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Short Communication

Monitoring Antibodies against FMD Using ELISA in Vaccinated and Unvaccinated Cattle in Gresik Regency, Indonesia

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Abstract

Background: Indonesia is currently experiencing a Foot and Mouth Disease (FMD) outbreak in livestock caused by FMD virus. The Indonesian government has implemented a vaccination program for all FMD-susceptible animals using imported vaccines. This study aimed to determine the presence of antibodies against vaccination and field infections by examining SP-O and NSP antibodies. **Methodology**: Cattles serum samples were collected in Gresik Regency after the first booster vaccine, were tested using ELISA structural protein O and ELISA non structural protein at the National Center for Veterinary Biologics, Indonesia. **Results**: The results of 325 serum samples showed, that the SP-O antibody seroprevalence was 93.8%, whereas it was 100% in the vaccinated cattle samples and 50% in unvaccinated cattle. The results of the NSP antibody test showed that seroprevalence of FMD was 14.19% and 7.71% in vaccinated, and 60% in unvaccinated cattle. **Conclusion:** The study showed that vaccination can induce SP-O antibodies up to 100% in cattle in Gresik, the seroprevalence of FMD infection in cattle in Gresik was 14.19%, regional differences and vaccination significantly affected the prevalence of SP-O antibodies, age, region, and vaccination had a significant effect on NSP antibodies.

Keywords

Foot and mouth disease (FMD); vaccination; ELISA; structural protein-O antibody; non struktural protein antibody

1. Introduction

Foot and mouth disease (FMD) is a contagious and acute infectious disease that affects animals with even or split hooves. The disease is caused by a single-stranded positive-sense RNA virus from the genus Aphthovirus in the Picornaviridae family, which has seven serotypes: O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3. The virus comprises four structural proteins, VP1, VP2, VP3, and VP4, as well as eight non-structural proteins, Lpro, 2A, 2B, 2C, 3A, 3B, 3Cpro, and 3Dpol. These proteins regulate RNA replication, protein folding, and virus assembly, and they play a role in the formation of an antibody response [1].

In May of 2022, an outbreak of FMD was officially announced in several provinces of Indonesia. The virus responsible for this outbreak is known as FMD virus serotype O,topotype Middle East-South Asia (ME-SA) lineage Ind-2001, subliniege - e, or O/ISA/1/22. According to [2], this virus shares 95.3% nucleotide sequence similarity with the Ind-2001 virus that has been previously identified in FMD cases in Asia. To control the spread of FMD, the government has implemented a vaccination program. The government has implemented a vaccination program to control the spread of FMD. The vaccines currently used in Indonesia are imported inactivated vaccines that are compatible with the FMD virus serotypes found in the country. Vaccination is an essential strategy for controlling FMD, and is expected to cause a strong and consistent immune response in vaccinated animals. This

response will help build herd immunity and prevent recurring infections [3].

According to [4], animals that receive FMD vaccination produce antibodies for structural proteins (SP), whereas naturally infected with FMD field viruses generate antibodies for both structural and non-structural proteins
(NSP). To determine the effectiveness of the vaccination program, SP and NSP antibody diagnostics were utilized as screening measures for FMD cases. It is essential to establish a seromonitoring program in countries that implement vaccination policies to assess the success of vaccination program [5]. In Gresik Regency, an inactivated vaccine using the FMD virus isolates Strain O-3039, O1 Manisa, and Strain A22 IRAQ is one of the vaccines administered. Previous research has indicated that the O-Manisa strain of the FMD vaccine offers substantial protection against the O/ ME-SA strain of the FMD virus in North Africa [6]. However, there is no research available on the efficacy of PMK vaccination in the field, particularly in Gresik Regency. This study aimed to identify the presence of antibodies caused by both vaccination and field infections through NSP antibody examination. This was done by conducting antibody tests using serum samples that were examined by SP and NSP ELISA tests. This study aimed to estimate the level of vaccine-induced herd immunity in the population and the occurrence of field infections. The analyzed serosurveillance data were used to assess the outcomes of vaccination, estimate post-vaccination antibodies, and evaluate herd immunity among the livestock population in Gresik Regency.

