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RESEARCH ARTICLE

Gastrointestinal parasitic infections of buffaloes (*Bubalus bubalis*) in Sarawak Borneo: Prevalence, risk factors, and farming practices

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ABSTRACT

The objectives of this study were to investigate the prevalence and associated risk factors for gastrointestinal (GI) parasites in buffaloes from various areas of Sarawak, and to assess current management practices of GI parasites among farmers. Faecal samples were collected from 15 farms and 129 animals, as well as data on farm and animal-based characteristics. A total of 129 faecal samples were examined for GI parasites using a modified McMaster and sedimentation. Association between potential risk factors and the prevalence of GI parasites was investigated using Chi-square statistic. The prevalence of *Paramphistomum* sp., strongyles, and coccidia were 75.2% (95% CI±7.5), 52.7% (95% CI±8.6) and 48.1% (95% CI±8.6), respectively. Farms which had a grazing area less than 50 acres in size had significantly higher prevalence of strongyles (70.5%, χ^2 = 8.34, P = 0.004) and paramphistomes (88.6%, χ^2 = 6.46, P = 0.01) relative to farms with a larger grazing area (43.5% and 68.2%, respectively). Prevalence of strongyles was lower in farms that did not implement a cut- and-carry system (45.6%, χ^2 = 4.17, P = 0.04) in comparison to those that did (64%). The prevalence of paramphistomes was higher on farms with more than 40 animals (80.6%, χ^2 = 3.18, P = 0.05) relative to farms with fewer animals. The majority of farmers surveyed (67.9%) showed awareness of GI parasite infection and reported that they recognized the associated symptoms. Most farmers practised deworming, and ivermectin was the most commonly used anthelminthic (60.4%); only 1.9% of farmers used albendazole. Overall this study revealed a high prevalence of GI parasites in buffalo in Sarawak. Although farmers report they are aware of parasitic diseases, further education is still required. This could include how they can successfully implement on-farm changes to reduce the prevalence of GI parasites in their herds.

Keywords: Buffalo; gastrointestinal parasites; risk factors; farmers; Sarawak.

INTRODUCTION

Gastrointestinal (GI) parasites are ubiquitous and they contribute significantly to reduced productivity in the buffalo industry (Gorsich *et al.*, 2014). The effects of GI parasites on their hosts are related to the depletion of host resources through reduced intestinal function (Stewart & Penzhorn, 2004), or limiting the capacity of the host to control co-infecting parasites (Gorsich *et al.*, 2014). Such effects are manifested at different degrees depending on the immune status and immune response of the host (Graham-Brown *et al.*, 2018). Economic losses in the buffalo industry, particularly in smallholder farming systems, have also been attributed to the deleterious effects of GI parasites (Raza *et al.*, 2007). However, the precise economic impact of these infections is unknown.

Parasitism is considered to be the second most significant disease in Malaysia's livestock industry with high morbidity and mortality (Nor-Azlina *et al.*, 2011). The commonly reported parasites in buffaloes include strongyles (Karim *et al.*, 2016; Nurhidayah *et al.*, 2019), *Trichuris* sp., *Moniezia* sp., *Toxocara vitulorum* (Dorny *et al.*, 2015; Gunathilaka *et al.*, 2018), and *Theileria* sp. (Rohaya *et al.*, 2017); as well as trematodes such as *Fasciola gigantica* (Das *et al.*, 2018; Zhang *et al.*, 2019), Paramphistomes (Gunathilaka *et al.*, 2018) and *Gigantocotyle explanandum* (Malik *et al.*, 2017). The

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Malaysian water buffalo*, Bubalus bubalis* is an important component of livestock production for the inhabitants of Borneo Sarawak. Most buffalo farmers in Sarawak practice extensive management systems. Buffaloes often co-graze with other species and this may increase their exposure to parasitic helminths. However, several studies have stated that a deeper understanding of feeding behaviour and the potential for cross-nematode species transmission could provide a better comprehension of the host-parasite relationship (Hoste *et al*., 2010; Moco *et al*., 2014).

Currently, there is no available data on GI parasitic infections and associated risk factors in Malaysian water buffalo, as most of the previously published studies were conducted in cattle and goats (Chandrawathani *et al*., 2009; Nor-Azlina *et al*., 2011; Basripuzi *et al*., 2012). Furthermore, there is limited data on the socio-demographics of buffalo farmers, as well as herd health control strategies and specific management practices used by these farmers to manage GI parasites of buffaloes in Sarawak. The present study was designed to investigate, 1) the prevalence of GI parasites in buffalo located in the major buffalo rearing areas of Sarawak, namely Bintulu, Limbang, and Lawas, 2) describe the socio-demographic characteristics, knowledge, and management practices of GI parasites of buffalo farmers in these areas.

