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Research article

Relationship between parasitism with milk yield and body weight of Mafriwal cattle

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Abstract

Mafriwal cattle have good characteristics, but parasitism effects on their production have never been explored. This study aimed to determine the relationship between phenotypic markers of parasitism, namely packed cell volume (PCV), peripheral eosinophils count (PEC), nematode eggs (EPG), and coccidia oocyst (OPG) with the milk yield and body weight of Mafriwal cattle. Fecal and blood samples were collected from 242 cattle between 2021 and 2022. PCV and PEC were obtained from blood samples, while EPG and OPG were obtained from fecal samples. PCR was conducted to identify haemoparasite species. Milk yield data and the body weight of calves were also recorded. Spearman's rank correlation showed that body weight has a negative correlation with PCV (r=-0.4; P<0.01) and a positive correlation with PEC (r=0.39; P<0.01) in 2022. Multiple linear regression models were used to determine the effect of phenotypic markers and year of sampling on milk yield and body weight. The best models were selected based on the lowest Akaike Information Criterion. Milk yield decreased significantly (P<0.001) from 2021 to 2022. An increase in PEC led to a significant reduction (P<0.001) in milk yield. Conversely, body weight increased significantly (P<0.001) from 2021 to 2022 and an increase in PCV caused a significant (P<0.001) reduction in body weight. Elevated EPG and OPG led to significant (P<0.01) reductions in body weight but the interaction between EPG and OPG has a significant positive (P<0.001) impact on body weight. Overall, the findings suggest that milk yield and body weight of Mafriwal cattle were significantly affected by parasitism thus necessitating the implementation of parasite control approaches.

Keywords: Body weight, Mafriwal cattle, Milk yield, Parasitism, Phenotypic markers

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INTRODUCTION

The ruminant industry in Malaysia faces numerous challenges, despite efforts by the government, farmers, and stakeholders to boost local milk and meat supply. The industry remains underdeveloped (Faghiri et al., 2019). The Food and Agriculture Organization (FAO, 2021) reported a 4.5% drop in the average price of whole milk powder in 2020 due to reduced sales in Asian countries, including China, Bangladesh, Malaysia, and Singapore. This decline was driven by decreased demand during the economic crisis caused by the Covid-19 pandemic. Additionally, Malaysia has not reached self-sufficiency in milk and meat production, preventing exports to other countries (Hashim, 2015). Therefore, it is crucial to investigate issues such as parasitism in the ruminant industry, as they may hinder Malaysia's ability to achieve self-sufficiency in milk and meat production (Syamsul et al., 2020). Gastrointestinal parasites namely *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., *Oesophagustomum* spp., and *Paramphistomum* spp. are known to cause severe gastroenteritis in cattle worldwide (Yusof, 2019). Parasite infections are very common among ruminants in the Southeast Asia region due to the suitable climate for the development and spreading of the infective parasite stage. The parasites are transmitted through the environment contaminated with parasites' eggs, oocysts, or cysts (Ananta et al., 2014). Generally, the clinical signs during parasitism may differ according to parasite burden, species involved, and individual immunity. Symptoms of gastrointestinal parasite infections include decreased weight gain, diarrhea, rough coat, body weakness, and anemia (Pulido-Medellin et al., 2022). In addition, coccidiosis caused by *Eimeria* spp. has become one of the most economically important diseases in the livestock industry that brings about both clinical and subclinical losses, especially in young cattle (Lopez-Osorio et al., 2020). Clinical infection may result in diarrhea, anorexia, weakness, dehydration, and occasional deaths (Tomczuk et al., 2015).

Babesiosis, theileriosis, and anaplasmosis are important tick-borne diseases of cattle around the world (Adjou Moumouni et al., 2015). These haemoparasites often cause sub-clinical infections that lead to a large number of vectors and asymptomatic carriers which act as a source of infection for susceptible ruminants in the population (Bell-Sakyi et al., 2004; Ola-Fadunsin et al., 2017). The prevalence of multiple haemoparasite species such as *Anaplasma* spp., *Babesia* spp., *Mycoplasma* spp, *Theileria* spp., and *Trypanosoma* spp. in the cattle farms in Peninsular Malaysia has been reported previously (Ola-Fadunsin et al., 2017; Agina et al., 2021) and anaplasmosis is known to be endemic in Malaysia (Tay et al., 2014).

According to Brito et al. (2020), phenotypic markers can be defined as a variable that can be measured continuously and represents a biological mechanism at a specific time. The studies conducted by Basripuzi et al. (2018) and Hayyan et al. (2020) highlighted packed cell volume (PCV), peripheral eosinophils count (PEC) and nematode eggs per gram (EPG) of feces as phenotypic markers of parasitic gastroenteritis in small ruminants. PCV is commonly measured to evaluate blood-sucking parasite infections as low red blood cell count indicates anemia. PEC represents an immune response against parasite infection in the circulating blood (Abd-Majid et al., 2022).

Eosinophilia can occur during certain infections or early stages of infection, uremia, acute hemolysis, and stressful circumstances (Abramowicz et al., 2019). Hence, hematological parameters such as PCV and PEC are important for the assessment of general health conditions as well as early diagnosis of many diseases in animals. EPG is the most frequently used parameter to assess the gastrointestinal parasite burden in ruminants and is reliable for the identification of resistant animals (McRae et al., 2015).

The Friesian-Sahiwal crossbreeding program was established by the Department of Veterinary Services (DVS) Malaysia since 1978 (Panandam et al., 2005). The program aimed to develop a dairy cattle breed with high milk yield and adaptability to the hot and humid local environment. The program also aimed to involve local farmers in dairy cattle production to improve the industry. As a result, the Mafriwal cattle breed with 60-75% Friesian genes was developed, possessing dual-purpose traits such as good dairy production, manageability, resilience to local climate and diseases, and adaptability to local feed resources (Mastura et al., 2019). Until to-date, no research has been conducted to determine if the milk and meat production of Mafriwal cattle could be affected by parasitism.

