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Hematological and Molecular Health Indicators of Beef Cattle in a High Density Livestock Area in Indonesia

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Abstract | Beef cattle play a role in national food security through the provision of meat. Regular livestock health evaluation is essential to ensure productivity and animal welfare through hematological parameter testing. This study aims to provide information on the hematological profile of cattle in Blora. Blood samples were obtained via the jugular vein or coccygeal vein into a 5 mL tube containing EDTA. A total of 51 cattle were taken for blood smear and PCR, while 39 cattle in Blora were chosen for haematological testing. Thin blood smears and PCR techniques were employed for the detection of *Trypanosoma evansi*. Analysis of blood samples using the VETSCAN® HM5 haematology analyser. The extraction of DNA from the blood samples was performed using the Thermo Scientific K0721 GenJET Genomic DNA Purification Kit. This study documented the subsequent blood test results: WBC: 5.97 x 10^9/L, LYM: 3.36 x 10^9/L, MON: 0.29 x 10^9/L, NEU: 1.39 x 10^9/L, EOS: 0.83 x 10^9/L, BAS: 0.09 x 10^9/L, LYM: 55.36%, MON: 4.69%, NEU: 23.94%, EOS: 14.55%, BAS: 1.46%, RBC: 7.11 x 10^{12}/L, HGB: 9.79 g/dL, HCT: 36.00%, MCV: 51.15 fL, MCH: 13.93 pg, MCHC: 27.27 g/dL, RDWc: 19.05%, RDWs: 40.00 fL. The findings of the study revealed that the cattle in Blora exhibited good health, with neither thin blood smears nor PCR testing identifying any blood parasites (*T. evansi*). The hematology parameters of cattle in Central Java are within the established normal range. It is advisable to conduct periodic haematology monitoring to assess the health status of the cattle, to pursue further research on blood parasites utilizing PCR multiplex techniques, and to enhance surveillance efforts in regions with substantial livestock populations, such as Blora.

Keywords | Beef cattle, Blora, Food security, Hematological, Livestock health, PCR

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INTRODUCTION

Beef cattle are an important part of Indonesia's livestock system because they provide most of the country's meat. According to the 2023 agricultural census, Blora sub-district has the most cows in Central Java. It is important to keep an eye on the health of cattle to make sure that production and animal welfare are both good. This is because both depend on physiological conditions and environmental factors. A way to check an animal's health and physiology is to look at its blood parameters (Handayani *et al.*, 2020). Various factors such as hemoglobin, hematocrit, white blood cell (WBC), red blood cell (RBC), and additional elements reflect an animal's nutritional condition, infection

level, stress degree, or metabolic irregularities (Majid *et al.*, 2023).

Environmental factors such as temperature, humidity, and different husbandry systems in Blora can influence the hematological parameters of cattle (Kristanto and Septiyani, 2023). The hematological values of cattle can be influenced by health issues during blood parasite infections; thus, blood smear and PCR tests are essential to assess the condition of the cattle. Earlier research has investigated the blood characteristics of Aceh cattle (Sofyan *et al.*, 2020).

Environmental factors such as temperature and humidity, as well as different methods of raising cattle in Blora, can alter the blood parameters of cows (Kristanto and Septiyani, 2023). Health problems arising from blood parasite infections can alter the haematological parameters of cattle. This is why blood smear and PCR tests are important for assessing a cow's health. Previous research have analyzed the blood characteristics of Aceh cattle (Sofyan *et al.*, 2020).

Nevertheless, there is still a significant knowledge gap regarding the blood characteristics of cattle in Blora. The current global literature may inadequately represent the genetics, nutrition, and environmental conditions of Indonesian livestock, underscoring the need for localized data to formulate precise benchmarks. This study aims to provide insights into the blood parameters of Blora cattle. The results will be utilized in Blora to assess animal health, enhance livestock health management programs, and assist in strategizing methods to boost livestock productivity.

MATERIALS AND METHODS

The study was conducted from July to December 2023. Through the jugular vein or coccygeal vein, A 5 mL blood sample was extracted from the jugular vein or coccygeal vein and placed in an EDTA tube for haematological testing, PCR, and thin blood smear. 39 Blora cattle were chosen for haematological testing, while 51 cattle were selected for blood smear and PCR.. The Parasitology Laboratory, Department of Parasitology, Faculty of Veterinary Medicine, Gadjah Mada University, was the site of the blood sample evaluation. The Department of Clinical Pathology's Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Gadjah Mada University, performed a blood haematological analysis.

