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Research Article

Hematological and Biochemical Profile of Bali Cattle Affected by Foot and Mouth Disease at Different Infection Stages

Fika Yuliza Purba[1,4*], Andi Magfira Satya Apada[1], Andi Ariyandy[2], Irwan Ismail[1,4], Subaedy Yusuf[3,4]

[1]Veterinary Medicine Study Program, Faculty of Medicine, Universitas Hasanuddin, Jalan Perintis Kemerdekaan KM.10 Makassar 90245, Sulawesi Selatan, Indonesia; [2]Physiology Department, Faculty of Medicine, Universitas Hasanuddin, Jalan Perintis Kemerdekaan KM.10 Makassar 90245, Sulawesi Selatan, Indonesia; [3]Agricultural System Study Program, Postgraduate School, Universitas Hasanuddin, Jalan Perintis Kemerdekaan KM.10 Makassar 90245, Sulawesi Selatan, Indonesia; [4]Center for Research and Development of Livestock Resources and Tropical Animals, Universitas Hasanuddin, Jalan Perintis Kemerdekaan KM.10 Makassar 90245, Sulawesi Selatan, Indonesia.

Abstract | Foot and Mouth Disease (FMD) has an adverse effect on numerous animals with cloven hooves, including cattle, sheep, goats, buffalo, pigs, etc. Therefore, this study aimed to examine hematological and biochemical profile of cattle, based on different stages of FMD infection. A total of 24 blood samples were collected from Bali cattle, then grouped into 4, based on the estimated age of lesion inflicted on the animals: Group A (no lesions), Group B (early stage), Group C (advanced stage), and Group D (recovery stage). The results showed that the white blood cell and granulocyte count in Group B was significantly higher than in Group A, while the lymphocyte and mid-cell count in Group B were higher compared to the other groups. The concentration of hemoglobin and hematocrit in Group A was significantly higher compared to the other groups. In contrast, the mean corpuscular volume and mean corpuscular hemoglobin in Group A were significantly higher compared to Groups B and C. The serum concentration of albumin in Group A was significantly higher than in other groups, and the serum concentration of aspartate aminotransferase in Group D was higher than in the other groups. The serum concentrations of creatinine and blood urea nitrogen in Group A were significantly higher than in Groups C and D. In conclusion, hematological and biochemical profile of FMD-infected cattle differed during early, advanced, and recovery stages. Therefore, the treatment of FMD among various stages of infection must be considered.

Keywords | Foot and mouth disease, Hematological profile, Biochemical profile, Bali cattle, Lesions age, South Sulawesi

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***Correspondence** | Fika Yuliza Purba, Veterinary Medicine Study Program, Faculty of Medicine, Universitas Hasanuddin, Jalan Perintis Kemerdekaan KM.10 Makassar 90245, Sulawesi Selatan, Indonesia; **Email:** fikapurba@med.unhas.ac.id
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INTRODUCTION

Foot and Mouth Disease (FMD) is classified by the World Organization for Animal Health (OIE) as a transmissible disease of international concern, with the potential for significant socioeconomic consequences and disruptions to international trade (OIE, 2019). This disease is endemic in many countries including parts of

Asia, Africa, South America, and the European Union's periphery, and it is caused by the FMD-virus (FMDV), which belongs to the genus Aphtovirus of the family Picornaviridae (Brito *et al*., 2017). FMD leads to delayed sales of animals and animal products due to its direct impact on reducing animal reproduction and productivity. On the other hand, the indirect effects include denied access to animals and animal products in marketplaces, culled animals, higher costs associated with treatment and vaccination, and restricted mobility (Knight-Jones and Rushton, 2013).

