

Seroprevalence and Risk Factor of Post Vaccination Antibodies for Foot and Mouth Disease Serotype O in Cattle and Goat, in Tanah Laut District, South Borneo 2022-2023

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Abstract

Foot and Mouth Disease (FMD), caused by the Foot Mouth Disease virus (FMDV), is an animal disease that spreads quickly and causes substantial economic losses. The Indonesian government established the vaccination program as a form of national FMD management in 2022. This study was conducted to determine the success and post-vaccination seroprevalence of FMD in Tanah Laut District, South Borneo, and to analyze the factors that influence the success of vaccination. The research method is observational analytic with a cross-sectional study approach. Samples were sera of cows and goats vaccinated, then tested serologically with SP ELISA serotype O and NSP ELISA. Results were analyzed using the chi-square test (X^2), odds ratio (OR), relative risk (RR), and spatial analysis (average nearest neighbor). The seroprevalence of immunity to FMD in Tanah Laut Regency was 95.94%. Factors that influence the formation of antibodies after FMD vaccination were the type of animal (OR = 3.781), age (OR = 6.106), sex (OR = 2.801), rearing system (OR = 3.848), feed (OR = 3.448), type of vaccine (OR = 5.508), and number of vaccinations (OR = 0.109). Spatial analysis shows a clustered pattern (Nearest Neighbor Ratio = 0.005457). The seroprevalence of immunity to FMD in Tanah Laut Regency was 95.94%. Goat immune seroprevalence has a higher value when compared to cattle. Factors that influence the formation of FMD vaccination antibodies are animal type, age, sex, feed, rearing system, type of vaccine, and number of vaccinations.

Keywords: foot and mouth disease; monitoring; post-vaccination

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Introduction

Foot and Mouth Disease (FMD) is an animal disease that spreads quickly and attacks cloven hoofs. Animals infected with FMD virus show pathognomonic clinical signs in the form of blisters or lesions in the mouth and all over the paws. FMD morbidity reaches 80-100%, has a wide range of hosts, more than 70 animal species are known to be susceptible to FMD, and causes substantial economic losses. Foot and Mouth Disease has a high ability to survive in the environment (1). The Ministry of Agriculture of the Republic of Indonesia established the status of an FMD outbreak in May 2022 after being declared free without

vaccinations in 1990. Based on the Ministry of Agriculture's policy, the Indonesian government established the vaccination program as one of the efforts to deal with National FMD (2,3). To carry out efficient disease control, vaccination uses vaccines with FMD viruses that are homologous at the subtype level or immunologically similar to viruses circulating in the field (4). Serotype O FMD virus is one of the leading causes of global epidemics and causes significant economic losses (5).

The FMD vaccination program aims to form immunity at the group level progressively. Vaccinations can fail, so antibody monitoring is

carried out using serological tests to determine the success of the vaccination program (6). FMD vaccination was monitored by the Structural Protein (SP) Enzyme-Linked Immunosorbent Assay (ELISA) serology test. Serologically, SP-ELISA can detect animals that are not vaccinated. Structural protein ELISA can also determine immunological compatibility in determining viral serotypes. ELISA can detect antibodies faster than the virus neutralization test (VNT) and is independent of cell culture. ELISA can be performed with inactivated or recombinant antigens, thus requiring less complicated biocontainment facilities (7)(25). The use of ELISA is generally to detect FMD infection status in the population or for epidemiological studies, as was done by (8)(18). This study was conducted to determine the success and post-vaccination seroprevalence of FMD in Tanah Laut District, South Kalimantan, and to analyze the factors that influence vaccination success, such as host factors, vaccination and vaccines through the results of FMD antibody testing with ELISA SP. Mapping and patterns of seropositive distribution were carried out using spatial analysis to show areas with a high post-vaccination seroprevalence.

Materials and methods

Ethical clearance

Data-based research taken from the Disease Investigation Centre of Banjarbaru, ethical clearance based on moral eligibility approved by the Faculty Veterinary Medicine of Gadjah Mada University research ethics commission no: 001/EC-FKH/Eks/2023

Research design, time, and place

This type of research is an observational analysis with a cross-sectional type of study. This research will be conducted in Tanah Laut Regency, South Borneo Province, May 2022 - March 2023.

