

## Peripheral Blood Smear Analysis for Cattle with Foot and Mouth Diseases in Lembang, West Bandung

Septiyani<sup>1\*</sup>, Rully Abiyoga Majid<sup>2</sup>, Resty Gradia<sup>1</sup>, Iyus Setiawan<sup>3</sup>,  
Puji Yantini<sup>4</sup>, Arindita Niatazaya Novianti<sup>5</sup>

<sup>1</sup>Department of Basic Medical Sciences, Faculty of Medicine, Padjadjaran University, Jatinangor, Sumedang, Indonesia, <sup>2</sup>Veterinary Study Program, Faculty of Medicine, Padjadjaran University, Jatinangor, Sumedang, Indonesia, <sup>3</sup>Koperasi Peternak Sapi Bandung Utara (KPSBU) Lembang, Indonesia, <sup>4</sup>Medika Satwa Animal Clinic, Lembang, Indonesia, <sup>5</sup>Division of Basic Veterinary Medicine Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

\*Corresponding author: [drh.septiyani@unpad.ac.id](mailto:drh.septiyani@unpad.ac.id)

### Abstract

Foot and Mouth Disease (FMD) is an infectious disease that is endemic and has a significant impact on dairy farming in Indonesia. In reducing the impact caused by FMD, proper handling by veterinarians is necessary. The peripheral blood smear can be used as a data reference to give priority to the right treatment for cattle with FMD. This study aimed to describe the appearance of peripheral blood smears in cattle with FMD. The study data were obtained from 15 cattle with FMD at KPSBU Lembang, West Bandung, West Java, Indonesia. The morphological profile of the blood was examined using the peripheral blood smear method. Morphological abnormalities found in the peripheral blood smear of red blood cells were anisocytosis (microcytosis and macrocytosis), poikilocytosis (achanthocytosis and echinocytosis), and hypochromasia. Different morphological abnormalities of leukocytes can be caused by anorexia due to FMD secondary to lack of nutritional intake. To determine an effective and efficient treatment, it is recommended that veterinarians refer to the results of this study.

Keywords: dairy cattle, foot and mouth disease, peripheral blood smear

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### INTRODUCTION

The dairy industry continues to grow from year to year to meet the demand for milk in Indonesia. Milk productivity is influenced by the health status of livestock (Aisyah, 2011; Yuniarti, 2017). The low status of animal health due to infectious diseases is one of the factors that can reduce the production of fresh milk in Indonesia (Agustina *et al.*, 2020). One of the infectious diseases in livestock that is currently hitting Indonesia is Foot and Mouth Disease (FMD). FMD is an infectious disease caused by the Aphthovirus virus in split hooved animals such as cattle, buffalo, sheep, goats, camels, and wild animals such as elephants, bison, and giraffes (Jamal and Belsham, 2013; World Organization for Animal Health, 2021). According to Rushton and Knight-Jones (2015) and Tawaf (2017), the impact of FMD can include weight loss, decreased milk production, decreased fertility,

increased costs of culling animals affected by FMD, vaccination costs, lost income costs, prolong the calving interval, or even death can occur in an area. The simulation of economic losses due to FMD in Indonesia was carried out by Naipospos and Suseno (2017) with the cost to be incurred by the government due to the impact of the FMD outbreak around 9.9 trillion Indonesian Rupiahs. These losses can be minimized by carrying out effective and efficient treatment (Oktanella *et al.*, 2023).

Blood examination in the form of a peripheral blood smear can be a reference to determine the health status of animals to help monitor the incidence of the disease (Mayulu *et al.*, 2012). Viral infections can change the cell numbers and morphology in the peripheral blood smear. There has been no data about the morphological changes in peripheral blood smears in cattle with FMD, especially in Indonesia. In West Java, KPSBU Lembang whose

territory is not free from FMD infection. This study aimed to examine the characteristics of the cells detected in the peripheral blood smear on cattle with FMD. The results were expected to be a reference for further suitable treatment of cattle affected by FMD so that losses can be reduced.

