Research Article

Risk Factors and Blood Profile Changes Associated with *Haemonchus contortus* Infection in Fat-tailed Sheep in a Tropical Highland Area

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Abstract |Haemonchosis, caused by the gastrointestinal nematode *Haemonchus contortus*, is a major parasitic disease affecting small ruminants in tropical regions and leads to substantial economic losses and mortality in sheep. This study aimed to identify risk factors associated with *H. contortus* infection and evaluate the relationship between fecal egg counts (eggs per gram; EPG) and both hematological and blood chemistry profiles in fat-tailed sheep managed under highland tropical conditions. A total of 150 sheep exhibiting clinical signs suggestive of haemonchosis including dull hair coat, weakness, pallor, and diarrhea were examined. Fecal samples were collected per rectum and analyzed using sedimentation, flotation, and McMaster techniques, which identified 98 animals as positive for helminth infection. Based on a minimum sample size calculation performed using G*Power software, blood samples were collected from the jugular vein of 42 animals for hematological and biochemical analysis using ABX Micros 60 and ABX Pentra C200 analyzers, respectively. Multivariate logistic regression analysis revealed that ambient temperature was the only variable significantly associated with infection status; sheep raised under lower temperature conditions exhibited higher odds of infection (OR = 3.50, $p < 0.05$). No significant correlations were detected between EPG counts and blood chemistry parameters ($p > 0.05$). In contrast, EPG counts demonstrated significant associations with several hematological variables, including positive correlations with leukocyte and lymphocyte counts and a negative correlation with basophil counts ($p < 0.05$). However, the low coefficients of determination indicated that these associations had limited explanatory power. Taken together, these findings indicate that hematological parameters exhibit only weak associations with infection intensity and may offer limited diagnostic support under field conditions. Furthermore, EPG counts alone serve as a poor predictor of overall physiological variation in naturally infected sheep.

Keywords: Blood chemistry; EPG counts; *Haemonchus contortus;* Hematology; Risk factors, Sheep

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INTRODUCTION

Sheep are among the most commonly raised small ruminants in Indonesia, favored due to their relatively low capital requirements, ready availability of feed resources, simple management practices, and high reproductive potential. However, sheep production is often constrained by parasitic disease, particularly helminthiasis, which remains a significant problem in many smallholder farms, including those in Malang Regency, Indonesia. Helminth infections, which spread through contaminated environments or feed, can cause substantial economic losses due to reduced body weight (up to 38%), decreased productivity, impaired immunity, and mortality rates reaching 17% (Mahartina *et al*., 2020).

Haemonchosis is a parasitic disease caused by *Haemonchus contortus*, a blood-feeding gastrointestinal nematode that predominantly infects goats and sheep in tropical regions (Arifin *et al*., 2019). The parasite attaches to the abomasal mucosa and feeds on host blood, leading to mucosal irritation, hemorrhage, and anemia in infected animals (Edwards *et al*., 2016). Severe infections may result in significant blood loss and even death (Mohamed *et al*., 2024). Infection with *H. contortus* can induce alterations in hematological and blood biochemical parameters in small ruminants (Baihaqi *et al*., 2020). Previous studies have reported decreases in packed cell volume (PCV), hemoglobin concentration, and red blood cells (RBC) counts in infected animals. In addition, changes in several white blood cell (WBC) populations, including lymphocytes, eosinophils, and monocytes, have also been observed (Awad *et al*., 2023). Other reports describe alterations in serum protein concentrations and liver enzyme activities associated with haemonchosis (Bandhaiya *et al*., 2019).

However, most previous investigations were conducted under lowland conditions or within controlled experimental settings, which may not fully represent the complex dynamics of natural infection under field conditions. Information regarding naturally infected fat-tailed sheep raised in tropical highland environments remains limited. Highland areas are characterized by lower ambient temperatures, higher humidity, and different management systems, which may influence parasite survival, transmission patterns, host immune responses, and the clinical manifestation of haemonchosis. These distinct environmental and host-related factors may result in different patterns of infection intensity and physiological responses compared to those observed in sheep raised in lowland regions. Therefore, this study aimed to analyze the risk factors associated with *H. contortus* infection and to evaluate the relationship between EPG counts and hematological as well as blood biochemical profiles in fat-tailed sheep raised under tropical highland conditions in Indonesia.

