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RESEARCH ARTICLE

Parasitic coinfections among selected smallholder goat flocks in Malaysia

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ABSTRACT

This paper describes the occurrence of multiple parasitic infection with special reference to emerging haemotropic *Mycoplasma ovis*. A cross-sectional survey of four selected goat flocks was conducted to collect samples and management information. Blood samples were processed using microhaematocrit centrifugation to determine the packed cell volume (PCV). Detection and morphological identification of blood protozoa and haemotropic *Mycoplasma ovis* from Giemsa-stained smears were done microscopically. *M. ovis* infection was classified mild (1-29% infected cells), moderate (30-59% infected cells), or severe (above 60% infected cells). Faecal floatation and McMaster faecal egg count were used to detect and classify strongyle infections as negative (no eggs/oocysts), light (< 500 epg), Moderate (500 – 1000 epg), or severe (>1000 epg) and coccidia infection as light (<1800 opg), moderate (1800 – 6000 opg), or severe (>6000 opg). There were 149 goats with blood protozoa (57.98%; 95% CI: 51.87 – 63.85) and 204 goats with GI parasites (79.38%; 95% CI: 74.02 - 83.87) involved in single (15.8%; 95% CI: 11.7 – 21.0) or multiple (84.2%; 95% CI: 79.0 – 88.3) infections. The risk of Strongyles increases by 2.49 (95% CI: 1.24 – 4.99) in females versus males and 6.79 (95% CI: 3.25 – 14.18, p =0.000) in adults versus young. The risk of *Eimeria* species increases by 7.32 (95% CI: 3.45 – 15.50, p =0.000) in adults versus young, while *M. ovis* coinfection risk increases by 4.51 (95% CI: 1.40 – 14.50, p =0.000) in female versus males. Thin animals had a significantly higher ($p<0.05$) mean burden of Strongyle (1370.37 ± 345.49) and *Eimeria* (1594.12 ± 695.26) than the moderate and fat goats. The PCV was negatively associated with mean faecal egg count (FEC) ($p<0.05$) such that a lower PCV was recorded in animals with a higher Strongyle epg output. A severe burden of *M. ovis* was accompanied by an increased nematode FEC and decreased haematocrit ($p<0.05$). Coinfections of Strongyles, or *Eimeria* species involving *M. ovis* were associated with a higher parasitaemia compared with single infections ($p<0.05$). This study highlights the importance of *M. ovis* and Strongyle or *Eimeria* species coinfections among goat flocks and provides valuable data for developing and implementing an integrated herd health management program for parasite control among low-input smallholder flocks.

Keywords: Blood protozoa; coinfection; gastrointestinal parasites; goats; parasite burden.

INTRODUCTION

Parasitism is a significant factor limiting the productivity of small ruminants worldwide (Urquhart *et al*., 1996; Bhat *et al*., 2012). In Malaysia, helminthosis, coccidiosis, and haemoparasitism are common causes of production losses due to reduction in weight gain, growth retardation, decrease in productivity and mortality amongst small ruminants (Dorny *et al*., 1995; Sani *et al*., 2004; Chandrawathani *et al*., 2009; Zainalabidin *et al*., 2015; Tan *et al*., 2017; Khor *et al*., 2018; Jesse *et al*., 2019; Paul *et al*., 2020b). Strongyle nematodes, *Eimeria*, *Moniezia*, and *Trichuris* species are endemic problems among small ruminants in Malaysia due to grazing practice in semi-intensive management and the presence of a favourable humid climate, which ensures succession of environmental stages of the parasites (Ikeme *et al*., 1987; Chandrawathani *et al*., 2009; Paul *et al*., 2020b). Haemoprotozoa of the genus *Theileria*, *Babesia, Anaplasma*, and *Ehrlichia* (Bilgic *et al*., 2017) and Haemoplasma species / haemotropic *Mycoplasma ovis*, formerly *Eperythrozoon ovis* (Neimark *et al*., 2004) are frequently associated with production losses among small ruminants in Malaysia (Paul *et al*., 2020a, 2021) due to anaemia, slow weight gain, poor milk production, mortality, and economic burden due to the cost of treatment and control of parasites and vectors (Rohaya *et al*., 2017; Azima *et al*., 2020).

The coinfection of red blood cells with various species of blood protozoa is a common phenomenon in small ruminants worldwide (Kocan *et al*., 2004; Glaji *et al*., 2014; Ait Lbacha *et al*., 2015; Rohaya *et al*., 2017). Coinfection of haemotropic *M. ovis* and piroplasms is known to increase the severity of anaemia in sheep (Neimark & Kocan, 1997). Haemotropic *M. ovis* was also associated with piroplasmid infection in sheep in Turkey (Aktas & Ozubek, 2017). Multispecies coinfection with haemoprotozoa and GI parasites frequently occurs in grazing animals (Chandrawathani *et al*., 2009; Ait Lbacha *et al*., 2015). Moreover, the coinfections of *Haemonchus contortus* and haemotropic *M. ovis* are known to increase the severity of anaemia and pathology of clinical disease in sheep and goats presented with chronic diarrhoea and wasting in Malaysia (Jesse *et al*., 2013, 2015, 2017).

Although there is significant clinical evidence linking the increased disease burden in concurrent infections involving haemotropic *M. ovis* and *H. contortus* in both sheep and goats in Malaysia, the field situation remains unexplained. Based on the available clinical evidence, we hypothesised that coinfections involving *M. ovis* are associated with an increased burden of parasitaemia in goats. This paper describes the occurrence of multiple parasitic infection with reference to the severity of emerging haemotropic *M. ovis* in goats observed under field conditions.