Therefore, this study aimed to determine the relationship between phenotypic markers of parasitism namely PCV, PEC, EPG, and coccidia oocyst per gram (OPG) of feces with the milk yield and body weight of Mafriwal cattle.

MATERIALS AND METHODS

Study site, duration, and animal ethics approval

This research was carried out on a government farm located in Kluang, Johor, Malaysia in December 2021 and June 2022. This study was conducted after approval from the Animal Ethics Committee of Universiti Malaysia Kelantan (UMK/FPV/ACUE/RES/003/2021).

Animals and sample collection

A total of 242 Mafriwal cattle consisting of less than 1-year-old calves, yearlings, lactating cows, and dry cows were sampled in this study (Table 1). The less than 1-year-old calves were managed under an intensive system while the other groups of cattle were managed under a semi-intensive system on the pasture. Blood samples were collected from the coccygeal vein of each cattle using ethylenediaminetetraacetic (EDTA) coated blood collection tubes and fecal samples were collected from the rectum using gloved hands. Data on the body weight of less than 1-year-old calves and yearlings; as well as the milk yield of lactation cows in December 2021 and June 2022 were collected from DairyCHAMP, a computerized dairy herds database system. DairyCHAMP database system has been used in the farm since the 1990s to keep animals' pedigree records along with phenotypic data such as sex, date of birth, birth weight, body weight, and milk yield.

Table 1 Number of sampled Mafriwal cattle of different age groups in 2021 and 2022

Group/Year	Number of Mafriwal cattle
Less than 1-year-old calves	
2021	30
2022	30
Yearlings	
2021	31
2022	30
Lactating cows	
2021	31
2022	30
Dry cows	
2021	30
2022	30

Screening of haemoparasites

Thin blood smears were prepared in a preliminary study to screen the presence of haemoparasites microscopically following a standard procedure by the Department of Veterinary Services Malaysia (Chandrawathani et al., 2019). The air-dried thin blood smears were fixed with methanol and dipped in eosinophilic and basophilic stainings of Diff-Quick solution following the procedures by the manufacturer (Labchem Sdn. Bhd, Malaysia) and screened for the presence of haemoparasites under 100x magnification of a light microscope (Olympus CX21, Japan).

DNA extraction and amplification

Deoxyribonucleic acid (DNA) of haemoparasites was extracted from each blood sample using the Geneaid GsyncTM DNA extraction kit following the manufacturer's protocol (Geneaid Biotech Ltd. New Taipei City, Taiwan) and stored at -20°C until further use. Polymerase chain reaction (PCR) amplification was conducted using MyCyclerTM thermocycler (Bio-Rad, USA) to amplify nuclear and ribosomal gene fragments of the haemoparasites. PCR amplifications were performed using published primers and thermocyclic profiles of bovine haemoparasites in Peninsular Malaysia (Ola-Fadunsin et al., 2017) as shown in Table 2. PCR was carried out in a 25 µL reaction comprising 12.5 µL of 1x GoTaq Green Master Mix (Promega Madison, USA), 1 µL of each primer, 1.5 µL of nuclease-free water, and 5 µL of DNA template.

Table 2 Primer sets for identification of haemoparasites

Parasite	Primer Sequence 5'-3'	Thermocycler profile	Length (bp)
Anaplasma marginale	Fw: CATCTCCCATGAGTCACGAAGTGGC Rv: GCTGAACAGGAATCTTGCTCCAAG	ID: 95°C/5mins D: 95°C/1mins A: 65°C/2mins E: 72°C/1min No of cycles: 40 FE: 72°C/10mins	761
Babesia bigemina	Fw: TACTGTGACGAGGACGGATC Rv: CCTCAAAAGCAGATTCGAGT	ID: 95°C/5mins D: 95°C/30secs A: 59°C/1min E: 72°C/1min No of cycles: 40 FE: 72°C/10mins	211
Theileria orientalis	Fw: CTTTGCCTAGGATACTTCCT Rv: ACGGCAAGTGGTGAGAACT	ID: 94°C/4mins D: 94°C/1min A: 63°C/1min E: 72°C/1min No of cycles: 40 FE: 72°C/7mins	776

Fw=Forward; Rv=Reverse; ID=Initial denaturation D=Denaturation; A=Annealing E=Extension; FE=Final extension

Gel electrophoresis and purification

The DNA products were electrophoresed on 2% agarose gel (Promega Madison, USA) at 100 V for 40 minutes. Tris-acetate-EDTA (TAE) buffer was stained with Midori Green Dye (Nippon Genetics, Europe) and visualization of DNA fragments was performed using the GelDocTM EZ Imager. Representative amplicons from each haemoparasite species were purified using Gel/PCR DNA Fragment Extraction Kit (Geneaid Biotech Ltd. New Taipei City, Taiwan) according to the manufacturer's protocol and submitted for Sanger sequencing (Apical Scientific Sdn. Bhd, Malaysia). Representative sequences of each haemoparasite were then compared with known haemoparasite gene fragments sequences curated by the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST).

Modified McMaster technique

The original McMaster technique to count gastrointestinal nematodes eggs and coccidia oocysts in ruminants' feces (Gordon et al., 1939) was modified following Abd-Majid et al. (2022). Three grams of feces were homogenized in 45 ml of saturated sodium chloride solution (S.G=1.2) then filtered through a fine-mesh sieve. The filtrate was pipetted to fill two chambers of the McMaster slide and left to stand for a few minutes. The nematode eggs and coccidia oocysts were observed and counted microscopically at 10x magnification (Olympus CX21, Japan) within the grids of both McMaster chambers.