MATERIALS AND METHODS

Study design, sample size estimation and sampling protocol

A cross-sectional study was conducted involving buffalo farms in Bintulu, Lawas, and Limbang between October 2017 and May 2019 (Figure 1). These locations were selected since 86% (5,500) of the buffalo in Sarawak are located within these regions. The farmers' details were provided by the Department of Veterinary Service (DVS), Sarawak. On initial contact, the farmers were briefed about the study objectives and the inclusion criteria. The inclusion criteria included, 1) animals should not have been treated with anthelmintics for at least 3 months prior to sampling, 2) farms were required to have up to date records on animal health, 3) the farm should be registered with the DVS, and 4) farmers should be available during the scheduled time for farm visits. A total of 15 farms fulfilled the inclusion criteria and were enrolled in the study. Only one farm agreed to participate in Bintulu.

Farm and animal assessment

Information was collected regarding the characteristics of the farm including: management system, the number of pens, herd size, the number of adult and young animals, size of wallowing area and grazing area, source of livestock, deworming practices, method of feed preparation, and the source of feed. The sex, breed, and age of the animals were recorded. Domestic water buffalo are classified into two different types: swamp ('Kerbau Sawah') and river ('Murrah') buffalo. Swamp buffaloes are mainly used for labour and are raised for meat purposes, whereas Murrah buffaloes are used for milk production. Buffaloes were classified by age as either calf (less than 1 year), adults (1–5 years), or old (more than 5 years).

The buffaloes from Limbang and Lawas were owned by smallholder farmers who had small herd sizes, ranging from two to 16 animals. Farms from these regions mainly practiced extensive management systems. In Bintulu, only one farm fulfilled the inclusion criteria. The buffaloes at this farm

