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Hemotropic *Mycoplasma ovis* infection in goats with concurrent gastrointestinal parasitism in Malaysia

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ABSTRACT

Hemotropic Mycoplasmosis is common in sheep and goats worldwide, which leads to huge economic losses. In this study, ten goats each were sampled from five herds belonging to the Ladang Angkat, Faculty of Veterinary Medicine (FVM) for the assessment of *Mycoplasma ovis* infection and concomitant intestinal parasites burden. Giemsa stain and Modified McMaster techniques were used to study the hemotropic mycoplasmosis and gastrointestinal parasite burden, respectively. Questionnaires were equally administered to each farmer and a fly trap was used to trap biting flies around the goat herds. Out of 50 samples analyzed, 94.0% (n=47/50) were positive for *M. ovis* infection. Among the positive samples, 93.6% (n=44/47) were mild infection while 6.4% (n=3/47) were moderate infection, with highest infection rate of 38.5% parasitemia. There was a significant association (*P*<0.05) between infection status and parasites burden. However, there was a weak positive correlation (r=0.107, *P*=0.460) between *M. ovis* infection rates and parasitic burden. Though a high occurrence rate of *M. ovis* was observed among the infected goats, the levels of parasitemia were generally mild.

Keywords

Mycoplasma ovis, infection rate, parasites burden, goats

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INTRODUCTION

Hemotropic mycoplasmas were previously identified as *Eperythrozoon* or *Haemobartonella* but are now reclassified as *Mycoplasma* genus based on 16S rRNA gene sequencing (Neimark et al., 2004). Hemotropic mycoplasma species are regarded as emerging or re-emerging zoonotic pathogens that have an effect on livestock, companion animals, wildlife, and also humans worldwide (Maggi et al., 2013a). More recently, *M. ovis* or infection with a *M. ovis* -like organisms has been reported to infect dogs (Varanat et al., 2011) and humans (Sykes et al., 2010). Hemotropic mycoplasma infection was considerably greater prevalence affecting veterinarians, veterinary technicians, and others that are exposed to arthropod or frequent contact with affected animal (Maggi et al., 2013b). Animals affected with Hemotropic mycoplasma are often chronic; nevertheless, hemolytic anemia of different degree of severity has been reported in animals (Willi et al., 2007; Willi et al., 2010). Clinical responses in affected animals are often associated with immunosuppression, stressors such as poor nutrition, pregnancy, lactation, or with concurrent infection with another more virulent pathogen (Novacco et al., 2010; Sykes et al., 2010; Tanahara et al., 2010).

The first report on *M. ovis* infection in Malaysia was reported in a sheep concurrently suffering from copper toxicity (Fatimah et al., 1994). In another study, Abdullah et al. (2013) reported hemotropic mycoplasmosis in a goat with concurrent case of parasitic gastro-enteritis in Malaysia. This disease was reported to be mildly pathogenic in Malaysia (Marina, 2002). *M. ovis* was diagnosed on the basis of

history, clinical signs, and identification of antigens using microscopic examination of blood smear stained with Giemsa. *M. ovis* appears as bluish or pinkish violet organism either on the surface of erythrocytes or free in the plasma, with the size of 0.4 to 2.5 µm in diameter (Ershaduzzaman, 2001). There are several methods of scoring of parasitemia caused by *M. ovis* using blood smears stained with Giemsa or Wright stain under light microscopy based on the number of infected cells or number and location of organism found in blood smears. According to scoring method described by Gulland et al. (1987), parasitemias were classified as mild (1-2%), moderate (30-59%) and severe (60% or more).

There is paucity of information on prevalence of hemotropic mycoplasmosis among goat population in Malaysia. The level of parasitemia and contributing factors towards occurrence of the disease in Malaysia is not known. Therefore, this study was designed to provide a preliminary data related to hemotropic *M. ovis* infection rate among goat population from selected goat farms in Malaysia and the possible contributing factors to the occurrence of the disease.

MATERIALS AND METHODS

Sample collection: Blood and fecal samples were collected from 10 goats each from five goat farms under Ladang Angkat Program of Faculty of Veterinary Medicine, Universiti Putra Malaysia. All the goat farms are located in two states of Selangor and Negeri Sembilan. Blood samples (3 mL) were collected aseptically from each goat by jugular venipuncture using 21G vacutainer needle. The collected blood samples were immediately put into sterile sodium heparin blood collection tube. Fecal samples (approximately 5 gm) were collected per rectum via digital evacuation. All the samples were labeled accordingly and transported in an ice box to the laboratory for further processing and analysis.

