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High Prevalence Fluke Infection at Four Cattle Farms Located in Kuala Terengganu, Malaysia

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Running title: Stomach Fluke and Liver Fluke Infection in Cattle

Abstract. A survey was conducted to determine the presence of parasitic worm infection in cattle at four farms located in Kampung Surau Haji Daud, Kampung Kubang Jela, Kampung Gemuruh and Kampung Peradong, Kuala Terengganu. Thirty-three (33) faecal samples and blood samples were obtained from each cattle involved in this survey. Faecal samples were subjected to sedimentation method, modified McMaster method and faecal culture to detect trematode eggs, nematode eggs, and to identify the third stage of larvae. Blood samples were subjected to Packed Cell Volume (PCV) method to determine if the cattle are anaemic, normal or dehydrated. Result indicated that out of 33 cattle examined 17% of the cattle were positive for liver fluke, 67% were positive for stomach fluke and 42% were positive for nematode infection. PCV value indicated that all the cattle examined are not anaemic or dehydrated. The results obtained showed that trematode and nematode infections are common problem in all the four farms. The results were submitted to Department of Veterinary Services to plan further action and treatment.

Keywords: Paramphistomum, Fasciola, cattle, Malaysia

INTRODUCTION

Parasites infection is one of the most important causes of the production losses of meat and milk in most cattle-producing countries of the world (Chiu et al., 2014). The economic impact of gastrointestinal parasite infection results in mortality losses and retarded in growth and production of food (Rajakaruna and Warnakulasooriya, 2011). Effects on animal productivity are common following parasite infection, affecting weight gain and milk production. Clinical effects following parasite infections are anaemia, oedema and diarrhoea (Bowman, 2014).

There were 90,480 cattle recorded in Terengganu in 2014 (Department of Veterinary Services, 2017). Parasitic infection may affect the health and productivity of cattle which eventually leads to considerable economic losses. Several studies (Khadijah et al., 2015; 2017; Rita et al., 2017) on parasitic infection in cattle have been carried out in Terengganu, Malaysia. This survey was conducted with the aim to determine the current prevalence of parasitic worm infection in Kuala Terengganu, specifically stomach fluke and liver fluke infection, in order to add more information on the current status of parasitic infection of cattle in Kuala Terengganu.

MATERIALS AND METHODS

Farms Location and Information

The survey was performed at four cattle farms in Kuala Terengganu, namely Kampung Surau Hj Daud (Farm A), Kampung Kubang Jela (Farm B), Kampung Gemuruh (Farm C) and Kampung Peradong (Farm D), Kuala Terengganu (Figure 1). Animals at all the farms were allowed to graze in the morning until late afternoon in open pastures. For farm D, the animals were also allowed to graze under rubber trees.

Animals

Thirty-three (33) cattle were selected in this survey and the breed involved was Kedah-Kelantan. The cattle were aged between 1½ to 5 years old and at each farm, at least 30% of the cattle were randomly sampled.

Sample Collections

Faeces

Faeces were collected individually from the rectum of the cattle following the guidelines by Ministry of Agriculture, Fisheries and Food (1986). These faecal samples were brought back to the laboratory and were kept at 4°C until processing for different tests. The samples were

subjected to tests for trematode egg count using sedimentation method (Ministry of Agriculture, Fisheries and Food, 1986) nematode worm egg count using McMaster method (Ministry of Agriculture, Fisheries and Food, 1986) and faecal culture (Ministry of Agriculture, Fisheries and Food, 1986) for third stage larvae identification of nematodes (Ministry of Agriculture, Fisheries and Food, 1986).

Blood

Blood samples were withdrawn from the cattle (Kerr, 2002) either from the tail vein or the jugular vein using needles sized 18G into 3ml blood tubes with Ethylene-diamine-tetraacetic acid (EDTA) anticoagulant (Vacutest Kima brand, Italy). The whole blood was subjected to the Packed Cell Volume method (Rosenberger et al., 1979) to determine the proportion of erythrocytes in blood.

