



UNIVERSITI PUTRA MALAYSIA

***HEMOTROPIC MYCOPLASMA OVIS INFECTION RATE AMONG GOATS
IN LADANG ANGKAT, FACULTY OF VETERINARY MEDICINE,
UNIVERSITI PUTRA MALAYSIA***

NURUL HAFIZAH BT ABU JAZID

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FPV 2015 50**

**HEMOTROPIC *MYCOPLASMA OVIS* INFECTION RATE AMONG GOATS
IN LADANG ANGKAT, FACULTY OF VETERINARY MEDICINE,
UNIVERSITI PUTRA MALAYSIA**

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A project submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
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DEGREE OF DOCTOR OF VETERINARY MEDICINE

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It is hereby certified that we have read this project paper entitled “Hemotropic *Mycoplasma Ovis* Infection Rate among Goats in Ladang Angkat, Faculty of Veterinary Medicine, UPM”, by Nurul Hafizah Bt. Abu Jazid and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.



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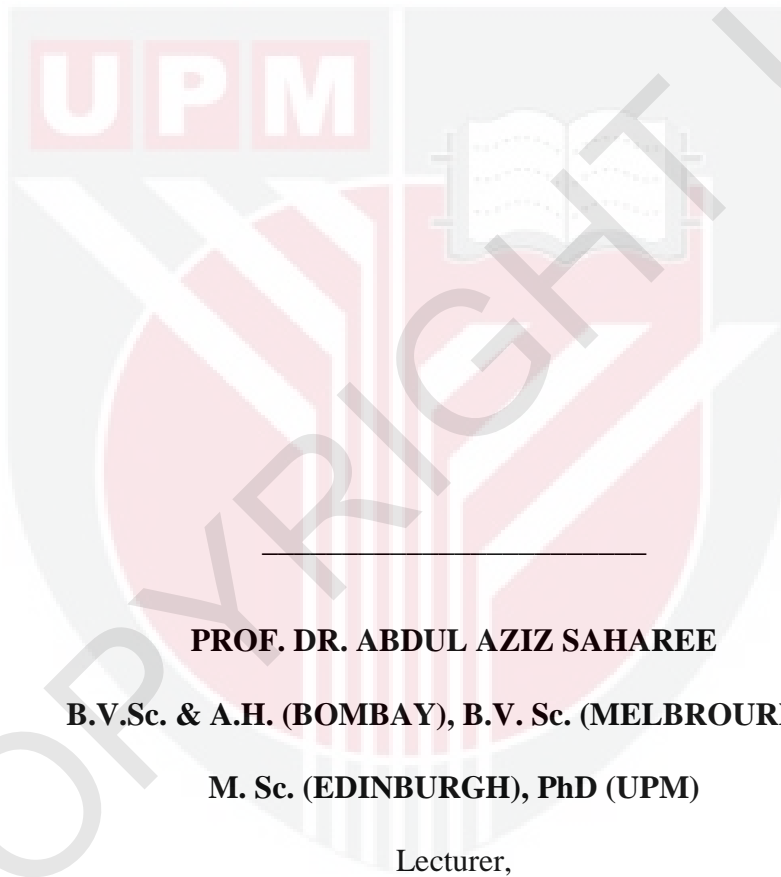
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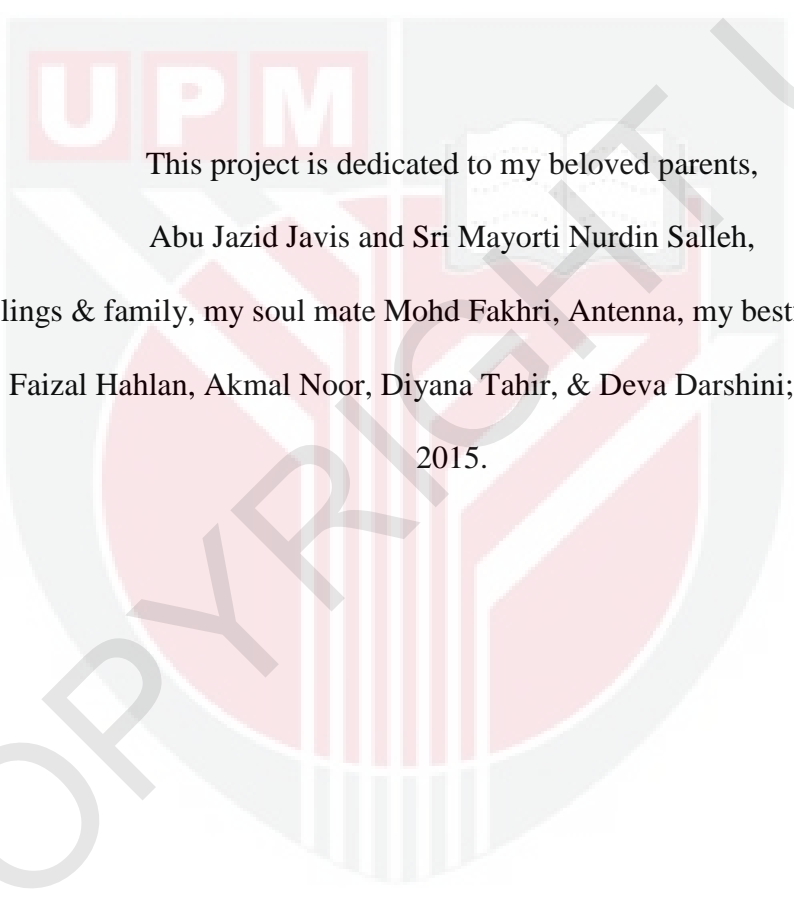
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DEDICATION



This project is dedicated to my beloved parents,
Abu Jazid Javis and Sri Mayorti Nurdin Salleh,
my siblings & family, my soul mate Mohd Fakhri, Antenna, my bestfriends: Izdihar
Ishak, Faizal Hahlan, Akmal Noor, Diyana Tahir, & Deva Darshini; & DVM class
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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir

**KADAR JANGKITAN HEMOTROPIK MYCOPLASMA OVIS DALAM
KALANGAN KAMBING DI LADANG ANGKAT, FAKULTI PERUBATAN
VETERINAR, UPM**

Oleh

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2015

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Mikoplasmosis hemotropik menjangkiti kambing dan biri-biri di serata dunia, yang juga mendatangkan kerugian ekonomi. Di Malaysia, masih terdapat kekurangan maklumat bertulis mengenai jangkitan *M. ovis* dalam kalangan kambing. Dalam kajian ini, sampel diambil daripada 10 ekor kambing dari lima buah Ladang Angkat, Fakulti Perubatan Veterinar (FPV), dan jangkitan *M. ovis* dan beban parasit gastrousus masing-masing ditentukan menggunakan pewarnaan Giemsa dan teknik modifikasi McMaster. Perangkap lalat dipasang untuk menangkap lalat menggigit dan kertas soal selidik diberikan kepada setiap ladang. Semua data dianalisa secara

statistik. 47 sampel (94.0%) daripada 50 sampel adalah positif bagi jangkitan *M. ovis*. Antara sampel-sampel positif, 44 sampel (93.6%) merupakan jangkitan ringan, dan 3 sampel (6.4%) merupakan jangkitan sederhana dengan kadar jangkitan tertinggi dicatatkan adalah 38.5% parasitemia. Tiada lalat mengigit ditangkap; kertas soal selidik mendedahkan semua ladang terletak di kawasan endemik, dan kewujudan haiwan-haiwan pembawa. Analisa statistik menyimpulkan bahawa tiada perbezaan nyata antara telur per gram dan oosis per gram dibandingkan tahap parasitemia, dan tiada korelasi nyata antara kadar jangkitan *M. ovis* dengan telur dan oosis per gram. Kesimpulannya, kadar kejadian *M. ovis* adalah tinggi dalam kalangan kambing di Ladang Angkat FPV tetapi tahap parasitemia adalah ringan secara umumnya.