Culture and identification of gastrointestinal parasite larvae from fecal

The culture was prepared following Hayyan et al. (2020). The identification and enumeration of infective stage larvae (L3) of gastrointestinal parasites were conducted by observing their head and sheath tail morphology according to the Ministry of Agriculture, Fisheries and Food of Great Britain (1986). In this study, the identification of gastrointestinal parasites was only conducted microscopically due to the lack of DNA extraction from detected parasites for molecular analysis.

Peripheral eosinophil counts

Carpentier's eosinophil counting solution was prepared by mixing 3 mL of 40% formaldehyde saturated with calcium carbonate, 2 mL of 2% aqueous solution of Eosin Y, and 95 mL of distilled water. A total of 10 µL of each blood sample was preserved in 90 µL of Carpentier's solution and kept at room temperature for diagnosis. The PEC was enumerated microscopically in a hemocytometer (LO-Laboroptik Ltd, Germany) in which one eosinophil cell was counted as 5.6 cells per µL of whole blood (Stear et al., 2002).

Packed cell volume

The PCV was measured by filling the blood into glass capillary tubes and sealed with Cristaseal (Hawskley and Sons Ltd, United Kingdom). The tubes were centrifuged at 220 rcf for 5 min and the volume was measured by a rotoreader (Hawskley and Sons Ltd, United Kingdom) (Basripuzi et al., 2018).

Statistical analysis

R software version 4.1.1 was used for statistical analysis. The percentage of samples detected with a particular parasite from the total number of observed samples was calculated to determine the prevalence of each parasite. The milk yield of lactation cows and body weight of yearlings were analyzed for descriptive statistics such as means, standard deviations, variance, minimum values, maximum values, and skewness. Shapiro-Wilk test was used to check the normality of the data. Correlations between the means of phenotypic markers namely PCV, PEC, EPG, and OPG as well as the average of Mafriwal cattle production such as milk yield and body weight in each year of sampling were analyzed using Spearman's rank correlation. Data on milk yield and body weight were only collected from lactation cows and below 2-year-old calves (i.e. both groups of calves), respectively.

Multiple linear regression models were used to study the interaction between PCV, PEC, EPG, OPG, and year of sampling which were fitted as explanatory variables while milk yield (Model 1) and body weight of calves (Model 2) were fitted as response variables in two separated models. The phenotypic markers for gastrointestinal parasites (i.e. EPG and OPG) were not included in Model 1 as nematode eggs and coccidia oocysts were absent in the feces of lactation cows. On the other hand, all phenotypic markers (i.e. PCV, PEC, EPG, and OPG) were included as explanatory variables in Model 2. The insignificant explanatory variables in each model were removed one by one according to the largest *P*-value. The best model that fits the data was selected based on the lowest Akaike Information Criterion (AIC) for each modeling analysis.

RESULTS

Detection of haemoparasites and gastrointestinal parasites

Genera of haemoparasites that were detected microscopically from the blood smears during preliminary screening were *Babesia* sp*., Anaplasma* sp*.,* and *Theileria* sp. Subsequently, the haemoparasite species were confirmed by PCR as *Anaplasma marginale, Babesia bigemina*, and *Theileria orientalis* [GenBank accession numbers: *A. marginale* (2021: OP965393-OP965396; 2022: OP965397-OP965400), *B. bigemina* (2021: OP965401-OP965404; 2022: OP965405-OP965408), and *T. orientalis* (2021: OP965409-OP965411; 2022: OP965412-OP965414)]. Most of the sequences were found to be 100% similar to sequences obtained from Cuba (MK809387.1) and Sri Lanka (LC438499.1). Meanwhile, *T. orientalis* shared a 100% similarity with other sequence references from India (MK874825.1, AB562562.1) and Thailand (KU886279.1). As expected, the microscopic examination of fecal samples and fecal cultures detected nematode eggs, coccidia oocysts as well as the L3 of *Haemonchus* sp. and *Trichostrongylus* sp. The coccidia oocysts were suspected to belong to *Eimeria* spp.

Prevalence of haemoparasites and gastrointestinal parasites

The prevalence of parasites among Mafriwal cattle in 2021 and 2022 (Figure 1) revealed that *B. bigemina* was the most predominant haemoparasite species with 80% and 90% prevalence respectively; followed by *Anaplasma marginale* (62%, 39%) and *T. orientalis* (25%, 29%). Meanwhile, the most prevalent gastrointestinal parasite throughout the study was *Eimeria* sp. with 34% and 17% prevalence in 2021 and 2022, respectively. This was followed by *Haemonchus* sp. (7%, 3%) while the least prevalent was *Trichostrongylus* sp. with 2% prevalence in both years.

Unlike the other age groups, yearlings have been infected by all detected haemoparasites and gastrointestinal parasites in this study (Figure 2). Most animals in all groups were infected with *B. bigemina* followed by *A. marginale*; except in the calves' group which was followed by infection of *Eimeria* sp. Nonetheless, no infection of gastrointestinal parasites has been detected in the lactation group for both years. There was also a consistent absence of infection caused by *Haemonchus* sp. and *Trichostrongylus* sp. among less than 1-year-old calves throughout this study. Furthermore, infections caused by gastrointestinal parasites such as *Haemonchus* sp., *Trichostrongylus* sp., and *Eimeria* sp. were considerably low in the dry cows' group in comparison to the infections caused by haemoparasites namely *B. bigemina*, *A. marginale* and *T. orientalis*.

