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RESEARCH ARTICLE

Retrospective prevalence and associated risk factors of *Mycoplasma haemofelis* infection in owned cats

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ABSTRACT

Data on the prevalence and associated risk factors of naturally occurring haemoplasmosis in owned cats in Malaysia is limited. Being the most pathogenic of the three known feline haemoplasma species, *Mycoplasma haemofelis* (Mhf) infection was analysed from 2016 to 2019 to determine the periodical prevalence and associated risk factors in Northeastern Malaysia – Kelantan. Archived patient data of 77 clinically ill cats suspected of having *M. haemofelis* infection were reviewed in this study. Out of the 77 suspected cases, 53 (68.8%) were clinically diagnosed with haemoplasmosis amongst which 46 (59.7%) of the subpopulation were further confirmed with polymerase chain reaction (PCR). Risk factors for *M. haemofelis* infection (age, breed, ectoparasitism, household condition, roaming status, and sex) were analysed. There was no significant association of breed, ectoparasitism, household condition (number of cats) and occurrence of clinical signs with feline mycoplasmosis. Young, male and roamer cats were more likely to be diagnosed of mycoplasmosis than other categories of cats in this study. There was also a significant association between cats infected with '*Candidatus* Mycoplasma haemominutum' with *M. haemofelis*. Thus, the coinfection of these two haemoplasma species is not uncommon. This study indicates that infection by *M. haemofelis* in anaemic cats is a common find in client-owned cats from Northeastern Malaysia. As the natural mode of transmission of haemoplasma infection remain unestablished, information in this study may highlight the importance of this disease and contribute to effective prevention and control strategies to minimize feline infectious anaemia (FIA) caused by *M. haemofelis*.

Keywords: Feline haemotropic mycoplasmosis; haemoplasmosis; period prevalence; associated risk factors; household cat.

INTRODUCTION

Feline haemotropic mycoplasmosis (haemoplasmosis) is an acute to chronic haemolytic diseases of the feline species caused by Gram negative epierythrocytic bacteria of the Genus Mycoplasma. They are said to be vector-borne through the Cat flea (*Ctenocephalides felis*), but reports are abounding of other means of transmission such as via broken skin. Dogs can also be affected. Of the three haemoplasma (HM) species affecting cats; '*Candidatus* Mycoplasma haemominutum' (CMhm), *Mycoplasma haemofelis* (Mhf) and '*Candidatus* Mycoplasma turicensis' (CMt); *M. haemofelis* is the most pathogenic. It causes severe and often fatal, haemolytic anaemia following acute infection in some cats. Preliminary studies compiling laboratory detected evidence of PCR-detection of *Mycoplasma haemofelis* infections in stray cats in Malaysia, reveals a prevalence of about 11.7% (11/60) prevalence (Aklilu *et al*., 2016). Despites worldwide distribution of the diseases, the prevalence of haemoplasma species is characterised by geospecific variations (Díaz-Regañón *et al*., 2018). To date, most of the studies on the prevalence of feline haemoplasmosis were conducted in European countries viz. Switzerland (9.9%), Germany (9.4%), Italy (13.2%), Spain (10.6%) (Willi *et al*., 2006; Bergmann *et al*., 2017; Ravagnan *et al*., 2017; Díaz-Regañón *et al*., 2018) and in non-European countries such as South Africa (38.5%), Canada (4%), US (27%), Japan (26.4%), Iran (32.7%.), South Korea (47.9%), Brazil (35.5%) and Thailand (38.1%) (Lobetti & Tasker, 2004; Kamrani *et al*., 2008; Sykes *et al*., 2008; Tanahara *et al*., 2010; Rassouli, 2016; Hwang *et al*., 2017; Munhoz *et al*., 2018; Do *et al*., 2020). Data on the prevalence of the infections in many parts of the world are still scarce (Aklilu *et al*., 2016). There is thus a paucity of information regarding the epidemiology and characterization of *M. haemofelis* infection in cats in Southeast Asia; a tropical climate region in Asia. Aside the dearth of information on the prevalence of feline haemoplasmosis, the risk factors in the Southeast Asian region are also understudied. The

predominant understanding surrounds the role of *Ctenocephalides felis* (Cat flea) as a vector for the disease. This study was therefore being conducted to enrich the data which are still scarce with regards of the retrospective prevalence and associated risk factors of *M. haemofelis* infection in owned cats in this region.