MATERIALS AND METHODS

Study period and location

This study was conducted over an 8-month period at a mixed livestock farm (comprising goats, sheep, and cattle) located in Wagir District, Malang Regency. Laboratory analyses were performed at the Clinical Pathology Laboratory (Faculty of Medicine) and the Parasitology Laboratory (Faculty of Veterinary Medicine), Universitas Brawijaya.

Sample collection

This study aimed to evaluate risk factors associated with *H. contortus* infection and to assess correlations between EPG counts, hematological profile, and blood chemistry parameters in sheep under field conditions. The study involved 150 fat-tailed sheep, aged 1 to 3 years, raised under a tropical highland production system. The selected animals exhibited clinical signs suggestive of haemonchosis, including dull hair coat, weakness, reduced body condition, pallor, pale conjunctival mucosa, and diarrhea, reflecting chronic anemia and gastrointestinal disturbance. The risk factors analyzed included body condition score (BCS), ambient temperature, age, sex (male and female), stocking density, light intensity, and humidity. Fecal samples were collected per rectum into labeled containers for parasitological examination. For blood chemistry analysis, blood was collected from the jugular vein into serum separator tubes with clot activator and gel (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA), whereas samples for hematological analysis were drawn into K_2EDTA anticoagulant tubes (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). The overall study workflow is summarized in Figure 1.

Blood sample selection

Of the 98 sheep that tested positive for helminthiasis, 42 animals were selected for blood sampling based on feasibility consideration and minimum sample size estimation using G*Power version 3.1.9.7 to ensure adequate statistical power for correlation analysis. Blood samples were selected randomly from the positive group with stratification based on EPG count intensity. Due to logistical constraints and animal welfare considerations, blood sampling was not conducted on all positive animals. This sampling strategy may introduce selection bias and is therefore acknowledged as a limitation of the study.

Fecal analysis

Fecal samples were examined using sedimentation, flotation, and McMaster methods. Sedimentation and flotation methods were used to identify the worm eggs found, while McMaster was used to calculate the EPG counts of the identified nematode eggs. The sedimentation test was carried out by taking 3 grams of feces, grinding it, and adding enough water. The feces were then filtered into a falcon tube and centrifuged for 5 minutes at 1500 rpm. After that, the supernatant was discarded and replaced with water so that it could be centrifuged a second time. Next, the sediment was taken for observation.

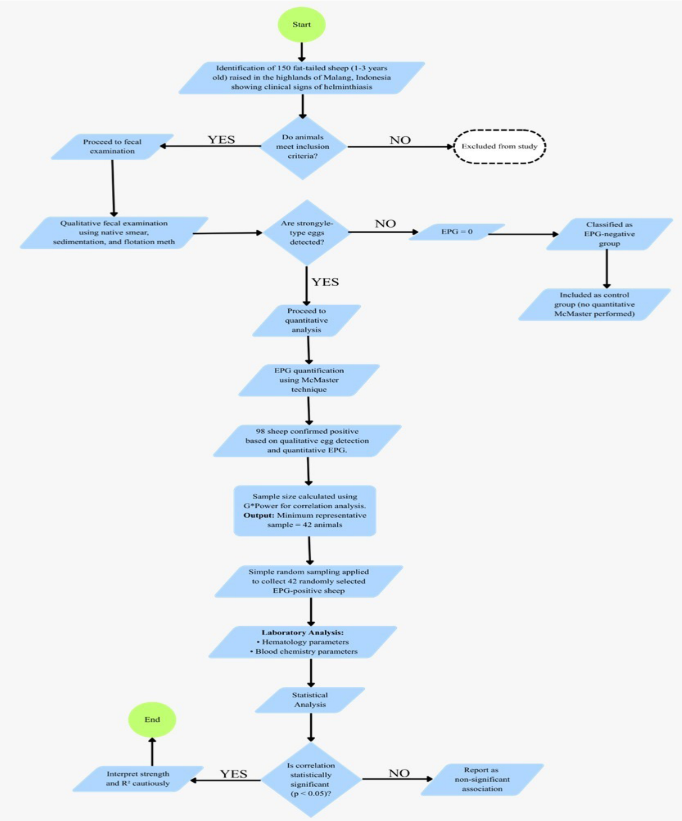

Figure 1: Flowchart of the study design illustrating sample selection and analysis procedures.

