PREVALENCE AND RISK FACTOR ANALYSIS OF FELINE BLOOD-BORNE PATHOGENS IN THAILAND

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ความชุกและการวิเคราะห์ปัจจัยเสี่ยงของโรคที่ติดเชื้อผ่านทางกระแสเลือดในแมวในประเทศไทย

นายนนทรี รอดมณี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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นนทรี รอดมณี : ความชุกและการวิเคราะห์ปัจจัยเสี่ยงของโรคที่ติดเชื้อผ่านทางกระแสเลือดในแมวในประเทศไทย. (PREVALENCE AND RISK FACTOR ANALYSIS OF FELINE BLOOD-BORNE PATHOGENS IN THAILAND) อ.ที่ปรึกษาหลัก : รศ. สพ.ญ. ดร.สุกัลยา ฤทธิกุลประเสริฐ

โรคติดเชื้อที่ส่งผ่านทางกระแสเลือดในแมวเป็นโรคสำคัญ การเลือกตรวจคัดกรองโรคดังกล่าวต้องมีความรู้ด้านความชุกและปัจจัยเสี่ยงต่อการติดเชื้อ เมื่อนำตัวอย่างเลือดที่เก็บจากแมวชุดใหม่ (กลุ่ม A) จำนวน 298 ตัวอย่างและจากแมวชุดก่อนหน้า (กลุ่ม B) จำนวน 112 ตัวอย่าง โดยทั้งหมดเป็นแมวที่มีเจ้าของที่มารับบริการที่โรงพยาบาลสัตว์ของจุฬาลงกรณ์มหาวิทยาลัยและโรงพยาบาลสัตว์เอกชนในเขตกรุงเทพฯ และปริมณฑลมาทำการทดสอบหากลุ่มโรคติดเชื้อที่สนใจศึกษา พบว่าตัวอย่างเลือดจากกลุ่ม A จำนวน 55 ตัวอย่าง และจากกลุ่ม B จำนวน 12 ตัวอย่างนั้นได้แสดงผลบวกต่อเชื้อ hemoplasma โดยพบว่า สายพันธุ์ของเชื้อที่ตรวจพบมากที่สุดคือ '*Candidatus* Mycoplasma haemominutum' และรองลงมาคือสายพันธุ์ *Mycoplasma haemofelis* สำหรับเชื้อ *Bartonella* นั้นได้ผลบวกทั้งหมด 10 และ 12 ตัวอย่าง จากกลุ่ม A และ B ตามลำดับ โดยระบุสายพันธุ์ของเชื้อได้เป็น *Bartonella henselae* และ *Bartonella clarridgeiae* เมื่อศึกษาวิเคราะห์ถึงปัจจัยเสี่ยงต่อการติดเชื้อ '*Candidatus* Mycoplasma haemominutum' พบว่าปัจจัยเสี่ยงสำคัญได้แก่ เพศผู้ สายพันธุ์แมวพื้นเมือง แมวที่มีอาการเจ็บป่วย รวมถึงการติดเชื้อไวรัสเอดส์แมว ในขณะที่ลูกแมวอายุน้อยกลับมีปัจจัยเสี่ยงลดลง นอกจากนี้ความเสี่ยงต่อการติดเชื้อ hemoplasma สายพันธุ์ใดก็ตามได้ผลเช่นเดียวกับปัจจัยเสี่ยงต่อการติดเชื้อ '*Candidatus* Mycoplasma haemominutum' และเช่นเดียวกัน ลูกแมวมีโอกาสติดเชื้อลดลง สำหรับปัจจัยเสี่ยงอื่นที่เป็นไปได้อีกได้แก่ แมวที่ออกนอกบ้าน แมวโตเต็มวัยหรือชรามีโลหิตจาง และติดเชื้อไวรัสลิวคีเมียร่วมด้วย การศึกษานี้จึงสนับสนุนให้ทำการตรวจหาเชื้อ hemoplasma และ *Bartonella* ในแมวตัวให้เลือดก่อนการถ่ายเลือดทุกครั้ง เพื่อที่จะยับยั้งการส่งถ่ายเชื้อก่อโรคที่ส่งผ่านกระแสเลือดไปสู่แมวตัวรับเลือดต่อไป

สาขาวิชา	อายุรศาสตร์สัตวแพทย์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2566	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KEYWORD: Anaplasma, Babesia, Bartonella, Cytauxzoon, Ehrlichia, feline blood-borne infection, hemotropic mycoplasmas

Nonsee Rodmanee : PREVALENCE AND RISK FACTOR ANALYSIS OF FELINE BLOOD-BORNE PATHOGENS IN THAILAND. Advisor: Assoc. Prof. SUKULLAYA RITTHIKULPRASERT, D.V.M., M.Sc., Ph.D., D.T.B.V.M.

Feline blood-borne pathogens are infectious agents which knowledge of prevalence and risk factors for infection is important to recognize pathogens that should be screened in blood. A total of 298 samples (GROUP A) and 112 archived samples (GROUP B) were collected from client-owned cats attending the veterinary hospital of Chulalongkorn University, and private veterinary hospitals in Bangkok and vicinity. A total of 55 and 12 samples were positive for hemoplasmas in GROUP A and B, respectively. The species were '*Candidatus* Mycoplasma haemominutum' and *Mycoplasma haemofelis*. DNA of *Bartonella* were detected in 10 and 12 samples from GROUP A and B, respectively. *Bartonella henselae* and *Bartonella clarridgeiae* were revealed. Risk factor analysis showed that male sex, Domestic Shorthair breed, sickness, and feline immunodeficiency virus infection were risk factors of '*Candidatus* Mycoplasma haemominutum' infection; however, kitten was a protective factor. In addition, risk factors of any hemoplasma species infection were similar to those of '*Candidatus* Mycoplasma haemominutum'. Moreover, possible risk factors for hemoplasma infection were outdoor access, mature adult or senior, anemia, and feline leukemia virus infection. The current study advocates for screening hemoplasmas and *Bartonella* spp. in all feline blood donors prior to blood transfusion.

Field of Study: Veterinary Medicine Student's Signature .....

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Nonsee Rodmanee

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LIST OF ABBREVIATIONS

AAHA	American Animal Hospital Association
AAFP	American Association of Feline Practitioners
Bp	Base pair
^{o}C	Degree Celsius
^{o}F	Degree Fahrenheit
CMhm	'*Candidatus* Mycoplasma haemominutum'
cPCR	Conventional polymerase chain reaction
DNA	Deoxyribonucleic acids
DSH	Domestic Shorthair
EDTA	Ethylenediaminetetraacetic acid
E-value	Expect value
FCVRP	Feline calicivirus, viral rhinotracheitis, and panleukopenia
FeLV	Feline leukemia virus
FIV	Feline immunodeficiency virus
IFAT	Immunofluorescence antibody test
IMHA	Immune-mediated hemolytic anemia
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
TBE	Tris-borate-ethylenediaminetetraacetic acid
USA	United States of America

CHAPTER I INTRODUCTION

1.1 Objectives of study

1) To investigate the prevalence of feline blood-borne pathogens including feline hemotropic mycoplasmas, *Bartonella* spp., *Ehrlichia* spp., *Anaplasma* spp., *Babesia* spp., and *Cytauxzoon* spp. in client-owned cats
2) To evaluate the risk factors including signalment, lifestyle, prevention history, health status, clinical signs, laboratory abnormalities and co-infection of these pathogens.

1.2 Hypothesis

Cats are commonly infected with feline hemotropic mycoplasmas and *Bartonella* spp., while *Ehrlichia* spp., *Anaplasma* spp., *Babesia* spp., and *Cytauxzoon* spp. are less likely to infect client-owned cats in Thailand.

1.3 Keyword (Thai)

อะนาพลาสมา บาบีเซีย บาร์โทเนลล่า ไซทอกซูน เออร์ลิเชีย โรคที่ติดเชื้อผ่านทางกระแสเลือดในแมว ไมโครพลาสมาในเลือด

1.4 Keyword (English)

Anaplasma, *Babesia*, *Bartonella*, *Cytauxzoon*, *Ehrlichia*, feline blood-borne infection, hemotropic mycoplasmas

1.5 Advantages of study

The study will provide the prevalence of specific blood-borne pathogens together with the risk factors among client-owned cats which, in the future, can be applied clinically as the screening tests and selection protocol for feline blood donors.

CHAPTER II LITERATURE REVIEW

2.1 Feline blood-borne infections and screening guideline for blood transfusion

In clinical settings, blood transfusion in cats is a common procedure manipulated in most veterinary clinics or hospitals. Feline blood donors should be completely free from any infection that can be transmitted via blood transfusions. Therefore, screening of blood-borne pathogens is essential before blood donation. According to a review from 3 feline blood transfusion guidelines, different lists of blood-borne infections to be screened are provided which vary among geographical regions (Marenzoni et al., 2018). The pathogens which are recommended to be tested in all guidelines are feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), and *Mycoplasma haemofelis*. The infectious agents which are considered for being tested based on geographical distribution include '*Candidatus* Mycoplasma haemominutum', '*Candidatus* Mycoplasma turicensis', *Bartonella* spp., *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia* spp., *Cytauxzoon felis*, *Babesia* spp., and *Leishmania infantum*. Another pathogen which may be considered is *Neorickettsia risticii*. However, due to unavailable laboratory testing, the guideline states that it may be unnecessary to identify this pathogen before blood donation (Wardrop et al., 2016). Finally, the blood-borne infections which are not recommended for testing prior blood donation are coronavirus, *Rickettsia* spp., and *Toxoplasma gondii* (Marenzoni et al., 2018).

2.2 Situations of feline blood-borne diseases in Thailand

In Thailand, retroviral infections which routinely diagnosed by commercially available test kits are well documented in client-owned and stray cat populations (Sukhumavasi et al., 2012; Noosud et al., 2014; Nedumpun et al., 2015; Aiyaranoi et

al., 2018; Kengradomkij et al., 2018; Chaiyasak et al., 2020; Piyarungsri et al., 2020; Rungsuriyawiboon et al., 2022; Moyadee et al., 2023). Hemotropic mycoplasmosis is a common feline infectious disease. However, the analysis of risk factors among cats in Thailand is still limited (Assarasakorn et al., 2012; Do et al., 2020; Kaewmongkol et al., 2020; Do et al., 2021a; Zarea et al., 2022). *Bartonella* spp. infection, including *B. henselae* and *B. clarridgeiae* are common in cats in this region, and less common species of *Bartonella* spp. including *B. koehlerae* and *B. vinsonii* subsp. *berkhoffii* have been reported (Inoue et al., 2009; Assarasakorn et al., 2012; Srisanyong et al., 2016; Saengsawang et al., 2021; Zarea et al., 2022). *Anaplasma* spp., *Ehrlichia* spp., and *Babesia* spp. are common infections in dogs which have been identified in all regions of Thailand (Pinyoowong et al., 2008; Liu et al., 2016; Nambooppha et al., 2018; Huggins et al., 2019). To date, there is no report of feline ehrlichiosis, and few reports of *A. platys* infection in a cat and *B. canis vogeli* infection in stray cats have been published (Simking et al., 2010; Salakij et al., 2012; Do et al., 2021a). Feline cytauxzoonosis has recently been identified in Asia. Many cats in China and one cat in Korea were diagnosed with feline cytauxzoonosis (Zou et al., 2019; Choi et al., 2020). In Thailand, there was only one report of feline cytauxzoonosis which was diagnosed by cytology (Jittapalapong and Jansawan, 1993).

2.3 Feline hemotropic mycoplasmosis

2.3.1 Introduction

Feline hemotropic mycoplasmosis is one of the most common feline infectious diseases worldwide. There are three major species, including *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum', and '*Candidatus* Mycoplasma turicensis'. The most common species is '*C.* M. haemominutum', followed by *M. haemofelis,* and '*C.* M. turicensis'. Each species has different

pathogenicity in which *M. haemofelis* is more pathogenic than '*C.* M. haemominutum' and '*C.* M. turicensis' (Gentilini et al., 2009; Lobetti and Lappin, 2012). There are significant risk factors of *Mycoplasma* infection such as male gender and domestic shorthair (DSH) breed (Ravagnan et al., 2017; Latrofa et al., 2020; Zhang et al., 2021). Infection of *M. haemofelis*, '*C.* M. haemominutum', and '*C.* M. turicensis' has been proven by intravenous inoculation of infected blood in experimental studies (Flint, 1958; Foley et al., 1998; Alleman et al., 1999). Aggressive interaction such as biting or fighting between cats is supposed to be the most possible route of natural transmission. Transmission of *M. haemofelis* via fleas was proven for *M. haemofelis* infection, but transmission of '*C.* M. haemominutum', and '*C.* M. turicensis' among cats via arthropods has not been validated (Woods et al., 2005; Woods et al., 2006). The diagnostic tests are cytology which has low sensitivity and molecular techniques including conventional PCR, nested PCR, and real-time PCR.

2.3.2 Prevalence

Feline hemoplasmosis has been reported worldwide. The prevalence rates of the infection depend on hemoplasma species, population, and geographical distribution. Moreover, the sensitivity of diagnostic tests also affects the potential to identify the pathogen. The infection rates of hemoplasma species are between 9.1-44.9% in Africa, 4.9-85.2% in Asia, 15.3-31% in Australia, 3.7-43.5% in Europe, 3.5-23.9% in North America, and 6.5-44% in South America (Lobetti and Tasker, 2004; Lappin et al., 2006; Watanabe et al., 2008; Barrs et al., 2010; Nibblett et al., 2010; Bortoli et al., 2012; Jenkins et al., 2013; Mesa-Sanchez et al., 2020; Mifsud et al., 2020; Mesquita et al., 2021; Santos et al., 2021; Zhang et al., 2021).

Common hemoplasma species in cats include *M. haemofelis*, '*C.* M. haemominutum', and '*C.* M. turicensis'. Most of the studies report that '*C.* M.

haemominutum' is the most prevalent species among feline hemoplasmas. The prevalence of '*C.* M. haemominutum' varies due to geographical distribution. By PCR method, the prevalence is 7.5-38.5%, 2.6-46.7%, and 15.3-25% in Africa, Asia, and Australia, respectively (Lobetti and Tasker, 2004; Fujihara et al., 2007; Barrs et al., 2010; Jenkins et al., 2013; Alho et al., 2017; Mesquita et al., 2021). Additionally, the prevalence of the infection is 2.3-43.5% in Europe, 0-21.7% in North America, and 0-25% in South America (Lappin et al., 2006; Levy et al., 2008; Nibblett et al., 2010; Firmino et al., 2016; Mesa-Sanchez et al., 2020; Mifsud et al., 2020). The infection rate in Thailand is 19.6-22.9% for this species (Assarasakorn et al., 2012; Kaewmongkol et al., 2020).

The high prevalence of this species among cats may be due to low pathogenicity of this species compared to *M. haemofelis* which do not prevent socialization between cats (Tanahara et al., 2010). Additionally, this species may maintain persistent infection in feline hosts. All cats in one study were detected with '*C.* M. haemominutum' more than a year after inoculation (Willi et al., 2006a). Even the treatment of hemoplasmosis can only temporarily yield low or negative copies in feline blood samples, but then hemoplasma DNAs can be amplified afterward.

M. haemofelis infection is usually reported as the second most prevalent feline hemoplasmosis. The infection rate is lower than *'C.* M. haemominutum' infection except in some studies which are suspected to be the study of the outbreak of the species among the feline colonies (Watanabe et al., 2003; Kewish et al., 2004; Kamrani et al., 2008; Watanabe et al., 2008; Criado-Fornelio et al., 2009; Gentilini et al., 2009; Makino et al., 2018).

Due to its lower, or reported to be the lowest, in pathogenicity as well as lower in bacteriemia, *'C.* M. turicensis' is the least common hemoplasma among three major feline hemoplasmas (Fujihara et al., 2007; Lobetti and Lappin, 2012; Cho

et al., 2016; Rosenqvist et al., 2016; Jikuya et al., 2017; Kaewmongkol et al., 2020). In addition, one of the most commonly used conventional PCR assay cannot differentiate between the band size of *'C.* M. turicensis' and *M. haemofelis* (Jensen et al., 2001). The amplicons must be sequenced; otherwise, all positive amplicons would be acknowledged as *M. haemofelis*. The prevalence rates by continent are 0-26.1% in Africa, 0-10% in Asia, 0.9-10.2% in Australia, 0-9.5% in Europe, 0.1-1.4% in North America, and 0.5-13.5% in South America (Willi et al., 2006b; Fujihara et al., 2007; Kamrani et al., 2008; Barrs et al., 2010; Assarasakorn et al., 2012; Miceli et al., 2013; André et al., 2014; Spada et al., 2014a; Persichetti et al., 2016; Manvell et al., 2021; Mesquita et al., 2021).

Furthermore, uncommon hemoplasma species have been identified in cats which these species commonly infect the other specific hosts beside cats. DNA of '*Candidatus* Mycoplasma haematoparvum' were amplified from cats in Chile, Portugal, and the United States with the highest prevalence of 4.38% in Chile (Sykes et al., 2007; Martínez-Díaz et al., 2013; Vergara et al., 2016). All cats with '*C.* M. haematoparvum' infection were co-infected with other hemoplasma species except 2 cats with *'C.* M, haematoparvum'-like infection from the United States. Additionally, a 10-month-old British Shorthair from Germany was infected with *Mycoplasma haemocanis* which the cat appeared healthy (Bergmann et al., 2016). Moreover, *Mycoplasma wenyonii* which is the species commonly infect cattle was identified in an apparently healthy cat co-infected with *Bartonella henselae* from Spain (Álvarez-Fernández et al., 2022).

Co-infection of hemoplasmas is common (Ravagnan et al., 2017; Munhoz et al., 2018; Sarvani et al., 2018). Moreover, co-infection with triple species is found worldwide (Peters et al., 2008; Martínez-Díaz et al., 2013; Santis et al., 2014; Duarte et al., 2015; Cetinkaya et al., 2016; Raimundo et al., 2016; Vergara et al., 2016; Weingart

et al., 2016; Sarvani et al., 2018). Interestingly, one study found more cats with co-infection than those with single infection (Aquino et al., 2014). Another study reported a quadruple infection with *M. haemofelis*, '*C.* M. haemominutum', '*C.* M. turicensis', and '*C.* M. haematoparvum' in a cat (Martínez-Díaz et al., 2013).

There are studies of the prevalence among feline blood donor candidates. These studies reported the prevalence of 3.7% and 18.4% for any hemoplasma species infection (Duarte et al., 2015; Mesa-Sanchez et al., 2020).

2.3.3 Risk factors

2.3.3.1 Signalment

2.3.3.1.1 Male

Male is one of the most frequently reported risk factors of feline hemoplasmosis. The odds ratio in all hemoplasma species infection ranges from 1.75 to 20.4 with the majority of the studies reported odds ratio between 2 to 4 (Willi et al., 2006a; Roura et al., 2010; Tanahara et al., 2010; Lobetti and Lappin, 2012; Jenkins et al., 2013; Ghazisaeedi et al., 2014; Santis et al., 2014; Silaghi et al., 2014; Duarte et al., 2015; Bergmann et al., 2016; Vergara et al., 2016; Hwang et al., 2017; Ravagnan et al., 2017; Hwang et al., 2018; Makino et al., 2018; Sarvani et al., 2018; Latrofa et al., 2020; Demkin and Kazakov, 2021; Do et al., 2021a). In '*C.* M. haemominutum' infection, the odds ratio is between 1.94 to 4.84 (Tasker et al., 2003; Luria et al., 2004; Willi et al., 2006a; Bauer et al., 2008; Silaghi et al., 2014; Bergmann et al., 2016; Raimundo et al., 2016; Makino et al., 2018). While the odds ratio in *M. haemofelis* infection is between 3.11 to 6.13 (Luria et al., 2004; Spada et al., 2014a; Makino et al., 2018).