Kata kunci: *Mycoplasma ovis*, pewarnaan Giemsa, kadar jangkitan, teknik Modifikasi McMaster, kambing

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project

**HEMOTROPIC MYCOPLASMA OVIS INFECTION RATE AMONG
GOATS IN LADANG ANGKAT, FACULTY OF VETERINARY MEDICINE,**

UPM

By

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2015

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Hemotropic Mycoplasmosis infects sheep and goats worldwide, which also lead to economic losses. For Malaysia, there is still lack of information documented for *Mycoplasma ovis* infection among goats. In this study, 10 goats from five Ladang Angkat, Faculty of Veterinary Medicine (FVM) were sampled, *M. ovis* infection and intestinal parasites burden was determined using Giemsa stain and Modified McMaster technique respectively. Fly trap was used to trap biting fly and questionnaire was given to each farm. All the data were statistically analysed. Out of 50 samples, 47 samples (94.0%) were positive with *M. ovis* infection. Among the positive samples, 44 samples (93.6%) were mild infection and three samples (6.4%)

were moderate infection with highest infection rate of 38.5% parasitemia. No biting fly was trapped; questionnaire revealed that all farms located in endemic area, and presence of carrier animals. Statistically, there were no significant difference in egg per gram (e.p.g) and oocyst per gram (o.p.g) with level of parasitemia, and there were no significant correlation between infection rate of *M. ovis* with e.p.g and o.p.g. As conclusion, occurrence rate of *M. ovis* is high among Ladang Angkat FVM but the parasitemia levels were generally mild.

Keywords: *Mycoplasma ovis*, Giemsa stain, infection rate, Modified McMaster technique, goats

1.0 INTRODUCTION

Mycoplasma ovis (*M. ovis*) or previously known as *Eperythrozoon ovis* is a wall-less, and pleomorphic bacterium that parasitizes the surface on erythrocytes of sheep and goats worldwide. *Mycoplasma ovis* causes chronic disease with low mortality but high morbidity in the host. Hemotropic mycoplasmosis is characterized by ill-thrift, anemia, icterus, depression and reduced weight gain, which eventually lead to economic losses to the small ruminant industry (Burroughs, 1988; Ershaduzzaman, 2001).

Parasitemia caused by *M. ovis* infection is often chronic, which persists up to 16 weeks and some cases demonstrated parasitemia up to 5 years (Daddow, 1981). Predisposing factors of this disease are pathogenicity of *M. ovis*, sheep breed susceptibility, concurrent diseases and management aspects (Sheriff, 1979). Ovine hemotropic mycoplasmosis was clinically seen in sheep of all age range (Neitz, 1940), and the infection remained persist for life (Sheriff, 1978).

The diagnosis of *M. ovis* organism in infected animals is based on the manifestation of either antigen or antibodies. Detection of antigen can be accomplished using morphological, cultural, biochemical, or molecular techniques. Example of method of detection of *M. ovis* organism based on morphology is thin blood smears stained with Giemsa which is the oldest, easiest and cheapest method of *M. ovis* identification (Ershaduzzaman, 2001).

The first report on *M. ovis* infection in Malaysia was in a sheep concurrently suffering from copper toxicity (Fatimah et al., 1994). The previous study of morphology characteristic of *M. ovis* in sheep and goats in Malaysia revealed that the organism as being coccoid and rod-like shape (Mariah et al., 1997). Prevalence of *M. ovis* infection in sheep in Malaysia was studied by Azman (1995) in several states of Malaysia, which revealed 50% of sampled farms were positive with this hemoparasite.

Abdullah et al. (2013) reported a clinical case of goat was diagnosed with Parasitic Gastro-Enteritis concurrent with hemotropic mycoplasmosis infection. According to the author there is still no study have been carried out related to *M. ovis* infection among goat population in Malaysia. Due to lack of documented information related to prevalence of this disease among goat population in Malaysia, the parasitemia level and contributing factors towards occurrence of this disease. Therefore this study was designed to have preliminary data related to hemotropic *Mycoplasma ovis* infection rate among goat population in selected goat farms.

The objectives of this study were to determine the hemotropic *Mycoplasma ovis* infection rate among goats, contributing factors of this disease, and correlation between contributing factors (presence of biting flies and intestinal parasites burden) with severity of parasitism of *M. ovis* infection among goats in farms under Ladang Angkat Program, Faculty Veterinary Medicine, UPM.

2.0 LITERATURE REVIEW

2.1 *Mycoplasma ovis*

Mycoplasma ovis which previously known as *Eperythrozoon ovis* is a pleomorphic, gram negative, wall-less bacterium that only attach to surface of sheep and goats erythrocytes (epierythrocytic parasites) and do not penetrate the blood cell (Neimark *et al.*, 2004). Hemotropic mycoplasmosis or formerly known as eperythrozoonosis was first documented in South Africa by Neitz *et al.*, (1934) and infection of this disease is first reported in Malaysia by Fatimah *et al.*, (1994). Hemotropic mycoplasmosis is a chronic disease that posed as a threat to the small ruminant industry which leads to economical losses caused by mild to severe anemia, poor growth and weight gain, depression, and even death (Mason & Statham, 1991; Neimark, 2004).

2.2 Pathogenicity of *M. ovis*

Mycoplasma ovis is known to have variable pathogenicity and it is influenced by the strain of the parasite and the contribution of environmental factors. Clinical manifestation of *M. ovis* infections varied from no obvious effect to ill thrift, and in severe cases it may lead to death due to severe anemia (Harbutt, 1969; Sutton, 1970). The effects caused by *M. ovis* on the erythrocytes are closely related to the duration of parasitemia (Gulland *et al.*, 1987). According to study by Marina, (2002), *M. ovis* strain in Malaysia is suggested to be mildly pathogenic which may not caused the effect of disease solely, but the organism proliferates in large quantity with anemic signs in sheep flock under stressful conditions.

2.3 Life cycle *M. ovis*

Hemotropic mycoplasmosis is chronic in nature and the infection may persist up to 16 weeks and some cases reported the infection in the host could be as long as 2 to 5 years (Daddow, 1977). According to Sheriff (1976), sheep that was previously infected with *M. ovis* possibly remain infected for life and served as carrier of this disease.

Study made by Marina (2002) revealed that the parasitemia patent period caused by *M. ovis* infection in sheep were longer than in goats. In goats, parasitemia were patent for 3 to 4 weeks, while the parasitemia in sheep were patent for 4 to 6 weeks. Several studies suggested variable incubation periods for *M. ovis* infection, ranges between 3 days to 28 days (Littlejohns, 1960; Foggie and Nisbet, 1964; Marina, 2001). The differences in the duration of parasitemia and the effects of *M. ovis* infection on erythrocytes were correlated with the degree of the host's immunity (Gulland *et al.*, 1987).

2.4 Pathogenesis of *M. ovis* infection

Generally, *M. ovis* infections are clinically unnoticeable and usually as incidental findings during diagnosis of blood smears. According to Kanabathy (2004), several forms of stresses have been identified to influence the development of parasitemia and carrier status of hemotropic mycoplasmosis. The primary site of proliferation of *M. ovis* is the bone marrow which the organism will be found in the peripheral blood circulation in one to four weeks later (prepatent period). The proliferation resumes on the surface of erythrocytes by budding, thus causing deformation of the surfaces of the infected erythrocytes. Pleomorphic characteristics

manifested by *M. ovis* were based on the level of parasitemia. In light and moderate parasitemias, characteristic of *M. ovis* were predominately in single cocci; and chain forms were observed in cases of severe parasitemia (Scott *et al.*, 1993). The attachment of *M. ovis* to the surface of erythrocytes leads to alteration in the infected cells membrane thus causing it to be more predisposed to extravascular hemolysis (Zachary and Basgall, 1985).

