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RESEARCH ARTICLE

Assessment of *Fasciola* and Paramphistomes co-infection in large ruminants through faecal egg counts around Taiping, Malaysia

Che-Kamaruddin, N.[1], Isa, N.M.M.[1*]

[1]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Malaysia
*Corresponding author: nurmahiza@upm.edu.my

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ABSTRACT

Emerging cases of *Fasciola* and Paramphistomes co-infection have been reported, especially in tropical regions. This is due to *Fasciola* and Paramphistomes sharing biological factors which influence the pattern of transmission, especially in faecal egg shedding due to interaction and competition in the definitive host. Most reports surveyed the occurrence of fasciolosis in ruminants with a lack of observation of faecal egg distribution. Therefore, present study is aimed to assess the distribution of *Fasciola* and Paramphistomes faecal egg count (fec) in co-infected large ruminants in Larut, Matang, and Selama areas (Taiping). A total of 371 faecal samples were collected at random from 23 ruminant herds. Flukefinder® sedimentation was used to quantify the *Fasciola* and Paramphistomes eggs. Descriptive analyses were performed to determine the prevalence of co-infections, and Spearman correlation analysis was used to correlate the fec. Overall, the prevalence of *Fasciola* and Paramphistomes co-infection was 23.7% (n=89/371) in Taiping. Prevalence of paramphistomosis was always higher than fasciolosis in overall and single infection, with 46.9% (n=174/371) and 22.9% (n=85/371) compared to 36.9% (n=137/371) and 12.9% (n=48/371) respectively. Egg per gram (epg) of both parasites were positively skewed with a median of 1.5 epg in fasciolosis and 10.5 epg in paramphistomosis. Spearman correlation analysis of the epg in co-infected bovine was found to have a moderately positive correlation with r_s=0.39 (p-value<0.01). The recent study observed a moderate prevalence of *Fasciola* and Paramphistomes co-infection in a large ruminant population from Taiping, with the prevalence of paramphistomosis being higher than fasciolosis. Hence, this suggests that infection with one of these parasites increases the chance of infection with another. There is a need to integrate fec in parasite surveillance to monitor the trend of parasite transmission. Findings in the present study could tailor control strategies, especially for fasciolosis to limit the economic loss and prevent zoonotic transmission.

Keywords: Agriculture; fasciolosis; helminth; livestock; paramphistomosis.

INTRODUCTION

Multiparasitism is common in ruminant livestock (May *et al*., 2019). The interaction of different parasite species in parasitising hosts may cause repercussions to livestock health, especially in breeding and animal production (May *et al*., 2019). In different animal populations, the likelihood of parasitic infection, burden, and parasite community dynamics are significantly varied (Barber & Dingemanse, 2010). The differences are due to various factors, such as the variational environmental and the intrinsic host factors (Barber & Dingemanse, 2010).

During the inspection of ruminant fasciolosis, the Paramphistomes (rumen flukes) are often observed to co-infect with *Fasciola* through coproscopical diagnoses, such as the faecal sedimentation during the patent infection (Aragaw & Tilahun, 2019). The co-infection with these parasites has been reported globally with increasing occurrences as the result of common risk factors that promote their transmission (Aragaw & Tilahun, 2019). Compared to fasciolosis, paramphistomosis in ruminants is frequently neglected as it is traditionally considered less pathogenic and insignificant towards livestock production (Sarmah *et al*., 2014; Aragaw & Tilahun, 2019). However, the migration phase of juvenile Paramphistomes through the ruminant's tissues can result in significant clinical manifestation in the infected ruminants (Sarmah *et al*., 2014; Munita *et al*., 2019).

The susceptibility and infectivity of the same snail species to both parasites have been reported in temperate regions during the intermediate host life stage of *Fasciola* and Paramphistomes (Sargison *et al*., 2016; Huson *et al*., 2017; Naranjo-Lucena *et al*., 2018; Munita *et al*., 2019). Both *Fasciola* and Paramphistomes can infect sympatric freshwater snails, resulting in successful cercarial shedding with a synchronised shedding peak (Elelu *et al*., 2016; Vignoles *et al*., 2017). As a result, the infective metacercariae of both parasites can contaminate within the same geographical area, exposing the ruminant concurrently (Elelu *et al*., 2016; Vignoles *et al*., 2017).

