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Prevalence and Hematological Profile of *Paramphistomum* spp. Infection in Bali Cattle at the Pesanggaran Abattoir, Bali, Indonesia

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ABSTRACT

This study aimed to assess the prevalence and hematological profile of *Paramphistomum* spp. infection in Bali cattle (*Bos sondaicus*) at the Pesanggaran abattoir in Bali. A total of 154 post-slaughter Bali cattle were used in this study. *Paramphistomum* spp. were identified based on their morphology in the rumen and reticulum. The level of infection was determined as slight (<25% of the rumen and reticulum infected), moderate (25–50% infected), or severe (>50% infected). Mann-Whitney U and Kruskal-Wallis statistical analyses were used to determine differences in hematological profiles. The results showed that 49.35% (76/154) of the cattle were infected with *Paramphistomum* spp. Most cattle had a moderate infection (26.62%, 41/154). The percentage of monocytes was significantly higher (P<0.05) in Bali cattle infected with *Paramphistomum* spp. There was a significant difference (P<0.05) in the percentages of monocytes and red blood cells (RBCs), and in mean corpuscular hemoglobin (MCHC), across infection levels. The observed changes in the hematological profile were leucocytosis, lymphopenia, monocytosis, monocytopenia, neutrophilia, and anaemia. *Paramphistomum* spp. In Bali cattle at the Pesanggaran abattoir, infection is high, and the more severe the infection, the lower the RBC and MCHC values. Changes in the leukocyte profile due to infection were dominated by neutrophilic leukocytosis.

Keywords: *Bos sondaicus*, Complete blood count, Paramphistomiasis, Rumen flukes, Slaughterhouse.

INTRODUCTION

Paramphistomiasis is a neglected trematode disease (Nyagura et al. 2024). The disease is caused by *Paramphistomum* spp. Fischoeder, 1901, infects the rumen and reticulum of ruminants through an intermediate host of freshwater snails (Forstmaier et al. 2021). This trematode is classified in the order Plagiorchiida, family Paramphistomidae, which has a conical morphological characteristic resembling a pear with a length of approximately 1cm and a width of 3-5mm, and is pink when fresh (Otranto and Wall 2024). Although classified as neglected diseases, paramphistomiasis and fascioliasis are estimated to cause economic losses of more than 3 billion USD per year worldwide (Rinca et al. 2019; Hambal et al. 2020). These economic losses result from reduced milk and meat production, poor nutritional conversion, weight loss, and decreased fertility. Paramphistomiasis results in high morbidity and mortality rates, especially in young cattle (*Bos* spp.) (Nyagura et al. 2024). Slight infection of *Paramphistomum* spp. does not cause serious damage, but a massive infection can have a severe impact, especially on the newly ingested metacercaria phase and into the duodenum, resulting in gastroenteritis (Huson et al. 2017; Chongmobmi and Panda 2018). Adult phase infections in the rumen and reticulum can cause various pathological conditions, including erosion, bleeding, hemorrhage, necrosis, and shortening of the rumen papillae (Lestari et al. 2017; Sapkota et al. 2021). The damage can result in ruminant body conditions, including anemia due

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to blood loss, diarrhea, anorexia, and nutritional deficiencies (Chongmobmi and Panda 2018; Khan et al. 2021). The postmortem diagnosis of *Paramphistomum* spp. involves the morphological examination of worms in the rumen and reticulum (Chongmobmi and Panda 2018). In live animals, diagnosis is achieved by detecting worm eggs using the sedimentation method, which is stained with methylene blue (Otranto and Wall 2024).

Paramphistomum spp. infection in ruminants can cause changes in their hematological profile. Several studies have been conducted to determine these changes. Khan et al. (2021) reported an increase in leukocyte levels, whereas the levels of erythrocytes, hemoglobin, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and total platelets decreased significantly due to infection by *Paramphistomum epiclitum* in cattle and buffaloes (*Bubalus* spp.). A study by Chauhan et al. (2015) In buffaloes infected with amphistomes in Gujarat, India, mean hemoglobin, total leukocytes, total erythrocytes, and packed cell volume (PCV) decreased, while total neutrophils and eosinophils increased. Similarly, Yadav et al. (2019) reported that *Paramphistomum cervi* and *Cotylophoron cotylophorum* infections in buffaloes caused anemia characterized by decreased hemoglobin, packed cell volume (PCV), and total erythrocyte values, but increased eosinophil and neutrophil values. Recent investigations into the alterations in hematological profiles resulting from *Paramphistomum* spp. infection in cattle are limited. Similarly, data concerning Bali cattle (*Bos sondaicus* Blyth, 1842 syn. *Bos javanicus domesticus* Wilckens, 1905) remain unavailable. This study is an important step toward filling the knowledge gap and supporting the optimal health of Bali cattle.