Sampling design

The animals used in the study were cows or goats with a history of vaccination at least 30 days before. The sample taken was blood serum to be tested for antibodies against FMD. Determination of the sample size is calculated using Martin's formula (9), namely:

$$Equals = \frac{4 PQ}{L^2}$$

Sampling using Stratified random cluster sampling method, with Probability Proportional to Size stages.

Data collection

A total of 2,264 bovine and goat serum samples were used in this study. All cases were confirmed using structural protein ELISA and nonstructural protein ELISA tests from the Banjarbaru Veterinary Center. The coordinates were taken from the farm coordinates using the Garmin Etrex 10 GPS (Global Positioning System).

Testing methods

Testing for antibodies to assess SP antibodies for the vaccinated FMD was carried out using the antibody structural protein ELISA (ELISA-SP) method with the ID Screen® FMD Type O Competition ELISA Kit from IDVet™. Sera was tested with nonstructural protein (NSP) antibodies with the ID Screen® FMD NSP Competition ELISA Kit from IDVet™. The procedure was carried out according to the instructions of the test kit.

Data analysis

The implementation of data analysis includes three steps, namely univariate analysis (seroprevalence) and bivariate analysis (Chi-square, odds ratio, and relative risk) using Statistics for Windows 9 software.

Spatial analysis

Data processing was carried out using the Geographic Information System (GIS) and ArcMap (ESRI) version 10.8.2 software, and analysis of distribution patterns was carried out using average nearest neighbor analysis.

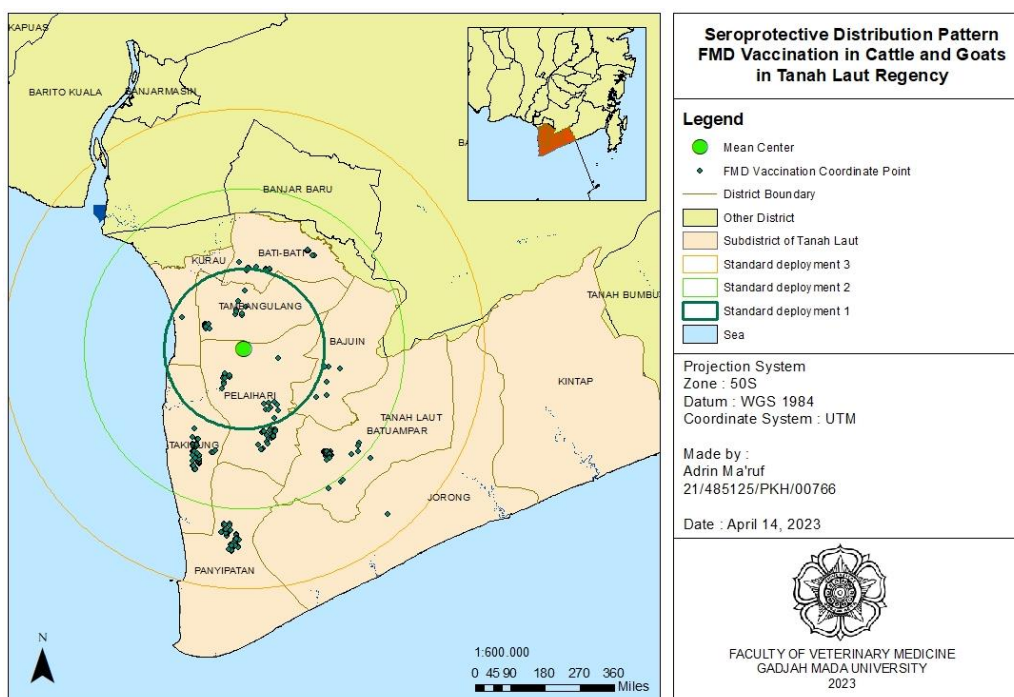


Figure 1. Map of the dot distribution pattern of FMD vaccination seropositive antibodies in cattle and goats in Tanah Laut District showing a clustered pattern

Result

NSP-positive cattle and goats were excluded. The remaining samples were 2.264, consisting of 910 cattle and 1.354 goat samples. The results of the SP ELISA test and description of livestock seroprevalence in Tanah Laut District are shown in Table 1. Seroprevalence of

immunity FMD disease in Tanah Laut District was 95,94% (2.172/2.264) and 4,06% seronegative (92/2.264). Goat immune seroprevalence has a higher value of 98,01% (1.327/1.354) than cattle, namely 92,86% (845/910).