Multiple studies suggested the possibility that male cats might be more aggressive than the female cats, leading to an increase in cat fights and bites. Male

cats were reported to initiate the fighting between cats (Lindell et al., 1997). Additionally, more male cats had been diagnosed with aggression as behavior problem than the female ones (Bamberger and Houpt, 2006).

2.3.3.1.2 Domestic shorthair

Domestic shorthair or non-pedigree cats are at more risk to be infected with feline hemoplasmosis. The odd ratio in all hemoplasma species ranges from 3.03 to 5.55 (Jenkins et al., 2013; Rosenqvist et al., 2016; Sarvani et al., 2018; Zhang et al., 2021). One study reported the significant statistics in any hemoplasma species and '*C.* M. haemominutum' (Sarvani et al., 2018). In contrast, another study found only significance in '*C.* M. haemominutum' infection but not in any hemoplasma species (Makino et al., 2018). The reported odds ratios from both studies for '*C.* M. haemominutum' were 3.04 and 11.04 (Makino et al., 2018; Sarvani et al., 2018). Zhang et al. (2021) described that domestic shorthair cats might be allowed outside than the purebred ones, and there was the possibility to adopt the cats from places where they first lived together with infected cats. In addition, purebred cats which were more expensive than DSH cats might have better quality of life than those of DSH cats from breeding cattery.

2.3.3.1.3 Age

Being a kitten or young adult is reported to be a protective factor for feline hemoplasmosis. Likewise, age is identified as a risk factor by multivariable logistic analysis in multiple studies (Attipa et al., 2017; Sarvani et al., 2018; Latrofa et al., 2020). Cats older than 1 year had greater risk of infection with the odds ratio between 2.45 to 5.41 (Ravagnan et al., 2017; Do et al., 2020; Salim et al., 2020; Do et al., 2021a). Similarly, those cats with the age more than 2 years had odds ratio

between 2.21 to 3.42 to be detected with feline hemoplasmosis (Tanahara et al., 2010; Vergara et al., 2016).

Older age as the risk factor may be multi-factorial. Cats may become chronic carriers of disease especially in *'C.* M. haemominutum' infection (Tasker et al., 2003). In addition, older cats may be exposed to the infection via various routes. Also, those cats may have immunosuppressive conditions including chronic diseases, neoplasia, or retroviral infections, which may increase their likelihood of getting infected as well as lower possibility to clear the natural infection.

Conversely, other studies revealed controversial results regarding senior cats as the risk factors. Cats aged than 8 years is considered to be the risk factor (Rosenqvist et al., 2016). On the other hand, another study found cats aged more than 10 years had a lesser likelihood to be infected compared to the cats aged between 1-10 years (Zhang et al., 2021). The latter study suspected that geriatric cats might be less aggressive, suffer from other diseases, or spend less time roaming outside, resulting in lesser exposure to the infection.

2.3.3.1.4 Weight

Only one study analyzed the body weight, and they found that hemoplasma-positive cats were heavier than uninfected cat (Manvell et al., 2021). Additionally, they found that positive males were also heavier than negative male, but not for the female.

2.3.3.2 Housing condition

2.3.3.2.1 Outdoor access

Frequently reported, cats with outdoor access are at greater risk for being infected. The reported odds ratios were between 1.88 to 9.29 in any hemoplasma

species infection (Willi et al., 2006a; Roura et al., 2010; Bergmann et al., 2016; Vergara et al., 2016; Attipa et al., 2017; Sarvani et al., 2018; Do et al., 2020; Imre et al., 2020). For '*C.* M. haemominutum' infection, the odds ratios were between 2.7 to 13.5 (Willi et al., 2006a; Bergmann et al., 2016; Sarvani et al., 2018). One study disclosed that the frequency of outdoor access was not associated with increased risk of infection (Tanahara et al., 2010). The straightforward explanation of outdoor access is that those cats have more possibility to become infected due to the contact with infected cats.

2.3.3.2.2 Household size and contact with other cats

Multiple household cats have a significant chance to be infected with hemoplasmosis than single household cat in one study (Bergmann et al., 2016). Cats which contact other cats were also reported to have higher infection rate than cats which had no contact at all (Munhoz et al., 2018; Celik et al., 2021).

2.3.3.2.3 Stray or shelter cats

Stray cats were more likely to be infected with hemoplasma species than owned cats in a study (Kamrani et al., 2008). In addition, shelter or shelter-feral cats were more likely to be PCR-positive for '*C.* M. haemominutum' than client-owned cats (Bergmann et al., 2016; Attipa et al., 2017). Duarte et al. (2015) found that shelter cats had higher prevalence in hemoplasma infection than client-owned, stray, or blood donor cats. However, multiple studies did not identify that either stray or shelter cats are at risk for infection (Juvet et al., 2010; Nibblett et al., 2010; Santis et al., 2014; Zhang et al., 2021). This suggests that close contact or interaction between stray or shelter cats may be associated with the infection.

2.3.3.3 Prevention

Ectoparasitic prevention and deworming could not decrease the risk of hemoplasma infection (Attipa et al., 2017; Do et al., 2020; Salim et al., 2020; Zhang et al., 2021). Similarly, desexing was also not a protective factor for infection (Gentilini et al., 2009; Martínez-Díaz et al., 2013; Vergara et al., 2016; Celik et al., 2021). A previous study found that non-vaccinated cats were likely to be infected with *M. haemofelis* or '*C.* M. turicensis' than the vaccinated cats (Attipa et al., 2017). This finding appeared unrelated to the hemoplasma infection, but vaccinated cats might be in good take care by the owners than non-vaccinated ones.

2.3.3.4 Health status

Most of the studies did not reveal the association between any hemoplasma species infection and the presence of clinical signs in cats (Barrs et al., 2010; Roura et al., 2010; Tanahara et al., 2010; Martínez-Díaz et al., 2013; Vergara et al., 2016). In contrast, two studies reported that symptomatic cats had a higher chance of being infected with any hemoplasma infection (Attipa et al., 2017; Sarvani et al., 2018). Due to differences in pathogenicity and distribution, those studies might not find significant statistics because of including numerous cats with less virulent hemoplasma infection.

When compared to the apparently healthy cats, those with the presence of clinical signs had more chance to be infected with *'C.* M. haemominutum' (Ural et al., 2009; Attipa et al., 2017; Sarvani et al., 2018; Alanazi et al., 2021). In contrast, the other studies did not find the association in *'C.* M. haemominutum' infection (Tasker et al., 2003; Spada et al., 2014a). There was no significant difference in number of cats presenting with or without clinical signs in *M. haemofelis* or *'C.* M. turicensis'

infection (Spada et al., 2014a; Attipa et al., 2017). These might be due to the low number of infected cats in each study.

A low body condition score was associated with the infection in a study (Spada et al., 2014a). One study also found that cats presented with bite wounds after fighting had a greater risk to be infected with hemoplasma (Tanahara et al., 2010). The presence of clinical signs including pallor, icterus, and vomiting was not identified as the risk factor for feline hemoplasmosis (Salim et al., 2020; Celik et al., 2021). One study found that cats with high temperature or fever were significant to be infected with *M. haemofelis* (Salim et al., 2020).

2.3.3.5 Co-infection

2.3.3.5.1 FIV infection

FIV infection is the common co-infection of feline hemoplasmosis. For any hemoplasma species infection, cats with the presence of positive FIV antibody had the odds ratio of infection between 2.4 to 26.8 (Gentilini et al., 2009; Roura et al., 2010; Tanahara et al., 2010; Jenkins et al., 2013; Duarte et al., 2015; Bergmann et al., 2016; Vergara et al., 2016; Attipa et al., 2017; Ravagnan et al., 2017; Díaz-Regañón et al., 2018; Persichetti et al., 2018; Sarvani et al., 2018; Latrofa et al., 2020).

The most possible explanation for this co-infection is that FIV infection shares the same route of transmission with feline hemoplasmosis which is via biting or fighting between cats as well as transfusion of contaminated blood product (Magden et al., 2013). Additionally, cats with FIV infected may be in the state of immunosuppression from the viral infection which these cats may not be able to clear the hemoplasma infection.

2.3.3.5.2 FeLV infection

Co-infection with FeLV is less likely to be identified as the risk factor. Only two studies found cats with progressive FeLV infection (diagnosed by the antigen test kit) were at a greater risk to be infected with any hemoplasma species with the odds ratio of 3.07 and 8.04 (Tanahara et al., 2010; Díaz-Regañón et al., 2018). Unlike FIV infection, biting between cats is not likely to be considered as the common route of FeLV infection. However, cats may be immunocompromised from the FeLV infection, and cannot clear the hemoplasma infection.

2.3.3.5.3 Other hemoplasma infection

As described above in section 2.3.2, co-infection among hemoplasma infection is common. A study found that cats infected with either '*C.* M. haemominutum' or *M. haemofelis* had higher chances to be detected with '*C.* M. turicensis' with the odds ratio of 5.27 and 3.20, respectively (Willi et al., 2006b). Moreover, cats with '*C.* M. turicensis' infection had 5.12 times higher risk to be infected with '*C.* M. haemominutum' (Munhoz et al., 2018).

2.3.3.5.4 Ectoparasitic infestation

Only *Mycoplasma haemofelis* infection was proved to be transmitted through hematophagous activity of fleas (Woods et al., 2005; Woods et al., 2006). However, none of the studies identified the presence or history of ectoparasitic infection (including fleas, ticks, or lice) as the risk factor for feline hemoplasmosis (Martínez-Díaz et al., 2013; Sarvani et al., 2018; Do et al., 2020; Imre et al., 2020; Salim et al., 2020; Do et al., 2021a). Detailed information will be discussed in section 2.3.4.3.

2.3.3.6 Laboratory abnormalities

Laboratory abnormalities were frequently reported in the prevalence studies; however, few studies analyzed the risk factor from the laboratory profile. Therefore, some risk factors were further analyzed by calculating the Chi-square on this review. The presence of laboratory abnormalities in each hemoplasma infection will further be discussed in section 2.3.6.

One study identified anemia as the risk factor in any hemoplasma species infection (Raimundo et al., 2016). In contrast, many studies did not find the association between anemia and hemoplasma infection (Gentilini et al., 2009; Ural et al., 2009; Roura et al., 2010; Jenkins et al., 2013; Martínez-Díaz et al., 2013; Persichetti et al., 2016; Attipa et al., 2017; Ravagnan et al., 2017; Sarvani et al., 2018; Celik et al., 2021; Zhang et al., 2021). Furthermore, cats with *'C.* M. turicensis' infection had significant lower hematocrit, red blood cell count, and hemoglobin concentration lower than non-infected ones; however, cats in this study had numerous co-infection which might cause anemia by these diseases as well as increase the pathogenicity of hemoplasma infection (Willi et al., 2006b).

Thrombocytopenia is the risk factor of any hemoplasma species infection in one study (Do et al., 2021a). For white blood cell profile, cats with monocytosis, lymphocytosis, or presence of activated monocytes were likely to be PCR-positive for *M. haemofelis* infection, compared to those without the abnormalities of these white blood cell (Raimundo et al., 2016). Additionally, Persichetti et al. (2016) reported cats with either elevated creatine kinase or elevated creatinine were more likely to be infected with any hemoplasma species.

2.3.4 Modes of transmission

2.3.4.1 Blood transfusion

Feline hemotropic mycoplasmas have been proved to be transmitted via experimental intravenous inoculation of hemoplasma-contaminated blood. The experiments of intravenous inoculation of pathogens were successful in cats (Flint, 1958; Foley et al., 1998; Alleman et al., 1999). By natural transmission, transfusion of infected blood product which had been stored for 1 week could cause the infection (Gary et al., 2006).

2.3.4.2 Biting and fighting

Direct transmission by biting has been strongly indicated to be the major mode of transmission among cats. Even though there is no experimental design of demonstrating direct biting from cat to cat, multiple scientific evidence supports this route of transmission.

Firstly, the risk factors for being infected include the presence with abscess, being bitten, or having bitten wound after fight which these reflect the fighting between cats (Grindem et al., 1990; Tanahara et al., 2010). In addition, male cats are considered as the major risk factor, which these cats are likely to be more aggressive than female ones. Therefore, fighting is common among male cats. Another risk factor which supports the evidence is the co-infection of FIV which this retroviral infection is documented to be transmitted via aggressive interactions among cats.

The experiments of transmitting *M. haemofelis* and '*C.* M. turicensis' via subcutaneous or peritoneum inoculation of bloods or blood components are successful (Museux et al., 2009; Baumann et al., 2013). This may support the infection via blood through the ruptured skin; however, large volume of blood

contamination to wound is not common during cat fight. Thus, transmission through saliva is questioned.

'C. M. haemominutum' has been identified in oral mucosa, saliva, and salivary gland (Dean et al., 2008; Bennett et al., 2011). Moreover, '*C.* M. turicensis' has been detected from the saliva in early phase after experimental inoculation as well, despite the low copy number (Willi et al., 2007). These may suggest that cats can transfer pathogens via inoculation of saliva to others. However, subcutaneous inoculation of '*C.* M. turicensis'-infected saliva was not successful in immunosuppressed cats which were induced with corticosteroid (Museux et al., 2009). There is no study for subcutaneous inoculation of *'C.* M. haemominutum' and *M. haemofelis* to cats via saliva. Therefore, further studies are required to prove this route of transmission.

2.3.4.3 Vector-borne transmission

In the past, hemotropic mycoplasmosis had been believed to be a vector-borne disease. However, numerous studies are controversial. The most studied vectors are fleas, followed by ticks. The cat flea (*Ctenocephalides felis*) is the most studied vector, but the transmission via this route is less likely.

Ectoparasitic infestation as well as ectoparasitic prevention was not the risk factor for the infection in multiple studies (Lappin et al., 2006; Kamrani et al., 2008; Assarasakorn et al., 2012; Martínez-Díaz et al., 2013; Sarvani et al., 2018; Do et al., 2021a). Numerous hemoplasma-infected cats were also identified in the flea-free environment. Persichetti et al. (2016) found that none of fleas in the study was positive for hemoplasma while the prevalence of hemoplasmosis in cat population was 26.2%.

Furthermore, the experimental transmission via infected fleas was inconclusive despite the DNA of pathogens were amplified from fleas (Shaw et al., 2004; Woods et al., 2005; Woods et al., 2006; Willi et al., 2007; Kamrani et al., 2008; Barrs et al., 2010). In a study, one cat was transiently positive for *M. haemofelis* DNA after being caged with infected fleas (Woods et al., 2005). Cats were not successfully infected by ingestion of infected fleas from the experiment (Woods et al., 2006). Therefore, the infected fleas may not be the major route of transmission, but rather reflect the infestation of hemoplasma in feline hosts (Barrs et al., 2010).

For tick as the vector of the infection, hemoplasma DNAs were amplified from ticks including *Rhipicephalus sanguineous* and *Ixodes* species, either fed or unfed on cats (Taroura et al., 2005; Willi et al., 2007; Fyumagwa et al., 2008). The same study as in fleas showed that none of ticks were hemoplasma-positive even they were collected from many hemoplasma-positive cats (Persichetti et al., 2016). The information on tick is limited since there is no experimental study.

Additionally, few studies were interested in transmission via mosquito as the vector of the feline hemoplasmosis. Reagan et al. (2017) found one mosquito positive for *M. wenyonii* which was the hemotropic mycoplasma in cattle, and none of the mosquitoes collected near the hemoplasma-positive stray cat colony was positive for hemoplasma. Additionally, hemoplasma was only detected shortly after feeding of blood meal in the mosquitos, but none of the cats in the study was infected with hemoplasmosis when being kept with the mosquitoes which previously fed on infected cats.

2.3.4.4 Vertical transmission

The supporting evidences for vertical transmission from mothers to offspring in other animal species have been reported vastly including *Mycoplasma*

haemocanis in dogs, *Mycoplasma suis* in pigs, *'Candidatus* Mycoplasma haemobos' in cows, and *Mycoplasma haemolamae* in llamas (Almy et al., 2006; Girotto-Soares et al., 2016; Lashnits et al., 2019; Stadler et al., 2019). The transmission may be either in utero or lactogenic. Hemoplasma DNAs can be amplified from feline ovary and testicle tissue; however, this might be falsely positive because these organs were highly vascularized, leading to contaminated of samples with blood (Manvell et al., 2021). There is no study to verify this route of transmission among cats.

2.3.4.5 Oral, oronasal, and oral-fecal transmission

The earlier study demonstrated oral inoculation of 5 mL of infected blood resulted in the infection, which diagnosed by cytology (Flint et al., 1959). Subsequently, experimentally oral ingestion of *'C.* M. turicensis'-infected blood (not exceed 500 μL) did not result in infection (Museux et al., 2009). Thus, this route of transmission may need further investigation, but it is an uncommon route because cats will not intentionally feed on blood meals. Possibly, cats may be infected if they ingest enough amount of contaminated blood during biting and fighting.

As described above, DNA of hemoplasma can be detected in saliva. Therefore, the oronasal transmission via sharing the same feed bowl or grooming may be possible but the data is still limited. Experiment oronasal inoculation with saliva did not result in infection (Museux et al., 2009). This might be due to the low numbers of shedding in saliva, the survival capacity of pathogen in saliva and outside the hosts, and the contact or dehiscence of epithelium as well which required further investigation.

'*C.* M. turicensis can be detected in feces in early phase (Willi et al., 2007). However, there is no study about oro-fecal transmission in cats.

2.3.5 Clinical signs

Each hemoplasma species causes different severity of clinical signs in feline host due to their differences in pathogenicity. The most pathogenic species is reported to be *M. haemofelis*, followed by *'C.* M. haemominutum'. While *'C.* M. turicensis' is reported to be the least virulent hemoplasma species in cats. The following part will be discussed about the reported clinical signs of feline hemoplasmosis. Note in some studies, cats might have concurrent diseases which can worsen the severity or result in uncommon clinical presentation. Additionally, the other infections or diseases have not been ruled out.

Even though *M. haemofelis* is considered as the most pathogenic species, infected cats in many studies were reported to be apparently healthy (Kamrani et al., 2008; Nibblett et al., 2010; Bergmann et al., 2016; Pedrassani et al., 2019). In contrast, there were studies which found multiple cats infected with this species were present of clinical signs (Maher et al., 2010; Rosenqvist et al., 2016; Salim et al., 2020; Sushma, 2021). Cats with *M. haemofelis* infection had been reported to have non-specific clinical signs such as anorexia, lethargy, or fever (Maher et al., 2010; Sushma, 2021). Fever was reported in 16.7% to 60% in some feline population (Watanabe et al., 2003; Salim et al., 2020). Few cats were present with specific signs such as icterus, vomiting, and red urine (Inokuma et al., 2004; Salim et al., 2020). Cats experimentally inoculated with *M. haemofelis* were present with depression, dehydration, or fever (Foley et al., 1998; Tasker et al., 2009).

As mentioned above, multiple studies identified asymptomatic '*C.* M. haemominutum'-infected cats (Kamrani et al., 2008; Nibblett et al., 2010; Sarvani et al., 2018; Alanazi et al., 2021). Moreover, these infected cats which had co-infection with FIV or FeLV were found to have no clinical signs, as well (Bergmann et al., 2016). On the other hand, numerous cats with '*C.* M. haemominutum' infection were also

present with the clinical signs in several epidemiological studies (Maher et al., 2010; Rosenqvist et al., 2016; Attipa et al., 2017; Sarvani et al., 2018; Sushma, 2021). These cats were present with non-specific clinical signs including depression, anorexia, or fever (Watanabe et al., 2003; Ghazisaeedi et al., 2014; Salim et al., 2020). In addition, '*C.* M. haemominutum'-infected cats with possible FIV, FeLV, or *Toxoplasma gondii* co-infection were found to be present with lymphadenomegaly, pallor, stomatitis, signs of respiratory tract infection or ocular infections; even though, these clinical signs might be caused by other diseases beside '*C.* M. haemominutum' infection (Spada et al., 2014a). Experimental inoculation with *'C.* M. haemominutum' caused no or mild clinical signs in infected cats (Foley et al., 1998; Tasker et al., 2009).