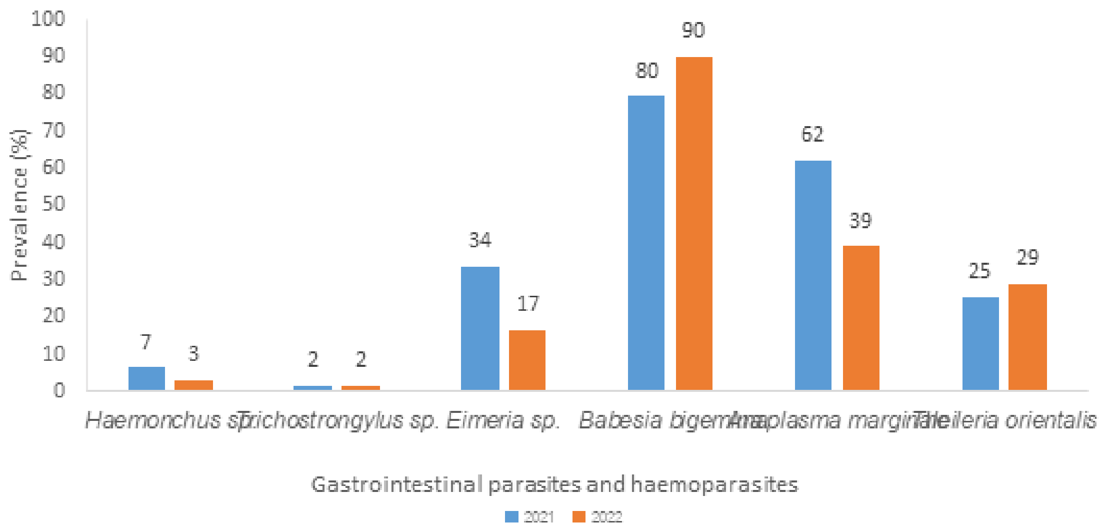

Figure 1 Prevalence of parasites among Mafriwal cattle in 2021 and 2022

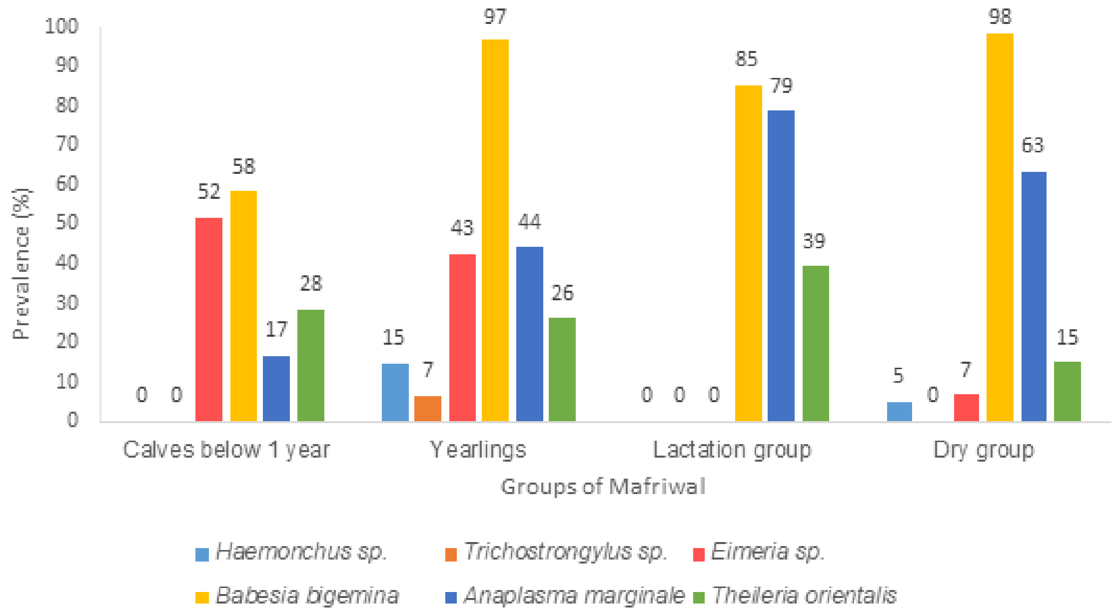

Figure 2 Prevalence of parasites among groups of Mafriwal cattle in 2021and 2022

Phenotypic traits of Mafriwal cattle

Table 3 shows the summary statistics of lactating cow's milk yield and body weight of less than 2-year-old calves. The mean milk yield decreased from 2021 (233.6 liters/kg±23.52) to 2022 (196.1 liters/kg±16.07) while the mean body weight of calves below 2-year-old increased from 2021 (130.1kg±6.62) to 2022 (146.1kg±8.74). The milk yield ranged from 60 to 480 liters/kg in 2021 and 90 to 390 liters/kg in 2022. The body weight of less than 2-year-old calves in 2021 and 2022 ranged from 58 to 257 kg and 51 to 259 kg, respectively. The milk yield of lactation cows in 2021 showed no significant difference from a normal distribution (P<0.05), on the contrary with milk yield in 2022 which showed a significant difference from a normal distribution (P<0.05). The body weight of less than 2-year-old calves showed significant differences from the normal distribution (P<0.01) in 2021 and 2022. Both milk yield and body weight traits have been estimated throughout the study.

Table 3 Summary statistics for milk yield of lactation cows and body weight of less than 2-year-old calves of Mafriwal breed in 2021 and 2022

Variable/Year	n	Mean±SE	SD	Minimum	Maximum	Prob (Norm)
Milk yield						
2021	31	233.6±23.5	124.5	60.0	480.0	0.0797
2022	30	196.1±16.1	85.0	90.0	390.0	0.0248
Body weight						
2021	61	130.1±6.6	50.4	58.0	257.0	0.0073
2022	60	146.1±8.7	62.4	51.0	259.0	0.0074

SE=standard error; SD=standard deviation; Prob (Norm)=probability that the distribution was significantly different from normal distribution by Shapiro- Wilk test (P<0.05)

The distributions of PCV (Figure 4) and PEC (Figure 5) among Mafriwal cattle are shown according to the age groups and the years of sampling. The range of PCV percentages was almost consistent among all groups in 2021 but decreased as the age group of cattle increased in 2022.

