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The prevalence of helminthiasis in cattle, Terengganu, Peninsular Malaysia

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Abstract. A study was conducted to determine the prevalence of helminthiasis among cattle in seven districts of Terengganu, namely: Kuala Terengganu, Hulu Terengganu, Setiu, Dungun, Kemaman, Marang and Besut. The periods of samplings were from March 2015 to January 2016 conducted between 0800-1400h. A total of 219 faecal samples and 214 blood samples were collected from the animals. The faecal samples were analyzed by faecal sedimentation method, modified McMaster method, culture techniques and identification of third stage larvae morphologically. The blood samples were centrifuged for sera that were used for serological test. Results showed that the overall helminthiasis was made of 55.0% trematodes and 14.0% nematode infection. Out of 55.0% of trematode infection, 41.0% was liver fluke infection, 8.0% was rumen fluke infection and 52.0% was co-infection of liver fluke and rumen fluke. A significant difference in the distribution of trematode eggs was recorded among the animals, $\chi^2(2, N=120) = 41$, $p<0.05$. Faecal egg count (FEC) for gastrointestinal (GI) nematodes were ranged from 0 – 800 e.p.g. The mean faecal egg count was 21.5 ± 4.5 e.p.g. Faecal culture revealed that the most prevalent nematodes identified was *Haemonchus* (81.0%), followed by *Trichostrongylus* (15.0%) and *Oesophagostomum* (4.0%), $\chi^2(2, N=100) = 104$, $p<0.05$. Sandwich ELISA test revealed 82.0% of the sero samples were positive for liver fluke infection. It can be concluded that trematode infection is an on-going problem in cattle located in Terengganu, which can lead to economic losses for the farmers.

INTRODUCTION

Helminthiasis is considered as one of the main problem for livestock industry which can lead to lost productivity, increased mortality, reduced milk yield, fertility problems and eventually it can lead to economic losses to the farmers (Gray *et al.*, 2008).

Trematode infection especially by liver flukes and gastrointestinal (GI) nematode infection are common among cattle in South East Asia (Saleha, 1991; Holland *et al.*, 2000). The warm and humid climate condition throughout the year in Malaysia is favourable for the development of infective larvae found on the pasture for grazing animals (Geurden *et al.*, 2008; Chandrawathani *et al.*, 2009).

Fascioliasis is caused by liver fluke species of genus Fasciola: *Fasciola gigantica* and *Fasciola hepatica*. However, *F. gigantica* is the only liver fluke that has been recorded in Peninsular Malaysia (Rajamanickam *et al.*, 1996). Among the Asian countries, Cambodia (Dorny *et al.*, 2011), Pakistan (Bhutto *et al.*, 2012) and Thailand (Woodruff *et al.*, 1992) have reported cases of ruminant fascioliasis. According to Rajamanickam *et al.* (1996), bovine fascioliasis was reported in all the states in Malaysia except Langkawi Island. Ariff (2014) and Sakinah (2014) reported prevalence of fascioliasis in Kuala Terengganu as 74.2% and 62.0% respectively.

The objective of this study was to determine the prevalence of helminthiasis among cattle in seven districts of Terengganu,

Peninsular Malaysia using coprological and serological tests.

MATERIALS AND METHODS

Sampling site

The sampling was conducted in seven districts of Terengganu namely: Kuala Terengganu, Hulu Terengganu, Besut, Dungun, Kemaman, Marang and Setiu (Table 1). Two farms were selected in Besut, Dungun, Hulu Terengganu, Kemaman, Marang and Setiu, while four farms were selected in Kuala Terengganu. Farm selection was made by the officers from the Department of Veterinary Services based on health screening schedule. Therefore, a total of 16 farms were selected for the samplings (Figure 1). From each of the farms, at least 15 animals were sampled. This is due to time limitation and to avoid sampling in the noon in order to reduce stress in animals. For farms which have less than 15 animals all the animals were selected. Therefore, a total of 219 cattle were randomly chosen from male and female cattle of different age groups (less than 5 years, 5 to 10 years and above 10 years). Most of the breeds were Kedah-Kelantan cross.