## MATERIALS AND METHODS

### Ethical Approval

This study has been approved on research ethics No. 835/UN6.KEP/EC/2022, which was submitted to the Ethics Committee of the Faculty of Medicine, Padjadjaran University.

### Study Period and Location

The study was conducted at the KPSBU Lembang area and samples were analyzed at Medika Satwa Petshop and Petclinic.

### Sampling Methods

The samples in this study were dairy cattle with FMD in the KPSBU Lembang area which were selected by purposive sampling technique. The number of samples used was 15 dairy cattle with FMD. The criteria for selecting the sample used were female adult cattle aged 2 years, cattle with mild to moderate clinical symptoms, and cattle that had FMD and had not been treated with systemic-acting drugs such as antibiotics, vitamins, anti-inflammatory, analgesics, and anesthesia.

### Blood Smears Procedure

Blood smear preparation was carried out according to laboratory procedures and then evaluated using a microscope by evaluating the morphology of erythrocyte, neutrophil, eosinophil, basophil, lymphocyte, and monocyte.

### Data Analysis

The data obtained were analyzed descriptively.

## RESULTS AND DISCUSSION

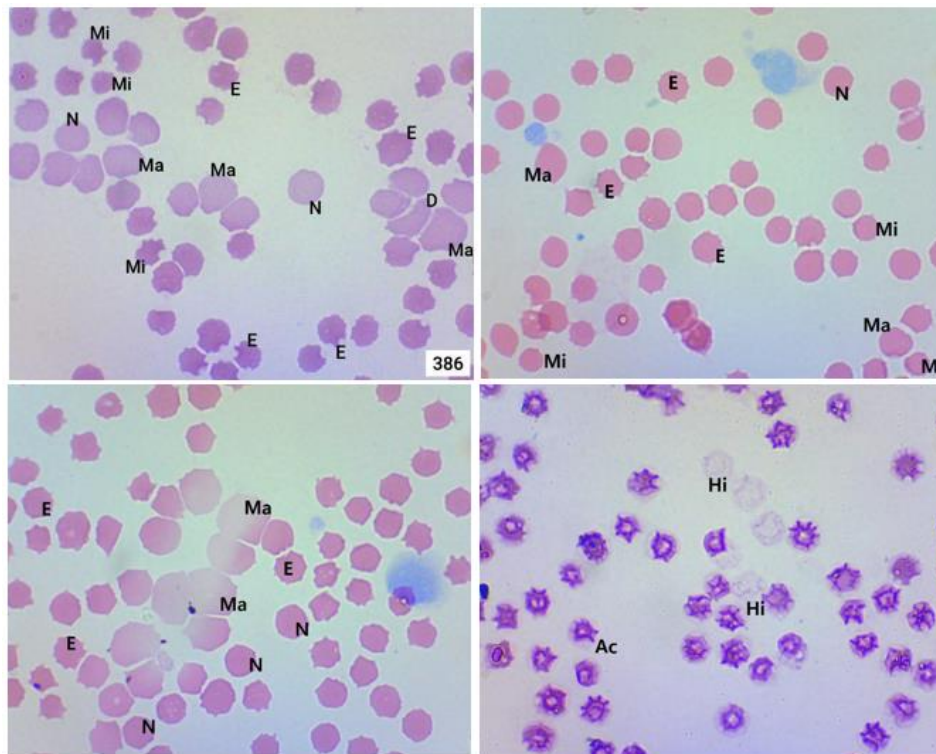
The results of the observation of peripheral blood smear of cattle infected with FMD showed

some morphological abnormalities in erythrocytes and different types of leucocytes. According to Weiss and Wardrop (2010), there are at least four parameters that must be evaluated from erythrocytes i.e. color, size, deformities, and presence of inclusion body. The study showed the abnormalities of erythrocytes consist of size abnormalities, i.e. microcytosis and macrocytosis, color abnormalities in the form of hypochromasia, and echinocytosis. No inclusion body was found (Figure 1).