'*C.* M. turicensis' had been frequently reported to be the least virulent species among common feline hemoplasma. Cats which were experimentally infected with '*C.* M. turicensis' showed no or mild clinical signs such as inappetence and apathy (Museux et al., 2009; Tasker et al., 2009; Novacco et al., 2012). In most of the epidemiological studies, *'C.* M. turicensis'-infected cats without co-infection or other diseases were found to be clinically healthy (Kamrani et al., 2008; Ghazisaeedi et al., 2014). In contrast, a study identified several symptomatic cats with '*C.* M. turicensis' infection, but concurrent diseases were possible (Attipa et al., 2017).

2.3.6 Laboratory findings

2.3.6.1 Red blood cell abnormality

M. haemofelis is reported to be the most virulent hemoplasma to cause anemia. Cats infected with *M. haemofelis* were significantly lower in red blood cell count, hematocrit, and hemoglobin concentration than those with *'C.* M. haemominutum' infection or without hemoplasma infection (Lobetti and Tasker, 2004). Additionally, DNA load of *M. haemofelis* was reversely correlated with

hematocrit in one study but absence of this finding was found in another study (Lobetti and Tasker, 2004; Maher et al., 2010). Cats with *M. haemofelis* infection had a significant lower hematocrit than that of uninfected cats, but those of *'C.* M. haemominutum'-infected cats were not found to be at risk (Lobetti and Lappin, 2012). Similarly, cats infected with *M. haemofelis*, but not with *'C.* M. haemominutum' had lower red blood cell count than uninfected cats (Gentilini et al., 2009). In this study, *M. haemofelis*-infected cats with or without co-infection with other species of hemoplasma did not have a difference in red blood cell count. In contrast, a study of naturally *M. haemofelis*-infected cats did not find the difference in erythrocytic abnormality (Braddock et al., 2004). An experimental study revealed that all cats that were infected with *M. haemofelis* became anemic, and most of the cats were considered to have severe anemia (Tasker et al., 2009).

'*C.* M. haemominutum' and *'C.* M. turicensis' were reported to be less virulent than *M. haemofelis*, and cats with these infections were likely to be non-anemic in many studies. *'C.* M. haemominutum'-infected cats did not have the difference in red blood cell profile compared to uninfected cats (Lobetti and Tasker, 2004; Rosenqvist et al., 2016). Bacterial load was not correlated with hematocrit in cats infected with '*C.* M. haemominutum' (Maher et al., 2010). Cats with single infection of *'C.* M. turicensis' did not have a difference in hematocrit, compared to uninfected cats (Willi et al., 2006b). Tasker et al. (2009) revealed that none of the cats infected with either '*C.* M. haemominutum' or '*C.* M. turicensis' had anemia. Another experimental study found that '*C.* M. turicensis' only caused mild and transient anemia (Novacco et al., 2012).

For any hemoplasma species, a study reported that infected cats had lower hematocrit than uninfected cats, but another reported no difference between these groups of cats (Ghazisaeedi et al., 2014; Vergara et al., 2016). The difference in this

finding might be due to the prevalence of each hemoplasma species in the population which contributed to various levels of anemia. In addition, anemic cats were not usually infected with hemotropic mycoplasmas; however, a study reported 20.2% of cats with anemia were diagnosed with hemoplasmosis (Ishak et al., 2007; Korman et al., 2013).

Co-infection between hemoplasma species has strong evidence to increase the severity of anemia. Co-infection of *'C.* M. haemominutum' and *M. haemofelis* caused hematocrit, hemoglobin concentration, and red blood cell count of these cats to be lower than the uninfected (Lobetti and Tasker, 2004). Cats which were infected with '*C.* M. turicensis' together with either *'C.* M. haemominutum' or *M. haemofelis* had abnormalities in red blood cell profile compared to negative cats; on the other hand, those that had single infection with *'C.* M. turicensis' had no significant difference of red blood cell profile than uninfected ones (Willi et al., 2006b). Furthermore, dual or triple infection was a risk factor of anemia, compared to single infection of any species of hemoplasma (Sarvani et al., 2018). Nevertheless, one study did not reveal that cats with either single infection or co-infection had more risks to be anemic than uninfected cats (Aquino et al., 2014).

In addition, retroviral infection might be an aggravating factor that increases the extent of anemia in hemoplasma-positive cats. The cats co-infected with retrovirus had a greater risk to have severe anemia than those without retroviral infection (George et al., 2002). Additionally, Firmino et al. (2016) found that cats infected with hemoplasma and FeLV had lower packed cell volume (PCV) than uninfected cats, but hemoplasma-infected cats without FeLV infection did not have the difference in PCV compared to the uninfected cats. In the same study, cats with co-infection between *M. haemofelis* and FeLV had lower PCV than cats with single infection with *M. haemofelis* (Firmino et al., 2016). The increase in pathogenicity may

be caused by various pathologies including 1) immunosuppressive effect of retroviral infection which leads to inadequate control of the hemoplasma, resulting in the increase in hemoplasma load, and 2) myelosuppression from the retroviral infection itself.

Red blood cell abnormalities as well as other abnormalities in feline hemoplasmosis may not only depend on the species of infection, but also may be due to the different phase of infection i.e., acute, or chronic phase. Most of the prevalence studies cannot distinguish between the phases of infection; thus, the abnormalities may not be significantly different if there are multiple chronic subclinical infected cats in the study. Furthermore, there is no test that differentiates and compares the phases of infection. Measurement of hemoplasma copy number by PCR is still controversial (Lobetti and Tasker, 2004; Tasker et al., 2004).

2.3.6.2 White blood cell abnormality

Studies regarding the white blood cell abnormalities of feline hemoplasmosis showed diverse outcomes. Cats with any hemoplasma had lower white blood cell count than uninfected cats (Ghazisaeedi et al., 2014; Do et al., 2021a). In another study, those two groups of cats were not different in white blood cell count (Vergara et al., 2016). Cats with *M. haemofelis* infection might have low or high white blood cell count compared to uninfected cats, but '*C.* M. haemominutum'-infected cats had no difference in white blood cell count to cats without hemoplasma infection (Gentilini et al., 2009; Lobetti and Lappin, 2012). Cats with *M. haemofelis* infection was found to be lower in monocyte count (Lobetti and Tasker, 2004). Leukopenia was also be identified in *'C.* M. haemominutum'-infected cats with FeLV infection (Firmino et al., 2016). Leukocytosis was diagnosed in cats experimentally infected with 'C. *M. turicensis*' (Novacco et al., 2012).

White blood cell abnormalities are not specific, and these may only reflect on the response to any bacterial infection. Therefore, these abnormalities may not be suitable to use as ancillary parameters to diagnose hemoplasmosis in cats.

2.3.6.3 Platelet abnormality

Thrombocytopenia is controversial. Cats infected with any hemoplasma might have lower or equivocal platelet count compared to uninfected cats (Ghazisaeedi et al., 2014; Vergara et al., 2016; Do et al., 2021a). However, cats infected with *M. haemofelis* was reported to have significantly lower platelet count than non-infected cats (Lobetti and Tasker, 2004; Lobetti and Lappin, 2012). On the other hand, cats with *'C.* M. haemominutum' did not have difference in platelet count when compared to uninfected cats (Lobetti and Lappin, 2012).

2.3.6.4 Blood chemistry abnormality

A few studies discussed the changes in biochemical profiles of hemoplasma-infected cats. In a group of sick cats, *'C.* M. haemominutum'-infected cats were found to have higher blood urea nitrogen, creatine, protein, and lipase than uninfected cats (Willi et al., 2006a). In addition, Persichetti et al. (2016) found elevated creatine kinase and creatinine as significant risk factors for any hemoplasma infection, but not for hypoalbuminemia, hyperglobulinemia, and elevated alkaline phosphatase. Blood glucose concentrations were not different among cats with either *M. haemofelis*, '*C.* M. haemominutum', and no hemoplasma infection (Tasker et al., 2009).

2.3.7 Diagnosis

2.3.7.1 Cytology

Cytology can be used as a diagnostic test for feline hemoplasmosis. This method is still being used in many laboratories in Thailand; this is because it is 1) easy to perform as in-house laboratory; 2) less expensive than PCR assay; 3) fast turnaround time within an hour; and 4) minimal equipment and instruments required. Nevertheless, both sensitivity and specificity of cytology are poor.

Most epidemiological studies showed higher number of hemoplasma-positive cases detected by PCR assay than cytology (Jensen et al., 2001; Watanabe et al., 2008; Ural et al., 2009; Nibblett et al., 2010; Jenkins et al., 2013; Silaghi et al., 2014; Cetinkaya et al., 2016; Cho et al., 2016). Additionally, many studies reported the discrepancy in detection between cytology and PCR detection (Ghazisaeedi et al., 2014; Aklilu et al., 2016). When compared to PCR assay, cytology to detect any hemoplasma species has the sensitivity ranging from 9.7% to 69.6%, and the specificity ranging from 56.8% to 97.8% (Tasker et al., 2003; Jenkins et al., 2013; Ghazisaeedi et al., 2014). For detection of '*C.* M. haemominutum' by cytology, the sensitivity and specificity compared to PCR technique were reported to be 10.3%-70.73% and 87.1%-100%, respectively (Bauer et al., 2008; Ural et al., 2009).

In any hemoplasma species infection, low levels of bacteremia can result in false negative especially in cats with the chronic state. In addition, '*C.* M. turicensis' may not be visible on light microscopic examination due to its tiny size and low bacterial load in blood, compared to *M. haemofelis* and '*C.* M. haemominutum' (Jenkins et al., 2013). Regarding to the time preparation after blood collection, most of the agents may dislodge from red blood cells after 2 hours of incubation (Alleman et al., 1999).

Inexperienced examiners may not be able to distinguish artifacts from hemoplasma such as extracellular bacteria, stain crystals, and other intra-erythrocytic pathogens which may result in false positives. Also, some abnormalities in RBC such as Howell Jolly bodies, Heinz body, or protein precipitate can be mistaken as hemoplasmas. In conclusion, the sensitivity and specificity of cytology is not high enough to be used as a sole diagnostic tool for feline hemoplasmosis.

2.3.7.2 Polymerase chain reaction

Conventional PCR (cPCR) technique can be used to diagnose this disease. This technique is still widely used in clinical diagnosis and numerous studies. Primers can be designed to detect different levels of pathogens.

Before the discovery of '*C.* M. turicensis', traditional cPCR technique was used to distinguish between the two species of major feline hemoplasma based on the product size on electrophoresis (Jensen et al., 2001; Criado-Fornelio et al., 2003). However, the latter discovered species '*C.* M. turicensis' had a similar size of PCR product to *M. haemofelis*; therefore, specific PCR detection of '*C.* M. turicensis' must be performed individually, or the PCR product should be further submitted for nucleotide sequencing.

Real-time PCR (qPCR) is usually reported to have higher diagnostic sensitivity than cPCR, due to higher analytical sensitivity which the technique can detect lower bacterial load (Rosenqvist et al., 2016). However, qPCR was reported to detect less hemoplasma-positive cats than conventional PCR in a study (Sykes et al., 2007). In addition, the use of qPCR as a screening tool may be limited due to slightly divergent strain of hemoplasma exhibiting mutation in the probe target region. This leads to failure of attachment of probe to this region, and eventually results in false negative of detection. For example, qPCR failed to detect *M. haematoparvum*-like species in

feline blood sample, but conventional PCR was able to detect this species (Sykes et al., 2007). This suggests that both PCR techniques must be performed concurrently to maximize the detection of species.

2.4 Feline bartonellosis

2.4.1 Introduction

Bartonellosis is one of the most neglected infectious diseases in cats because most of the infected cats are subclinical and become chronic carriers of the microorganism. *Bartonella* are gram-negative bacilli in the family Bartonellaceae. They infect and reside in red blood cells. The prevalence of *Bartonella* infection in humans is reported to be the highest among feline zoonotic blood-borne diseases. There are multiple species which are specific for each mammalian reservoir.

2.4.2 Prevalence

Feline bartonellosis has been reported worldwide. The most common species of feline bartonellosis are *B. henselae, B. clarridgeiae*, and *B. koehlerae*. These species have been identified worldwide. Most studies found *B. henselae* is the most common species, followed by *B. clarridgeiae* and *B. koehlerae*, respectively (Raimundo et al., 2019). Uncommon species of *Bartonella* spp. in cats include *B. vinsonii* subsp. *Berkhoffii* and *B. quintana*.

The prevalence of feline bartonellosis are 0.9-7.8% in Africa, 4.3-63.9% in Asia, 5.2-16.2% in Australia, 0-70.6% in Europe, 1.7-57.7% in North America, and 6.7-29.7% in South America (Bergh et al., 2002; Hackett et al., 2006; Kamrani et al., 2008; Barrs et al., 2010; Pennisi et al., 2010; Lobetti and Lappin, 2012; Switzer et al., 2013; André et al., 2014; Gutiérrez et al., 2015; Bessas et al., 2016; Dybing et al., 2016; Sacristan et

al., 2019). The seroprevalence of feline bartonellosis is reported to be higher than the PCR detection from most of the studies all over the world.

In countries located in East, South, and Southeast Asia besides Thailand, there are reports in detecting feline *Bartonella* species from China, Japan, Korea, Malaysia, Philippines, and Taiwan (Chomel et al., 1999; Tsai et al., 2011; Yuan et al., 2011; Hassan et al., 2017; Sato et al., 2017; Hwang et al., 2018; Zarea et al., 2022). The commonly reported species of *Bartonella* include *B. henselae* and *B. clarridgeiae* (Sato et al., 2017; Zarea et al., 2022).

In Thailand, the reported species of *Bartonella* species which are detected among cats included *B. henselae, B. clarridgeiae, B. koehlerae*, and *B. vinsonii* subsp. *berkhoffii* (Maruyama et al., 2001; Inoue et al., 2009; Assarasakorn et al., 2012; Srisanyong et al., 2016). *B. henselae* is the most frequently identified species with the prevalence of 5.7% in mixed-cat population and 16.0% in stray cats (Inoue et al., 2009; Srisanyong et al., 2016). The other species of *Bartonella* species have been reported to be less than 6% among sample cats. Recent study reports the prevalence of any *Bartonella* infection as 16.1% in client-owned cats (Boonaramrueng et al., 2022).

Co-infection between *Bartonella* species is common but not as frequent as co-infection between hemoplasmas. For instance, there are reports of co-infection between *B. henselae* and *B. clarridgeiae*, as well as *B. clarridgeiae* and *B. koehlerae* (Sato et al., 2017; Raimundo et al., 2019; Zhang et al., 2021; Raimundo et al., 2022). Co-infection of *Bartonella* spp. is also identified from cardiac tissue samples in one study (Donovan et al., 2018).

The prevalence of feline bartonellosis among feline blood donors is between 0.2% to 1.7% (Hackett et al., 2006; Mesa-Sanchez et al., 2020). The prevalence of blood donors in human is reported about 3.2% (Pitassi et al., 2015; Diniz et al., 2016).

2.4.3 Risk factors

2.4.3.1 Signalment

2.4.3.1.1 Age

For risk factors related to signalment, younger age is most likely to be the significant risk factor. Many studies found the increase in prevalence of infection in younger cats comparing to the older cats especially those who were younger than 1 year old (Chomel et al., 1995; Maruyama et al., 2001; Birtles et al., 2002; Barrs et al., 2010; Assarasakorn et al., 2012; Bergmann et al., 2017; Drummond et al., 2018; Persichetti et al., 2018; Cruz et al., 2022). Additionally, previous studies revealed that older cats were less likely to be infected with bartonellosis compared to younger cats (Maruyama et al., 2000; Birtles et al., 2002; Hassan et al., 2017; Zhang et al., 2021). Young cats had a higher degree of bacteremia than adult cats (Chomel et al., 1995). Similarly, cats aged under 2 years old were at higher risk to be detected for *Bartonella* antibodies (Álvarez-Fernández et al., 2022).

The most speculation is young cats may have the immature immune system which cannot control the infection. Unlike in adult cats, these cats can terminate or suppress the infection as well as those cats also have higher antibody titer (Bergmann et al., 2017; Hassan et al., 2017). Additionally, kittens can easily be infected with different strains of *Bartonella* despite maternal immunity is present (Fleischman et al., 2015). Another support evidence is that fleas collecting from kittens are likely to be PCR positive than those collected from adult cats (Tsai et al., 2011). This will contribute to a higher chance of infection in juvenile cats. This risk indicates that cats are likely to be infected within the first year of life.

On the other hand, some studies found that cats aged more than 1 year or those with increasing age were at a significantly greater risk to be detected with anti-*Bartonella* antibodies (Yuan et al., 2011; Boonaramrueng et al., 2022). This finding may indicate that older cats may have no bacteriemia despite the seropositivity of bartonellosis.

2.4.3.1.2 Breed

Mixed breed of cats was identified as the significant risk factor in one study; however, other studies reported no relationship between breed and *Bartonella* infection (Maia et al., 2014; Attipa et al., 2017; Hassan et al., 2017; Mazurek et al., 2020; Shamshiri et al., 2022).

2.4.3.1.3 Sex

Similar to breed, sex is not a significant risk factor as well, albeit reported significance in one study (Inoue et al., 2009). They speculated that male cats may be more aggressive to be bitten or scratched than female while protected in limited areas like in Bangkok Metropolitan.

2.4.3.2 Housing condition and lifestyle

2.4.3.2.1 Ownership

Stray or former stray cats were more likely to be infected with *Bartonella* species than client-owned cats; notably, one study reported the odds ratio more than 40 for being stray (Chomel et al., 1995; Arvand et al., 2001; Kamrani et al., 2008; Yuan et al., 2011; Gutiérrez et al., 2013; Drummond et al., 2018). In addition, a previous study reported the increase in antibody detection of feline bartonellosis among colony cats (Álvarez-Fernández et al., 2022). Stray cats are more likely to be exposed to other infected cats, pathogens, and pathogen-burden vectors. Fighting with other cats may result in scratches and wounds which the bacteria may be

inoculated via this route (Celebi et al., 2009). Fleas are also considered as the route of transmission for the disease.

Cats raised in shelters were also likely to be positive for the infection (Bergmann et al., 2017; Shamshiri et al., 2022). Even though multiple-household condition when cats are expected to be stressed does not result in higher positivity, shelter cats may additionally have more chances to be exposed to vectors as well as, in some circumstances, poorer environmental condition (Pennisi et al., 2010; Srisanyong et al., 2016; Bergmann et al., 2017).

2.4.3.2.2 Access to outdoor

Access to outdoor was reported to be the significant risk in numerous studies (Chomel et al., 1995; Raimundo et al., 2019; Mazurek et al., 2020; Cruz et al., 2022). The straightforward explanation is that those cats have more chance to contact other infected cats or vectors.

2.4.3.2.3 Other housing conditions and lifestyle

Frequent contact with other cats, such as cats in multiple households was not reported to have a greater risk for the infection (Pennisi et al., 2010; Srisanyong et al., 2016; Bergmann et al., 2017; Cruz et al., 2022). Moreover, contact with other animals such as dogs was not considered as a significant risk factor (Chomel et al., 1995; Raimundo et al., 2019; Cruz et al., 2022). One study reported that cats with history of fighting were at more risk of feline bartonellosis in shelter condition (Raimundo et al., 2019).