Paramphistomidae is a family of rumen worms widely distributed in Asia. A total of 14 countries in Asia were found to be infected by Paramphistomidae (61.5%). *Paramphistomum* spp. are highly prevalent in most tropical and sub-tropical countries, such as Bangladesh, Thailand, Indonesia, and India (Chongmobmi and Panda 2018; Tookhy et al. 2022). Based on a recent meta-analysis, the overall prevalence rate of *Paramphistomum* spp. in cattle in Indonesia ranged from 12-20.4% (Ninditya et al. 2024). Prevalence studies on *Paramphistomum* spp. in Indonesia have been conducted on various cattle breeds. Prevalence studies of *Paramphistomum* spp. have been reported in Banda Aceh city, with 81% in female Aceh cattle (*Bos indicus*) and 72% in males from 103 cattle (Hambal et al. 2020). Rinca et al. (2019) reported a prevalence of 47% (47/100) in Ongol (*Bos indicus*), Simmental (*Bos taurus*), and Limousin (*Bos taurus*) crossbred cattle in the Progo River Basin, Yogyakarta. In addition, amphistomes invaded local cattle more frequently than crossbred cattle in Lampung, with prevalence rates of 27.1% and 29.8%, respectively (Martindah et al. 2023).

Bali cattle are among the breeds susceptible to *Paramphistomum* spp. infection. (Purwaningsih et al. 2018). Investigations into the prevalence of *Paramphistomum* spp. infection in Bali cattle within Bali Province have been conducted at the Suwung landfill in Denpasar, revealing a prevalence rate of 11% (Dwinata et al. 2018), and at the Pesanggaran abattoir in Denpasar, where a prevalence rate of 15% was observed (Lestari et al. 2017). While the prevalence of *Paramphistomum* spp. infection at the Pesanggaran abattoir is established; there is a lack of documented research concerning the prevalence categorized by slight, moderate, and severe infection levels, as well as the hematological profile associated with *Paramphistomum* spp. infection in Bali cattle. Therefore, this study was conducted to determine the prevalence of *Paraphistomum* spp. infection based on the severity of infection and hematological profile of Bali cattle slaughtered at the Pesanggaran abattoir in Denpasar. The results of this study are useful for the early detection of disease impact and monitoring the severity of *Paraphistomum* spp. infection on the health condition of Bali cattle. Thus, treatment should not only focus on causative therapy but should also consider the overall health condition of cattle.

MATERIALS AND METHODS

Study period and location

The study sample comprised organs, specifically the rumen and reticulum, and blood collected from 154 Bali cattle. The samples were obtained through purposive sampling at the Pesanggaran slaughterhouse in Denpasar, Bali Province, Indonesia (-8.71128°S, 115.21715°E). The study was conducted from July 2023 to November 2023.

Experimental design

This study used an observational cross-sectional design to determine the prevalence and hematological changes associated with *Paramphistomum* spp. infection in Bali cattle (*Bos sondaicus*) at the Pesanggaran abattoir, Bali. The study combined parasitological and hematological examinations to identify infected animals and assess their blood profiles in relation to the severity of the infection.

Identification of *Paramphistomum* spp. in the rumen and reticulum

Paramphistomum spp. were identified by direct observation of the parasites during postmortem examination of the rumen and reticulum mucosa (Chongmobmi and Panda, 2018). Worms were determined based on the morphology described by Otranto and Wall (2024). Bali cattle positive for *Paramphistomum* spp. infection were divided into three categories based on their infection levels. The determination of slight, moderate, and severe infection categories was based on the distribution of worms in the rumen and reticulum. Slight infection was defined as <25% of the organ infected, moderate if 25-50% was infected, and severe if >50% was infected.

Blood sample collection

Blood samples (2–3mL) were obtained post-slaughter from Bali cattle at the Pesanggaran slaughterhouse. Blood was collected during the slaughter process and placed in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant to prevent coagulation. Each tube was labelled with the corresponding cow identity. The blood samples were then stored at 4°C until they were transported to the Denpasar Veterinary Center (BBVet) for hematological profile analysis.