In the definitive host life stage, paramphistomosis was more common in large ruminants such as cattle than in small ruminants such as goats (Naranjo-Lucena *et al*., 2018). This is because Paramphistomes' prepatent period is shorter in cattle hosts, suggesting that this animal group is more susceptible to paramphistomosis (Naranjo-Lucena *et al*., 2018). The common Paramphistomes found to co-infect with *Fasciola* in temperate regions are *Calicophoron daubneyi* and *Paramphistomum cervi* (Gordon *et al*., 2013; Toolan *et al*., 2015). Whilst in tropical regions, several Paramphistomes genera were identified, such as *Fischoedurious* spp., *Gastrothylax* spp., *Carmyerius* spp., and *Paramphistomum* spp. in ruminant (Anucherngchai *et al*., 2020). While the co-infection of *Fasciola* and Paramphistomes in ruminant livestock is emerging in temperate countries such as Europe (Gordon *et al*., 2013; Toolan *et al*., 2015), this event is common and prevalent in tropical and sub-tropical countries (Yabe *et al*., 2008; Nzalawahe *et al*., 2015; Elelu *et al*., 2016; Aragaw & Tilahun *et al*., 2019). It is attributable to the varied parasite community structure amongst definitive host populations due to environmental stochasticity (Barber *et al*., 2010). Accordingly, the co-infection pattern of *Fasciola* and Paramphistomes is understudied in some regions in different climates.

Thus, understanding the dynamics of interactions between *Fasciola* and Paramphistomes could help better comprehend the parasitic reciprocation and the effects of co-infection on ruminant livestock (Vamourin *et al*., 2015). The present study hypothesised that infection with Paramphistomes in large ruminants is a risk factor for ruminant fasciolosis, leading to higher exposure to *Fasciola*, jeopardising livestock production and economic growth. To our knowledge, there is a lack of studies evaluating the correlation of *Fasciola* with Paramphistomes in co-infected ruminant livestock from Southeast Asian regions, particularly in Malaysia. Hence, present study aimed to assess the *Fasciola* and Paramphistomes co-infection in naturally co-infected bovine through faecal egg count (fec) by using Flukefinder® sedimentation assay to better understand the inter-specific interaction among these parasites.

MATERIALS AND METHODS

Ethical statement

Ethical approval was obtained from the Universiti Putra Malaysia (UPM). The work described in this study involved the use of non-experimental animal and non-invasive sampling was performed. The faecal samples collected were non-invasive and non-painful procedures following the clearance number UPM/IACUC/AUP-007/2019.

Study design

The sampling was conducted from February until August 2020 during a survey of ruminant fasciolosis by Universiti Putra Malaysia (UPM) in Larut, Matang, and Selama areas (Taiping), Malaysia. Taiping has favourable climatic conditions for the growth of freshwater snails, with an average yearly rainfall of more than 4,000mm. Livestock farms are mostly family businesses, and both semi-intensive and extensive housing systems are adopted in the study site. The livestock is often grazed on communal pastures.

Farm selection was performed randomly from the farm registration list by the local Department of Veterinary Services. Farms without anthelmintic treatment for at least 6 months through the local anthelmintic usage record were selected for this study. Verbal consent was taken through a phone call prior to the farm visit. During the farm visit, rectal faecal samples were collected from large ruminants such as beef cattle, dairy cattle, and buffalo at random. Samples were stored in 4°C and transported to the Veterinary Parasitology Laboratory, Faculty of Veterinary Medicine, UPM, for diagnosis. Demographic data such as sex, estimated animal age, and body condition score (BCS) were collected during the sampling.

The sample size estimation for the observational study was carefully determined following the guidelines presented in Thrusfield (2007). Utilising the recommended table, a minimum sample of 367 was determined to ensure adequate statistical power and robustness of the study findings.

Coproscopical analysis

Faecal egg count (fec) was performed using a modified Flukefinder® sedimentation method (Richard Dixon, ID, USA). Flukefinder® is more sensitive to recovering parasite eggs with more than 95% compared to other diagnostics such as Kato-Katz, FLOTAC, and conventional sedimentation (Zárate-Rendón *et al*., 2019; Reigate *et al*., 2021). Briefly, 2 grams of faecal sample was mixed with 30mL treated water before being poured into the Flukefinder®. The filtered materials were sedimented into a 5cm vial for 2 minutes, and the supernatant was discarded. The sediment was then transferred into a gridded petri dish with three drops of 10% methylene blue and was observed under a stereomicroscope at 25X magnification for the parasite egg count. The differentiation between *Fasciola* and Paramphistomes eggs was based on their shape, colouration, and operculum morphology following Gordon *et al*. (2010). The eggs of *Fasciola* are elongated and more ellipsoidal, and relatively larger compared to Paramphistome eggs. The shell morphology of these parasite groups is often distinct, with *Fasciola* eggs having a distinctive golden colour, while Paramphistome eggs are usually colourless. In *Fasciola* eggs, a prominent "shoulder spine" is often present in the operculum, whereas Paramphistome eggs possess a small operculum at the anterior part of the egg.

