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FULL PAPER

Detection of *Schistosoma spindale* ova and associated risk factors among Malaysian cattle through coprological survey

Tiong Kai Tan[1)], Van Lun Low[2)], Soo Ching Lee[1)], Chandrawathani Panchadcharam[3)], Sun Tee Tay[4)], Romano Ngui[1)], Premaalatha Bathmanaban[3)], Kai Ling Kho[4)], Fui Xian Koh[4)], Reuben Sunil Kumar Sharma[5)], Tariq Jaafar[6)], Quaza Nizamuddin Hassan Nizam[6)] and Yvonne Ai Lian Lim[1)]*

[1)]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

[2)]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

[3)]Veterinary Research Institute, 59, Jalan Sultan Azlan Shah, 31400, Ipoh, Perak, Malaysia

[4)]Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

[5)]Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[6)]Department of Veterinary Services, Ministry of Agriculture and Agro-Based Industry Malaysia, Federal Government Administrative Center, 62630 Putrajaya, Malaysia

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Abstract

The present study was conducted to determine the occurrence of *Schistosoma spindale* ova and its associated risk factors in Malaysian cattle through a coprological survey. A total of 266 rectal fecal samples were collected from six farms in Peninsular Malaysia. The overall infection rate of *S. spindale* was 6% (16 of 266). *Schistosoma spindale* infection was observed in two farms, with a prevalence of 5.4% and 51.9%, respectively. This trematode was more likely to co-occur with other gastro-intestinal parasites (i.e., *Dicrocoelium* spp., *Paramphistomum* spp., strongyle, *Eimeria* spp. and *Entamoeba* spp.). Chi-square analysis revealed that female cattle are less likely to get *S. spindale* infection as compared to male cattle (OR = 0.3; 95% CI = 0.08–1.06; $p < 0.05$), and cattle weighing lower than 200 kg, were significantly at higher risk than those higher than 200 kg (OR = 5; 95% CI = 1.07–24.79; $p < 0.05$) to the infection. Multivariate analysis confirmed that among the cattle in Malaysia, the age (cattle with two year old and higher: OR = 21; 95% CI = 2.48–179.44; $p < 0.05$) and weight (weighing 200 kg and lower: OR = 17; 95% CI = 3.38–87.19; $p < 0.05$)

*Corresponding author: Yvonne Ai Lian Lim, Ph.D., Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
Phone: +603-7967-4748. Fax: +603-7967-4754. E-mail: limailian@um.edu.my
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were risk factors for *S. spindale* infection among Malaysian cattle.

Key Words: *Schistosoma spindale*, ova, formalin-ether concentration technique, risk factors, Malaysian cattle

Introduction

Schistosomiasis is a disease caused by parasitic flukes of the genus *Schistosoma*. These parasites have been estimated to infect 700 million cattle worldwide, with more than 165 million cattle affected in the regions of Africa and Asia[8]. To date, a total of 10 *Schistosoma* species are infectious to cattle. *Schistosoma spindale*, *Schistosoma indicum*, *Schistosoma nasale* and *Schistosoma japonicum* are the most common species infecting cattle in Asia[8,18]. With the exception of *S. nasale* which inhabit the veins of nasal mucosa, the rest of the mentioned species inhabit the mesenteric veins (i.e., *S. spindale*), portal veins, urogenital veins and may cause several clinical signs such as diarrhea (sometimes with blood traces and mucoserous secretion), anemia, edema, excessive thirst, anorexia and emaciation[3,18].

Schistosomes are prevalent and widely distributed among animals in Asia[18,22,24].The prevalence of *Schistosoma* infections in cattle ranged from 0.2% in Thailand to 72.7% in India[19,23]. In Malaysia, there has been a paucity of research on these parasites over the past 20 years. The occurrence of *S. nasale* has been intermittently recorded in buffaloes[14], *S. spindale* in cattle[9], buffaloes and goats[15]; and *S. incognitum*-like eggs in swine[16]. However, there has been no report of schistosome infection among the diagnosed animal samples (through fecal examination or observation of the internal organs during post-mortem) by animal disease diagnostic laboratories in Malaysia: Veterinary Research Institute or VRI and Regional Veterinary Laboratories. Previous reports demonstrated the presence of adult schistosomes from internal organs[9,15]. For routine animal health monitoring, it is not feasible to diagnose *Schistosoma* infection by means of slaughtering. Fecal examination (i.e., formalin-ether concentration technique) could therefore be the alternative way to detect a wide range of gastro-intestinal (GI) parasites from protozoan (oo)cysts to helminth ova. The aims of this study were (1) to detect *Schistosoma* ova in cattle fecal samples by the formalin-ether concentration technique; (2) to determine the co-occurrence of *Schistosoma* spp. with other intestinal parasites (protozoa or helminthes); and (3) to determine the risk factors associated with schistosome infection in Malaysian cattle.

