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ANALYSIS OF ANTIBODY STATUS OF BALI CATTLE BEFORE AND AFTER FOOT AND MOUTH DISEASE (FMD) VACCINATION IN RENDANG VILLAGE, KARANGASEM

Analisis Status Antibodi Sapi Bali Sebelum dan Sesudah Vaksinasi Penyakit Mulut dan Kuku (PMK) di Desa Rendang, Karangasem

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Abstract

Bali cattle (*Bos sondaicus*) are an indigenous cattle breed originating from Indonesia and widely distributed across various regions of Indonesia. However, in 2022, the population declined due to an outbreak of Foot and Mouth Disease (FMD). The main strategy to control FMD is through vaccination, and the effectiveness of vaccination can be evaluated by detecting the presence of antibodies using serological tests, such as Enzyme-Linked Immunosorbent Assay (ELISA). This study aimed to analyze the difference in antibody status in Bali cattle before and after FMD vaccination using a competitive ELISA test. The samples were divided into two groups of male Bali cattle calves aged under one year. The first group was seronegative calves (n=12), and the second group was seropositive calves (n=7) based on the pre-vaccination ELISA test. Data were analyzed using the McNemar test and Paired t-test after the data were confirmed to be normally distributed (Shapiro-Wilk, $P > 0.05$). The statistical results showed that in the first group, there was no significant difference ($P > 0.05$) in antibody status before and after vaccination. In contrast, a significant difference ($P < 0.05$) in antibody levels was found in the second group before and after vaccination. These findings indicate that vaccination in the first group was not fully capable of triggering the desired immune response, while in the second group, booster vaccination could maintain and enhance the existing immune response. Therefore, it is recommended to implement the existing vaccination protocol and conduct accurate data collection regarding livestock vaccination status.

Keywords: bali cattle, competitive ELISA, FMD, McNemar test, vaccination.

Abstrak

Sapi bali (*Bos sondaicus*) merupakan jenis sapi asal Indonesia yang tersebar luas di berbagai wilayah Indonesia, namun pada tahun 2022 terjadi penurunan populasi akibat wabah penyakit mulut dan kuku (PMK). Strategi utama untuk mengendalikan PMK adalah melalui vaksinasi, dan efektivitas vaksinasi dapat dievaluasi dengan mendeteksi keberadaan antibodi menggunakan uji serologis, seperti *Enzyme-Linked Immunosorbent Assay* (ELISA). Penelitian ini bertujuan untuk menganalisis perbedaan status antibodi pada sapi bali sebelum dan sesudah vaksinasi PMK menggunakan uji ELISA kompetitif. Sampel penelitian ini dibagi menjadi dua kelompok pedet sapi bali jantan yang berumur di bawah satu tahun. Kelompok pertama merupakan kelompok pedet yang seronegatif (n=12), dan kelompok kedua merupakan pedet yang seropositif (n=7) berdasarkan uji ELISA sebelum vaksinasi. Data dianalisis menggunakan uji McNemar dan uji t berpasangan setelah data dinyatakan berdistribusi normal (Shapiro-Wilk, $P > 0.05$). Hasil statistik menunjukkan bahwa pada kelompok pertama tidak terdapat perbedaan yang signifikan ($P > 0.05$) pada status antibodi sebelum dan sesudah vaksinasi. Sebaliknya, pada kelompok kedua ditemukan perbedaan yang signifikan ($P < 0.05$) pada kadar antibodi sebelum dan sesudah vaksinasi. Temuan ini mengindikasikan bahwa vaksinasi pada kelompok pertama belum sepenuhnya mampu memicu respons imun yang diinginkan, sementara pada kelompok kedua, vaksinasi ulangan yang dilakukan dapat mempertahankan dan meningkatkan respons imun yang telah ada. Oleh karena itu, disarankan untuk menerapkan protokol vaksinasi yang ada dan melakukan pendataan yang akurat terkait status vaksinasi ternak.