Hematological profile

Hematological profile examination was performed using a Hematology Analyzer Rayto RT-7600 for Vet machine. The white blood cell parameters studied were white blood cell (WBC) count, differential percentage of leukocytes (eosinophils, basophils, lymphocytes, monocytes, and neutrophils), red blood cells (RBC), hemoglobin (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH).

Statistical analysis

Prevalence data were obtained by dividing the number of positive samples by the total number of samples examined, multiplied by 100. The prevalence data and types of hematological changes were analyzed descriptively. The hematological profiles of Bali cattle, both positive and negative for *Paramphistomum* spp., were compared using the Mann-Whitney U test. Differences in hematological profiles based on the level of infection (slight, moderate, and severe) were tested with Kruskal-Wallis; if there was a significant difference, it was followed by the Mann-Whitney U test. All analyses were considered statistically significant at P<0.05. All statistical analyses were performed using IBM SPSS Statistics version 26.

RESULTS

The results showed that the prevalence of *Paramphistomum* spp. infection in Bali cattle at the Pesanggaran abattoir in Denpasar, through direct identification of worms in the rumen and reticulum, was 49.35% (76/154). In this study, we categorized the infection level based on the distribution of worms in the rumen and reticulum. Slight infection was defined as <25% of the rumen and reticulum infected, moderate infection as 25%-50% infected, and severe infection as >50% infected. Moderate infection (26.62%, 41/154) was the most common, followed by slight (15.58%, 24/154) and severe (7.14%, 11/154).

Infection with *Paramphistomum* spp. in Bali cattle may alter leukocyte profiles. Table 1 shows that the percentage of monocytes was significantly higher (P<0.05) in Bali cattle not infected with *Paramphistomum* spp. than in those infected. However, this value was still within the reference range, as suggested by Brooks et al. (2022). In addition, Bali cattle examined for hematological profiles, both infected and uninfected, experienced leukocytosis. Based on the comparison of the worm infection levels (Table 2), there was a significant difference (P<0.05) in the percentage of monocytes based on the infection level of *Paramphistomum* spp. The percentage of monocytes was lower in moderate infection levels than in mild and severe infections (P<0.05). Bali cattle infected with *Paramphistomum* spp. exhibited several alterations in their leukocyte profiles, including leukocytosis (78.95%), lymphopenia (65.79%), monocytosis (9.21%), monocytopenia (19.74%), and neutrophilia (80.26%) (Table 3).

Table 1: Comparison of hematological profiles of Bali cattle non and infected with *Paramphistomum* spp

Parameters	The mean of hematology value (mean)		P-value	Normal reference value (Brooks et al. 2022)
	Negative parasite (n=78)	Positive parasite (n=76)		
WBC (10^3/µL)	16.45	17.46	0.179	4.4-10.8
Eosinophils (%)	2.72	2.29	0.305	0-20
Basophils (%)	0.00	0.00	1.000	0-2
Lymphocytes (%)	36.41	37.58	0.493	45-75
Monocytes (%)	5.71	3.67	0.012*	2.0-7
Neutrophils (%)	54.71	56.25	0.505	15-45
RBC (10^3/µL)	7.98	7.04	0.840	4.8-7.6
HB (g/dL)	15.17	15.06	0.921	8.2-13
PCV (%)	36.73	36.44	0.760	24-39
MCV (fL)	51.81	52.36	0.967	41.2-58.7
MCHC (g/dL)	41.62	41.87	0.941	32.4-35.8
MCH (pg)	21.57	21.79	0.835	14.3-19.67

Note: *Significance determined by the Mean Whitney U test. WBC: white blood cells, RBC: red blood cells, HB: hemoglobin, PCV: packed cell volume, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin, SD: standard deviation.