Material and methods

Ethical consideration: The study protocol 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 on animal farms was obtained from owners prior to sample collection.

Coprological survey: The coprological survey of GI parasitic infection in Malaysian cattle was carried out in six government farms located in East Coast (Farm A, Kuantan, Pahang state), Northern (Farm B, Sungai Siput, Perak state), Central (Farm C, Serdang, Selangor state; Farm D, Jerantut, Pahang state) and Southern (Farm E, Jelai Gemas, Negeri Sembilan state; Farm F, Air Hitam, Johore state) parts of Peninsular Malaysia. These farms were routinely monitored by the Department of Veterinary Services, Ministry of Agriculture and Agro-Based Industry, Malaysia. All cattle in the studied farms were allowed to graze freely around the farming areas and likely to be exposed to high risk of cross-GI

Table 1. Sampling locations and cattle breeds (based on 248 individuals with complete information)

Farms	Breed			
	Pure breed	Total of individual	Cross-breed	Total of individual
Farm A (Kuantan, Pahang)	Brahman, Nellore	40	NA	NA
Farm B (Sungai Siput, Perak)	NA	NA	FriesianXJersey	4
Farm C (Serdang, Selangor)	Braford, Brangus, Herefold, Jersey, Kedah-Kelantan, Simmental	19	BrafordXBrangus, BrafordXFriesian-Sahiwal, BrafordXSimmental, BrangusXFriesian-Sahiwal, BrangusXSimmental, FriesianXJersey, FriesianXSahiwal, HerefordXFriesian-Sahiwal, JerseyXFriesian, Kedah-KelantanXBraford, Kedah-KelantanXFriesian, Kedah-KelantanXSimmental, Santa GertrudisXBrangus, SimmentalXBrangus, SimmentalXFriesian, SimmentalXFriesian-Sahiwal	98
Farm D (Jerantut, Pahang)	Drakensberger, Kedah-Kelantan, Mafriwal	37	NA	NA
Farm E (Jelai Gemas, Negeri Sembilan)	Nellore	12	NA	NA
Farm F (Air Hitam, Johor)	Jersey, Mafriwal,	38	NA	NA

NA=Not Applicable

parasitic transmission. A total of 28 breeds of cattle were studied (Table 1) with ages ranging from five months to 12 years, with weights from 102 kg to 694 kg.

Fecal samples were collected per rectum from each animal and kept at 2 to 8°C immediately post sampling and short-term storage at 4°C until further analysis. Formalin-ether concentration technique was performed on the samples to concentrate any GI parasite ova and (oo)cysts present in the feces[1]. The sediment was smeared on the clean glass slide, stained with Lugol's iodine and microscopically examined at 100X total magnification for the detection of helminth ova and 400X for protozoan parasites. Modified Zeihl-Neelsen stain was used for the detection of the *Cryptosporidium* sp. at 1000X total magnification[5]. The identification of the GI parasites was based on morphological characteristics described by Taylor *et al.*[24] and Kaufmann[12]. The samples were considered as *Schistosoma* positive when at least one *Schistosoma* ova was detected.

Statistical analysis: Statistical analysis was carried out using the statistical program (SPSS statistical program, SPSS Inc., Chicago, IL). The samples with missing data were excluded from the statistical analysis. A Pearson's Chi-square test was applied to determine the association between the dependent variable (i.e., *S. spindale* infections) and independent variables (i.e., age, breed, gender and weight). Independent variables that generated a p value of less than 0.25 in the univariate model were included in the logistic

regression analysis using forward likelihood ratio (LR) in order to identify the risk factors for *S. spindale* infections. The risk factors were tested using Odds-Ratio (OR) and the significance was analyzed using a 95% confidence interval and p value less than 0.05.