Table 1. Seroprevalence of immunity to FMD in Tanah Laut District in Cattle and Goats

| Type of Animal | Result | | | | Total | |
|----------------|--------------|--------------|--------------|-------------|--------------|------------|
| | Seropositive | % | Seronegative | % | Σ | % |
| Cattle | 845 | 92,86 | 65 | 7,14 | 910 | 100 |
| Goat | 1.327 | 98,01 | 27 | 1,99 | 1.354 | 100 |
| Total | 2.172 | 95,94 | 92 | 4,06 | 2.264 | 100 |

The type of animal is very significantly associated with the formation of FMD vaccination immunity with a P value of 0,0001 (Table 2). This type of animal has an RR value of 1,055 (CI = 1,035-1,076) and an OR value of 3,781 (CI = 2,394-5,971).

The age of livestock (cows and goats) was highly associated with forming FMD vaccination immunity with a P value of 0,0001 (Table 2). Livestock age has a RR value of 1,134 (CI = 1,081-1,190) and an OR of 6,106 (CI = 3,959-9,418).

The sex of the livestock was highly associated with the formation of FMD vaccination immunity with a P value of 0,0001

(Table 2). The gender variable has an RR value of 1,062 (CI = 1,021-1,105) and an OR value of 2,801 (CI = 1,714-4,578).

The rearing system was highly associated with forming FMD vaccination immunity with a P value of 0,0001 (Table 2). The rearing system has an RR value of 1,096 (CI = 1,039-1,156) and an OR value of 3,848 (CI = 2,323-6,375). The type of feed is significantly associated with the formation of FMD vaccination immunity with a P value of 0,0001 (Table 2). This type of feed has an RR value of 1,060 (CI = 1,035-1,085) and an OR value of 3,448 (CI = 2,257-5,269).

Table 2. Bivariate analysis of host factors, vaccines, and vaccinations on the formation of FMD vaccination immunity in cattle and goats in Tanah Laut District

| Variable | Identification | | P-Value | X ² | OR | 95% CI | RR | 95% CI |
|--------------------------------|----------------|-------|----------|----------------|-------|--------------|-------|-------------|
| | Sero+ | Sero- | | | | | | |
| Host | | | | | | | | |
| Type of animal | | | | | | | | |
| a. Goat | 1.327 | 27 | 0,0001** | 37,008 | 3,781 | 2,394-5,971 | 1,055 | 1,035-1,076 |
| b. Cattle | 845 | 65 | | | | | | |
| Age | | | | | | | | |
| a. Adult (> 18 months) | 1.929 | 52 | 0,0001** | 84,140 | 6,106 | 3,959-9,418 | 1,134 | 1,081-1,190 |
| b. Young (6-18 months) | 243 | 40 | | | | | | |
| Sex | | | | | | | | |
| a. Female | 1.941 | 69 | 0,0001** | 18,285 | 2,801 | 1,714-4,578 | 1,062 | 1,021-1,105 |
| b. Male | 231 | 23 | | | | | | |
| Rearing system | | | | | | | | |
| a. Cage | 2.008 | 70 | 0,0001** | 31,337 | 3,848 | 2,323-6,375 | 1,096 | 1,039-1,156 |
| b. Mix (Pasture and cage) | 164 | 22 | | | | | | |
| Feed | | | | | | | | |
| a. Grass mix concentrate | 1.558 | 39 | 0,0001** | 36,560 | 3,448 | 2,257-5,269 | 1,060 | 1,035-1,085 |
| b. Grass or foliage only | 614 | 53 | | | | | | |
| Vaccine and vaccination | | | | | | | | |
| Type of vaccine | | | | | | | | |
| a. Vaccine A | 2.078 | 73 | 0,0001** | 46,916 | 5,508 | 3,197-9,489 | 1,153 | 1,064-1,250 |
| b. Vaccine B | 98 | 19 | | | | | | |
| Number of vaccination | | | | | | | | |
| a. 1 | 45 | 15 | 0,0001** | 69,299 | 0,109 | 0,058-0,203 | 0,777 | 0,671-0,900 |
| b. 2 | 2.127 | 77 | 0,0001** | 69,299 | 9,208 | 4,919-17,237 | 1,287 | 1,112-1,489 |