Livestock and wildlife with cloven hooves are susceptible to FMD, which is characterized by fever, hypersalivation, foot lesions, lameness, and ruptured vesicular lesions in mouth and mammary gland (Ismail *et al*., 2023; Jamal and Belsham, 2013). FMD can spread more easily during an acute disease due to virus leakage from ruptured vesicles and bodily secretions such as milk, semen, and breath. Susceptible ruminants can be exposed to very low concentrations of inhaled virus through direct means, such as encountering the breath of an animal with an acute infection, and indirectly, by inhaling aerosols resuspended from contaminated surfaces (Alexandersen *et al*., 2003).

Case mortality rates are generally modest, except for young stock, but high productivity losses and control costs are associated with FMD. The challenge is compounded by the protracted, asymptomatic, persistent disease observed in certain ruminant species, making FMD control more difficult (Stenfeldt *et al*., 2016b). The percentage of seropositive animals exhibiting clinical symptoms of disease is rarely recorded and remains unknown. This variability depends on factors such as age, breed, maternal immunity, immunization status, and the viral strain (Knight-Jones *et al*., 2017), Nonetheless, the initial diagnosis of disease is typically made primarily on clinical manifestations, particularly in non-endemic regions.

Assessing blood and serum profiles during infection is crucial for developing corrective actions that lessen the animal's biological stress. Several investigations have explored the blood and serum characteristics of cattle, considering both natural and artificial infections with FMD (Barkakati *et al*., 2015; Hashem *et al*., 2018; Kamal *et al*., 2018; Kar, 2015; Saravanan *et al*., 2020; Zaman and Faruk, 2021). There is limited documentation on hematological and biochemical alterations in Bali cattle spontaneously infected with FMD in Indonesia. Additionally, native Bali cattle in Indonesia are believed to be tolerant of a variety of infectious diseases (Mohamad *et al*., 2012); however, no systematic study has been carried out to establish the resistance of Bali cattle to FMD.

This study aimed to examine the effect of FMD infection on certain clinical, hematological, and serum biochemical parameters in Bali cattle at early, advanced, and recovery stages of infection.

MATERIALS AND METHODS

Animals

A total of 24 blood samples were collected from Bali cattle in smallholder farms of Pangkajene dan Kepulauan Regency and Maros Regency, South Sulawesi. Among these, 17 samples were obtained from FMD-infected cattle and 7 were obtained from healthy cattle. FMD-diagnosed cattle, aged 1 to 7 years and of various sexes, have never received any treatment. They are managed under a traditional system based on pasture, following a semi-intensive farming approach. This study was approved and conducted following the guidelines of the Universitas Hasanuddin Animal Research Committee.

Estimation of FMD lesions age

Animals infected with FMD were identified based on the characteristics and estimated age of lesions observed in mouth or on foot. The types of lesions that can be expected in common locations in many animals include vesicle production (blisters), rupture, and healing. After vesicle rupture, various factors can influence the speed of healing. Therefore, determining age of lesion can usually only be carried out approximately. The process of the lesion can be precisely dated within a day, specifically between days 0 and 5. Table 1 shows the accurate estimation of age of vesicular lesions.

Table 1: Descriptions for estimating the age of foot and mouth disease lesions.

Clinical disease	Appearance of lesion
Day 1	Blanching of epithelium followed by formation of fluid-filled vesicle
Day 2	Freshly ruptured vesicles characterized by raw epithelium, a clear edge to the lesion, and no deposition of fibrin
Day 3	Lesions start to lose their sharp demarcation and bright red color. Deposition of fibrin starts to occur.
Day 4	Considerable fibrin deposition has occurred, and regrowth of epithelium is evident at the periphery of the lesion.
Day 7	Extensive scar tissue formation and healing have occurred. Some fibrin deposition is usually still present.

Source: Foot and Mouth Disease Ageing of Lesions, 2005. Department for Environment, Food and Rural Affairs UK, adapted from Kitching and Mackay, 1995. Foot and Mouth Disease Ageing of Lesions. State Veterinary Journal, Vol. 5(3), pp. 4–8.