MATERIALS AND METHODS

A retrospective analysis of all cats suspected of having haemoplasmosis at the Veterinary Clinic, Universiti Malaysia Kelantan (UMK) was conducted between March 2016 to December 2019. These cases were also corroborated with laboratory confirmation from the Molecular Laboratory of the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (UMK). Selected cases were often presented with clinical signs of haemoplasmosis including jaundice and with low packed cell volume (PCV). The definitive diagnosis of haemoplasmosis (HM) was determined by validated polymerase chain reaction (PCR) tests for detection of the 16S rRNA gene of '*Candidatus* Mycoplasma haemominutum' (CMhm) and *Mycoplasma haemofelis* (Mhf). Patients' data were also datamined for signalment (age, breed, household condition, roaming status, and sex) and history of ectoparasites infestation to establish risk factors. These cases represented the Northeastern parts of Peninsular Malaysia with a majority from Kelantan and some from Terengganu (Figure 1).

Obtained data were entered, validated, and explored in a database (Excel 2016; Microsoft), and exported into statistics software (SPSS, version 22.0, Woking) for Chi-square analysis at 95% confidence interval (CI).

RESULTS

A convenient sampling approach was utilized for this study because of the low number of suspected haemoplasmosis cases in the study area. In addition, interest in feline haemoplasmosis in Kelantan began to rise from 2016 onwards. During the 4-year period, 77 cats were identified with anaemia (low PCV) and suspected to be HM-induced. PCR results revealed that 68.8% (53/77) were positive for haemoplasmosis and 59.7% (46/77) of the PCR positive cases diseases were caused by *Mycoplasma haemofelis* (Mhf) (Table 1). Out of these 77 cats, 36 cats were tested for '*Candidatus* Mycoplasma haemominutum' (CMhm) using PCR and the results showed that 88.9% (32/36) of the cats were positive for CMhm. Cats concurrently infected with both Mhf and CMhm accounted for 32.5% (25/77) of the study population. This study observed a year-on-year increasing trend of haemoplasmosis between 2016 and 2019. The increase in prevalence was steep (about two folds) in 2017 and 2018 (Figure 2). Collectively the periodic prevalence of haemoplasmosis were highest in the latter months of the year, peaking in October (Figure 3) annually for three years.

Figure 1. The geographical location on the study of the prevalence of *M. haemofelis* in cats showing Kelantan and Terengganu states of Malaysia.