Kata kunci: sapi bali, PMK, ELISA kompetitif, uji McNemar, vaksinasi.

INTRODUCTION

The fulfilment of animal product needs in Indonesia largely depends on the livestock sector. Local livestock, such as bali cattle, contribute significantly as meat suppliers and demonstrate the competitiveness of local livestock. Bali cattle (*Bos sondaicus*) are an indigenous Indonesian cattle breed (Satrija, 2024) originating from Bali Island and are the result of the domestication of wild cattle (Saleh *et al.*, 2023). Currently, bali cattle are widely distributed across various regions in Indonesia with a fairly large population. However, based on data from Central Statistics Agency (Badan Pusat Statistik, 2024), there was a decrease in the population of meat cattle in 2022, both nationally and in the province of Bali due to the outbreak of Foot and Mouth Disease (FMD).

FMD is a contagious viral infection caused by the foot and mouth disease virus, which is a ssRNA⁺ belonging to the genus *Aphthovirus* and the family *Picornaviridae*. This virus is classified as a small virus, non-enveloped virus with a strong capsid (Wong *et al.*, 2020). This disease is characterized by the appearance of vesicles and only affects cloven-hoofed animals, such as cattle, buffalo, sheep, goats, deer, and pigs. There are seven serotypes that cause FMD, namely serotypes O, A, C, Asia-1, South African Territories (SAT) 1, 2, and 3. Meanwhile, only one serotype of FMD circulates in Indonesia, which is type O (Mahapatra & Parida, 2018; Rohma *et al.*, 2022). In general, FMD can be detected through observation of clinical signs in the form of vesicular lesions on the hooves, tongue, mouth, and teats, with morbidity rates up to 100%. These clinical signs are influenced by various factors, such as species, age, immune system, virus strain, and the amount of virus (Wong *et al.*, 2020). To address this, the main strategy that can be implemented is animal vaccination in accordance with the Minister of Agriculture's Decree (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2024) on emergency vaccination and blanket vaccination to boost animal immunity.

Therefore, to determine the effectiveness of this vaccination program, vaccination monitoring can be carried out by detecting the presence of antibodies in animals post-vaccination. The method for detecting the immune response can be done through serological testing, namely the Enzyme-Linked Immunosorbent Assay (ELISA) (Sari *et al.*, 2025). This study aims to determine the antibody status before and after FMD vaccination in two groups of Bali calves: seronegative calves that had never been vaccinated and seropositive calves that had been previously vaccinated, using competitive ELISA.

MATERIALS AND METHODS

Animal Testing Ethics Approval

This study has obtained an animal ethics approval certificate from the Animal Ethics Committee of Veterinary Medicine, Udayana University, Bali, Indonesia (B/16/UN14.29/PT.01.04/2026).

Research Object

The objects used in this study were serum samples from nineteen male Bali cattle calves aged under one year in Rendang Village, Karangasem Regency, Bali. The calves selected were those that had never been vaccinated against FMD, based on information from the farmer. However, after being tested using ELISA to obtain pre-vaccination data, it was found that only twelve calves were seronegative, while the other seven were seropositive.

Research Design

This study is an experimental study with a longitudinal prospective design. The purpose of this study was to describe and analyze changes in antibody status in Bali cattle calves before and after FMD vaccination in Rendang Village, Karangasem Regency. This study was conducted from December 2025 to January 2026. Sampling was carried out in Rendang Village, Karangasem Regency, while ELISA testing was performed at the Virology Laboratory of the Denpasar Regional Veterinary Office (Balai Besar Veteriner Denpasar).

Research Variables

The variables examined in this study consisted of independent, dependent, and controlled variables. The independent variable was the administration of the FMD vaccine. The dependent variable was the antibody status of Bali cattle calves before and after FMD vaccination. Meanwhile, the controlled variables included breed, age, sex, and vaccination status to minimize potential confounding effects on the study outcomes.

