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Occurrence of gastro-intestinal parasites among small ruminants in Malaysia: highlighting *Dicrocoelium* infection in goats

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Abstract. The aim of the present study was to determine the gastro-intestinal (GI) parasitic infections among small ruminants (i.e., goats, sheep, deer) in Malaysia through formalin-ether concentration technique. Overall, 70.9% or 302 out of 426 small ruminants (79.4% or 251/316 goats; 87.5% or 35/40 sheep; 22.9% or 16/70 deer) were infected with at least one species of GI parasites. Overall, ten types of GI parasites [Helminth: strongyle (57.7%), *Moniezia* spp. (5.4%), *Paramphistomum* spp. (4.5%), *Strongyloides* spp. (4.2%), *Dicrocoelium* spp. (2.3%), *Trichuris* spp. (2.3%); Protozoa: *Eimeria* spp. (23.7%), *Entamoeba* spp. (18.8%), *Giardia* spp. (1.9%), *Cryptosporidium* spp. (0.2%)] were detected in this study. Among the studied animals, goats harboured the highest diversity of GI parasites (ten types), followed by sheep (six types) and deer (two types). Polyparasitism was observed in goats (43.7% or 138 of 316) and sheep (15.0% or 6 of 40). Cumulatively, a total of 32 combinations of co-infections (Helminth+Helminth: 8 combinations; Helminth+Protozoa: 20 combinations; Protozoa+Protozoa: 4 combinations) between detected parasites with up to quintuple infections were reported. Among these parasites, "strongyle + *Eimeria* spp." and "*Moniezia* spp. + strongyle" were the commonest infections in goats (13.5% or 34 of 251) and sheep (5.7% or 2 of 6), respectively. This study is a comprehensive documentation on multiple GI parasitisms among small ruminant in Malaysia, and the findings are crucial for effective farm management, especially for the formulation of parasitic control and elimination strategies.

INTRODUCTION

Livestock industry in Malaysia is one of the key contributors to local economic development. In 2015, the livestock population reached approximately three million heads, of which 17.7% comprised small ruminants [estimated by Department of Veterinary Services (DVS), 2016]. Consumption of mutton has increased by 152.8% from 15,072 million tons in 2004 to 38,107 million tons in 2015 (DVS, www.dvs.gov.my).

Occurrence of GI parasites has threatened agriculture sector, leading towards lower productivity and higher mortality of livestock (i.e., small ruminants), particularly in young animals (Ayana *et al*., 2009). In veterinary parasitology, one of the greatest concerns has been on GI helminthic infections (i.e., strongyles) because it can cause greater severity of health problems in animals. Furthermore, a variety of GI helminths have also been reported in small ruminants all over the world (Jittapalapong *et al*., 2012; Ibrahim *et al*., 2014; Tan *et al*., 2014; Singh *et al*., 2015). In Malaysia, the commonly reported GI helminths in small ruminants were restricted to strongyle, *Moniezia* spp. and *Trichuris* spp.

Of these common GI helminths, strongyle (nematode group) is the most pathogenic parasite in small ruminants, resulting in severe losses in agriculture industry. Therefore, elimination and prevention of the strongyle infections in livestock in Malaysia must be taken into consideration when establishing effective farm management strategies. In addition, the importance of any other GI helminths such as flukes should not be disregarded.

In the field, animals are always exposed to more than one type of parasitic infections and occurrence of multi-infections can aggravate the health problems of the animals (Steel *et al*., 1982; Kaufmann, 1996; Abakar *et al*., 2001). It is vital that efforts are being made to improve understanding of the significance of multi-GI parasitic infections in small ruminants for better treatment management. Furthermore, the data gathered will be useful for the formulation of effective parasitic control strategies. Therefore, the aim of the present study was to determine the GI parasitic infections among small ruminants (i.e., goats, sheep and domesticated deer) in Malaysia through a qualitative coprological method (i.e., formalin-ether concentration technique).

MATERIALS AND METHODS

Ethical Consideration

The study was approved by the Ethics Committee of the University Malaya Medical Center, Malaysia (MED Ref. No. 896.36). Permission for the study to be conducted in animal farms was obtained from owners prior to sample collection.