Thin blood smear

The slide undergoes a cleaning process using 70% alcohol before being left to dry. A single drop of blood from an infected or potentially infected animal is positioned on the tip of a clean slide. A thin blood smear is then formed by angling the clean slide at 45 degrees to the drop of blood on the specimen slide, allowing the blood to spread evenly across the entire width of the slide. At the same time, it is pushed rapidly and smoothly. The thin blood smear is air-dried, followed by the application of absolute methanol (100%), which is allowed to dry completely for 5 minutes. Subsequently, the smear is dripped or soaked with Giemsa solution for 30 minutes. The blood smear is cleaned with tap water and dried. The blood sample, after generating a thin blood smear, is dyed with Giemsa and then utilized for microscopic investigation of blood parasites.

Polymerase chain reaction (PCR)

DNA extraction from blood was performed using the Thermo Scientific K0721 GenJET Genomic DNA Purification Kit. PCR test method to detect *T. evansi* with forward primer 5'-CGT TTG ACA TGG GAG ATG AG-3' and reverse primer 5-'GCC TTT CCC ATT TCT CTT CC-3'. DNA amplification by PCR was carried out under the following conditions: initial denaturation at 94 °C for 5 minutes, followed by denaturation at 94 °C for 30 seconds, annealing at 56 °C for 30 seconds, elongation at 72 °C for 30 seconds for 35 cycles, and then ended with a final extension for 5 minutes at 72 °C (Qudsiyati *et al.*, 2024).

Hematology test

The selected blood samples were examined for a complete blood count (CBC) using the VETSCAN® HM5 hematology analyzer with the impedance method. Hematological parameters were observed, including WBC (10^9/L), LYM (10^9/L), MON (10^9/L), NEU (10^9/L), EOS (10^9/L), BAS (10^9/L), LYM (%), MON (%), NEU (%), EOS (%), BAS (%), RBC (10^{12}/L), HGB (g/dL), HCT (%), MCV (fL), MCH (pg), MCHC (g/dL), RDWc (%), RDWs (fL).

RESULTS AND DISCUSSION

Blood smear examination and PCR detection

The blood smears from 51 cattle from Blora, we found 0% (51/0), which means that no blood parasites were found. The PCR test for *T. evansi* came back negative, which means that the sample was not contaminated with *T. evansi*. The diagnostic procedure of Giemsa staining of blood smears is straightforward to apply, affordable, and requires minimal equipment. However, the thin blood smear approach has a drawback in that the test results are not very sensitive when parasitemia is low. Therefore, molecular analysis using Polymerase Chain Reaction (PCR) is an alternative to establish a diagnosis that is precise, accurate, and more sensitive when parasitemia is low compared to the thin blood smear method, because small amounts of parasite DNA can be amplified to detectable levels if sufficient

cycles are carried out (Qudsiyati *et al*., 2024; Dyahningrum *et al*., 2019).

Hematology test

Hematological parameters are indicators of livestock physiological condition because they identify changes in an animal's health and physiological status that are not obvious during a physical examination but have an impact on animal fitness (Alfarisi *et al*., 2025). There are many reasons why animal hematological parameters can vary so much. This study examines these factors and their impact on blood parameters to assist in determining appropriate physiological baseline values for livestock populations. An autocounter can find good and exact blood parameters better than counting red blood cells by hand. Table 1 displays the findings of this study, which juxtaposed hematological parameter data for cattle from Blora with reference hematological parameters from the international journal Mohteshamuddin *et al*. (2022). The data illustrated in Table 1 indicates that most hematological values of Blora cattle fall within the normal reference range set by Mohteshamuddin *et al*. (2022), implying that the health status of the cattle is stable.

Table 1: Hematological parameters of beef cattle in Blora.

Haematological parameter	Reference range (as per Cornell University Veterinary Hospital) (Mohteshamuddin et al., 2022)	Beef cattle in Blora
WBC (10^9/L)	4-12	5.97
LYM (10^9/L)	2.5-7.5	3.36
MON (10^9/L)	0.00-0.84	0.29
NEU (10^9/L)	0.60-6.70	1.39
EOS (10^9/L)	0.10-1.0	0.83
BAS (10^9/L)	0.00-0.50	0.09
LYM %	22-64	55.36
MON %	0-10	4.69
NEU %	27-72	23.94
EOS %	0-12	14.55
BAS %	0-3	1.46
RBC (10^{12}/L)	5-10	7.11
HGB (g/dl)	8-15	9.79
HCT %	24-46	36.00
MCV (fL)	40-60	51.15
MCH	11-17	13.93
MCHC	30-36	27.27
RDWc %	16-22	19.05
RDWs fl	15-94	40.00

