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

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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 haemoparsiates 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, Cytauxzoonosis, 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 immunocompromised people from Croatia (Bosnic *et al.*, 2010) and Inner Mongolia (Yang *et al.*, 2000). Another report documents an HIV-

positive human patient coinfected with *Bartonellahenselae* 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*is has been recognized as haemoparsites 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. heamofelis* 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. heamofelis* 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).

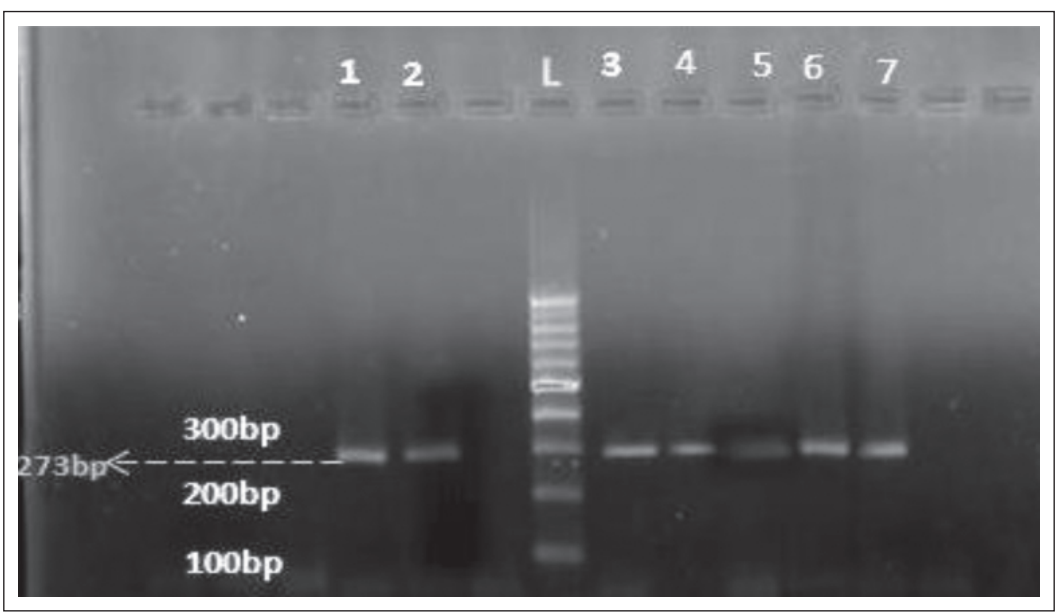

Figure 1. Agarose gel electrophoresis of PCR product showing a *273bp* expected band size for *M. haemofelis* (1: K004, 2: K019, 3: K022, 4: K033, 5: K044, 6: K046, 7: K053, L: 100bp plus DNA ladder).

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. haemoflis* 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

AAFP, American Association of Feline Practitioner (2004). AAFP Feline Behavior Guidelines report. American Association of Feline Practitioners. Retrieved from: http://www.catvets.com/public/PDFs/PracticeGuidelines/FelineBehaviorGLS.pdf.

Bosnic, D., Baresic, M., Anic, B., Sentic, M., Cerovec, M., Mayer, M. & Cikes, N. (2010). Rare zoonosis (hemotrophic mycoplasma infection) in a newly diagnosed systemic lupus erythematosus patient followed by a *Nocardia asteroides* pneumonia. *Brazilian Journal of Infectious Diseases*, **14**: 92-95.

Braga, M., André, M., Freschi, C.,Teixeira, M. & Machado, R. (2012). Molecular detection of hemoplasma infection among cats from São Luís island, Maranhão, Brazil.São Paulo 2012 June: *Brazilian Journal of Microbiology*, **43**: 2.

dos Santos, A.P., dos Santos, R.P., Biondo, A.W., Dora, J.M., Goldani, L.Z., de Oliveira, S.T., de Sa Guimaraes, A.M., Timenetsky, J., de Morais, H.A. & other authors (2008). Hemoplasma infection in HIV-positive patient, Brazil. *Emerging Infectious Diseases*, **14**: 1922-1924.