2. Materials and Methods

2.1 Samples and Study Design

This study is a descriptive observational study using exploratory research. Cattle blood samples were collected from four areas in Gresik Regency: Dapet Village and Babadan Village, in Balongpanggang Sub-district, Karangrejo Village in Ujung Pangkah Sub-district, and Ngimboh Village in Panceng Sub-district. A map of Gresk Regency, East Java Province, Indonesia, is shown in Figure 1. SPCE SP-O and SPCE NSP testing was carried out in the biosafety level 2 laboratory at the National Center for Veterinary Biologics, Ministry of Agriculture, Surabaya. Samples were collected from cattles that had received PMK vaccination, imported inactivated vaccine, FMD virus isolates Strain O-3039, O1 Manisa, and Strain A22 IRAQ, had an ear tag or vaccine card from the local animal health officer, and were at least 21 days to 5 weeks after the second vaccination. The animal population under study was divided into different age groups: young (≤2 years), adult (> 2 years -> 5 years), and old (≥ 5 years).

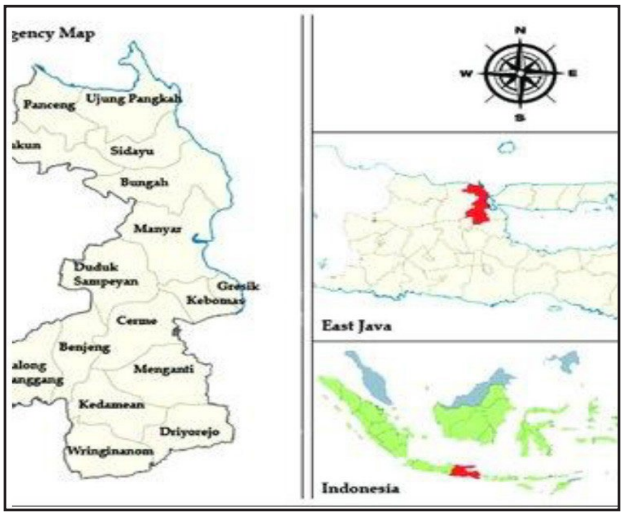

Figure 1. Gresik Regency Map [7].

The number of samples taken for one district was calculated using the Lemeshow formula. The sample calculation is based on seroprevalence, which is the calculation of the number of individuals in a population who show positive results for a particular disease based on serological specimens (blood serum). The seroprevalence of FMD in cattle is 19.8% [8].

$$n = \frac{4PQ}{LxL}$$

Information
P = seroprevalence 19,8 %
Q = 1 – P
L = error rate 5%

$$n = \frac{4\ x\ 0{,}198x\ (1-0{,}198)}{0{,}05\ x\ 0{,}05}$$

n = 255
N = 255 + 20% population (for error standart)
N = 315 cattle sample minimum

2.2 Samples Collection, Interview and Samples Transport

The sample was collected from cattle blood serum, and the identity of each animal was documented. Blood was extracted from either the jugular vein or caudal vein using a 5 ml syringe or non-EDTA vacutainer tube and a G-20 or G-21 needle in a sterile manner. The vacutainer tube was then placed in a cooler during the transportation. After centrifugation, the serum was carefully transferred into a sterile cryotube, sealed securely with parafilm to prevent any leakage, and labeled. Finally, the sample tubes were packaged and stored in a freezer at -20 °C. To ensure successful vaccination, we obtained information from animal health officers about vaccine storage management, the cold chain, and the processes used to administer vaccines to animals. Proper storage, cold chain, and administration processes can have a significant impact on vaccination results in the field.

2.3 Solid Phase Competitive ELISA - Structural Protein O (SPCE SPO)

The ELISA SP-O (SPCE SPO) competitive test uses the ELISA Kit from ID Screen® FMD Type O Competition from ID-VET, Grabels, France. Samples and reagents were prepared at room temperature (21°C). The microplate from the ID Vet ELISA Kit which has been coated with the PMK virus antigen SP-O is filled with dilution buffer 14 per well. Positive controls, negative controls, and samples were added to the microplate then incubated for 45 minutes ±4 minutes at 21 °C ± 5 °C. The microplate was washed with washing solution. Conjugate was added to each well and then incubated for 30 minutes ± 3 minutes at 21 °C ± 5 °C. The microplate was washed again with washing solution. Substrate was added to each well, then incubated in the dark for 15 minutes ± 2 minutes at 21 °C ± 5 °C. Stop solution was added to each well, and the reaction results were read using an ELISA reader at a wavelength of 450 nm. Interpretation of the SPCE SPO results can be seen in Table 1.