Blood samples were grouped into 4, based on the estimated age of lesion inflicted on the animals, as previously described by Zaman and Faruk (2021). Group A (no lesion), referred to animals with normal physiological conditions and no clinical results, Group B (early stage), referred to early stage group where animals are affected with FMD of 1 to 2 days post infections, Group C (advanced stage), referred to advanced stage group where animals are affected with FMD of 3 to 7 days post infections, and Group D (recovery stage), referred to recovery stage group where animals are affected with FMD of 8 to 14 days post infections.

Collection of blood samples

In this study, 24 blood samples (5 mL) were collected directly from the jugular vein and transferred into a blood-collecting vial coated with the anticoagulant EDTA (ethylene diamine tetra acetic acid) for hematological analysis. In addition, another 24 samples were collected from the same site and put into blood blood-collecting vial without anticoagulant for biochemical analysis. Blood samples were preserved in a cool box during transport to the laboratory.

Hematological and biochemical analysis

Hematological parameters including white blood cell (WBC), lymphocyte, mid-cell and granulocyte, red blood cell (RBC), platelet count (PLT), hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured using a hematology analyzer (Licare Biomedical Technology Co., Ltd, Shenzhen, China). Additionally, biochemical parameters including serum contents of total protein (TP), albumin, globulin, total bilirubin (TB), aspartate transaminase (AST), alanine transaminase (ALT), creatinine kinase (CK), amylase (AMY), creatinine, blood urea nitrogen (BUN), calcium (Ca) and phosphorus (P) were measured using an automated chemistry analyzer (Seamaty SMT-120VP, Chengdu Seamaty Technology Co.Ltd., Chengdu, China).

Statistical analysis

The demographic and sample measurement data were analyzed using JMP®17 statistical analysis software and presented in tabulated form. In this study, the statistical analysis was ANOVA followed by Post hoc Duncan's Test for data with a normal and homogeneous distribution. For non-normally distributed or heterogeneous data, Kruskal-Wallis analysis was used. Results are expressed as mean ± standard deviation (SD). Differences between groups were considered significant if $p<0.05$, highly significant if $p<0.01$, and very highly significant if $p<0.001$.

RESULTS AND DISCUSSION

In this study, the demographic data of cattle used included age, sex, and estimated ages of FMD lesions, as shown in Table 2. Half of cattle were 2 to 3 years old (12/24), with 13 out of 24 being female. Based on the clinical signs of FMD and the estimated ages of lesions, 7 cattle appeared healthy without any lesions, 8 out of 24 were in the early stage of infections (Figure 1), while the remaining 5 were in the advanced stage (Figure 2), and 4 were in the recovery stage (Figure 3).

Table 2: Distribution of cattle based on age, sex, and estimated age of FMD lesions.

Characteristic	Number of cattle (head)
Age	
1-2 years	6
2-3 years	12
3-4 years	4
> 4 years	2
Total number of cattle	24
Sex	
Male	11
Female	13
Total number of cattle	24
Estimated lesions age	
No lesions	7
1-2 days	8
3-7 days	5
8-14 days	4
Total number of cattle	24

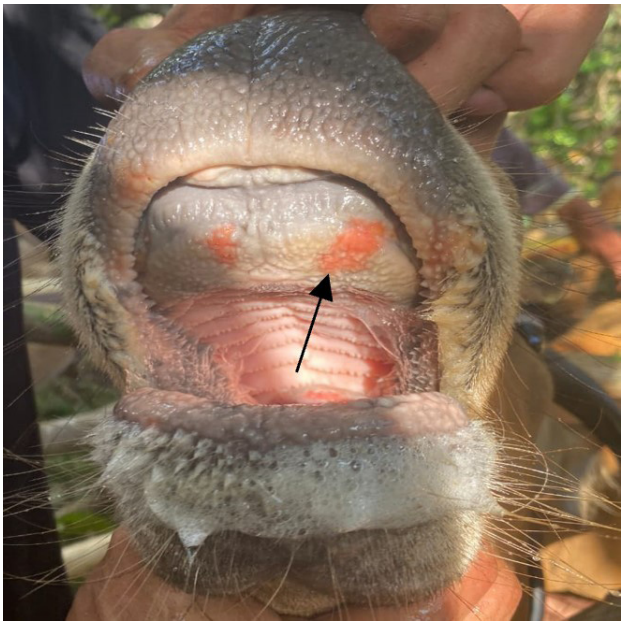

Figure 1: Characteristics of FMD lesions at the early stage of infection. The arrow shows the lesion.