Sample collection

Total of 219 fresh faecal samples from 44 male and 175 female cattle were collected directly from the rectum to avoid contamination of other parasites and to ensure the nematode eggs are not yet hatched. Samples were kept in plastic bags and stored in refrigerator at 4°C, to prevent the eggs from hatching before the examination.

From the 219 animals selected, only 214 blood samples were successfully collected due to the animal's aggressive behaviour. The blood samples were drawn from the jugular or tail veins of the cattle into 10 ml plain tube (BD Vacutainer, Belliver Industrial Estate Plymouth, UK) without anticoagulant to allow the blood to clot in order to get the serum. A new needle (Vacuette, Greiner Bio-One GmbH, Nipro Medical industries Ltd, Japan) size 18G was used for each animal to avoid cross contamination of blood. The collected blood was kept in polystyrene box with ice cubes to avoid haemolysis and then taken to the laboratory. In the laboratory, the blood samples were centrifuged for 10 minutes at 478×g. The supernatant, serum was transferred into 1.5ml eppendorf tube (Eppendorf AG, 22331 Hamburg, Germany) and stored at -20°C until ELISA tests were conducted as recommended by the manufacturer (Awad *et al*., 2009).

Parasitological and haematological examinations

Coprological examination

Faecal sedimentation method was conducted to detect the trematode eggs in faeces (Hansen & Perry, 1994).

Modified McMaster technique was conducted to estimate the number of nematode eggs per gram (e.p.g) of faeces where each egg counted was equated to 100 e.p.g.

Faecal culture in wide-mouth jars were conducted as described by Roberts & O'Sullivan (1949) technique to identify the genus of nematodes present. The head and tail of the larvae were examined and identified based on the identification key provided by Ministry of Agriculture, Fisheries and Food (1986).

Serological examination

Eighty five sera which yielded negative result in faecal sedimentation method were selected for Sandwich Enzyme Linked

Table 1. Number of farms and number of cattle screened per district

Districts	Number of farms	Number of cattle screened
Kuala Terengganu	4	49
Hulu Terengganu	2	29
Besut	2	18
Setiu	2	36
Marang	2	14
Kemaman	2	40
Dungun	2	33
TOTAL	16	219

