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# Seroprevalence of antibodies to non-structural protein of foot-and-mouth disease virus in vaccinated dairy cattle

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## Abstract

Foot-and-mouth disease (FMD), a highly contagious viral disease of livestock, is endemic in Iran. To investigate the prevalence of antibodies against 3ABC non-structural protein (NSP) of FMD virus, a cross-sectional study was conducted on dairy cattle in eight cities of Kurdistan Province from May to September 2016. Serum samples (n = 283), were collected from cattle vaccinated with the recommended dose of a commercial vaccine and tested by a Competition enzyme-linked immunosorbent assay. Results showed the overall seroprevalence of antibodies against NSP of FMD virus in the vaccinated cattle was 22.30% (95.00% CI: 17.40 - 27.20%). The seroprevalence of antibodies was affected by geographical regions, with the highest seroprevalence related to the samples of vaccinated cattle in the cities of Marivan 95.00% (95.00% CI: 92.50 - 97.50%) and Saqqez 38.50% (95.00% CI: 32.80 - 44.20%). In terms of age, the highest seroprevalence of antibodies to FMD virus 26.70% (95.00% CI: 21.60-31.80%) belonged to ≤ 24-month-old cattle. These findings suggest that the presence of NSP antibodies in vaccinated cattle indicates the risk of infection with FMD virus serotypes circulating in the west of the province, so further studies with a larger sample size are recommended.



## Introduction

Foot-and-mouth disease (FMD) is an *Aphthovirus* belonging to the *Picornaviridae* family, with 7 serotypes and several subtypes.[1] Infection with one serotype does not confer immunity against other serotypes in livestock.[2]

Severe epidemics of FMD occur due to a wide range of susceptible hosts in nature. Excessive numbers of viruses can be spread by infected animals in the environment, and even low numbers of viruses can lead to infection in healthy cattle.[1] The morbidity is very high in mature cows, but its mortality is low. The recovered cattle are unable to return to the economic production cycle for a long time due to excessive physical weakness.[2]

Analysis of exposure of cattle vaccinated against FMD to circulating viruses in endemic areas of the disease by serum methods is very important and helpful, and the significance of using nonstructural proteins (NSPs) to diagnose the animals infected with the virus is well-known in the circulation of FMD in the vaccinated cattle.[3] The animals infected with FMD produce antibodies against both structural and nonstructural FMD proteins, while the vaccinated animals only produce antibodies against structural proteins.[4] Conventional serological tests that can diagnose structural antibodies are suitable to analyze the prevalence of FMD.[2] However, in areas where vaccinated and infected animals exist, the production of structural antibodies in vaccinated and infected animals is induced through both inactive viruses (vaccines) and live viruses (in infected or carrier animals.[5] The serological tests capable of diagnosing NSPs due to infection or FMD viruses in circulation are used in such cases.[3]

In countries like Uganda, Loanda, Thailand, Cameroon, Egypt, Bhutan[5] and Cambodia[6] where FMD is endemic and vaccination programs are performed, the disease is investigated through this method.

The outbreaks of FMD are still considered a serious threat to the cattle population of Iran. The population of cattle vaccinated against FMD has increased since the implementation of the "FMD Control by Vaccination"

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program.[7] Serological analysis of antibodies against Foot and Mouth Virus (FMDV) was limited to the population of dairy cattle farms that were regularly vaccinated. Therefore, this study aimed to measure the presence of 3ABC antibodies to NSPs of foot-and-mouth disease virus in vaccinated cattle.

## Materials and Methods

**Study design and sampling.** To study the seroprevalence of antibodies against NSP of FMD virus, a cross-sectional study for sample collection was conducted from May to September 2016. Information about risk factors such as age and location was collected from the cattle owners. In total, 283 blood samples were obtained from dairy cattle farms in eight cities: Saqqez, Baneh, Marivan, Sarvabad, Kamyaran, Dehgolan, Qorveh, and Bijar. Sampling was based on geographical location, proximity to the livestock, trading places, and availability of cattle. Blood samples were obtained from uninfected dairy cattle immunized with the recommended dose of a commercial trivalent vaccine (Razi Vaccine and Serum Research Institute, Karaj, Iran) containing serotypes O, A, and Asia1.The serum samples were tested for detection of antibodies against 3ABC NSPs of FMD viruses using a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit (Rue Louis Pasteur, Grabels, France) according to the manufacturer's protocol. The optical density (OD) values were measured spectrophotometerically (Pharmacia Biotech, Cambridge, UK) at 450 nm and for each sample, and the calculation of the competition percentage (S/N %) was as follows:

$$S/N\ (\%) = OD\ sample / OD\ negative\ control \times 100$$

Samples with S/N % ≤ 50.00% were considered positive while samples with S/N % > 50.00% were considered negative.