Examination of Parasites and Packed Cell Volume (PCV)

Trematodes

Sedimentation method (Ministry of Agriculture, Fisheries and Food, 1986) was conducted to determine the trematode egg count in faecal samples. The number of eggs observed in the sediment was divided by 5 to get the value of eggs per gram faeces.

Nematodes

Modified McMaster Method was performed for the estimation of nematode eggs in 1 gram of faeces according to Ministry of Agriculture, Fisheries and Food (1986). The number of eggs observed was multiplied by 100 to get the value of eggs per gram faeces.

Faecal culture method was conducted to obtain the third stage larvae (L_3) from faecal samples. The L_3 was identified based on the identification keys provided by Ministry of Agriculture, Fisheries and Food (1986).

Packed Cell Volume (PCV)

Packed Cell Volume (PCV) was conducted according to Rosenberger et al. (1979). The PCV values obtained were recorded and then differentiated with the normal blood values of cattle in order to determine if they were either anaemic, normal or dehydrated.

Statistical Analysis

Normality test was conducted in order to know the distribution of data (number of worm egg). The data was not normally distributed. Thus, Kruskal-Wallis test was conducted to compare the WEC (for nematodes and trematodes) between four different farms. Chi-square test was performed to differentiate the frequency of third stage larvae present at the farms.

Statistical analyses were performed using SPSS version 22 (IBM Corporation, USA).

RESULTS

Prevalence of Trematode Infection

From 33 samples, six (18%) were found positive for liver flukes and 27 samples (81%) were found positive for stomach fluke.

Fasciola and Paramphistomum worm egg counts do not differ across the four farms, $\chi^2(3, N=33) = 1.269$, $p>0.05$ and $\chi^2(3, N=33) = 4.950$, $p>0.05$ respectively (Table 1).

Prevalence of Nematode Infection

The nematode egg count for all four farms ranged between 0-1000 eggs per gram (e.p.g). The highest nematode egg count was recorded in Kampung Kubang Jela with 1000 e.p.g while the rest of the farms were recorded with lowest nematode egg count of 0 e.p.g. There was no significant different of nematode egg count distribution between the four farms, $\chi^2(3, N=33) = 6.782$, $p>0.05$ (Table 2).

Third Stage Larvae (L_3) Identification

The most dominant nematode recorded was *Haemonchus* spp., followed by *Oesophagostomum* sp. There was no significant difference between the numbers of *Haemonchus* spp. on the four farms $\chi^2(3, N=393) = 0.374$, $p>0.05$ (Table 3).

Packed Cell Volume

The Packed Cell Volume recorded ranged between 24% - 48 % for all the cattle sampled.

DISCUSSIONS

This present survey recorded high prevalence of trematode and low prevalence of nematode infection in cattle at four farms in Kuala Terengganu, Malaysia. All four farms reported the presence of stomach fluke while three farms reported the presence of liver fluke.

The prevalence of stomach fluke infection in this current survey was high (81%) compared to liver fluke infection. It is also reported to be higher than the findings of Khadijah et al. (2017) and Rita et al. (2017) which recorded the percentage of stomach fluke infection in cattle Terengganu, Malaysia as 15% and 59% respectively. The report of stomach fluke incidences coincides with other reports which suggested that stomach fluke is highly reported in areas with sub-tropical and tropical climate, such as in Asia, Africa and Russia (Horak, 1971;

Gupta et al., 1978). However, studies on stomach fluke infection in Terengganu and Malaysia are very limited. Therefore, more studies on prevalence of stomach fluke infection in Malaysia and the epidemiology are suggested, due to the fact that this parasite was reported to cause production losses in cattle and buffalo (Saleha, 1991).

The prevalence of liver fluke infection in this present survey was lower (18%) than the those reported by Khadijah et al. (2015; 2017) and Rita et al. (2017) where the prevalence were recorded as 94.6% and 67.8% respectively. However, the infection of liver flukes in this present survey was supported by previous researchers who reported that liver fluke infection affected cattle (Saleha, 1991; Rajamanickam et al., 1996) and buffaloes in Malaysia (Khadijah et al., 2017; Rosilawati and Saipul Bahari, 2017). Similar to stomach fluke studies, studies on liver fluke infection in Terengganu and Malaysia are very limited, with very few literatures published after the findings of Rajamanickam et al. (1996). Therefore, more studies on liver fluke infection in Malaysia and the epidemiology are suggested, due to the fact that this parasite was reported to cause production losses in cattle, sheep and goats (Saleha, 1991).