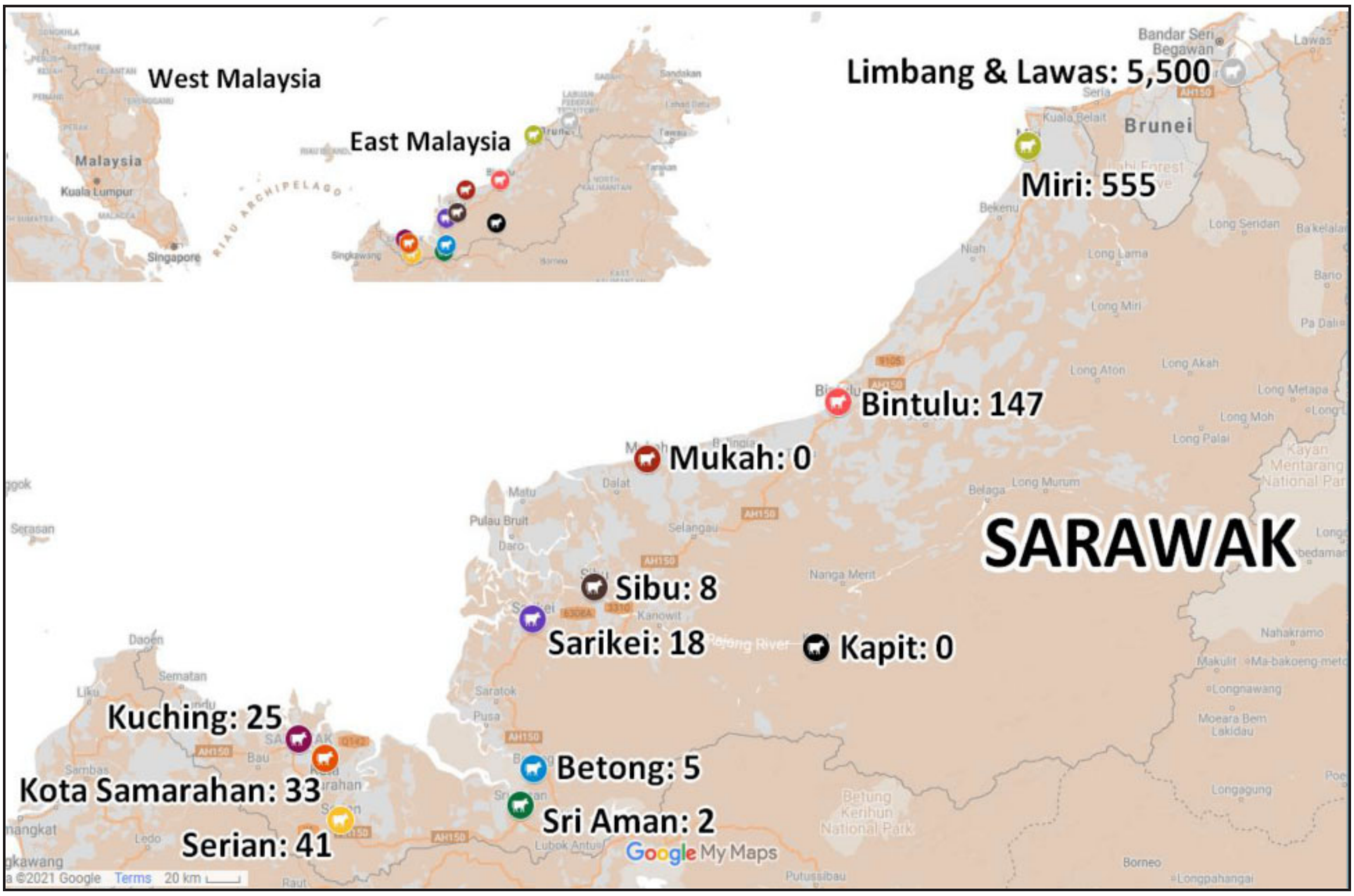

Figure 1. Distribution of buffalo in Borneo Sarawak, East Malaysia, 2020 (Source: Department of Veterinary Services, DVS).

were managed semi-intensively and provided with grazing areas at the University Agriculture Park (Livestock Unit) all year round. The animals were fed using a 'cut-and-carry' feeding system which entailed the harvest and preparation of Napier grass within given farmland.

Sampling of animals

On farms with large herd sizes, animals were randomly selected from the different pens. If a farm had more than one pen, an equal number of animals were sampled from each. Sampling was conducted to reflect the proportion of adult and young animals in each herd. In farms with less than 10 buffaloes, all animals were sampled.

Each animal was immobilized in a standing position and a single researcher collected the faecal samples per rectum using a clean, gloved hand (Jolles *et al*., 2008). Faecal samples were transferred to a sampling bottle and labelled. Samples were stored in iceboxes and transported to the office (20 to 45 minutes distance) where they were then stored at 4°C. After completion of each sampling session (3 to 4 days), the faecal samples were stored in iceboxes and transported to the laboratory which took approximately 9 hours and refrigerated at 4°C until analysis. The time between sampling animals and testing at the laboratory ranged from 1 to 2 weeks.

Parasitological techniques

Faecal oocyst/egg counts were performed at the Parasitology Laboratory, Faculty of Agriculture Science and Forestry, Universiti Putra Malaysia, Bintulu, Sarawak. A modified McMaster technique was used to perform the oocyst and egg count (Kamaludeen *et al*., 2010). The results were recorded as strongyle eggs per gram of feces (epg) or coccidia oocysts per gram of feces (opg). Approximately 2 g of feces were used to determine the number of oocysts and nematode eggs. For trematodes (i.e., flukes), approximately 5g of feces was analysed using a sedimentation technique as described by Kamaludeen *et al*. (2019). Parasite intensity and infection burden were evaluated based on the counts of nematode or trematode eggs and coccidia oocysts (Ezenwa & Jolles, 2008; Gorsich *et al*., 2014).

Questionnaire design and administration

A structured questionnaire was developed to assess farmer demographics, current management practices, and knowledge of GI parasites. To assist design we (1) reviewed previous surveys of buffalo farmers that focuses on health and welfare management, (2) we reviewed specific practices relating to buffalo farming in the Malaysian context to ensure the questionnaire was appropriate, and (3) the questionnaire was assessed by staff from the DVS Sarawak.

The questionnaire was split into three sections and comprised of 27 questions. The first section focused on the demographics of the farmers. The second section was about farm details and management practices, and included questions on the size of the farm, animal feed, water sources, herd size, and management system. The final section collected information on health management such as control of parasitic infection, use of anthelminthic drugs, deworming practices, and also included questions to assess farmers' knowledge of the symptoms of parasitic infections in animals.

The registry list provided by the DVS Sarawak were used to contact buffalo farmers within the study location. A total of 150 farmers that were registered with the DVS were contacted to participate in the survey. Each farmer was informed about the purpose of the study, methodology, confidentiality of all information provided, and their voluntary consent to take part in the survey. Fifty-three (35%) of the farmers agreed to take part. They were asked to sign a consent form, and then complete the questionnaire.

Data analysis

All statistical analyses were conducted using IBM SPSS (version 23.0). A Kolmogorov-Smirnov normality test was conducted for the dependent (prevalence of GI parasites and coinfection) and independent variables (age, sex, breed, and location) to determine the appropriate descriptive statistic (mean and standard deviation, or median and interquartile range).