2.4.3.3 Prevention history

Prevention of ectoparasites was a protective measure for the disease (Srisanyong et al., 2016; Raimundo et al., 2019; Mazurek et al., 2020; Zhang et al.,

2021). Previous studies found that intact cats were prone to be detected with *Bartonella* (Barrs et al., 2010; Raimundo et al., 2019; Cruz et al., 2022). The authors suspected that these intact cats might be likely to wander around especially during heat or response to female pheromones, and consequently contact with other infected cats and vectors. Raimundo et al. (2019) reported that vaccinated cats were less likely to be infected with feline bartonellosis than non-vaccinated ones in shelter environment. This might reflect poor disease prevention by owners, as well as those cats might be infected with other infectious diseases that caused immunosuppression.

2.4.3.4 Health status

Cats with the presence of clinical signs or sick cats were not at greater risk to be PCR positive for *Bartonella* than apparently healthy cats (Fabbi et al., 2004; Pennisi et al., 2010; Srisanyong et al., 2016; Attipa et al., 2017; Raimundo et al., 2019; Mazurek et al., 2020). Moreover, presence of non-specific clinical signs (e.g., pale mucous membranes and enlarged lymph nodes) were not considered as a significant risk factor (Pennisi et al., 2010; Hassan et al., 2017; Da Silva et al., 2019; Zarea et al., 2022). In a previous study, cats with fever were less likely to be detected with *Bartonella* antibodies compared to those without fever (Lappin et al., 2009). However, other studies did not find the significance (Barrs et al., 2010; Assarasakorn et al., 2012; Zarea et al., 2022).

The presence of uveitis and ocular discharge was a significant risk factor in 2 studies (Powell et al., 2010; Hassan et al., 2017). However, in the study regarding uveitis, less number of cats without uveitis were included, and also cats with uveitis were previously stray cats (Powell et al., 2010). One study found that infected cats were more likely to be present with stomatitis; in contrast, other 2 studies did not find the significance (Pennisi et al., 2010; Sykes et al., 2010; Hassan et al., 2017).

Respiratory-associated problems or skin lesions were not considered as the significant findings in cats with bartonellosis (Hassan et al., 2017; Cruz et al., 2022).

2.4.3.5 Co-infection

2.4.3.5.1 Ectoparasitic infestation

Present or recent history of flea infestation was considered as the significant risk factor in prevalence studies (Tsai et al., 2011; Raimundo et al., 2019; Cruz et al., 2022). Additionally, cats with exposure to fleas were more likely to be detected with *Bartonella* antibody (Dowers et al., 2010; Álvarez-Fernández et al., 2022). Conversely, numerous studies did not find the correlation between feline bartonellosis and the presence of fleas (Chomel et al., 1995; Chomel et al., 2002; Fabbi et al., 2004; Pennisi et al., 2010; Cicuttin et al., 2014; Hassan et al., 2017; Zarea et al., 2022). These may indicate the various routes of transmission of feline bartonellosis. Tick and louse infestation was not considered as a significant risk factor (Raimundo et al., 2019).

2.4.3.5.2 FeLV and FIV infestation

A study found cats with FeLV infection were at a greater risk to be infected with bartonellosis albeit there was no significance in 2 studies (Chomel et al., 1995; Bergmann et al., 2017; Sato et al., 2017).

2.4.4 Modes of transmission

2.4.4.1 Blood transfusion

Transfusion of blood from bacteremic cats to naïve ones was successful to cause the infection, either via intravenous or intramuscular administration (Kordick and Breitschwerdt, 1997). Additionally, previous studies showed that *B. henselae* and *B. bacilliformis* could survive in the human blood product which stored at 4 °C more than a month (Magalhães et al., 2008; Ruiz et al., 2012). Infection to other mice by transfusion of *Bartonella*-contaminated blood was successful as well (Silva et al.,

2016). Subcutaneous administration of blood obtained from infected cats also resulted in an infection (Foil et al., 1998). Moreover, intravenous administration of *Bartonella* colony-suspended fluid was able to cause the infection in cats (Abbott et al., 1997).

2.4.4.2 Vector-borne transmission

2.4.4.2.1 Flea-borne transmission

Common feline *Bartonella* species including *B. henselae*, *B. clarridgeiae*, and *B. vinsonii* subsp. *berkhoffii* had been vastly detected in *Ctenocephalides felis* fleas obtained from cats worldwide (Mokhtar and Tay, 2011; Assarasakorn et al., 2012; Gracia et al., 2015; Bessas et al., 2016; Fontalvo et al., 2017; Raimundo et al., 2019; Mifsud et al., 2020; Furquim et al., 2021; Razgunaite et al., 2021; Raimundo et al., 2022; Zarea et al., 2022; Alias et al., 2023). Rare species of *Bartonella* infection in fleas was reported as well such as *Bartonella schoenbuchensis*-like species in Lithunia (Razgunaite et al., 2021). Additionally, *Ctenocephalides canis* were found to be infested cats and also positive for *Bartonella* DNAs (Razgunaite et al., 2021).

As discussed in section 2.4.3.5.1, present or previous history of flea infestation is considered as the significant risk factor in prevalence studies; meanwhile, ectoparasitic prevention is the protective factor in other studies. Moreover, cats infested with *Bartonella*-infected fleas were more likely to be infected with *Bartonella* species than cats infested with non-infected flea (Zarea et al., 2022). Vice versa, fleas obtained from bacteremic cats were significantly higher in detection rate of *Bartonella* than those collected from non-bacteremic cats (Tsai et al., 2011).

A study found that specific-pathogen free cats experimentally infested with *B. henselae*-infected fleas were eventually bacteremic; on the other hand, those lived with bacteremic cats in the flea-free environment had not been detected for bartonellosis (Chomel et al., 1996). Another experimental study also succeeded in

the transfer of *Bartonella* species via infected fleas from infected to non-infected cats (Finkelstein et al., 2002).

The mechanism of transmission via flea is believed to be from the inoculation of *Bartonella*-infected flea feces through the scratch of the broken skin, rather than from flea bite. Viable *B. henselae* had been detected in feces of the flea (Higgins et al., 1996; Foil et al., 1998; Finkelstein et al., 2002). Cats which experimentally injected intradermally with *B. henselae*-infected feces became bacteremic (Foil et al., 1998). Additionally, Cruz et al. (2022) detected *Bartonella* DNA from claws of infected cats, as well as flea infestation was analyzed to be a statistically significant risk factor in the study.

There were studies regarding ingestion of infected fleas. One study demonstrated the transmission of *B. henselae* by flea ingestion in very young kittens; however, another study failed to transmission via this route in cats aged between 4 to 7 months (Guptill et al., 1997; Foil et al., 1998).

2.4.4.2.2 Tick-borne transmission

Tick-borne transmission of feline bartonellosis is still questionable. DNA of *Bartonella* were identified in *Ixodes* ticks attached to cats (Duplan et al., 2018). Transstadial transmission of *B. henselae* by *Ixodes ricinus* ticks was possible, but transovarial transmission was unlikely (Cotté et al., 2008). From the experiment in mice, there was a report of successful transmission of *Bartonella birtlesii* to naïve mice via *I. ricinus* ticks (Reis et al., 2011). There is no experimental transmission of *B. henselae*, *B. clarridgeiae*, and *B. koehlerae* via ticks which these species cause the disease primarily in cats.

2.4.4.3 Biting and fighting in flea-free environment.

Bartonella DNA had been detected in saliva or samples from oral swabs (Kim et al., 2009; Namekata et al., 2010; Oskoueizadeh et al., 2010; Fard et al., 2016; Alias et al., 2023). Additionally, some studies found that PCR-positive oral samples were collected from the non-bacteremic cats (Oskoueizadeh et al., 2010; Alias et al., 2023). The detection of *Bartonella* spp. from nail bed had been identified (Kim et al., 2009; Fard et al., 2016; Cruz et al., 2022). One study found fighting history as a significant risk factor (Raimundo et al., 2019). In addition to successful intradermal inoculation of *Bartonella*, this route of transmission is possible, and further investigation is required to prove.

2.4.4.4 Vertical transmission

Bartonella spp. DNA had been amplified from various reproductive tissues including fetus and placenta in cats (Manvell et al., 2021). Nevertheless, two studies failed to demonstrate trans-placentally or transmission via colostrum or milk suckling (Abbott et al., 1997; Guptill et al., 1998). In addition, none of the experiment queens which were inoculated with *B. henselae* gave birth to bacteremic kittens (Fleischman et al., 2015). Thus, this route of transmission may be less likely.

2.4.5 Clinical signs

The main pitfall in identifying clinical signs associated with feline bartonellosis is the requirement of excluding other diseases. Due to the low pathogenicity of *Bartonella* in nature, the diagnosis of this disease can be made when all other possible diseases are ruled out. For the study of bartonellosis, numerous studies only detect *Bartonella* without identifying other co-infection or co-morbidity of each cat. Thus, the clinical signs of bartonellosis are difficult to identify and are still under debate.

The clinical signs of feline bartonellosis which will be discussed further are obtained from the studies which detect *Bartonella* by PCR or bacterial isolation (not including antibody detection). Most of the *Bartonella*-infected cats are present without clinical signs. As discussed above, presence of clinical signs is not likely to be considered as a significant risk factor. Multiple studies reported asymptomatic cats more than half of the infected sample population, or even all of the cats in the studies (Pons et al., 2005; Solano-Gallego et al., 2006; Ishak et al., 2007; Srisanyong et al., 2016; Bergmann et al., 2017; Sacristan et al., 2019; Boonaramrueng et al., 2022; Shamshiri et al., 2022). Barrs et al. (2010) did not find the association between the presence of clinicals sign and the presence of *Bartonella* DNA; additionally, DNA of *B. clarridgeiae* were also amplified more in the healthy cats than the sick cats. However, due to different sample population and inclusion criteria, some studies reported the presence of clinical signs from numerous infected cats in their studies (Birtles et al., 2002; Pennisi et al., 2010; Muz et al., 2021).

For symptomatic cats, the most reported clinical signs are non-specific signs such as anorexia, dehydration, fever, lymphadenomegaly, and pallor (Kordick et al., 1999; Birtles et al., 2002; Pons et al., 2005; Lappin et al., 2009; Pennisi et al., 2010; Hassan et al., 2017). Lymphadenopathy can be detected in 6.3% and 54.9% of infected cats (Pennisi et al., 2010; Hassan et al., 2017).

To further explain the relationship between clinical signs and bartonellosis, each specific sign has been studied exclusively. One of the most studied clinical presentations of feline bartonellosis is uveitis. *Bartonella* spp. may cause uveitis in cats when other possible causes are ruled out. However, a study found that most of the cats with uveitis were not detected with either *Bartonella* antibody or DNA in aqueous chamber (Lappin et al., 2000). Additionally, cats which successfully inoculated with *B. henselae* were detected with *Bartonella* antibodies and DNA in aqueous humor without uveitis which the lesions were confirmed by histopathology (Lappin et al., 2000). Another clinical finding about ocular diseases is the presence of

ocular discharge which was significantly higher in infected cats than non-infected cats in one study (Hassan et al., 2017).

For neurological signs, cats with specific signs such as seizure had been detected with *B. henselae* DNA in cerebrospinal fluid; however, there was no healthy control group to be compared in this study (Leibovitz et al., 2008). Experimental transmission of infected blood to specific-pathogen free cats caused one cat to have focal seizure, nystagmus, and rigidity which resolved spontaneously (Kordick et al., 1999).

Gingivostomatitis is also one of the most studied clinical presentations. There were no differences in the prevalence of feline bartonellosis between cats with or without gingivostomatitis (Dowers et al., 2010; Namekata et al., 2010). In addition, DNA of *Bartonella* species was rarely detected in tissue from cats with gingivostomatitis, unlike feline calicivirus (Dowers et al., 2010). Notably, only *B. clarridgeiae* DNA were detected in oral tissues from this study. Another found that oral swab samples were PCR positive for *Bartonella* from bacteremic cats rather than non-bacteremic cats (Namekata et al., 2010). In this study, there was no association between presence of oral lesions and cats that were PCR positive in blood or oral swab samples; however, those cats with seropositive to either *B. henselae* or *B. clarridgeiae* were significant at higher risk to have oral lesions.

For endocarditis, *Bartonella* spp. is considered as the most common cause of endocarditis in humans (Edouard et al., 2015). Additionally, cases of canine endocarditis caused by *Bartonella* spp. were reported (Pesavento et al., 2005). Feline cases of endocarditis related to feline bartonellosis had been reported periodically (Chomel et al., 2003; Perez et al., 2010). DNA of *Bartonella* spp. were identified in cardiac tissue samples from cats with feline endomyocarditis-left ventricular endocardial fibrosis complex (Donovan et al., 2018).

2.4.6 Laboratory findings

Multiple studies reported controversial findings in blood profile. Hematological findings of infected cats were within normal limits; although, there were significantly lower hematocrit, red blood cell count, hemoglobin concentration, plasma protein, as well as significantly higher in white blood cell count and neutrophil count than non-infected cats in (Souza et al., 2017). Another study found that infected cats had higher platelet count than non-infected cats, but the number of platelet counts in both groups were normal (Cruz et al., 2022). Infected cats also had no difference in red blood cell and white blood cell profile (Cruz et al., 2022). There was no difference in complete blood count and biochemical profile in another study (Da Silva et al., 2019). However, some studies found a change in hematological profile of the infected cats. An experimental study found that anemia was found in cats with acute infection, but not found in those with relapsing bacteremia (Kordick et al., 1999). Hassan et al. (2017) found neutrophilia and monocytosis as risk factors for any *Bartonella* spp. infection. Neutrophilia was reported in human with bacillary angiomatosis, and monocytosis was reported in infected dogs which might indicate the chronic process of inflammation; however, monocytosis and neutrophilia may represent the stress leukogram in cats (Hassan et al., 2017). Another study disclosed that cats with bartonellosis were likely to have eosinophilia, but other possible causes were not ruled out (Raimundo et al., 2019). Additionally, they also revealed that these infected cats had lower in total plasma protein concentrations and higher in platelet count than non-infected, but the values of these blood parameters were within normal limits. Another study found that cats with presence of antibodies had significantly lower in blood glucose concentration and higher in globulin concentration (Whittemore et al., 2012). Therefore, blood parameters may not be useful in diagnosing feline bartonellosis.

2.4.7 Diagnosis

Various genes (such as *bat*R, *glt*A, *gro*EL, *fts*Z, *nlp*D, *rib*C, *rpo*B) have been used to be as template of *Bartonella* sequences in cats (Pretorius et al., 1999; Diniz et al., 2007; Billeter et al., 2011; Yuan et al., 2011; Srisanyong et al., 2016; Pedrassani et al., 2019; Saengsawang et al., 2021). Detailed analysis of nucleotide sequence is obtained from multiple gene sequencing; however, the most common genes used in detection by PCR are *16s* rRNA or internal transcribed spacer (*ITS*).

PCR can be used as a diagnostic tool for feline bartonellosis because bacteremia can persist to several months (Kordick et al., 1999). However, false negative result of PCR can be found in the case of cyclic bacteremia which the level of infection is below the detection of PCR (Kordick et al., 1999). Conventional PCR assay is reported to have lower assay sensitivity than nested PCR and real-time PCR.

Antibody detection tests such as immunofluorescence antibody test (IFAT) are used in prevalence studies (Maruyama et al., 2003; Luria et al., 2004; Sykes et al., 2010; Guzel et al., 2011; Maden et al., 2015; Spada et al., 2016; Hwang et al., 2018). Due to its high sensitivity but low specificity, the test is mainly used as screening test rather than diagnosis test. Pre-enrichment culture and isolation is also used to diagnose feline bartonellosis in the studies (Yuan et al., 2011; Drummond et al., 2018; Furquim et al., 2021). Pre-enrichment culture is the step to temporarily grow *Bartonella* in the media especially liquid culture to allow these to multiply and later detected by the PCR technique. Meanwhile, isolation culture is the process to grow these pathogens on media especially the agar and then later identified by the microbiological bacterial tests or PCR.

Cats may have controversial results between PCR, bacterial culture, and antibody testing (Chomel et al., 1995; Lobetti and Lappin, 2012). There are many

cases positive for either PCR or bacterial culture, but negative for antibody detection (Chomel et al., 1995; Lobetti and Lappin, 2012). This may be explained by the infection course is early which the presence of *Bartonella* antibodies in some cats began to be detected approximately 6 months post-infection (Kordick and Breitschwerdt, 1997; Kordick et al., 1999). Another possible cause is that these cats are in the state of immunosuppression from other disease e.g., retroviral infection or chronic disease which suppresses or delays the production of antibodies by lymphocytes.

Vice versa, some cats are present with antibody detection but negative for PCR and/or bacterial culture. Seropositivity may not prove the current infection, but rather indicate that these cats are exposed to *Bartonella*. Antibodies can persist up to several months or even years; therefore, the antibody detection is not suitable for screening in blood donors. Additionally, several cats after some periods after infection may have level of bacteremia below the detection threshold but can still be found to be antibody positive (Kordick et al., 1999).

When comparing PCR techniques to bacterial culture, both methods have their advantages and disadvantages. Several studies found that numerous cats were positive for pre-enrichment liquid culture but negative for PCR from blood (Yuan et al., 2011; Drummond et al., 2018; Furquim et al., 2021). This may be due to low bacterial loads of *Bartonella* which are lower than the minimum detection threshold of the PCR. In addition, pre-enrichment liquid culture technique improves the value of quantification of the bacteria.

In contrast, multiple studies reported the samples which were PCR positive but negative for the bacterial culture. Most of the studies did find more PCR-positive samples than ones which positive for pre-enrichment liquid culture or bacterial isolation (Yuan et al., 2011; Drummond et al., 2018; Furquim et al., 2021). This might

indicate non-viable bacteria in blood which unable to grow in the media. Drummond et al. (2018) reported the diagnostic test which had the most assay sensitivity was nested PCR from blood, followed by nested PCR from liquid culture and conventional PCR from liquid culture, respectively. Due to difference in positivity for each sample, PCR and bacterial culture should be used concurrently to increase the diagnostic sensitivity.

2.5 Feline anaplasmosis

2.5.1 Introduction

Anaplasmosis is one of the common infectious diseases in dogs; however, cats can also be infected with *Anaplasma* spp. as well. This pathogen belongs to the family Anaplasmataceae along with *Ehrlichia*, *Wolbachia*, and *Neorickettsia*. These are gram-negative rod bacteria. There are multiple species of *Anaplasma* spp., but the frequently reported species which cause clinical disease in both dogs and cats are *Anaplasma phagocytophilum* and *Anaplasma platys*. The first species infects white blood cells mainly granulocytes; meanwhile, the latter one resides within platelets. This disease is usually vector-borne, which tick is a major vector among the infections in dogs. The diagnosis can be performed by cytology, but the sensitivity is low. PCR has higher diagnostic accuracy and has been used more frequently than in the past. In Thailand, the species which diagnosed in dogs is only *A. platys* which may be due to the absence of *Ixodes* tick, a major vector of *A. phagocytophilum*.

2.5.2 Prevalence

In contrast to the common feline blood-borne bacterial diseases such as feline hemotropic mycoplasmosis, cats which are diagnosed with anaplasmosis are negligible. Nevertheless, *Anaplasma* spp. infection in cats has been detected in

various countries. Due to the absence of species detection or DNA sequencing in several studies, only genus of *Anaplasma* is reported. Multiple studies did not detect any cat with anaplasmosis; however, regardless to the different sample population, the prevalence of any *Anaplasma* species in cats were reported by PCR up to 1% in Africa (Oliveira et al., 2018), 13% in Asia (Ahmed et al., 2020), 31.1% in Europe (Zobba et al., 2015), 6.9% in North America (Galemore et al., 2018), and 13.2% in South American (Correa et al., 2011). There is no detection of feline anaplasmosis in Australia (Barrs et al., 2010).