2.4 Solid Phase Competitive ELISA – Non Structural Protein (SPCE NSP)

Competitive test ELISA NSP (SPCE NSP) ELISA kit from ID Screen® FMD NSP Competition from ID-VET, Grabels, France All preparations were made at room temperature (21 °C). Microplates that had been labeled with the PMK virus antigen NSP were filled with dilution buffer 18 in each well. Positive controls, negative controls, and samples were added to the microplate and then incubated for 2 hours ± 10 minutes at 37 °C ± 3 °C. Afterward, the microplate was washed with washing solution. Conjugate was added to each well and then incubated for 30 minutes ± 3 minutes at 21 °C ± 5°C. The microplate was washed again with a washing solution. Substrate was added to each well, then incubated in the dark for 15 minutes ± 2 minutes at 21 °C ± 5 °C. Stop solution was added to each well, and the reaction results were read using an ELISA reader at a wavelength of 450 nm. Interpretation of the SPCE NSP results can be seen in Table 1.

Table 1 Interpretation of SPCE SPO and SPCE NSP Test Results

Result	Interpretation
SPCE - SPO	
S/N % ≤ 35%	Positive
35% < S/N % ≤ 45%	Doubtful
S/N % > 45%	Negative
SPCE - NSP	
S/N % ≤ 50 %	Positive
S/N % > 50%	Negative

2.5 Statistical Analysis and Data Analysis

The data obtained from laboratory analyses were recorded in a Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using SPSS version 25. Th2.5e prevalence of antibodies against FMD virus in individual animals was calculated by dividing the number of animals with a positive ELISA test by the total number of animals tested. Logistic regression was used to test for associated risk factors for FMD virus seroprevalence. The *R*-Square test was used to determine the percentage of influence the variable had on the results. The odds ratio was used to calculate the ratio between two odds from two different groups. All analyses were conducted at a 95% confidence level with a *p*-value of < 0.05 considered statistically significant. The correlation value was used to determine the level of significant influence between the results and the influencing variables.

3. Results

Post-vaccination monitoring is a process that involves determining the antibodies formed after vaccination, as well as evaluating the spread of infection in the population and the herd immunity that is established after vaccination. Blood serum samples were collected from 325 cattle for testing. These samples were collected from 158 farms in four different areas of Gresik Regency. SPCE SP-O and SPCE NSP tests were used to test the 325 serum samples. The results of the SPCE SP-O test are presented in Table 2, whereas the results of the SPCE NSP test are presented in Table 3 and Table 4.

Table 2 Result of the SPCE SP-O test

	Total number	SP-O Positive	(%)	ODDR	Confidence Interval 95% (CI)	*R*-square	Chi square	**Phi value**
Babatan	93	92	98.9	1	-	0.173	0.00	0.235
Dapet	61	61	100	1	-			
Karangrejo	57	53	92.9	0.14	0.01 – 1.32			
Ngimboh	114	99	86.8	0.07	0.009 – 0.55			
Total	325	305	93.8					
Vaccination status								
Vaccinated	285	285	100	1	-	0.684	0.00	0.684
Unvaccinated	40	20	50	0.04	0.00 – 0.03			
Total	325	305	93.8					
Age								
Old (≥ 5 years)	56	55	98.2	1		0.028	0.187	0.092
Adult (> 2 - > 5 years)	119	112	94.1	4.78	0.61 – 37.66			
Young (≤2 years)	150	138	92	1.39	0.53 – 3.65			
Total	324	305	93.8					

Table 3 Result of the SPCE NSP test

	Total number	NSP Positive	(%)	ODDR	Confidence Interval 95% (CI)	*R*-square	Chi square	Phi value
Babatan	93	1	1.07	1		0.228	0.00	0.343
Dapet	61	2	3.27	1.53	0.094 - 24.9			
Karangrejo	57	11	19.29	10.88	2.31 – 51.15			
Ngimboh	114	32	28.07	17.75	4.12 – 76.41			
Total	324	46	14.19					
					Vaccination status			
Vaccinated	285	22	7.71	1	0.285 0.00	0.493		
Unvaccinated	40	24	60	17.93	8.32 – 38.64			
Total	324	46	14.19					
					Age			
Old (≥ 5 years)	56	1	1.78	1		0.065	0.002	0.162
Adult (> 2 - > 5 years)	119	20	16.81	11.11	1.45 – 85.04			
Young (≤2 years)	150	25	16.67	11.00	1.45 – 83.23			
Total	325	46	14.19					