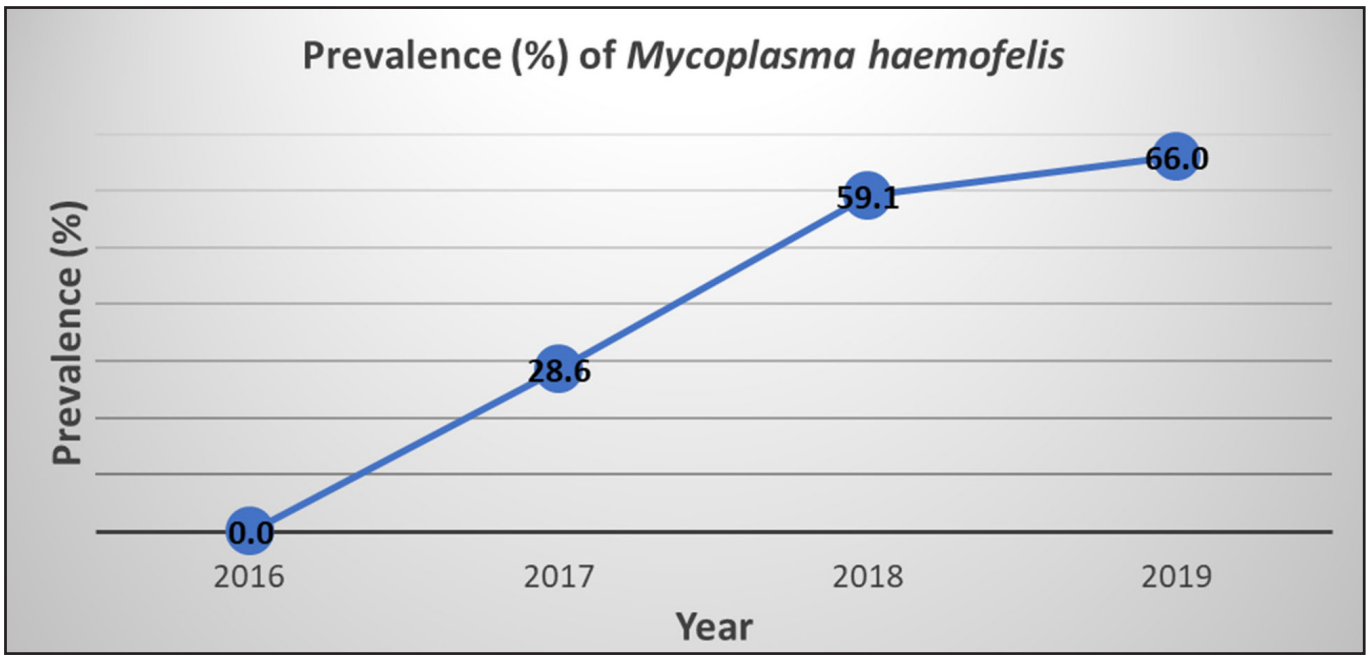

Figure 2. Prevalence of *Mycoplasma haemofelis* (Mhf) infection among cats with suspected haemoplasmosis in Malaysia.