Anisocytosis, hypochromasia, and poikilocytosis abnormalities in dairy cattle with FMD at KPSBU Lembang were thought to be caused by deficiency of iron, vitamin B6 (pyridoxine), vitamin B12, and minerals such as copper, as well as liver dysfunction (Scott and Stockham, 2013; Pisestyani *et al.*, 2023). This theory was proposed because anorexia in cattle with FMD causes a lack of adequate nutritional intake, causing abnormalities in erythrocyte morphology.

The most typical poikilocytosis disorder seen in cattle with FMD at KPSBU Lembang was echinocytosis. One of the causes of echinocytosis is dehydrated conditions in livestock (Scott and Stockham, 2013). This is per the condition of farmers in KPSBU Lembang who have a habit of not providing drinking water directly, while only mixing it through concentrate. There is also a possibility of echinocytosis caused by a technical error that makes crenate erythrocytes. Sick animals are prone to crenation in erythrocytes (Brooks *et al.*, 2022; Scott and Stockham, 2013). Echinocytes usually represent an artifact, resulting from the aging of the blood and exposure to excessive concentrations of EDTA as occurs when the sample tube is significantly underfilled (Kristanto and Septiyani, 2023).

The occurrence of anemia may be attributed to a depression of erythropoiesis (Hasyem, *et al.*, 2018). Bunga *et al.* (2019) stated that anisocytosis disorders, poikilocytosis, and hypochromasia caused by impaired erythropoiesis bone marrow that occur due to the presence of nutritional deficiencies such as vitamin B12, or cobalt. The disorder is also possibly caused by an infectious disease bone



**Figure 1.** Description of erythrocyte abnormalities in dairy cattle with FMD at KPSBU Lembang. (Mi) Microcytosis, (Ma) Macrocytosis, (E) Echinocytosis, (N) Normal Erythrocytes, (Hi) Hypochromasia, and (A) Acanthocytosis (Wright staining, 100x magnification).

marrow. In this study, hypochromasia was also found. According to Harvey (2012), hypochromasia occurs when the presence of erythrocytes with decreased hemoglobin concentration. Increased hypochromasia is due to iron deficiency anemia.