The pathologic attribute of hemotropic mycoplasmosis is anemia and serous effusion, and in some cases a moderate icterus with presence of yellowish staining of fat tissue (Valli, 1993). The spleen of the infected animal will be enlarged and soft; splenomegaly persists for some period of time even after clinical recovery. In most cases, demonstration of *M. ovis* in the blood smears was difficult as the parasitemia has declined by the time severe anemia is developed. Hemogram interpretation of macrocytic or normocytic, normochromic anemia with appropriate history and clinical signs are suggested to be diagnostic of hemotropic mycoplasmosis (Kanabathy, 2004).

2.5 Transmission of *M.ovis*

According to Smith (1986), any inoculation of a drop of blood from *M. ovis* infected animal into a susceptible host will initiate the infection. Spread of *M. ovis* infection from one sheep to another were observed in management practices and procedure that inflict bleeding and sharing of equipment such as ear-tagging, shearing, mulesing, and vaccination (Wilkinson, 1981).

Natural spread of *M. ovis* is caused by biting insects that served as mechanical vectors of this disease (Nikolskii *et al.*, 1969; Avakian *et al.*, 1973; Howard, 1975; Daddow, 1980). Studies done by Mason and Statham (1991) demonstrated that minimal infective dose for *M. ovis* was equal to one parasitized erythrocytes. The studies further indicate that a mosquito could transfer one to two erythrocytes and a biting fly may transfer approximately 400 erythrocytes in a single meal, thus can be concluded that biting fly serve as most effective vector of transmission.

2.6 Diagnosis of *M. ovis* infection

Diagnosis of *M. ovis* can be done based on the history, clinical signs, and identification of antigens using microscopic examination of blood smear stained with Giemsa. *Mycoplasma 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). First documentation of *M. ovis* morphology under light microscopy using Giemsa modification of Romanowsky's stain was made by Neitz *et al.* (1934), with the findings of multiple forms including rods, commas, rings, and irregular shapes, which have been verified by other authors. Although diagnosis of *M. ovis* using Giemsa stain under light microscopy was straightforward, easy and cheap method, the disadvantages of this method are the specificity and sensitivity of this method is low (>60% sensitivity, and >65% specificity), difficulty to differentiate between hemoplasma and artifacts such as stain deposit or dust particles, especially in cases of low parasitemia (Neitz *et al.*, 1934; Henry, 1979).

Polymerase Chain Reaction (PCR) is a molecular biology procedure with high sensitivity (>96% sensitivity) and specificity (>85% specificity) for detection of antigens from samples (Gwaltney and Oberst, 1993). Compared to conventional diagnosis method, advantages of using PCR in which this method requires only small amount of samples and does not require the presence of viable organism (Peter, 1991; Ershaduzzaman, 2001).

2.7 Scoring of parasitemia caused by *M. ovis* infection

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 of parasitemia described by Gulland *et al.*, (1987), parasitemias were classified as mild, moderate, and severe; as in table 1.

Table 1: Parasitemia scoring according to Gulland *et al.*, 1987

| Score | Percentage of infected cells |
|----------|------------------------------|
| Mild | 1 to 29% |
| Moderate | 30 to 59% |
| Severe | 60% or more |

Another parasitemia scoring described by Daddow *et al.*, (1977) based on the range of 0 to 4 (table 2).

Table 2: Parasitemia scoring according to Daddow *et al.*, 1977

| Score | Description of parasitemia |
|-------|---|
| 0 | No organism seen |
| + | Occasional ring form organism seen on careful searching |
| +1 | One ring-form organism was associated with most cells |
| +2 | Two to four ring-form organism in extracellular spaces |
| +3 | Four to eight ring-form organisms associated with cells together with organisms in the extracellular spaces |
| +4 | More than eight ring-form organisms associated with cells and may organisms found free in plasma |

2.8 Hemotropic mycoplasmosis in stressed animals

Hemotropic mycoplasmosis is usually occurs secondary to stress factors such as social stress, malnutrition and helminth infestation, but it has also been documented that ill thrift and poor weight gain can be caused by *M. ovis* solely when all other aspects such as mineral and nutritional insufficiency and gastro-intestinal parasites infestation have been eliminated (Colin, 1981). Well-fed and managed sheep flocks appeared to maintain the degree of parasitemia at low levels, in comparison to the emaciated and mismanaged flocks which observed to have consistently high level of parasitemia score and showing anemic signs (Kanabathy, 2004).

2.9 *Mycoplasma ovis* infection in Malaysia

Hemotropic mycoplasmosis was first reported in Malaysia in a sheep concurrently suffering from copper toxicity (Fatimah *et al.*, 1994). According to a study of this disease made by Mariah *et al.* (1997), the morphology of *M. ovis* in sheep and goats in Malaysia as being coccoid and rod-like shape. Study done by Ershaduzzaman (2001) indicated that *M. ovis* isolated from sheep and goat were similar.

The incubation period of *M. ovis* infection in sheep in Malaysia is unknown and the host remains infected, without showing any clinical signs. High levels of parasitemia caused by *M. ovis* was seen only when emaciating factors were present (Marina, 2002). The author also concluded that *M. ovis* infection in sheep flocks in Malaysia were not severe, where *M. ovis* in sheep flocks in Malaysian environment were mildly pathogenic and produced smallest changes in whole blood and plasma parameters.

Prevalence study on *M. ovis* among sheep by Azman (1995) revealed that 50% of sampled farms in certain areas in Malaysia were found to be positive for hemotropic mycoplasmosis, and these animals did not show any clinical signs. *Mycoplasma ovis* infection in Malaysia was localized in several states such as Selangor and Negeri Sembilan, but the possible source of infection was unidentified (Azman, 1995). Sheep imported into Malaysia may be the source of *M. ovis* carriers (Marina, 2002).

The case report on hemotropic mycoplasmosis in a goat in Malaysia was reported by Abdullah *et al.* (2013) concurrent with Parasitic Gastro-Enteritis

infection in which the goat showed moderate normocytic, normochromic anemia.

The economical losses of hemotropic mycoplasmosis in small ruminant industry in Malaysia are still unknown and there is still lack of knowledge related to this disease in goats in Malaysia.



3.0 MATERIALS AND METHODS

3.1 Sample collection

Blood and fecal samples were collected from 10 goats in five different goat farms under Ladang Angkat Program of Faculty of Veterinary Medicine, UPM. All the goat farms are located in Selangor and Negeri Sembilan (Farm A: Semenyih, Farm B: Balakong, Farm C: Dengkil, Farm D: Hulu Langat, Farm E: Labu).

Each goat was physically restrained, and the site of jugular venipuncture was disinfected using 70% alcohol swab. 21G vacutainer needle was used and the blood sample (approximately 3ml) was collected in sodium heparin blood collection tube. Then, fecal sample (approximately 5g) was collected transrectally via digital evacuation. All the samples were labelled accordingly and the samples were transported in an ice box to laboratory for further procedures.

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 should be clear of any vegetation. Centre pole was placed in the middle of trap site, as a guide in setting up poles on the back and both left and right sides of trap. The trap was tied firmly onto the poles to make sure the entrance faces the suitable direction. Then Nzi trap was erected. The opening of netting on top of the Nzi trap was placed with bottle for fly sample collection. Captured flies were transferred into small plastic bottles and were transported to laboratory for further analysis.

Questionnaire related to management, biosecurity, and medical history of each sampled farm was given to the farm owner or worker (Appendix 1).

3.2 Diagnosis of *M. ovis*

3.2.1 Thin blood smear

A small drop of uncoagulated blood sample was placed on the near end of a labelled glass slide. Edge of a cover slip was placed onto the drop of blood with angle of 30°, the cover slip also acted as the spreader. Then, the spreader was carefully dragged along entire length of sample slide. A good thin blood smear will end in a “feathered edge” which is the region to be examined under light microscopy as the blood cells were individually distributed on the slide. The sample slides were then air dried before conducting the procedure for Giemsa stain.

3.2.2 Giemsa stain

The thin blood smear was fixed by flooding the samples with absolute ethanol for 20 minutes or methanol for 1 minute. Then, the smear was stained using freshly prepared 10% Giemsa stain for duration of 30 minutes. After that, the smear was rinsed with distilled water and air dried before the slides were viewed under light microscopy.