Coprological survey

The present study was carried out from year 2012 to 2015 at ten livestock farms (i.e., seven goat farms; two goat+deer farms; and one sheep farm) in several states in Malaysia. The goat rectal faecal samples were collected from nine farms situated in Serdang, Selangor [Farm GDF1 (N=51)]; Sungai Siput, Perak [Farm GDF2 (N=98)]; Parit, Perak [Farm GF3 (N=8) and GF4 (N=15)]; Kuala Kangsar, Perak [Farm GF5 (N=24)]; Chenderiang, Perak [Farm GF6 (N=21)]; Tanjung Rambutan, Perak [Farm GF7 (N=22)]; Bau, Sarawak [Farm GF8 (N=40)] and Kuala Pah, Negeri Sembilan [Farm GF9 (N=37)]. In addition, faecal samples in sheep were collected in Pokok Sena, Kedah [Farm SF1 (N=40)] and deer in Farm GDF1 (N=54) and Farm GDF2 (N=16). These farms were routinely monitored by the Department of Veterinary Services, Malaysia. With the exception of the goats in farm GF6 and sheep in farm SF1 which were reared under intensive farming system, the rest of the goats were allowed to graze freely around farming areas and kept in pen during night time; whereas the deer were pasture raised.

The rectal faecal sample was collected from each studied animal and kept in 4°C for short-term storage until analysis. Formalin-ether concentration technique (Allen & Ridley, 1970) was conducted on the faecal samples. The processed samples were stained using Lugol's iodine solution and viewed under compound microscope at lower power (100X total magnification) for detection of helminth ova and protozoan (oo)cysts at higher power (400X total magnification). In addition, modified Ziehl-Neelsen stain (Casemore, 1991) was also performed to detect the presence of *Cryptosporidium* oocysts in the faecal samples at 1000X total magnification. The GI parasite identification was based on the morphological characteristics described by Kaufmann (1996) and Taylor *et al.* (2007). The animals were considered as GI parasite positive when at least one helminth ova and/or protozoan (oo)cysts were observed in the faecal samples.

RESULTS

A total of 426 rectal faecal samples were collected from goats (316; 74.2%), sheep (40; 9.4%) and deer (70; 16.4%). Microscopic detection on the 426 faecal samples showed 70.9% of the animals (95% CI: 66.59–75.21) were infected with at least one species of GI parasite. Overall, helminth and protozoan infections were 58.9% (or 251; 95% CI: 54.23–63.57) and 35.7% (or 152; 95% CI: 31.15–40.25), respectively. Sheep showed the highest prevalence of parasites (87.5% or 35; 95% CI: 77.25-97.75), compared to goats (79.4% or 251; 95% CI: 74.94–83.86) and deer (22.9% or 16; 95% CI: 13.06-32.74). In the present study, a total of ten GI parasites were observed among small ruminants. More than half of the collected samples were infected with strongyle (57.7% or 246), followed by *Eimeria* spp. (23.7% or 101), *Entamoeba* spp. (18.8% or 80), *Moniezia* spp. (5.4% or 23), *Paramphistomum* spp. (4.5% or 19), *Strongyloides* spp. (4.2% or 18), *Dicrocoelium* spp. (2.3% or 10), *Trichuris* spp. (2.3% or 10), *Giardia* spp. (1.9% or 8) and *Cryptosporidium* spp. (0.2% or 1) (Table 1). The presence of *Dicrocoelium* in this study is unexpected given that this parasite has not been reported in the Malaysian veterinary records for several decades.

In goats (total examined samples: 316), strongyle (66.5% or 210) was the only parasite with the infection rate exceeding 50%, while the rest of the parasites were observed in lower frequencies ranging from 31.3% *Eimeria* spp., 20.6% *Entamoeba* spp., 6.0% *Moniezia* spp. and *Paramphistomum* spp., 5.4% *Strongyloides* spp., 3.2% *Dicrocoelium* spp., 2.8% *Trichuris* spp., 2.5% *Giardia* spp. to 0.3% *Cryptosporidium* spp. (Table 1). With regards to sheep (total examined samples: 40), a total of six GI parasites (four helminths and two protozoa) were detected. Similarly, in goats, strongyle (80.0%) was the highest infection rate in sheep, followed by *Moniezia* spp. (10.0%), *Entamoeba* spp. (7.5%), *Eimeria* spp. (5.0%), *Strongyloides* spp. (2.5%) and *Trichuris* spp. (2.5%). Contrastingly, only *Entamoeba* spp. (75.0%) and strongyle (25.0%) were recorded in deer (total examined samples: 70).