Meanwhile, the flotation test was continued using the previous fecal sample. Saturated sugar solution was added to 3/4 of the falcon tube and centrifuged again. Saturated sugar was added to the tube until it was convex, and a cover glass was attached to the top of the tube for 5 minutes before finally being placed on an object glass for observation. Observations were made using an Olympus CX32 Microscope with the aid of Optilab Advance Plus. The McMaster method is carried out by mixing 1 gram of feces with 15 ml of saturated sugar solution in a mortar and grinding it. The mixture is filtered into a falcon tube and left for 5 minutes. Next, 0.5 ml of the filtrate on the surface is taken using a Pasteur pipette and placed in a McMaster chamber. Then, observation and calculation of eggs per gram of feces. Nematode eggs were identified as *H. contortus* based on morphological characteristics consistent with standard parasitological references. However, molecular confirmation using PCR was not performed, which is acknowledged as a limitation of this study.

Hematology and blood chemistry analysis

Serum was separated through centrifugation and analyzed using ABX Pentra C200 biochemical analyzer to measure blood chemistry parameters, while whole blood was used to analyze the complete hematological profile using ABX Micros 60 hematology analyzer at the Clinical Pathology Laboratory of the Faculty of Medicine, Universitas Brawijaya. The parameters observed in the blood hematology examination in this study were leukocyte, erythrocyte, hemoglobin, hematocrit, platelet, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC). Meanwhile, the blood chemistry parameters used in this study were albumin, globulin, total protein, total bilirubin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), glucose, and total cholesterol. In addition to sample examination, other examinations in the form of risk factors carried out at the research site include gender, age, density, BCS, temperature, humidity, and light.

Data analysis

This study focused on naturally infected sheep under field conditions; non-infected animals were also included in the correlation analysis where applicable. Data analysis included normality, homogeneity, and linearity tests to determine the appropriate statistical methods. Pearson correlation analysis was applied to normally distributed data with linear relationships, while Spearman rank correlation analysis was used for non-normally distributed or ordinal data. To identify potential risk factors associated with *H. contortus* infection, univariate analysis was first performed using Chi-square tests. Variables evaluated included sex, BCS, age, stocking density, ambient temperature, humidity, and light intensity. Variables identified in the screening stage were subsequently included in a multivariate logistic regression model to control for potential confounding effects and to determine independent predictors of infection. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 29 with a significance level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Prevalence and study population characteristics

Fecal examination of 150 sheep revealed that 98 animals (65.3%) were positive for helminth infection, indicating a relatively high level of parasite exposure within the study population. The selected animals displayed a constellation of signs consistent with chronic infection, including dull hair coat, generalized weakness, reduced body condition, pallor, pale conjunctival mucosa, and diarrhea collectively reflecting gastrointestinal disturbance and the early stages of anemia. This prevalence is consistent with reports from

Table 1: Univariate (Chi-square) analysis of potential risk factors for *H. contortus* infection.

Risk Factors		Total Animals	Infected Animals (%)	OR	95% CI	p-value
Sex	Female	94	56 (59.6)	2.04	0.98-4.23	0.055
	Males	56	42 (75.0)			
BCS	<3	47	22 (46.8)	3.20	1.55-6.58	0.001*
	≥3	103	76 (73.8)			
Age (years old)	<2	93	59 (63.4)	1.25	0.62-2.51	0.534
	≥2	57	39 (68.4)			
Density (heads)	<20	142	93 (65.5)	0.88	0.23-3.83	0.863
	≥20	8	5 (62.5)			
Ambient temperature (°C)	<33.2	68	37 (54.4)	2.43	1.22-4.84	0.010*
	≥33.2	82	61 (74.4)			
Humidity (%)	<41	39	27 (69.2)	0.79	0.36-1.73	0.552
	≥41	111	71 (64.0)			
Light (lux)	<1939	72	48 (66.7)	0.89	0.46-1.75	0.742
	≥1939	78	50 (64.1)			

*Significant at p<0.05; OR: Odds Ratio; 95% CI: 95% Confidence Interval.

other smallholder production systems in Indonesia, where helminthiasis remains one of the primary constraints on sheep productivity capable of reducing body weight by up to 38% and contributing to mortality rates reaching 17%. Based on the minimum sample size estimation, 42 animals were subsequently selected for hematological and biochemical analysis. Stratified random sampling based on EPG count intensity was employed to ensure an adequate range of infection burden was represented; however, this approach and the non-exhaustive sampling of positive animals are acknowledged as potential sources of selection bias. The study was conducted at a livestock farm situated in a tropical highland environment, providing a field context that remains relatively under-investigated in the haemonchosis literature and offering novel insights into infection dynamics under highland conditions.