3.2.3 Light microscopy and calculation of infection rate of *M. ovis*

Thin blood smear was examined under light microscope under 100x objective lens with oil immersion. The organisms attached to surface of infected erythrocytes were identified to be *M. ovis* if it is appeared as bluish or pinkish violet and morphologically in comma, rod, cocci, ring, chain and irregular shape. The infection rate or parasitemia percentage was calculated using the formula as below:

Infection rate (%) = (no. of organism / 1000 erythrocytes) x 100

The severity of parasitemia was determined using scoring method by Gulland *et al.* (1987), as stated in table 1.

3.3 Fecal egg count using Modified McMaster technique

Modified McMaster technique is a fecal egg floatation method for determining number of nematode eggs and coccidian oocysts per gram of feces for estimation of gastro-intestinal parasites burden in an animal.

2 grams of feces was weighed and mixed with 28 ml of saturated salt solution. The mixture was then sieved through into a flask and then was stirred vigorously. Sample of mixture with amount of 0.15ml was then pipetted and filled into McMaster chamber. The slides were subjected to stand for about 5 to 10 minutes for floatation process. The process of viewing and counting of oova and oocyst were performed under light microscopy.

Light microscope using objective lens of 10x was used to focus the lines of the grid to count presence of oova and oocyst in each lane of both chambers, counting of each different types of parasites should be done individually. The calculation of eggs or oocysts per grams are as following:

$$\frac{\text{Egg / oocysts counted}}{\text{Weight of feces}} \times \frac{\text{Volume of saturated salt solution}}{2 (0.15\text{ml})}$$

= total number of eggs/ oocysts per gram of feces (e.p.g or o.p.g)

3.4 Correlation between severity of *M. ovis* parasitemia and gastro-intestinal parasites burden

The results obtained related to the infection rate and gastro-intestinal parasites burden this study were subjected to statistical analysis using IBM SPSS version 20.

3.5 Identification of *Stomoxys calcitrans*

Flies that were trapped were identified individually based on their morphological structures (Masmeatathip *et al.*, 2006).

3.6 Questionnaire data

The data obtained from the questionnaire (Appendix A) were subjected to statistical analyses to know the frequency and similarities shared by sampled farms in comparison to overall infection rate of *M. ovis*.

4.0 RESULTS

4.1 Infection rate and parasitemia levels of *M. ovis*

Thin blood smear stained with Giemsa revealed that morphology of *M. ovis* found in blood samples were bluish epierythrocytic predominantly rod, and coccoid shape (Figure 1).

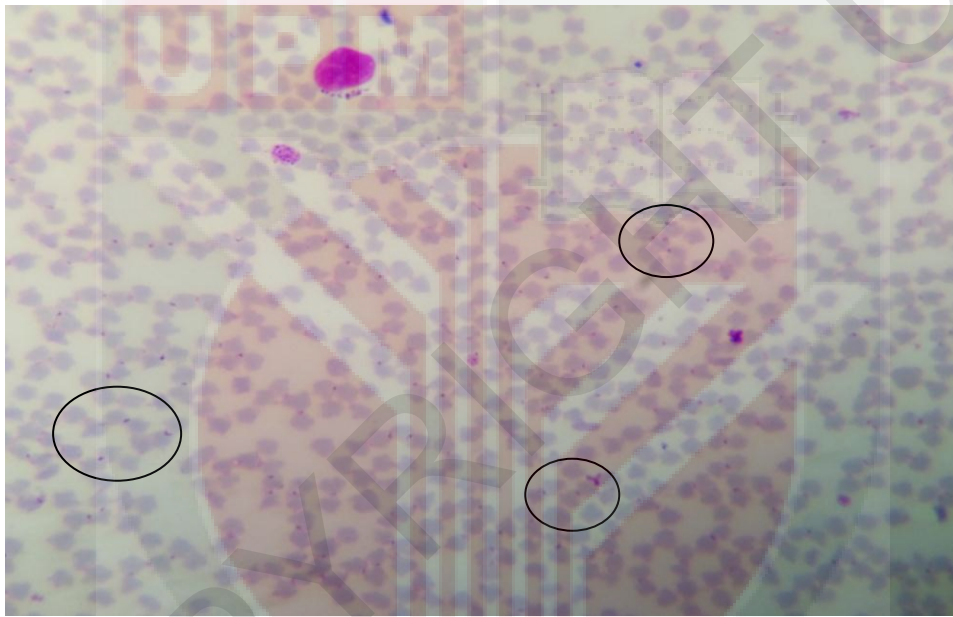


Figure 1: Giemsa stained blood smear showing bluish rod and coccoid shape epierythrocytic (black circle) organism indicative of *M. ovis* under light microscopy (under 100x objective lense with oil immersion)

From 50 blood samples, 47 samples (94%) were found to be positive with *M. ovis*; and 3 samples (6%) were negative with *M. ovis* infection. Highest value of *M. ovis* infection was 38.5% infection in a goat from Farm D in Hulu Langat. Based on the scoring system of parasitemia by Gulland *et al.* (1987), among the 47 positive samples, 44 samples were scored to be mild parasitemia (1 to 29% infection), and

another 3 samples were scored to be moderate parasitemia (30 – 59% infection); as shown in Table 3.

Table 3: Infection rate and parasitemia levels of *M. ovis* among blood samples using thin blood smear stained with Giemsa

| Animal id | <i>M. Ovis</i> infection | Infection | |
|-----------|--------------------------|-----------|--------------|
| | | Rate (%) | Parasitemia |
| A1 | Positive | 22.4 | Mild |
| A2 | Positive | 1.8 | Mild |
| A3 | Positive | 5.1 | Mild |
| A4 | Positive | 1.5 | Mild |
| A5 | Positive | 2.6 | Mild |
| A6 | Positive | 2.2 | Mild |
| A7 | Positive | 1.4 | Mild |
| A8 | Positive | 15 | Mild |
| A9 | Positive | 1.2 | Mild |
| A10 | Negative | 0 | No infection |
| B1 | Positive | 0.8 | Mild |
| B2 | Positive | 1.7 | Mild |
| B3 | Positive | 31.4 | Moderate |
| B4 | Positive | 0.3 | Mild |
| B5 | Positive | 1.7 | Mild |
| B6 | Negative | 0 | No infection |
| B7 | Positive | 2.3 | Mild |
| B8 | Positive | 2.3 | Mild |
| B9 | Positive | 2.1 | Mild |
| B10 | Positive | 2.1 | Mild |
| C1 | Positive | 5.4 | Mild |
| C2 | Positive | 10.5 | Mild |
| C3 | Positive | 2.5 | Mild |

Table 3 (cont.)

| Animal id | <i>M. Ovis</i> infection | Infection | |
|-----------|--------------------------|-----------|--------------|
| | | Rate (%) | Parasitemia |
| C4 | Positive | 4.5 | Mild |
| C5 | Positive | 1.5 | Mild |
| C6 | Positive | 1.8 | Mild |
| C7 | Positive | 2.2 | Mild |
| C8 | Positive | 2.5 | Mild |
| C9 | Positive | 1.3 | Mild |
| C10 | Positive | 4.2 | Mild |
| D1 | Positive | 13.7 | Mild |
| D2 | Positive | 26.6 | Mild |
| D3 | Positive | 29.7 | Moderate |
| D4 | Positive | 5.2 | Mild |
| D5 | Positive | 38.5 | Moderate |
| D6 | Positive | 20.1 | Mild |
| D7 | Positive | 5.8 | Mild |
| D8 | Positive | 1.7 | Mild |
| D9 | Positive | 2.1 | Mild |
| D10 | Positive | 15.8 | Mild |
| E1 | Positive | 0.7 | Mild |
| E2 | Positive | 1 | Mild |
| E3 | Positive | 5.7 | Mild |
| E4 | Positive | 15.6 | Mild |
| E5 | Positive | 1.5 | Mild |
| E6 | Positive | 1.2 | Mild |
| E7 | Positive | 2.3 | Mild |
| E8 | Positive | 0.9 | Mild |
| E9 | Negative | 0 | No infection |
| E10 | Positive | 1.4 | Mild |

4.2 Fecal egg count using Modified McMaster technique

The result of nematodes oova and coccidian oocyst count per gram of feces (Table 4) showed that Farm B which located in Semenyih have the lowest gastro-intestinal eggs count. Farm C have the highest fecal egg count among their goats, 8 out of 10 goats which managed under intensive management system were determined to have fecal egg count more than 500 e.p.g. (indicate moderate to high nematode burden in intensive management system). Only Farm A was managed in semi-intensive system. Name of anthelmintic and date of last administration of anthelmintic for each farm are as stated in Table 5.