Table 1. Infection rate of gastrointestinal parasites by species in small ruminants

Animal type / Parasitic Infection	Goats			Sheep			Deer			Total		
	n	%[a]	%[b]	n	%[a]	%[b]	n	%[a]	%[b]	n	%[c]	%[d]
Helminths												
Dicrocoelium spp.	10	3.2	4.0	–	–	–	–	–	–	10	2.3	3.3
Moniezia spp.	19	6.0	7.6	4	10.0	11.4	–	–	–	23	5.4	7.6
Strongyle	210	66.5	83.7	32	80.0	91.4	4	5.7	25.0	246	57.7	81.5
Strongyloides spp.	17	5.4	6.8	1	2.5	2.9	–	–	–	18	4.2	6.0
Trichuris spp.	9	2.8	3.6	1	2.5	2.9	–	–	–	10	2.3	3.3
Paramphistomum spp.	19	6.0	7.6	–	–	–	–	–	–	19	4.5	6.3
Protozoa												
Cryptosporidium spp.	1	0.3	0.4	–	–	–	–	–	–	1	0.2	0.3
Eimeria spp.	99	31.3	39.4	2	5.0	5.7	–	–	–	101	23.7	33.4
Entamoeba spp.	65	20.6	25.9	3	7.5	8.6	12	17.1	75.0	80	18.8	26.5
Giardia spp.	8	2.5	3.2	–	–	–	–	–	–	8	1.9	2.6

[a]Frequency was calculated based on total infected animals for each species (n) divided by total animal sampled (316 goats, 40 sheep and 70 deer).
[b]Frequency was calculated based on total infected animals for each species (n) divided by total infected animals (251 goats, 35 sheep and 16 deer).
[c]Frequency was calculated based on the total infected animals for each species (n) divided by 426 studied animals.
[d]Frequency was calculated based on the total infected animals for each species (n) divided by 302 infected animals.

Monoparasitism was observed in most of the sheep, of which 74.3% of 35 had single strongyle infection, and three respective individuals (2.9% of 35) had single *Moniezi*a spp., *Eimeria* spp. and *Entamoeba* spp. infections. However, there were six individuals (15.0% of 35) were observed with polyparasitism which involved "helminth+helminth" (50.0% of 6) and "helminth+protozoa" (50.0% of 6) infections. All examined deer were observed with monoparasitism, of which 75.0% of 16 had single infection of *Entamoeba* spp. and 25.0% of 16 had single strongyle infection. (Table 2).

In goats, polyparasitism (43.7% of 251 GI parasite positive samples) was slightly more prevalent than monoparasitism (35.8% of 251). Specifically, single strongyle infection was more prevalent with a rate of 35.5% of 251 GI parasite positive goats. In contrast, a total of 32 types of concurrent infections were observed in goats (Table 2). These combinations were divided into three categories, "Helminth+Helminth" (17.4% of 251), "Helminth+Protozoa" (73.2% of 251) and "Protozoa+Protozoa" (9.4% of 251). Among these categories, "Helminth+Protozoa" infections comprised 20 types of parasite combinations from double to quintuple infections. The concurrent infection of strongyle and *Eimeria* spp. (13.5% of 251) exhibited the highest infection rate among the types of polyparasitism in goats. In addition, the high prevalence of co-GI parasitic infection in "Helminth+Helminth" was represented by "*Paramphistomum* spp. + strongyle" (3.2% of 251); "Protozoa+ Protozoa" was "*Eimeria* spp. + *Entamoeba* spp." (3.2% of 251) (Table 2).