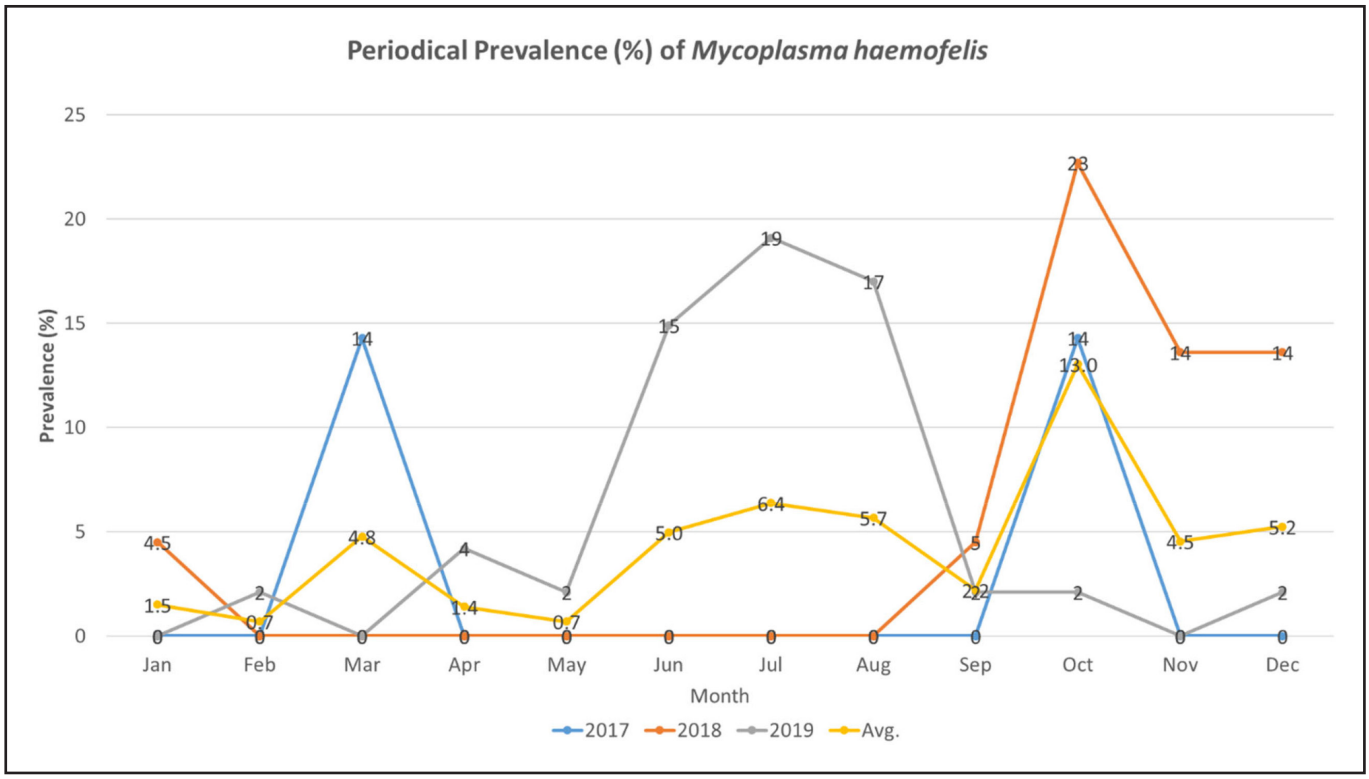

Figure 3. Periodic Prevalence of *Mycoplasma haemofelis* (Mhf) infection among cats with suspected haemoplasmosis in Malaysia.

Clinical signs: In total there were 42/77 cats exhibiting the clinical signs of haemoplasmosis. However, clinical signs alone did not correlate with the haemoplasmosis cases, X^2 (1, N = 77) = 2.08, p = 0.15. Only 22 cats out of the 46 HM-infected ones had apparent clinical signs. The common clinical signs presented for these cats were jaundice (7/22; 32%) and anaemia (6/22; 27%); while inappetence were the most common complaints of these positive cases (9/22; 41%). Although haemoplasmosis is usually associated with jaundice and anaemia, this study showed that there was no significant association between these clinical signs and haemoplasmosis caused by Mhf, X^2 (1, N = 42) = 0.65, p = 0.72. Other than the jaundice and anaemia, negative results were obtained for four highly suspected cases with clinical signs of respiratory disorders (N = 3) and one positive case that was presented for wound management.

Signalment: Young cats (< 2 years-old) were more likely to be infected with Mhf than older cats (e" 2 years-old), X^2 (2, N = 77) = 6.21, p = 0.04. Male cat was more likely (1.9 times) to contract haemoplasmosis than female cats, X^2 (2, N = 77) = 14.71, p <0.001. Both domestic breeds (domestic short hair and domestic long hair) and pure breeds had equal risks of contracting haemoplasmosis in Malaysia, X^2 (2, N = 77) = 1.78, p = 0.41. Important in this finding, is the non-association of ectoparasitism (flea infestation) with the detection and occurrence of Mhf infection in cats, X^2 (2, N = 77) = 1.80, p = 0.41. Rather, the prevalence of Mhf infection was much higher in cats without ectoparasitism compared to those with flea infestation. (flea / flea-dirt).

Other factors that were thought to influence the likelihood in acquiring Mhf infection were roaming status and the number of cats in the household. Roaming status had a significant association with Mhf infection. Roamers were 1.9 times more likely to be infected with Mhf compared to non-roamers, X^2 (2, N = 77) = 8.13, p = 0.02. Meanwhile, the number of cats in a household played no significant role in the prevalence of haemoplasmosis; Mhf, X^2 (2, N = 77) = 1.04, p = 0.59. Results showed that 80% of the cats (4/5) living in a single cat household were infected with Mhf compared to only 60.6% of the cats (20/33) living in a multi-cat household. Of the 36 cats tested for both CMhm and Mhf DNA, there were only 2 cats negative for both haemoparasites; there were 7 (19.4%) cats positive for only CMhm; 2 (5.6%) cats were positive for only the DNA of Mhf and 25 (69.4%) cats were positive for both haemoplasma. Infection with CMhm occurred concurrently with Mhf infection in cats Mhf, X^2 (2, N = 77) = 7.72, p = 0.02.

Table 1. Potential risk factors for infection with *Mycoplasma haemofelis* (Mhf) among cats with possible haemoplasmosis

	Negative	Positive	Total	Prevalence (%)
Age				
< 2 yrs.	10	25	35	71.4*
≥ 2 yrs.	19	15	34	44.1
Unknown	2	6	8	75.0
		46	77	
Breed				
Domestic	21	32	53	60.4
Pure	3	8	11	72.7
Unknown	7	6	13	46.2
		46	77	
Sex				
Male	8	32	40	80.0*
Female	12	9	21	42.9
Unknown	11	5	16	31.3
		46	77	
Roaming Status				
Roamer	2	14	16	87.5*
Non-Roamer	20	17	37	45.9
Unknown	9	15	24	62.5
		46	77	
Household Condition				
Single cat	1	4	5	80.0
Multi-cats	13	20	33	60.6
Unknown	17	22	39	56.4
		46	77	
Ectoparasites Infestation				
Absent	3	10	13	76.9
Fleas	6	6	12	50.0
Unknown	22	30	52	57.7
		46	77	
Clinical Signs				
With clinical signs	20	22	42	52.4
Without / Unknown	11	24	35	68.6
		46	77	
CMhm Infection				
Positive	7	25	32	78.1*
Negative	2	2	4	50.0
Unknown	22	19	41	46.3
		46	77	