A. phagocytophilum is the most prevalent species in Europe and North America. It had been detected by PCR up to 4.3% in Germany (Krücken et al., 2013), 17.7% in Italy (Spada et al., 2014c), and 1.7% in United Kingdom (Shaw et al., 2005). Additionally, cases of feline anaplasmosis in Finland, Poland, and Switzerland were reported as well (Schaarschmidt-Kiener et al., 2009; Heikkilä et al., 2010; Adaszek et al., 2013; Gorna et al., 2013). Notably, the reports of detection of antibodies to *A. phagocytophilum* were much higher than PCR detection in these countries. On the other hand, cats from several countries such as Greece and Ireland were still tested negative for feline anaplasmosis (Juvet et al., 2010; Diakou et al., 2017; Mylonakis et al., 2018). Before 2015, no case of feline anaplasmosis detected by PCR had been reported in the USA, then after 2015 onward, the prevalence of *A. phagocytophilum* had been reported between 0.9-6.9% (Hegarty et al., 2015; Savidge et al., 2016; Galemore et al., 2018). Besides countries in Europe and North America, *A. phagocytophilum* had also been detected in Korea and Brazil (André et al., 2014; Lee et al., 2016; Pedrassani et al., 2019).

A. platys has been reported to be the second most prevalent species of feline anaplasmosis. Unlike *A. phagocytophilum*, the confirmed cases of *A. platys* had not been detected in Europe, except a report in Italy of which there was an

outbreak of *A. platys*-like strains (Qurollo et al., 2014; Hegarty et al., 2015; Zobba et al., 2015). It has been sporadically detected in Asia including Saudi Arabia and Thailand, and more frequently reported from countries in South America such as Brazil and Chile (Lima et al., 2010; Correa et al., 2011; Salakij et al., 2012; Guimarães et al., 2019; Sacristan et al., 2019; Alanazi et al., 2021). Only a few cases of *A. platys* infection in cats had been detected in USA (Qurollo et al., 2014; Hegarty et al., 2015).

Another reported species of feline anaplasmosis was *Anaplasma bovis* in Angola and Japan (Sasaki et al., 2012; Oliveira et al., 2018).

In Thailand, *A. platys* infection was the only *Anaplasma* species reported in a cat (Salakij et al., 2012). In contrast, *A. platys* infection in dogs have been commonly reported with the prevalence of 3.7% to 30.61% (Liu et al., 2016; Kaewmongkol et al., 2017; Piratae et al., 2019; Rucksaken et al., 2019; Sarker et al., 2021). Additionally, *A. bovis* was detected in rats, and *A. bovis*-like strain was also reported in ticks (Parola et al., 2003; Malaisri et al., 2015; Panthawong et al., 2020).

There are multiple proposed hypotheses to explain the low prevalence of feline anaplasmosis. First, ticks which may play the role as vectors in transmission of the disease rarely infest cats, and they are prone to fall off due to the grooming behavior of the cats (Lee 2016; Bergman 2015). However, *Babesia* spp. which is also a vector-borne disease and transmitted mainly by ticks is diagnosed more frequently than *Anaplasma* in cats. In addition, the study reveals that the duration of tick attachment for the transmission of *Anaplasma* spp. is shorter than *Babesia* spp. in multiple host species (Richards et al., 2017). Therefore, the grooming behavior may not play the major role in this case.

Moreover, the seroprevalence of this disease in cats is higher than the molecular detection. However, seropositive titers only indicate the previous exposure

of the pathogen. There are 2 possible explanations: 1) *Anaplasma* species may sequester in other tissues which may not be detected in blood sample, or 2) cats may rapidly clear the infection. Studies suggest that cats may be more resistant to the infection than dogs (Lappin et al., 2006; Ebani and Bertelloni, 2014). Multiple *Anaplasma* PCR-positive cats after antimicrobial treatment became PCR-negative within a month onwards; however, they had persistently positive antibody titers for several months (Lappin et al., 2004).

Additionally, low prevalence of feline anaplasmosis may be contributed to the pathogen and clinical setting such as genetic heterogeneity of *A. phagocytophilum* which feline hosts might respond to these infections in the different degree of immunity (Silaghi et al., 2014). In addition, demographic and environmental distribution of cat population, vectors, and veterinary procedures to the animals may also be the possible factors contributing to the prevalence of feline anaplasmosis.

2.5.3 Risk factors

Due to absence of risk factor analysis in some studies despite the provision of detailed information, odds ratios of risk factors are calculated later in this literature review. Additionally, significant risk factors have not been identified in several studies due to the low number of cases.

2.5.3.1 Signalment

Symptomatic cats which aged more than 1 year were more likely to be detected with *A. phagocytophilum* infection than symptomatic kittens (Muz et al., 2021). Age of cats was not considered as a risk factor of *Anaplasma* spp. infection in studies of stray cats, client-owned cats, or sick cats (Spada et al., 2014c; Ahmed et al., 2020; Muz et al., 2021). Breed of cat was not identified as a risk factor (Ahmed et

al., 2020). Similarly, there was no sex predisposing for feline anaplasmosis (Spada et al., 2014c; Ahmed et al., 2020; Muz et al., 2021).

2.5.3.2 Housing condition

Ahmed et al. (2020) found that client-owned cats in poor house hygiene had significantly higher risk of infection than those in good house hygiene. The authors stated that these cats with poor house hygiene were prone to be exposed to infectious agents as well as vectors. However, outdoor access was not considered as a risk factor (Ahmed et al., 2020).

2.5.3.3 Prevention and health condition

Ectoparasitic infestation was considered as protective factor in one study (Ahmed et al., 2020). Presence of clinical signs as well as poor body condition score was not identified as risk factor (Ahmed et al., 2020).

2.5.3.4 Co-infection

Tick infestation or the history of tick exposure was considered as a risk factor for feline anaplasmosis (Ahmed et al., 2020). FeLV, FIV, rickettsial, and *Toxoplasma gondii* infection was not found to be a significant risk factor (Spada et al., 2014c).

2.5.4 Modes of transmission

2.5.4.1 Blood-borne infection

There is no experimental study of infection via blood transfusion in cats. However, the human cases which had been infected with *A. phagocytophilum* via contaminated blood transfusion were reported in USA (Annen et al., 2012; Alhumaidan et al., 2013; Fine et al., 2016; Goel et al., 2018) and Slovenia (Jereb et al., 2012). There was a study demonstrated transmission of *A. phagocytophilum* via

experimentally transfusion of infected blood in horses as well (Franzén et al., 2005). Thus, infection via blood transfusion in cats is likely to be possible.

2.5.4.2 Vector-borne transmission

Tick is a major vector of anaplasmosis in dogs, but the possibility of tick as a vector of anaplasmosis in cats is questioned. First, DNA of *A. phagocytophilum* were detected in *Ixodes hexagonus* and *I. ricinus* ticks which harbored on cats in the United Kingdoms (Duplan et al., 2018). Additionally, cats with *A. phagocytophilum* infection were reported to be previously infested with ticks in Finland and Poland (Heikkilä et al., 2010; Adaszek et al., 2013). Presence of ticks as well as previous history of tick exposure was considered as risk factor for feline anaplasmosis in a study (Ahmed et al., 2020). A major experiment was published in 2015 in which wild *I. scapularis* ticks were caught and then transferred to non-infected cats; eventually, these cats were consequently detected with *A. phagocytophilum* DNA in their blood (Lappin et al., 2015). Therefore, ticks, especially *Ixodes* species, are likely to be vectors for *A. phagocytophilum* infection in cats.

Flea as a vector for feline anaplasmosis is less likely. Multiple studies reported no detection of *Anaplasma* species in fleas collected from cats (Lappin et al., 2006; Alves et al., 2009; Barrs et al., 2010; Nguyen et al., 2020b). However, a study detected *A. phagocytophilum* DNA in both dog and cat fleas (Pawełczyk et al., 2019). Hence, this route of transmission requires further investigation.

2.5.4.3 Other routes

Transplacental (vertical) transmission of canine anaplasmosis in dogs is possible in which an experimental study found that tissues from uterus, ovaries, and placenta as well as fetuses were PCR-positive for *A. platys* (Latrofa et al., 2016). There is no study illustrating transplacental transmission in cats.

2.5.5 Clinical signs

Various clinical signs of cats with *A. phagocytophilum* infection have been reported in numerous studies. Those cats might be present with non-specific clinical signs such as anorexia, lethargy, weakness, lymphadenomegaly, and fever (Foley et al., 2003; Shaw et al., 2004; Schaarschmidt-Kiener et al., 2009; Heikkilä et al., 2010; Adaszek et al., 2013; Gorna et al., 2013; Spada et al., 2014b; Hegarty et al., 2015; Savidge et al., 2016; Schäfer et al., 2021). Regardless to co-morbidities, several infected cats were reported to have different clinical presentations including painfulness, joint pain, icterus, gingivitis, and presence of ocular discharge (Heikkilä et al., 2010; Hamel et al., 2012; Adaszek et al., 2013; Gorna et al., 2013; Spada et al., 2014b; Hegarty et al., 2015; Savidge et al., 2016). In contrast, a prevalence study reported no clinical signs detected in infected cats as well (Galemore et al., 2018). Additionally, there was no clinical abnormality of *A. phagocytophilum*-infected cats from an experimental study in which those cats got infected by ticks (Lappin et al., 2015).

Cats with *A. platys* infection were usually detected with other co-morbidities; thus, exact clinical signs caused by this species were difficult to clarify. Non-specific clinical signs were reported in those cats including depression, anorexia, and fever (Lima et al., 2010; Salakij et al., 2012; Hegarty et al., 2015; Sacristan et al., 2019; Muz et al., 2021). *A. platys*-infected cats were reported to be asymptomatic as well (Sacristan et al., 2019; Alanazi et al., 2021).

In the study of Sasaki et al. (2012), both cats with *A. bovis* infection were co-infected with retrovirus; one with FIV infection and the other one with both FIV and FeLV infection. The first cat was only present with stomatitis, while another had clinical signs of anorexia, fever, and diarrhea.

2.5.6 Laboratory findings

Hematological abnormalities have been infrequently reported in *A. phagocytophilum*-infected cats. Anemia was reported in approximately 15.4-45.9% of infected cats (Gorna et al., 2013; Spada et al., 2014b; Hegarty et al., 2015; Savidge et al., 2016; Guimarães et al., 2019; Schäfer et al., 2021). Additionally, both leukocytosis and leukopenia were reported in 8.1-45.5% and 5.4-23.1% of infected cats, respectively (Heikkilä et al., 2010; Hamel et al., 2012; Salakij et al., 2012; Spada et al., 2014b; Hegarty et al., 2015; Lappin et al., 2015; Savidge et al., 2016; Guimarães et al., 2019; Schäfer et al., 2021). Thrombocytopenic cats were detected approximately 8.1-72.7% of cats with *A. phagocytophilum* infection as well (Hamel et al., 2012; Gorna et al., 2013; Spada et al., 2014b; Hegarty et al., 2015; Schäfer et al., 2021). Another study reported none of the infected cats were present with hematological abnormality (Galemore et al., 2018). For biochemical profile, hyperproteinemia, hyperalbuminemia, and hyperglobulinemia were reported in several studies (Hamel et al., 2012; Savidge et al., 2016; Schäfer et al., 2021). Some cats were icteric and present with hyperbilirubinemia (Gorna et al., 2013; Schäfer et al., 2021).

For feline *A. platys* infection, complete blood count abnormalities included anemia, leukocytosis, and thrombocytopenia (Salakij et al., 2012; Hegarty et al., 2015; Guimarães et al., 2019). Hyperproteinemia was frequently reported as well (Salakij et al., 2012; Hegarty et al., 2015; Guimarães et al., 2019).

2.5.7 Diagnosis

Diagnosis of *Anaplasma* species can be performed by cytology, mainly in dogs. However, the sensitivity and specificity are low. In contrast, PCR can be used to diagnose these infections which have higher sensitivity and specificity than cytology. Primers of PCR can be designed to be specific for various levels of taxonomy

including species-specific, genus-specific, or family-specific. Most of the case reports used family-specific PCR coupling with DNA sequencing to detect the pathogens in which this technique can detect both *Anaplasma* and *Ehrlichia* at the same time as well as further analysis for species identification by DNA sequencing.

2.6 Feline ehrlichiosis

2.6.1 Introduction

Ehrlichiosis is one of the most prevalent infectious diseases in dogs worldwide. However, the infection in cats is less likely, and the significance of this disease is undetermined. Similar to *Anaplasma*, *Ehrlichia* is one member of the family Anaplasmataceae.

2.6.2 Prevalence

The disease is less identified compared to the common blood-borne infectious diseases such as hemoplasmosis or bartonellosis. The prevalence is usually less than 5% except in some countries or population which may be found up 20% (De Oliveira et al., 2009; Pedrassani et al., 2019). There is no publication of feline ehrlichiosis in Thailand.

Due to the similar pathogenesis and routes of transmission, the low prevalence of feline ehrlichiosis may be explained in the similar trends as for feline anaplasmosis. The copy number of *Ehrlichia* in feline blood samples was shown to be less than in canine blood samples which may be contributed to false negative in detection (Eberhardt et al., 2006).

2.6.3 Risk factors

Abbas et al. (2023) reported numerous significant risk factors of feline ehrlichiosis including unsanitary hygiene status, anemia, no use of ectoparasitic prevention, previous tick-borne disease, and humane society (compared to house/flat type). Additionally, one study found that mixed-bred cats older than 1 year old had a greater risk to be detected with *Ehrlichia* antibodies than those younger than 1 year old (Ebani and Bertelloni, 2014). In contrast, a study reported no statistically association between the *Ehrlichia* PCR positivity and numerous risk factors, including age, sex, complete blood count abnormality, or retroviral status (Braga et al., 2012; Santos et al., 2013; Spada et al., 2014b; André et al., 2015).

2.6.4 Modes of transmission

Cats have been sporadically diagnosed with *E. canis* worldwide. The main route of transmission is believed to be transmitted via tick vector especially *Rhipicephalus sanguineous* or brown dog ticks which this route of transmission has been proved in dogs (Lewis Jr et al., 1977). In contrast, there is no experimental study of tick transmission in cats; however, DNA of *E.* canis were detected from *R. sanguineous* tick collected from cats in Indonesia and Philippines (Nguyen et al., 2020b). In addition, other tick vectors might be possible to transmit *Ehrlichia* among cats. There was a study reporting an infected cat was infested with *Amblyomma* tick (Pedrassani et al., 2019). Additionally, there might be other possible routes of transmission for feline ehrlichiosis. A study found numerous cats with ehrlichiosis, but ticks or other vectors could not be examined on the cats (Braga et al., 2014). Due to rare prevalence of *E. chaffeensis* infection in nature, the study of the infection in cats is limited. Multiple studies are required to understand the route of transmission for feline ehrlichiosis.

2.6.5 Clinical signs

Most of the cats infected with either *E. canis* or *E. chaffeensis* remained subclinical (Hegarty et al., 2015; Braga et al., 2017; Oliveira et al., 2018; Pedrassani et al., 2019). On the other hand, cats might show several clinical signs including lethargy, fever, and inappetence, regardless to co-morbidities (Breitschwerdt et al., 2002; Braga et al., 2017).

2.6.6 Laboratory findings

E. canis-infected cats had lower in white blood cell count, lymphocyte count, eosinophil count, red blood cell count, hemoglobin concentration, and platelet count than uninfected cats; however, anemia is the only significant risk factor in the study (Abbas et al., 2023). In addition, cats with ehrlichiosis had higher serum alkaline phosphatase, alanine transaminase, bilirubin, and lower in total protein than uninfected ones (Abbas et al., 2023). Other studies reported various abnormalities ranging from normal complete blood count to anemia or thrombocytopenia (Santos et al., 2013; Braga et al., 2017).

2.6.7 Diagnosis

PCR is mainly used in epidemiological studies due to high sensitivity and specificity; however, antibody detection techniques including IFAT and test kit have been used as well. In contrast, numerous cats have been detected with DNA of *Ehrlichia* in blood by PCR, despite the negative result of antibody detection (Braga et al., 2012). The discordant results of PCR detection and antibody testing can be explained in a similar way as canine anaplasmosis in which dogs might be detected only for *Ehrlichia* antibodies in situations following 1) early course of the infection, 2) exposure to the pathogen and not causing the disease, and 3) chronic infection. The

main hypothesis is the difference in phases of infection which is well documented in dogs.

2.7 Feline babesiosis

2.7.1 Introduction

Babesia is an intra-erythrocytic pathogen which infects both humans and animals. Dogs have been diagnosed with babesiosis more frequently than cats. There are multiple species and variants of *Babesia* species, leading to differences in virulence and pathogenicity. Transmission via tick vector is the main route of transmission among dogs; however, this pathogen can be transmitted via blood transfusion as well. *Babesia* mainly resides in red blood cells and usually destroys these cells, leading to hemolysis, and eventually resulting in anemia and clinical signs.

2.7.2 Prevalence

Feline babesiosis has been diagnosed in all continents. Prevalence of the disease which detected by PCR technique were reported up to 51.9% in Africa (Bosman et al., 2007), 39.5% in Asia (Do et al., 2021a), 24% in Europe (Muz et al., 2021), 19.3% in North America (Kelly et al., 2017), and 16.2% in South America (André et al., 2014).

Additionally, there are various species of *Babesia* detected in feline species. The reported species in cats include *Babesia vogeli, Babesia canis, Babesia microti, Babesia felis, Babesia bigemina, Babesia leo, Babesia lengau, Babesia hongkongensis, Babesia panickeri,* and novel *Babesia* species such as *Babesia* sp. Western Cape (Wong et al., 2012; Bosman et al., 2019; Panicker et al., 2020). Prevalence of each species depends on the geographic distribution of each species.

To authors' knowledge, the first report of feline babesiosis in Thailand was published in 1993 which 2 cats with *Babesia* were diagnosed by cytology (Jittapalapong and Jansawan, 1993). The following report was published in 2010 in which nested PCR was used to detect *B. vogeli* in 1.4% of stray cats (Simking et al., 2010). Surprisingly, Do et al. (2021a) reported the prevalence of *B. vogeli* infection as 39.5% among stray cats, which was much higher than the previous study. Nevertheless, the species reported in dogs from Thailand included *B. vogeli*, *B. canis*, and *B. gibsoni* (Suksawat et al., 2001; Buddhachat et al., 2012; Piratae et al., 2015; Rucksaken et al., 2019; Do et al., 2021b).

2.7.3 Risk factor

2.7.3.1 Signalment

Client-owned cats aged between 0.4-1.5 years were at a higher risk to be infected with *B. vogeli* than those aged more than 7 years (Vilhena et al., 2013). In contrast, stray cats which were older than 1 year was reported to be at a greater risk to be co-infected with *Babesia* spp. and hemoplasma than ones which was younger than 1 year, but not for single infection with *Babesia* (Do et al., 2021a). Otherwise, no other studies identified age as a risk factor (Simking et al., 2010; Maia et al., 2014; Akram et al., 2019; Muz et al., 2021). Numerous cats with feline babesiosis were reported to be younger than 1 year (Zhang et al., 2019; Wang et al., 2020). Being kitten as a risk factor of feline babesiosis may be due to less mature immune status than adult cats; however, it is still required further investigation (Vilhena et al., 2013).

Maia et al. (2014) found that DSH cats were considered as a protective factor for feline babesiosis, while another study did not identify breed as the risk factor (Vilhena et al., 2013). This is controversial to other blood-borne diseases, and further studies are needed to confirm this finding.

Female gender as a significant risk factor had been reported in several studies (Simking et al., 2010; Maia et al., 2014; Akram et al., 2019). However, there is no clear explanation for this finding, but it may be due to the longer lifespan of female cats or the higher likelihood of tick infestation in the female than male ones (Simking et al., 2010).

2.7.3.2 Housing condition and lifestyle

Access to outdoor or environmental condition is not a risk factor (Simking et al., 2010; Vilhena et al., 2013). Interestingly, client-owned cats tend to be burden with the pathogens than the stray cats in one study (Maia et al., 2014).