Information : * (significant), ** (very significant)

The vaccine type was significantly associated with forming FMD vaccination immunity in cattle and goats with a P value 0.0001 (Table 2). This type of vaccine has an RR value of 1,153 (CI = 1,064-1,250) and an OR value of 5,508 (CI = 3,197-9,489). The number of vaccinations or repeated vaccinations was highly associated with forming FMD vaccination immunity in cattle and goats with a P value of 0,0001 (Table 2). The number of vaccinations has an RR value of 1,287 (CI = 1,112-1,489) and an OR value of 9,208 (CI = 4,919-17,237).

Based on ANN analysis, the distribution pattern of FMD vaccination in Tanah Laut Regency is clustered. The results of the ANN analysis show that Z score = -88,960439; p-value = 0,0000001; Nearest Neighbor Ratio = 0,022700. The estimated distance of vaccination distribution in Tanah Laut Regency is 13,10-39,94 km (circle 1: 13,10 km, circle 2: 26,36 km, and circle 3: 39,94 km).

Discussion

The goal of zero surveillance is to detect animals that have been infected, but in vaccinated animals, the goal is to monitor the effectiveness of the vaccination campaign. Serum samples from vaccinated animals can be tested to check for antibodies produced by the

vaccine (1). Research conducted by Abdela (10) showed that small ruminants had a 4-11% lower disease prevalence than large ruminants, namely 5.6-42.7%. FMD vaccination provides immunity in goats to fight the clinical disease even with a low "antigen payload" of 1.88 µg (11). Research conducted by Elbe et al. (12) showed that vaccinated goats showed a lower risk of disease transmission at 21 days post-vaccination and an R0 of less than one (R0 < 1, p = 0.013). These results align with research by Berek et al. (13), who found that female cattle have a higher protective antibody response than male cattle.

Female cattle are also much more resistant to infection because they have the hormone estrogen, which plays a role in disease resistance. These hormones can stimulate phagocytic cell activity through macrophage activation. Activated macrophages become more active in phagocytes against foreign materials that enter the body (5). The proportion of female livestock in the population is also much more significant because female livestock is directed for production, and broodstock is used to produce livestock. Male livestock are more directed at supplying meat needs, and a few of them are used for natural mating males. According to Sarsana and Merdana (14), the bulls found were mostly calves to young cows

under one year old. Adult bulls that function as natural mating are few, less than 2% of the population, because breeders have used artificial insemination (AI). Cattle that meet the selection for sires will be reared once they have sufficient body weight as beef cattle.

The maintenance system factor also supports worm infection with an extensive rearing system, early morning grazing, and dewy grass conditions. The high prevalence rate in grazing livestock is related to the high level of grazing contamination and the high biological potential of snails as intermediate hosts (15). This study differs from Munsey et al. (16), which stated that penned cattle had an OR value of 1.24, more diminutive than cattle released to pasture with an OR value of 19.43 for FMD seroprevalence. Selection of the type of feed is important because it plays a role in forming antibodies. Vaccination has the effect of reducing growth performance due to decreased absorption or digestibility of nutrients in ruminants. Animals that experience nutritional deficiencies can become immunocompromised to delay the immune response time (17).