* Statistically significant at the level of P < 0.05 with 95% confidence interval (CI).

DISCUSSION

The 68.8% overall prevalence of haemoplasmosis in this retrospective study was higher than previous reports of 9.4% to 47.9% (Assarasakorn *et al*., 2012; Jenkins *et al*., 2013; Martínez-Díaz *et al*., 2013; Bergmann *et al*., 2017; Hwang *et al*., 2017; Díaz-Regañón *et al*., 2018; Sacristán *et al*., 2019; Do *et al*., 2021). This comparatively lower total prevalence from previous reports over the last decade were for studies conducted on both healthy and sick cats. The prevalence of Mhf-induced haemoplasmosis in this study was also higher than previously reported in Malaysia, in which only 11.7% for randomly sampled cats tested with PCR (Aklilu *et al*., 2016) compared to the current result with 59.7% of the reviewed cats were infected with Mhf. However, our results were coherent with that from Japan (58.3%) (Fujihara *et al*., 2007) and Canada (60%) (Kamrani *et al*., 2008). A narrower sampling criterion that included presentation of clinical signs indicative of haemoplasmosis in this study may have accounted for the recorded high prevalence in Malaysia. Nevertheless, comparison among different studies is challenging as population definitions are variable. Variability in infection rates may reflect differences in both the geographic distribution of *Mycoplasma* species and the population sampled (Nibblett *et al*., 2009).

The increased prevalence in the recent years might also be due to the upsurge of awareness among cat owners in taken care on the wellbeing of their companion animals (McConnell *et al*., 2019). The alertness of clinicians towards this disease might also play a major role in the increase cases of reported haemoplasmosis in Malaysia. In the past, haemoplasmosis was relatively an uncommon infection of cats. Many cases of haemoplasma infections in cats went undetected. Some sub-clinically infected cats remain carriers of the bacteria and spread the bacteria to other cats (Barker, 2019). Besides these, the advancement in detecting the etiological agents through the highly sensitive PCR technique is also contributing to the rise of haemoplasmosis detection (Martínez-Díaz *et al*., 2013). This underscores the fact that blood film examinations have about 50% false negative rate (Stokes, 2011), the application of PCR in haemoplasma detection significantly increases the sensitivity in detection of the agents.

Our results showed that there were periodical fluctuations in the occurrence of feline haemotropic mycoplasmosis between 2017 and 2019. Reported cases tended to surge consistently in the months of

March, July, and October. The spike in caseloads for haemoplasmosis were much lower in July compared to March and October during the period in review. This finding may suggest seasonal influence in the epidemiology of haemoplasmosis in Kelantan. The months of March and October are the transition months of the monsoon season. During October to March there are higher precipitation in the East-coast of Malaysia (Fakaruddin *et al*., 2019). Increased occurence of haemoplasmosis during summer has been previously reported by Salim *et al*. (2020) in Pakistan. Noting that the ambient temprature variation in Malaysia is quite low (32±1°C). Whether this seasonal influence is associated with the proliferation of the fleas (Raimundo *et al*., 2016), though found to be insignificant ($p = 0.41$). Hence the authors postulate that based on the significant ($p = 0.02$) association of haemoplasmosis with roaming status of cats seen in this study, there may be a behavioural peculiarity of the cats that exposes them to frequent contact at the available feeding sites or breeding (particularly roamer cats thereby increasing the chances of brawls) during the Northeast monsoon season.