Table 4 Result of SPCE NSP test for vaccinated animals

	Total number	NSP Positive	(%)	ODDR	Confidence Interval 95% (CI)	*R*-square	Chi square	Phi value
Babatan	92	2	2.17	1		0.137	0.01	0.235
Dapet	61	1	1.63	0.75	0.06 – 8.45			
Karangrejo	50	8	16	8.57	1.74 – 42.12			
Ngimboh	82	11	13.41	6.97	1.49 – 32.46			
Total	285	22	7.71					
				Age				
Old (≥ 5 years)	55	1	1.81	1		0.083	0.06	0.182
Adult (> 2 - > 5 years)	98	4	4.08	2.298	0.25 – 21.08			
Young (≤2 years)	132	17	12.87	7.983	1.03 – 61.54			
Total number	285	22	7.71					

4. Discussion

To evaluate the effectiveness of vaccination programs, it is important to monitor the post- vaccination period, especially for countries experiencing FMD outbreaks for the first time and implementing vaccination-based disease control [9]. Post-vaccination monitoring (PVM)is critical in evaluating the effectiveness of vaccines, vaccination policies and charting out plans [10]. Exposure to FMD serotype O vaccination results in animals developing specific antibodies for structural protein O (SP-O), whereas field FMD virus infection leads to antibodies against the corresponding structural proteins (SP) and non-structural proteins (NSP) [11].

The structural proteins in the FMD virus consist of VP4, VP2, VP3, and VP1 which form the icosahedral capsid of the virus particle. VP1 plays an important role in FMD infection, such as in inducing neutralizing antibodies, mediating cellular and humoral immunity, induces host cell apoptosis, and promotes FMD replication. The neutralizing antibodies that are expected to be produced by vaccination are antibodies from structural proteins, especially antibodies from VP1. Most of the antigen sites that induce immune responses are found in the GH Loop of VP1 [Peng 2020]. The seroprevalence of SP-O antibodies in the entire sample was 93.8%, whereas it was 100% in vaccinated cattle. This study revealed that the vaccination induced a fairly good antibody response in the Gresik Regency area. Vaccination of 80% of the population is expected to be able to induced herd immunity [12] [13]. These results indicate that vaccination can induce structural protein antibodies. According to the research results of [5] scheduled vaccination is effective in building herd immunity and routine biennial vaccination will produce better immunity.

Population immunity was assessed in the subpopulations to identify potential risk factors. The populations were categorized by region, vaccination status and age. Based on statistical analysis, regional differences in the prevalence of SP-O antibodies showed a weak relationship (phi value = 0.235). Regional differences only have an effect of 17.3% on the seroprevalence of SP-O antibodies, while from the ODDR results, cattle in the Babatan and Dapet areas have the potential to have the highest SP-O antibodies compared to the Karangrejo and Ngimboh areas. In the results of examination of cattle that had received vaccination, all samples showed positive results for SP-O antibodies. These results are similar to the research of [5]. In which there was no difference in the seroprevalence of SP-O antibodies in vaccinated animals between the regions studied.

All of 285 samples of vaccinated cattle had SP-O antibodies, the SP-O seroprevalence was 100%. Of the 40 samples of cattle that were not vaccinated, 20 cattle had SP-O antibodies (50%), which indicated FMD infection status or antibodies obtained from the cow through colostrum or milk. Statistical analysis showed that vaccination significantly influenced SP-O antibody results ($P<0.005$) and had a strong relationship with SP-O antibody seroprevalence (phi values = 0.684). The seroprevalence of SP-O antibodies is 68.4% higher in infected cattle, while unvaccinated cattle have 0.04 times or less the potential to have SP-O antibodies compared to vaccinated cattle according to ODDR calculations.

In the group of animals that did not receive vaccination, 50% had SP-O antibodies, indicating that unvaccinated animals may have contracted antibodies from field infections. In unvaccinated cattle, the possibility of SP-O antibodies is lower because antibodies are obtained only from field infections. Infection can induce SP antibodies according to circulating serotypes [4]. Antibody diagnostics against NSP and SP in FMD cases can be used as a screening in FMD cases to determine whether the antibodies obtained came from vaccination or infection [14]. To determine whether these antibodies were obtained from vaccination or infection, it is necessary to perform a combined SP and NSP test as well as a clear vaccination history [4].

