping) and guinea pig (detector) antiserum were obtained from the Institute for Animal Health, Pirbright Laboratory, UK. Cell culture derived FMDV O and A serotype antigens, and horseradish peroxidase conjugated rabbit anti-guinea pig immunoglobulin were obtained from the Institute for Foot and Mouth Disease, Turkey.

*Solid-phase competition ELISA*

The solid-phase competition ELISA was carried out as described by Mackay et al (2001). ELISA plates were coated with serotype specific (O and A) rabbit anti-FMDV serum, and held overnight at 4°C. Plates were washed with PBS containing 0.05% Tween-20 (PBST), and FMDV antigen (50 µL) homologous to the rabbit antiserum was added to each well. Then, plates were incubated at 37°C for 1 hour. Next, 50 µL of test sera and control sera (twofold dilutions of an initial serum dilution of 1:2.5 through 1:20), in blocking buffer (PBST

containing 10% normal bovine serum and 5% normal rabbit serum), and serotype specific guinea pig antiserum were added. After incubation at 37°C for 1 hour, optimal concentration of anti-guinea pig immunoglobulin conjugated with horseradish peroxidase in blocking buffer was added to all wells and the plates were incubated at 37°C for 1 hour. Then, substrate/chromogen (OPD+ 0.05% $H_2O_2$) was added to each well of the plates. The reaction was stopped after 15 min by adding 1.25 M sulphuric acid, and OD values at 492 nm wavelength were read using a spectrophotometer (Molecular Devices, Sunnyvale, CA). Sera giving ≥60% inhibition were considered positive (OIE 2008). This represents a titres ≥1:7.5 (log2=2.9). ELISA titres of 1:15 (log2=3.9) or more were considered protective (Berinstein et al 2000, Sap Institute 2009).

*Statistical analysis*

Wherever possible, descriptive statistics were used. The Fisher's exact 2-tailed test was used for nonparametric analysis. All statistical analyses were performed with GraphPad InStat version 3.10 (GraphPad Software, San Diego, CA, USA).

## Results

*Antibody responses of female and male animals*

Serotype O antibody was detected in 86 (68.2%) and serotype A antibody in 90 (71.4%) of 126 male sera. On the contrary, serotype O antibody was detected in 106 (84.1%) and serotype A antibody in 112 (88.8%) of 126 female sera (Table 1). The differences between the seropositivity rates against both serotype O (P=0.0047) and serotype A (P=0.0008) in female and male animals were statistically significant. In the age groups of 12-35 months and older than 35 months, females had a significantly higher seropositivity rates against both serotype O (P=0.0133 for 12-35 months, P=0.0033 for older than 35 months) and serotype A (P=0.0071 for 12-35 months, P=0.0016 older than 35 months) than males at the same age (Table 1).

*Protective antibody levels of female and male animals*

Protective level of antibody against serotype O was detected in 89 (70.6%) and serotype A in 98 (77.7%) of 126 female sera. The level of protective antibody was significantly lower for both serotype O (P=0.0063) and serotype A (P=0.0259) in males than that of female animals (Table 1). A higher percentage of cattle with protective level of antibody titres against both serotype O and serotype A were found in 12-35 months (83.3%) and older than 35 months (90.4%) of female animals (Figure 1 and 2).

## Discussion

Geographically, Turkey is the connection between Europe, and Asia where the disease is endemic. The FMD vaccination programme in Turkey has been implemented since 1962. Considerable success in the control of FMD has been achieved by the effective use of oil adjuvanted vaccines (Sap Institute 2013).

Three serotypes of FMDV have been isolated from Turkey field samples. Serotypes A and O have been observed since their first identification in 1952, whereas Asia-1 is epidemic and no case of Asia-1 has been reported since 2002. After 9 years new incursion by Asia-1 (Sub lineage Asia $1^{AFG-07}$) occurred in 2011 (FAO 2013). Genetic analysis of serotypes O and A during 1964 and 2003 revealed that the evolutionary rates were 0.6% and 1% nucleotide substitution per year, respectively (Gilbert et al 2005). Turkish isolates were closely matched with isolates from the Middle-East. Two different subtypes of serotype A (A Iran 96 and A Iran 99) have been circulating in Turkey since 1999 (Klein et al 2006). The O/ME-SA/PanAsia-$2^{ANT-10}$ and A-Iran-$05^{AFG-07}$ lineages continue to dominate in Turkey (WRLFMD 2013). Comparison of genetic diversity between immunogenic region of the Turkish type O strains and the serotype O vaccine strain, $O_1$ Manisa, reveals significant similarity (Klein et al 2006). Also, serum neutralization assays demonstrated a closer relationship between $A_{22}$ and A/IRN/2005 subtype (Paton 2006). Therefore