Statistical analysis

Raw data was processed in a database and was later exported into R statistical software, where analysis was performed. The prevalence for the single- and co-infection of the parasite was calculated from the percentage of total positive samples and divided by the total number of the sampled individuals and between groups. The faecal samples observed with at least one parasite egg count were considered positive.

The descriptive analyses of egg per gram (epg) of *Fasciola* and Paramphistomes were performed to summarise the overview of parasite egg distribution. The Kolmogorov-Smirnov (KS) normality test was done following the skewness of the epg distribution before proceeding with the correlation test for *Fasciola* and Paramphistomes co-infection. Spearman correlation analysis was used to correlate the epg of *Fasciola* and Paramphistomes while log-transformed [log(epg+1)] of the epg was performed in the linear association analysis to associate the trend of epg from both parasites. Analyses were considered statistically significant when the p-value was less than 0.05.

RESULTS

Figure 1 shows *Fasciola* and Paramphistomes eggs. The distinct differences between these parasite eggs were the colour and shape, as the *Fasciola* egg is golden-coloured and round egg shape, while Paramphistomes eggs are silver-coloured and more oval. Briefly, *Fasciola* eggs are approximately 180 to 210 µm in length and 95 to 120 µm in width, whereas Paramphistomes eggs measure 113 to 185 µm in length and 73 to 112 µm in width.

Table 1 shows the overview of fasciolosis and paramphistomosis prevalence through Flukefinder® sedimentation. The overall prevalence of Paramphistomes is higher than *Fasciola,* with 46.9% (n=174/371) compared to 36.9% (n=137/371), respectively. Similar trend was observed in the single infection where Paramphistomes was found in 22.9% (n=85/371) of the examined bovine, which was higher than *Fasciola* with 12.9% (n=48/371). The coprological examination showed the co-infection prevalence of *Fasciola* and Paramphistomes in bovine was 23.7% (n=89/371). Of animals

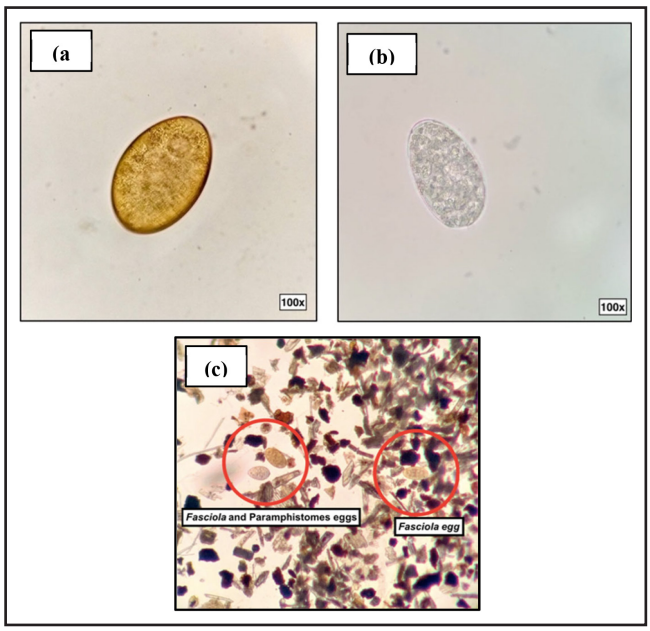

Figure 1. Parasite eggs from Flukefinder® sedimentation. (a) *Fasciola* egg, (b) Paramphistomes egg, (c) Both *Fasciola* and Paramphistomes eggs from co-infected bovine.

Table 1. Overview of *Fasciola* and Paramphistomes prevalence according to overall, single-, and co-infection through coproscopical examination

Infection	Parasite group	Prevalence, % (95% C.I.)
Overall (N=371)	*Fasciola*	36.9 (± 4.9)
	Paramphistomes	46.9 (± 5.2)
Single infection	*Fasciola* (n=48)	12.9 (± 3.2)
	Paramphistomes (n=85)	22.9 (± 4.2)
Co-infection (n=88)	*Fasciola* ~ Paramphistomes	23.7 (± 4.2)

infected with at least one parasite, 39.5% (n=88/223) were co-infected with *Fasciola* and Paramphistomes.