Table 2: Comparison of the Bali cattle hematological profile based on the level of *Paramphistomum* spp. infection

Parameters	Infection level (mean)			P-value	Normal reference value (Brooks et al. 2022)
	Slight (n=24)	Moderate (n=41)	Severe (n=11)		
WBC (10^3/µL)	19.43	16.41	17.05	0.367	4.4-10.8
Eosinophils (%)	2.58	1.98	2.82	0.789	0-20
Basophils (%)	0.00	0.00	0.00	1.000	0-2
Lymphocytes (%)	38.58	38.46	32.09	0.276	45-75
Monocytes (%)	4.38^A	3.12^B	4.18^A	0.027^*	2.0-7
Neutrophils (%)	53.46	56.44	61.64	0.288	15-45
RBC (10^3/µL)	7.65^A	6.75^B	6.76^B	0.032^*	4.8-7.6
HB (g/dL)	16.01	14.62	14.64	0.158	8.2-13
PCV (%)	38.55	35.11	36.84	0.105	24-39
MCV (fL)	51.07	52.53	54.58	0.220	41.2-58.7
MCHC (g/dL)	42.18^A	42.24^A	39.82^B	0.024^*	32.4-35.8
MCH (pg)	21.59	21.92	21.72	0.352	14.3-19.67

Note: *Significance determined by the Kruskal Wallis test, Different letters A and B in the row indicate significance (P<0.05), WBC: white blood cells, RBC: red blood cells, HB: hemoglobin, PCV: packed cells volume, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin.

Table 3: The number of Bali cattle with an altered hematological profile due to *Paramphistomum* spp. infection

Parameter	Number of Bali cattle (n) (%)		
	Above normal value	Below normal value	Within normal value
WBC (10^3/µL)	60 (78.95)	0	16 (21.05)
Eosinophils (%)	0	0	76 (100)
Basophils (%)	0	0	76 (100)
Lymphocytes (%)	0	50 (65.79)	26 (34.21)
Monocytes (%)	7 (9.21)	15 (19.74)	54 (71.05)
Neutrophils (%)	61 (80.26)	0	15 (19.74)
RBC (10^3/µL)	22 (28.95)	3 (3.95)	51 (67.11)
HB (g/dL)	62 (81.58)	0	14 (18.42)
PCV (%)	24 (31.58)	4 (5.26)	48 (63.16)
MCV (fL)	12 (15.79)	5 (6.58)	59 (77.63)
MCHC (g/dL)	74 (97.37)	2 (2.63)	0
MCH (pg)	61 (80.26)	3 (3.95)	12 (15.79)

Note: The normal hematological values used are based on Brooks et al. (2022), WBC: white blood cells, RBC: red blood cells, HB: hemoglobin, PCV: packed cell volume, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin.

Paramphistomum spp. infection causes changes in erythrocyte profiles. The average HB, MCHC, and MCH values in positive- and negative-infected cattle were higher than the normal reference values according to Brooks et al. (2022) (Table 1). Based on the comparison of parasite infection levels (Table 2), there was a significant difference ($P<0.05$) in RBC and MCHC values depending on the infection level of *Paramphistomum* spp. RBC values were higher in mild infection than in moderate and severe infections. Meanwhile, MCHC values were lower in severe infection than in slight and moderate infections. This indicates that the more severe the *Paramphistomum* spp. infection, the lower the RBC and MCHC values were. However, only 3.95% of the Bali cattle had decreased RBC counts, and 5.26% had reduced PCV below the normal reference values (Table 3). A decrease in RBC and PCV values below the normal reference values indicates anemia. Of the 76 Bali cattle infected with *Paramphistomum* spp., only five (6.58%) were anemic. The types of anemia included hyperchromic normocytic anemia in two cows (2.63%), hyperchromic microcytic in two cows (2.63%), and hyperchromic macrocytic in one cow (1.32%) (Table 3). Although *Paramphistomum* spp. can affect the blood profile of Bali cattle, only a few cattle had a decrease in RBC count that was serious enough to be considered anemic. Most infected Bali cattle still showed blood profile results within normal limits, although there were slight changes in the results.

DISCUSSION

The prevalence we found (49,35%) was higher than the range Ninditya et al. (2024) reported, which was 12-20.4%. In addition, our prevalence was higher than that reported by Lestari et al. (2017) in the same location where this study was conducted, which was 15% (30/200). This may be due to the different sampling times and sources of slaughtered cattle. Dwinata et al. (2018) also reported a lower prevalence of 11% (11/100) due to differences in detection methods. Dwinata et al. (2018) used the fecal worm egg detection method to detect *Paramphistomum* spp. Another postmortem study conducted in Punjab, Pakistan, reported a prevalence of *Paramphistomum* spp. infection in cattle of 69.09%, the highest prevalence compared to other animals examined (Rizwan et al. 2022). A study in Gondar Efora abattoir, Ethiopia, reported 51.92% of local and breed cattle suffering from paramphistomiasis (Ayalew et al. 2016). Furthermore, a study in Zabol abattoir, Iran, found a prevalence of 36.9% among 1000 cattle examined during postmortem examination (Khedri et al. 2015). Postmortem examination is considered highly accurate, as it allows for the direct observation of adult *Paramphistomum* spp. in the rumen and reticulum. This method can provide a definitive and reliable diagnosis to confirm infection (Tasse 2024). The high prevalence of *Paramphistomum* spp. in Bali cattle that we found in the Pesanggaran abattoir, compared to a previous study (Lestari et al. 2017). This indicates a possible trend of increased infection in farms that supply cattle for slaughter to abattoirs. The origins of the farms that supply Bali cattle need to be traced and researched.

