Molecular investigation of *Mycoplasma haemofelis* in stray cats in Kota Bharu, Kelantan

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Received 12 October 2015; received in revised form 30 August 2016; accepted 24 October 2016

**Abstract.** *Mycoplasma haemofelis* (*M. haemofelis*) is a gram-negative, epicellular bacteria that infects feline red cells (RBC). The pathogen appears as small blue cocci, rings, or rods on the edges or across the surface of RBCs and often causes haemolytic anemia. However, the epizootiology of *M. haemofelis* is still poorly understood. So far, there are only a few studies that have been carried out to determine the prevalence of *M. haemofelis*. Most of the studies were conducted in Europe and other continents and the epidemiology of *M. haemofelis* in Malaysia, particularly in stray cats have not been reported. In this study, 60 blood samples were collected from stray cats in Kota Bharu and were examined by using thin blood smear and polymerase chain reaction (PCR) methods. This study showed that seven out of the 60 blood samples were positive for *M. haemofelis* by using PCR. Thus the prevalence rate of *M. haemofelis* in stray cats in Kota Bharu from this study was 11.7%. However, more studies with larger sample size and diverse sample distribution should be conducted to better understand the occurrence of this pathogen in both housed and stray cats. Moreover, the genetic variability of *M. haemofelis* infecting domestic and wild animals need to be conducted to verify the relationship among geographic distribution, genetic diversity and the potential threats to animal and human health.

**INTRODUCTION**

Feline tick-borne diseases are of worldwide concern. The tropical sub-tropical regions are known for their favorable climatic conditions that promote the survival and proliferation of vectors. Being a tropical paradise, Malaysia is a home to a wide range of vectors as well as the pathogens that they harbor (Watanabe, 2012). This in turn implies the heightened risks posed by vector borne animal and human diseases in the country. Furthermore, increasing affluence and pet ownership has led to dramatic increase in a pet population. Coupled with this, increasing number of stray cats has resulted in rapid increase of vector-borne diseases in companion animals in Southeast Asia (Irwin & Jeffries, 2004). Among the vector-borne pathogens, haemoparasites have been known to cause different human and animal diseases worldwide. These parasites can range from single-celled protozoa to more complex bacteria and rickettsiae. The method of transmission of haemoparasitae is often through the bites of fleas, ticks or flies (Lyght et al., 1956). There are several feline diseases that are caused by blood parasites including Babesiosis, Cyttauxzoonosis, Hepatozoonosis and Hemobartonellosis. Among these, *Hemobartonella felis* is a bacterial parasite of the red blood cells of cats and is found worldwide.

Apart from the occurrence of eperythrozoonosis in animals, there are reports of human eperythrozoonosis in different contries (Huang et al., 2012). There are also rare reports of hemotrophic mycoplasma infection in immuno-compromised people from Croatia (Bosnic et al., 2010) and Inner Mongolia (Yang et al., 2000). Another report documents an HIV-
positive human patient coinfected with *Bartonella henselae* and a hemoplasma which are genetically similar to *M. haemofelis* (dos Santos et al., 2008). This individual owned five cats and had multiple scratch and bite wounds. All five cats were PCR positive for *Bartonella* spp and two were positive for *M. haemofelis*, suggesting the possibility of zoonotic transmission (Aiello & Moses, 2012). Eventhough *M. haemofelis* has been recognized as haemoparasites of cats worldwide and has become of public health concerns, studies describing the prevalence of infections in cats in different parts of the world were limited. Despite it being a major cause of anaemia in cats there are still a paucity of information regarding the epidemiology and characterization of *M. haemofelis* from cats in Malaysia. Moreover, according to Ministry of Health Malaysia 28.8% out of every 100,000 Kelantanese have been infected by the incurable diseases including immune compromising diseases (Ubaidah, 2011). However, there are no studies reported regarding the prevalence of *M. haemofelis* and its molecular detection in Kota Bharu, Kelantan. Therefore, this study was conducted to investigate the occurrence and prevalence of *M. haemofelis* in stray cats in Kota Bharu, Kelantan by using Polymerase Chain Reaction (PCR) and to determine the risk factors associated with *M. haemofelis* infection of stray cats.

**MATERIALS AND METHODS**

**Study area, Subjects and Sample Collection**

The study was conducted in several locations (Pengkalan Chepa, Panji, Pantai Cahaya Bulan, Kubang Kerian and Wakaf Che Yeh) in Kota Bharu, Kelantan. Blood samples were collected from 60 stray cats regardless of their age, sex and breed to determine the prevalence of *M. haemofelis*. Once the cat was restrained, typical signs of illness were observed and recorded in and then, blood samples were collected. From each cat, 3-5 mL blood was collected from jugular or cephalic vein aseptically by using 23 gauge needle, disposable syringe and EDTA for anticoagulant. All the relevant information such as gender, age (age determination was done by examining the teeth), breed and presence of ectoparasite were recorded for further analysis.

**Examination of blood sample**

A single drop of blood from sample container (EDTA tube) was taken and placed on one end of a labeled clean glass slide. Then, the drop was spread by using another slide (spreader). The slide was moved in forward direction allowing the blood to spread as thin layer on the surface of the slide. The smear was allowed to air dry. Cytological examination of a blood smear for the presence of the organism was carried out using Giemsa Stain and Diff Quick Stain.