Table 4: Result for gastro-intestinal parasite eggs count using modified McMaster technique and level of parasitemia

| Animal id | Epg | Opg | Parasitemia (<i>M. ovis</i>) |
|------------------|------------|------------|-------------------------------------|
| A1 | 2000 | 1208 | Mild |
| A2 | 326 | 326 | Mild |
| A3 | 0 | 744 | Mild |
| A4 | 279 | 94 | Mild |
| A5 | 0 | 5440 | Mild |
| A6 | 930 | 558 | Mild |
| A7 | 558 | 186 | Mild |
| A8 | 1162 | 94 | Mild |
| A9 | 46 | 46 | Mild |
| A10 | 46 | 280 | No infection |
| B1 | 0 | 186 | Mild |
| B2 | 0 | 46 | Mild |
| B3 | 0 | 604 | Moderate |
| B4 | 0 | 280 | Mild |

Table 4 (cont.)

| Animal id | Epg | Opg | Parasitemia (<i>M. ovis</i>) |
|------------------|------------|------------|-------------------------------------|
| B5 | 0 | 0 | Mild |
| B6 | 0 | 0 | No infection |
| B7 | 0 | 0 | Mild |
| B8 | 0 | 94 | Mild |
| B9 | 0 | 0 | Mild |
| B10 | 0 | 232 | Mild |
| C1 | 604 | 326 | Mild |
| C2 | 1023 | 280 | Mild |
| C3 | 94 | 512 | Mild |
| C4 | 372 | 930 | Mild |
| C5 | 1581 | 977 | Mild |
| C6 | 1349 | 3302 | Mild |
| C7 | 1163 | 1395 | Mild |
| C8 | 1442 | 186 | Mild |
| C9 | 512 | 233 | Mild |
| C10 | 1953 | 1674 | Mild |
| D1 | 94 | 512 | Mild |
| D2 | 326 | 326 | Mild |
| D3 | 186 | 512 | Moderate |
| D4 | 94 | 1023 | Mild |
| D5 | 94 | 1163 | Moderate |
| D6 | 94 | 1906 | Mild |
| D7 | 372 | 140 | Mild |
| D8 | 186 | 326 | Mild |
| D9 | 186 | 140 | Mild |
| D10 | 558 | 186 | Mild |
| E1 | 150 | 200 | Mild |
| E2 | 150 | 94 | Mild |
| E3 | 1750 | 1674 | Mild |
| E4 | 150 | 200 | Mild |

Table 4 (cont.)

| Animal id | Epg | Opg | Parasitemia (<i>M. ovis</i>) |
|------------------|------------|------------|-------------------------------------|
| E5 | 100 | 0 | Mild |
| E6 | 400 | 200 | Mild |
| E7 | 200 | 150 | Mild |
| E8 | 150 | 100 | Mild |
| E9 | 0 | 46 | No infection |
| E10 | 0 | 94 | Mild |

Table 5: Name of anthelmintic and date of last administration of anthelmintic for each farm

| Farm | Name of anthelmintic | Duration of last administration before sampling |
|-------------|----------------------------------|--|
| A | Nilverm oral drench (Levamisole) | 1 month |
| B | Unknown | 6 months |
| C | Ivermectin | 6 months |
| D | Ivermectin | 4 month |
| E | Unknown | 9 months |

4.3 Presence of biting flies (*Stomoxys calcitrans*)

No biting flies (*Stomoxys calcitrans*) were trapped during the sampling session using Nzi trap.

4.4 Questionnaire data

The answers obtained from the respondents which served as description of study herd were tabulated by frequency and overall percentage (Table 6).

Based on the answers regarding the farm's management, the location of the study herds located 80% (4/5) in Selangor and 20% (1/5) in Negeri Sembilan. All of

the farms were private-owned goat farms; 80% (4/5) of farms practice intensive management system, and 20% (1/5) farm was managed semi-intensively. 40% (2/5) farms also reared Katjang breed, 20% (1/5) farms reared Saanen breed, 20% (1/5) farms reared Jamnapari breed; alongside Boer breed which were reared by all farms. The purpose of goat was primarily reared for meat; milk and breeder purpose were only in 40% (2/5) farms. 80% (4/5) farms have goat with oldest age range of 2 to 4 years, and 20% (1/5) farm have goat older than 4 years old. 40% (2/5) farms have herd size of less than 100 goats, 40% (2/5) farms have herd size of 100 to 200 goats, and 20% (1/5) farm have herd size of more than 200 goats. All of the farms in this study were housed in raised floor with slatted flooring goat house. 80% (4/5) farms have other species of animal namely sheep in their goat farm, and 20% (1/5) farm do not reared goat alongside other animal species.

For the questions related to the biosecurity of the farm. 60% (3/5) farms do not practice importation of animal, and 40% (2/5) farms did import animal from abroad. The origin country of imported animals was from Australia. 60% (3/5) farms did exchanged or purchased goats with nearby farms, and 40% (2/5) farms did not exchanged or purchased goats with nearby farms. All of the farms have isolation or quarantine facilities in their farm, 100% (5/5) farms quarantined newly arrived and sick animals. 20% (1/5) farm quarantined under-performed animals, 40% (2/5) farms quarantined pregnant animals. 60% (3/5) farms practice quarantine of young animals. 100% (5/5) farms did not vaccinate their goats, but endoparasite control (anthelmintic drugs) was practiced in 100% (5/5) of sampled farms. 40% (2/5) farms practiced ectoparasite control, 60% (3/5) farms do not practiced ectoparasite control. 80% (4/5) farms cleaned their goat pens in daily basis, 20% (1/5) farm cleaned their

goat farm intermittently; 100% (5/5) farms did not use disinfectant when cleaning the farms. 60% (3/5) farms did not practice fly or mosquito control in the goat farms, 40% (2/5) farms did employed fly or mosquito control in their farms; namely usage of smoke or effective microorganism. 80% (4/5) farms did have fly or mosquito problem, 20% (1/5) farm did not have fly or mosquito problem. Levels of disturbance caused by fly or mosquito was 20% (1/5) farm tolerable, 40% (2/5) did not sure about level of disturbance, 40% (2/5) claimed to have no disturbance caused by fly and mosquito.

For the previous medical history, 100% (5/5) farms did observed diarrhea cases, 80% (4/5) farms claimed cases occurred among young kids, 80% (4/5) farms responded that the condition resolved and another 20% (1/5) farm claimed the condition of some of the affected animal became worsen and died. Only 20% (1/5) farm did observe jaundice cases among their goats, another 80% (4/5) did not know if there any jaundice animals in their farms. Sudden death did not occur in 40% (2/5) farms, while 60% (3/5) farms witnessed at least one case of sudden death among their herds, which mostly occurred in kids. 60% (3/5) farms did have animal with poor weight gain which occurred in adult goats. 100% (5/5) farms observed depressed looking goat among their herds. 100% (5/5) farms seek treatment for their animals from veterinarian from FVM, UPM, but 80% (4/5) farms also self-medicate their sick animals.