The high prevalence of *Paramphistomum* spp. infections can be attributed to environmental and host factors. The prevalence of infection is influenced by environmental conditions, such as rainfall and temperature (Rafiq et al. 2022). The prevalence of *Paramphistomum* spp. infection was highest during the rainy season, and animal origin was significantly correlated with infection (Njoku-Tony et al. 2017; Aragaw and Tilahun, 2019; Tasse 2024). A study in Tyumen, Russia, also found a positive correlation between average temperature and the average number of cattle infected with *Paramphistomum* spp. (Siben et al. 2018). Host factors that influence the prevalence of infection include age, with older cattle having a higher potential for infection (Kebede et al. 2023). Body condition, breed, and husbandry system of cattle have been associated with the prevalence of *Paramphistomum* spp. infection (Aragaw and Tilahun 2019). However, no significant difference was observed between male and female animals regarding infection prevalence (Preethi et al. 2020; Tasse 2024). Environmental factors also affect the survival of snails as the intermediate host. Weather plays an important role in *Paramphistomum* spp. infection, parasite reproduction, vector growth, and survival. The highest prevalence was found in summer and fall (countries with four seasons), which may be influenced by favorable temperature, humidity, and rainfall (Rafiq et al. 2022).

The categorization of *Paramphistomum* spp. infection levels can also be based on the number of worms infecting a certain area, as previously reported. Slight infection was defined as 10–20 worms per 5cm², moderate infection as 20–40 worms per 5cm², and severe infection as > 41 worms per 5cm². The number of worms found correlated with a decrease in epithelial thickness in the rumen and reticulum of the animals. The most significant decrease in epithelial thickness occurred in the moderate and severe infection categories (Rizwan et al. 2022). In this study, moderate infection (26.62%) dominated the infection level of *Paramphistomum* spp. in the Pesanggaran abattoir. Similarly, Rizwan et al. (2022) reported that approximately 38% of the animals showed a moderate infection burden of *Paramphistomum* spp., although with different measurement parameters. Infection levels are most commonly categorized by quantitative determination of eggs per gram (EPG) of feces in live cattle, where a higher EPG indicates a more severe infection. Bosco et al. (2023) and Che-Kamaruddin and Isa (2023) have categorized EPG values for trematodes in cattle as < 10EPG as slight infection, 10-50EPG as moderate, and > 50EPG as severe. Various studies have revealed a close relationship between the number of adult *Paramphistomum* spp. parasites in the rumen and reticulum, and the EPG values. For example, a study conducted in Peru showed a proportional direct relationship between the number of adult parasites and EPG, with a correlation coefficient of 0.971. This implies that a higher EPG value indicates more parasites (Coronado et al. 2024). The more worms that infect, the greater the damage caused, which results in increased blood consumption by the worms and anemia in the host.

Paramphistomum spp. can damage the small intestine during its juvenile phase in the small intestine and the rumen and reticulum during its adult phase (Chongmobmi and Panda 2018; Huson et al. 2021). Once infected, newly excysted juveniles (NEJs) of *Paramphistomum* spp. invade the duodenal submucosa of the host. This invasion can lead to significant pathology, particularly in cases of severe infection. This attachment is facilitated by ventral suckers. The larvae then migrate upward along the digestive tract to the rumen, where they develop into adults (Huson et al. 2021). Infiltration of inflammatory cells was observed in the mucosa and submucosa of the duodenum. This includes aggregates or follicles of inflammatory cells and sparse infiltration of eosinophils, globular leukocytes, mast cells, and macrophages in the lamina propria. The larvae cause tissue damage, characterized by epithelial acanthosis and hyperkeratosis. This damage is more severe in areas with higher parasite concentrations (Fuertes et al. 2015). Juvenile flukes in the duodenum can cause severe clinical signs, such as diarrhea and weight loss. These symptoms are more pronounced in the juvenile than in the adult stage (Morariu et al. 2023). In chronic infection in the adult phase, flukes generally do not cause severe symptoms and can still be tolerated by cattle. However, pathological changes in the rumen and reticulum were still observed. Ruminants with higher parasite loads had significantly decreased epithelial thickness, shortened rumen papillae, and thinning of the rumen wall. In addition, mucosal cells in the rumen papillae showed eruptions, and there were changes in cells that function for epidermal adhesion (Rizwan et al. 2022). Comparable observations have been documented in Bali cattle, specifically necrosis, and inflammation, reduction in the length of the rumen papilla, erosion, cellular apoptosis, and infiltration of eosinophilic inflammatory cells (Lestari et al. 2017; Iqbal et al. 2024).