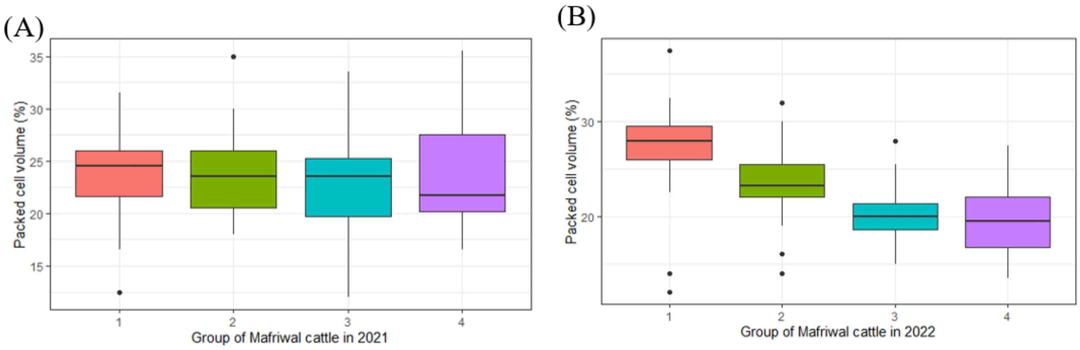

Figure 4 Packed cell volume in 2021 (A) and 2022 (B) of different age groups of Mafriwal cattle (1=less than 1-year-old calves, 2=yearlings, 3=lactation cows, 4=dry cows). The horizontal line gives the median values and the boxes indicate the 25th and 75th percentile.

The PEC range of both calves' groups was almost similar but lower than the older age groups in 2021 (Figure 5a). Nevertheless, the PEC of all age groups in 2021 was considerably higher than the PEC of all age groups in 2022 (Figure 5b).

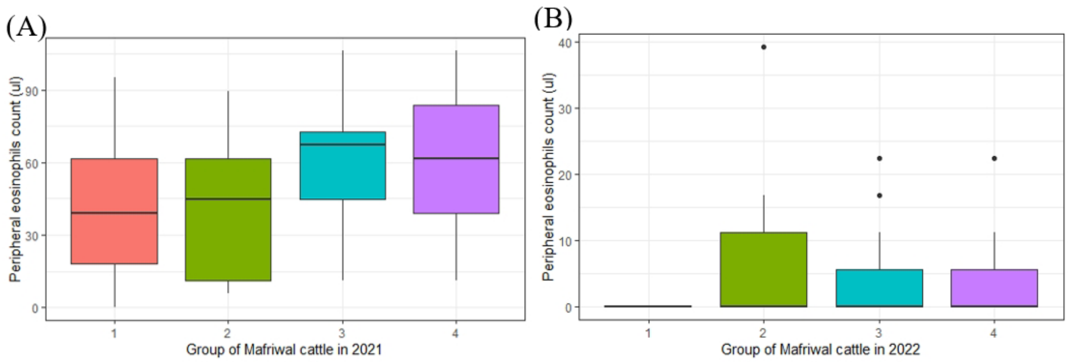

Figure 5 Peripheral eosinophils count in 2021 (A) and 2022 (B) of different age groups of Mafriwal cattle (1=less than 1-year-old calves, 2=yearlings, 3=lactation cows, 4=dry cows). The horizontal line gives the median values and the boxes indicate the 25th and 75th percentile.

Correlation analysis between Mafriwal cattle's production and phenotypic markers of parasitism

No significant correlations were observed between milk yield and phenotypic markers of parasitism, namely PEC and PCV, in the same year of sampling (Figure 6). The only significant correlation was observed between PEC and PCV (r=0.56; P<0.01) in different years of sampling thus not relevant to be taken into consideration for this study.

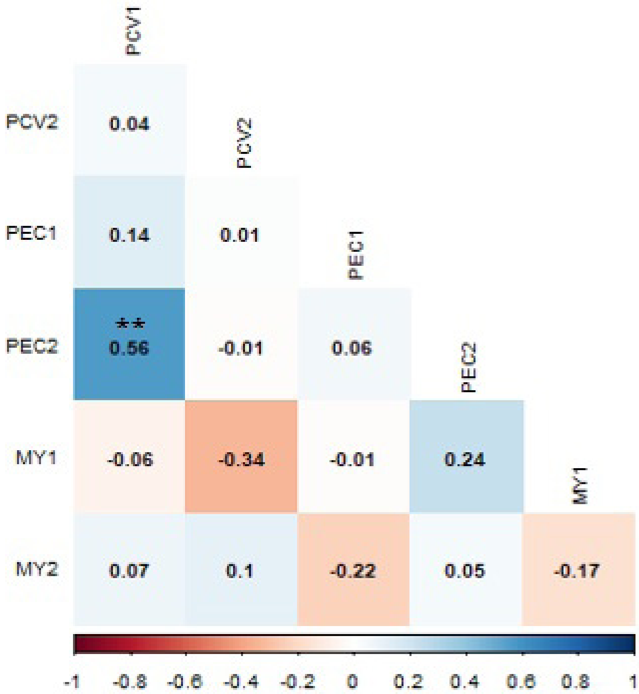

Figure 6 Spearman's rank correlations among packed cell volume, peripheral eosinophil counts, and milk yield in each year of sampling

*PCV=packed cell volume; PEC=peripheral eosinophil counts; MY=milk yield; 1=2021; 2=2022; *=P< 0.05; **=P<0.01, ***=P<0.001*

Significant correlations were observed between body weight and the other phenotypic markers of parasitism in 2022 (Figure 7). The body weight that represented meat production has a significant negative correlation with PCV (r=-0.4; P<0.01) and a positive correlation with PEC (r=0.39; P<0.01). In the same year, EPG showed a highly significant positive correlation with OPG (r=0.51; P<0.001).