The prevalence of SP-O antibodies was determined in each age group. The results showed that the seroprevalence of SP-O antibodies in the younger age group was 92%, adult cattle groups had a seroprevalence 94.1%, and in the older cattle group, was 98.2%. Statistical analysis of the data revealed that the differences in seroprevalence based on age group were not significant ($p> 0.05$). A weak relationship (phi value = 0.0926) was observed between age and SP-O antibody levels. The influence of age on SP-O antibody results was 2.8%. Moreover, there was no significant

difference in the SP-O antibody results between vaccinated and non-vaccinated cattle. This finding contradicts that of a previous study [5], where the seroprevalence of SP-O was higher in young animals than in adult animals. This difference can be attributed to the fact that the adult cattle had already received cumulative doses of the vaccination program [5]. In contrast, the FMD outbreak in Indonesia was the first of its kind in the last 32 years. Therefore, the vaccination program in Indonesia was the first of its kind for both young and old animals.

In the overall results of the SP-O antibody examination, vaccination can induce the formation of SP-O antibodies. Theseroprevalence of SP-O was quite good, namely 93.8% in the entire sample. In the group of animals that had received vaccination, all samples (100%) had SP-O antibodies. The SP-O antibody results obtained did not show a neutralizing antibody titer. To determine the protective efficacy of SP-O antibodies from vaccination against field infections, an Liquid Phase Blocking (LPB) ELISA or VNT test can be conducted [4]. FMD vaccination will cause an increase in neutralizing antibody titers to prevent FMD infection in the field [15]. IgG antibodies, as important neutralizing antibodies, are produced 4 to 7 days after vaccination and will reach maximum levels at 3 weeks post-vaccination [16]. Vaccination is expected to be able to induce herd immunity of up to 80% so that the vaccine can stop the circulation of the FMD virus in the environment [13] [15].

Livestock infected with FMD can be identified by the presence of NSP antibodies, which are also used to differentiate between infected and vaccinated animals (DIVA) [17]. In this study, it was not possible to determine whether the infection occurred before or after vaccination, and when the samples were taken, the cattle did not show symptoms of FMD. Clinical symptoms that appear in animals infected with FMD include fever up to 40 °C, lameness, vesicular wounds in the oral cavity, lips, feet, and hypersalivation. These clinical symptoms appear during the first 14 days after the virus infects susceptible animals [18].The NSP antibody examination revealed that 14.19% of the entire sample was infected, whereas in the vaccinated cattle group, it was 7.71%. This suggests that the prevalence of FMD cases in Gresik is lower than prevalence of FMD cases of cattle in Ethiopia [19].

The prevalence of FMD was determined to be 1.07%, 3.27%, 19.29%, and 28.07% in the four regions, respectively. Notably, the Ngimboh region had the highest prevalence rate of FMD at 28.07%. Analysis revealed a moderate correlation of 0.34 between regions and infection cases, with 22.8% of FMD prevalence influenced by regions. Further statistical analysis indicated that the Dapet area had a potential risk of FMD infection 1.53 times higher than other regions, while the Karangrejo area had a potential risk 10.88 times higher, and the Ngimboh area had a potential risk 28.07 times higher than the Babatan area. Based on the vaccinated population, regional differences were found to significantly impact infection cases ($P<0.05$). However, the relationship between regional differences and infection cases was weak, with a correlation value of 0.235. Specifically, the Karangrejo area was found to have an OODR value indicating a potential risk of FMD infection 8.57 times higher than other regions.

It is important to note that FMD spreads through the movement of infected animals and direct contact between infected and susceptible animals via contact, inhalation, abrasion of the skin or mucous membranes, and the digestive tract. The virus can also travel easily through the air, allowing for rapid spread over long distances following the movement of animals. Moreover, the virus can be transmitted within an area with a radius of ±1-16 km [20] movement of infected animals, followed by direct contact between infected animals and susceptible animals through touch, inhalation, and abrasion of the skin or mucous membranes and the digestive tract [21]. The FMD virus can move easily and quickly through the air, making it possible to spread long distances in a short time by following the movement of wind. FMD virus can spread up to 16 km from its source [20].