According to the reference values set by Mohteshamuddin et al. (2022), the RBC, HGB, HCT, MCV, and MCH levels in Blora cattle were all normal (no significant anemia, the quantity and quality of red blood cells are generally healthy). Cattle in Nigeria's Borno state naturally infected with T. evansi show a decline in PCV, Hb, and RBC counts, while leucocyte and WBC count rise (Bello et al., 2025). The findings of this study did not support it. The findings are consistent with the results of thin blood smears, which revealed no blood parasites, and PCR assays, which did not detect T. evansi. The food regimen also influences the quantity of red blood cells in cows (Adam et al., 2015).

The study found that the number of red blood cells in Blora cattle was normal. This could be because they were well-fed and well-managed, with access to grass and pens. Several values fall beyond the normal reference range of Mohteshamuddin *et al*. (2022), including neutrophils below the normal reference, eosinophils above the normal limit, and MCHC below the normal limit. Neutropenia is a reduction in the body's circulating neutrophil count. This disorder appears in shock, bone marrow depression, excessive bacterial/viral infections, and vitamin B12 and folic acid deficits. High eosinophil counts (eosinophilia) are commonly caused by allergic disorders (such as drug sensitivity, asthma, allergic rhinitis, and atopic dermatitis), parasitic infections (parasites that invade tissue), and cancer (Hodgkin's lymphoma, leukemia, and myeloproliferative neoplasms) (Saxena and Srivastava, 2021; Indiastari *et al*., 2025). Eosinophilia can develop when the syndrome is accompanied by type I hypersensitivity reactions and parasite infections (Roland *et al*., 2014; Syafar, 2017).

It differs from the hematological value of neutrophils (%) reported by Saxena and Srivastava (2021), who found the usual range of neutrophils in cattle (15-33%), therefore it remains within the normal limit. This also occurs for eosinophils compared to Saxena and Srivastava (2021), who revealed the typical range of eosinophils in cattle (0-20%), therefore it is still within the normal range. This research is also supported by the absence of blood parasites in thin blood smears and no detection of *T. evansi* in PCR, so that the possibility of other blood parasite infections is low parasitemia/ beef cattle are in healthy condition. This confirms that, despite minor variations in value of neutrophils and eosinophils (%), the cattle in Blora are generally healthy.

The most prevalent reason of a slightly lower MCHC is severe regenerative anemia, in which immature red blood cells (polychromatophils) have fewer than the full complement of HGB. Iron deficiency anemia also lowers MCHC, and a blood smear may reveal hypochromasia (pale red blood cells) (Jones and Allison, 2007; Hardiansyah *et al*., 2024). A low MCHC value implies a decrease in color or a quantity increase of hypochromic erythrocytes. Because the MCHC value was below normal and the MCV value

was normal, the samples were also suspected of having hypochromic normocyctic anemia. It can be the result of a technical error that occurred during sample storage (cooler box temperature was not maintained) or during sampling (hemolysis) (Majid *et al*., 2023). Nevertheless, according to Du *et al*. (2020) show that the factors such as breed, breeding environment and climatic variables can trigger haematological and biochemical variations.

CONCLUSION

The hematological parameters of beef cattle in Blora can be utilized to assess local livestock health in Central Java.

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NOVELTY STATEMENT

Information on the haematological values of healthy cattle free from blood parasites (*T. evansi*) in Blora using thin blood smear and PCR methods. The haematological values are used as indicators of the health of local livestock in Central Java.

AUTHOR'S CONTRIBUTION

NQ: Writing original draft, conceptualization, writing review and editing. RWN: Funding acquisition, supervision, project administration. DP: Methodology, supervision, data curation. SI: Validation, supervision, formal analysis.

Ethical approval

This research has been permitted by the LPPT Ethical Committee, UGM No. 00014/04/LPPT/V/2023.

Generative AI and AI-assisted technology statement

1. The authors used artificial intelligence–based tools to assist in language polishing and readability improvement. No AI tools were used in data generation, data analysis, image creation, or drawing scientific conclusions. All results and interpretations were conducted and verified by the authors.
2. Artificial intelligence tools were used only for language refinement and editorial support. All scientific content and interpretations are the responsibility of the authors.
3. The authors declare that artificial intelligence tools were used solely to assist with language editing, grammar checking, and improving clarity of the manuscript. The scientific content, data analysis, interpretation, and conclusions were fully developed and validated by the authors. The authors take full responsibility for the content of this manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

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