**Detection of *M. haemofelis***

Direct PCR for haemotropic *Mycoplasma* spp. using feline whole blood without DNA extraction was performed according Watanabe et al. (2007) with minor modification. The PCR products were electrophoresed on 1.5% agarose gel containing Midori Green DNA Stain and formation of 273-bp DNA bands is regarded to be positive result for *M. haemofelis*.

**Statistical Analysis**

Data summaries and descriptive analyses were calculated in Excel (Microsoft Corporation) and Epi Info™. The Chi-square test was used to compare prevalence of *M. Haeofelis*. The significance level for all statistical tests was set to p-value ≤ 0.05.

**RESULTS**

Seven out of sixty blood samples collected from sixty stray cats around Kota Bharu (11.7%) were positive for *M. haemofelis*. Laboratory detections were done using both blood smear and PCR amplification of *M. haemofelis* specific gene.

**Microscopic Examination**

Microscopic examination using both diff quick stain and giemsa stain showed that 10% (6/60) were presumptively positive for
M. haemofelis that appeared on the surface of erythrocytes as a small (0.5–3 µm) basophilic round, rod, or ring-shaped structures present on erythrocytes individually or in chains. However, microscopic examination alone is insufficient to confirm the diagnosis as it tends to lead to false positive and negative result. Figure 2 and 3 (below) are the references for further explanation.

**Polymerase Chain Reaction (PCR) Result**
Polymerase chain reaction detection of M. haemofelis revealed that 11.7% (7/60) of the samples were positive for M. haemofelis specific gene (Fig. 1).

**DISCUSSIONS**
This is the first study in Malaysia to detect M. haemofelis infection in stray cats and is the first study to be carried out in Kota Bharu, Kelantan. From the sixty blood samples collected, seven cats (11.7%) were positive for M. haemofelis as detected by PCR. Few similar studies conducted in other countries recorded M. haemofelis prevalence of 2.5%, by Braga et al. (2012) in Brazil, 66% in study by Kathryn et al. (2004) in Saskatchewan and Alberta, Canada, 1.4% in United Kingdom (Tasker et al., 2003), 7.5% by Jenkins et al. (2013) in New Zealand and 3.9% by Lappin et al., 2012) in South Africa.

In this study, microscopic examination revealed that 10% (6/60) of the samples were positive for M. haemofelis. However, four of the results were negative upon PCR detection. On the other hand, another five samples which were presumed to be negative upon microscopic examination were shown to be positive upon PCR amplification. Such discrepancies can be attributed to staining or smear artifacts (Tasket et al., 2003) and the possible presence of other structures such as Howell Jolly bodies, Heinz body and impurities in the stain that can be mistakenly interpreted as M. haemofelis. In earlier reports by Lapin (2003), it was mentioned that false negative results might be due to the fact that the organism may be present in very low levels, especially in chronically infected cats. Besides, it was also found that M. haemofelis may literally fall off the surface of erythrocyte when blood sample is collected in blood tubes containing EDTA anti-coagulant (Lappin, 2003).

![Figure 1. Agarose gel electrophoresis of PCR product showing a 273bp expected band size for M. haemofelis (1: K004, 2: K019, 3: K022, 4: K033, 5: K044, 6: K046, 7: K053, L: 100bp plus DNA ladder).](image-url)
The current study also showed that most of the cats were heavily infested with ectoparasites, 81.7% infested with fleas, 56.7% infested with lice, and 30% with mites. This might suggest that fleas Ctenocephalides felis (C. felis) could be playing a major role in transmitting M. haemofelis. However, no significant association (P > 0.05) was found between the positive results for M. haemofelis and the presence of fleas or other extoparasites. It has long been suspected that transmission of these organisms has been via the bite of infected fleas. Similar suggestion has been made by (Lappin et al., 2003) who experimentally demonstrated that fleas can transmit the pathogen. However, a study by Woods et al. (2006), reported that M. haemofelis is not transmitted through flea (C. felis) bites.

The current result also shows that male stray cats are 1.4 times more prone to get infected with M. haemofelis as compared to the female. This is probably due to male’s physiological, sociological and behavioural activity. According to Feline Behavioural Guidelines from The American Association of Feline Practitioners (AAFP), cats get mature socially at 2-3 years of age (AAFP, 2004). Hence, since these cats are strays, they would probably develop different types of aggressive behaviours including fighting that may result in wounding. According to study conducted in Switzerland, It was reported that direct transmission through saliva and feces at the early phase of infection could play a role in the epizootiology of feline hemotropic mycoplasmas (Willi et al., 2007). Since study proved that M. haemofelis can be transmitted through saliva, thus it is highly likely that the higher prevalence in male cats may be attributed to aggressive behaviour- fighting, biting.

In conclusion, the prevalence of M. haemofelis in stray cats in Kota Bharu is higher as compared some of the reports from other similar studies. However, more studies with larger sample size and diverse sample distribution should be conducted to better understand the occurrence of this pathogen in both housed and stray cats. Moreover, the genetic variability of M. haemofelis infecting domestic and wild animals need to be conducted to verify the relationship among geographic distribution genetic diversity and threat to animal and human health.

REFERENCES