We found a significantly higher percentage of monocytes in infected cows compared to uninfected cows, although it was still within the reference range. Monocytes play various roles in the elimination of parasites in the host body. Monocytes play a particularly important role in the detection and engulfment of pathogens. They move towards the site of infection and then differentiate into macrophages and dendritic cells, both of which are important for clearing pathogens. Monocytes and macrophages have two main roles: first, they promote inflammation to fight infection, and second, they play a role in tissue repair after infection. They also secrete molecules that aid in the healing of parasite-damaged tissues. However, helminths can manipulate monocyte function to evade the host's immune system. They secrete molecules that induce an anti-inflammatory response, which helps the parasite survive for a longer time in the host (Rajamanickam and Babu 2024). In this study, Bali cattle infected with *Paramphistomum* spp. showed an increase in monocytes, possibly due to an inflammatory reaction and tissue repair due to the worm infection. However, this inflammatory reaction was relatively chronic and tolerated by the Bali cattle; therefore, no severe clinical signs were observed. Chronic *Paramphistomum* spp. infection generally causes no symptoms, and cattle can still compensate for the situation despite pathological changes in the rumen and reticulum organs (Huson et al. 2017; Rizwan et al. 2022). Bali cattle with moderate infection showed lower percentages of monocytes than those with slight and severe infections. This may be because, in a slight infection, the cow's body can control the number of parasites with a controlled immune response; therefore, monocytes play a more significant role in dealing with pathogens without much change in their distribution. Severe infection triggers a stronger immune response, increasing monocyte recruitment and changing the percentage of immune cells due to more intense inflammation. Meanwhile, in moderate infections, the immune response is sufficient to reduce the number of parasites but not completely eliminate them, which might lead to a decrease or alteration of monocyte regulation through a counter-regulatory mechanism to prevent tissue damage due to excessive inflammation (Gonzalez-Granado et al. 2023; Grandoni et al. 2023; Grandoni et al. 2022).

Leukocytosis in infected and uninfected Bali cattle with *Paramphistomum* spp. may result from infection with this fluke or other factors, such as stress. Khan et al. (2021) reported that *Paramphistomum* spp. infections can cause leukocytosis. The average WBC count of 28 infected cows was $9.35\pm0.67/mm^3$ compared to $7.20\pm1.96/mm^3$ in the control group. However, Khan et al. (2021) did not conduct research on leukocyte differentials. In this study, WBC in the infected group showed a value of $17.46\times10^3/\mu L$ compared to the uninfected group, which was $16.45\times10^3/\mu L$, when compared to the normal reference by Brooks et al. (2022), both infected and uninfected groups experienced leukocytosis. Leukocytosis in Bali cattle was predominantly characterized by neutrophilia (80.26%). This is in line with the findings of Nath (2007),who reported that the number of neutrophils and eosinophils increased significantly in animals infected with *Paramphistomum* spp. In general, neutrophils play a crucial role in helminthiasis. Neutrophils are essential in the defense against helminth infection in cattle through mechanisms such as neutrophil extracellular trap (NET) formation, reactive oxygen species (ROS) production, and immune regulation mediated by interleukin-10 (IL-10) (Mendez et al. 2018; Li et al. 2019). Stress is another factor most likely to cause leukocytosis in infected and uninfected Bali cattle. Slaughter and transport stress can increase the number of leucocytes, especially neutrophils, contributing to leucocytosis. Cattle slaughtered after prolonged transport showed significant haematological changes, including increased neutrophils (neutrophilia) and decreased lymphocytes (lymphopenia), indicating stress-induced leukocytosis (Burns et al. 2022). This suggests Bali cattle slaughtered at the Pesanggaran abattoir may experience stress during transport and/or slaughter. However, this requires further research. Lymphopenia was observed in 65.79% of Bali cattle infected with *Paramphistomum* spp. (Table 3). This was also followed by Nath (2007), who reported a significant decrease in lymphocytes in crossbred cattle infected with natural paramphistomiasis. Lymphocytes play a role in infiltrating the rumen tissue and reticulum to overcome infection in these areas due to *Paramphistomum* spp. infection (Khan et al. 2021), which may decrease the number of lymphocytes in the blood.