Fly trapping: Nzi fly trap was placed near the goat house to trap biting flies (*Stomoxys calcitrans*). Before setting up the Nzi trap, the selected trap site was cleared of any vegetation. A centre pole was placed in the middle of the trap site to serve as a guide in setting up poles on the back and both left and right sides of the trap. The trap was tied firmly onto the poles to make sure that the entrance faces the suitable direction. The opening of netting on top of the Nzi trap was placed with a bottle for fly sample collection. Captured flies were transferred into small plastic bottles and were transported to the laboratory where they were identified individually based on their morphological structures (Masmeatathip et al., 2006).

Administration of questionnaires: Questionnaire related to management, biosecurity, and medical history was administered to each farmer of the selected farms.

Thin blood smear and Giemsa staining: A small drop of the non-coagulated blood sample was placed on a clean labeled glass slide and a thin blood smear was made and allowed to air dry. The thin smear was fixed by flooding the slide with methanol for 1 min. The smear was then stained using freshly prepared 10% Giemsa stain for 30 min. The slide was rinsed with distilled water and air dried before viewing under light microscope at × 100 objective.

Light microscopic evaluation and calculation of infection rate of ***M. ovis*:** The organisms attached to surface of infected erythrocytes were identified as *M. ovis* when it was appeared as bluish or pinkish violet and morphologically in comma, rod, cocci, ring, chain and irregular shaped. The infection rate or parasitemia percentage was calculated using the following formula:

$$infection\ rate(\%) = \frac{No.of\ organisms}{1000\ erythrocytes} \times 100$$

The severity of parasitemia was determined using the scoring method of Gulland et al. (1987).

Fecal egg count: The modified McMaster technique was used for determining the number of nematode eggs and coccidian oocyst per gram of feces for the estimation of gastro-intestinal parasites burden. Two grams of feces was weighed and mixed with 28 mL of saturated salt solution. The mixture was then sieved into a flask and stirred vigorously. A volume of 0.15 mL was then pipetted and filled into McMaster chamber. The slides were allowed to stand for about 5 to 10 min for floatation process. The process of viewing and counting of ova and oocyst were performed under light microscopy using the objective lens of 10× to focus the lines of the grid and counted the presence of ova and oocyst in each lane of both chambers, counting of each different type of parasites was done individually. The calculation of eggs or oocysts per gram was done as follows:

$$\frac{egg\ or\ oocyst\ counted}{weight\ of\ feces} \times \frac{volume\ of\ saturated\ salt\ solution}{2(0.15\ mL)} = \text{total number of eggs per gram of feces (epg or opg).}$$

Table 1: Hemotropic *Mycoplasma ovis* infection rate among goats (n = 50).

	Infection rate			
Infection status	Not infected	Mildly infected	Moderately infected	Total
Positive	0	44	3	47
Negative	3	0	0	3
Total	3	44	3	50

$X^2 = 50.00$, $P<0.005$, $df = 2$

Table 2: The prevalence and mean fecal egg count among goat farms.

	EPG		OPG	
Farms	Prevalence (%)	Mean	Prevalence (%)	Mean
Farm A	80	583.30	100	902.60
Farm B	0	ND	60	144.20
Farm C	100	1009.30	100	981.50
Farm D	100	219.00	100	623.40
Farm E	80	305.00	90	275.80

Data analysis: All the data collected were analyzed using IBM SPSS statistics version 20 software. Pearson Chi-square was used to measure association between infection status and infection rate. Pearson's correlation coefficient was used to measure the strength of the relationship between *M. ovis* infection rate and worm burden.

RESULTS

Farm management and characteristics

All of the farms surveyed were private-owned goat farms with 80% of them practicing intensive management system. Goat breeds were predominantly local in all the farms surveyed. The purpose of goat rearing was primarily for meat; milk and breeding purpose. About 40% of the farms have herd size of less than 100 goats, housed in raised floor with slatted flooring goat house. Sheep were also reared with goat in 80% of the surveyed farms. All of the farms had isolation or quarantine facilities in their farm for the sick animals. All the farms did not practice scheduled deworming program. Previous medical history were mainly diarrhea among goat kids, jaundice, sudden death and poor weight gain among adult goats.