2.7.3.3 Health status and prevention

The clinical findings of infected cats may be similar to non-infected cats in the aspect of health condition, body temperature, mucous membrane, or vomiting (Akram et al., 2019). Prevention of ectoparasite especially tick is a protective factor; however, the specific vectors for transmission of this disease among cats are still unknown (Maia et al., 2014). Other prevention including vaccination and deworming does not prevent the transmission of feline babesiosis (Akram et al., 2019).

2.7.3.4 Co-infection

There is no association between PCR positivity and ectoparasitic infestation (Simking et al., 2010; Akram et al., 2019; Do et al., 2021a). In addition, cats with FeLV or FIV infection are not in a greater risk to be infected with feline babesiosis (Vilhena et al., 2013; Maia et al., 2014).

2.7.4 Modes of transmission

2.7.4.1 Blood transfusion

Even though transmission via blood transmission has not been experimented in cats, yet there are studies demonstrating transmission of *Babesia* via blood transfusion in dogs and humans (Perdrizet et al., 2000; Stegeman et al., 2003; Van Ngo, 2009). Therefore, blood transfusion is likely to be the plausible route of transmission among cats.

2.7.4.2 Vector-borne infection

Ticks such as *Rhipicephalus* and *Dermacentor* have been proved to be the major vectors in dogs; however, the studies about transmission via ticks in cats are still limited (Bashir et al., 2009). Other tick species which *B. microti* has been identified are *Ixodes ricinus* and *Hyalomma marginatum* (Katargina et al., 2011; Usluca et al., 2019).

Ixodes ricinus ticks obtained from cats were reported to be PCR-positive for *Babesia* spp. in Belgium (Lempereur et al., 2011). In addition, DNA of *Babesia* were detected in *I. ricinus* ticks collected from cats in Poland (Król et al., 2016). Similarly, soft ticks collected from cats in the United Kingdoms were found to harbor *Babesia* in which DNA of *Babesia venatorum* were detected in *I. ricinus* ticks, and DNA of *B. microti*-like species were detected in *I. hexagonus* ticks (Davies et al., 2017). Recent studies reported detection of *Babesia conradae* in *Dermacentor albipictus* ticks from United States and *B. canis* in *Dermacentor reticulatus* ticks from Hungary in which all ticks were collected from cats (Geurden et al., 2018; Duncan et al., 2021). In contrast, other studies did not detect DNA of *Babesia* in ticks collected from cats which tick species included *R. sanguineous* and *Haemaphysalis longicornis* (Iwakami et al., 2014;

Nguyen et al., 2020b). There is no study illustrating transmission of *Babesia* among cats via tick vectors.

2.7.4.3 Other routes of transmission

Transplacental transmission of *Babesia* may be possible in cats. Experimental transmission of *B. gibsoni* via transplacental route in dogs was successful (Fukumoto et al., 2005). This route of transmission has also been demonstrated in other *Babesia* species and hosts such as *B. microti* in mice, and *B. equi* in mares (Chhabra et al., 2012; Sudan et al., 2015; Tufts and Diuk-Wasser, 2018).

2.7.5 Clinical signs

Cats infected with *Babesia* were reported to be present in either healthy, fair, or poor condition which might indicate the different states of the infection (Simking et al., 2010). Cats with non-specific clinical signs were less likely to be infected with *Babesia* (Vilhena et al., 2013; Maia et al., 2014). In contrast, numerous cats with babesiosis were present with clinical signs (Schoeman et al., 2001). Due to various species of *Babesia*, the pathogenicity of the disease varies among cats infected with each species. Reported clinical signs ranged from non-specific signs such as weakness, anorexia, fever to severe life-threatening clinical conditions such as neurological signs, shock or death (Bosman et al., 2013). The following discussion will be about the clinical signs in cats with *Babesia* infection which are present in Thailand including *B. vogeli*, *B. canis canis*, and *B. gibsoni*.

Cats infected with *B. vogeli* were reported to have mild clinical signs including non-specific signs such as fever (Vilhena et al., 2017; Zhang et al., 2019). In addition, numerous cats infected with this species were found to be asymptomatic (Simking et al., 2010; Kelly et al., 2017; Vilhena et al., 2017).

B. gibsoni-infected cats were also found to be apparently healthy (Kelly et al., 2017; Yin et al., 2022). However, there was a fatal case in which a cat with *B. gibsoni* infection was suspected to have immune-mediated anemia and thrombocytopenia, and eventually died from fatal hemorrhage (Almendros et al., 2023).

B. canis canis had been reported to cause fatality in a cat (Remesar et al., 2022). This feline case showed non-specific signs and icterus; moreover, the hematological abnormalities including hemolytic regenerative anemia, thrombocytopenia, and leukocytosis.

2.7.6 Laboratory findings

Anemia was the most reported hematological abnormalities from cats with babesiosis (Schoeman et al., 2001; Baneth et al., 2004; Wells, 2012; Bosman et al., 2019). Most of the anemia was regenerative with some cases present with immune-mediated hemolytic anemia. In addition, thrombocytopenia was also found in multiple cats with babesiosis (Schoeman et al., 2001; Baneth et al., 2004; Wells, 2012).

2.7.7 Diagnosis

Babesiosis can be diagnosed by various methods. Cytology can be used to identify the pathogen from blood smear examination; however, the sensitivity is lower than PCR technique. Moreover, species identification might not be obtained solely based on cytology. False positive might be possible due to other artifacts including stain precipitates, inclusion bodies, and feline hemotropic mycoplasmas.

PCR technique has been more frequently used recently. PCR can distinguish *Babesia* spp. based on multiple levels which may be piroplasm-specific, genus-specific, or species-specific. Due to various species of *Babesia*, piroplasm-specific or genus-specific are more suitable for being used as a screening test for *Babesia*.

Further, species-specific PCR or DNA sequencing will be used to identify the species of *Babesia*.

2.8 Feline cytauxzoonosis

2.8.1 Introduction

Feline cytauxzoonosis is one of the well-documented feline infectious diseases. *Cytauxzoon* belongs to the Order Piroplasmida which includes *Babesia* and *Theileria*. It is strongly recommended to test for the infection in feline blood donor especially those who can access to the outdoor in the endemic area (Wardrop et al., 2016).

2.8.2 Prevalence

There are 2 common species of *Cytauxzoon* in cats including *Cytauxzoon felis* and *Cytauxzoon manul*. The most common species is *C. felis*. Meanwhile, *C. maul* has been sporadically reported mainly in Europe (Alho et al., 2016; Legroux et al., 2017).

The detection of *Cytauxzoon* in cats has been frequently reported in North America, and sporadically in Asia, Europe, and South America. The prevalence of feline cytauxzoonosis varies from location to location as well as from the different sample population. In the United States, *Cytauxzoon* can be detected up to 30.3% in asymptomatic cats (Haber et al., 2007; Brown et al., 2010; Levy et al., 2011; Rizzi et al., 2015; Nagamori et al., 2016; Jacobs, 2018; Wikander et al., 2020; Chan et al., 2021). The pathogen can be detected up to 0.8% in France (Criado-Fornelio et al., 2009), 2.5% in Italy (Ebani et al., 2020), 1.2% in Spain (Díaz-Regañón et al., 2017), and 6.6% in Turkey (Muz et al., 2021). The prevalence of feline cytauxzoonosis is reported up to 3.3% in Brazil (André et al., 2015; Braga et al., 2016; Pedrassani et al., 2019).

In Asia continent, the study from China identified the pathogen as high as 21.5% among cats (Zou et al., 2019). Additionally, one stray cat was detected by cytology in India, and more recently, another Maine Coon cat with the clinical signs of acute dyspnea and lethargy from Korea was diagnosed with feline cytauxzoonosis by cytology and real-time PCR (Varshney et al., 2009; Choi et al., 2020). In Thailand, the first study identified *Cytauxzoon* in cats by microscopic examination was published in 1993; nonetheless, there was no confirmation by PCR technique in this study (Jittapalapong and Jansawan, 1993). Afterwards, no case of feline cytauxzoonosis has been reported in Thailand.

The difference in prevalence rate may be further explained by different clinical presentation between asymptomatic carriers and fatal cases. Due to the reports of the severe clinical manifestations in some cats, these cats were not included in the prevalence studies because of death. Most of the cross-sectional studies detected asymptomatic carriers of the disease. In contrast, deceased cats had been reported in interviews of veterinarians, the retrospective studies, or the necropsy reports. Therefore, the prevalence reported from each study is difficult to compare.

2.8.3 Risk factors

Due to the low number of infected cats, the difference in clinical manifestations, and the absence of detailed information about lifestyle and medical history, risk factor analysis in the disease is limited. Moreover, most of the studies sampled cats from one or few areas, some risk factors regarding the geographical distribution may be significant.

Signalment including age, breed, or sex is not considered as a significant risk factor in any studies (Díaz-Regañón et al., 2017; Zou et al., 2019; Moghaddam et al., 2020; Muz et al., 2021).

Stray cats have a significantly greater risk of infection more than client-owned cats in one study (Zou et al., 2019). This may be due to the poor sanitation and higher chance of contact with ticks. The presence of specific tick vectors or wild animals in the area may be the risk of infection to domestic cats (Spada et al., 2014c). Further studies or systematic reviews may disclose these factors.

Medical history such as ectoparasitic prevention or health condition of cats is also not a significant risk factor. Co-infection with FIV has been reported as significant finding in infected cats (Díaz-Regañón et al., 2017).

Anemia is considered as a significant risk factor in one study (Moghaddam et al., 2020). A study in Iran found hypoproteinemia and hyperglobulinemia as significant risk factors (Moghaddam et al., 2020).

2.8.4 Modes of transmission

2.8.4.1 Blood transfusion

Blood transmission had been proved to be the route of transmission in the experimental study which cats became PCR-positive for *C. manul* or *C. felis* after being transfused with infected blood from Pallas' cats (Joyner et al., 2007). Additionally, there was a report which *Cytauxzoon* was detected after blood transfusion from a domestic cat to another; however, there was no testing for *Cytauxzoon* before the transfusion (Nentwig et al., 2018).

2.8.4.2 Vector-borne transmission

Tick species have been proved to be the route of transmission of feline cytauxzoonosis. Most of the experimental studies revealed that *Amblyomma americanum* is a major vector of the disease (Reichard et al., 2009; Reichard et al., 2013; Thomas et al., 2018; Allen et al., 2019; Reichard et al., 2019). Additionally, successful transmission by *Dermacentor variabilis* was reported in previous studies (Blouin et al., 1984; Kocan et al., 1992). DNAs of *C. felis* were amplified in *D. variabilis* tick (Shock et al., 2014). However, recent study could not identify the transmission of *Cytauxzoon* by *D. variabilis* tick (Allen et al., 2019). It has been proved that the pathogens can be transferred via transstadial transmission by *A. americanum* tick species (Allen et al., 2019).

However in some countries with high prevalence of feline cytauxzoonosis such as China and Italy, there is no report of existence of the previously described tick species which this may indicate that there might be other potential tick vectors or even other route of transmission among cats (Zou et al., 2019). To the author's knowledge, there is no experimental study demonstrating the infection of feline cytauxzoonosis transferring via other tick species besides *A. Americanum* and *D. variabilis*.

2.8.4.3 Other routes of transmission

There is a study investigating transplacental transmission of feline cytauxzoonosis. A dead pregnant cat with confirmed *Cytauxzoon* parasitemia was not detected for pathogens in muscle, bone, or placenta (Weisman et al., 2007). Another study found that kittens born from chronically *C. felis*-infected cats were PCR negative until 3 weeks of age when there was PCR positivity in *C. felis* (Lewis et al.,

2012). Therefore, transplacental or perinatal transmission is inconclusive and requires further investigation.

2.8.5 Clinical signs

Due to cross-sectional study, most prevalence studies of feline cytauxzoonosis identified numerous apparently healthy infected cats. This might be due to the less severity of the disease which most of them did not suffer the disease in life-threatening conditions. Eventually, these cats recovered but were unable to clear the infection, leading to become carriers of the infection (Haber et al., 2007; Jacobs, 2018). However, there were case reports describing deadly conditioned cats (Alho et al., 2016; Legroux et al., 2017). Interview from clinicians in endemic areas revealed fatal condition of the disease in cats as well (Rizzi et al., 2015).

In deceased cases, there were multiple clinical presentations of infected cats. A cat was present with anorexia, lethargy, fever, and respiratory distress which finally died (Jackson and Fisher, 2006). Another cat which was pregnant was found to abort, then present with seizure and die the next day after abortion (Weisman et al., 2007). Alho et al. (2016) reported fatal case of feline cytauxzoonosis with *C. manul* which this cat was diagnosed with pleural and abdominal effusion. Last example of fatal cytauxzoonosis was a cat from France that clinical presentations before death included prolonged fever, abdominal pain, and hemorrhagic diarrhea (Legroux et al., 2017).

As stated above, most of the prevalence studies identified asymptomatic carriers rather than cats with severe clinical presentation. Multiple studies reported none of the cats was present with clinical sign, as well as another study reported 84% of cats with feline cytauxzoonosis were asymptomatic (Haber et al., 2007; Karaca et al., 2007; Brown et al., 2008; Brown et al., 2010; Jacobs, 2018; Moghaddam et al., 2020). Additionally, some of the clinical signs might be due to other infections or diseases which had not been diagnosed concurrently; for instance, diarrhea in a

feline case might be caused by toxocariasis rather than cytauxzoonosis (Carli et al., 2014). Therefore, cats with co-infection or concurrent disease might find it difficult to clarify the exact clinical signs of feline cytauxzoonosis.

Cytauxzoon-infected cats might be present with non-specific clinical signs including fever, dehydration, and lethargy (Meinkoth et al., 2000; Díaz-Regañón et al., 2017; Nentwig et al., 2018; Moghaddam et al., 2020). These cases were usually non-fatal and eventually became chronic carriers of feline cytauxzoonosis. However, pathological lesions in feline cytauxzoonosis have been reported regarding multiple systems e.g., neurological, pulmonary, and ocular systems.

Fatal *C. felis*-infected cases were found to have pathological changes in central nervous tissues; however, only one cat showed signs related to neurological abnormalities (Clarke and Rissi, 2015). Other necropsy reports revealed pulmonary lesions in cats with *C. felis* infection such as interstitial pneumonia, neutrophilic infiltrates, and vascular occlusion (Snider et al., 2010; Frontera-Acevedo and Sakamoto, 2015). Additionally, ocular lesions caused by *C. felis* infection in fatal feline cases included schizonts-laden macrophages distributed within blood vessels of the uveal tracts (Meekins and Cino-Ozuna, 2018)

Cats with *C. manul* infection were reported to show more severe clinical presentations than cats infected with *C. felis*. As described above, one cat showed signs of prolonged fever, abdominal pain, and hemorrhagic diarrhea; meanwhile, another cat had pleural effusion, splenomegaly, abdominal effusion (Alho et al., 2016; Legroux et al., 2017). Both cases eventually died. Notably, further diagnosis for other diseases had not been performed in both case studies, so concurrent diseases could not be ruled out. In conclusion, due to low cases with definitive *C. manul* infection compared to *C. felis*, it cannot be stated that *C. manul* has more severity.

2.8.6 Laboratory findings

Anemia is expected to be due to hemolysis from intraerythrocytic schizont which these infected red blood cells eventually rupture. Anemia can be either non-regenerative or regenerative. Non-regenerative anemia may be from an early course of disease or infection of erythrocytic progenitor cells. Regenerative anemia may be from the response to normal hemolysis or even immune-mediated hemolysis. Several studies reported most of the cats in their studies to be anemic (MacNeill et al., 2015; Nentwig et al., 2018). On the other hand, some studies found few or none of the cats were anemic (Karaca et al., 2007; Díaz-Regañón et al., 2017).

Immune-mediated hemolytic anemia (IMHA) is also reported in 2 cases; one is the Maine Coon cat in Korean, and another one is pregnant cat in the United States (Weisman et al., 2007; Choi et al., 2020). However, the first cat was also diagnosed with FIV, and another had not been tested for retroviral infection; thus, other possible causes of IMHA cannot be ruled out, and diagnosis of IMHA caused by *C*ytauxzoon cannot be clarified.

Leukocytosis which may expected to be the common response to any infection have been reported in numerous cats with feline cytauxzoonosis (Carli et al., 2014; Rassouli et al., 2015; Alho et al., 2016; Díaz-Regañón et al., 2017; Moghaddam et al., 2020). Elevated white blood cell count included neutrophilia, monocytosis, and lymphocytosis (Carli et al., 2014; Rassouli et al., 2015; Legroux et al., 2017; Choi et al., 2020). Erythrophagocytic macrophages was identified in feline case (Weisman et al., 2007). In contrast, a study reported numerous *C. felis*-infected cats with leukopenia (MacNeill et al., 2015).

Few cases of feline cytauxzoonosis were present with thrombocytopenia (Weisman et al., 2007; MacNeill et al., 2015; Alho et al., 2016; Moghaddam et al., 2020). There might be other co-morbidities which caused thrombocytopenia in these cases.

One of the most reported biochemical test changes was hypoalbuminemia (Choi et al., 2020; Moghaddam et al., 2020; Panait et al., 2020). A study in Iran reported hypoproteinemia and hyperglobulinemia as significant risk factors for feline cytauxzoonosis (Moghaddam et al., 2020). This finding might be due to (1) dehydration (2) increased production of acute phase protein which responds to the infection.

2.8.7 Diagnosis

Cytology can be used for diagnosis. The presence of piroplasm-infected erythrocytes is more common than the presence of schizont-laden macrophages from peripheral blood smear (Sleznikow et al., 2022). Inclusion bodies in erythrocytes should be carefully interpreted due to similar findings such as *Babesia*, hemotropic mycoplasma, or other erythrocytic inclusion bodies; nevertheless, this might lead to misdiagnosis. Therefore, examination of pathogens in both erythrocytes and macrophages would increase the accuracy of cytology.

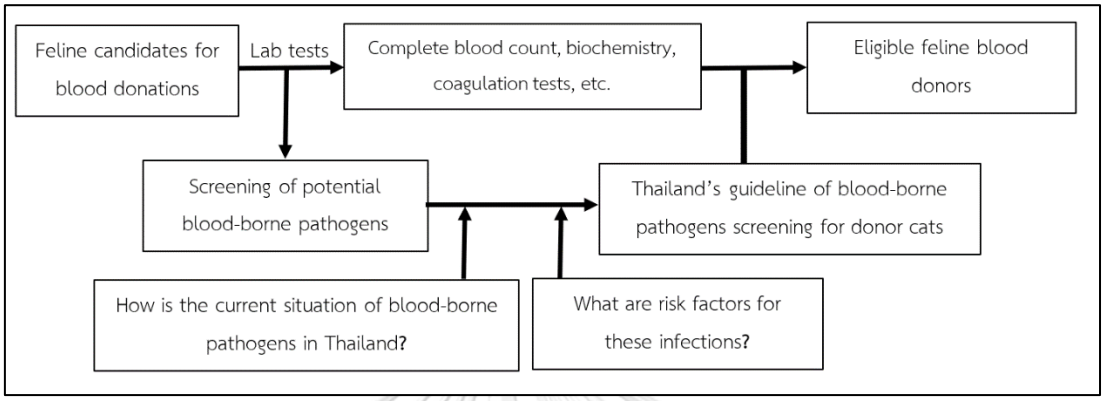
For PCR, primers can be designed for amplification of piroplasmids which include *Babesia*, *Cytauxzoon*, and *Theileria*. More species-specific PCR to amplify *Cytauxzoon* has been used as well. The primers targeting *18s* rRNA are more widely used than *ITS2* primers (Birkenheuer et al., 2006; Haber et al., 2007; Carli et al., 2012; Nagamori et al., 2016; Mylonakis et al., 2018).