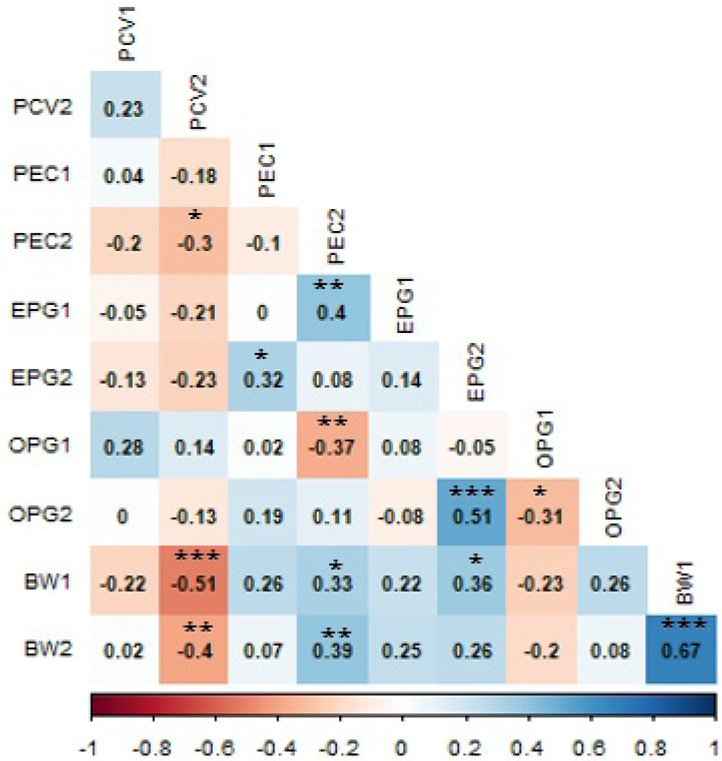

Figure 7 Spearman's rank correlations among packed cell volume, peripheral eosinophil counts, nematode eggs per gram (EPG) and coccidia oocyst per gram (OPG), and body weight in each year of sampling

*PCV=packed cell volume; PEC=peripheral eosinophil counts; EPG=nematode eggs per gram; OPG=coccidia oocysts per gram; BW=body weight; 1=2021; 2=2022; *=P< 0.05; **=P<0.01, ***=P<0.001.*

Multiple linear regression model of milk yield

In this model, PCV, PEC, and year of sampling were fitted as the explanatory variables while milk yield was fitted as the response variable. The year 2021 was fitted as the baseline for milk yield data to be compared with milk yield in 2022. There were three different models of milk yield analyzed in this study consisted of Model 1(a) (i.e. full model with all explanatory variables; AIC=3234.1), Model 1(b) (i.e. removal of PCV-PEC interaction from the explanatory variables; AIC=3232.1) and Model 1(c) (removal of PCV and the PCV-PEC interaction; AIC=3233.6). Therefore, Model 1(b) was selected as the best model that fit the milk yield data (Figure 6) based on the lowest AIC value. In the model, the milk yield of lactation cows decreased significantly ($P<0.001$) from 2021 to 2022 and an increase in PEC led to a significant reduction ($P<0.001$) in the milk yield. In the meantime, there was no significant effect of PCV observed on the milk yield ($P=0.061$).

Table 4 Model coefficients of association of milk yield with PCV, PEC, and year of sampling from lactation cows based on the lowest AIC value

Variable	Estimate	Standard Error	*P*-value	
Intercept	$6.178x10^{2}$	$6.434x10^{1}$	$< 2x10^{-16}$	***
PCV	$4.390x10^{-3}$	$2.341x10^{-3}$	0.061	
PEC	$-2.635x10^{-3}$	$4.807x10^{-4}$	$4.25x10^{-8}$	***
Year	$-3.030x10^{-1}$	$3.182x10^{-2}$	$< 2x10^{-16}$	***

*PCV=packed cell volume; PEC=peripheral eosinophil counts; EPG=nematode eggs per gram of feces ; OPG=coccidia oocysts per gram of feces; Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Multiple linear regression model of calves' body weight

In this model, PCV, PEC, EPG, OPG, and year of sampling were fitted as the explanatory variables, and body weight was fitted as the response variable. There were three different models of calves' body weight consisting of Model 2(a) (i.e., the full model with all explanatory variables; AIC=2954.6), Model 2(b) (i.e., removal of PCV-PEC interaction; AIC=2955.1) and Model 2(c) (removal of PEC and PCV-PEC interaction; AIC=2983.2).

Model 2(a) has been chosen as the best model that fits the body weight data (AIC=2954.6) as shown in Table 5. The body weight of less than 2-year-old calves increased significantly ($P<0.001$) from 2021 to 2022. PCV ($P<0.001$), EPG ($P<0.01$), and OPG ($P=0.001$) have negative significant relationships with body weight hence their increase caused a reduction in the body weight of the calves. An interaction between EPG and OPG has a significant positive ($P<0.001$) impact on body weight. There was no significant effect of PEC on body weight as shown in this model ($P=0.717$).

Table 5 Model coefficients of association of calves' body weight with PCV, PEC, nematode eggs (EPG), coccidia oocyst (OPG), and year of sampling (Model a).