Table 6: Herd description based on questionnaire (Appendix A) responses

| VARIABLES | CATEGORY | FREQUENCIES | PERCENTAGE |
|-----------|----------|-------------|------------|
|-----------|----------|-------------|------------|

| | | (n=5) | (%) |
|----------------------|-----------------------------------|-------|-----|
| MANAGEMENT | | | |
| State | Selangor | 4 | 80 |
| | Negeri Sembilan | 1 | 20 |
| Type of farm | Government's owned | 0 | 0 |
| | Private / smallholder | 5 | 100 |
| System of production | Intensive | 4 | 80 |
| | Semi-intensive | 1 | 20 |
| Breed | Boer | 5 | 100 |
| | Katjang | 2 | 40 |
| | Saanen | 1 | 20 |
| | Jamnapari | 1 | 20 |
| Purpose | Meat | 5 | 100 |
| | Milk | 2 | 40 |
| | Breeder | 2 | 40 |
| Oldest age range | <2 years | 0 | 0 |
| | 2- 4years | 4 | 80 |
| | >4 years | 1 | 20 |
| Herd size | <100 heads | 2 | 40 |
| | 100 – 200 heads | 2 | 40 |
| | >200 heads | 1 | 20 |
| Housing | Raise floor with slatted flooring | 5 | 100 |
| | Others | 0 | 0 |

Table 6 (cont.)

| VARIABLES | CATEGORY | FREQUENCIES (n=5) | PERCENTAGE (%) |
|-----------|----------|----------------------|-------------------|
|-----------|----------|----------------------|-------------------|

| | | | |
|---|------------------|---|-----|
| Other species in farm | No species | 1 | 20 |
| | Sheep | 4 | 80 |
| BIOSECURITY | | | |
| Importation of animals | Yes | 2 | 40 |
| | No | 3 | 60 |
| Origin of animals | Malaysia | 3 | 60 |
| | Australia | 2 | 40 |
| Exchanged or purchased of animal from nearby farm | Yes | 3 | 60 |
| Last introduction of new animal | Nov 2014 | 1 | 20 |
| | Dec 2014 | 2 | 40 |
| | January 2015 | 1 | 20 |
| | Not sure | 1 | 20 |
| Quarantine/isolation facilities | Yes | 5 | 100 |
| | No | 0 | 0 |
| Criteria of quarantine animals | New animal | 5 | 100 |
| | Under-performed | 1 | 20 |
| | Pregnant/kidding | 2 | 40 |
| | Young | 3 | 60 |
| | Sick | 5 | 100 |
| Vaccination status | None | 5 | 100 |
| | Up-to-date | 0 | 0 |
| Endoparasite control | Yes | 5 | 100 |
| | No | 0 | 0 |

Table 6 (cont.)

| VARIABLES | CATEGORY | FREQUENCIES (n=5) | PERCENTAGE (%) |
|-----------|----------|----------------------|-------------------|
|-----------|----------|----------------------|-------------------|

| | | | |
|---|-------------------------|-------------------|-----|
| Last administration of anthelmintic | 1month ago | 1 | 20 |
| | 4months ago | 1 | 20 |
| | 6months ago | 2 | 40 |
| | 9months ago | 1 | 20 |
| Ectoparasite control | Yes | 2 | 40 |
| | No | 3 | 60 |
| Last administration of ectoparasite control drug | 2months ago | 1 | 20 |
| | 6months ago | 1 | 20 |
| Frequency for cleaning of pen | Daily | 4 | 80 |
| | Any other days | 1 | 20 |
| Use of disinfectant | Yes | 0 | 0 |
| | no | 5 | 100 |
| Fly or mosquito control | Yes | 2 | 40 |
| | No | 3 | 60 |
| Method of fly / mosquito control | Smoke | 1(out of 2 farms) | 50 |
| | Effective microorganism | 1(out of 2 farms) | 50 |
| Fly / mosquitoes problem | Yes | 4 | 80 |
| | No | 1 | 20 |
| table 1Level of disturbance caused by fly/ mosquitoes | Tolerable | 1 | 20 |
| | Not sure | 2 | 40 |
| | No disturbance | 2 | 40 |

Table 6 (cont.)

| VARIABLES | CATEGORY | FREQUENCIES (n=5) | PERCENTAGE (%) |
|--------------------------------|-----------------|----------------------|-------------------|
| MEDICAL HISTORY | | | |
| Diarrhea | Yes | 5 | 100 |
| | No | 0 | 0 |
| | Young | 4 | 80 |
| | Adult | 1 | 20 |
| | Resolved | 4 | 80 |
| | Died | 1 | 20 |
| Jaundice | Yes | 1 | 20 |
| | No | 0 | 0 |
| | Unknown | 4 | 80 |
| Sudden death | Yes | 3 | 60 |
| | No | 2 | 40 |
| | Young | 2 (out of 3 farms) | 67 |
| | Adult | 1 (out of 3 farms) | 33 |
| | | | |
| Poor weight gain | Yes | 3 | 60 |
| | No | 0 | 0 |
| | unknown | 2 | 40 |
| | Young | 0 | 0 |
| | Adult | 3 (out of 3 farms) | 100 |
| | | | |
| Depressed-looking | Yes | 5 | 100 |
| | no | 0 | 0 |
| | Young | 1 | 20 |
| | Adult | 4 | 80 |
| How do treat your sick animals | Self-medicate | 4 | 80 |
| | UPM Vet/student | 5 | 100 |

4.5 Statistical analysis between infection rate with the severity of gastrointestinal parasites burden

All the data was analysed using IBM SPSS version 20. Based on the normality test using Shapiro-Wilk (as the sample size was small, $n=50$), null hypothesis stated that data was normally distributed, and alternate hypothesis stated that data was not normally distributed. P-value for the data of this study was less than $\alpha=0.05$, therefore null hypothesis was failed to be accepted and the data was not normally distributed (Table 7).

Table 7: Test of normality

| | Shapiro-Wilk | | |
|----------------|--------------|----|-------|
| | Statistic | df | Sig. |
| Infection rate | 0.668 | 50 | 0.000 |
| e.p.g | 0.739 | 50 | 0.000 |
| o.p.g | 0.590 | 50 | 0.000 |

As the data was not normally distributed and non-parametric data, Kruskal Wallis test was employed. Null hypothesis for Kruskal Wallis stated that there is no significant difference between means of samples, and alternate hypothesis of this test stated that there is a significant difference between means of samples. The p-value of e.p.g and o.p.g were both more than $\alpha=0.05$, thus failed to reject null hypothesis. Kruskal Wallis test concluded that there is no significant difference in e.p.g and o.p.g with levels of parasitemia (Table 8).

Table 8: Kruskal Wallis test for e.p.g and o.p.g with levels of parasitemia

| | e.p.g | o.p.g |
|-------------|-------|-------|
| Chi-Square | 4.684 | 4.791 |
| Df | 2 | 2 |
| Asymp. Sig. | 0.096 | 0.091 |

Correlation and regressions test were also done to the data with null hypothesis stated that there is no significant correlation between samples, and alternate hypothesis stated that there is a significant correlation between samples. Based on the Pearson Correlation, the p-value for e.p.g (0.0508) and o.p.g (0.107) were more than $\alpha=0.05$. Therefore, null hypothesis were failed to be rejected, and there is no significant correlation between infection rate with e.p.g and o.p.g (Table 9).

Table 9: Correlation test using Pearson Correlation

| | Infection rate | e.p.g | o.p.g |
|----------------|---------------------|-------|-------|
| Infection rate | Pearson Correlation | 1 | 0.058 |
| | Sig. (2-tailed) | | 0.687 |
| | N | 50 | 50 |
| e.p.g | Pearson Correlation | 0.058 | 1 |
| | Sig. (2-tailed) | 0.687 | 0.046 |
| | N | 50 | 50 |
| o.p.g | Pearson Correlation | 0.107 | 0.283 |
| | Sig. (2-tailed) | 0.460 | 0.046 |
| | N | 50 | 50 |

Regressions test (Table 10) was also done to the data using the following calculation:

$$\text{Infection rate (IR)} = B_0 + B_1 (\text{e.p.g}) + B_2 (\text{o.p.g})$$

B_0 = constant; B_1 = coefficient value for ep.g; B_2 = coefficient value for o.p.g

$$\text{IR} = 5.707 + 0.001 (\text{e.p.g}) + 0.001 (\text{o.p.g})$$

$$R^2 = 0.012 = 1.2\% \text{ (very low)}$$

This test concluded that e.p.g and o.p.g were not good predictor variables for infection rate of hemotropic mycoplasmosis.