The prevalence of each parasite was computed using descriptive statistics. Chi-square statistics were used to determine the univariate associations between potential risk factors (i.e., animal and farm factors) and the prevalence of strongyles, coccidia, paramphistomes, and co-infection. A P-value less than 0.05 was considered significant. Since only one farm was sampled from Bintulu, the association between farm location and prevalence of parasitic infection was only calculated for farms in Limbang and Lawas.

Data from the completed questionnaires were inputted into Microsoft Excel, before transfer to the statistical software. Descriptive statistics were used to analyse the responses based on the socio-demographic characteristics of farmers, management practises, and their knowledge on the effect of GI parasites to buffalo.

RESULTS

Prevalence and intensity of GI parasites

A total of 129 faecal samples were collected from Limbang, Lawas, and Bintulu District. The majority of the sampled buffaloes were swamp type (83.7%) with fewer Murrah buffaloes (16.3%) sampled. Three parasitic species were identified: *Paramphistomum* sp. with egg counts ranged from 0 to 1440 epg, coccidia oocyst counts ranged from 0 to 500 opg, and *Strongyle* sp. egg counts ranged from 0–250 epg (Table 1). The highest prevalence across all farms was *Paramphistomum* sp. (75.2%). Co-infection with multiple GI parasites was seen at nine farms (Table 1). Only one farm showed no evidence of GI parasitic infection (Farm 3; Table 1).

Factors associated with the prevalence of strongyle, coccidia and paramphistomes

Farms with a grazing area less than 50 acres had significantly higher prevalence of strongyles (70.5%, χ^2 = 8.34, degree of freedom [df] = 1, P = 0.004) than farms where the grazing area was more than 50 acres (43.5%; Table 2). There was a lower prevalence of strongyles on farms that did not practise the cut-and carry-systems (45.6%, χ^2 = 4.17, P = 0.04) compared to those that did (64%; Table 2).

Buffaloes from Limbang had a lower prevalence of paramphistomes (56.1%, χ^2 = 8.7, df =1, P = 0.003) that those from Lawas. Farms with a grazing area less than 50 acres in size had significantly higher prevalence of paramphistomes (88.6%, χ^2 = 6.46, df = 1, P = 0.01) relative to farms with more than 50 acres (68.2%). Similarly farms with more than 40 animals also had significantly higher prevalence of paramphistomes (80.6%, χ^2 = 3.18, P = 0.05) than farms with less than 40 animals.

None of the animal factors was significantly associated with the prevalence of any of the GI parasites. The prevalence of co-infection was higher in farms engaging in self-acquisition of livestock (58.7%, χ^2 = 4.09, df =1, P = 0.04)

Table 1. Animals positive with infection, prevalence of strongyle, coccidia, *Paramphistomum* sp. and co-infections of parasitic infections (%, 95% CI); egg/oocytes counts (Mean±SE) from 15 buffalo farms in Bintulu, Limbang and Lawas, Sarawak, Malaysia