Figure 2: Characteristics of FMD lesions at the advanced stage of infection. The arrow shows the lesion.

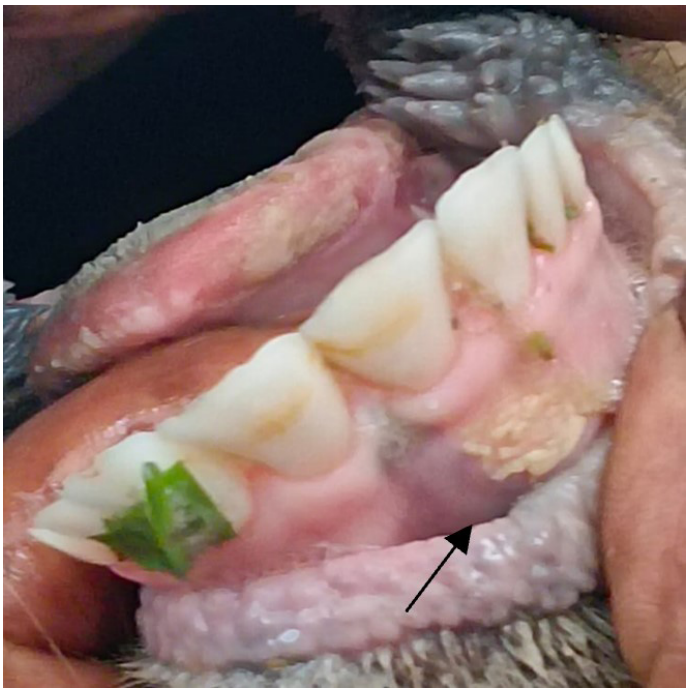

Figure 3: Characteristics of FMD lesions at the recovery stage of infection. The arrow shows the lesion.

Clinical signs of FMD include vesicular lesions, erosions, and ulcers on the muzzle, teats, coronary band, mouth, and interdigital spaces (Smith, 2015). The estimation of the age of the lesion primarily relied on mouth lesions due to foot lesions frequently being obscured by mud. Ruptured vesicular lesions with well-defined margins signaled the early stage of infection. At the advanced stage, fibrin deposition is visible, and lesion edge is experiencing epithelial development. During the healing phase, scar tissue forms and becomes apparent as lighter-colored epithelium.

This study had several limitations, which might cause some potential biases. First, the primary method for determining age of FMD lesions relied on visual assessment by a veterinarian, introducing an inherent approximation. Second, the number of samples in each group was not equal because it depended on the number of cases in the field. Third, animals with natural FMD infections may have different physiological conditions that can influence hematological and blood biochemical profiles. Using animals experimentally infected with FMD would offer a clearer demonstration of physiological differences between infection stages. Despite the discrepancy, this study showed variations in hematological and biochemical parameters among FMD-infected animals.