We observed that *Paramphistomum* spp. Infection in bali cattle causes changes in the erythrocyte profile. Several previous studies have found mixed results for both cattle and buffalo. Khan et al. (2021) reported that RBC, MCHC, HB, MCV, and platelet values were lower in cattle infected with Paramphistomum spp. Nath (2007) found a significant decrease in PCV, RBC, and HB values in infected cattle. Yadav et al. (2019) found decreased PCV, HB and RBC levels in buffaloes infected with amphistomes. Chauhan et al. (2015) found reduced RBC, HB, and PCV values, but MCV, MCHC, and MCH did not differ between infected and uninfected buffaloes. Khurana et al. (2023) reported decreased HB, RBC, and PCV values in infected buffaloes. However, González-Garduño et al. (2019) reported no significant difference in RBC, PCV, MCV and HB values between cattle infected with trematodes (Fasciola spp. and Paramphistomum spp.) and those without. Our results are in line with those of González-Garduño et al. (2019), who found no significant differences in RBC, HB, PCV, MCH, MCHC, and MCV values between cattle infected with *Paramphistomum* spp. and those without (Table 1).

RBC counts in uninfected and infected Bali cattle with *Paramphistomum* spp. showed no significant difference (P>0.05). This is different from the findings of Khan et al. (2021) and Nath (2007), who studied cattle, as well as Yadav et al. (2019), Chauhan et al. (2015), and Khurana et al. (2023) in buffalo, where RBC values were lower in infected animals, but in line with González-Garduño et al. (2019). The difference in results may be influenced by the type of animal (cattle and buffalo) or the breed of cattle used in the study. In this case, Bali cattle may have a more efficient defense mechanism against *Paramphistomum* spp. infection that prevents greater haematological changes, such as decreased RBC, even though they are infected. This is consistent with the incidence of anemia that we found in only five Bali cattle (6.58%) (Table 3), where RBC values were lower in Bali cattle with severe and moderate levels of infection (Table 2). This difference in findings could also be due to several other factors, such as differences in research methods or parameters used to measure RBC, differences in the genus or species of amphistomes that infect, or in combination with other infections, different levels of infection, and different research locations. Further research considering these factors will provide a clearer understanding of the relationship between *Paramphistomum* spp. infection and haematological changes in Bali cattle. In addition to RBC, MCHC values were lower in severe infection than in mild and moderate infections (Table 2). This indicates that the more severe the infection, the lower the hemoglobin concentration in red blood cells. However, no significant difference was observed between those infected with *Paramphistomum* spp. and those without infection (Table 1). This contrasts with the findings of Khan et al. (2021), who found the opposite, but agrees with González-Garduño et al. (2019).

Conclusion

This study focuses on the high prevalence of paramphistomiasis in Bali cattle on Pesanggaran abbatoir, with an infection rate of approximately 49.35%, predominantly moderate infections. Leukocytosis, particularly neutrophilia, was the most common leukocyte change observed in *Paramphistomum* spp. infection. In addition, there was a decrease in the RBC and MCHC counts, particularly in severe infections. Given the increasing prevalence of infections, it is imperative to conduct further research on the factors contributing to the increase in *Paramphistomum* spp. infections. The prevention and eradication of paramphistomiasis in Bali cattle, particularly those sourced from farms supplying the Pesanggaran abattoir, is essential. Moreover, addressing anemia and leukocytosis resulting from *Paramphistomum* spp. infections is crucial for enhancing the health status of Bali cattle.

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Author's Contribution: IAPA: conceptualization, methodology, supervision and writing – original draft. NAS and NWTI: investigation, data curation, and validation. INPAP, GSDY, and IPGSN: investigation and data curation. IPCP: methodology, software, formal analysis, visualization, and writing - original draft. All authors contributed to reviewing and editing the manuscript to improve the quality of writing, clarify the content, and ensure formatting compliance.

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