Division	Farm ID	n (positive animals)	Percentage (95% CI) of each species; EPG/OPG range (Mean±SE)						
			Strongyle	EPG range (Mean±SE)	Coccidia	OPG range (Mean±SE)	*Paramphistomum* sp.	EPG range (Mean±SE)	Co-infection
Bintulu	Teaching farm	12 (12)	91.7 (±15.7)	0-200 (83.3±14.2)	75.0 (24.5)	0-150 (54.2±13.0)	100.0 (-)	219-634 (452.8±40.4)	66.7 (26.7)
Limbang	Farm 1	9 (4)	33.3 (±30.8)	0-100 (22.2±12.1)	22.2 (±27.2)	0-50 (16.7±11.8)	11.1 (±20.5)	0-233 (25.9±25.9)	0 (-)
	Farm 2	5 (1)	20.0 (±35.1)	0-150 (30.0±30.0)	0 (-)	0	0 (-)	0	0 (-)
	Farm 3	5 (0)	0 (-)	0	0 (-)	0	0 (-)	0	0 (-)
	Farm 4	6 (6)	50.0 (±40.0)	0-50 (25.0±11.2)	6.7 (±37.8)	0-500 (125.0±78.3)	6.7 (±12.6)	21-820 (240.7±121.4)	50.0 (±40.0)
	Farm 5	16 (16)	56.3 (±24.4)	0-100 (37.9±9.7)	30.0 (±23.7)	0-150 (56.3±14.3)	100.0 (-)	154-544 (346.6±30.2)	43.8 (±24.4)
Lawas	Farm 6	15 (3)	13.3 (±17.2)	0-100 (13.3±9.1)	40.0 (±12.6)	0-50 (3.3±3.3)	100.0 (-)	0-258 (17.2±17.2)	0 (-)
	Farm 7	7 (7)	80.0 (±24.8)	0-250 (105.0±28.3)	66.7 (±28.4)	0-150 (30.0±17.0)	100.0 (-)	136-1440 (466.0±139.6)	30.0 (±28.4)
	Farm 8	10 (10)	50.0 (±14.0)	0-250 (40.0±24.5)	90.0 (±30.4)	0-150 (30.0±15.3)	100.0 (-)	120-693 (339.0±51.5)	20.0 (±24.8)
	Farm 9	10 (10)	77.8 (±27.2)	0-200 (83.3±25.0)	66.7 (±30.8)	0-200 (72.2±25.2)	100.0 (-)	23-934 (500.1±101.9)	55.6 (±32.5)
	Farm 10	10 (10)	60.0 (±30.4)	0-100 (35.0±10.7)	62.5 (±18.6)	0-100 (60.0±10.0)	100.0 (-)	91-541 (311.4±39.0)	50.0 (±31.0)
	Farm 11	8 (8)	62.5 (±33.5)	0-100 (37.9±12.5)	87.5 (±22.9)	0-100 (56.3±14.8)	100.0 (-)	198-884 (518.4±19.1)	37.5 (±33.5)
	Farm 12	10 (10)	70.0 (±28.4)	0-100 (50.0±12.9)	50.0 (±31.0)	0-150 (40.0±16.3)	100.0 (-)	169-683 (347.8±46.7)	30.0 (±28.4)
	Farm 13	2 (2)	50.0 (±50.0)	0-50 (25.0±25.0)	50.0 (±50.0)	0-50 (25.0±25.0)	100.0 (-)	21-953 (487.0±466.0)	0 (-)
	Farm 14	2 (2)	0 (-)	0	50.0 (±50.0)	0-150 (75.0±75.0)	100.0 (-)	127-358 (242.5±115.5)	0 (-)
Mean (%) / Total	**15 farms**	**129 (103)**	**52.7±8.6**	**0-250 (44.2±5.0)**	**48.1±8.6**	**0-500 (41.9±5.7)**	**75.2±7.5**	**0-1440 (292.0±23.6)**	**30.2±7.9**

n=number of animals sampled

compared to those assisted by DVS, and farms practising cut-and carry-system (62.0%, χ^2 = 5.01, df =1, P = 0.02) relative to farms not practising this system.

Buffalo farming in Sarawak: characteristics and management practises assessed by questionnaire

There was a regional variation in the survey response rates, as most of the responses were from farmers in Lawas (44/53; 83%). Only eight responses were from farmers in Limbang (15.1%), and there was only one response from Bintulu (Table 3).

The majority of the farmers were male (77.4%) compared to females (3.8%, Table 3). Most of the respondents were above 60 years old (52.8%) and only 11.3% were below 50 years old. Most of the respondents were full-time farmers (60.4%) and 73.6% had more than 20 years of experience working in the buffalo farming industry. However, it can be noted that the results in Table 3 shows missing data. This was unavoidable as farmers declined to answer some of the questions, this was due to the sensitivity of the questions and an unwillingness to provide such information.

The majority of respondents (90.6%) practised an extensive management system and depended mostly on grazing natural pastures. Seventy per cent of the participants raised their buffaloes for replacement, whereas 7.5% of them obtained their buffalo from overseas. The herd size of most of the respondents (86.8%) was either less than or equal to 30 buffaloes, and 96.2% of them had land sizes ranging from 0 to 200 acres. The main source of drinking water for animals on the farms was from streams (67.9%) (Table 3).

Ivermectin was the most frequently used anthelminthic (60.4%), whilst only 1.9% of the respondents used albendazole (Table 3). The majority of respondents (67.9%) were aware of helminth infection in their animals, and they were familiar with associated symptoms. However, only half of the respondents believed that parasite infection occurs mostly in calves, whereas 20.7% of them thought that adults were more susceptible to GI parasites.

DISCUSSION

Little is known about GI parasite infections in livestock from Borneo Island, therefore our findings serve to describe the importance of such infections in buffalo in the studied locations. We identified high prevalence of all the parasites studied: strongyles, coccidia and *Paramphistomum* sp.; indeed on ten of the fifteen farms we visited every animal sampled was positive for at least one of these groups of parasites.