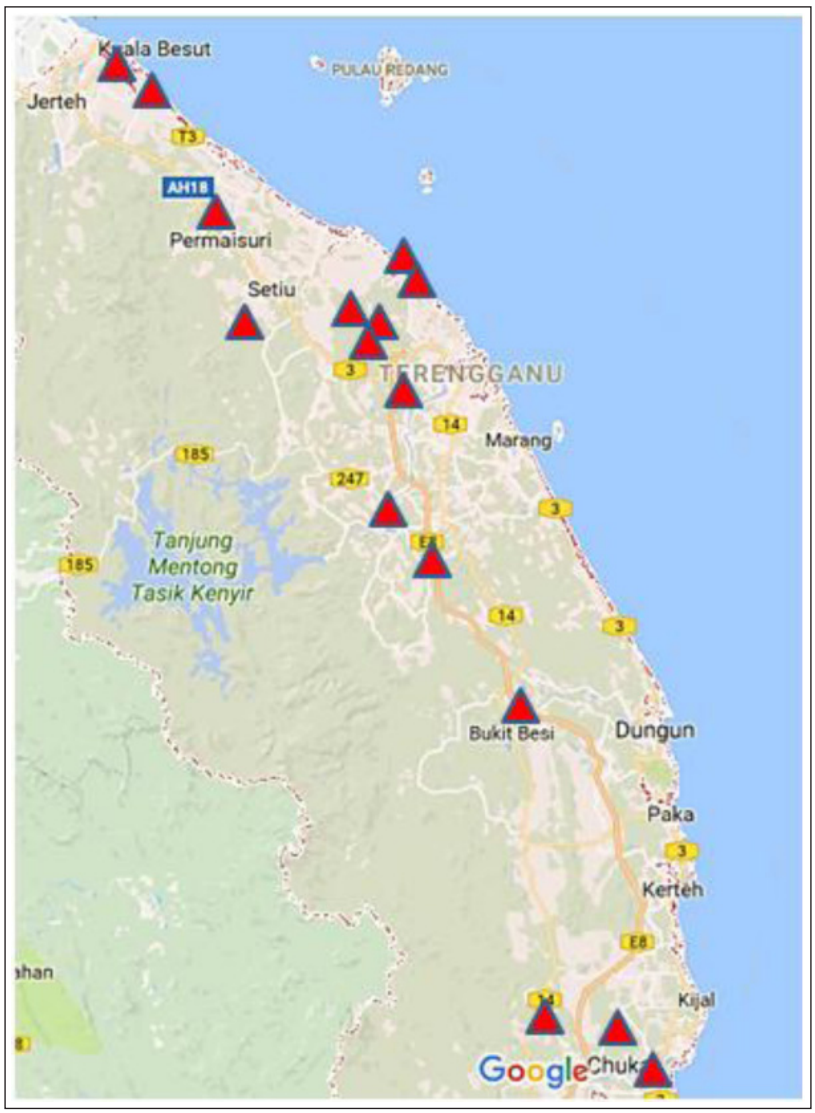

Figure 1. Map showing the sampling site of the seven districts in Terengganu, Google map, 2015.

Immunosorbent Assay (ELISA) to detect antibodies of specific antigens of liver fluke (Hillyer *et al*., 1992). The sandwich ELISA was performed on 96 well microplates which were coated with antigen-affinity purified sheep anti-Bovine lgG (KOMA BIOTECH INC, Republic of Korea).

Statistical analysis

Data was analysed by SPSS version 20 (IBM Corporation) at confidence level 95%. Chi-square (χ^2) test was performed to compare the distribution of trematode eggs and the species of nematodes present in the samples. Chi-square (χ^2) test was also performed to study the effect of age and gender on the prevalence of fascioliasis in cattle.

The data of faecal egg count (FEC) for liver fluke infection was not normally distributed. Thus, Kruskal-Wallis test which is equivalent to the one-way between groups analysis of variance (ANOVA) was performed to study the distribution of FEC among seven districts of Terengganu.

Table 2. Number of farms, number of cattle screened and the prevalence (%) of trematodes and nematodes in seven districts of Terengganu (Kuala Terengganu, Hulu Terengganu, Besut, Setiu, Marang, Kemaman and Dungun)

Districts	Number of farms	Number of cattle screened	Trematodes (%)	Nematodes (%)
Kuala Terengganu	4	49	49	24
Hulu Terengganu	2	29	7	10
Besut	2	18	100	0
Setiu	2	36	100	22
Marang	2	14	57	21
Kemaman	2	40	38	5
Dungun	2	33	52	0
TOTAL	16	219	55	14

RESULTS

Prevalence of helminth infection of cattle in Terengganu

Fifty-five percent (55.0%) of trematode infection and fourteen percent (14.0%) of nematode infection recorded in Terengganu (Table 2).

The prevalence of trematodes

From 219 cattle screened, 120 samples (55.0%) were found positive for trematode eggs. Out of this, 49 animals were positive for liver fluke only (41.0%), 9 animals were positive for rumen fluke only (8.0%) and 62 animals were diagnosed with co-infection of liver fluke and rumen fluke (52.0%). The frequency of liver fluke, rumen fluke and co-infection of liver fluke and rumen fluke were significantly different across the farms, $\chi^2(2, N=120) = 41$, $p<0.05$. Faecal egg count (FEC) for liver fluke ranged between 0-104 e.p.g while for rumen fluke the FEC were ranged between 0–73.4 e.p.g. Out of 16 farms screened, 12 (75.0%) farms were positive for trematode infection.

