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**COMPARISON OF LYMPHOCYTE AND MONOCYTE COUNTS IN BALI CATTLE  
(*BOS SONDAICUS*) BEFORE AND AFTER FOOT AND MOUTH DISEASE  
VACCINATION**

**Perbandingan Jumlah Limfosit dan Monosit Sapi Bali (*Bos Sondaicus*) Sebelum dan  
Setelah Vaksinasi Penyakit Mulut dan Kuku**

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**Abstract**

Bali cattle (*Bos sondaicus*) are an indigenous Indonesian breed with good resilience to tropical climates, yet they are susceptible to Foot-and-Mouth Disease (FMD). Vaccination is carried out to enhance the immune system, which can be observed through changes in lymphocyte and monocyte counts. This study aimed to compare lymphocyte and monocyte counts before and after FMD vaccination. A total of 15 blood samples from healthy Bali cattle were examined before and after FMD vaccination at Disease Investigation Center Denpasar. Hematological examinations were performed manually using Giemsa-stained blood smear preparations for differential leukocyte counts. Data were analysed using a paired t-test. The results showed that the mean lymphocyte count increased significantly from  $43,07 \pm 10,559\%$  to  $63,47 \pm 7,745\%$  ( $p < 0.05$ ). Furthermore, the mean monocyte count also showed a significant increase from  $3.60 \pm 2,354\%$  to  $6,07 \pm 2,738\%$  ( $p < 0.05$ ). Thus, FMD vaccination in Bali cattle can stimulate the immune response, primarily the humoral immune response through antibody production, supported by the cellular immune response in combating FMD virus infection.

Keywords: bali cattle, FMD vaccination, lymphocytes, monocytes

### Abstrak

Sapi bali (*Bos sondaicus*) merupakan plasma nutfah asli Indonesia yang memiliki daya tahan baik terhadap iklim tropis, namun rentan terhadap Penyakit Mulut dan Kuku (PMK). Vaksinasi dilakukan untuk meningkatkan sistem imun yang dapat diamati melalui perubahan jumlah limfosit dan monosit. Penelitian ini bertujuan untuk membandingkan jumlah limfosit dan monosit sebelum dan sesudah vaksinasi PMK. Sebanyak 15 sampel darah dari sapi bali sehat diperiksa sebelum dan sesudah vaksinasi PMK di Balai Besar Veteriner (BBVet) Denpasar. Pemeriksaan hematologi dilakukan secara manual menggunakan preparat ulas darah yang diwarnai Giemsa untuk pemeriksaan diferensial leukosit. Data dianalisis menggunakan uji t berpasangan. Hasil penelitian menunjukkan bahwa rata-rata jumlah limfosit meningkat secara signifikan dari  $43,07 \pm 10,559\%$  menjadi  $63,47 \pm 7,745\%$  ( $p < 0,05$ ). Selain itu, rata-rata jumlah monosit juga mengalami peningkatan signifikan dari  $3,60 \pm 2,354\%$  menjadi  $6,07 \pm 2,738\%$  ( $p < 0,05$ ). Dengan demikian, vaksinasi PMK pada sapi bali dapat menstimulasi respons imun, terutama imun humoral melalui produksi antibodi, serta didukung oleh respon imun seluler dalam melawan infeksi virus PMK.

Kata kunci: limfosit, monosit, sapi bali, vaksinasi PMK

### INTRODUCTION

Bali cattle (*Bos sondaicus*) are an indigenous genetic resource of Indonesia that has undergone domestication and plays an important role in the national livestock sector. These cattle are well known for their adaptability to tropical environments, resistance to certain environmental stressors, and their significant economic value for local farmers. Their existence contributes to food security and supports the livelihoods of rural communities. Therefore, maintaining the health and productivity of Bali cattle is essential for the sustainability of livestock production systems (Borithnaban, 2021; Purba *et al.*, 2024).

However, Bali cattle remain vulnerable to infectious diseases, particularly Foot and Mouth Disease (FMD), a highly contagious viral disease affecting cloven-hoofed animals such as cattle, goats, sheep, pigs, and buffaloes (Grubman & Baxt, 2004; WOA, 2025). FMD is characterized by vesicular lesions in the mouth, feet, and other body parts, leading to pain, fever, reduced feed intake, and decreased productivity. The disease also causes significant economic losses due to mortality, decreased productivity, and restrictions on animal movement and trade. For example, the 2001 FMD outbreak in the United Kingdom resulted in estimated economic losses of approximately £8 billion, highlighting the substantial impact of the disease on the livestock sector and the broader economy (Alexandersen *et al.*, 2003). Vaccination is one of the primary strategies for controlling FMD, as it stimulates the immune system to recognize and respond to viral antigens. The effectiveness of vaccination can be evaluated through hematological parameters, particularly leukocyte profiles such as lymphocytes and monocytes. Lymphocytes play a crucial role in adaptive immunity through antibody production by B cells and cellular immune responses by T cells, while monocytes function as phagocytic cells and antigen-presenting cells that support immune activation. Changes in these cell populations after vaccination reflect immune system activation and the development of immunological memory (Hashem *et al.*, 2023; Jamal & Belsham, 2022).