Table 2. Association between prevalence of strongyle, coccidia and paramphistomes and associated animal and farm factors in the 15 buffalo farms in Bintulu, Limbang and Lawas, Sarawak, Malaysia

Variables	Number of animals/ farm[a]	Strongyles			Coccidia			Paramphistomes			Co-infection		
		n (%)	χ^2	P-value	n (%)	χ^2	P-value	n (%)	χ^2	P-value	n (%)	χ^2	P-value
Age													
Adult	46	35 (77.8)	0.01	0.91	27 (60.0)	1.29	0.25	43 (95.6)	2.96	0.24	25 (55.6)	0.92	0.32
Old	30	23 (76.7)			14 (46.7)			30 (100.0)			20 (66.7)		
Breed													
Murrah	21	9 (42.9)	0.97	0.32	10 (47.6)	0.001	0.97	16 (76.2)	0.01	0.90	10 (47.6)	0.04	0.84
Swamp	108	59 (54.6)			51 (47.2)			81 (75.0)			54 (50.0)		
Sex													
Female	88	48 (54.5)	0.37	0.54	41 (46.6)	0.05	0.82	69 (78.4)	1.53	0.21	44 (50.0)	0.01	0.89
Male	41	20 (48.8)			20 (48.8)			28 (68.3)			20 (48.8)		
Location[b]													
Limbang	6	16 (39.0)	2.37	0.17	16 (39.0)	0.75	0.38	23 (56.1)	8.70	**0.003***	16 (39.0)	0.75	0.38
Lawas	8	41 (53.9)			36 (47.4)			62 (81.6)			36 (47.4)		
Herd size (in acres)													
<40	7	32 (51.6)	0.05	0.81	28 (45.2)	0.21	0.64	43 (69.4)	3.18	**0.05**	31 (50.0)	0.007	0.93
>40	8	36 (53.7)			33 (49.3)			54 (80.6)			33 (49.3)		
Source[c]													
Self	10	38 (60.3)	2.85	**0.09**	34 (54.0)	2.20	0.13	50 (79.4)	1.14	0.28	37 (58.7)	4.09	**0.04***
Vet	5	30 (45.5)			27 (40.9)			47 (71.2)			27 (40.9)		
Cut and carry													
No	9	36 (45.6)	4.17	**0.04***	33 (41.8)	2.48	0.11	55 (69.6)	3.39	**0.06**	33 (41.8)	5.01	**0.02***
Yes	6	32 (64.0)			28 (56.0)			42 (84.0)			31 (62.0)		
Deworming[d]													
<8 week	2	1 (25.0)	0.34	1.27	2 (50.0)	0.01	0.91	4 (100.0)	1.36	0.24	2 (50.0)	0.001	0.98
>8 week	13	67 (53.6)			49 (47.2)			93 (74.4)			62 (49.6)		
Grazing area (in acres)													
<50	6	31 (70.5)	8.34	**0.004***	23 (52.3)	0.66	0.41	39 (88.6)	6.46	**0.01***	26 (59.1)	2.40	0.12
>50	9	27 (43.5)			38 (44.7)			58 (68.2)			38 (44.7)		
Water source													
Pond	6	40 (58.8)	2.15	0.14	33 (48.5)	0.08	0.76	54 (79.4)	1.37	0.24	36 (52.9)	0.42	0.63
Stream	9	28 (45.9)			28 (45.9)			43 (70.5)			28 (45.9)		

Note: n (%) = number of positive animals (prevalence percentage); χ^2 = chi-square statistics
Statistical significant associations at $P < 0.05$ are presented in bold and asterisk (*), while $P < 0.1 > 0.05$ are presented in bold only
[a]Number of animals was presented for the animal-based variables, while number of farms was presented for farm-level variables
[b]The farm and animals in Bintulu were not included in the location analysis due to insufficient numbers
[c]Source refers to farms engaging the DVS in acquisition of new stocks and those without such practice
[d]When the animals last treated

Table 3. Socio-demographics of the respondents, farm characteristics and respondents' management practices related to gastrointestinal parasites