Table 2 shows the prevalence of *Fasciola* and Paramphistomes co-infection according to host factors. Ruminant group, age group, and body condition score (BCS) of the bovine are significantly correlated with the co-infection through chi-square analysis. Sex of the bovine was not significantly correlated with co-infection, thus suggesting both male and female animals were equally exposed to co-infection in Taiping.

Table 3 shows the descriptive statistics of *Fasciola* and Paramphistomes egg per gram (epg) in co-infected bovine (n=88), and Figure 2 illustrates the epg distribution in a single infection. The median egg count of *Fasciola* and Paramphistomes in co-infected bovine was 1.5 and 10.5 per gram of faecal samples, with the range of 0.5 to 33.5 and 0.5 to 51.5 epg, respectively. In general, the parasite egg count in this study was positively skewed and relatively low in naturally co-infected bovine. The present study showed the Paramphistomes epg was consistently higher than *Fasciola* epg in co-infected bovine. In contrast, in the single infection, the range of epg for *Fasciola* (n=48) was 0.5 to 56.5 epg, while for Paramphistomes (n=85) was 0.5 to 51.5 epg, respectively.

Table 2. Occurrence of *Fasciola* and Paramphistomes co-infection between host factors from examined bovine (N = 371)

Factor	Co-infection % (n)	P-value*
Ruminant group		< 0.001
Beef cattle (*Ref*)	15.9 (41/258)	
Dairy cattle	34.8 (23/66)	
Buffalo	51.1 (24/47)	
Sex	0.782	
Female (*Ref*)	29.1 (59/203)	
Male	17.3 (29/168)	
Age group (Years)		< 0.001
< 1 (*Ref*)	0 (0/35)	
1 - 3	21.8 (55/252)	
> 3	39.3 (33/84)	
Body condition score		< 0.001
1 (*Ref*)	81.3 (13/16)	
2	47.7 (41/86)	
3	16.4 (33/201)	
4	2.1 (1/47)	
5	0 (0/21)	

**P-value derived from the chi-square of each factor to co-infection.*

Table 3. Descriptive statistics of *Fasciola* and Paramphistomes egg per gram (epg) in co-infected bovids (n=89)

Co-infection	*Fasciola* epg	Paramphistomes epg
Mean (95% Confidence Interval)	2.60 (± 0.87), *S.E.: 0.44*	12.135 (± 2.02), *S.E.: 1.02*
Median	1.50	10.50
Variance	16.93	92.0
Std. Deviation	4.11	9.59
Minimum epg	0.50	0.50
Maximum epg	33.50	51.50
Interquartile Range	2.30	8.80
Skewness	5.46, *S.E.: 0.26*	1.77, *S.E.: 0.26*
Kurtosis	37.35, *S.E.: 0.51*	4.11, *S.E.: 0.51*

The Spearman correlation analysis showed a statistically significant positive correlation between *Fasciola* and Paramphistomes in co-infected bovine (r_s=0.39, p-value<0.01). The present study simulates the *Fasciola* response on Paramphistomes exposure in the fec distribution to understand the association while assuming there is a linear association between these parasites. From the regression, the present study showed that if log(Paramphistomes epg + 1) increases by 10%, then log(*Fasciola* epg + 1) also increases by 5.5%.

Figure 3 shows the distribution of *Fasciola* and Paramphistomes faecal egg count (fec) according to the body condition score (BCS) of examined bovine. Considering BCS significantly correlates with co-infection (p-value<0.01), the present study plotted the fec distribution to observe the pattern between scores. The plot shows higher BCS in bovine with fewer fec, which regards lower infection.