Based on this background, this study aims to analyze the differences in lymphocyte and monocyte counts in Bali cattle before and after FMD vaccination as indicators of immune response. The results of this study are expected to provide scientific evidence regarding the effectiveness of FMD vaccination in stimulating both humoral and cellular immune responses, as well as to support disease control strategies and improve the health and productivity of Bali cattle.

## RESEARCH METHODS

### Ethical Approval

The use of animals in this study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, under approval number B/29/UN14.2.9/PT.01.04/2026.

### Research Subjects and Sample Collection

This study used blood samples obtained from 15 healthy adult Bali cattle (*Bos sondaicus*), consisting of both males and females with relatively uniform age and management conditions. A total of 30 blood samples were collected, as each animal was sampled twice: before vaccination and 21 days after FMD vaccination. Blood samples were collected via the jugular vein on the ventrolateral side of the neck using an 18 G needle (Figure 1A). Prior to sampling, animals were properly restrained to minimize stress. The puncture site was disinfected using cotton soaked in 70% alcohol. Approximately 3 mL of blood was collected and placed into EDTA tubes to prevent coagulation, followed by gentle homogenization. Samples were stored in a cool box and transported to the Disease Investigation Center Denpasar for further analysis (Figure 1CD).

### Experimental Design

This study employed an analytical experimental design using a paired pre–post (before–after) approach. Sampling was conducted using purposive sampling, where each animal served as its own control. Blood samples were collected before vaccination and 21 days after administration of a commercial inactivated Foot-and-Mouth Disease (FMD) vaccine (Aphthovet®, 25 doses per vial), following standard vaccination procedures. This design allowed the evaluation of changes in leukocyte components, particularly lymphocytes and monocytes, as indicators of immune response following vaccination.

### Hematological Examination

Hematological examination was performed manually using blood smear preparations stained with Giemsa. Differential leukocyte counts were determined microscopically by counting 100 leukocytes and recording the percentages of lymphocytes and monocytes. The results were recorded as quantitative data for both pre-vaccination and post-vaccination conditions.

### Data Analysis

The obtained data were tabulated and presented in tables. Descriptive analysis was conducted by calculating the mean and standard deviation to describe data distribution. Statistical analysis was performed using a paired t-test with SPSS software to determine significant differences between pre- and post-vaccination values. A significance level of  $P < 0.05$  was applied. The paired t-test was selected because the measurements were taken from the same subjects at two different time points.

## RESULTS AND DISCUSSION

### Results

The results of this study are presented in Table. The mean lymphocyte count increased from 43.07% before vaccination to 63.47% after vaccination, while the mean monocyte count increased from 3.60% to 6.07%. Furthermore, statistical analysis using a paired t-test as shown in Table revealed that both lymphocyte and monocyte counts differed significantly before and after FMD vaccination ( $P < 0.05$ ). The mean lymphocyte count increased from  $43.07 \pm 10.559\%$  to  $63.47 \pm 7.745\%$ , while the mean monocyte count increased from  $3.60 \pm 2.354\%$  to

$6.07 \pm 2.738\%$ . Referring to the normal reference values for Bali cattle, lymphocyte counts (45–75%) and monocyte counts (2–7%) remained within normal ranges after vaccination. This indicates that the observed increase reflects a physiological immune response rather than a pathological condition.

## DISCUSSION

### Lymphocytes

Based on the results presented in Table, lymphocyte counts significantly increased after vaccination ( $P < 0.05$ ). Lymphocytes play a central role in adaptive immunity, including antigen recognition, antibody production, and immunological memory formation (Tizard, 2018; Weiss & Wardrop, 2010). The lower lymphocyte count observed before vaccination may be influenced by physiological factors such as stress, nutritional status, or environmental conditions, which can suppress immune function through increased cortisol levels (Andini *et al.*, 2018; Putra *et al.*, 2016). However, since no clinical symptoms were observed, this condition is considered non-pathological. After vaccination, lymphocyte counts increased to within the normal range, indicating activation of the adaptive immune system. This increase reflects stimulation of B lymphocytes, which produce specific antibodies, and T lymphocytes, which regulate immune responses and eliminate infected cells (Rodriguez-Habibe *et al.*, 2020; Jamal & Belsham, 2022).

Previous studies have reported similar findings, where FMD vaccination stimulates lymphocyte proliferation, antibody production, and immunological memory formation, leading to improved protection against viral infection (Doel, 2003; Cox & Barnett, 2009; Rodriguez-Habibe *et al.*, 2020). Therefore, the results of this study are consistent with existing theories and previous research. Furthermore, the increase in lymphocyte counts may be associated with the use of an inactivated FMD vaccine (Aphthovet<sup>®</sup>). Inactivated vaccines contain viral antigens that cannot replicate but are still capable of stimulating the immune system. Following vaccination, these antigens are recognized and processed by antigen-presenting cells, leading to lymphocyte activation, antibody production, and the development of immunological memory.