MATERIALS AND METHODS

Ethics approval

The study design, sampling, data collection and laboratory protocols of this study were approved by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (UPM/IACUC/AUP-R037/2023). The Department of Veterinary Services (DVS) Negeri Sembilan approved the selection of farms and sampling. The farmers also consented to participate in the study before collecting samples and data.

Study area

Negeri Sembilan is located on the southwest coast of Peninsular Malaysia (2.8 2.7258°N, 101.9424°E). There are approximately 16,094 individual semi-intensively managed goats kept by mostly smallholder farmers in the state (DVS, 2022). A total of 257 individual goats from four smallholder flocks: Farm A (91, 35.4%), Farm B (55, 21.4%), Farm C (75, 29.2), and Farm D (36, 14.0%) were included in the study. All the farms were individual smallholders owned by the local farmers that integrate with plantation agriculture. The farmers practised a low input semi-intensive production system that grazed animals on small lands at the fringes of forests and undergrowth in plantations. The system also provides limited housing facilities and feed supplementation but there is no organised herd health program for disease control.

Study design

The sample size for this study was calculated based on a previous cross-sectional survey on GI parasites and haemotropic *Mycoplasma ovis* among small ruminants in Negeri Sembilan, Malaysia (Paul *et al*., 2021, 2020b) based on 88% expected prevalence (p) (Mohammed *et al*., 2016), 5% absolute precision (d), and $Z_{1-\alpha/2}$ (standard normal variate at 5% type 1 error, p<0.05) (Charan & Biswas, 2013). Thus, $n = Z_{1-\alpha/2}{}^2$. $p (1 - p)/d^2 = 162$, but 257 samples were collected to increase accuracy and minimise sampling error. The individual goats were randomly selected within each flock regardless of age, gender, or physiological status. The selected goats were examined once to determine the body condition score (BCS) by palpation in the lumbar and sternum region and graded as emaciated (1), thin (2), average (3), fat (4), or obese (5) according to Jackson and Cockcroft (2007). Blood (5 mL) was collected in EDTA vacutainer tubes by jugular puncture and 5g of faeces was collected in plastic containers by rectal evacuation using a gloved finger. The demographic information of goats and farm management data were also collected on a sampling form.

Laboratory examination of samples

Determination of PCV

The blood samples were processed to determine the packed cell volume (PCV) by microhaematocrit centrifugation technique, and the height of haematocrit was read on a micro haematocrit reader and recorded as the value of PCV (%) (Grindem, 2011).

Microscopic examination of blood smear

Thin and thick blood smears were prepared and routinely stained in 10% Giemsa solution (pH 7.2) for 30 and 45 minutes, respectively for the detection and morphological identification of blood protozoa and haemotropic *M. ovis* under oil immersion (100×) objective of a compound microscope (Paul *et al*., 2021). The per cent infection of *M. ovis* was calculated as the number of *M. ovis* infected cells/800RBC ×100, and the severity was reported as mild (1-29% infected cells), moderate (30-59% infected cells), or severe (above 60% infected cells) (Gulland *et al*., 1987a).

Faecal examination

Sodium chloride floatation was used to detect parasite eggs or oocysts in faces, and the modified McMaster faecal egg count with a sensitivity of 50 eggs or /oocysts per gram faeces was used for quantitative faecal egg or oocyst count (FEC/FOC) (Urquhart *et al*., 1996). The FEC was classified as negative (no eggs), light (< 500 epg), Moderate (500 – 1000 epg), or severe (>1000 epg) while FOC results were classified as negative (no oocysts), light (<1800 opg), moderate (1800 – 6000 opg), or severe (>6000 opg) (Lambertz *et al*., 2018).

Statistical analysis

The proportions of different categories of endoparasites and their respective 95% CI was calculated using the EpiTools® statistical calculators based on the binomial exact method and presented descriptively (Sergeant, 2018). Other statistical analyses were performed using the Statistical Package for Social Sciences Software (SPSS) version 25.0. The association between prevalence of different types of endoparasites and explanatory variables (gender, age, and body condition) was determined using the Chi-square test statistic by Fishers exact method. A binary logistic regression model ($Y = B_0 + B_1X_1 + \ldots + B_KX_K$) was used to predict the relationships between the occurrence of major types of endoparasites and explanatory variables of goats. The one-way analysis of variance (ANOVA) and Tukey's HSD test were used to determine the association between parasite burden (epg and opg) and haematocrit level at 95% CI and 5% significance level. Finally, the Independent Samples *t*-Test was computed at a 95% CI and 5% significance level and assuming equal variances (p>0.05 Levene's test for equality of variance) to determine the association between single or mixed haemotropic *Mycoplasma ovis* infection and the level of parasitaemia in the goats.

RESULTS

Characteristic of the selected goat flocks

The distribution of goats according to their breed, gender, age, farm location, production purpose and type of management system is presented in Table 1. The main breeds of the goats were Boer (224, 87.2%) and Saanen (33, 12.8%) reared for meat and milk production. There were more female (189, 73.5%) than male (68, 26.5%), and more adult (203, 79.0%) than young (54, 21.0%) goats in the population.