Table 10: Regression test for e.p.g and o.p.g with infection rate

| Model | R | R Square | Adjusted R Square | Std Error of the Estimate |
|-------|-------|----------|-------------------|---------------------------|
| 1 | 0.111 | 0.012 | -0.030 | 9.35040 |

| Model | Unstandardized Coefficients | |
|------------|-----------------------------|------------|
| | B | Std. Error |
| (Constant) | | |
| e.p.g | 5.707 | 1.743 |
| | 0.001 | 0.002 |
| o.p.g | 0.001 | 0.002 |

5.0 DISCUSSION

From this study, there were 47 positive samples out of 50 samples (94%) for *M. ovis* infection among goats from farms in Ladang Angkat Program of Faculty of Veterinary Medicine UPM. The predominant features of *M. ovis* in this study were bluish rod and coccoid-like shape of organism at the surface of the erythrocytes. These findings were in agreement with Neitz *et al.* (1934), Mariah *et al.* (1994), and Ershaduzzaman (2001).

The findings of this study revealed that Farm D which located in Hulu Langat revealed highest infection rate at 38.5%, where as the parasitemia level was found to be moderate. These findings were in agreement with Marina (2002) stated that *M. ovis* infection in Malaysia was mildly pathogenic. Furthermore, the findings from Marina (2002) were accord with our findings where 44 samples in this study revealed mild parasitemia level.

For the findings of gastro-intestinal parasites infection, Farm B showed lowest gastro-intestinal parasite egg count where as Farm C showed highest gastro-intestinal parasite egg count. Farm B and Farm C both had mild parasitemia infection although Farm C showed higher in the burden of gastro-intestinal parasite. These results were not in accord with Colin (1981) and Abdullah *et al.* (2013) stated that hemotropic mycoplasmosis infection usually occurs due to secondary stress factor such as helminth or Parasitic Gastro-Enteritis infection. This may be due to Farm B and C practiced good feeding and sanitary management that may lead to good immunity build up in the host and thus reduce the stress factor in the host itself.

In this study, there was no biting fly was trapped during the sampling period at the selected farms. The factors that may contribute to the inability to trap the mechanical vector of *M. ovis* are inappropriate orientation, location, and duration of Nzi trap was placed, climate changes during sampling period and vegetation at the sampling site, and these factors were in accord with Mihok (2006). According the Hennekeler (2008) stated that Nzi trap will be efficient in trapping biting flies with using bait and thus may be one of the reasons of failure in trapping biting flies in this study as the bait was not used.

All the selected farms in this study were predisposed to hemotropic mycoplasmosis infection according to the location of the farms which situated in endemic areas: Selangor and Negeri Sembilan (Azman, 1995). The data of questionnaire showed that the risk of *M. ovis* infection may due to presence of carriers or infected animals in the farm due to importation of animal from endemic country such as Australia (Little Johns, 1960) and exchanged of animals with other farms. Another factor that contributes to *M. ovis* infection is poor quarantine facilities in the farm where the location of the quarantine pens in all the sampled farms were observed to be in close proximity to the herds of animals. Poor deticking program practice could be another factor contributing *M. ovis* infection, as this disease transmission was mainly caused by stable flies (Mason and Statham, 1991). In this study, only 2 farms practiced ectoparasite control and poor practiced of ectoparasite control may contribute to transmission of *M. ovis* infection among goat in the selected farms. From the questionnaire, all the sampled farms showed clinical signs of hemotropic mycoplasmosis such as poor weight gain, depression and jaundice as mentioned by Burroughs (1988). However, these clinical signs of

hemotropic mycoplasmosis were not specific and may due to other concurrent disease as well.

As a conclusion, from this study there was no significant difference in severity of gastro-intestinal parasites infestation with the severity of parasitemia. The result of this study is not in agreement with Colin (1981) and Abdullah *et al.* (2013) stated that *M. ovis* infection occurred as secondary to stress factors such as helminth infestation. This may be due to small sample size, short duration of the study and only one month of the year was involved in this study.

6.0 CONCLUSION AND RECOMMENDATIONS

As a conclusion, the occurrence rate of hemotropic mycoplasmosis among goats from selected farms in Ladang Angkat Program of Faculty of Veterinary Medicine, UPM is 94% (47/50), which is high occurrence but the parasitemia levels were generally mild.

Recommendations for future research in hemotropic mycoplasmosis are to increase the sample size and longer duration of study with repeated sampling on the animals in different month or seasons, usage of more sensitive and specific diagnostic method for *M. ovis* such as Polymerase Chain Reaction (PCR) method, and identify hematology values and health status of the sample animals for better comparison with the infection rate or effects of this disease.

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8.0 APPENDIX

Appendix A: Questionnaire related to management, biosecurity, and medical history
for each sampled farm

| Farm's background: | | | |
|---|---|---------------------------------------|---|
| Farm's owner: | | | |
| Farm's address: | | | |
| Year of farm's established: | | | |
| Type of farm (please tick): | <input type="checkbox"/> Government's owned farm | <input type="checkbox"/> Small holder | <input type="checkbox"/> Others(please specify): |
| Purpose of animals (please tick): | <input type="checkbox"/> Meat | <input type="checkbox"/> Dairy | <input type="checkbox"/> Others(please specify): |
| Breed of goats: | | | |
| Farm's population: | Total: | | |
| | Female: | | |
| | Male: | | |
| | Young/kids: | | |
| Performance of animals in farm: | | | |
| In general, how do you think the performance level of your goats? (please tick) | | | |
| <input type="checkbox"/> Poor <input type="checkbox"/> Fair <input type="checkbox"/> Good <input type="checkbox"/> Very Good <input type="checkbox"/> Excellent | | | |
| No. of kidding per year: | | | |
| Range of age: | Youngest: | Oldest: | |
| Average Daily Gain (g/day): | | | |
| Market weight & age: | Market weight (kg): | Market age: | |
| Since last year, what is the percentage of your animals was: | Sold for cash: | | |
| | Slaughtered because of sickness: | | |
| | Exchanged but not sold for cash: | | |
| | Disposed of other reason: (Please specify reason): | | |
| Milk production (L/day): | | | |

Appendix A (cont.)

| Housing and feeding management: | | |
|--|--|---|
| Type of housing: | <input type="checkbox"/> Raised house with slatted flooring | <input type="checkbox"/> Others (please specify): |
| Management system: | <input type="checkbox"/> Intensive system <input type="checkbox"/> Semi – intensive system <input type="checkbox"/> Extensive system | |
| Feeding management: | <input type="checkbox"/> Pellets + cut & carry <input type="checkbox"/> Pellets + free graze <input type="checkbox"/> Pellets only <input type="checkbox"/> Cut & carry only <input type="checkbox"/> Free graze only <input type="checkbox"/> Others (please specify): | |
| Type of pellets: | | |
| Frequency & amount of feeding with pellets: | Frequency: | Amount (g): |
| Type of grass: | | |
| Frequency & amount of feeding with pellets: | Frequency: | Amount (g): |
| Time of grazing: | From: To: | |
| Feed supplement: | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify (if yes): |
| Farm biosecurity and disease management or control: | | |
| Any nearby farm: | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify (if yes) Distance (km): Species: |
| Importation of animals: | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify (if yes) Country of origin: |
| Exchange or buy animal from nearby farm: | | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| Last introduction of new animal (dd/mm/yy): | | |
| Do you quarantine your animal? | | <input type="checkbox"/> Yes <input type="checkbox"/> No |
| Where did you quarantine the animal? Please specify: | | |
| Which condition of animals do you quarantine your animal? | | <input type="checkbox"/> New animals <input type="checkbox"/> Underperform / ill thrift <input type="checkbox"/> Pregnant <input type="checkbox"/> Young <input type="checkbox"/> Sick / diseased |
| Do you vaccinate your animal? | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify types of vaccine: |