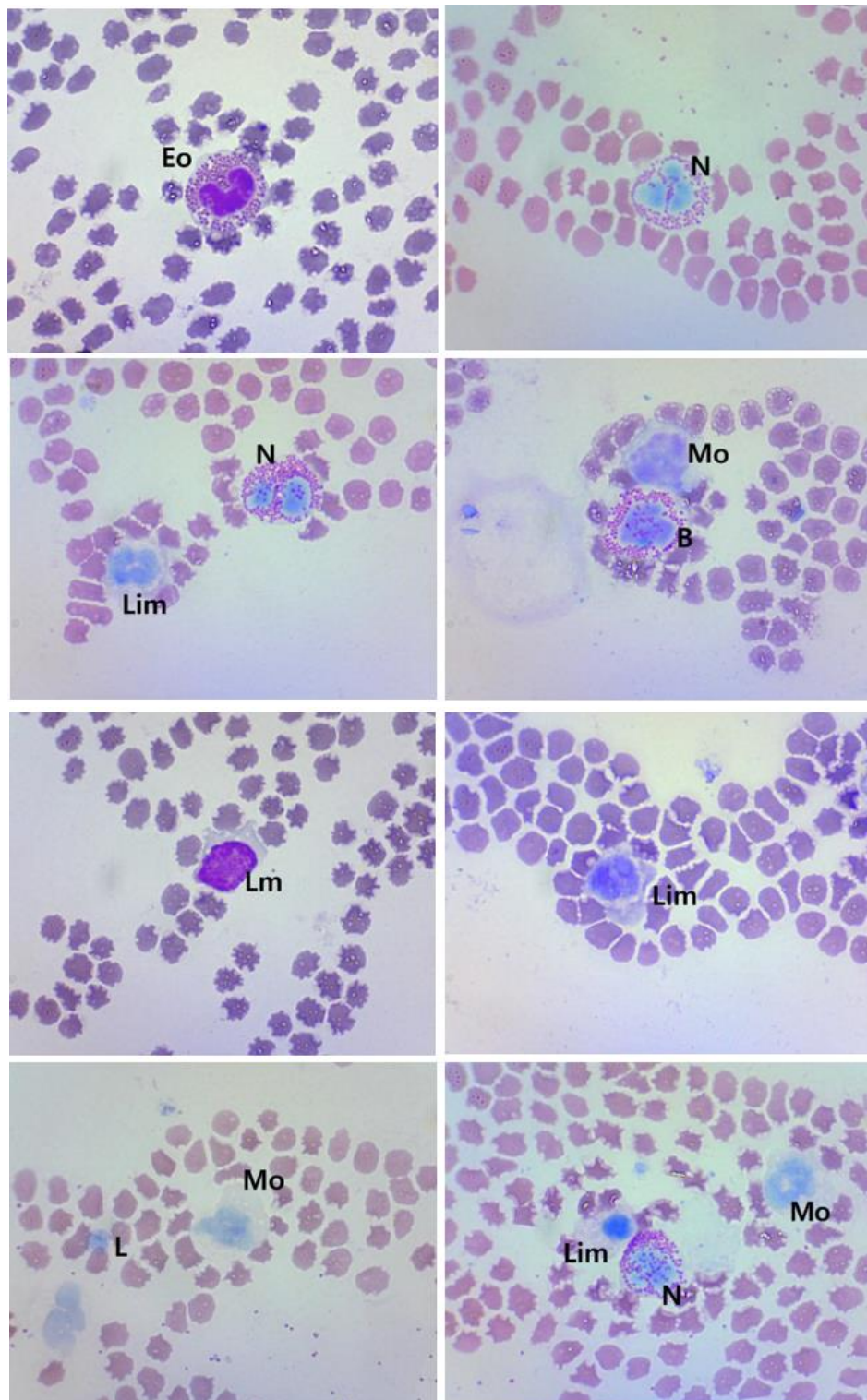
Leukocytes or white blood cells are components of the body's defense system. Based on their morphology, leukocytes are divided into granulated and non-granulated forms. The differentiation of leukocytes includes neutrophils, eosinophils, and basophils as granulated leukocytes, as well as lymphocytes and monocytes as non-granulated leukocytes (Ganong, 2008). In the results of blood smears of cattle with FMD, the morphological structure of neutrophils showed that the chromatin nucleus looks pale and almost fills the entire cytoplasm. The fine granules in the cytoplasm were visible. This is different from the morphological picture in the reference where the chromatin nucleus looks dense and clustered, although the cytoplasm has fine granules (Rahayu *et al.*, 2016).

The morphological structure of eosinophils showed a nucleus with two lobes. The cytoplasm

was visible in red. This is in accordance with the statement (Rahayu *et al.*, 2016) that eosinophils in ruminants are bright red because they are acidophilic. The morphological structure of basophils showed an irregular nucleus with granules filling the cytoplasm. The nucleus looked quite pale with a bright purple granule color. This is in accordance with the study by Putra *et al.* (2015), where the granules in the cytoplasm of basophils are basophilic and cover the nucleus so that they have a dark blue or slightly bright purple color. Eosinophils have a role in the body's defense against infectious parasites. The observation in a sample of FMD blood smear did not show a large number of eosinophils.