**Statistical analysis.** The animals were divided into two age categories: > 24 months and ≤ 24 months of age. The chi-square test was used for univariate analysis to assess the association between FMD seropositivity and different locations and age groups. The confidence level of the test was set at 95.00% and a $p$ value of < 0.05 was considered significant. Basic statistics were analyzed using SPSS software (version 22.0; IBM Corp., Armonk, USA) for biomedical research.

## Results

In competitive ELISA for the detection of antibodies against NSPs of FMD 22.30% (95.00% CI: 17.40 - 27.20%) of the samples were positive. The lowest rate of antibody to FMDV NS proteins was found in the serum of cattle in Bijar and Sarvabad cities, whereas the highest level was observed in the cattle of Marivan and Saqqez cities (Table1).

According to Table 2, the seroprevalence of NSP-Ab of the FMD virus was significantly ($p < 0.05$) higher in the age group ≤ 24 months (26.70%, 95.00% CI: 21.60 - 31.80%) compared to the age group > 24 months (19.70%, 95.00% CI: 15.00 - 24.40%).

## Discussion

Foot-and-mouth disease is endemic in Iran and causing highly economic losses. Systematic vaccination campaigns covering large domestic ruminants have been in place under the Veterinary Organization. Serosurveillance is an efficient and practical method for the retrospective analysis of FMD prevalence and the production of epidemiological information about the prevalence of the disease in a region.[7] From April 2014 to March 2015, 1381 cases of FMD were reported among cattle in Iran, a large proportion of which (1076) were related to the prevalence

**Table 1.** Seroprevalence of FMD virus NSP-Ab in different location.

| Location | Number of samples | Number of positive samples | Seroprevalence (%) 95.00% CI | *p*-value |
|---|---|---|---|---|
| **Baneh** | 40 | 9 | 22.50 (17.50 - 27.50) | 0.000 |
| **Bijar** | 16 | 0 | 0 | 0.998 |
| **Dehgolan** | 47 | 8 | 17.00 (12.70 - 21.30) | 0.522 |
| **Sarvabad** | 27 | 2 | 7.40 (4.50 - 10.30) | 0.119 |
| **Saqqez** | 39 | 15 | 38.50 (32.80 - 44.20) | 0.126 |
| **Qorveh** | 60 | 5 | 8.30 (5.20 - 11.40) | 0.053 |
| **Kamyaran** | 34 | 5 | 14.70 (10.60 - 18.80) | 0.397 |
| **Marivan** | 20 | 19 | 95.00 (92.50 - 97.50) | 0.000 |
| **Total** | 283 | 63 | 22.30 (17.40 - 27.20) | 0.001 |

$\chi^2 = 83.679$, $p < 0.05$.

**Table 2.** Seroprevalence of FMD virus NSP-Ab in different age groups.

| Age category | Number of samples | Number of positive samples | Seroprevalence (%) 95.00%CI | *p*-value |
|---|---|---|---|---|
| **≤ 24 months** | 105 | 28 | 26.70 (21.60 - 31.80) | 0.028 |
| **> 24 months** | 178 | 35 | 19.70 (15.00 - 24.40) | 0.156 |
| **Total** | 283 | 63 | 22.30 (17.30 - 27.30) | 0.005 |

$\chi^2 = 8.013$, $p < 0.05$.

of the disease.[7] Iran's policy toward FMD control is based on the mass vaccination of livestock. The role of carrier animals in the occurrence of clinical infections among vaccinated livestock is still debatable. Vaccinated populations are highly susceptible to repeated subclinical infections. However, it is sometimes possible to produce antibodies against NSPs in animals under a systematic vaccination program in the absence of infection.[2] In many countries, vaccination plays a pivotal role in FMD control, and vaccine manufacturers and distributors only distribute a vaccine when there are standard indices in the given vaccine.[8] Moreover, in many areas around the world, NSP-ELISA kits are used as an integral part of vaccination control and surveillance programs.[5]

In this study, the titer of antibody against NSPs of FMD virus in the vaccinated cattle was 22.30%, indicating the risk of circulating serotypes in the province and an insufficient level of protection from the vaccine.