Data Collection Method

Data collection was carried out in two stages. The first stage was the collection of the first blood sample before vaccination to obtain baseline antibody status data. At 21 days post-vaccination, the second stage involved taking a second blood sample to measure the immune response. Blood samples were centrifuged until the serum was separated from the blood.

The serum from both samples was analyzed using a competitive ELISA kit (ID Screen® FMD Type O Competition, IDvet) to obtain optical density (OD) values according to the manufacturer's protocol (IDvet, n.d). The kit uses microwells coated with inactivated FMDV type O antigen. The principle of the test is that antibodies against FMDV type O, if present in the sample, will bind to the antigen and block the binding of the conjugate.

All reagents were brought to room temperature ($21^{\circ}\text{C} \pm 5^{\circ}\text{C}$) and homogenized before use. First, 50 μL of Dilution Buffer 14 was added to each well of the ELISA microplate. Then, 20 μL of Positive Control was added to wells A1 and B1, 20 μL of Negative Control was added

to wells C1 and D1, and 20 μ L of each serum sample was added to the remaining wells. The microplate was covered and incubated for 45 minutes \pm 4 minutes at 21°C (\pm 5°C). After incubation, the liquid was discarded, and each well was washed five times with 300 μ L of Wash Solution (1X). The microplate was tapped dry on tissue paper after washing.

The Conjugate 1X was prepared by diluting the Concentrated Conjugate (10X) with Dilution Buffer 13 in a 1:10 ratio. Then, 100 μ L of Conjugate (1X) was added to each well. The microplate was covered and incubated for 30 minutes \pm 3 minutes at 21°C (\pm 5°C). The liquid was then discarded, and each well was washed five times with 300 μ L of Wash Solution (1X), followed by tapping the microplate dry on tissue paper.

Next, 100 μ L of Substrate Solution was added to each well. The microplate was covered and incubated for 15 minutes \pm 2 minutes at 21°C (\pm 5°C) in the dark. Finally, 100 μ L of Stop Solution was added to each well in the same order as the substrate addition. The optical density (OD) was read and recorded at 450 nm using an ELISA reader.

Validation of the Test

The test was considered valid if the following criteria were met according to the kit manual (IDvet, n.d): the mean OD of the Negative Control (ODNC) was greater than 0.700, and the mean OD of the Positive Control (ODPC) was less than 30% of ODNC ($ODPC/ODNC < 0.3$)

Interpretation

The S/N ratio (%) was calculated using the following formula: $S/N (\%) = (OD \text{ sample} - OD \text{ positive control}) / (OD \text{ negative control} - OD \text{ positive control}) \times 100$. The antibody status was determined according to the kit criteria (IDvet, n.d). Samples with $S/N \leq 35\%$ were classified as seropositive (antibodies present), samples with $35\% < S/N \leq 45\%$ were classified as dubious, and samples with $S/N > 45\%$ were classified as seronegative (antibodies absent).

Data Analysis

Prior to statistical analysis, the numerical data (S/N ratio percentage from the seropositive group) were tested for normality using the Shapiro-Wilk test. The data were considered normally distributed if $P > 0.05$. This study used two different tests for each data set using IBM SPSS Statistics 26 software. The first data set, which consisted of the seronegative group ($n=12$) with nominal scale data (negative and positive), was analyzed using a nonparametric test, namely the McNemar test. The second data set, which consisted of the seropositive group ($n=7$) with numerical data (S/N ratio percentage), was analyzed using a Paired t-test after the normality assumption was met.

RESULT AND DISCUSSION

Result

Field observations identified two different groups of data based on pre-vaccination ELISA results. The first group consisted of calves that were seronegative prior to vaccination ($n=12$), and the second group consisted of calves that were seropositive prior to vaccination ($n=7$). All calves in the second group still received the vaccine despite their seropositive status.