Results

A total of 266 rectal fecal samples were collected from Farm A (N = 40), Farm B (N = 4), Farm C (N = 120), Farm D (N = 37), Farm E (N = 27), and Farm F (N = 38) (Table 1). From the total number of cattle sampled, only 248 animals had complete information on age, weight, gender and breeds. The information of (i) gender, (ii) age and (iii) weight were summarized according to farm A [(i) male: 20, female: 20; (ii) range: 7 months–1 year 7 months, median: 11 months 5 days (interquartile range: 8 months–1 year 3 months); (iii) range: 102 kg–200 kg, median: 150 kg (interquartile range: 139.0 kg–171.5 kg)], farm B [(i) male: 2, female: 2; (ii) range: 5 months–7 months, median: 6 months (interquartile range: 5 months 5 days–6 months 5 days); (iii) range: 183 kg–196 kg, median: 192 kg (interquartile range: 185.5 kg–196.0 kg)], farm C [(i) male: 8, female: 109; (ii) range: 5 months–11 years, median: 4 years (interquartile range: 3 years–6 years); (iii) range: 90.0 kg–694 kg, median: 409Qkg (interquartile range: 302.0 kg–475.0 kg)], farm D [(i) male: 20, female: 17; (ii) range: 8 months–11 years, median: 2 years (interquartile range: 2 years–8 years); (iii) range: 105.0 kg–421.0 kg, median: 180.0 kg (interquartile range: 129.0 kg–222.0 kg)], farm E [(i) male: 7, female: 5; (ii) range: 2 years–3 years, median: 3 years (interquartile range: 2 years–3 years); (iii) range: 139.0 kg–214.0 kg, median: 190.5 kg (interquartile range: 173.0 kg–200.5 kg)], and farm F [(i) male: 21, female: 17; (ii) range: 1 year–12 years, median: 1 year (interquartile range: 1 year–2 years); (iii) range: 102.0 kg–435.0 kg, median: 195.5 kg (interquartile range: 159.0kg–250.0kg)], respectively. The overall infection rate of *S. spindale* was 6% (16 of 266). *S. spindale* was observed in Farm D (5.4% or 2 of 37) and Farm E (51.9% or 14 of 27). The ova of *S. spindale* observed among the positive samples were within the range of 270 to 320 μm long, elongated and spindle shape with a terminal spine (Figure 1).

Fig. 1. *Schistosoma* ova (≈280 μm) viewed under light microscope (100X total magnification) aided by iodine stain.

With regards to multi-parasitism, *S. spindale* was more likely to co-occur with other GI parasites in both farms. Up to quadruple infection (1 of 16) (*S. spindale* + *Dicrocoelium* spp. + *Paramphistomum* spp. + strongyle) was observed among the co-helminth infections (3 of 16). Contrastingly, a total of 13 of 16 *S. spindale* positive samples were found more commonly co-occurring with helminth and protozoa parasites (Table 2). The co-occurrence between *S. spindale*, *Dicrocoelium* spp., *Paramphistomum* spp., strongyle, *Eimeria* spp. and *Entamoeba* spp. (sextuple infection) had higher prevalence as compared to different combination of helminth and protozoa infections (*S. spindale* + helminthes + protozoa) (Table 2). In addition, a total of 248 samples with complete information (i.e., breed, gender, age and weight) were included in the statistical analysis. Chi-square analysis of the four independent variables (breed, gender, age and weight) with *S. spindale* infection

Table 2. Type of multi-parasitism between *Schistosoma spindale* and other helminthes and protozoa

Co-parasitic infections	Farms				Total (N = 16)	
	D		E			
	n	%	n	%	n	%
***Schistosoma spindale* + Helminth**						
Double Infections						
Paramphistomum sp.	–	–	1	3.7	1	6.3
Triple Infections						
Paramphistomum sp. + strongyle	–	–	1	3.7	1	6.3
Quadruple Infections						
Dicrocoelium sp. + *Paramphistomum* sp. + strongyle	–	–	1	3.7	1	6.3
***Schistosoma spindale* + Helminth + Protozoa**						
Triple Infections						
Paramphistomum sp. + *Entamoeba* sp.	–	–	1	3.7	1	6.3
Strongyle + *Eimeria* sp.	1	2.7	1	3.7	2	12.5
Strongyle + *Entamoeba* sp.	–	–	1	3.7	1	6.3
Quadruple Infections						
Paramphistomum sp. + strongyle + *Eimeria* sp.	1	2.7	–	–	1	6.3
Paramphistomum sp. + strongyle + *Entamoeba* sp.	–	–	2	7.4	2	12.5
Paramphistomum sp. + *Eimeria* sp. + *Entamoeba* sp.	–	–	1	3.7	1	6.3
Quintuple Infections						
Dicrocoelium sp. + *Paramphistomum* sp. + strongyle + *Entamoeba* sp.	–	–	1	3.7	1	6.3
Paramphistomum sp. + strongyle + *Eimeria* sp. + *Entamoeba* sp.	–	–	1	3.7	1	6.3
Sextuple Infections						
Dicrocoelium sp. + *Paramphistomum* sp. + strongyle + *Eimeria* sp. + *Entamoeba* sp.	–	–	3	11.1	3	18.8