The results of a study showed that FMD seroprevalence was 7.71% in vaccinated cattle and 60% in unvaccinated cattle. According to statistical analysis, unvaccinated cattle had a significantly higher risk of contracting FMD, almost 18 times higher than vaccinated animals. The study also found that vaccination status was a significant factor in FMD infection cases ($P<0.05$). The correlation between vaccination and FMD infection was moderate (phi value = 0.493), and vaccination status had an influence

of 28.5% on FMD infection. Livestock infection result in the production of NSP and SP antibodies. It was observed that unvaccinated livestock produce a stronger antibody response to NSP [12]. However, vaccinated animals that do not experience infection do not contain NSP antibodies. This is because NSP can be damaged during the vaccine formulation process; thus, the vaccine that is distributed has been purified and does not contain non-structural protein [22] [23].

In this study, it was difficult to determine whether the infection occurred before or after vaccination, because the cattle did not show any signs of FMD when the samples were collected. However, it's typical for clinical symptoms to emerge within the first two weeks of the virus infecting vulnerable animals [18]. Although vaccinated animals can still contract the virus, the clinical symptoms are usually less severe. Probang samples can detect the virus up to 2-3 weeks following exposure to FMD. When unvaccinated animals are exposed to the virus, they typically exhibit more obvious FMD lesions on their tongue and feet [22]. It's important to understand that even when an animal shows no signs of FMD, the virus could still be present in nasopharyngeal cells for up to three weeks undetected. While some animals may eliminate the virus through their immune system, others, particularly ruminant hosts, may harbor the virus in certain nasopharyngeal epithelial sites and lymphoid tissues, thereby becoming carriers [24] The NSP competitive ELISA test can detect NSP antibodies up to 1118 days following infection [25].

NSP antibody examination results were categorized by age: young, mature, and old cattle. The seroprevalence of FMD in young cattle was 16.67%, in adult cattle 16.81%, and in old cattle 1.78%. The statistical analysis indicates that age has a significant effect on FMD incidence ($p < 0.005$) and younger animals tended to have a higher infection rate, but this could be a result of passive immunity from colostral antibodies [13]. According to statistical analysis, the relationship between age differences and FMD infection in Gresik has a weak relationship with a correlation value of 0.162. Age differences only have an effect of 6.5% on the incidence of FMD in Gresik Regency, Indonesia. In the group of cattle that received vaccination, the age difference did not have a significant effect ($p > 0.05$), with a weak correlation value (Phi value: 0.182). This result is in line with the research results of [19] where there was no significant relationship between FMD seropositivity and cattle age in the Bench Maji zone of southern Ethiopia.

While vaccination is an important step in controlling FMD outbreaks, it alone is not sufficient. In order to effectively curb the spread of the disease, it is essential to implement a range of other measures. These include monitoring livestock movements, ensuring sanitation and biosecurity, maintaining proper cold chain processes, managing vaccine distribution, educating and training stakeholders and field technical personnel, and ensuring the efficient supply and logistics distribution. It is also crucial for the government responsible for national animal health to play an active role in controlling and ultimately eradicating FMD. By working together, breeders and the government can successfully implement these control measures and prevent further FMD outbreaks.

5. Conclusions

The study showed that 100% of the sampled cattle tested positive for SP-O antibodies after vaccination, while the overall seroprevalence of SP-O antibodies was 93.8%. The present study showed that vaccinated animals not display any discernible regional or age-specific differences in their antibody production. However, vaccination status can affect SP-O antibody formation. In a recent study conducted on cattle in Gresik, a seroprevalence of 14.19% for FMD was observed. Regional disparities and vaccination status were identified as risk factors for FMD in the cattle population. Therefore, further investigation is necessary to assess the efficacy and protective qualities of the vaccine. This will enable a comprehensive evaluation of the vaccination program and the type of vaccine employed.

Abbreviations

FMD, foot and mouth disease; NSP, non structural protein; SP-O, struktural protein-O; SPC ELISA, solid phase competitive ELISA; LPB ELISA, Liquid phase blocking ELISA.

Author Contributions

Conceptualization, R.R.P., S.W.R.N., and J.R.,; Methodology, R.R.P., and D.K.L.,; Investigation, R.R.P.,; Writing – Original Draft, R.R.P., S.W.R.N., and J.R.,; Writing – Review & Editing, R.R.P., and S.W.R.N.,; Funding Acquisition, R.R.P.,; Resources, R.R.P., and D.K.L.,; Supervision, S.W.R., J.R., and D.K.L.

Ethics Approval and Consent to Participate

This research has passed the animal ethics test at the Animal Ethics Commission, Faculty of Veterinary Medicine, Airlangga University with number 01.001.KEH.TE.05.2023.

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Conflict of Interest

The authors declare no conflict of interest.

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