Table 1. General characteristic of the selected goat flocks in Negeri Sembilan

Variables	Categories	Frequency	Percentage (%)
Farms	Farm A	91	35.4
	Farm B	55	21.4
	Farm C	75	29.2
	Farm D	36	14.0
Breed	Boer	224	87.2
	Saanen	33	12.8
Production purpose	Meat	224	87.2
	Milk	33	12.8
Gender	Female	189	73.5
	Male	68	26.5
Age	Adult	203	79.0
	Young	54	21.0
Management system	Semi-intensive*	257	100
Total	**All goats**	**257**	**100**

*Reared on pasture with limited supplemented feeding and housing

Categories of parasitic infection

The variety of endoparasites identified among smallholder goats is presented in Table 2. There were total counts of 149 (57.98%; 95% CI: 51.87 – 63.85) goats with blood protozoa and 204 (79.38%; 95% CI: 74.02 – 83.87) goats with GI parasites in single or multiple infections. For mixed infections, there were 77 (30%;95% CI = 24.7 – 35.8) goats with *M. ovis* + Mixed GIP, 72 (28%; 22.9 – 33.8) goats with Mixed GIP, 25 (9.7%; 95% CI = 6.7 – 14.0) goats with *M. ovis* + Strongyle, 11 (4.3%; 95% CI = 2.4 – 7.5) goats with *Anaplasma* + Strongyle, 6 (2.3%; 95% CI = 1.1 – 5.0) goats with *M. ovis* + *Anaplasma*, and 6 (2.3%; 95% CI = 1.1 – 5.0) goats with *Anaplasm*a + Mixed GIP. For single infections, there were 19 (7.4%; 95% CI = 4.8 – 11.3) goats with haemotropic *Mycoplasma ovis*, 8 (3.1%; 95% CI = 1.6 – 6.0) goats with Strongyle eggs, 5 (1.9%; 95% CI = 0.8 – 4.5) goats with *Coccidia* oocysts, 3 (1.2%; 95% CI = 0.4 – 3.4) goats with *Babesia* spp., and 2 (0.8%; 95% CI = 0.2 – 2.8) goats with *Anaplasma*.

Association between coinfection and intrinsic factors of sheep and goats

Univariable analysis by Chi-square test revealed that the detection rate of the major types of parasites were associated with the gender and age of the goats (Table 3). There was an association between the incidence of Strongyle eggs with the gender (OR = 5.073; 95% CI: 2.79 - 9.24; $P < 0.001$) and age (OR = 10.096; 95% CI: 5.13 - 19.89; $P < 0.001$) of the goats. The incidence of *Eimeria* oocysts was also associated with the gender (OR = 3.127; 95% CI: 1.76 - 5.56; $P < 0.001$) and age (OR = 8.63; 95% CI: 4.38 - 17.02; $P < 0.001$) of the goats. Similarly, the incidence of haemotropic *M. ovis* coinfection with other parasites was associated with gender (OR = 7.156; 95% CI: 2.72 - 18.82; $P < 0.001$) and age (OR = 5.928; 95% CI: 2.22 - 15.83; $P < 0.001$) of the goats.

For Strongyle species, the coefficients of Wald ratios (Chi-square) for the gender (female, $X^2 = 6.565$, df = 1, n = 257, $P = 0.010$) and the age (adult, $X^2 = 26.026$, df = 1, n = 257, $P < 0.001$) were significantly different from those in the even odds (null) model and the risk of GI Strongyles increases by 2.49 (95% CI: 1.24 - 4.99) in females versus males and increases by 6.79 (95% CI: 3.25 - 14.18) in adults versus young. For *Eimeria* species, the coefficient of Wald ratio for age (adult, $X^2 = 27.013$, df = 1, n = 257, $P < 0.001$) was significantly different from those in the even odds model and the risk of *Eimeria* species infection increased by 7.32 (95% CI: 3.45 - 15.50) in adults versus young. For *M. ovis* coinfection, the coefficient of the Wald ratio for gender (female, $X^2 = 6.395$, df = 1, n = 257, $P = 0.011$) was significantly different from those in the even odds model, and the risk of *M. ovis* coinfection increases by 4.51 (95% CI: 1.40 - 14.50) in female versus males. We conclude that gender and age were significant predictors of the major types of endoparasite infections detected among the goats (Table 4).

Severity of endoparasites in goats

Intensity of GIP infection versus BCS of goats

Based on the overall FEC, the goats were moderately infected with Strongyle eggs (mean epg = 716.30 ± 69.69) and lightly infected with *Eimeria* species (mean opg = 1460.70 ± 353.51). Although there was no association between the occurrence of different endoparasites and the body condition score of the goats, the mean burden of Strongyle eggs was significantly different among the goats, F (2,181)

Table 2. Proportions of different types of endoparasites detected among smallholder goats (n=257, CI = confidence interval, L = lower boundary, U = upper boundary)

Parasitic infection	No. of goats positive	Prevalence (%)	95% CI	
			L	U
Blood protozoa (total positive)	149	58.0	51.9	63.9
GI parasites (total positive)	204	79.4	74.0	83.9
Single infections				
Total number	37	15.8	11.7	21.0
Single M. ovis	19	7.4	4.8	11.3
Single Strongyle	8	3.1	1.6	6.0
Single *Coccidia*	5	1.9	0.8	4.5
Babesia spp.	3	1.2	0.4	3.4
Anaplasma spp.	2	0.8	0.2	2.8
Coinfections				
Total number	197	84.2	79.0	88.3
M. ovis + Mixed GIP	77	30	24.7	35.8
Mixed GIP	72	28	22.9	33.8
M. ovis + Strongyle	25	9.7	6.7	14.0
Anaplasma + Strongyle	11	4.3	2.4	7.5
M. ovis + *Anaplasma*	6	2.3	1.1	5.0
*Anaplasm*a + Mixed GIP	6	2.3	1.1	5.0