The data from the first group (seronegative calves) are presented in Table 1. Based on the data, nine calves showed a negative antibody status both before and after vaccination. Meanwhile, three calves (calves number 4, 5, and 9) showed a change in antibody status from negative to positive after vaccination. A McNemar test was conducted to analyze differences in antibody status in the seronegative group. The test yielded a P-value of 0.250 ($P > 0.05$). This indicates

that vaccination had no significant effect on antibody status before and after FMD vaccination in this group (Table 3).

Further analysis was conducted on the second group (seropositive calves), as shown in Table 2. These data were analyzed using a paired t-test after the normality assumption was met (Shapiro-Wilk, $P > 0.05$). The paired t-test yielded a P-value of 0.015 ($P < 0.05$). The significant P-value indicates a difference in antibody levels before and after FMD vaccination in the seropositive group. Therefore, vaccination successfully increased antibody levels in calves that were already seropositive prior to vaccination. The statistical summary is presented in Table 3.

Discussion

Based on data in Table 1, twelve calves were seronegative prior to vaccination, indicating that these calves had never been vaccinated against FMD. Twenty-one days post-vaccination, only three calves tested seropositive for antibodies, while the other nine remained seronegative. This indicates that an antibody response to FMD had developed only in some individuals as a general post-vaccination response, which is not uncommon given that vaccine effectiveness is influenced by multiple factors. According to Tizard (2018), host-related factors include the health status of the animal, stress, immunodeficiency, an immature immune system, waning immunity over time, and the presence of maternally derived antibodies (MDA). Vaccine-related factors include low vaccine potency and antigen mismatch with field virus strains. Additionally, failure to vaccinate can be caused by incorrect administration routes or doses, lack of booster doses, disruption of the vaccine cold chain, and expired products (Lyons *et al.*, 2016; Tizard, 2018)

The findings in Table 1 can be attributed to two factors: technical aspects of vaccination guidelines and individual immunological factors. First, the Technical Guidelines of the Indonesian Minister of Agriculture's Decree (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2024) require the administration of two vaccine doses with a four-week interval between the primary and booster doses, followed by a booster every six months. This requirement indicates that a single dose is insufficient to induce a uniform protective antibody response within a population. According to Wiedermann *et al.* (2016), low post-vaccination antibody levels result from the first dose's inability to fully stimulate antibody production.

The second influencing factor is immunological status. Tizard (2018) states that an individual's immune response is a biological process that does not provide absolute protection and is never identical among individuals within a population. Some animals respond well to vaccination, while others have a poor response. With only three out of twelve calves showing seroconversion, this illustrates a normal response within a population where a small proportion are rapid responders, while the majority require more time or repeated antigen exposure to achieve protective status.

This study only evaluated antibody response following a single dose of FMD vaccination without assessing the booster dose. The decision to limit the study to a single vaccination was based on the study's primary objective, which was to evaluate the early immune response to primary vaccination in calves with different pre-vaccination serostatuses. Future studies should include post-booster antibody evaluation to provide a more comprehensive assessment of vaccine effectiveness.

This study found that seven out of nineteen calves showed seropositive antibody status prior to FMD vaccination, despite farmer reports that they had never been vaccinated. This indicates that farmer reports do not always align with serological status. Since this test used only ELISA

to detect antibodies against Structural Proteins (SP), it is not possible to determine whether the seropositive status was caused by previous vaccination or by natural viral infection. Antibodies against SP can be induced by both vaccination and natural infection (Sari *et al.*, 2025; Tewari *et al.*, 2021).

To differentiate between vaccination and natural infection, additional testing using a Non-Structural Protein (NSP) ELISA is recommended. NSP antibodies are only produced during viral replication in natural infection and are not induced by vaccination. Therefore, future studies should consider using NSP ELISA to confirm whether seropositive samples originate from previous vaccination or natural FMD infection, as suggested by Kashem *et al.* (2024).