Variable	Estimate	Standard Error	P- value	
Intercept	$5.192x10^{0}$	$5.799x10^{-2}$	$< 2x10^{-16}$	***
PCV	$-1.778x10^{-2}$	$2.288x10^{-3}$	$7.83x10^{-15}$	***
PEC	$-7.404x10^{-4}$	$2.042x10^{-3}$	0.717	
EPG	$-6.343x10^{-4}$	$2.456x10^{-4}$	0.009	**
OPG	$-1.516x10^{-4}$	$4.730x10^{-5}$	0.001	**
Year	$2.277x10^{-1}$	$2.442x10^{-2}$	$< 2x10^{-16}$	***
PCV-PEC	$1.342x10^{-4}$	$8.556x10^{-5}$	0.117	
EPG-OPG	$5.270x10^{-6}$	$7.838x10^{-7}$	$1.78x10^{-11}$	***

*PCV=packed cell volume; PEC=peripheral eosinophil counts; EPG=nematode eggs per gram of feces ; OPG=coccidia oocysts per gram of feces; Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

DISCUSSION

The objective of this study to determine the relationship between phenotypic markers (i.e. PCV, PEC, EPG, OPG) and Mafriwal cattle production (i.e. milk yield, body weight) has been achieved. The findings were crucial in providing information to control and prevent infections caused by gastrointestinal parasites and haemoparasites that could affect the production of Mafriwal cattle. According to Basripuzi et al. (2018), it is necessary to investigate the distributions of the measured variables to figure out the most effective way to represent the data and the most appropriate analysis to be conducted.

Haemoparasite species namely *A. marginale, B. bigemina* and *T. orientalis* were detected in all groups of Mafriwal cattle. In terms of prevalence, the most predominant haemoparasite species among the cattle in 2021 (80%) and 2022 (90%) was *B. bigemina*. This finding is supported by Adjou Uilenberg (2006) and Moumouni et al. (2015) in which *B. bigemina* is known as the most prevalent haemoparasite species and more widespread than other *Babesia* spp. In contrast, a study by Ola-Fadunsin et al. (2017) found *A. marginale* (72.6%) as the most prevalent bovine haemoparasite in Peninsular Malaysia followed by *T. orientalis* (49.8%) and *B. bigemina* (30.5%).

The least common haemoparasite species identified in this study was *T. orientalis* with a prevalence of 25% and 29% in 2021 and 2022, respectively. This finding differs from the previous study on cattle, sheep, and goats in Malaysia with 100% *Theileria* spp. detection rate (Kho et al., 2017). According to Gebrekidan et al. (2016), the variation in the prevalence of *T. orientalis* among different countries could be due to numerous factors such as the presence and abundance of ticks or other vectors, susceptibility of animal breeds, the presence of wildlife reservoirs and other ecological or climatic factors.

In the present study, the gastrointestinal parasite infections represented by EPG and OPG were detected in all groups of Mafriwal cattle except the lactation group in both years of sampling. Co-infection of gastrointestinal parasites was expected to occur among Mafriwal cattle in this study as it has commonly occurred among small ruminants in natural infections which also affect their productivity (Basripuzi et al., 2020). Nevertheless, species identification of gastrointestinal parasites could not be performed in this study due to the lack of DNA that could be extracted from the parasites, most possibly due to the low intensity of infections.

The most predominant gastrointestinal parasite detected in this study was coccidia which was suspected as *Eimeria* sp. with a prevalence of 34% in 2021 and 17% in 2022. Compared to nematodes, coccidia have a shorter pre-patent period thus their infections manifested earlier in the infected animals (Kimeli et al., 2020). The reduction of *Eimeria* sp prevalence was observed in all infected groups (i.e. less than 1-year-old calves, yearlings, and dry cows).) which could be due to an improvement in the farm hygiene management in 2022. Chlorine and disinfectant liquid for livestock have been used in the farm for cleaning and disinfecting purposes while the timetable for cleaning was also fixed to be conducted twice a day which presumably contributed to the reduction in *Eimeria* spp. infections among cattle in 2022. According to Andrew (2023), all calves should be kept in a clean and disinfectant environment with good ventilation to avoid fecal contamination that could lead to gastrointestinal parasite infections.

The age group with the highest infection rate of gastrointestinal parasites consisted of yearlings. These findings were inconsistent with Makau et al. (2017) and Lopez-Osorio et al. (2020) that reported less than 1-year-old calves were usually the most susceptible age group to *Eimeria* spp. infections. Nonetheless, the calves typically become asymptomatic hosts after recurrent infections as they age, serving as a source of infections to younger and more susceptible animals. Although previous studies found that immunoglobulins obtained from the colostrum were insufficient to prevent coccidiosis caused by *Eimeria* sp. (Faber et al., 2002; Olivares-Muñoz et al., 2022), the infection rate among Mafriwal calves of less than 1-year-old in this study was lesser than the yearlings.

The youngest group of calves on this farm were managed intensively before they were let to graze in the paddocks when they reached 1 year old. Since then, the calves were managed under the semi-intensive system with rotational grazing on the pasture hence exposed to gastrointestinal parasite infections from the contaminated pasture while still developing acquired immunity against infections. For lactation cows, the negative findings of gastrointestinal parasites may be due to the good feeding management on the farm. Basically, the ingredients used in the feeds for the cows include palm kernel cake, mixed wheat and corn grains, Signal and Guinea grasses as well as mineral blocks. Furthermore, lactation cows have been provided with special feed such as dairy cattle pallets and minerals premix to enhance milk production and maintain their health during the lactation period (Garamu, 2019).

Besides, the lactation cows were only released to the pasture after milking at 3 pm. Therefore, the duration of their exposure to the contaminated pasture was shorter than other groups. This suggests that the occurrence of gastrointestinal parasite infections on this farm was influenced by the environment and management practices. High tropical temperatures, humidity, and rainfall are known to favor the epidemiology of parasites by ensuring optimal development, survival, and availability of infective stages of gastrointestinal parasites on the pasture (Paul et al., 2020).