demonstrated that female cattle are less likely of being infected with *S. spindale* as compared to the male cattle (OR = 0.3; 95% CI = 0.08–1.06; $p < 0.05$). In addition, cattle weighing less than 200kg are more likely to get infected (OR = 5; 95% CI = 1.07–24.79; $p < 0.05$) with this trematode parasite (Table 3). Multivariate analysis confirmed that among the Malaysian cattle sampled, age and weight (significant correlation between age and weight; R = 0.785, $p < 0.05$) were risk factors for infection: adult cattle with two year old or older (OR = 21; 95% CI = 2.48–179.44; $p < 0.05$) and weight less than 200kg (OR = 17; 95% CI = 3.38–87.19; $p < 0.05$) were at higher risk of being infected with *S. spindale* (Table 4).

Discussion

The present study has demonstrated a relatively low prevalence of *S. spindale* infection (6%) among the studied Malaysian cattle. This infection rate was lower than that reported for other countries in Asia, such as 74.0%, 57.3% and 30.7% in India[11,20,23], 31.3% in Sri Lanka and 15% in Pakistan[7,17]. Over the years, there has been a limited number of reports on the prevalence and epidemiology of schistosomes infecting livestock in Malaysia. In 1986, the first case of *S. incognitum* infection in swine was reported[16]. Subsequently, the first description of the morphology of *S. spindale* collected from goats and buffaloes were reported and *S. nasale* was found to infect the buffaloes (29% or 6 of 21)

Table 3. Association between *Schistosoma spindale* infections and risk factors among the Malaysia cattle

Variables	Total (N = 248)	*Schistosoma spindale*		OR (95% CI)	*p* value
		Number	%		
Breed					
Pure	146	10	6.8	–	–
Cross breed	102	0	0	–	
Gender					
Female	170	4	2.4	0.3 (0.08–1.06)	0.047
Male	78	6	7.7	1	
Age (years)					
Adult (≥2)	162	9	5.6	5 (0.61–39.41)	0.099
Young (<2)	86	1	1.2	1	
Weight (kg)					
≤200	112	8	7.1	5 (1.07–24.79)	0.024
>200	136	2	1.5	1	

CI Confidence interval
OR Odd ratio

in the country[14,15]. Apart from the present study, there remains only a single report on *Schistosoma* infection in cattle, where internal organs of 24 cattle from Alor Setar, Kedah (northern Malaysia) were inspected revealing a prevalence of 16.7% for *S. spindale*[9].

In the present study, *S. spindale* was more likely to co-occur with other GI parasites (up to sextuple infections), such as helminthes (*Dicrocoelium* spp., *Paramphistomum* spp., and strongyle) and protozoa (*Eimeria* spp., and *Entamoeba* spp.). Among the detected parasites, *Paramphistomum* spp. was the most common species which displayed concurrent infection with *S. spindale* in the positive individuals (81.3% of 16) and present from double to sextuple mixed-GI parasitic infections. Several researchers have reported *Paramphistomum* spp. and *Schistosoma* spp. infections among cattle[3,21,25], however, limited studies focused on the co-occurrence of these two species in these ruminants. It is important to understand the co-occurrence of these two parasites because of the overlapping clinical signs of visceral schistosomiasis and paramphistomiasis[24]. As such; the importance of schistosomiasis in cattle might be overlooked and underestimated.

In addition to *S. spindale*, other detected flukes during the coprological survey such as *Dicrocoelium* spp. (15 or 100% of 15 *Dicrocoelium* positive samples) and *Paramphistomum* spp. (62 or 86.1% of 72 *Paramphistomum* positive samples) were also likely to co-occur with other GI helminthes and protozoa (data not shown). Besides, *S. spindale* negative samples revealed 26 co-occurrence of GI parasitic infection combinations with up to quintuple infections among helminthes (i.e., *Dicrocoelium* spp., *Moniezia* spp., *Nematodirus* spp., *Paramphistomum* spp., strongyle, *Trichuris* spp.) and protozoa (i.e., *Eimeria* spp., *Entamoeba* spp.) (data not shown).