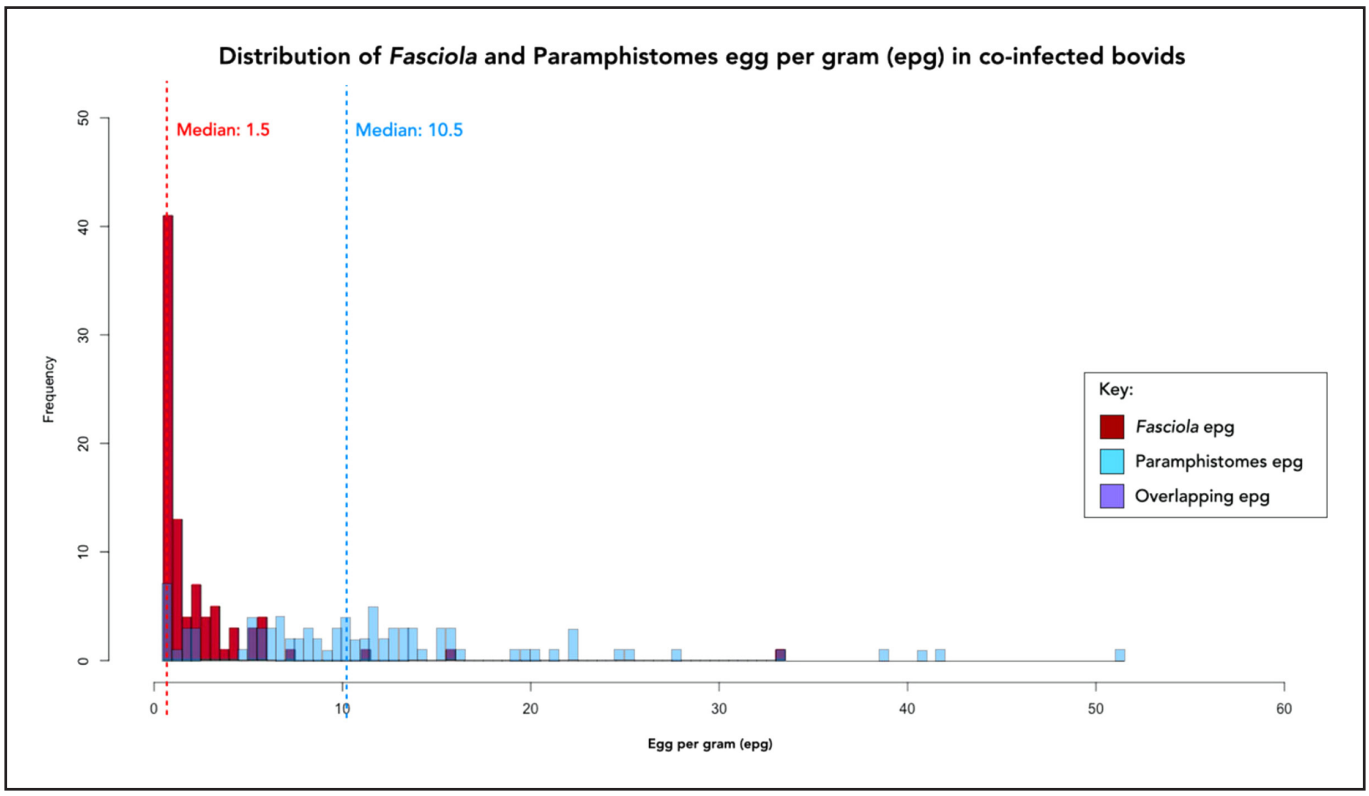

Figure 2. Distribution of egg per gram (epg) count in *Fasciola* and Paramphistomes from co-infected bovine.