CI = confidence interval, L = lower boundary, U = upper boundary

Table 3. Contingency table and odds ratios for the association of intrinsic factors with infection status (CI = confidence interval, X^2 = Chi-square)

	Variables	Examined	Positive	Prevalence	Odds Ratio (95%CI)	P value (X^2)
Strongyle eggs						
Gender	Male	68	31	45.6	1.00	
	Female	189	153	81	5.073 (2.79 – 9.24)	0.000*
Age	Young	54	17	31.5	1.00	
	Adult	203	167	82.3	10.096 (5.13 – 19.89)	0.000*
BCS	Fat	22	17	77.3	1.00	
	Thin	34	27	79.4	1.134 (0.31 – 4.16)	0.849
	Average	201	140	69.7	0.675 (0.24 – 1.91)	0.460
***Eimeria* spp. oocyst**						
Gender	Male	60	32	47.1	1.00	
	Female	189	139	73.5	3.127 (1.76 – 5.56)	0.000*
Age	Young	54	15	27.8	1.00	
	Adult	203	156	76.8	8.63 (4.38 – 17.02)	0.000*
BCS	Fat	22	15	68.2	1.00	
	Thin	34	24	70.6	1.12 (0.35 – 3.58)	0.814
	Average	201	132	65.7	0.893 (0.35 – 2.29)	0.096
***M. ovis* coinfections**						
Gender	Male	34	20	58.8	1.00	
	Female	101	92	91.1	7.156 (2.72 – 18.82)	0.000*
Age	Young	26	15	57.7	1.00	
	Adult	109	97	89	5.928 (2.22 – 15.83)	0.000*
BCS	Fat	11	9	81.8	1.00	
	Thin	15	14	93.3	3.111 (0.25 – 39.54)	0.382
	Average	109	89	81.7	0.989	0.989

Table 4. Binary logistic regression for parasitic infection of goats (B = regression coefficient, S.E = standard error, Wald = Wald's Chi Square Test Statistic, df = Degree of Freedom, Sig. = p values for Wald's Chi square test, AOR = Adjusted Odds Ratios, CI = Confidence Interval, X^2 = Chi-square)

Variables	B	S.E.	Wald	df	Sig.	AOR (95% CI)
Strongyle*s*						
Gender (female)	0.912	0.356	6.565	1	0.010*	2.49 (1.24 – 4.99)
Age (adult)	1.916	0.375	26.026	1	0.000*	6.79 (3.25 – 14.18)
Constant	-1.113	0.331	11.28	1	0.001	0.329
Eimeria						
Gender (female)	0.344	0.357	0.927	1	0.336	1.41 (0.70 – 2.84)
Age (adult)	1.990	0.383	27.013	1	0.000*	7.32 (3.45 – 15.50)
Constant	-1.076	0.332	10.529	1	0.001	0.341
***M. ovis* coinfection**						
Gender (female)	1.507	0.596	6.395	1	0.011*	4.51 (1.40 – 14.50)
Age (adult)	0.875	0.618	2.005	1	0.157	2.40 (0.71 – 8.06)
Constant	0.15	0.422	0.001	1	0.972	1.015

The regression equation for predicting infection is expressed as $L_i = B_0 + B_1X_1 + B_2X_2$, where X_1 and X_2 represent the gender and age of the goats, respectively.

= 3.781, P = 0.025. The FEC was higher among thin (1370.37 ± 345.49 epg) than the moderate (613.21 ± 56.75 epg) and fat (526.47 ± 112.23 epg) goats. Similarly, the mean burden of *Eimeria* oocysts was significantly different among the goats, F (2,168) = 4.204, P = 0.039. The opg was higher (P<0.05) in thin (1594.12 ± 695.26 opg) and moderate (1543.28 ± 436.0 opg) than in fat (500 ± 180.55 opg) goats (Table 5).

Intensity of GIP *infection versus haematocrit of goats*

The average PCV of goats with different levels of Strongyle egg burden are shown in Figure 1. There was a significantly lower (P < 0.05) PCV value among goats with a severe nematode burden (24.6 ± 5.0; 95% CI = 22.9 - 26.3; P < 0.05) compared with the light burden (28.1 ± 5.4; 95% CI = 27.1 - 29.0) and uninfected goats (29.8 ± 5.5; 95% CI = 28.5 - 31.1; P < 0.01). Conversely, there were no

Table 5. Proportion of goats with different severity of GI parasite and the relationships between their BCS and the mean FEC or FOC

Strongyles	No. (%) of goats				
	Negative	Light	Moderate	Severe	FEC (Mean ± SE)
Thin BCS	7 (2.7)	9 (3.5)	11(4.3)	7 (2.7)	1370.37±345.49[a]
Moderate BCS	61 (23.7)	81(31.5)	28 (10.9)	31(12.1)	613.21±56.75[b]
Fat BCS	5 (1.9)	9 (3.5)	5 (1.9)	3 (1.2)	526.47±112.23[b]
Total	73 (28.4)	99 (38.5)	44 (17.1)	41 (16.0)	716.30± 69.69
Eimeria species	No. (%) of goats				
	Negative	Light	Moderate	Severe	FOC (Mean ± SE)
Thin BCS	10 (3.9)	17 (6.6)	5 (1.9)	2 (0.8)	1594.12±695.26[a]
Moderate BCS	69 (26.8)	99 (38.5)	25 (9.7)	8 (3.1)	1543.28±436.0[a]
Fat BCS	7 (2.7)	12 (4.7)	2 (0.8)	1 (0.4)	500±180.55[b]
Total	86 (33.8)	128 (49.8)	32 (12.5)	11 (4.3)	1460.70±353.51