Table 1. Antibody response by SPCE in different age groups of male and female animals.

| Sex | Age (Month) | No. tested | Number of FMD Vaccination | No. positive | | No. protective | |
|---|---|---|---|---|---|---|---|
| | | | | Serotype O | Serotype A | Serotype O | Serotype A |
| Female | 0-11 | 42 | 1-2 times | 26 | 30 | 16 | 25 |
| | 12-35 | 42 | 3-6 times | 40[a] | 41[a] | 35 | 35 |
| | ≥36 | 42 | ≥ 6 times | 40[a] | 41[a] | 38[a] | 38[a] |
| | | | Total | 106 | 112 | 89 | 98 |
| Male | 0-11 | 42 | 1-2 times | 26 | 28 | 14 | 20 |
| | 12-35 | 42 | 3-6 times | 31[b] | 32[b] | 28 | 32 |
| | ≥36 | 42 | ≥ 6 times | 29[b] | 30[b] | 25[b] | 29[b] |
| | | | Total | 86 | 90 | 67 | 81 |

[a, b]:Statistically different in the same age groups of different sexes (P<0.05).

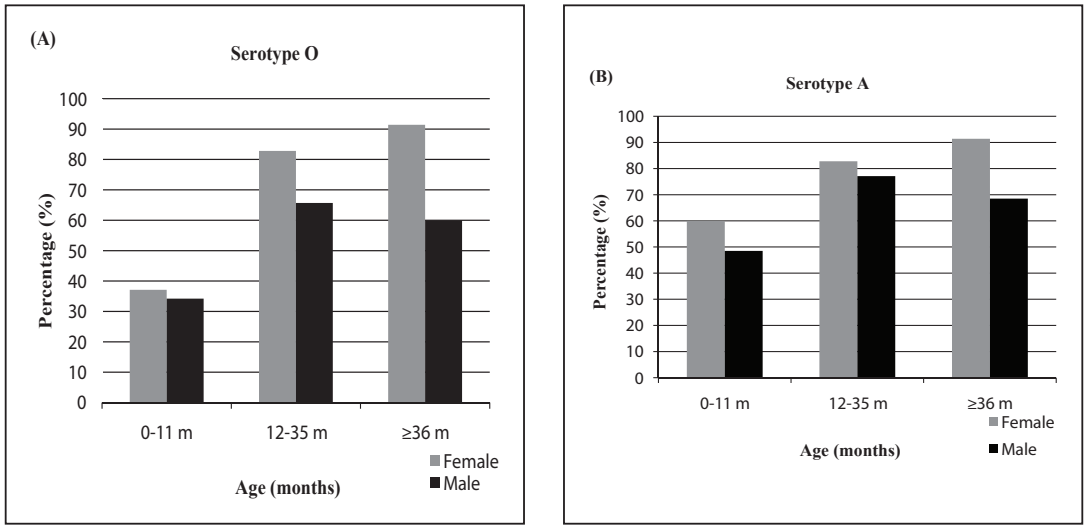


Figure 1. Protective level of antibody against serotype O and serotype A according to age in female and male cattle.

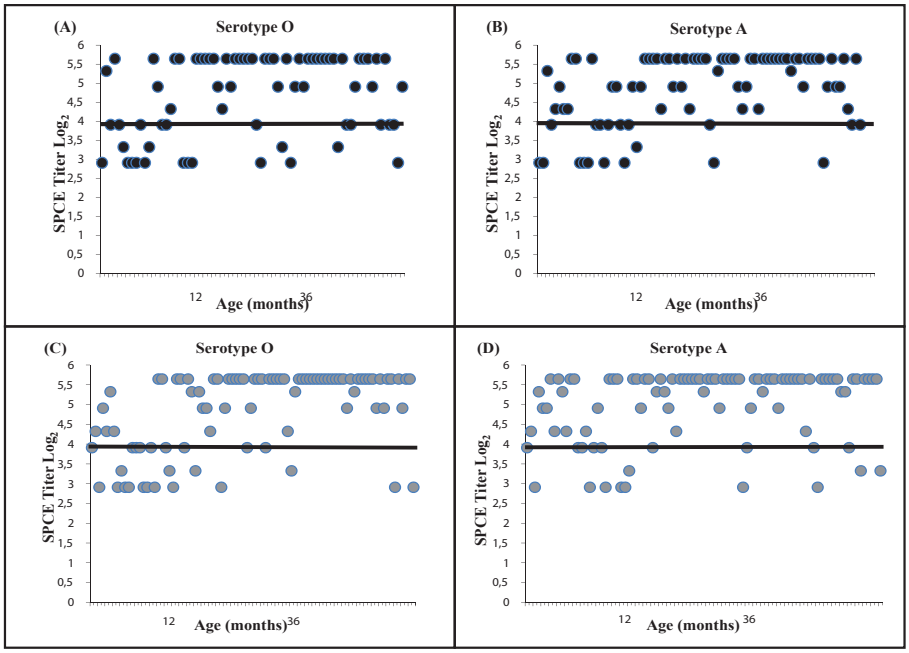


Figure 2. Comparison of protective antibody titres in seropositive male (A-B) and female (C-D) cattle.