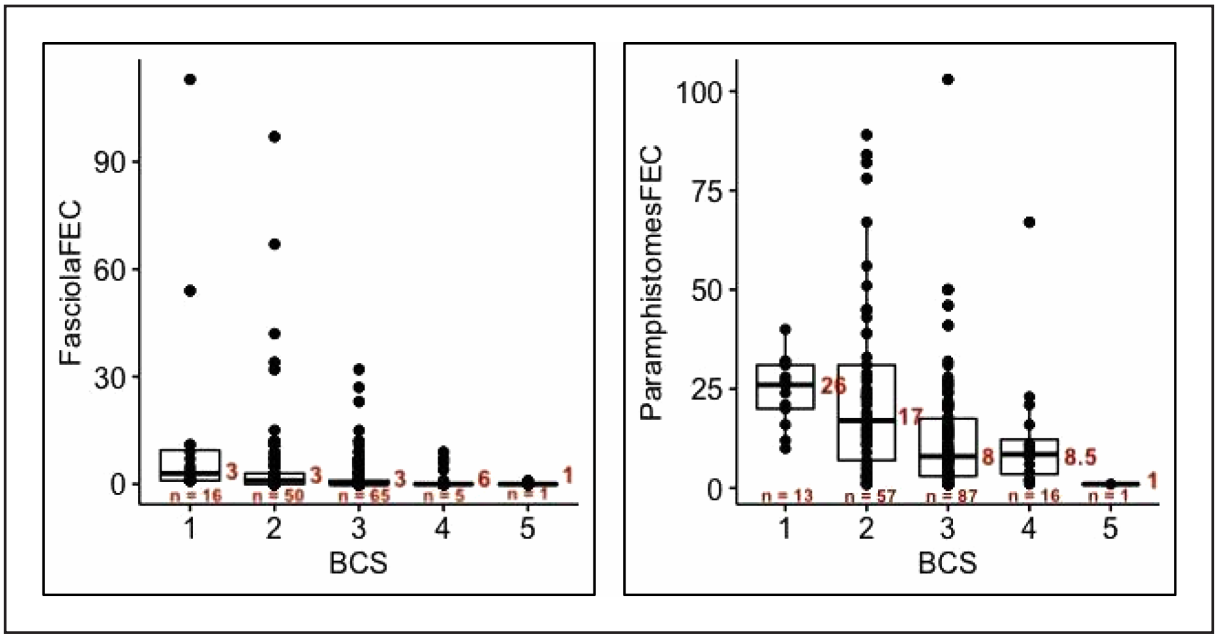

Figure 3. Boxplot with median value of faecal egg count (FEC) of fasciolosis and paramphistomosis according to body condition score (BCS).

DISCUSSION

Multiple parasites often infect a single host and compete for resources which could modify the parasite's fitness (Morgan *et al*., 2019). There are increasing reports of *Fasciola* co-infecting with Paramphistomes in ruminant livestock (Yabe *et al*., 2008; Sargison *et al*., 2016; Elelu *et al*., 2016; Jones *et al*., 2017; Naranjo-Lucena *et al*., 2018; Aragaw & Tilahun, 2019; Munita *et al*., 2019). Co-infection of *Fasciola* and Paramphistomes could be explained by the fact that both parasites have similar biological characteristics and share intermediate hosts. Therefore, both parasites share risk factors for exposure to ruminant livestock (Keyyu *et al*., 2005; Madsen & Hung, 2014; Nurhidayah *et al*., 2019). Taken together, the occurrence of paramphistomosis could affect the exposure to fasciolosis.

In the present study, paramphistomosis was more prevalent than fasciolosis in bovine, with 46.9% compared to 36.9%. This trend is consistent with a local study in Terengganu, which reported 4% (n=10/267) of the examined small ruminants being positive for Paramphistomes eggs. At the same time, none tested positive for the *Fasciola* egg (Mursyidah *et al*., 2017). Similar co-prevalence trends have been observed in other climatic regions, including temperate regions where paramphistomosis in ruminants is emerging (Yabe *et al*., 2008; Nzalawahe *et al*., 2015; Aragaw & Tilahun, 2019; Japa *et al*., 2020). In tropical climatic regions, the prevalence of paramphistomosis is consistently higher than fasciolosis in definitive hosts (Japa *et al*., 2020). Present study shows there was a moderate prevalence of *Fasciola* and Paramphistomes co-infection in a large ruminant population from Taiping with 23.7%, which was comparable to studies from sub-tropical regions such as Tanzania and Zambia with 24.9% (Nzalawahe *et al*., 2015) and 34.0% (Yabe *et al*., 2008). In the European ruminant population, this trend has also been reported to signify a syndemic infection of both parasites (Gordon *et al*., 2013; Huson *et al*., 2017). This is due to multiple reasons, including the paramphistomosis treatment is often neglected, leading to higher paramphistomosis prevalence than fasciolosis (Sargison *et al*., 2016). Historically, adult Paramphistomes which reside in the forestomach of ruminants are regarded as non-pathogenic to definitive hosts and lack pathologic effect in animal production (Sargison *et al*., 2016). Although the significant pathogenicity induced by adult Paramphistomes has not been extensively studied, clinical manifestations of the Paramphistomes-infected animals have been reported in several studies (Mason *et al*., 2012; Devos *et al*., 2013; Fuertes *et al*., 2015; Huson *et al*., 2017). This includes the inflammatory response in localised infections such as rumenitis, abomasitis, and rumen papillae atrophy (Fuertes *et al*., 2015; Mason *et al*., 2012). The pathogenicity of paramphistomosis is associated with the migration phase of juvenile Paramphistomes such as *Calicophoron daubneyi* that excyst in duodenum and burrow into the intestinal submucosa, predominantly parasitising proximal small intestine which can cause significant economic loss (Devos *et al*., 2013; Huson *et al*., 2017) especially in tropical and sub-tropical regions (Huson *et al*., 2017). Furthermore, narrow-spectrum drugs targeting only *Fasciola* result in persistent paramphistomosis and contribute to higher occurrence (Jones *et al*., 2017). In contrast, during the cercariae life stage, the occurrence of *C. daubneyi* in co-infected snail intermediate host is lower compared to *Fasciola*, which signifies the need for further investigation of the interaction between these parasites (Jones *et al*., 2017). Other factors which influence the emergence of paramphistomosis in ruminant includes high species diversity within the Paramphistomidae family (Nurhidayah *et al*., 2019; Anucherngchai *et al*., 2020), the endurance of encysted Paramphistomes metacercariae which resilient towards heavy rain or floods (Nurhidayah *et al*., 2019), and a generalist group that infects a variety of snail species during their intermediate host life stages (Nzalawahe *et al*., 2015).