Risk factors associated with H. contortus infection

Univariate risk factor analysis (Table 1) identified two variables significantly associated with *H. contortus* infection. Sheep with a BCS < 3 had higher odds of infection (OR = 3.20) compared to animals with BCS ≥ 3 (OR = 3.20; 95% CI: 1.55-6.58; p = 0.001), suggesting that poorer body condition may increase susceptibility to infection rather than merely reflect its consequences. This finding aligns with previous research indicating that poor BCS is often associated with reduced resistance to gastrointestinal parasitism. Similar associations have been reported previously (Seyoum *et al*., 2018). Additionally, sheep raised under lower ambient temperature conditions exhibited an increased infection risk (OR = 2.43; 95% CI: 1.22-4.84; p = 0.010), highlighting the potential role of cooler highland microclimates in supporting parasite transmission and larval survival. Other variables, including age, stocking density, humidity, and light intensity, were not significantly associated with infection (p > 0.05), indicating a limited contribution of these factors within the observed ranges.

To control for potential confounding effects among risk factors, multivariate logistic regression analysis was performed. The multivariate model was statistically significant (Omnibus test, χ^2 = 19.14, p = 0.008), indicating that the included predictors collectively contributed to explaining infection status. The goodness-of-fit test showed that the model adequately fitted the data (Hosmer-Lemeshow test, p = 0.405). However, the explanatory power of the model was relatively modest, with a Nagelkerke R^2 of 0.165, suggesting that approximately 16.5% of the variability in infection status was explained by the examined variables.

However, after adjustment using multivariate logistic regression (Table 2), only ambient temperature remained a statistically significant predictor of infection (OR = 3.50, p = 0.017). Sheep raised under lower ambient temperature conditions showed higher odds of infection compared with those maintained at higher temperature ranges. This finding suggests that environmental conditions may play a more important role than the host-related variables examined in determining infection occurrence in the studied population. Environmental temperature is well-documented to influence the development and survival of gastrointestinal nematode eggs and larvae. Moderate temperature ranges typically support larval persistence and development, whereas excessively high temperatures can reduce larval viability and survival rates (Arsenopoulos *et al*., 2021; Wirawan *et al*., 2022).

Body Condition Score (BCS) showed a borderline association in the multivariate model but was no longer statistically significant after adjustment for other variables. This pattern suggests that the apparent relationship observed in univariate analysis may partly reflect confounding effects related to environmental conditions or management practices rather than a direct causal relationship. Similar inconsistencies have been reported in field studies of gastrointestinal nematodes, where the relationship between nutritional status and parasite infection is often complex and influenced by multiple interacting factors, including host adaptation, feeding practices, and grazing management (Seyoum *et al*., 2018). Other variables, including sex, age, stocking density, humidity, and light intensity, were not statistically significant after adjustment in the multivariate analysis ($p > 0.05$).

Relationship between EPG counts and blood profiles

The present study evaluated associations between infection intensity, as measured by EPG counts, and various hematological and biochemical parameters in sheep naturally infected with gastrointestinal nematodes under tropical highland conditions. Due to the cross-sectional design employed, the results describe statistical relationships rather than establishing causal mechanisms between parasite burden and physiological responses.

Pearson correlation analysis (Table 3) revealed no significant relationships between EPG counts and any biochemical variables, including total protein and AST ($p > 0.05$). The low coefficients of determination ($R^2 < 0.1$) indicated that infection intensity explains only a small proportion of the variation in blood chemistry profiles. These findings suggest that, under tropical highland conditions, parasite burden alone may not strongly influence systemic biochemical parameters (Das *et al*., 2025; Mannan *et al*., 2017; Samadie *et al*., 2016).

In contrast, several hematological parameters were significantly associated with infection intensity (Table 4). Leukocyte, lymphocyte, and basophil counts showed significant correlations with EPG counts ($p < 0.05$), suggesting an immune response related to parasite burden. However, the relatively low coefficients of determination ($R^2 < 0.3$) indicate that parasite load explains only a limited proportion of hematological variation under field conditions.

Therefore, while these associations are statistically detectable, they should be interpreted cautiously and do not necessarily indicate strong biological dependence between parasite burden and circulating leukocyte levels

Table 2: Multivariate logistic regression analysis of potential risk factors for *H. contortus* infection.