Hematological analysis is useful not only in diagnosing diseases of hematological system but also in diagnosing numerous organ and systemic diseases. Hematological results are presented in Table 3. The total white blood cell count in Group B was significantly higher than in Group A. The lymphocyte and mid-cell count in Group B were higher than in the other groups, while the granulocyte count in Group B was significantly higher than in Group A. The results showed that the elevated white blood cell count typically reflects the normal response of bone marrow to an early inflammatory process, predominantly by elevated granulocytes, mid-cells, and lymphocytes. Partially, these results are in line with earlier discoveries (Hashem *et al*., 2018; Kar, 2015; Zaman and Faruk, 2021). Leukocytes, neutrophils in particular, are quickly drawn from the blood at the site of infection in viral diseases to aid in immunity and infection resistance (Ma *et al*., 2021). Local replication of the FMD virus occurs in the lungs or nasopharynx during primary infection. It then travels to secondary replication sites such as foot and mouth, as well as occasionally to other sites, through circulation (Stenfeldt *et al*., 2018). Subsequently, there is a speculation that this inflammation-associated leukocytosis in the early stage of infection also occurs due to vesicular lesions, erosions, and ulcers resulting in pain and discomfort in cattle.

The result showed that lymphocyte count and percentage were lower in Groups C and D than in Groups A and B. Accordingly, cattle commonly exhibit lymphopenia, or the depletion of lymphocytes in the peripheral blood, after FMDV infection (Saravanan *et al*., 2020). This lymphopenia plays a crucial role in FMDV's evasion of the host immune response and subsequent immune suppression (Stenfeldt *et al*., 2016a). It is also well-established that lymphopenia is associated with the severity of disease and an increased predisposition to various infections (Guo *et al*., 2021; Joshi *et al*., 2009).

Table 3: Hematological parameters (mean ± SD values) in FMD at early, advanced, recovery stage group and group without lesions

Parameter	Descriptions (Mean±SD)				Normal reference	P value
	A	B	C	D		
WBC (×10³/µL)	10.75±2.49[a]	17.28±4.74[b]	13.02±2.73[ab]	13.72±2.66[ab]	5.1–13.3	0.015
Lymph (×10³/µL)	5.43±1.25	6.91±1.81	3.94±1.26	3.95±0.68	1.8–8.1	0.005
Mid (×10³/µL)	0.61±0.12	1.22±0.56	0.7±0.2	0.85±0.26	0.1–0.7	0.038
Gran (×10³/µL)	4.71±3.89[a]	9.15±3.46[b]	8.38±3.53[ab]	8.92±2.34[b]	1.7–6.0	0.039
Lymph (%)	51.42±8.58[a]	40.61±6.17[ab]	31.76±4.33[b]	29.35±4.974[b]	NA	0.002
Mid (%)	5.83±1.70	7.012±2.70	5.76±2.32	6.07±1.10	NA	0.677
Gran (%)	42.75±9.06[a]	52.37±7.76[ab]	62.48±15.37[b]	64.55±5.59[b]	NA	0.005
RBC (×10⁶/µL)	5.92±1.17	6.14±1.10	5.15±0.45	4.74±0.50	4.9–7.5	0.110
HGB (g/dL)	9.9±7.67[a]	7.67±1.24[b]	7.02±1.0963[b]	7.65±0.87[b]	8.4–12.0	0.000
HCT (%)	41.61±1.13[a]	33.25±3.54[b]	31.02±4.15[b]	33.8±1.77[b]	21–30	0.000
MCV (fL)	72.28±11.81[a]	54.95±5.54[b]	61.3±7.77[bc]	71.12±4.12[ac]	36–50	0.002
MCH (pg)	17.07±2.47[a]	12.51±1.15[b]	13.76±2.17[bc]	15.97±1.46[ac]	14–19	0.001
MCHC (g/dL)	24.12±11.68	22.90±14.56	22.42±9.89	22.55±16.44	38–43	0.127
PLT (×10³/µL)	362.57±153.02	420.62±112.24	350.2±268.23	465±260.09	160–650	0.809

Values at $p<0.05$ are statistically significant, highly significant at $p<0.01$, and very highly significant at $p<0.001$. A: no lesions, B: early stage, C: advanced stage, and D: recovery stage. WBC: White Blood Cell, Lymph: Lymphocyte, Mid: Mid cell, Gran: Granulocyte, RBC: Red Blood Cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, PLT: Platelet. Normal Reference: Wood and Quiroz-Rocha (2014).