Similar to other blood-borne pathogens, detection by cytology shows lower sensitivity and specificity compared to PCR technique (Birkenheuer et al., 2006). A study in Iran showed that 5% of population positive by cytology while 19% positive by PCR. Additionally, only one case was positive by both techniques while other positive cats by cytology showed negative results by PCR (Moghaddam et al., 2020).

CHAPTER III MATERIALS AND METHODS

3.1 Conceptual framework

Figure 1 Flow chart of conceptual framework



3.2 Animals

The animal study was approved by the Institutional Animal Care and Use Committee of Faculty of Veterinary Science, Chulalongkorn University (protocol number 2031069). A total of 298 cats were included in the study (GROUP A). Client-owned cats without any age, sex, or breed predilection were recruited from the Small Animal Teaching Hospital, Chulalongkorn University in addition to 7 private animal clinics and hospitals located in Bangkok and vicinities. Any cat which had been treated with doxycycline or fluoroquinolones within 30 days or had a transfusion with any blood product prior to the day of presentation was excluded from the study.

Consent forms were signed by the owners. Detailed history taking and physical examination were performed to all cats. Attending veterinarians completed the questionnaires including lifestyle (access to outdoor, size of household, contact to other animals), medical history (reason of visit, clinical signs on the day of presentation), medical records (physical examination, temperature), history of a

previous transfusion, and preventives (vaccination, prevention of ectoparasites). Additionally, data of requested blood tests would be further added to the form.

In addition, 112 blood samples (GROUP B) were retrieved from archived samples from a previous study of feline blood typing (Sangkaew et al., 2021). Only information of signalment and health history was available for the analysis. Feline blood types and packed red cell volumes were available to all archived samples.

3.3 Sample collection and blood tests

The biosafety protocol was approved by the Institutional Biosafety Committee of Faculty of Veterinary Science, Chulalongkorn University (protocol number 2031045). Feline blood samples were collected via venipuncture from a peripheral or central vein and placed in EDTA and heparin tubes on the day of presentation. Heparinized blood was submitted for biochemical tests requested by attending veterinarians. EDTA-anticoagulated blood samples were aliquoted into 2 portions for complete blood count and PCR assays. The volume of blood for PCR tests was at least 100 μL. Blood samples were immediately frozen at -20 °C until further use.

3.4 DNA extraction

Sets of laboratory equipment were provided separately for each procedure including DNA extraction, PCR assay, and gel electrophoresis. Equipment and working areas were disinfected with 70% ethanol before and after usage. Whole blood samples were thawed at room temperature. Total nucleic acid extraction was performed using a commercially available kit (DNeasy® Blood & Tissue Kits, Qiagen, Hilden), according to manufacturer's instructions. A final eluted volume was 200 μL. The extracted DNA were then frozen at -20 °C until further process.

3.5 Conventional PCR and gel electrophoresis

Conditions of individual conventional PCR assays have been shown in table 1, following the protocol described in previous studies. Primer pairs for hemoplasma, *Bartonella*, and Anaplasmataceae were designed in previous studies which confirmed to be able to detect these pathogens in cats (Jensen et al., 2000; Parola et al., 2000; Jensen et al., 2001; Attipa et al., 2017). On the other hand, the primers for piroplasm in our study were novel primers primarily designed to detect piroplasm in buffaloes (Nguyen et al., 2020a). We had tested that these primers could be used to amplify the DNA of *Babesia* in canine blood samples. Since we could not obtain the sample for *Cytauxzoon* spp. from any host species in Thailand, the possible sequences were aligned using statistical software (BioEdit Sequence Alignment Editor version 7.2.5) to assess the compatibility to the novel primers. We found that the primers were compatible to DNA of feline *Cytauxzoon*, and the expected product size was approximately 1,230 base pairs which around the size of the product of *Babesia*. Positive controls for these PCR assays were DNA products of '*C.* M. haemominutum' and *B. henselae* from feline blood samples, as well as *A. platys* and *B. vogeli* from canine blood samples which were confirmed by DNA sequencing. Sterile distilled water was served as negative control. PCR mixture preparation was performed in a specific hood which was treated with ultraviolet light before and after use. The PCR reactions were run in a PCR thermocycler (SureCycler 8800, Agilent Technologies).

Table 1 Primer sets for PCR amplification and PCR conditions

Pathogen	Gene	PCR primers	PCR condition	Product size (bp)	Reference
Feline hemotropic mycoplasma	*16S* rRNA	F: ACGAAAGTCTG ATGGAGCAATA R: ACGCCCAATA AATCCGRATAAT	95°C 2 min, 45 cycles (95°C 1 min, 60°C 1 min, 72°C 30 sec), 72°C 5 min	170, 193	(Jensen et al., 2001)

Table 1 Primer sets for PCR amplification and PCR conditions (continued)

Pathogen	Gene	PCR primers	PCR condition	Product size (bp)	Reference
Bartonella	*16S-23S ITS* rRNA	F: (C/T)CTTCGTT TCTCTTTCTTCA R: AACCAACTGA GCTACAAGCC	95°C 2 min, 45 cycles (95°C 1 min, 60°C 1 min, 72°C 30 sec), 72°C 5 min	172-260	(Jensen et al., 2000)
Anaplasmataceae	*16S* rRNA	F: GGTACCYAC AGAAGAAGTCC R: TAGCACTCA TCGTTTACAGC	95°C 5 min, 35 cycles (94°C 30 sec, 55°C 30 sec, 72°C 90 sec), 72°C 5 min	345	(Parola et al., 2000)
Piroplasmida	*18S* rRNA	F: GCAAATTACCCA ATCCTGACACAGG R: CCGAATAATT CACCGGATCACTCG	94°C 2 min, 40 cycles (98°C 10 sec, 60°C 30 sec, 68°C 90 sec), 68°C 5 min	1,221	(Nguyen et al., 2020a)

After DNA amplification, PCR products were distinguished using agarose gel electrophoresis with the gel concentration of 2-3%. Calculated agarose gel powder was mixed with 1X Tris-borate-ethylenediaminetetraacetic acid (TBE), and then stained with a DNA staining dye (RedSafe® DNA staining dye, Thermo Fisher Scientific). The mixture was microwaved to dissolve the powder, and then poured into the gel cast. Set gel was submerged under the 1X TBE buffer in the electrophoresis device. PCR products were prior mixed with loading dye and applied into the wells. Specific ladders were used based on the expected size of amplicons. The condition of gel electrophoresis was set to the voltage of 220 volts for 50-60 minutes. Finally, gels were visualized under ultraviolet light. The size of amplicons was compared with a specific size of DNA ladder.

3.6 PCR product purification and sequencing

The positive PCR products were extracted by DNA extraction kit (NucleoSpin® Gel and PCR Clean-up, Macherey-Nagel), following the manufacturer's instruction. The extracted DNA products were submitted to a commercial laboratory to perform Sanger's sequencing (Macrogen, South Korea).

3.7 DNA sequence analysis

The nucleotide sequences were obtained from a commercial laboratory. The sequences were manually aligned using commercial program (BioEdit Sequence Alignment Editor version 7.2.5). Then, the DNA sequences were compared with available nucleotide sequences provided by the NCBI GenBank™ database using nucleotide Basic Local Alignment Search Tool (nBLAST). Most possible species were reviewed from percentage of identity, coverage, and e-value.

3.8 Statistical analysis

The data were arranged in Microsoft Excel format (Microsoft Excel 2019). Statistical analysis was performed using statistical program SPSS for Windows (version 22, SPSS Inc., Chicago, IL, USA). The prevalence of each species was reported in percentage. Epidemiological variables obtained from questionnaires, physical examination and blood parameters were reported for each pathogen and statistically analyzed to identify the risk factors. Initially, the variables were tested for multi-collinearity. Categorical variables were tested using Pearson's chi-square test followed by Phi and Crammer's V test. Continuous variables were tested using Pearson's correlation test. If the collinearity of two variables met, one which had more biological plausibility was chosen. Next, variables were tested for possible association using univariable logistic regression. Variables which had *p*-value less than 0.2 were further included in multivariable logistic regression. To minimize the effect of missing data on the analysis, only variables which had information on both blood

sample groups were used in analysis of all blood samples including age, sex, breed, hematocrit, and presence of illness. Besides these variables, the rest will be used in the analysis of GROUP A or GROUP B samples. Additionally, continuous data such as age, temperature, and blood parameters will be converted into categorical data to simplify the use in clinical practice. Any variable which had a *p*-value less than 0.05 in the multivariable logistic regression analysis was considered as a risk factor for infection. Odds ratio was obtained from exponential calculation of beta-coefficient in the analysis.

CHAPTER IV RESULT

4.1 Population distribution of GROUP A samples

A total of 298 blood samples were collected from client-owned cats attending the veterinary hospitals and clinics in Bangkok and its vicinity. By place of collection, 111 blood samples were obtained from the Small Animal Teaching Hospital, Chulalongkorn University; additionally, 187 blood samples were obtained from the other 6 veterinary hospitals and clinics elsewhere. By year of collection, 121 cats were collected in 2020, and 177 were collected in 2021.

The ages of 234 cats were known by the owners, while the other 53 cats' ages were estimated by the owners and attending veterinarians into generation. There was no information of age in 11 cats. The range of ages of the population was between 1 month and 20 years. According to the 2021 AAHA/AAFP feline life stage guideline (Quimby et al., 2021), the ages of cats in the study were classified into 55 kittens, 190 young adults, 27 mature adults, and 15 senior cats.

For the other signalment, there were 112 females, 164 males, and 22 cats with unknown sex. Breeds were known in 273 cats; most of the cats in the study were Domestic Shorthair (195 cats) and the rest of the cats (78 cats) were purebred, including Scottish Fold (22), Persian (19), Main Coon (11), British Shorthair (7), American Shorthair (5), Siamese (4), Bengal (2), Munchkin (1), Ragdoll (1), and other purebred-crossed cats (6).

Sixty-five cats had a single household lifestyle, while 177 cats were in multiple households. The additional cats in the multiple households ranged between one cat and up to 70 cats which these cats lived in the large colony such as cattery. For the cohabitation with the other animal species, 8 cats in the study

lived with dogs, ranging from 1 to 4 dogs per household. Strictly indoor lifestyle was documented in 116 cats; in contrast, 113 cats were able to access the outdoor area.

Questionnaires regarding preventives included vaccination of core vaccines in Thailand which were feline viral rhinotracheitis-calicivirus-panleukopenia (FVRCP) vaccine, and rabies vaccine. Furthermore, the owners were asked about the non-core vaccines including feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), and feline coronavirus vaccines as well as ectoparasitic preventives. For FVRCP vaccines, 15 cats were vaccinated once, 14 cats were vaccinated twice, 35 cats were vaccinated for 3 times, 94 cats were annually vaccinated, and 55 cats had never been vaccinated. Moreover, 117 cats were vaccinated against rabies, while 63 cats had never been vaccinated against rabies. For non-core vaccines, 163 cats had never been vaccinated against any non-core vaccines. Number of cats vaccinated against FeLV and FIV were 46 and 8, respectively. None of the cats had been vaccinated against feline coronavirus. Forty-two cats were regularly administered the ectoparasitic preventives; on the other hand, 71 cats had not been regularly administered the prevention, and 100 cats had never been prevented for ectoparasitic infestation. Two of the cats in the study were used to be blood recipients only, and one cat was used to be both donor and recipient.

For other infectious diseases screening, 183 cats were previously tested for FeLV and FIV using a commercially available test kit (WITNESS FELV-FIV, Zoetis, USA, or SNAP FELINE TRIPLE, Idexx, Netherlands), while 68 had never been tested. Twenty-two cats were tested positive for FeLV antigen, and 15 cats were tested positive for FIV antibody. Two cats in the study were tested positive on both retroviruses. No further testing was performed to confirm the infection of these pathogens. Ectoparasitic infestation was recorded in 246 cats. The number of cats which were infested with fleas, ticks, and ear mites were 9, 1, and 2, respectively.

Considering on reasons of veterinary visit, 58 apparently healthy cats visited for wellness check-up, 14 apparently healthy cats visited for wellness check-up in which these cats had already recovered from the illness, 36 cats visited for check-up before gonadectomy, 103 cats were recently sick within a week before visit, and 65 cats had been suffered from the diseases which prolonged more than a week. Categorizing by presence of illness, 159 cats were sick, while 117 cats appeared healthy. Moreover, 7 cats were present with wounds or abscess after biting.

Rectal temperature was recorded in most of the cats. Temperature more than 102.5 °F was considered elevated temperatures which might be from fever or hyperthermia. Twenty-one cats were recorded having elevated rectal temperature; in contrast, 219 cats had rectal temperatures within normal range.

There were available laboratory records of blood tests in 175 cats. Anemia was diagnosed in 71 cats; in contrast, 99 cats were considered non-anemic.

4.2 Population distribution of GROUP B samples

Data of GROUP B samples were retrieved in the study from Sangkaew et al. (2021). A total of 112 feline blood samples were used in the current study. Categorizing by year of sample obtaining, 4 blood samples were collected in 2018, while 108 samples were collected in 2019. By location, 42 feline bloods were collected from the Small Animal Teaching Hospital, Chulalongkorn University, and 70 samples were collected in veterinary hospitals or clinics elsewhere.

All cats in GROUP B which had data regarding age were between 1 and 8 years old. Eighty-five cats were young adults, while 11 cats were mature adults. Age was not documented in 16 cats. There were 45 females and 67 males. Domestic shorthair was the most common breed in the study (84 cats); meanwhile, the other

28 cats were other breeds, including Persian (12), Scottish Fold (6), British Shorthair (3), Siamese (2), Exotic Shorthair (1), Sphynx (1), Ragdoll (1), and crossbred cats (2).

Cats with blood type A were identified in 107 cats, followed by 5 cats of blood type B. None of the cats was present with blood type AB. Twenty-two cats were present with anemia (hematocrit 22-34%; average 30.43%); on the other hand, 89 cats were considered non-anemic (hematocrit 35-55%; average 42.28%). Total protein of all cats ranged from 7 to 10 g/dL with the average of 8.35 g/dL. Reasons for veterinary visit were present in 69 cats. Forty-eight cats were considered apparently healthy, while 21 cats were considered sick.

4.3 Prevalence of blood-borne pathogens

The distribution of prevalence of each infection in GROUP A, GROUP B, and all blood samples is shown in table 2. Due to limitations of the study, not all positive PCR samples are submitted for DNA sequencing; therefore, the diagnosis of the infection is based on the PCR technique. For hemoplasma, the PCR assay can distinguish '*C.* M. haemominutum' from the other common feline hemoplasma including *M. haemofelis* and '*C.* M. turicensis' from product size. Thus, all PCR products which are positive at 190 bp are assumed to be '*C.* M. haemominutum'. However, the 170-bp PCR products cannot distinguish *M. haemofelis* from '*C.* M. turicensis'. Likewise, PCR assay for *Bartonella* can differentiate *B. henselae* from the other species due to the difference in band size; therefore, all 172-bp size PCR products are assumed to be *Bartonella henselae*.

Table 2 Prevalence of infection, reported by species and sample group

Infection	GROUP A	GROUP B	All samples
Any infection	61/298 (20.5%)	23/112 (20.5%)	84/410 (20.5%)
Any hemoplasma species	55/298 (18.5%)	12/112 (10.7%)	67/410 (16.3%)
- '*C.* M. haemominutum'	48/298 (16.1%)	10/112 (8.9%)	58/410 (14.1%)
- Other species	7/298 (2.3%)	2/112 (1.8%)	9/410 (2.2%)

Table 2 Prevalence of infection, reported by species and sample group (continued)

Infection	GROUP A	GROUP B	All samples
Any *Bartonella* species	10/298 (3.4%)	12/112 (10.7%)	22/410 (5.4%)
- *B. henselae*	6/298 (2.0%)	10/112 (8.9%)	16/410 (3.9%)
- Other species	4/298 (1.3%)	2/112 (1.8%)	6/410 (1.5%)
Ehrlichia or *Anaplasma*	0/298 (0%)	0/112 (0%)	0/410 (0%)
Babesia or *Cytauxzoon*	0/298 (0%)	0/112 (0%)	0/410 (0%)
Co-infection between *'C.* M. haemominutum' and *B. henselae*	2/298 (0.7%)	0/112 (0%)	2/410 (0.5%)
Co-infection between *'C.* M. haemominutum' and other *Bartonella* species	2/298 (0.7%)	1/112 (0.9%)	3/410 (0.7%)

In GROUP A, positive PCR results were noted in 61 blood samples (20.5%). The number of cats which were PCR-positive for feline hemoplasmas was 55 (18.5%); out of these, 48 (16.1%) cats were infected with '*C.* M. haemominutum', while 7 (2.3%) cats were infected with other hemoplasma species including *M. haemofelis*. For *Bartonella* spp. infection, 10 (3.4%) cats were detected with any *Bartonella* species; *B. henselae* was identified in 6 (2.0%) cats, and other *Bartonella* species including *B. clarridgeiae* were identified in 4 cats (1.3%). No cats from GROUP A were detected for *Anaplasma*, *Ehrlichia*, *Babesia*, or *Cytauxzoon*. There was no co-infection of the same genus of pathogen, but the co-infection between hemoplasma and *Bartonella* was identified. There were 2 cats with *'C.* M. haemominutum' and *B. henselae* infection as well as other 2 cats with *'C.* M. haemominutum' and other *Bartonella* infection besides *B. henselae* (no species identification).

In GROUP B, the number of cats with any infection was 23 (20.5%). In contrast to GROUP A, the most common pathogen was *Bartonella* instead of hemoplasma. The prevalence of any *Bartonella* species, *B. henselae*, and other *Bartonella* infection was 12/112 (10.7%), 10/112 (8.9%), and 2/112 (1.8%), respectively. The prevalence of hemoplasmas was lower than the prevalence in the GROUP A which

there were only 12 cats (10.7%) with any hemoplasma species infection i.e., 10 (8.9%) cats with '*C.* M. haemominutum' infection and 2 (1.8%) cats for other hemoplasma species infection. There was only one cat which had the co-infection between *'C.* M. haemominutum' and *Bartonella* infection. Likewise, none of the cats were PCR-positive for *Ehrlichia*, *Anaplasma*, *Babesia*, and *Cytauxzoon*.

4.4 DNA sequences of hemoplasma and *Bartonella*

Overall, DNA sequences were performed in 30 selected positive samples which included 25 hemoplasma-positive samples and 5 *Bartonella*-positive samples. The nucleotide BLAST analysis for each sample of hemoplasma is shown in table 3. For nucleotide sequences of hemoplasma, 18 out of 20 samples which had the band size approximately 190 bp from hemoplasma PCR showed strongly positive results as *'C.* M. haemominutum' which had the identity of 97.95-100% to sequences from accession number MF281072, MN240865, and MN240867. In addition, the remaining 2 samples of 190-bp band size had slightly poor signals in DNA sequence analysis, resulting in lower e-value, but were likely to be '*C.* M. haemominutum' with the identify of 94.57-96.67% to sequences from accession number MN240864 and MN240865. Moreover, the other 5 hemoplasma-positive samples which had the band size around 170 bp were identified as *M. haemofelis* with the identity between 99.41-100% to the accession number AM748929 and MN240855 sequences.