DISCUSSION

In the present study, strongyles were the most prevalent GI parasites among goats (66.5%) and sheep (80.0%). Strongyle infections have been one of the main persisting problems in the livestock industry. For effective disease control, one of the key factors that have been neglected for some reasons was the accurate identification of the strongyle species implicated. Based on traditional faecal culture method, *H. contortus* and *Trichostrongylus* spp. were the main strongyle species infected in small ruminants (Chandrawathani *et al.*, 1999, 2003; Khadijah *et al.*, 2006; Basripuzi *et al.*, 2012). A subset of 99 goat samples from the present study has been used for molecular identification (Tan *et al.*, 2014), further confirming the presence of *H. contortus* and *Trichostrongylus* spp. in our samples.

In Malaysia, McMaster faecal egg count (i.e., saturated salt solution as media) was the only routine diagnostic tool performed on small ruminants resulting in under-reporting of fluke infections. In the past few decades, occurrence of *Paramphistomum* has only been reported in an abattoir study among Malaysian indigenous goats (Amin Babjee *et al.*, 1990). By using formalin ether concentration technique, we successfully detected *Paramphistomum* and *Dicrocoelium* in our studied goats. In fact, there was a serious paucity of information on *Dicrocoelium* infection among small ruminants in Malaysia. Their prevalence and abundance are not systematically recorded nor are they made available in local or international publications. *Dicrocoelium* infection in Malaysian goats was only filled by a series of methodical checking of the list on helminths of domestic animals in West Malaysia published approximately 26 years ago (Lee *et al.*, 1991; Shanta, 1982). In addition, Shanta (1982) has further categorized *Dicrocoelium* into the "rarely found, or recorded but not found anymore". While we demonstrate the usefulness of formalin ether concentration technique in diagnosing flukes, our results also expose the limitation of routine diagnosis works (single coprological detection method) performed in veterinary diagnosis laboratories. Hence, results from the present study are significant and provide crucial knowledge on the occurrence of *Dicrocoelium* spp. among the goats.

Mixed-infection of strongyle and *Eimeria* spp. was commonly found in the present study. Our previous molecular work (Tan *et al.*, 2014) for detection of *H.*

Table 2. Types of gastrointestinal parasites in small ruminants with monoparasitism and polyparasitism