Appendix A (cont.)

| | | |
|---|---|--|
| Last vaccination date (dd/mm/yy): | | |
| Age at first vaccination: | | |
| Do you deworm your animal: | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify types of anthelmintic: |
| Last deworming date (dd/mm/yy): | | |
| Who did the deworming: | <input type="checkbox"/> your self <input type="checkbox"/> worker | <input type="checkbox"/> DVS AVO / VO <input type="checkbox"/> Others (please specify): |
| Criteria of deworming: | <input type="checkbox"/> Every 3 or 4 months <input type="checkbox"/> Only young or pregnant | <input type="checkbox"/> Based on FEC <input type="checkbox"/> Others (please specify): |
| Do you perform any ectoparasite (eg: lice, mites) control to your animal: | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify types of ectoparasite control: |
| Last ectoparasite control date (dd/mm/yy): | | |
| Who did the ectoparasite control: | <input type="checkbox"/> your self <input type="checkbox"/> worker | <input type="checkbox"/> DVS AVO / VO <input type="checkbox"/> Others (please specify): |
| Criteria of ectoparasite control: | <input type="checkbox"/> Every 3 or 4 months <input type="checkbox"/> Only young or pregnant | <input type="checkbox"/> Based on ectoparasite burden <input type="checkbox"/> Others (please specify): |
| Do your goats experience any of following condition? | <input type="checkbox"/> Diarrhea | Date: Age/gender: Severity: Progress: |
| | <input type="checkbox"/> Jaundice | Date: Age/gender: Severity: Progress: |
| | <input type="checkbox"/> Poor weight gain | Date: Age/gender: Severity: Progress: |

Appendix A (cont.)

| | | |
|---|--|---|
| Do your goats experience any of following condition? | <input type="checkbox"/> Sudden death | Date: Age/gender: Severity: Cause of death: |
| | <input type="checkbox"/> Depressed | Date: Age/gender: Severity: Progress: |
| How do you treat your sick animals? | <input type="checkbox"/> Self medicate <input type="checkbox"/> DVS AVO | <input type="checkbox"/> DVS VO <input type="checkbox"/> Others (please specify): |
| Do you aware of disease of haemotropic mycoplasma? | <input type="checkbox"/> Yes <input type="checkbox"/> No | |
| Farm hygiene and waste disposal management: | | |
| Do you dispose farm waste properly? | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| How do you dispose the goat manure? | <input type="checkbox"/> Just flush with water to push it into drain | <input type="checkbox"/> collect and sell it |
| Does your farm have fly/ or mosquitoes problem? | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify method of control: |
| Frequency for cleaning of goats pen? | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly | <input type="checkbox"/> Monthly <input type="checkbox"/> Others (please specify): |
| What do you use to clean our farm? | <input type="checkbox"/> Disinfectant <input type="checkbox"/> Others (please specify): | <input type="checkbox"/> Detergent |
| In general, how would you say the hygiene level of your goats farm? (please tick) <input type="checkbox"/> Poor <input type="checkbox"/> Fair <input type="checkbox"/> Good <input type="checkbox"/> Very Good <input type="checkbox"/> Excellent | | |
| In general, how would you say the disturbance level of your goats caused by flies / mosquitoes? (please tick) <input type="checkbox"/> No disturbance <input type="checkbox"/> Tolerable <input type="checkbox"/> Not Sure <input type="checkbox"/> Severely Disturbed | | |

Appendix A (cont.)

| Farm biosecurity and disease management or control: | | |
|---|---|---|
| Any nearby farm: | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify (if yes) Distance (km): Species: |
| Importation of animals: | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify (if yes) Country of origin: |
| Exchange or buy animal from nearby farm: | <input type="checkbox"/> Yes <input type="checkbox"/> No | |
| Last introduction of new animal (dd/mm/yy): | | |
| Do you quarantine your animal? | <input type="checkbox"/> Yes <input type="checkbox"/> No | |
| Where did you quarantine the animal? Please specify: | | |
| Which condition of animals do you quarantine your animal? | | <input type="checkbox"/> New animals <input type="checkbox"/> Underperform / ill thrift <input type="checkbox"/> Pregnant <input type="checkbox"/> Young <input type="checkbox"/> Sick / diseased |
| Do you vaccinate your animal? | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify types of vaccine: |
| Last vaccination date (dd/mm/yy): | | |
| Age at first vaccination: | | |
| Do you deworm your animal: | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify types of anthelmintic: |
| Last deworming date (dd/mm/yy): | | |
| Who did the deworming: | <input type="checkbox"/> your self <input type="checkbox"/> worker | <input type="checkbox"/> DVS AVO / VO <input type="checkbox"/> Others (please specify): |
| Criteria of deworming: | <input type="checkbox"/> Every 3 or 4 months <input type="checkbox"/> Only young or pregnant | <input type="checkbox"/> Based on FEC <input type="checkbox"/> Others (please specify): |
| Do you perform any ectoparasite (eg: lice, mites) control to your animal: | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify types of ectoparasite control: |
| Last ectoparasite control date (dd/mm/yy): | | |
| Who did the ectoparasite control: | <input type="checkbox"/> your self <input type="checkbox"/> worker | <input type="checkbox"/> DVS AVO / VO <input type="checkbox"/> Others (please specify): |
| Criteria of ectoparasite control: | <input type="checkbox"/> Every 3 or 4 months <input type="checkbox"/> Only young or pregnant | <input type="checkbox"/> Based on ectoparasite burden <input type="checkbox"/> Others (please specify): |

Appendix A (cont.)

| | | |
|---|--|---|
| Do your goats experience any of following condition? | <input type="checkbox"/> Diarrhea | Date: Age/gender: Severity: Progress: |
| | <input type="checkbox"/> Jaundice | Date: Age/gender: Severity: Progress: |
| | <input type="checkbox"/> Poor weight gain | Date: Age/gender: Severity: Progress: |
| | <input type="checkbox"/> Sudden death | Date: Age/gender: Severity: Cause of death: |
| | <input type="checkbox"/> Depressed | Date: Age/gender: Severity: Progress: |
| How do you treat your sick animals? | <input type="checkbox"/> Self medicate <input type="checkbox"/> DVS AVO | <input type="checkbox"/> DVS VO <input type="checkbox"/> Others (please specify): |
| Do you aware of disease of haemotropic mycoplasma? | <input type="checkbox"/> Yes <input type="checkbox"/> No | |
| Farm hygiene and waste disposal management: | | |
| Do you dispose farm waste properly? | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| How do you dispose the goat manure? | <input type="checkbox"/> Just flush with water to push it into drain | <input type="checkbox"/> collect and sell it |
| Does your farm have fly/ or mosquitoes problem? | <input type="checkbox"/> Yes <input type="checkbox"/> No | Please specify method of control: |
| Frequency for cleaning of goats pen? | <input type="checkbox"/> Daily <input type="checkbox"/> Weekly | <input type="checkbox"/> Monthly <input type="checkbox"/> Others (please specify): |
| What do you use to clean our farm? | <input type="checkbox"/> Disinfectant <input type="checkbox"/> Others (please specify): | <input type="checkbox"/> Detergent |
| In general, how would you say the hygiene level of your goats farm? (please tick) <input type="checkbox"/> Poor <input type="checkbox"/> Fair <input type="checkbox"/> Good <input type="checkbox"/> Very Good <input type="checkbox"/> Excellent | | |
| In general, how would you say the disturbance level of your goats caused by flies / mosquitoes? (please tick) <input type="checkbox"/> No disturbance <input type="checkbox"/> Tolerable <input type="checkbox"/> Not Sure <input type="checkbox"/> Severely Disturbed | | |