Variable	Frequency	Percentage (%)	Variable	Frequency	Percentage (%)	Variable	Frequency	Percentage (%)
Socio-demographic characteristics			Farm characteristics			Management practices		
Sex			**Source of livestock**			**Deworming**		
Male	41	77.4	Self	37	69.8	2-4	1	1.9
Female	2	3.8	Trader/overseas	4	7.5	4-8	1	1.9
Age			Veterinarian	4	7.5	> 8	31	58.5
< 20	0	0	**Herd size**			Never	1	1.9
21-30	1	1.9	0-30	46	86.8	**Drugs used**		
31-40	0	0	31-60	4	7.5	Ivermectin	32	60.4
41-50	5	9.4	61-90	1	1.9	Albendazole	1	1.9
51-60	14	26.4	> 91-120	1	1.9	Fenbendazole	0	0.0
Location/region			**Grazing field size (acre)**			Others	1	1.9
Bintulu	1	1.9	0-200	46	90.6	**Awareness of worm infestation in buffalo**		
Lawas	44	83.0	201-400	0	0	Yes	36	67.9
Limbang	8	15.1	401-600	0	0	No	9	17.0
			> 600	1	1.9	**Infection signs**		
Experience (years)			**Cut and carry system**			Diarrhea	8	22.2
< 10	6	11.3	Yes	10	18.9	Thin	4	11.1
10-20	6	11.3	No	34	64.2	Lack of appetite	1	2.8
> 20	39	73.6	**Additional feed**			Bad fur coating	1	2.8
			Salt block	23	43.4	Death	0	0
			None	9	17.0	All	22	61.1
			Others/coarse salt/pellet	15	28.3			
			Wallowing area			**Occurrence of worm infestation**		
			Pond/lake	30	56.6	Calves	27	50.9
			Stream	15	28.3	Adult	11	20.7
			None	0	0.0			
			Others	1	1.9			
			Source of drinking water			**Plant-based treatment(s)**		
			Rainwater	4	7.5	Leaves of sweet flag plant, *Acorus calamus L.* ('jerangau'), Shoots of 'kalimpapa' (*Vitex cofassus*), Shoots and boiled water of tobacco, *Nicotiana* sp. ('tembakau'), Gliricidia plant, river tamarind or 'petai belalang' (*Leucaena leucocephala*)		
			Jabatan Bekalan Air	1	1.9			
			Pond/Lake	4	7.5			
			Stream	36	67.9			
			Others	0	0.0			
			Type of veterinary services					
			Vaccine	4	10.5			
			Drugs	3	7.9			
			Advice	5	13.2			
			All	30	78.9			

Rumen and liver fluke infections have been reported in ruminants in various farms in West Malaysia (Zainalabidin *et al*., 2015; Khadijah *et al*., 2017; Diyana *et al*., 2020), but reports from Borneo Island have been lacking. A lower prevalence of GI parasites (18.8%) has been reported in buffaloes and cattle from Sri Lanka (Gunathilaka *et al*., 2018), and 41.4% of the animals sampled in Bangladesh were positive for *P. cervi* (Bersissa *et al*., 2011). The egg counts for P*aramphistomum* sp. reported in the present study were relatively low, however, the overall prevalence was higher than that reported from other Asian countries. The current findings on FEC of strongyle and coccidia were notably lower than previous studies Bahrami and Alborzi (2013), Nath *et al*. (2016), and Nurhidayah *et al*. (2019). Our analysis of the faecal egg/oocyst counts from a total of 129 buffaloes on 15 farms detected a high number of zero counts, and the pattern of egg output did not follow a log-normal distribution pattern. It is important to remember that egg shedding is intermittent, and this limits the detection of infection via faecal sample analysis. Such distributions of zero egg counts for individual buffalo was influenced by the degree of pasture exposure and by time of sampling (Morgan *et al*., 2005). In cattle, for instance, the level of egg excretion is generally low and highly aggregated whereby most cattle have a low egg count in their feces although a few animals will shed a higher number of eggs (Levecke *et al*., 2012).

The prevalence of strongyles was higher on farms with grazing areas less than 50 acres, and in herds fed with the cut-and-carry system. The reason for these findings is not clearly understood; however, increased exposure to infection may be attributed to a higher concentration of eggs per grazing area and the density-dependent nature of the infection (Takeuchi-Storm *et al*., 2017). In the present study, all the animals were managed extensively and were moved in groups, grazed on the same pasture throughout the year, and used for production purposes. Contamination of feeds during the cut-and-carry process may contribute to a higher exposure to the GI parasites. Therefore, management practices may be influencing the prevalence of strongyles in the study area. It should be noted that grazing management recommendations such as pasture condition, forage quantity and quality as well as stoking density are needed to improve the nutritional status of the grazing grass. Excessive grazing will lead to certain forage folliage having inadequate regrowth time which in turn will negatively affect livestock welfare due to temporary nutritional stress. The nutritional value of grasses also plays an important part in maintaining the growth and condition of livestock. Previous studies have shown that the protein and fibre contents of 55 species of forage grasses selected from 16 countries ranged from 5 to 36% and 34 to 90%, respectively (Lee *et al*., 2017). The authors also showed that lower quality forage grasses were found frequently in warmer areas, and they contained higher portions of fibre (Lee *et al*., 2017). Higher percentages of fibre are generally tougher to digest. On top of that, animals with low protein diets are more susceptible to parasite infection due to less immunoglobulin IgA-produced. Thus, mineral blocks and vitamin supplements in animal diets may be required in the studied farms (Table 3).