The seven samples that tested positive from the start successfully maintained their seropositive status following vaccination and showed a decrease in OD and S/N ratio. This decrease indicates an increase in the number of specific antibodies in the samples, as antibodies in the serum compete with the conjugate to bind to the antigen (Kashem *et al.*, 2024). This finding indicates that vaccination not only maintains but also has the potential to enhance existing immunity. This finding is in line with Satrija (2024), who reported that animals vaccinated more than once have higher antibody levels, and also supports Tizard (2018) assertion that booster vaccination significantly enhances vaccine effectiveness. Furthermore, Sharma *et al.* (2017) added that repeated FMD vaccination can equalize individual antibody levels within a population, thereby establishing stronger herd immunity. On the other hand, Gunasekara *et al.* (2022) demonstrated that absence of booster vaccination can lead to heterogeneity in population immunity.

CONCLUSION AND SUGGESTIONS

Conclusion

This study found no significant difference in antibody status before and after FMD vaccination in seronegative calves ($P > 0.05$), indicating that a single dose of FMD vaccine is insufficient to induce a detectable antibody response in most calves within 21 days. In contrast, a significant difference was found in seropositive calves ($P < 0.05$), indicating that booster vaccination can maintain and enhance pre-existing immunity. These findings emphasize the importance of completing the full two-dose vaccination protocol to achieve adequate herd immunity against FMD.

Suggestions

Based on the findings, the authors recommend three things. First, the complete FMD vaccination protocol (primary dose followed by a booster dose) should be implemented to achieve optimal herd immunity, as a single dose alone is insufficient to induce a uniform protective antibody response. Second, farmers and veterinarians should rely on serological testing rather than farmer reports alone to determine the vaccination status of livestock. Third, future studies should include post-booster antibody evaluation and consider using Non-Structural Protein (NSP) ELISA to differentiate between antibodies induced by vaccination and those resulting from natural FMD infection.

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Tables

Tabel 1. Antibody status of seronegative bali calves (n=12) before and after FMD vaccination

Number	Antibody status pre-vaccination			Antibody status post-vaccination		
	OD	S/N Ratio (%)	Status	OD	S/N Ratio (%)	Status
1	1.242	104	N	1.203	84	N
2	1.295	109	N	1.238	86	N
3	1.278	89	N	0.775	53	N
4	0.873	59	N	0.142	7	P
5	0.952	65	N	0.383	28	P
6	1.398	108	N	1.292	98	N
7	1.272	89	N	1.099	91	N
8	1.521	107	N	0.847	69	N
9	0.784	53	N	0.137	6	P
10	1.322	93	N	0.723	58	N
11	1.423	100	N	0.908	74	N
12	1.594	113	N	0.668	53	N

Note: OD = optical density; S/N ratio (%) = sample-to-negative ratio percentage; N = seronegative; P = seropositive. Seropositive criteria: S/N ratio \leq 35% (IDvet, n.d).

Table 2. Antibody status of seropositive bali calves (n=7) before and after FMD vaccination

Number	Antibody status pre-vaccination			Antibody status post-vaccination		
	OD	S/N Ratio (%)	Status	OD	S/N Ratio (%)	Status
1	0.352	21	P	0.112	4	P
2	0.150	6	P	0.076	1	P
3	0.346	21	P	0.314	20	P
4	0.169	8	P	0.081	1	P
5	0.328	19	P	0.153	7	P
6	0.198	10	P	0.164	8	P
7	0.138	5	P	0.073	-1	P

Note: OD = optical density; S/N ratio (%) = sample-to-negative ratio percentage; N = seronegative; P = seropositive. Seropositive criteria: S/N ratio \leq 35% (IDvet, n.d).

Table 3. Summary of statistical analysis result

Group	n	Statistical test	P-value	Interpretation
Seronegative calves	12	McNemar test	0.250	Not significant (P > 0.05)
Seropositive calves	7	Paired t-test	0.015	Significant (P < 0.05)