Type of Parasitism	Goats*		Sheep*		Deer*	
	n	%	n	%	n	%
Monoparasitism						
Helminth						
Moniezia spp.	0	0	1	2.9	0	0
Strongyle	89	35.5	26	74.3	4	25.0
Strongyloides spp.	1	0.4	0	0	0	0
Protozoa						
Eimeria spp.	9	3.6	1	2.9	0	0
Entamoeba spp.	10	4.0	1	2.9	12	75.0
Giardia spp.	2	0.8	0	0	0	0
Polyparasitism						
Helminth + Helminth						
Double Infection						
Dicrocoelium spp. + Strongyle	5	2.0	0	0	0	0
Paramphistomum spp. + Strongyle	8	3.2	0	0	0	0
Moniezia spp. + Strongyle	1	0.4	2	5.7	0	0
Strongyle + *Strongyloides* spp.	4	1.6	0	0	0	0
Strongyloides spp. + *Trichuris* spp.	1	0.4	0	0	0	0
Triple Infection						
Dicrocoelium spp. + *Paramphistomum* spp. + Strongyle	2	0.8	0	0	0	0
Moniezia spp. + Strongyle + *Strongyloides* spp.	0	0	1	2.9	0	0
Strongyle + *Strongyloides* spp. + *Trichuris* spp.	3	1.2	0	0	0	0
Helminth + Protozoa						
Double Infection						
Paramphistomum spp. + *Eimeria* spp.	1	0.4	0	0	0	0
Strongyle + *Eimeria* spp.	34	13.5	1	2.9	0	0
Strongyle + *Entamoeba* spp.	18	7.2	1	2.9	0	0
Stronngyloides spp. + *Eimeria* spp.	1	0.4	0	0	0	0
Trichuris spp. + *Entamoeba* spp.	1	0.4	0	0	0	0
Triple Infection						
Dicrocoelium spp. + Strongyle + *Eimeria* spp.	2	0.8	0	0	0	0
Moniezia spp. + Strongyle + *Eimeria* spp.	11	4.4	0	0	0	0
Moniezia spp. + Strongyle + *Entamoeba* spp.	2	0.8	0	0	0	0
Paramphistomum spp. + Strongyle + *Eimeria* spp.	2	0.8	0	0	0	0
Paramphistomum spp. + Strongyle + *Entamoeba* spp.	3	1.2	0	0	0	0
Strongyle + *Eimeria* spp. + *Entamoeba* spp.	10	4.0	0	0	0	0
Strongyle + *Strongyloides* spp. + *Eimeria* spp.	2	0.8	0	0	0	0
Strongyle + *Trichuris* spp. + *Entamoeba* spp.	0	0	1	2.9	0	0
Quadruple Infection						
Moniezia spp. + Strongyle + *Eimeria* spp. + *Entamoeba* spp.	5	2.0	0	0	0	0
Paramphistomum spp. + Strongyle + *Eimeria* spp. + *Entamoeba* spp.	2	0.8	0	0	0	0
Paramphistomum spp. + *Strongyloides* spp. + *Eimeria* spp. + *Entamoeba* spp.	1	0.4	0	0	0	0
Strongyle + *Eimeria* spp. + *Entamoeba* spp. + *Giardia* spp.	2	0.8	0	0	0	0
Strongyle + *Strongyloides* spp. + *Eimeria* spp. + *Entamoeba* spp.	1	0.4	0	0	0	0
Strongyle + *Strongyloides* spp. + *Trichuris* spp. + *Eimeria* spp.	2	0.8	0	0	0	0
Quintuple Infection						
Dicrocoelium spp. + Strongyle + *Strongyloides* spp. + *Trichuris* spp. + *Eimeria* spp.	1	0.4	0	0	0	0
Protozoa + Protozoa						
Double Infection						
Eimeria spp. + *Entamoeba* spp.	8	3.2	0	0	0	0
Eimeria spp. + *Giardia* spp.	2	0.8	0	0	0	0
Cryptosporidium spp. + *Eimeria* spp. + *Entamoeba* spp.	1	0.4	0	0	0	0
Eimeria spp. + *Entamoeba* spp. + *Giardia* spp.	2	0.8	0	0	0	0

*Frequency was calculated based on total infected animals for each species (n) divided by total infected animals (251 goats, 35 sheep and 16 deer).

contortus and *Trichostrongylus* spp. on a subset of goat samples (47.1% of 210 microscopically strongyle positive samples from the present study) revealed that *Eimeria* spp. were co-infected with *H. contortus* (7 or 7.1% of 99) and *T. colubriformis* (6 or 6.1% of 99) (data not shown). In addition, triple infection among *Eimeria* spp., *H. contortus* and *T. colubriformis* was also observed (18 or 18.2% of 99) (data not shown). It is important to note that co-infections could have greater impact on the animal health, such as enteritis (*Eimeria* spp. + *T. colubriformis*); severe scouring, inappetence, reduction of body weight, and consequently accelerate the death of livestock (*Eimeria* spp. + *H. contortus*) (Kaufmann, 1996; Abakar *et al.*, 2001). In addition, the detected flukes (i.e, *Dicrocoelium* spp. and *Paramphistomum* spp.) in the present study were more likely to co-occur with other GI parasites. These findings were also supported by a study in Thailand, where the detected *Paramphistomum* spp. was co-infected with other GI parasites (i.e., strongyle, *Trichuris* spp., *Moniezia* spp., *Eurytrema pancreaticum*) (Sangvaranond *et al.*, 2010).

CONCLUSIONS

In conclusion, this study has updated the current status of GI parasitic infection in Malaysian small ruminants and this is the comprehensive documentation on the diversity and multiple GI parasitisms among small ruminant especially goats in Malaysia. The gathered GI parasitic infection data will be useful in providing information on prevalence, disease control and prediction of the future trends of the diseases (i.e., application of Geographical Information System) for an effective farm management. However, one limitation of the study is that the clinical signs assessment was not conducted because this study merely focused on parasite detection, and therefore its impact toward co-parasitic infections among small ruminants cannot be conclusively identified. Future works are required for the assessment of animal health in relation to parasitic infections, particularly multiparasitism.

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