The feed has a vital role in the development of livestock because feed is one of the determinants of the success of a livestock business besides the marriage factor and management factor; in the intensive livestock business, the feed factor has a percentage of up to 70% or even more of the total production cost of a modern livestock business. In general, ruminants require feed in the form of forage at least 10% of their body weight, while concentrates are 1-2%. Most livestock raising in Tanah Laut Regency is carried out extensively or traditionally, where all activities, starting from mating and breeding and feeding, are carried out in grazing areas and Needed to form antibodies. Forage contains crude fiber, a source of energy and protein needed by livestock, and forage content is highly dependent on the type of forage and soil nutrient content (18).

Inactivated vaccines with adjuvant mineral oil ingredients are vaccines that are widely used in the Southeast Asian region. The vaccine, given twice one month apart, can provide immunity approximately six months after the primary vaccination (6). Vaccine A is an inactivated vaccine of serotypes A and O with high potency. According to EuFMD (1), a high-potency vaccine with more antigens can elicit immunity more quickly and at a higher level. Vaccines with higher potency (≥ 6 PD50) are recommended for emergency vaccination in

previously accessible areas of FMD. The vaccine serotype used is adjusted to the serotype circulating in the field. Multivalent vaccines can form humoral immunity with antibody titers of more than 2.0 log 10 from the seventh day of vaccination. They can protect against clinical signs of infection for up to twelve months when animals are given one or two booster injections within six months of injecting the vaccine (19).

The formation of antibodies from the primary (first) vaccination is slower and as high as the following (second, and so on) re-vaccination in primary vaccination. Memory cells have not yet been formed, so the response to vaccination antibody formation is lower and takes longer than re-vaccination (booster) because memory cells have been created. Antibodies can be achieved after re-vaccination (booster) because the animal's body already knows the same immunogen, so antibodies are produced relatively faster than the primary vaccination and located on the surface of macrophages. The cells interact with APC via CD4 and TCR. Th cells are activated, proliferate, and release cytokines (IL-1), activating B cells, which become plasma cells that produce specific antibodies against these antigens (20). This study is in line with Park et al. (21), which stated that the status of cattle farms with herd immunity of less than 80% decreased from 24% to 13.1% during the second vaccination. Goat farming has a herd immunity status of less than 80%, from 25.3% to 10.2% during the 2nd vaccination. These results indicate that the 2nd vaccination program (biannual vaccination) effectively increases and maintains herd immunity in cattle and goats.

Booster or repeat vaccination can increase vaccination coverage and positively impact vaccination programs (21). A single, double booster dose of primary vaccination dramatically increases the degree and duration of immunity. In cattle vaccinated every six months, the majority (64% to 86%, depending on serotype) maintain antibody levels at protective levels (22). According to Agustini et al. (23), there is a tendency for the antibody titer to be higher in animals that have been vaccinated before compared to animals that have just been vaccinated for the first time. A booster is essential to maintaining protective antibody titers.

The spread distance from the results of this study can be a reference for estimating the level of the vaccination zone in preventing FMD transmission that has been carried out in Tanah Laut District. In the face of FMD outbreaks,

ring vaccination around the focus of infection (protection zone) should be carried out to prevent further spread of the disease. All susceptible animals in an imaginary ring with a size of about 10 km around the outbreak site or affected villages based on geographic terrain should be included in the vaccination ring zone to establish immunity around the focus of infection (24).

Vaccination is essential in controlling outbreaks or FMD transmission in susceptible animals. (There is a relationship between vaccination achievement and risk zoning, where the more significant the vaccination coverage in an area, the lower the disease risk zone status (25).

Conclusion

The seroprevalence of immunity to FMD in Tanah Laut Regency was 95.94%. The seroprevalence of goat immunity is higher when compared to cattle, namely, host factors (livestock and livestock) associated with forming FMD vaccination immunity in cattle and goats are animal type, age, sex, rearing system, and feed. Vaccine and vaccination factors associated with forming FMD vaccination immunity in cattle and goats are the type of vaccine and the number of vaccinations.

Spatial analysis shows that the distribution pattern of FMD vaccination in Tanah Laut Regency based on the ANN analysis is clustered with an estimated distance of vaccination spread in Tanah Laut Regency as far as 13.10-39.94 km.

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Conflict of Interest

There is no conflict of interest in this research.

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