Huang, D.S., Guan, P., Wu, W., Shen, T.F., Liu, H.L., Cao, S. & Zhou, H. (2012). Infection rate of *Eperythrozoon* spp. in Chinese population: a systematic review and meta-analysis since the first Chinese case reported in 1991. *BMC Infectious Diseases*. **12**: 171.

Irwin, P.J. and Jeffries, R. (2004). Arthropod-transmitted diseases of companion animals in Southeast Asia. *Trends Parasitology*, **20**: 27-34.

Jenkins, K.S., Dittmer, K.E., Marshall, J.C., Tasker, S. (2013). Prevalence and risk factor analysis of feline haemoplasma infection in New Zealand domestic cats using a real-time PCR assay. *Journal of Feline Medicine and Surgery*, **15**: 1063-1069.

Kathryn, E.K., Greg, D. & Marion, L.J. (2004). Mycoplasma haemofelis and Mycoplasma haemominutum detection by polymerase chain reaction in cats from Saskatchewan and Alberta. *Candadian Veterinary Journal*, **45**: 749-752.

Lappin, M.R. & Lobetti, R. (2012). Prevalence of *Toxoplasma gondii*, Bartonella species and haemoplasma infection in cats in South Africa. *Journal of Feline Medicine and Surgery*, **14**: 857-862.

Lappin, M.R. (2003). Update on Two Feline Parasites: Ehrlichia and Hemobartonella. Winn Feline Foundation 25th Annual Feline Symposium, June 19, 2003 St. Louis, MO.

Lobetti, R.G. & Tasker, S. (2004). Diagnosis of feline Haemoplasma infection using a real time PCR assay. *Journal of the South African Veterinary Association*, **75**: 94-99.

Lyght, C.E., Boger, W.P., Carden, G.A. & Gibson, A. (1956). The Merck Veterinary Manual, 9th ed. Merck & Co., Inc. Whitehoue Station, N.J., U.S.A. pp 25-26.

Tasker, S., Binns, M.J., Day, T.J. & Lappin, M.R., (2003). Use of a PCR assay to assess the prevalence and risk factors for Mycoplasma haemofelis and 'Candidatus Mycoplasma haemominutum' in cats in the United Kingdom. *Veterinary Record*, **152**: 193-198.

Ubaidah, W. (2011, October 20). Kelantan concerned over rise in HIV cases. Retrieved from http://www.mole.my/content/kelantan-concerned-over-rise-hiv-cases

Watanabe, M. (2012). Main Pet Arthropod-Borne Diseases in Asia: Canine tick-borne diseases of Malaysia. 9th Merial Symposium on Parasitosis & Arthropod-Borne Diseases, Tokyo 6th–9th November 2012, Japan.

Watanabe, M., Hisasue, M., Souma, T., Ohshiro, S., Yamada, T. & Tsuchiya, R. (2008). Molecular Detection of Mycoplasma haemofelis and 'Candidatus Mycoplasma haemominutum' Infection in Cats by Direct PCR Using Whole Blood without DNA Extraction. *Journal of Veterinary Medical Sciences*, **70**: 1095-1099.

Willi, B., Boretti, F.S., Meli, M.L., Bernasconi, M.V., Casati, S., Hegglin, D., Puorger, M., Neimark, H., Cattori, V., Wengi, N., Reusch, C.E., Lutz, H. & Hofmann-Lehmann, R. (2007). Real-Time PCR Investigation of Potential Vectors, Reservoirs, and Shedding Patterns of Feline Hemotropic Mycoplasmas. *Applied and Environmental Microbiology*, **73**: 3798-3802.

Woods, J.E., Wisnewski, N. & Lappin, M.R. "Attempted transmission of Candidatus Mycoplasma haemominutum and Mycoplasma haemofelis by feeding cats infected Ctenocephalidesfelis." *American Journal of Veterinary Research*, **67**: 494-497.

Yang, D., Tai, X., Qiu, Y. & Yun, S. (2000). Prevalence of *Eperythrozoon* spp. infection and congenital eperythrozoonosis in humans in Inner Mongolia, China. *Epidemiology and Infection*, **125**: 421-426.