Variable	B	Wald	p-value	Exp(B)	95% CI
Sex	0.850	3.843	0.05	2.34	1.00 – 5.49
Body Condition Score (BCS)	0.795	3.537	0.06	2.22	0.97 – 5.08
Age	0.160	0.169	0.68	1.17	0.54 – 2.52
Stocking Density	0.754	0.844	0.36	2.13	0.43 – 10.44
Ambient Temperature	1.252	5.714	0.01	3.50	1.25 – 9.82
Humidity	0.708	1.694	0.19	2.03	0.70 – 5.89
Light Intensity	-0.136	0.104	0.75	0.87	0.38 – 2.02

*Significant at $p<0.05$; B: regression coefficient (logit coefficient); Exp(B): exponentiated coefficient; 95% CI: 95% Confidence Interval.

Table 3: Correlation analysis between blood chemistry parameters and EPG counts.

Parameter	N	Result	Unit	p-value	r	R^2
Albumin†	42	2.61 ± 0.32	g/dL	0.55	-0.09	0.01
Total Cholesterol†	42	69.11 ± 19.82	mg/dL	0.42	0.13	0.02
AST‡	42	92.54 ± 24.31	IU/L	0.19	0.21	0.04
ALT†	42	16.5 ± 4.88	IU/L	0.41	0.13	0.02
Glucose†	42	49.09 ± 9.27	mg/dL	0.36	-0.14	0.02
Total Bilirubin†	42	0.31 ± 0.09	mg/dL	0.94	0.01	0.0001
Total Protein‡	42	7.17 ± 0.74	g/dL	0.30	0.16	0.03
Globulin†	42	4.60 ± 1.00	g/dL	0.51	0.10	0.01

*Significant at $p<0.05$; ‡: Pearson analysis; †: Spearman analysis; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; R^2: Coefficient of Determination; r: Correlation Coefficient.

Table 4: Correlation analysis between hematological parameters and EPG counts.

Parameter	N	Result	Unit	p-value	r	R^2
Leukocytes†	42	8.45 ± 4.16	10^9/L	0.003*	0.45	0.21
Erythrocytes†	42	10.69 ± 1.44	10^{12}/L	0.174	-0.21	0.01
Hemoglobin†	42	17.58 ± 23.15	g/dL	0.856	-0.03	0.01
Hematocrit†	42	36.23 ± 6.80	%	0.838	-0.03	0.01
Platelets†	42	708.87 ± 836.80	10^9/L	0.296	0.17	0.01
MCV†	42	33.49 ± 1.44	fl	0.196	-0.20	0.03
MCH†	42	11.27 ± 0.46	pg	0.962	-0.01	0.02
MCHC†	42	32.76 ± 0.65	g/dL	0.301	0.16	0.01
Neutrophils†	42	71.78 ± 5.79	10^9/L	0.101	-0.26	0.01
Lymphocytes†	42	14.43 ± 6.48	10^9/L	0.044*	0.31	0.01
Monocytes†	42	6.13 ± 2.00	10^9/L	0.161	-0.22	0.01
Eosinophils†	42	6.35 ± 3.88	10^9/L	0.592	-0.09	0.01
Basophils†	42	1.35 ± 0.77	10^9/L	0.009*	-0.40	0.06

*Significant at p<0.05; †: Spearman analysis; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; R^2: Coefficient of Determination; r: Correlation Coefficient.

(Arsenopoulos et al., 2021). Similar relationships have been reported by Baihaqi et al. (2020) and Das et al. (2025) who observed that higher parasite burdens tend to be accompanied by elevated leukocyte counts, reflecting host immune responses to nematode infection. Ceriac et al. (2017) also noted that *H. contortus* infection in sheep is commonly associated with increased lymphocyte levels, which correspond with the proliferation of T helper cells (CD4+) in the abomasal lymph nodes during early infection.

In contrast to leukocyte and lymphocyte counts, basophil counts showed a significant negative correlation with EPG values. This pattern indicates that higher parasite burdens were associated with lower circulating basophil levels. Given that basophils are known to participate in type-2 immune responses against helminths, this pattern may reflect the redistribution of basophils from peripheral blood to infection sites (e.g., abomasal mucosa) rather than a systemic decrease in cell production (Eberle and Voehringer, 2016; Obata-Ninomiya *et al.*, 2020). However, because tissue-level immune responses were not evaluated in this study, this interpretation remains speculative and requires further investigation.