There were only 22 out of the 46 Mhf positive cats recorded that presented clinical signs. Clinical signs associated with haemoplasmosis were otherwise non-specific. Commonly observed clinical signs in this study were inappetence (9/22; 40.9%), jaundice (7/22; 31.8%), and anaemia (6/22; 27.3%). Other less common signs include enlarged lymph node (2/22; 9.1%) and gingivitis (1/22; 4.5%). Anaemia characterised by paleness of mucous membrane and low haematological values is commonplace in Mhf infection pathology (Sykes, 2010; Raimundo *et al*., 2016). In the same manner, jaundice is also a known clinical sign of Mhf because of the haemolytic activity of this bacteria (Firmino *et al*., 2016). Several studies have associated these clinical signs with haemoplasmosis and are thus considered representative of this disease. However, it is not necessary to associate anaemia and jaundice with the disease (Willi *et al*., 2006; Roura *et al*., 2010; Santos *et al*., 2014) due to the wide range of aetiologies. This study observed a non-significant association between the signs (anaemia and jaundice) and Mhf-induced haemoplasmosis. This finding suggests that in the investigation of haemoplasmosis in cats, other causes of anaemia and jaundice should be considered and possibly ruled out. Such other causes may include nutritional deficiencies, haemorrhage, parasitism, haemolytic diseases, primary hepatic diseases, and toxicities. A low mean corpuscular haemoglobin (MCH) on haematology is a good pointer to suspect haemoplasmosis.

Younger cats (< 2 years-old) in this study were more likely to be infected with Mhf compared to those aged 2 years-old and above (71.4% vs. 44.1%). This result was in line with those of previous studies that reported that younger cats had a higher prevalence of Mhf-induced haemoplasmosis (Sykes *et al*., 2008; Nibblett *et al*., 2009; Salim *et al*., 2020). Conversely, there are studies reporting no association of age groups with Mhf infections. However, these studies usually have a relatively broad classification of the age groups that tend to pool or segregate ages too widely. For instance, grouping of up to 1 year-old and more than 1 year-old or juvenile (≤ 6 months-old) and adult (> 6 months-old) (Spada *et al*., 2014; Munhoz *et al*., 2018). The virulence of Mhf is more severe in younger age cats, because of the immature immune system, low maternally derived antibodies and the likelihood to be more aggressive or playful (Jenkins *et al*., 2013). Due to the pathogenic nature of different haemoplasma species, samples with more well-defined age groups should be obtained to determine the risk association of this factor for specific ages.

Due to the nature of male cats usually involved in more aggressive social contacts, the sex of cats exposes them to the risk of Mhf infection. Although not many studies showed statistical association between the sex of cats with haemoplasmosis. Generally speaking, authors agree with the trends regarding male cats being at relatively higher risk than female in acquiring haemoplasmosis (Sykes *et al*., 2008; Nibblett *et al*., 2009; Jenkins *et al*., 2013; Díaz-Regañón *et al*., 2018; Salim *et al*., 2020). Another risk factor that is loosely but repeatedly associated with haemoplasmosis is the outdoor access of the cats. Stray cats have a higher risk of exposure than than owned cats (Spada *et al*., 2014; Díaz-Regañón *et al*., 2018). In one study, 95% of the cats with HM-induced anaemia had known outdoor access (Nibblett *et al*., 2009) while a study in Spain, reported client owned cats with outdoor access was found to be a risk factor for infection (OR = 3.8) (Roura *et al*., 2010). Free-range cats may exhibit a higher fighting activity than owned and indoor cats; thus, leading to the subcutaneous inoculation of infected blood (Museux *et al*., 2009).

Although pure breeds or pedigree cats were reported less likely to be infected by haemoplasmosis. The low risk is not necessarily breed related but rather the tendency of pure-bred cats to be more strictly home managed than domestic breeds, the domestic breeds of cats in Malaysia are more often allowed outdoor access thereby increasing the risk of infection (Rosenqvist *et al*., 2016). Thus, the insignificant result in our study on the association between cat breed and Mhf infections might be because of the large sample size of domestic cats with strict indoor home management. Similar informed assumption is held for household condition on the number of cats in one house. Previous studies suggest multi-cat household would have higher risks of haemoplasma infection because of CMhm can probably transmitted through water, food, and via mutual grooming because haemoplasma species has been found in cats' saliva (Willi *et al*., 2007; Lappin, 2014). Nonetheless, our results on Mhf-induced haemoplasmosis were not significantly associated with the numbers of cats held under one roof. Additional reason might be because of the route of transmission of Mhf is believed to be through vectors and subcutaneous inoculation by bites (Museux *et al*., 2009).