In this study, the average milk yield of lactation cows decreased from 233.6kg/month in 2021 to 196.1kg/month in 2022. According to Panandam et al. (2005), the Mafriwal breeding population has an average milk yield of 2,337 kg/lactation and a lactation length of 260 days. However, not all lactating cows sampled in 2022 had been previously sampled in 2021. The new lactating cows were included for sampling in 2022 which could be a contributing factor to the observed reduction of milk yield in this study. During the early stages of lactation, high-yielding dairy cows often experience a negative energy balance, where the energy required for body tissue maintenance and milk production exceeds their normal energy consumption. Increased milk productivity can lead to metabolic stress, resulting in a decrease in milk yield and reproductive performance. Thus, mobilizing body energy reserves during early lactation helps bridge the gap between energy intake and the loss of milk production (Reist et al., 2002; Schoder et al., 2006; Souissi et al., 2019).

The average body weight of less than 2-year-old calves increased from 2021 to 2022 from 130.1kg to 146.1kg. However, these findings were slightly lower than the findings by Mastura et al. (2019) in which the average body weight of Mafriwal calves at 540 days to 720 days of age ranged between 215.34 kg and 257.33 kg. The birth weight and average daily gain of calves are important factors that contribute to the subsequent growth and development of beef production or milk production of the dam. According to Mastura et al. (2019), feeding and management of the farm may give a significant effect on the body weight of the calves. Likewise, Mohamad (2018) also emphasized the importance of feeding and management in rearing cattle as these factors will affect their performance including body weight gain. In the present study, less than 1-year-old calves were well-fed under intensive care; dissimilar to the yearlings that could be challenged by stressful conditions when they were moved to the pasture which could subsequently affect their body weight gain.

PCV was consistent and within the normal range in all age groups of Mafriwal cattle in 2021 but showed a decreasing trend in 2022 as the age of cattle increased with a marked reduction in dry cows (Figure 4). These findings could be related to the high infection rate among dry cows based on the slight decrease of gastrointestinal parasite and haemoparasite prevalence from 2021 to 2022 in comparison to the other age groups (Figure 2, Figure 3) which presumably led to anemia. According to Hayyan et al. (2020), mild parasite infections may not cause substantial anemia as shown in animals with heavier infections.

PEC showed an increasing trend according to age groups of Mafriwal cattle in 2021 but considerably low in 2022 for all age groups (Figure 5). In 2022, PEC could not be detected microscopically in less than 1-year-old calves but the highest PEC value was observed in yearlings. Presumably, eosinophilia occurred due to the high infection rate of gastrointestinal parasites in yearlings compared to the other age groups. This finding was consistent with the immune response of eosinophilia among induced animals that plays an important role to fight against gastrointestinal parasite infections (Hayyan et al., 2020; Jenvey et al., 2020; Abd-Majid et al., 2022).

There was no significant correlation observed between milk yield and phenotypic markers of parasite infections within the same year of sampling (Figure 6), but a significant (*P*<0.001) negative relationship was observed between PEC and milk yield (Table 4). Transition to the lactation period is challenging for dairy cows hence this will cause a decrease in immune functions (Gomes et al., 2019). Furthermore, the reduction of average milk yield from 2021 to 2022 could be due to many factors such as lactation cow age, heat stress, diseases, types of feed, and farm management. In this study, cows more than 10 years old were still used on the farm for milking. The length of service period in the same lactation also influences dairy animals' milk production capacity because an optimal service period is required to provide the animals with the necessary rest after calving (Singh et al., 2020). A previous study on the same farm has shown that the stage of lactation and parity influence the presence of sub-clinical mastitis in Mafriwal cattle which leads to a reduction in milk yield (Tan et al., 2014). Moreover, it has been suggested that the somatic cell count increases with parity and stage of lactation due to an increased prevalence of infection and glandular damage from previous infection (Syridion et al., 2012; Tan et al., 2014).

On the other hand, the body weight of Mafriwal calves in 2022 has a significant negative relationship with PCV in both correlation (r=-0.4; *P*<0.01) (Figure 7) and modeling (*P*<0.001) analyses (Table 5). A previous study conducted in South Africa demonstrated that indigenous Nguni and crossbred cattle exhibited their lowest PCV values during the hot-wet season and their highest values during the cool-dry season (Mapiye et al., 2009), which is consistent with this study. Additionally, even though body weight has a significant positive correlation with PEC (r=0.39; *P*<0.01) in 2022 (Figure 7), their relationship with each other throughout the study was not significant in the modeling analysis (Table 5).

The year of sampling was associated with the increase in body weight of Mafriwal calves (*P*<0.001) (Table 5). This suggested that the body weight of calves increased as they aged (Basri, 2019). Furthermore, gastrointestinal parasite infections as represented by EPG and OPG have a significant negative relationship with body weight (*P*<0.001). These findings were consistent with the previous studies that the body weight of ruminants could be negatively affected by gastrointestinal parasite infections (Johansson, 2017; Basri, 2019).

CONCLUSIONS

In conclusion, the Mafriwal cattle were infected with multiple species of gastrointestinal parasites and haemoparasites. This study also shows that milk yield and body weight of Mafriwal cattle were significantly affected by parasitism based on their relationships with the phenotypic markers of parasite infections. Therefore, prevention and treatment measures should be implemented on the farm to control the parasite infections and hence improve the cattle's production.

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AUTHOR CONTRIBUTIONS

Nur-Sabrina A.M.; Investigation, methodology, formal analysis, manuscript preparation, editing, and finalization
Muhamad-Ali H.A.M.; Investigation, methodology
Nur-Amalina N.; Investigation, methodology
Wan-Ladiana W.A.; Methodology
Hasimah H.; Methodology
C.W. Salma Wan, K.L.; Methodology
Basripuzi N. Hayyan; Conceptualization and design of the experiment, investigation, formal analysis, supervision, editing, and finalization.

CONFLICT OF INTEREST

We have no conflict of interest.

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