However, there are reports about the possibility of antibodies against NSPs in the cattle population under systematic vaccination against FMD.[2] Comparison of the results of this study with similar studies indicates that the seroprevalence of FMD is higher in Iran than in Ethiopia (14.90%)[9] and Libya (19.00%),[10] while the seroprevalence is higher in Tanzania (76.30%)[11] and Nigeria (72.62%).[12] It is interesting that among the cities of Kurdistan province, the amount of antibodies NSPs is very variant, which is indicative of extensive fluctuations in virus circulation in each city so that the amount of this antibody is higher in the western cities bordering Iraq where there is livestock transportation between the two countries. Other important reasons for the transmission and dispersion of FMDV are extensive livestock transportation, a high rate of contact between the cattle and pastures, and the livestock sales market. Comparison of the antibody against non-serum proteins in different age groups indicated that the highest levels of this antibody were found in the age group ≤ 24 months, because in this age group sensitivity is very high due to the absence of adequate immunity in the livestock against various serotypes of FMD, which is evident in other studies as well.[13] Meanwhile, higher nonstructural antibodies in the serum of livestock the age group > 2 years can be due to their easy transportation, more exposure to wandering viruses, as well as regular sales in such ages.[14] As the age of the animal increases, the chance of exposure to disease increases compared with young animals who are likely to have less prior exposure.[5] Despite significant information about the virus, disease, and FMD vaccines, this disease is still a major threat to the livestock industry worldwide. Sometimes the subtypes of FMD virus used to produce new vaccines have led to large epidemics in various parts of the world.[15] It can be argued that vaccination alone is not enough to control FMD unless the movement and transportation of animals and their transfer to different places of a region or country are controlled. However, various factors such as local customs,[16] religious occasions, and animal trade in live animal markets are major barriers to controlling this disease.[17] Understanding the epidemiology of FMD, actively supervising the implementation of surveillance programs, and monitoring the virus circulation trend in the country and region are the requirements for the implementation of every FMD control program. Additionally, determining the factors affecting the maintenance and development of the disease and having the necessary knowledge about the circulation of FMD subtypes are other factors influencing the control of the disease.[18] Implementing these control programs in many countries around the world is impossible due to limitations such as lack of knowledge and social awareness and shortage of laboratory and diagnostic facilities. Therefore, the information obtained from these regions and countries is not representative of the status, distribution, and severity of the disease in the region and country due to the failure to collect the required samples based on scientific principles. Consequently, it will be impossible or very difficult to make proper decisions about formulating and combating FMD.[17] A study conducted in South Sudan showed that economic damage due to reduced production and mortality of FMD was 11.50 times greater than the cost of vaccination.[19] In many countries, FMD has been successfully controlled despite the elimination of small ruminants from the vaccination program. It should be noted that nationwide vaccination programs in these countries have been regularly performed for the total population of large livestock with tight control and surveillance over their transportation. Hence, preventive vaccination in small ruminants will be a small step toward FMD control in line with the protection of large ruminants unless a similar level of proper vaccination and immunity is created in large ruminants.[20] Subclinical infections have been associated with secondary outbreaks; however, the role of the carrier animals in the natural transmission of the virus is still debated. An intensively vaccinated population is more prone to subclinical persistent infections. Detection of virus activity by serological methods is of great importance in areas where the outbreaks have been reduced progressively by vaccination.[5]

Considering the nationwide vaccination in this province, determining the exposure of vaccinated cattle to the circulating FMDV serotypes may lead to the implementation of more interventional programs to provide better safety for the livestock. Hence, further epidemiological studies with a greater number of samples are necessary to evaluate the status of FMDV circulation in the area. Additionally, in order to increase diagnostic reliability, the use of more than one non-structural antigen is recommended.

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**Conflict of interest**

The authors have no conflicts of interest to declare.

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