### Monocyte

As shown in Table, monocyte counts also increased significantly after vaccination ( $P < 0.05$ ). Monocytes are part of the innate immune system and function as phagocytic cells and antigen-presenting cells that link innate and adaptive immunity (Weiss & Wardrop, 2010; Tizard, 2018). The increase in monocyte counts observed in this study indicates activation of the early immune response following vaccination. Monocytes differentiate into macrophages that phagocytose antigens and present them to T lymphocytes, thereby initiating adaptive immune responses (Rodriguez-Habibe *et al.*, 2020; Hashem *et al.*, 2023). Importantly, monocyte levels remained within the normal range, suggesting that the immune response was controlled and not associated with pathological conditions (Andini *et al.*, 2018; Putra *et al.*, 2016). This indicates that vaccination induced a physiological immune response.

These findings are consistent with previous studies showing that FMD vaccination activates innate immunity, particularly through increased monocyte activity, which supports the development of adaptive immunity (Hashem *et al.*, 2023; Jamal & Belsham, 2022). Thus, the increase in monocyte counts reflects an effective early immune response to vaccination. The observed increase in monocyte counts may also be related to the administration of the inactivated FMD vaccine (Aphthovet<sup>®</sup>). Inactivated vaccines commonly require the involvement of monocytes and macrophages to recognize, phagocytose, and process vaccine

antigens before presenting them to lymphocytes. This mechanism helps bridge innate and adaptive immune responses and contributes to the development of protective immunity following vaccination.

## CONCLUSION AND SUGGESTIONS

### Conclusion

Based on the results of this study, it can be concluded that FMD vaccination in Bali cattle (*Bos sondaicus*) significantly increases lymphocyte and monocyte counts ( $P < 0.05$ ). The mean lymphocyte value increased from  $43.07 \pm 10.559\%$  before vaccination to  $63.47 \pm 7.745\%$  after vaccination, while the mean monocyte value increased from  $3.60 \pm 2.354\%$  to  $6.07 \pm 2.738\%$ . These findings indicate that FMD vaccination is able to stimulate the immune response, both adaptive and innate, without causing pathological conditions.

### Suggestions

Further studies are recommended using a larger sample size to improve data accuracy and reliability. Future research should also include a more comprehensive analysis of leukocyte profiles, not limited to lymphocytes and monocytes, to better describe the immune response following FMD vaccination. In addition, proper procedures in blood sampling, handling, and laboratory examination should be strictly maintained to ensure accurate and reliable results.

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### Table

Table 1. Results of Paired t-Test Analysis on Lymphocyte and Monocyte Counts in Bali Cattle Before and After Foot-and-Mouth Disease (FMD) Vaccination

Parameter	Mean ± SD		Sig. (2-tailed)	Reference Value
	Before Vaccination (n = 15)	After Vaccination (n = 15)		
Lymphocyte (%)	43,07 ± 10,559	63,47 ± 7,745	,000*	45-75
Monocyte (%)	3,60 ± 2,354	6,07 ± 2,738	,029*	2-7

Notes: \*Values with different superscripts are significantly different (P < 0.05). Reference values were adopted from Putra *et al.* (2016) and Andini *et al.* (2018).

## Figures



Figure 1. (A) Blood sampling procedure (B) Vaccination using Apthopet<sup>®</sup> (C) Sample before vaccination (D) Sample after vaccination

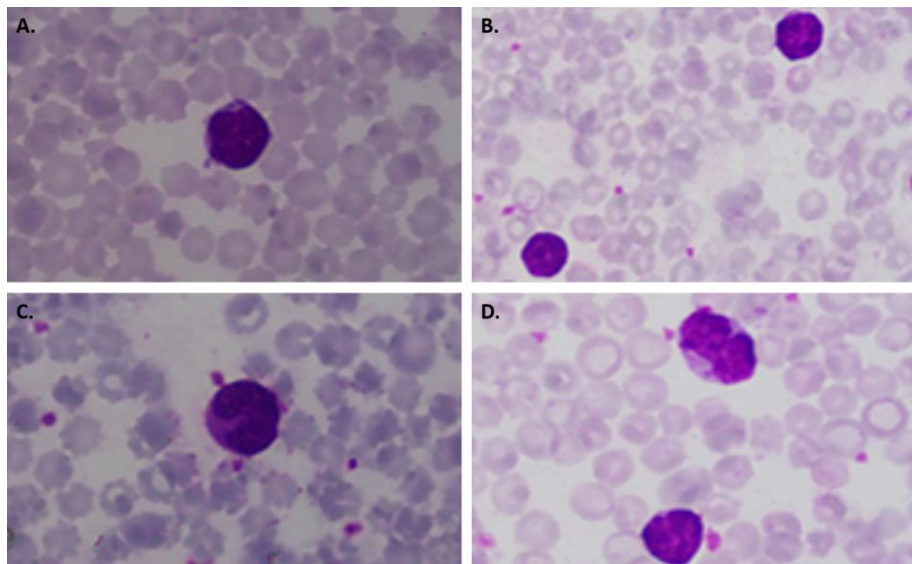


Figure 2. Peripheral blood smear showing lymphocytes (A, B) and monocytes (C, D). (A, C) before vaccination and (B, D) after vaccination (Giemsa staining).