Despite conclusions that blood-sucking arthropods including fleas, mosquitoes and ticks are thought to be the primary mode of dissemination of Mhf, only a few numbers of studies support the role of an arthropod vector in haemoplasma transmission (Willi *et al*., 2006; Sykes *et al*., 2007). The Mhf transmission by cat flea via the haematophagous activity were not induced in the recipient cat (Woods *et al*., 2005). Additionally, Lappin (2014) also found no evidence of haemoplasma transmission by fleas in an experiment involving the introduction of fleas into groups of cats housed together. Other arthropods such as mosquitoes and ticks have also no concrete evidence for their role in the transmission of Mhf (Reagan *et al*., 2017; Duplan *et al*., 2018). Our results in which ectoparasitism specifically on the evidence of fleas thus corroborates with the afore-mentioned reports by showing no significant association with the Mhf infection of the reviewed cats. Some authors have suggested that cat fights are important in transmission (Museux *et al*., 2009), however, this study did not include that criterion to for validation and there was only one cat in our review that had wounds.

Like previous studies, CMhm tends to be the most common species in the present study in which out of the 36 samples tested for both CMhm and Mhf, 7 samples were positive with CMhm (Assarasakorn *et al*., 2012; Jenkins *et al*., 2013; Bergmann *et al*., 2017; Díaz-Regañón *et al*., 2018; Sacristán *et al*., 2019; Do *et al*., 2021). The nature of CMhm causing a subclinical infection that would not render the cat lethargic so much so that it hinders the socialization with other cats might be the reason of widespread nature of this pathogen in cats (Rosenqvist *et al*., 2016). The CMhm species also maintains persistent infections for a longer time than other feline hemoplasmas (Barker & Tasker, 2013). Cats would usually succumb to full blown haemoplasmosis when immunosuppressed or secondary to other underlying diseases. Prevalence of coinfection with Mhf and CMhm was reported as low by previous studies (Macieira *et al*., 2008; Solano-Gallego *et al*., 2008; Miceli *et al*., 2013; Santos *et al*., 2014; Munhoz *et al*., 2018). Several studies on the other hand showed that almost 13% of tested samples were co-infected by various haemoplasma species,

with the most common co-infection being Mhf and CMhm (23.74%) (Martínez-Díaz *et al*., 2013) Our results showed that cats infected with CMhm were also more likely to be co-infected with Mhf. It may be that cats infected with one haemoplasma species could exhibit an increased susceptibility to other hemoplasma infections (Vergara *et al*., 2016).

CONCLUSION

This study indicates that the presence of feline hemotropic mycoplasmosis agents, Mhf and CMhm are common in Northeastern parts of Peninsular Malaysia (68.8%). There is an increasing prevalence of haemoplasmosis which could be due to the increasing awareness of the cat owner on the wellbeing of their cats. Periodic prevalence of haemoplasmosis throughout the year is not conclusive; larger sample size and active surveillance in the future might be able to give a confirmatory answer on the effects of monsoon season in this area. Besides, it was once again verified that Mhf infections in cats are not always associated with the clinical signs of anaemia and jaundice. Risk factors such as breed and number of cats in a household are most likely influenced by the management of the cats, whereby the accession to outdoor may affect the association of these two factors with the likeliness of haemoplasmosis. Indeed, younger male cats with outdoor access should be regularly monitored as they are at a higher risk of Mhf infections. The role of arthropods in vector transmission of feline haemoplasma is not being recognized in this study. Cats being infected by CMhm might subsequently increase the susceptibility toward Mhf-induced haemoplasmosis. Need in understanding the transmission of Mhf in cats might help to prevent this disease more effectively. Limitations of this retrospective study includes the low numbers of samples from cats, and the fact that historical data and final diagnoses of CMhm were not readily available for each cat. Others are the lack of serum biochemistry tests and age skewed to younger age of sampled cats may cause some drawbacks on the result obtained. Regardless of these limitations, we believe that this study provides useful information on feline haemoplasma infections in owned cats in Malaysia.

Conflict of interest

The author declares that they have no conflict of interests.

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