Prevalence of fascioliasis in different age groups of cattle

The age-wise distribution of fascioliasis revealed that 87.0% of infection was recorded in animals between 5 to 10 years of age group. There was a significant difference between the FEC and the age of the animals $\chi^2(2, N=112) = 144.1$, $p <0.05$.

Prevalence of fascioliasis in cattle by gender

The overall prevalence of fascioliasis in seven districts by gender was 55.0% in female and 36.0% in male. There was a significant difference between the gender of cattle and the prevalence of fascioliasis $\chi^2(1, N = 112) = 57.1$, $p <0.05$.

The prevalence of fascioliasis in cattle by districts

All the districts in Terengganu have recorded positive bovine fascioliasis except in Hulu Terengganu. The highest prevalence was in Setiu and Besut. The prevalence of fascioliasis is significantly differ across the districts $\chi^2(6, N = 219) = 163.0$, $p <0.05$.

Serological test

Out of 85 samples, 82.0% were found to be positive for IgG antibody detection for liver fluke infection.

The prevalence of GI nematodes

From the 16 farms, only 2 farms were positive with nematode infection and out of 219 faecal samples diagnosed, only 28 animals (14.0%) were found positive for nematodes eggs. The faecal egg counts of the total 16 farms were ranged from 0-800 e.p.g. The mean faecal egg count was 21.5 ± 4.5 e.p.g.

The third stage larval identification

The predominant genus recorded were *Haemonchus* (81.0%) followed by *Tricho-*

strongylus (15.0%) and *Oesophagostomum* (4.0%). There was a significant difference between genera of nematodes, $\chi^2(2, N=100) = 104$, $p < 0.05$.

DISCUSSIONS

This study showed current prevalence of trematode and nematode infections in Terengganu. From the results, it can be summarized that trematode infection is severe compared to nematode infection. In addition, the prevalence of fascioliasis in the current study was higher (93.0%) than amphistomiasis or rumen fluke infection (60.0%). The high prevalence of fascioliasis is an agreement with Khadijah *et al.* (2015) where 94.6% of the cattle screened at two farms in Kuala Terengganu were positive for fascioliasis. This indicates that fascioliasis is a serious problem in cattle. Therefore, this present study has extensively studied the occurrence of fascioliasis by districts and the factors affecting such as the age group and the gender of the cattle.

The present study recorded high prevalence of fascioliasis in animals aged between 5 to 10 years. These finding is supported by Molina *et al.* (2005) and Bhutto *et al.* (2012) who reported animals above 6 years were recorded to be highly infected with liver fluke. The higher infection rate among animals between 5 to 10 years of age group is due to long time exposure to disease which is also known as cumulative infection with age (Keyyu *et al.*, 2006). The management practice is also a reason where the young animals were let to graze around the farms while the adults were let to graze freely around the farm for longer period (Department of Veterinary Service officer, personal communication). This might result the adult cattle to expose to contaminated pastures compare to the young cattle.

The prevalence of fascioliasis was higher in female cattle (55.0%) when compared to male cattle (36.0%). These results are in agreement with Phiri *et al.* (2005) and Bhutto *et al.* (2012) who reported similar trend in Zambia and Pakistan respectively. In all the farms of the present study, the number of female cattle was high. The high female to male ratio is one of the reasons for the high prevalence of fascioliasis in female cattle in Terengganu. In addition, few studies have reported that gender does influence the helminth infection in the host (Larry *et al.*, 1981). Female cattle are usually weak and malnourished and they are more susceptible to helminth infection (Kuchai *et al.*, 2011). Thus, the high prevalence of fascioliasis in female cattle suggests that gender might affect the infection rate of fascioliasis in the animal.