Means in the same column represented by different superscripts ([a, b]) were significantly different (p<0.05)

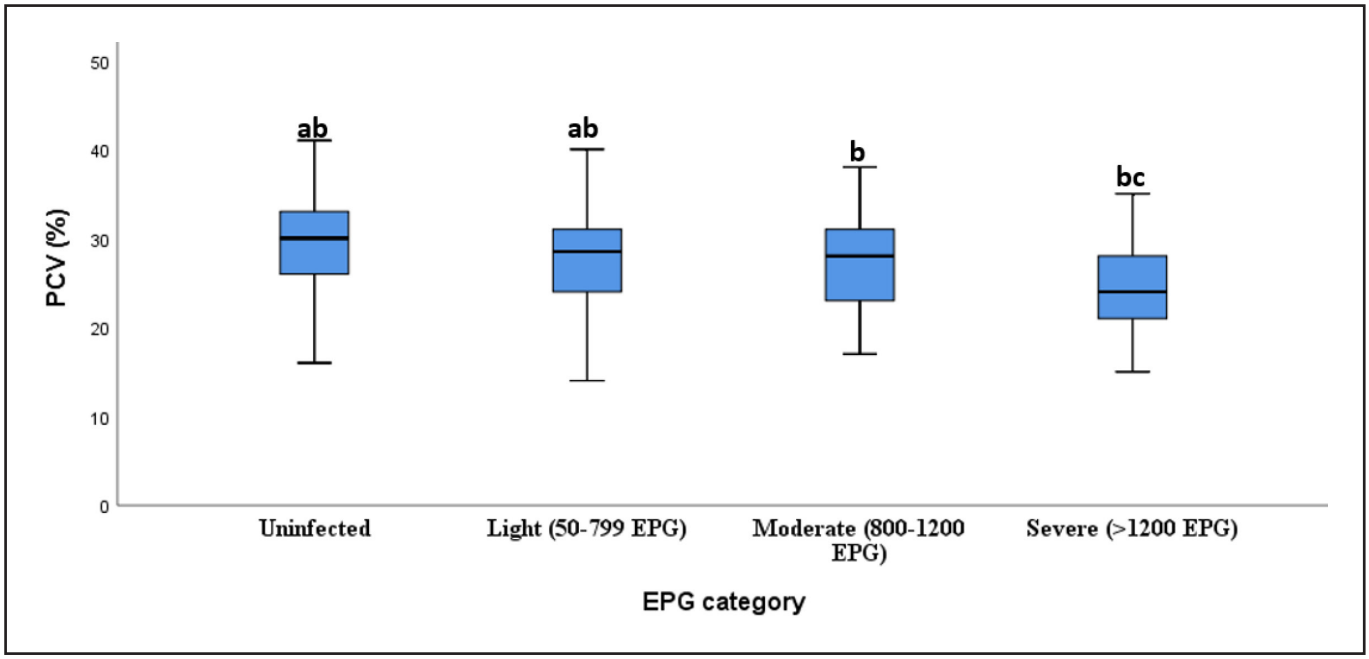

Figure 1. Relationship between Strongyle burden and PCV of goats.

significant differences in the PCV values of goats with a moderate burden (26.9 ± 5.3; 95% CI = 24.5 - 29.3) and the rest of the flock ($P > 0.05$).

Intensity of M. ovis infection versus haematocrit of goats

The average haematocrit values of smallholder goats with different levels of haemoplasma burden are shown in Figure 2. The average haematocrit was significantly lower ($P < 0.05$) among goats with a severe haemoplasma burden (21.4 ± 6.7; 95% CI = 16.6 - 26.2) compared with light (27.9 ± 5.3; 95% CI = 26.9 – 29.0; $P < 0.05$), moderate (28.9 ± 6.8; 95% CI = 26.3 - 31.5; $P < 0.01$), and uninfected (28.4 ± 5.0; 95% CI = 27.5 - 29.25; $P < 0.01$).

Intensity of Strongyles versus M. ovis severity in goats

The mean eggs per gram output (EPG) of goats with different levels of *M. ovis* infection is shown in Figure 3. There was a significantly lower ($P < 0.05$) mean EPG among goats with a severe *M. ovis* burden (1545.00 ± 412.95; 95% CI = 610.85 – 2479.15) compared with light (513.97 ± 51.20; 95% CI = 411.77 – 6.6.77; $P < 0.001$) and moderate (419.57 ± 90.34; 95% CI = 232.01 – 607.12; $P < 0.001$).

Magnitude of *M. ovis* parasitaemia in single and coinfections

There were significant differences {t (133) = -2.060, $P = 0.041$} in the mean parasitaemia, which was lower for single infection (17.1±4.5) than coinfection (24.9±5.2). The magnitude of the differences in the mean (-7.815, 95% CI: -15.32 to - 0.31) parasitaemia suggest that Strongyle coinfection increased the burden of *M. ovis* infection in goats.