$O_1$ Manisa and $A_{22}$ were used as vaccine strains in Turkey.

There is substantial evidence for a good correlation between the humoral immune response and protection against clinical sign of FMD (Goris et al 2008). Virus neutralization test (VNT), liquid-phase blocking ELISA (LPBE) and solid-phase competition ELISA (SPCE) are the internationally accepted tests for determining the FMD antibody status of cloven-hoofed animals (OIE 2008). VNT has high specificity and sensitivity, but it takes several days and requires cell culture systems. Although LPBE is highly sensitive, it can give false positive results. Compared to LPBE, SPCE has several advantages: it is more specific, simpler and quicker (Mackay et al 2001). In the current study, antibody titres were determined by SPCE.

There are accumulating data illustrating that rates of infection with bacteria and viruses are higher in male humans and mice than their female counterparts (Bouman et al 2005). Compared to males, females mount stronger innate and adaptive immune responses to pathogen challenge (Klein 2000). Additionally, vaccine studies demonstrated differences between males and females in response to immunization (Aaby et al 2006). Lorenzo et al (2011) reported that influenza vac-

cine induced antibody response was higher in female mice than males, and females exhibit greater cross protection to influenza viruses of different subtypes. Similarly, Klein et al (2010) reported that females had higher antibody responses to the yellow fever vaccine, hepatitis A and B vaccines, and combined measles, mumps, rubella vaccine than males. As it was shown in these studies, antibody responses to viruses and vaccines are higher in females than in males. There is little information on sex differences in FMD. In this study, for the first time I investigated the effects of sex on antibody serotype responses induced in cattle by oil-adjuvanted bivalent vaccine. To determine whether there is a difference in antibody response between male and female animals, cattle were classified according to age and number of vaccinations, and they were vaccinated with the same dose of FMD vaccine. I determined that seropositivity rates against both serotype O and serotype A in male animals were less than females of the same age (Table 1). Furthermore, protective levels of antibody increased, as expected, according to the age in female animals but slightly decreased in male animals older than 35 m (Figure 1). Researchers (Mannan et al 2009, Sarker et al 2011) reported that the prevalence of FMD was significantly higher in male than female in Bangladesh. Sil and Taimur (2000) were also obtained similar results, and they reported that bull/bullocks were more susceptible than cows. Results of this study were consistent with other researchers (Mannan et al 2009, Sarker et al 2011). Also, in this study I didn't find any statistically significant correlation between antibody response and sex at the age of 0-11 months. However, female animals older than 11 months had higher protective titres against both serotype O and serotype A than males at the same age (Figure 2). It can be explained by the hormonal activity. In the period between birth and puberty (8-14 months old) levels of androgen and oestrogen remain rather low and are nearly equal in both sexes (Haeberle 1983). It has been hypothesized that immunological differences between the sexes are linked with sex steroids, especially testosterone, 17β-oestradiol, prolactin, and progesterone, appear to stimulate immune cells (Kovats et al 2010). However, oestrogens affect the differentiation and functioning of dendritic cells, which has an important role in stimulating immune response to a vaccine (Carreras et al 2008). It is reported that male reproductive hormones including dihydrotestosterone and testosterone, appear to suppress the activity of immune cells, and have been shown to decrease immunoglobulin and cytokine production, and to limit lymphocyte proliferation (Rettew et al 2008). It has been also suggested that X chromosome genes are involved in immune responses, and have differential contributions on the immune systems of males and females (Fish 2008). X-linked genes mutations have been found that affect the immune responses. Compared to Y chromosome, X chromosome contains 10% of all microRNAs (miRNAs) which are the regulators of immune responses (Dai and Ahmed 2011, Pinheiro et al 2011).

## Conclusions

The results of this study suggest that female animals had higher antibody responses to bivalent (serotype O and serotype A) inactivated FMD vaccine than male animals. This difference in immune response between males and females can be used in development of vaccine strategies to control FMD. Also this difference has to be taken into consideration whether vaccination schedules should differ for male and female animals. In order to elicitate difference between immune response and susceptibility of male and female animals to FMD more studies are needed.

## Acknowledgements

I thank FMD Institute (Ankara, Turkey) for the use of laboratory facilities.

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