This study suggests that lymphopenia during the advanced and recovery stages of FMD might be important for viral clearance, showing immunosuppressive conditions. In the case of FMD in Indonesia, the veterinarian frequently administered systemic antibiotics and glucocorticoids. The investigation showed that cattle in the advanced and recovery stages may experience an immunosuppressive condition, which may be exacerbated by glucocorticoid administration. Therefore, the administration of antibiotics and glucocorticoids should be reconsidered.

The hemogram results showed no difference in red blood cell count among the groups, but Group A exhibited significantly higher hemoglobin and hematocrit concentrations than the other groups. The mean corpuscular volume and mean corpuscular hemoglobin in Group A were significantly higher than in Group B and C. Conversely, mean corpuscular hemoglobin concentration and platelet count showed no significant differences among groups. As one of the limitations of this study, animals with natural FMD infections may have different physiological conditions that can influence hematological and blood biochemical profiles. Samples were collected from cattle on several smallholder farms with semi-intensive farming systems, without knowing their previous medical history.

An absolute decline in hemoglobin concentration, hematocrit, or red blood cell counts is known as anemia (Roland *et al*., 2014). In accordance, significant alterations in hematological parameters in FMD-infected cows were presented by many previous studies, and it is related to depression of erythropoiesis (Ghanem and Abdel-Hamid, 2010; Mousa and Galal, 2013; Zaman and Faruk, 2021) and endocrinopathy (Radostits *et al*., 2007). In line with this study, the depletion of hemoglobin and hematocrit in FMD-infected groups and mean corpuscular volume and mean corpuscular hemoglobin in the early and advanced infection stage groups were related to the decrease of feed intake and dehydration due to anorexia caused by FMD. FMD causes vesicular lesions in and around mouth and on the feet, resulting in the reluctance of an animal to eat or move. Therefore, farmers must ensure animals receive adequate nutrition during infection.

Biochemical results in Table 4 showed that the serum concentration of albumin and the ratio of albumin to globulin in Group A were significantly higher than in other groups. While the concentration of total protein, globulin, and total bilirubin were not different among groups. The serum concentration of aspartate aminotransferase in Group D was higher compared to the other groups. In contrast, the concentration of alanine transaminase, creatinine kinase, and amylase was not different among groups. The serum concentrations of creatinine and blood urea nitrogen in Group A were significantly higher than in Groups C and D, however, the ratio of blood urea nitrogen to creatinine, calcium, and phosphorus concentration was not different among groups.

Table 4: Biochemical parameters (mean ± SD values) in FMD at early, advanced, recovery stage group and group without lesions.

Parameter	Descriptions (Mean±SD)				Normal reference	P-value
	A	B	C	D		
ALB (g/dL)	34.62±0.96[a]	28.47±0.89[b]	24.92±1.13[b]	24.40±1.46[b]	30-42	<0.000
TP (g/dL)	82.97±2.50	81.54±2.34	76.56±2.96	86.53±3.82	66-86	0.220
GLOB (g/dL)	48.32±2.18	53.06±2.04	51.64±2.57	62.13±3.32	30-53	0.213
A/G	0.73±0.03[a]	0.54±0.03[b]	0.48±0.04[bc]	0.39±0.05[c]	0.6-1.3	<0.000
TB (mg/dL)	3.57±0.74	4.61±0.69	3.78±0.87	2.20±1.12	0-5	0.345
AST (U/L)	92.29±13.10	134.62±43.76	126.2±18.81	233.33±138.48	44-153	0.001
ALT (U/L)	39.14±3.41	50.13±3.19	43.60±4.04	44.67±5.21	11-40	0.169
AMY (U/L)	12.00±2.25	14.25±2.10	14.40±2.66	18.67±3.43	14-50	0.466
CK (U/L)	99.57±12.65	117.13±11.83	117.40±14.96	104.00±19.32	44-211	0.713
Crea (mg/dL)	154.87±11.09[a]	105.50±10.38[ab]	101.86±13.12[b]	114.43±16.94[b]	34-88	0.014
BUN (mg/dL)	7.09±0.45[a]	6.12±0.42[ab]	4.79±0.53[b]	4.56±0.69[b]	3.0-8.0	0.009
BUN/Crea	47.00±8.20	61.88±23.30	48.66±13.25	39.95±9.24	NA	0.195
Ca (mg/dL)	2.16±0.11	1.96±0.57	1.94±0.29	2.09±0.07	2.10-2.80	0.346
P (mg/dL)	2.11±0.15	2.40±0.14	2.15±0.18	1.93±0.23	1.47-2.63	0.312