DISCUSSION

In Malaysia, most of the small ruminant flocks are held by individual smallholder farmers that practised a low input pasture-dependent semi-intensive production system without a herd health program for parasite control. This practice was associated with an increased risk of parasitic infections among small ruminants due to direct

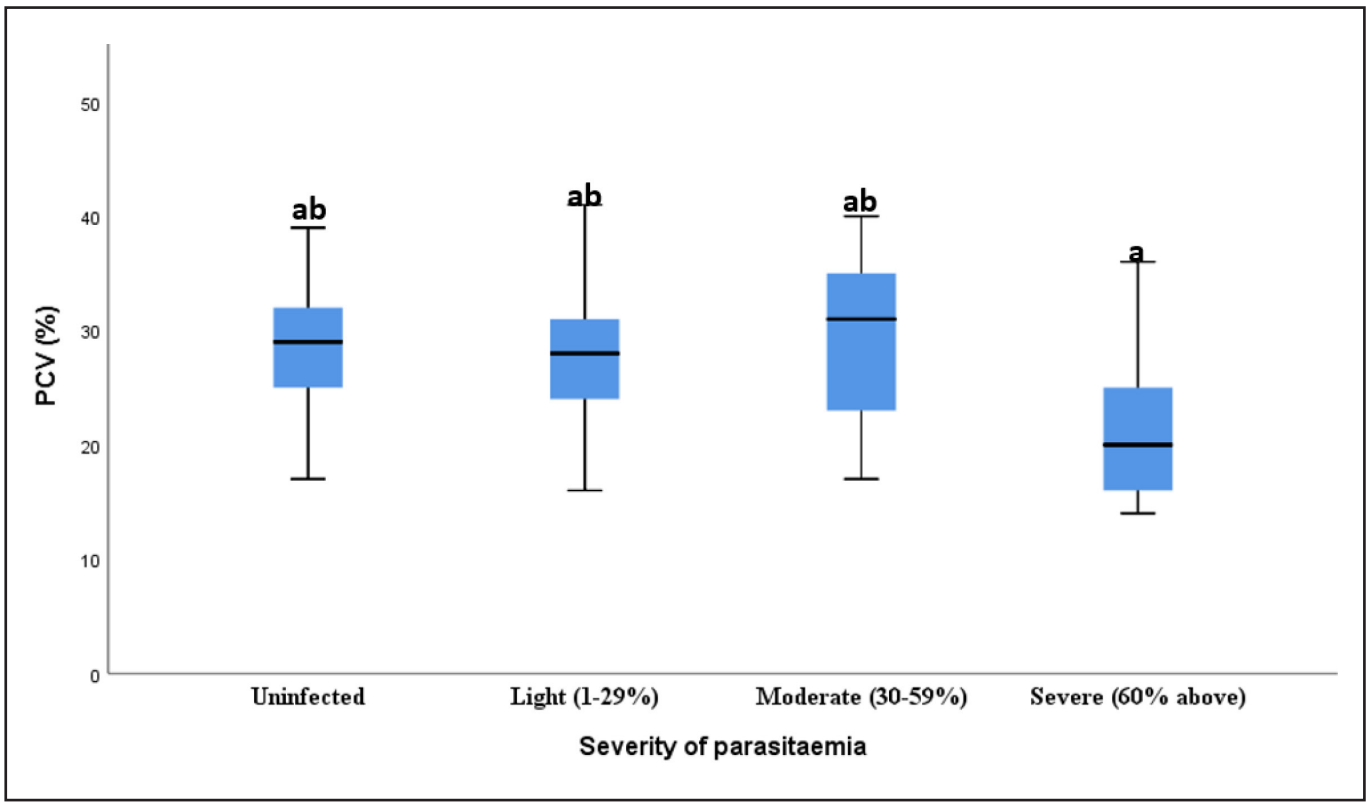

Figure 2. Relationship between *M. ovis* burden and PCV of goats.