Haemonchus spp. was found to be highly prevalent followed by *Oesophagostomum* sp. This is supported by Waruiru et al. (2001) who reported that *Haemonchus* spp. were recorded as the predominant species among the trichostrongylids in cattle.

Despite having *Haemonchus* spp. in abomasum and infected with flukes, percentage of PCV in all the animals were ranged between 24%-48%, indicating normal range as reported by Rajamanickam et al. (1987). This finding was not expected as the animals were infected by blood-sucking parasites, and PCV values should be significantly lower as reported by Rajamanickam et al. (1987). Packed Cell Volume (PCV) might not be affected by *Haemonchus* spp. infection as the infection was low, with mean worm egg counts were 100 and 275 e.p.g. Similar to this, mean Fasciola egg counts were 22 and 80 per gram, indicating low infection that might not be affecting PCV values. On the other hand, it was expected that infection by Paramphistomum with mean worm egg count ranged between 244-2110 e.p.g will affect the PCV value. However, this is not the case in this current survey. It was reported that the immature stages suck blood and are responsible for anaemia in cattle (Diaz et al., 2006) and it is probable that in this current survey the immature stages are low when compared to adults which produce eggs.

It is suggested that in future studies, observation and sampling of freshwater snails could be conducted to determine the source of fluke infections as in this current study the snails were not observed. Information on the availability of the snails as source of infection will help farmers to manage and control fluke infection.

CONCLUSION

Present survey recorded high prevalence of trematode infection and low prevalence of nematode infection. This survey revealed that the animals were infected with liver fluke, stomach fluke, *Haemonchus* spp., and *Oesophagostomum* sp. Although, the PCV value showed that all of the cattle sampled were in healthy condition, regular monitoring by Department of Veterinary Services is required. Similar survey with wider coverage of the farms in Terengganu and other states should be conducted to provide more information on trematode and nematode infection that will assist farmers and Department of Veterinary Services to improve animal's health.

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Map of study area (source: Google Earth and GIS version 2.14.1)

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Table 1: Number of egg count (mean ± standard deviation) for liver fluke (*Fasciola* sp.) eggs and stomach fluke eggs (*Paramphistomum* sp.) in cattle at four farms in Kuala Terengganu.

Farm Location	Number of Animal	Egg Count (e.p.g)	
		Fasciola sp.	*Paramphistomum* sp.
Kampung Surau Hj Daud	9	22.2 ± 44.1	1440 ± 1810.6
Kampung Kubang Jela	9	22.2 ± 44.1	244.4 ± 212.8
Kampung Gemuruh	10	80 ± 168.7	2110 ± 2373.2
Kampung Peradong	5	0	1560 ± 1128.3
Kruskal-Wallis		1.269	4.950
P value		0.737	0.176

Table 2: Mean worm egg count (mean ± standard deviation) of nematode eggs in cattle at four different farms in Kuala Terengganu.

Farm Location	Number of animals	Mean worm egg count (e.p.g)
Kampung Surau Hj Daud	9	100 ± 100.0
Kampung Kubang Jela	9	275 ± 369.9
Kampung Gemuruh	10	20 ± 42.0
Kampung Peradong	5	100 ± 141.4
Kruskal-Wallis		6.782
P value		0.079

Table 3: The percentage of *Haemonchus* and *Oesophagostomum* L_3 in cattle at four different farms in Kuala Terengganu.

Farm Location	Percentage (%)	
	Haemonchus spp.	*Oesophagostomum* sp.
Kampung Surau Hj Daud	100	0
Kampung Kubang Jela	100	0
Kampung Gemuruh	93	7
Kampung Peradong	100	0
Chi-square	0.374	
P -value	0.946	