Among the seven districts, all cattle sampled from Besut and Setiu were infected with liver fluke. Results from one of the farms in Besut recorded more than 500 eggs in coprological method which indicates severe infestation of fascioliasis. Based on the information gathered from the farmers, the last treatment given to the cattle in Besut and Setiu were in 2014. Lack of routine monitoring and lack of anthelmintic treatments might be the reason for high prevalence of fascioliasis in both of the districts.

Fascioliasis has been studied widely in Malaysia but information on amphistomiasis is still lacking. Previous studies by Ariff (2014), Nurlaili (2014) and Sakinah (2014) had recorded the prevalence of amphistomiasis in few farms of Kuala Terengganu as 17.0%, 18.0% and 13.0% respectively. The present study recorded amphistomiasis in eight farms (59.0%) in Terengganu and is thus in agreement with the previous findings. Since the intermediate host, freshwater snail plays an important role in rumen fluke infection, a comprehensive study on their prevalence, distribution and overall amphistomiasis in livestock is needed.

Out of the 55.0% of trematode infections, 52.0% were reported to be co-infection of liver fluke and rumen fluke. Ariff (2014) and Sakinah (2014) recorded co-infection of liver fluke and rumen fluke in cattle in Kuala Terengganu as 86.7% and 17.0% respectively but this information is not sufficient to conclude the status of co-infection of trematode in Malaysia. Massive numbers of liver flukes and immature rumen flukes can

prompt to death (Urquhart *et al*., 1996). Therefore, it is significant to know the current status of co-infection to conduct suitable treatment for liver fluke and rumen fluke respectively.

The coprological method has its limitation where it cannot detect the early infection (Salimi-Bejestani *et al*., 2005; Awad *et al*., 2009; Avcioglu *et al*., 2014). Therefore, sandwich ELISA test was conducted to detect bovine fascioliasis in Terengganu. Eighty-five cattle with negatively reported on the coproscopy sedimentation method yielded positivity (82.0%) in sandwich ELISA test. The antibody-ELISA test is more sensitive, it can detect the antibodies in the host present approximately 1-5 weeks before eggs discharge in faeces (Avcioglu *et al*., 2014). This annotates why the negative results of sedimentation method turned to be positive in sandwich-ELISA test. Although antibody-ELISA test is more sensitive and accurate, it has some limitations as well. The positive result only indicates the exposure of the fascioliasis at some time, but not necessarily current infection (Salimi-Bejestani *et al*., 2005; Avcioglu *et al*., 2014). Therefore, it is suggested both coprological method and antibody-ELISA test should be carried out simultaneously to evaluate the status of fascioliasis.

The present study indicated low prevalence of GI nematode infection in cattle, Terengganu. This is an agreement with the previous studies by Ariff (2014), Sakinah (2014) and Khadijah *et al*. (2015) on the low prevalence of nematode infection in Kuala Terengganu. It may have benefitted through continued anthelmintic treatment and routine monitoring by the Department of Veterinary Services, Terengganu. In addition, the common breed in Terengganu was Kedah-Kelantan cross. According to Johari and Yasmi (2009) Kedah-Kelantan breed is well known for its natural resistance towards parasites and this could be one of the reasons for the low prevalence of GI nematode infection among cattle in Terengganu.

All the GI nematodes present in this study have been recorded previously in Kuala Terengganu (Ariff, 2014; Sakinah, 2014; Khadijah *et al*., 2015) where the most predominant nematode in cattle was *Haemonchus*. The second most dominant nematode was *Trichostrongylus* followed by *Oesophagostomum*. The occurrence of mixed infection can prompt severe health problem to the cattle (Tan *et al*., 2014). Therefore, appropriate treatments should be planned to control this infection.

CONCLUSION

This study has signified the severity of helminth infection of cattle in Terengganu. Trematode infection in cattle is a growing problem in Terengganu and fascioliasis found to be highly prevalent. Therefore, more studies on helminthiasis should be conducted in Malaysia to improve the productivity of livestock in future.

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