A higher prevalence of paramphistomes was seen on farms with a grazing area less than 50 hectares and on farms with more than 40 animals. Small streams were present on most of the farms, especially around the pasture and grazing areas. Such areas are ideal habitats for the freshwater snail intermediate host of *Paramphistomum* sp., and likely explains the prevalence of paramphistomes. Factors such as a lack of fencing (to limit buffalo wallowing activity within snail habitats) and poor biosecurity measures may also contribute to infection with paramphistomes (Charlier *et al*., 2011). The cut-and-carry system may also be a potential source of rumen fluke infection, and high cutting of grass is recommended to avoid contamination by metacercariae (Suhardono *et al*., 2006). In previous studies, it was observed that calves had a lower prevalence of *Paramphistomum* sp. compared to adult buffaloes (Maqbool *et al*., 2002; Cauquil *et al*., 2016). Of note, in this study, calves were not considered in the analysis because data was only available for two animals. However, the adult animals were categorized using a cut value of 5 years old ($\leq$ 5 years = adult, > 5 years = old). Although there was no significant association between age and prevalence of each GI parasite, 100% of old buffaloes were infected with rumen flukes. Thus, this finding is consistent with previous reports showing a higher risk of rumen flukes in older buffaloes. For example, in a study from the Republic of Ireland, the prevalence of rumen fluke infection was lower in calves, which is expected based on their age and consequently shorter exposure time (Toolan *et al*., 2015). Further investigation is required to determine whether immunity to paramphistomes is acquired in the same way as to other GI parasites (Albuquerque *et al*., 2019).

The prevalence of co-infection with more than one type of parasite was significantly associated with the cut-and-carry system; poor biosecurity during the preparation of such feed may increase parasite exposure. Those farms that acquire animals themselves, rather than through the DVS, also had a higher prevalence of co-infection. This could indicate that the DVS plays a role in improving herd health management. We identified no clinical signs of parasitic diseases during our sampling visits. This highlights the importance of the identified GI parasites in subclinical infections, such infections can still result in significant economic losses (Bersissa *et al*., 2011). Co-infection may result in significant morbidity and reduced production in livestock (Bersissa *et al*., 2011). A particularly high prevalence of combined coccidia and nematode infection was seen and is recognized in other studies (Gorsich *et al*., 2014). This finding could be attributed to similarities in exposure, age patterns of infection, and susceptibility of the animals, rather than inter-parasitic interaction (Fenton *et al*., 2010). For instance, infection with coccidia and nematodes is via the faecal-oral route and involves development of infectious phases in the environment (Gorsich *et al*., 2014).

Although some farmers did not provide their age and gender, our study revealed that the majority of buffalo famers were male. This is in contrast to findings by Raney et al. (2011) who stated that over 40% of the agricultural labour force in the developing world is comprised of women. More than three-quarters of farmers surveyed were over 50 years old, and nearly three-quarters reported that they had more than 20 years of buffalo farming experience. This is in line with previous findings by Islam *et al*. (2017) who reported 53% of buffalo farmers had over 15 years' experience. It is likely that farmers' knowledge of buffalo management is based on experiences passed from generation to generation, which has implications for the future education of farmers.

There was good awareness of helminth infections in buffaloes among farmers, which may be related to their experience. However, only around fifty percent of farmers thought that GI parasite infections are more common in calves. Ivermectin was reported as the most common drug used by farmers to treat parasitic infections in buffaloes. This drug is effective at controlling the mature and immature stages of many species of GI parasites along with lungworms and insects (Dyary, 2016). We did not investigate the reason why most farmers preferred ivermectin, however over-reliance on a single drug may influence the development of drug resistance and is worth investigating further. Despite the high prevalence of *Paramphistomum* sp. in the sampled buffaloes, no drugs effective against these parasites were used, however flukicide drugs are not readily available in Malaysia. As this parasite is increasingly being recognized as clinically significant (Khadijah *et al*., 2017; Diyana *et al*., 2020), this could become more important in the future.

This study is the first of its kind to investigate the prevalence and distribution of GI parasites in buffalo in Sarawak. However, our study is not without its limitations, including the small sample size and the incomplete responses from survey participants. Due to the inclusion criteria used, it may not be possible to translate our findings to those farms that did not meet the criteria. Future studies will need to address such limitations, and should also include geographical and climate factors that could also influence prevalence of GI parasites. Although we attempted larval culture (data not shown) this was unsuccessful and means that species-level identification of the strongyle parasites was not possible.

In conclusion, a high prevalence of strongyles, coccidia and paramphistomes were observed in buffaloes in the sampled farms in Bintulu, Limbang and Lawas, Sarawak. This epidemiological data will be useful in providing information on prevalence, disease control and prediction of the future trends of the diseases for effective farm management. The findings suggest that control and prevention measures should be taken to reduce GI parasitic infections among buffaloes in Borneo, Sarawak. Likewise, there is a need to improve management practices and farmers' knowledge about GI parasites affecting buffaloes in the region.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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