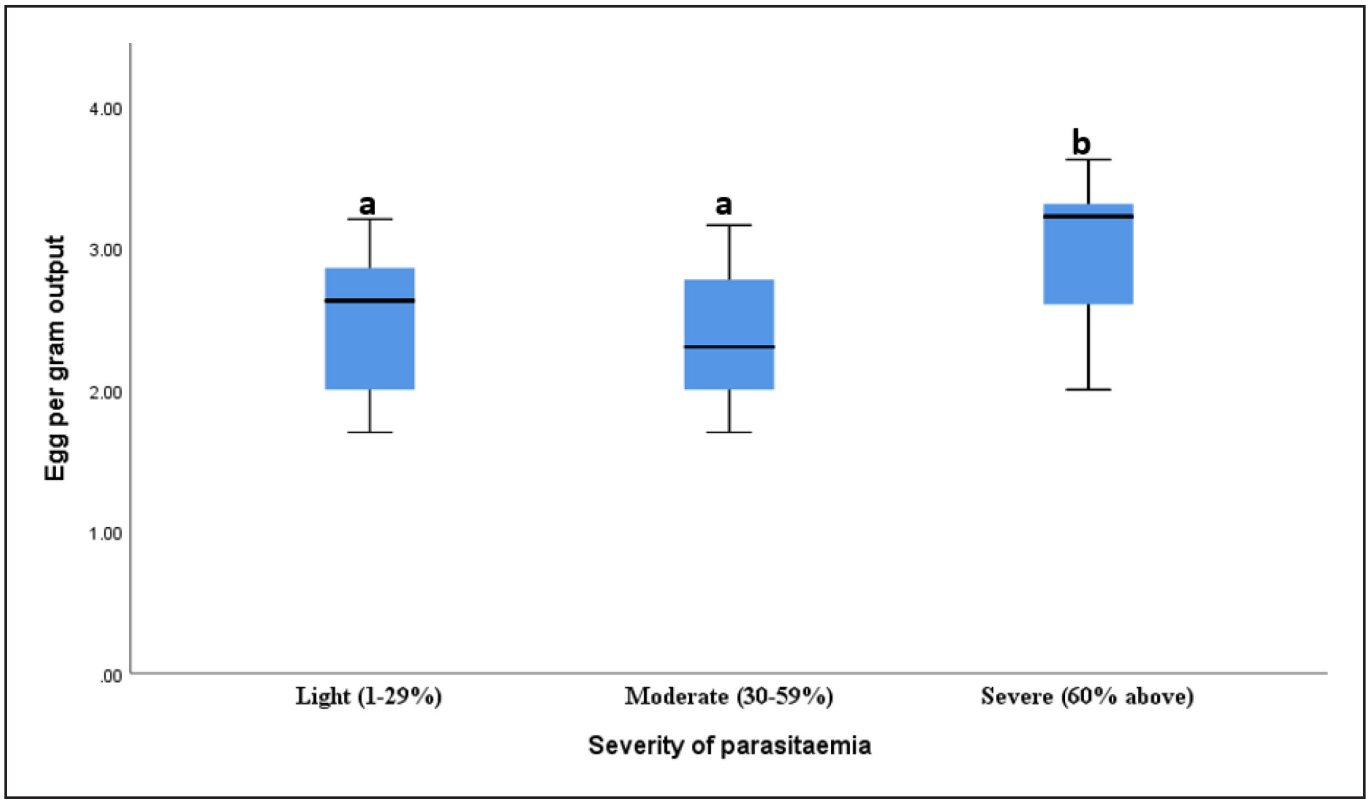

Figure 3. Relationship *M. ovis* severity and Strongyle burden in goats with coinfection.

exposure to the infective stages or indirect contact with vectors on pasture (Paul *et al*., 2020b). Although several studies have hinted at the importance of parasitic diseases on small ruminant production (Chandrawathani *et al*., 2009; Zainalabidin *et al*., 2015; Melissa *et al*., 2016; Paul *et al*., 2021), the knowledge of parasite coinfections, which is essential for efficient control, is very limited in Malaysia. Therefore, this study aimed to elucidate the pattern of parasitic infections amongst goats from selected smallholder farms.

There was a higher total count of goats with GI parasites (79.38%; 95% CI: 74.02 - 83.87) than blood protozoa (57.98%; 95% CI: 51.87 - 63.85). The current variety of endoparasites, including *M. ovis*, *Anaplasma*, *babesia, Eimeria species, and* Strongyles species, are common causes of anaemia, slow weight gain, poor milk production, and mortality among small ruminants in Malaysia (Rohaya *et al*., 2017; Azima *et al*., 2020). Early investigators have established their occurrence and importance amongst small ruminants (Dorny *et al*., 1995; Jalila *et al*., 1998; Chandrawathani *et al*., 2009; Nur Hazirah *et al*., 2016; Rohaya *et al*., 2017; Tan *et al*., 2017; Azima *et al*., 2020; Paul *et al*., 2020b, 2021). Strongyle nematodes, *Eimeria*, *Moniezia*, and *Trichuris* species are endemic problems among small ruminants in Malaysia (Ikeme *et al*., 1987; Chandrawathani *et al*., 1999; Paul *et al*., 2020b). Moreover, there are various reports on *Theileria*, *Babesia, Anaplasma*, and *Ehrlichia* (Bilgic *et al*., 2017) and haemotropic *Mycoplasma ovis* (Paul *et al*., 2020a, 2021) among sheep and goats from different parts of the country. The rate of GI parasites observed in the present study is similar to previous studies, which have suggested that most goats in Malaysia were chronic carriers due to favourable climatic conditions for parasite epidemiology and transmission (Hashim & Yusof, 2016; Paul *et al*., 2020b). The semi-intensive management practised by farmers in the present study which relies on pasture grazing and lacks a herd health program for parasite control, may be responsible for the increased risk of parasitic infection among goats. Previously, an increased risk of exposure to helminthosis among goats was attributed to grazing in semi-intensive rearing system (Rabbi *et al*., 2011). Additionally, poor sanitation of farms, as observed in the present study, is associated with a high incidence of parasitic infections among goats (Hashim & Yusof, 2016).