The morphological structure of small lymphocytes showed a relatively small nucleus with a round shape and a large cytoplasm. According to Jacobs (2019), the cytoplasm of small lymphocytes is in the form of a narrow circle around the nucleus, with a blue color due to its basophilic nature. The nucleus of small lymphocytes was round and pale in color with dense chromatin. Lymphocytes have a medium





**Figure 2.** Description of leucocyte morphology in dairy cattle with FMD at KPSBU Lembang. (N) Neutrophil, (Eo) Eosinophil, (B) Basophil, (M) Monocyte, (L) Lymphocyte, (Lm) Lymphocyte mature, and (Lim) Lymphocyte atypical/lymphocyte immature (Wright staining, 100x magnification).

cytoplasm so it looks relatively large nucleus. According to Jacobs (2019), medium-sized lymphocytes are several larger but have similar cytoplasmic. The nucleus of medium lymphocyte

was oval, dark in color with dense chromatin (Wilujeng *et al.*, 2020). Then the large lymphocytes had a very large nucleus with a fairly large cytoplasm as well. The color of the large

lymphocyte nucleus was slightly different from that of the small and medium lymphocyte, where the large lymphocyte nucleus had a more concentrated color. According to Jacobs (2019), the cytoplasm is pale blue, basophilic in nature, and often contains small vacuoles. The nucleus of large lymphocytes is round, oval, or kidney-like. Atypical lymphocytes in peripheral blood smear as seen in Figure 2 refer to immature lymphocytes. There were lymphocytes with a broad cytoplasm, loose chromatin network, and lobules appearance. Lymphocytes were larger than they should be. The cytoplasm of the lymphocytes was scattered looking adherent to the erythrocytes. Their appearance was particularly similar to stimulated lymphocytes seen in viral infections (Purnama *et al.*, 2019).

The morphological structure of monocytes showed a fairly pale nucleus with not very clear cytoplasmic walls. According to Jacobs (2019), monocytes have a nucleus shaped like a kidney or a bean, having the possibility of narrowing or indentation to the center of the nucleus that varies. The monocyte nucleus usually has little chromatin and the chromatin density varies widely. Monocyte cytoplasm was blue-grey. In general, viral infections are known to be manifested by atypical lymphocytes, inclusion bodies, monocytes with vacuole, and pyknosis in leukocytes of the peripheral blood smear. It is also possible that different leukocyte morphology can be caused by anorexia due to FMD in dairy cattle so that there is no incoming feed intake (Dinana *et al.*, 2023). This is because the feed given to dairy cattle with FMD can affect the morphology of leukocytes (Besung *et al.*, 2019). In addition, abnormal morphology such as pale core color, irregular cytoplasm, or unclear segmentation can indicate one of the disease conditions such as a viral case. The increase in the phagocytic cells could be due to tissue destruction resulting from virus infection (Hasyem *et al.*, 2018). The neutrophils and other phagocytic cells are the first line of defense against microbial and viral infections. Information about the morphological changes of FMD in peripheral blood smears is limited. Even so, other factors can affect the morphology of leukocytes in dairy cattle with

FMD. These factors include technical errors in the preparation of peripheral blood smears, the use of inappropriate staining, and the poor condition of the blood samples.

## CONCLUSION

A peripheral blood smear is a feasible method to help detect abnormalities in blood-related to clinical findings. Based on the results of peripheral blood smears obtained from cattle with FMD at KPSBU Lembang, abnormalities were found in the size, shape, and color of erythrocytes, such as anisocytosis (microcytosis and macrocytosis), poikilocytosis (achanthocytosis and echinocytosis), and hypochromasia. The appearance of leukocytes in the blood smear showed more neutrophils than other granular leukocytes, several atypical lymphocytes, and increased monocytes. Studies with a larger number of cattle are required to increase the reliability of this information and associate the findings of peripheral smears with the prognosis of FMD.

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## AUTHORS' CONTRIBUTIONS

S: Conceptualization and drafted the manuscript. RAM, RG, and IS: Collected and evaluated the blood samples. S, ANN, and RAM: Performed staining method. PY and ANN: Validation, supervision, and formal analysis. PY and IS: Performed the data analysis and the preparation of figures. All authors have read, reviewed, and approved the final manuscript.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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