Hemotropic mycoplasma infection rate

The results of the hemotropic *Mycoplasma ovis* infection rate among 50 goats studied from the five farms showed an overall prevalence of 94% (n=47/50). Out of the 47 infected goats, 44 (93.6%) were mildly infected while the remaining 3 (6.4%) were moderately infected. There was a significant association ($P<0.05$) between infection status of the goats and infection rate (**Table 1**).

There was a high prevalence of worm infestation in all the farms studied. The prevalence of worm infestation was 100% for both egg per gram (EPG) and oocyst per gram (OPG) in farms C and D. The highest mean fecal egg count of 1009.30 was recorded in farm C and the lowest 144.20 was recorded in farm B (**Table 2**).

Pearson's correlation coefficient test was done to measure the strength of the relationship between M. ovis infection rate and fecal egg count. There was no significant correlation (r=0.0508, $P=0.687$) between infection rate and EPG, however, a weak positive correlation (r=0.107, $P=0.460$) was observed between infection rate and OPG (**Table 3**).

Table 3: Pearson's correlation coefficient between infection rate and fecal egg count. Pearson's Correlation (n=50)

	Infection rate	EPG	OPG
Infection rate	1	0.058	0.107
		0.687	0.460
	50	50	50
EPG	0.058	1	0.283
	0.687		0.046
	50	50	50
OPG	0.107	0.283	1
	0.460	0.046	
	50	50	50

DISCUSSION

An overall prevalence of 94% hemotropic mycoplasmosis reported in goats in the present study is considered high. The findings in our reports that 93.6% of the infected goats having mild parasitemia concur with the report of Marina (2002) who reported that the disease among sheep in Malaysia is generally mildly pathogenic. The findings of our report concur with the study of Brun-Hansen et al. (1997) who reported that erythrocyte parasitemia can be as high as 100% in infected animals. The parasite has equally been reported to infect goats and produce more severe disease (Mason and Statham, 1991). The findings of hemotropic mycoplasmosis concurrently with gastrointestinal parasitism in goats have earlier been reported by Abdullah et al. (2013), where *M. ovis* infection was seen to occur as a secondary infection to stress factors such as helminth infestation. Likewise hemotropic mycoplasma infections have often been reported in relationship with other noninfectious and infectious diseases in animals (Willi et al., 2007; Willi et al., 2010).

The high prevalence of the disease observed among goats in this study can also be explained by the high concurrent gastrointestinal parasite burden (60-100%) recorded in almost all the farms studied. Though in our studies no positive correlations could be established between hemotropic mycoplasmosis and parasitic burden, there was a significant association (P<0.05) between infection status and hemotropic infection rate. This may not be unconnected with earlier reports (Novacco et al., 2010; Sykes et al., 2010; Tanahara et al., 2010), where the studies had stated that stressors such as poor nutrition, pregnancy, lactation, concurrent infection were the cause of animals getting infected with the disease. Poor ectoparasites control was observed in almost all the goat farms surveyed and may have contributed to the transmission of the disease in the farms. Thus, this supports the possibility that widespread infection of these species may be due to ticks, other vectors, direct contact of injured animals and possibility of vertical transmission (Grazziotin et al., 2011).

This organism is transmitted by blood-feeding arthropods (Neimark et al., 2004). Factors such as climate change during sampling period and vegetation at the sampling site (Mihok, 2006) may contribute to failure in trapping the biting flies in the present study. In short, emerging zoonotic pathogen such as Hemotropic mycoplasmosis may lead to serious public health concern in near future although the pathogenic potential of this organism as a cause of human disease has not been clearly defined (Maggi et al., 2013b).

CONCLUSION

This study recorded a high occurrence of hemotropic mycoplasmosis among goats of selected farms in Malaysia even though the parasitemia levels were generally mild. Considering the zoonotic potential of the organism, further studies with larger sample size and longer duration of study involving animals and their handlers with repeated sampling in different months or seasons is highly recommended. Use of more sensitive and specific diagnostic method for *M. ovis* detection such as polymerase chain reaction (PCR) based assays is imperative to elucidate the source and transmission routes of the organism.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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