Values at $p<0.05$ are statistically significant, highly significant at $p<0.01$, and very highly significant at $p<0.001$. A: no lesions, B: early stage, C: advanced stage, and D: recovery stage. ALB: Albumin, TP: Total Protein, GLOB: Globulin, TB: Total Bilirubin, AST: Aspartate Transaminase, ALT: Alanine Transaminase, AMY: Amylase, CK: Creatinine Kinase, Crea: Creatinine, BUN: Blood Urea Nitrogen; Ca: Calcium, P: Phosphorus. Normal Reference: Kaneko, Harvey, and Bruss (2008).

Decreased feed intake resulted in insufficient protein intake in cattle infected by FMD, showed by a lower concentration of albumin in the early stage of infection, and lower creatinine and BUN concentrations in the advanced and recovery stages of infection. These results are in line with previous investigations (Hashem *et al*., 2018; Kamal *et al*., 2018; Kar, 2015; Zaman and Faruk, 2021). Hypoalbuminemia, characterized by low albumin concentrations, is commonly associated with maldigestion, malabsorption, reduced feed intake, and abnormal liver metabolism (Mousa and Galal, 2013). Moreover, albumin is a negative acute-phase protein, and its concentration decreases in inflammation (Ghanem and Abdel-Hamid, 2010). The results showed that low blood levels of albumin, creatinine, and BUN in FMD-infected cattle may be associated with impaired muscle mass, malnourishment, insufficient protein intake, or liver disease.

This study also showed that the serum aspartate transaminase was higher in cattle with FMD lesions compared to cattle without lesions. This result is in line with previous studies (Ghanem and Abdel-Hamid, 2010; Hashem *et al*., 2018). Aspartate transaminase is used as a measure of liver function in several diseases, including heart, muscle, and liver diseases (Aktas *et al*., 2015). Since the rise in serum aspartate transaminase is not exclusive to liver disease, FMD-infected cattle might suffer muscle damage.

Considering that FMD is a viral disease, specific and effective treatments are lacking. Consequently, once animals contract FMD, prioritizing nutritional fulfillment becomes crucial to prevent additional complications. Treating symptoms, preventing secondary infection, and enhancing recovery capacity are necessary to control FMD, specifically in endemic regions (Brito *et al*., 2017).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, FMD resulted in a significant elevation of white blood cells in the early stage of infection, while serum aspartate transaminase increased in the recovery stage. Furthermore, FMD decreased the levels of hemoglobin, hematocrit, and albumin in all stages of infection, while simultaneously lowering the concentrations of creatinine and BUN in the advanced and recovery stages. Therefore, to improve the prognosis, these results can be used to help veterinarians be more successful in identifying and determining treatment priorities for cattle infected with FMD according to stage of infection. It can also be useful as a reference for further investigation in the development of diagnostic and prognostic tools involving hematological and biochemical changes.

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NOVELTY STATEMENT

This study found that hematological and biochemical profiles of FMD-infected cattle differed during early, advanced, and recovery stages. These findings can be used to help veterinarians identify and determine treatment priorities for Bali cattle infected with FMD according to the stage of infection. Thus, this will improve the prognosis of the disease.

AUTHOR'S CONTRIBUTION

All authors contributed to designing, collecting, and analyzing the data, as well as writing and editing the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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