The present study observed a low *Fasciola* and Paramphistomes egg count in the Taiping bovine population as the distribution of parasites epg was positively skewed. Reports from other studies have shown that the *Fasciola* epg count in bovine hosts was four times lower compared to goat and sheep, as reported by Sato *et al*. (2014) and Gueguen *et al*. (2016). This is possibly due to the differences in egg shedding among species groups and might be due to the bovine group being partially able to acquire immunity to *Fasciola* and Paramphistomes (Kassai, 1999). The variation in parasite fitness in generating eggs is expected among the different host individuals, translating to the differences in the parasite genotype (Sargison, 2013). High variance of the sporadic egg shedding distribution might be attributed to various factors, including the low fluke count, prepatent infection, and resistance towards the parasite (Flanagan *et al*., 2011; Morgan *et al*., 2019). In paramphistomosis, higher egg shedding from *C. daubneyi* has been observed in co-infected bovids, which is ten times greater than in *Fasciola* (Jones *et al*., 2017). Subsequently, higher egg count results in higher exposure to ruminant livestock (Jones *et al*., 2017). Moreover, the eggs of *Fasciola* and Paramphistomes can contaminate the environment and sustain the infection. This event is significant in areas with high livestock population density since these parasites can shed many eggs simultaneously (Bishop, 2012). In addition, the miracidia of both parasites can infect similar species of snail intermediate hosts and result in successful cercarial shedding (Jones *et al*., 2017). As a result of this event, both *Fasciola* and Paramphistomes genotypes are exposing the definitive mammalian hosts to co-infection, which impacts the epidemiological dynamic (Morgan *et al*., 2019). The present study highlights the egg per gram (epg) monitoring of fasciolosis and paramphistomosis is a useful tool to evaluate and predict the exposure and infection intensity.

Host factors such as ruminant group, age group, and body condition score (BCS) were found to be significantly correlated with co-infection in the present study (p-value<0.001). Ruminants are often exposed to high levels of parasite eggs when they graze on pastures. However, different ruminant species may exhibit differences in grazing behaviour and physiological factors that can affect their exposure and susceptibility to parasite infections (Gueguen *et al*., 2016; Huson *et al*., 2017). The differences can contribute to variations in *Fasciola* and Paramphistomes exposure and susceptibility among ruminant species. Age group was significantly correlated with co-infection, suggesting that older animals are more vulnerable to *Fasciola* and Paramphistomes co-infections (Gueguen *et al*., 2016; Huson *et al*., 2017). Similarly, the finding that body condition score was significantly correlated with co-infection could suggest that animals in poor condition are more correlated to parasitic infections, which could imply the negative effect of disease (Aragaw & Tilahun, 2019). However, it is essential to note that correlation does not necessarily imply causation. While the present study has identified significant correlations between host factors and co-infection, it does not prove that these host factors are causing co-infection. Additionally, it is important to consider other potential factors contributing to co-infection, such as environmental factors or the presence of other intermediate hosts in future studies.

Regardless, body condition scoring (BCS) of the large ruminant is a subjective assessment based on the visual and tactile evaluation that can monitor the overall health, which can indirectly provide information relevant to parasite control. Animals with poor body conditions are often susceptible to parasite infections due to weakened immune systems (Evering and Weiss, 2006). Therefore, regular monitoring of BCS allows early detection of declining body conditions and enables proactive control of parasite infections. Besides, BCS can be a valuable tool in guiding strategic parasite control interventions. Animals with a lower BCS, indicating compromised health, may necessitate prioritised treatment to address existing infections, such as deworming and pasture management strategies. It is crucial to acknowledge that the utilisation of BCS as a tool for parasite control should be complemented by integrating other diagnostic methods, such as faecal egg counts.