The lower incidence of haemoparasites observed in this study agrees with previous studies that detected low frequencies of *Theileria*, *Babesia*, and *Anaplasma* among sheep and goats in Malaysia (Rohaya *et al*., 2017; Tan *et al*., 2017). The occurrence of haemoparasites is associated with the abundance of vectors such as *Rh*. (*Boophilus*), *Dermacentor*, *Ixodes*, *Haemaphysalis* and *Rhipicephalus* in Malaysia (Khadijah *et al*., 2014). Generally, anaplasmosis, babesiosis and theileriosis are recognised as the leading tick-borne haemoparasitic diseases of sheep and goats worldwide (Kocan *et al*., 2004). The low rates of *Babesia* and *Anaplasma* in our study agrees with a previous study which reported 0.24% and 0.12% prevalence for *Babesia* and *Anaplasma* among goats in Malaysia (Rohaya *et al*., 2017). The presence of haemotropic *M. ovis* infection observed among goats in this study agrees with previous reports (Jesse *et al*., 2015, 2017; Paul *et al*., 2021). Haemotropic mycoplasmosis is currently an emerging haemoparasitic disease of economic and zoonotic concern in many sheep and goat producing areas of the world (Paul *et al*., 2020b), including Malaysia with 52% infection rate among small ruminants (Paul *et al*., 2021). The abundance of *M. ovis* may be linked to management practices and suitable agroecology for the arthropod vectors in the goat producing areas, which were situated close to the edges of forests (Paul *et al*., 2020a).

This study has further revealed a higher incidence of Strongyle and *M. ovis* coinfection (84.2% 95% CI: 79.0 - 88.3) than single species infection (15.8%; 95% CI: 11.7 - 21.0) with either GI or haemoparasites. This finding agrees with a previous study by Win *et al*. (2020) who reported 84.8% and 15.2% rates of mixed and single infections of parasites, respectively in Myanmar. Our result also agrees with other reports which suggest that most field infections comprise of a mixture of different species of endoparasites (Idris *et al*., 2012; Paul *et al*., 2020b; Wuthijaree *et al*., 2022). This result also agrees with the previously reported clinical case that suggest the coexistence of haemoplasma and GI parasites in small ruminants (Jesse *et al*., 2013, 2015, 2017). Moreover, Ait Lbacha *et al*. (2015) reported the coinfections of haemotropic *Mycoplasma* and *Anaplasma* species among small ruminant flocks in Morocco. The associations observed between gender and age of goats and the status of *Eimeria* or Strongyle infection agrees with previous reports (Nisbet *et al*., 2016; Zvinorova *et al*., 2016; Paul *et al*., 2020b) but this is the first report demonstrating the association between coinfection involving *M. ovis* and the gender of goats. The higher risk of *M. ovis* coinfection in female versus male goats mimics the pattern of *Eimeria* and Strongyle in the goats and corroborates clinical evidence that suggest potential association between coinfection and the severity of clinical disease in both sheep and goats. The higher risk of Strongyle infection in females was previously associated with the stress of pregnancy and lactation, which may suppress immunity and make females more susceptible (Paul *et al*., 2020). Moreover, there were 2.8 times more females in the sample and flocks under investigation, as smallholders usually kept them for longer periods than males for production purposes and may be subjected to cumulative exposure leading to acquired immunity, which allows them to thrive in the presence of a heavy worm burden.

There was a significantly lower mean haematocrit level and higher mean EPG output among goats with a severe degree of *M. ovis* parasitaemia compared to other categories of infection. While an increased nematode burden was associated with decreased haematocrit level. Haemotropic *M. ovis* (Paul *et al*., 2021) is associated with haemolytic anemia caused by the distortion of erythrocyte membrane (Gulland *et al*., 1987b), increased membrane fragility (Hashim & Yusof, 2016; Melissa *et al*., 2016), hemagglutination (Kanabathy & Nachiar, 2004), erythrophagocytosis (Philbey *et al*., 2006), oxidative injury, and enzymatic lysis or disruption of cell functions (Theiss *et al*., 1996). While GIP infection is associated with anaemia due to chronic intestinal haemorrhage and direct blood-feeding (Dorny *et al*., 1995). Moreover, we observed a significant higher degree of parasitaemia in goats diagnosed with a coinfection of *M. ovis* and GIP compared to single *M. ovis* infection among the goats. These observations support our hypothesis and existing evidence, which suggests that haemotropic *M. ovis* coinfection is important in the epidemiology of Strongyle nematodes among small ruminants. Even though Strongyles and haemotropic *Mycoplasmas* are common causes of anaemia and morbidities amongst small ruminants (Hornok *et al*., 2009), their co-morbidities under field conditions have been widely speculated because the exact mechanisms of increased pathogenicity in coinfections of parasites are not fully understood. But some workers have suggested that hemoplasmas may act in synergy with highly pathogenic nematodes such as *H. contortus* and contribute to the severity of disease in a concurrently infected flock (Souza *et al*., 2019). While other workers have observed an increased severity of *M. ovis* infection in small ruminants which were concurrently infected with *Anaplasma* and *Babesia* species (Neimark & Kocan, 1997; Ait Lbacha *et al*., 2015; Aktas & Ozubek, 2017). Moreover, a handful of clinical scenarios involving concurrent severe nematode EPG versus *M. ovis* were thought to be provoked by the immunosuppressive effects of the coinfecting parasites. To the best of our knowledge, stress due to heavy worm burden coupled with a nutritional deficiency, and protein-losing enteropathy may cause immunosuppression under field conditions and increase the severity of *M. ovis* infection.

CONCLUSION

Strongyle coinfection with *M. ovis* yields a higher level of parasitaemia among goats, suggesting an increased severity of *M. ovis* infection and degree of anaemia in small ruminants. This observation also suggest that the presence of nematode coinfection may likely confound the diagnosis of *M. ovis* due to similar pathogenic mechanisms. This paper highlights the importance of mixed parasitic infections among goat flocks in Malaysia and recommends the development and implementation of an integrated herd health program for endoparasite control among low-input smallholder flocks to ensure sustainability.

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Conflict of interest

The author declares that they have no conflict of interests.

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