The present study has indicated the adult cattle (≥ 2 years old) have higher risk to get infected by *S. spindale*. This phenomenon can be explained by the farm management. In Malaysia, the calves are normally housed in calf pens for easier feeding management to ensure sufficient food intake and to monitor their health condition to minimize the mortality rate. Therefore, calves are less likely to be exposed to parasite contaminated pastures. Contrastingly, adult cattle are allowed to graze freely on available pastures,

Table 4. Multivariate analysis of risk factors associated with *Schistosoma spindale* infection among Malaysian cattle

Variables		OR (95% CI)	*p* value
Age (years)	Adult (≥2)	21 (2.48–179.44)	0.005
Weight (kg)	≤200	17 (3.38– 87.19)	0.001

CI Confidence interval
OR Odd ratio

such as riversides, fields and along roadsides, as well as under oil palm or rubber plantations, thus increasing the possibility of fluke infections. In fact, there has been limited information available on the risk factors of schistosomiasis in cattle. However, previous studies elsewhere reported several risk factors of infection for other bovine flukes, for example, *Dicrocoelium dendriticum* – age, larger pastures[6]; *Fasciola* spp. – age, free grazing, mixed farming of small and large ruminants, stagnant pond and river bathing[13,26]. Since Malaysia is endowed with a hot, wet tropical climate, most grazing areas have puddles of water or streams which are running all the year round making it convenient for snail borne parasites to be transmitted.

The detection of the water-borne helminth, *S. spindale* in this study suggests the possibility of larvae contamination in water sources in the studied farms. It is acknowledged that *S. spindale* larval detection from water sources and intermediate hosts was not conducted in this study, nonetheless, the positive findings of larval *S. spindale* in its intermediate host, the snail *Indoplanorbis exustus*has been reported in Malaysia[4,10]. The occurrence of *S. spindale* is not only of veterinary concern, but is also important in the areas of zoonosis and public health (i.e., cercarial dermatitis), especially for the farmers who have close contact with the natural water sources in the farming area.

In Malaysia, the distribution of the schistosomes among livestock remains unclear. Over the last 20 years, there have been no reported cases of schistosomiasis from the samples received for routine diagnostics at the Regional Veterinary Laboratories. Furthermore, the limited research data available in the country suggests a low infection rate of schistosomes in livestock[9,14,16]. In fact, epidemiological studies on GI parasites among livestock have been given little attention in Malaysia, mainly due to the lack clinical cases and its relatively low impact to the local livestock industry.

The main techniques performed on the cattle/buffalo fecal samples received by the diagnostic laboratories are McMaster fecal egg counts and sedimentation. The sedimentation technique is preferred for the detection of large fluke ova. Nevertheless the negative detection of schistosomes reported during routine diagnostic work may be attributed to several factors: 1) Inexperience staff in the diagnostic laboratories who may be less familiar with the uncommon GIPs in livestock. In addition, while schistosome ova can be observed using the sedimentation method, the major focus of the detection are on the common flukes (i.e., *Fasciola* and *Paramphistomum*) sometimes resulting in other GI parasites like *Schistosoma* being overlooked; 2) Morphology of the schistosomes ova (i.e., *S. spindale*): the unique shape of the schistosomes (i.e., *S. spindale* ova) which differs from the typical helminth ova and may appear as fecal debris or artefacts (for inexperience staff); 3) Hosts (i.e., cattle): the clinical signs of schistosomiasis are rarely seen in cattle[24]. Generally, most of the samples sent to the diagnostic laboratories were collected from animals with clinical signs and those without clinical signs are ignored. In addition, for the routine monitoring of health in animals, farmers tend to submit little amount of fecal samples to the laboratories; therefore the possibility of detecting *S. spindale* might be low.

4) Biology of Schistosomes: The examination of the schistosome ova depends on the infection stage in the host. Fecal examination is feasible during the early stage of schistosome infection (high egg excretion rate). However, as the time goes by, egg counts will be reduced to few eggs per gram due to the suppression of egg production by the developed immune system of the host[2]. Therefore, the chances to get the schistosome ova in the feces are lowered. In addition, there are lower possibilities to detect schistosome eggs in animals with chronic infection as compared to one undergoing acute infection[12].

In fact, there are several techniques available for schistosome detection including serological techniques and post-mortem, however microscopic examination (i.e., McMaster egg count and sedimentation) remained the most economical diagnostic means for the laboratories in Malaysia, especially for those handling large number of samples. In order to overcome the factors mentioned above, training should be given to the staffs to understand the morphology and biology of the parasites (i.e., common and uncommon) and its interaction between hosts. In addition, authors would like to recommend that diagnostic laboratories to include the formalin-ether technique (qualitative detection technique) during the fecal examination. This technique commonly used in the diagnostic work in human fecal samples, has an advantage to detect a wide range of GI parasites (i.e., helminthes and protozoa) which is able to complement the limitations of McMaster fecal egg count and sedimentation.

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