The co-infection of *Fasciola* and Paramphistomes through fec in the present study showed a statistically positive correlation. A positive correlation between both parasites has also been observed in other studies from different regions and climates (Keyyu *et al*., 2005; Phiri *et al*., 2006; Nzalawahe *et al*., 2015; Elelu *et al*., 2016; Aragaw & Tilahun, 2019). The positive correlation might be due to the indirect competition in their resources for maturation and dissemination among these parasites. In addition, the linear regression showed an increment of Paramphistomes epg was observed in tandem with the increase in *Fasciola* epg. Therefore, suggesting that infection with one of these parasites increases the chance of infection with another. The positive correlation between *Fasciola* and Paramphistomes could be due to various factors, such as an immunological condition in the definitive host caused by infection with one parasite facilitating the infection with another (Karvonen *et al*., 2019). Both sequential and simultaneous infections are common since the metacercariae of both trematodes are found in the exact location (Jones *et al*., 2017; Vignoles *et al*., 2017; Karvonen *et al*., 2019). However, again, correlation findings in the present study do not intend to determine the causality event.

Present study, however, did not molecularly identify which *Fasciola* species was co-infecting with Paramphistomes. Nonetheless, *F. gigantica* is the only *Fasciola* present in Malaysia (Diyana *et al*., 2020), implying that the interaction with *F. hepatica* might differ, which needs further studies from tropical climates. There is a lack of information regarding the differences between *F. hepatica* and *F. gigantica* in modulating interspecific interaction with Paramphistomes in co-infected hosts. Studies from tropical and subtropical regions showed a significant positive correlation between *F. gigantica* with Paramphistomes in co-infecting hosts (Keyyu *et al*., 2005; Nzalawahe *et al*., 2015; Phiri *et al*., 2006). In contrast, *F. hepatica,* prevalent in temperate regions such as Europe, negatively correlated with Paramphistomes, particularly *C. daubneyi* (Jones *et al*., 2017). This is due to the differences in pathogenicity of different *Fasciola*, as *F. gigantica* is more pathogenic in definitive hosts than *F. hepatica* due to larger fluke size (Kassai, 1999; Valero *et al*., 2009). Therefore, the underlying hypothesis here is the phenotypic and genotypic differences between *F. gigantica* and *F. hepatica* could probably show differences in their interaction with Paramphistomes in co-infected hosts that need further attention in future studies.

In conclusion, the present study determined a moderate prevalence of co-infection of *Fasciola* and Paramphistomes in a large ruminant population from Taiping, Malaysia, with 23.7% (n=89/371). This finding signifies the presence of fasciolosis and paramphistomosis in Taiping's large ruminant population, although it is not yet considered widespread, and no human cases have been reported. Additionally, the prevalence of paramphistomosis was higher than that of fasciolosis. Considering the prevalence findings, the present study suggests the possibility of zoonotic transmission of fasciolosis in the Larut, Matang, and Selama areas (Taiping). Despite the moderate prevalence of ruminant fasciolosis observed in this study, it is important to note that each egg may develop into multiple infective metacercariae. Human exposure to these metacercariae can occur through consuming contaminated water and plants. Therefore, the study underscores the significance of effective surveillance, prevention, and control measures for fasciolosis. This study also presented a quantification of egg per gram (epg) of *Fasciola* and Paramphistomes to subjoin the information on the epidemiology of both parasites. The correlation analysis showed a significant positive correlation between *Fasciola* and Paramphistomes in co-infected bovids through parasite epg. Based on the study findings, it is highly recommended to incorporate regular monitoring and diagnostic procedures into the herd management plan. This includes conducting faecal egg counts, making clinical observations, closely collaborating with a veterinarian to ensure accurate and early parasite diagnosis, and implementing tailored control programs. By integrating the insights from ruminant parasite studies into their management practices, farmers can effectively manage livestock health, resulting in healthier animals and enhanced productivity within their herds. However, further research is needed to fully understand the underlying mechanisms and potential causative factors behind these correlations. Future studies should focus on fluke count in abattoirs and in vivo animal models to demonstrate whether the observed positive correlation through faecal egg count (fec) is the true parasite interaction.

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Conflict of Interest

The authors declare that they have no competing interests in the publication of this manuscript.

Data Availability Statement

The raw datasets which support the findings of this article are available from NCK and NMI upon reasonable request. Please send a request to naimchekamaruddin@gmail.com and nurmahiza@upm.edu.my to request access to the data.

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