Despite *H. contortus* being a blood-feeding nematode whose primary pathological effect in severe infection is hemorrhagic anemia, other hematological parameters including RBC, hemoglobin, hematocrit, MCV, MCH, and MCHC showed non-significant correlations with EPG counts (p > 0.05). Although anemia is a well-known consequence of severe haemonchosis, such alterations are not always detectable in naturally infected populations, particularly when infection intensity varies or when animals develop partial tolerance to chronic parasite exposure (Mannan *et al.*, 2017; Penago *et al.*, 2021).

Explanatory power and field implications

Overall, the low coefficients of determination observed in this study suggest that parasite burden alone is a weak predictor of hematological or biochemical variation in naturally infected sheep under field conditions. This likely reflects the multifactorial nature of physiological responses under field conditions, where host adaptation, nutritional status, environmental factors, and management practices interact with parasite exposure. Similarly, the low R^2 values from correlation analyses between EPG counts and hematological parameters further underscore the limited predictive value of individual blood markers in field settings. From a practical standpoint, these findings suggest that reliance on any single diagnostic indicator — whether EPG, leukocyte count, or blood chemistry profile — will be insufficient for accurate assessment of haemonchosis severity under field conditions. An integrated approach combining parasitological, hematological, and clinical indicators, such as the FAMACHA© conjunctival mucosa scoring system, may provide more robust field-level guidance for anthelmintic treatment decisions in resource-limited settings. The modest Nagelkerke R^2 of 0.165 in the logistic regression model reinforces this conclusion: even the combined suite of host and environmental risk factors examined here explains only a fraction of the observed variation in infection status.

Study limitations

Several limitations should also be considered when interpreting these findings. Blood sampling was performed on a subset of infected animals, and parasite identification relied on egg morphology without molecular confirmation. Because eggs of *H. contortus* are morphologically similar to those of other strongyle nematodes (e.g., *Trichostrongylus* spp.), the EPG counts may represent mixed strongyle infections rather than exclusively *H. contortus,* which could partially confound the correlation analysis. In addition, the absence of a non-infected control group limits comparative interpretation. Future studies incorporating larger sample size, molecular parasite identification, and immunological or histopathological analyses would help clarify the physiological responses associated with haemonchosis under tropical highland conditions.

CONCLUSIONS

This study demonstrated that ambient temperature was significantly associated with the occurrence of *Haemonchus contortus* infection in fat-tailed sheep raised under tropical highland conditions. Infection intensity was not associated with blood chemistry parameters but showed statistically significant, albeit weak, correlations with leukocyte, lymphocyte, and basophil counts. These findings suggest that while hematological parameters may offer limited supportive information in field settings, parasite burden itself is a poor predictor of overall physiological variation in naturally infected sheep. The absence of a non-infected control group and reliance on morphological identification remain key limitations of this study and restrict causal interpretation.

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NOVELTY STATEMENT

This study provides a field-based evaluation of *H. contortus* infection in fat-tailed sheep managed under a tropical highland production system by integrating risk factor analysis with quantitative correlations between EPG counts and blood profiles. Unlike previous studies that primarily focused on prevalence or single risk factors, this research simultaneously examines host condition, highland-specific environmental parameters, and physiological responses under natural infection settings. The findings demonstrate that lower ambient temperatures are significantly associated with increased infection risk, emphasizing the importance of highland microclimatic conditions in parasite transmission dynamics. Moreover, the study identifies specific hematological parameters particularly leukocyte, lymphocyte, and basophil counts as being more strongly associated with infection intensity than blood chemistry variables, suggesting differential sensitivity of hematological markers under field conditions. By focusing on naturally infected sheep managed in tropical highland systems, this work contributes context-specific evidence supporting the practical use of hematological indicators as complementary tools for monitoring haemonchosis in small ruminant production systems where laboratory resources are limited.

AUTHOR'S CONTRIBUTIONS

DK, AG, RH conceived the idea and planned the manuscript. AG, RH, SK, AEB contributed to sample preparation. DK, DP, TW, ARR and SKM have made significant scientific support and also contributed to the interpretation of the results. All authors provided significant contributions by giving feedback and help shape the manuscript.

Ethical approval

This study was approved by the Research Ethics Committee of Universitas Brawijaya, Indonesia, under the reference number 071-KEP-UB-2024.

Generative AI and AI-assisted technology statement

The authors declare that generative AI tools (Grammarly) were used solely for language editing and grammatical improvement. No AI tools were used for data analysis, interpretation, or scientific decision-making.

Conflicts of interest

The authors declared that there is no conflict of interest.

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