Table 3 Comparison of nucleotide sequences of hemoplasma to accession numbers

Sample No.	Accession No.	Species	Identity (%)	Query coverage (%)	E-value
1	MF281072	'*C.* M. haemominutum'	97.95	100	$2e^{-87}$
2	MF281072	'*C.* M. haemominutum'	100	100	$4e^{-95}$
3	MF281072	'*C.* M. haemominutum'	99.49	100	$5e^{-94}$
4	MF281072	'*C.* M. haemominutum'	99.49	100	$2e^{-93}$
5	MF281072	'*C.* M. haemominutum'	99.49	100	$5e^{-94}$
6	MF281072	'*C.* M. haemominutum'	100	100	$4e^{-95}$

Table 3 Comparison of nucleotide sequences of hemoplasma to accession numbers (continued)

Sample No.	Accession No.	Species	Identity (%)	Query coverage (%)	E-value
7	MF281072	'*C.* M. haemominutum'	100	100	$4e^{-95}$
8	MN240864	'*C.* M. haemominutum'	94.57	84	$9e^{-46}$
9	MN240865	'*C.* M. haemominutum'	100	99	$1e^{-94}$
10	MN240865	'*C.* M. haemominutum'	99.48	100	$2e^{-93}$
11	MN240865	'*C.* M. haemominutum'	100	99	$1e^{-94}$
12	MN240865	'*C.* M. haemominutum'	100	99	$1e^{-94}$
13	MN240865	'*C.* M. haemominutum'	100	99	$1e^{-94}$
14	MN240865	'*C.* M. haemominutum'	99.48	99	$2e^{-92}$
15	MN240865	'*C.* M. haemominutum'	99.48	99	$9e^{-92}$
16	MN240865	'*C.* M. haemominutum'	98.45	96	$5e^{-89}$
17	MN240865	'*C.* M. haemominutum'	100	99	$1e^{-94}$
18	MN240865	'*C.* M. haemominutum'	96.67	72	$8e^{-47}$
19	MN240867	'*C.* M. haemominutum'	100	100	$1e^{-94}$
20	MN240867	'*C.* M. haemominutum'	98.97	98	$1e^{-90}$
21	AM748929	*M. haemofelis*	92.35	98	$8e^{-67}$
22	MN240855	*M. haemofelis*	100	99	$8e^{-82}$
23	MN240855	*M. haemofelis*	100	99	$3e^{-81}$
24	MN240855	*M. haemofelis*	100	99	$8e^{-82}$
25	MN240855	*M. haemofelis*	99.41	98	$5e^{-79}$

For *Bartonella* PCR, the detail of nucleotide BLAST analysis is shown in table 4. All samples which had the band size approximately 172 bp were identified as *B. henselae*. These sequences showed the identity of 99.4-100% to sequences from accession number CP072904. The one remaining *Bartonella*-positive sample with the positive PCR band approximately 157 bp was acknowledged as *B. clarridgeiae* which had the identity of 100% to sequence from accession number CP116497. No other *Bartonella* species such as *B. koehlerae* or *B. vinsonii* subsp. *Berkhoffii* were present from the DNA sequencing.

Table 4 Comparison of nucleotide sequences of *Bartonella* to accession numbers

Sample No.	Accession No.	Species	Identity (%)	Query coverage (%)	E-value
1	CP072904	*B. henselae*	99.4	100	$7e^{-77}$
2	CP072904	*B. henselae*	100	100	$2e^{-78}$
3	CP072904	*B. henselae*	100	100	$2e^{-78}$
4	CP072904	*B. henselae*	100	100	$2e^{-78}$
5	CP116497	*B. clarridgeiae*	100	99	$1e^{-68}$

4.5 Risk factor analysis for feline hemoplasma infection

Univariable logistic analysis of each factor in cats with any hemoplasma species infection identified numerous risk factors. Cats in GROUP A (table 5) had the significant risk factors including Domestic Shorthair (OR 8.163; 95% CI 2.461–27.079), male sex (OR 2.394; 95% CI 1.679–7.346), kitten (OR 0.137; 95% CI 0.032–0.583), sickness or presence of clinical signs (OR 2.308; 95% CI 1.170–4.552), age (OR 1.143; 95% CI 1.040-1.256), co-infection with FIV (OR 11.887; 95% CI 3.548–39.831), and outdoor access (OR 3.136; 95% CI 1.576–6.240). In GROUP B (table 6), being sick was a significant risk factor for any hemoplasma species infection with the OR 4.400 (95% CI 1.091–17.741). Finally, considering from all samples (table 7), the significant risk factors of feline hemoplasmosis included age (OR 1.135; 95% CI 1.037-1.243), kitten (OR 0.172; 95% CI 0.041–0.726), male sex (OR 2.822; 95% CI 1.500–5.308), Domestic Shorthair (OR 4.279; 95% CI 1.786–10.252), anemia (OR 2.306; 95% CI 1.201–4.427), and sickness (OR 2.649; 95% CI 1.449–4.842).

Table 5 Univariable analysis of any hemoplasma species infection in GROUP A

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	1.143	1.040-1.256	0.005
Generation	Kitten	2	53	0.137	0.032-0.583	0.007
	Young adult	37	153	1.322	0.685-2.551	0.405
	Mature adult	19	8	2.067	0.851-5.020	0.109
	Senior	5	10	2.394	0.782-7.326	0.126

Table 5 Univariable analysis of any hemoplasma species infection in GROUP A (continued)

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Sex	Male	42	122	3.511	1.679-7.346	0.001
	Female	10	102	Reference		
Breed	DSH	48	147	8.163	2.461-27.079	0.001
	Other breeds	3	75	Reference		
Household	Multiple	32	145	0.804	0.397-1.626	0.544
	Single	14	51	Reference		
Cohabitation with dog	Yes	2	6	1.127	0.219-5.792	0.886
	No	42	142	Reference		
Outdoor access	Outdoor access	34	79	3.136	1.576-6.240	0.001
	Indoor only	14	102	Reference		
FVRCP vaccine	At least 1	27	131	0.604	0.290-1.258	0.178
	1-2	6	23	1.111	0.421-2.932	0.832
	3 or annual	21	108	0.622	0.313-1.236	0.175
Rabies vaccine	Yes	25	92	1.630	0.709-3.749	0.250
	No	9	54	Reference		
Non-core vaccine	Yes	9	37	0.958	0.421-2.181	0.919
	No	33	130	Reference		
FeLV vaccine	Yes	9	37	0.958	0.421-2.181	0.919
	No	33	130	Reference		
FIV vaccine	Yes	2	6	1.342	0.261-6.898	0.725
	No	40	161	Reference		
Ectoparasitic prevention	Used to	22	91	0.857	0.441-1.665	0.649
	Not regular	16	55	1.031	0.497-2.142	0.934
	Regular	6	36	0.583	0.229-1.488	0.259
Temperature	Continuous	-	-	1.002	0.776-1.294	0.986
Hyperthermia or fever	Yes	4	17	1.086	0.346-3.405	0.888
	No	39	180	Reference		
FeLV infection	Yes	8	14	2.084	0.808-5.377	0.129
	No	34	124	Reference		
FIV infection	Yes	11	4	11.887	3.548-39.831	<0.001
	No	31	134	Reference		

Table 5 Univariable analysis of any hemoplasma species infection in GROUP A (continued)

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Ectoparasitic infestation	Yes	4	9	1.807	0.533-6.127	0.342
	No	46	187	Reference		
Flea infestation	Yes	4	6	2.754	0.746-10.160	0.128
	No	46	190	Reference		
Hematocrit	Continuous	-	-	0.980	0.937-1.024	0.366
Anemia	Yes	17	54	1.763	0.813-3.823	0.151
	No	15	84	Reference		
Sick	Sick	40	128	2.308	1.170-4.552	0.016
	Apparently healthy	13	96	Reference		
Non-specific signs without obvious causes among sick cats	Yes	14	31	1.905	0.875-4.146	0.104
	No	23	97	Reference		
Bite wounds or abscess among sick cats	Yes	3	4	2.735	0.584-12.814	0.202
	No	34	124	Reference		

Table 6 Univariable analysis of any hemoplasma species infection in GROUP B

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	0.996	0.716-1.384	0.979
Sex	Male	8	59	1.390	0.392-4.923	0.610
	Female	4	41	Reference		
Breed	Others	3	25	1.000	0.251-3.986	1.000
	DSH	9	75	Reference		
Blood group	B	1	4	2.182	0.224-21.297	0.502
	A	11	96	Reference		
Hematocrit	Continuous	-	-	0.942	0.860-1.033	0.204
Anemia	Anemia	5	18	3.254	0.927-11.425	0.066
	Non-anemia	7	82	Reference		
Total protein	Continuous	-	-	0.965	0.392-2.375	0.939
Sick	Sick	6	15	4.400	1.091-17.741	0.037
	Apparently healthy	4	44	Reference		

Table 7 Univariable analysis of any hemoplasma species infection in all samples

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	1.135	1.037-1.243	0.006
Generation	Kitten	2	53	0.172	0.041-0.726	0.017
	Young adult	46	229	1.245	0.663-2.340	0.495
	Mature adult	8	30	1.469	0.639-3.379	0.365
	Senior	5	10	2.786	0.918-8.458	0.071
Sex	Male	50	181	2.822	1.500-5.308	0.001
	Female	14	143	Reference		
Breed	DSH	57	222	4.279	1.786-10.252	0.001
	Others	6	100	Reference		
Hematocrit	Continuous	-	-	0.965	0.928-1.003	0.070
Anemia	Anemia	22	72	2.306	1.201-4.427	0.012
	Non-anemia	22	166	Reference		
Sick	Sick	46	143	2.649	1.449-4.842	0.002
	Apparently healthy	17	140	Reference		

Univariable logistic analysis on GROUP A (table 8) for *'C.* M. haemominutum' positivity revealed the significant risk factors including age (OR 1.169; 95% CI 1.060–1.289), kitten (OR 0.077; 95% CI 0.010–0.571), mature adult (OR 2.460; 95% CI 1.005–6.019), male sex (OR 3.334; 95% CI 1.538–7.226), Domestic Shorthair (OR 6.863; 95% CI 2.060–22.864), outdoor access (OR 2.735; OR 1.338–5.591), FeLV infection (OR 2.653; 95% CI 1.016–6.928), FIV infection (OR 10.692; 95% CI 3.378–33.847), and sickness (OR 2.051; 95% CI 1.010–4.164). Additionally, anemia (OR 4.667; CI 1.222–17.823) and presence of clinical signs (OR 4.687; 95% CI 1.004–21.884) were statistically significant risk factors in GROUP B for *'C.* M. haemominutum' infection (table 9). The overall significant risk factors from all blood samples (table 10) for this species infection were age (OR 1.161; 95% CI 1.057–1.276), kitten (OR 0.098; 95% CI 0.012–0.726), senior (OR 3.333; 95% CI 1.092–10.171), male (OR 2.534; 95% CI 1.313–

4.888), Domestic Shorthair (OR 4.410; 95% CI 1.708–11.389), anemia (OR 2.553; 95% CI 1.277–5.103), and presence of clinical signs (OR 2.461; CI 1.300–4.659).

Table 8 Univariable analysis of '*C.* M. haemominutum' infection in GROUP A

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	1.169	1.060-1.289	0.002
Generation	Kitten	1	54	0.077	0.010-0.571	0.012
	Young adult	32	158	1.201	0.607-2.375	0.599
	Mature adult	8	19	2.460	1.005-6.019	0.049
	Senior	5	10	2.817	0.916-8.666	0.071
Sex	Male	37	127	3.334	1.538-7.226	0.002
	Female	9	103	Reference		
Breed	DSH	42	153	6.863	2.060-22.864	0.002
	Other breeds	3	75	Reference		
Household	Multiple	29	148	0.865	0.412-1.818	0.703
	Single	12	53	Reference		
Cohabitation with dog	Yes	2	6	1.324	0.257-6.830	0.737
	No	37	147	Reference		
Outdoor access	Outdoor access	29	84	2.735	1.338-5.591	0.006
	Indoor only	13	103	Reference		
FVRCP vaccine	At least 1	24	134	0.642	0.296-1.391	0.261
	1-2	5	24	1.028	0.364-2.903	0.958
	3 or annual	19	110	0.681	0.331-1.401	0.296
Rabies vaccine	Yes	23	94	1.957	0.789-4.855	0.147
	No	7	56	Reference		
Non-core vaccine	Yes	9	37	1.124	0.489-2.582	0.783
	No	29	134	Reference		
FeLV vaccine	Yes	9	37	1.124	0.489-2.582	0.783
	No	29	134	Reference		
FIV vaccine	Yes	2	6	1.528	0.296-7.879	0.613
	No	36	165	Reference		
Ectoparasitic prevention	Used to	19	94	0.760	0.382-1.514	0.436
	Not regular	13	58	0.843	0.390-1.822	0.664
	Regular	6	36	0.672	0.262-1.723	0.408

Table 8 Univariable analysis of '*C.* M. haemominutum' infection in GROUP A (continued)

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Temperature	Continuous	-	-	0.867	0.668-1.126	0.286
Hyperthermia or fever	Yes	1	20	0.246	0.032-1.890	0.178
	No	37	182	Reference		
FeLV infection	Yes	8	14	2.653	1.016-6.928	0.046
	No	28	130	Reference		
FIV infection	Yes	10	5	10.692	3.378-33.847	<0.001
	No	26	139	Reference		
Ectoparasitic infestation	Yes	3	10	1.405	0.370-5.331	0.617
	No	41	192	Reference		
Flea infestation	Yes	3	7	2.038	0.506-8.215	0.317
	No	41	195	Reference		
Hematocrit	Continuous	-	-	0.981	0.936-1.028	0.413
Anemia	Yes	15	56	1.772	0.784-4.005	0.169
	No	13	86	Reference		
Sick	Sick	34	134	2.051	1.010-4.164	0.047
	Apparently healthy	12	97	Reference		
Non-specific signs without obvious causes among sick cats	Yes	10	35	1.273	0.549-2.952	0.574
	No	22	98	Reference		
Bite wounds or abscess among sick cats	Yes	3	4	3.336	0.708-15.721	0.128
	No	29	129	Reference		

Table 9 Univariable analysis of '*C.* M. haemominutum' infection in GROUP B

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	0.984	0.678-1.428	0.932
Sex	Male	6	61	1.008	0.268-3.795	0.990
	Female	4	41	Reference		
Breed	Others	2	26	1.368	0.273-6.861	0.703
	DSH	8	76	Reference		
Blood group	B	1	4	2.722	0.274-27.021	0.392
	A	9	98	Reference		

Table 9 Univariable analysis of '*C.* M. haemominutum' infection in GROUP B (continued)

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Hematocrit	Continuous	-	-	0.922	0.834-1.019	0.113
Anemia	Anemia	5	18	4.667	1.222-17.823	0.024
	Non-anemia	5	84	Reference		
Total protein	Continuous	-	-	1.057	0.403-2.772	0.910
Sick	Sick	5	16	4.687	1.004-21.884	0.049
	Apparently healthy	3	45	Reference		

Table 10 Univariable analysis of '*C.* M. haemominutum' infection in all samples

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	1.161	1.057-1.276	0.002
Generation	Kitten	1	54	0.098	0.013-0.726	0.023
	Young adult	39	236	1.110	0.576-2.138	0.756
	Mature adult	8	30	1.778	0.767-4.120	0.180
	Senior	5	10	3.333	1.092-10.171	0.034
Sex	Male	43	188	2.534	1.313-4.888	0.006
	Female	13	144	Reference		
Breed	DSH	50	229	4.410	1.708-11.389	0.002
	Others	5	101	Reference		
Hematocrit	Continuous	-	-	0.962	0.923-1.002	0.062
Anemia	Anemia	20	74	2.553	1.277-5.103	0.008
	Non-anemia	18	170	Reference		
Sick	Sick	39	150	2.461	1.300-4.659	0.006
	Apparently healthy	15	142	Reference		

For multivariable logistic regression analysis of any hemoplasma infection in GROUP A (table 11), the risk factors which remained significance were FIV infection (OR 8.824; 95% CI 2.336–33.324), male (OR 4.534; 95% CI 1.776–11.573), and Domestic Shorthair (OR 8.886; 95% CI 1.991–39.658). None of the factors was significant from GROUP B. Overall, the significant risk factors for any hemoplasma species infection of all samples (table 12) were Domestic Shorthair (OR 4.344; 95% CI

1.784–10.574), male (OR 2.856; 95% CI 1.486–5.489), and kitten (OR 0.203; 95% CI 0.047–0.874).

Table 11 Multivariable analysis of any hemoplasma species infection in GROUP A

Variables	Odds ratio	95% CI	*p-value*
FIV infection	8.824	2.336-33.324	0.001
Male	4.534	1.776-11.573	0.002
DSH	8.886	1.991-39.658	0.004

Table 12 Multivariable analysis of any hemoplasma species infection in all samples

Variables	Odds ratio	95% CI	*p-value*
DSH	4.344	1.784-10.574	0.001
Male	2.856	1.486-5.489	0.002
Kitten	0.203	0.047-0.874	0.032

Multivariable logistic regression analysis showed that cats from GROUP A (table 13) had significantly greater risk to be infected with '*C.* M. haemominutum' when those cats were infected with FIV (OR 11.476; 95% CI 3.002–43.874), being male (OR 4.367; 95% CI 1.601–11.912), or Domestic Shorthair (OR 6.738; 95% CI 1.499–30.297). For GROUP B (table 14), sick cats (OR 4.687; 95% CI 1.004–21.884) had significantly high risk to be detected with '*C.* M. haemominutum'. In summary, the significant risk factors to be infected with '*C.* M. haemominutum' from all cats in the study (table 15) were male sex (OR 2.597; 95% CI 1.275–5.291), Domestic Shorthair (OR 3.721; 95% CI 1.396–9.915), sickness (OR 2.217; 95% CI 1.105–4.445), and kitten (OR 0.109; 95% CI 0.01–0.8200). In conclusion, summary of risk factors from either univariable or multivariable of each sample group is shown in table 16 and 17.

Table 13 Multivariable analysis of '*C.* M. haemominutum' infection in GROUP A

Variables	Odds ratio	95% CI	*p-value*
FIV infection	11.476	3.002-43.874	<0.001
Male	4.367	1.601-11.912	0.004
DSH	6.738	1.499-30.297	0.013

Table 14 Multivariable analysis of '*C.* M. haemominutum' infection in GROUP B

Variables	Odds ratio	95% CI	*p-value*
Sick	4.687	1.004-21.884	0.049

Table 15 Multivariable analysis of '*C.* M. haemominutum' infection in all samples

Variables	Odds ratio	95% CI	*p-value*
Male	2.597	1.275-5.291	0.009
DSH	3.721	1.396-9.915	0.009
Sick	2.217	1.105-4.445	0.025
Kitten	0.109	0.014-0.820	0.031

Table 16 Summary of significant risk factors of feline hemoplasma infection from univariable logistic analysis

Any hemoplasma species	GROUP A	DSH (8.163), male (2.394), kitten (0.137), sick (2.308), age (1.143), FIV (11.887), outdoor (3.136)
	GROUP B	Sick (4.400)
	All samples	DSH (4.279), male (2.822), kitten (0.172), sick (2.649), age (1.135), anemia (2.306)
'*C.* M. haemominutum'	GROUP A	DSH (6.863), male (3.334), kitten (0.012), mature adult (2.460), age (1.169), outdoor (2.735), FeLV (2.653), FIV (10.692), sick (2.051)
	GROUP B	Sick (4.687), anemia (4.667)
	All samples	DSH (4.410), male (2.534), kitten (0.098), senior (3.333), age (1.161), anemia (2.553), sick (2.461)

Table 17 Summary of significant risk factors of feline hemoplasma infection from multivariable logistic analysis

Any hemoplasma species	GROUP A	FIV (8.824), male (4.534), DSH (8.886)
	GROUP B	-
	All samples	DSH (4.344), male (2.856), kitten (0.203)
'*C.* M. haemominutum'	GROUP A	FIV (11.476), male (4.367), DSH (6.738)
	GROUP B	Sick (4.687)
	All samples	Male (2.597), DSH (3.721), sick (2.217), kitten (0.109)

4.6 Risk factors analysis for feline *Bartonella* infection

All the data of the risk factor analysis by univariable logistic analysis in any *Bartonella* species and *B. henselae* infection are shown in table 18–23. None of the factors was considered as the significant risk factor from the analysis in any blood sample group or the species of the *Bartonella*.

Table 18 Univariable analysis of any *Bartonella* infection in GROUP A

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	0.871	0.662-1.146	0.323
Generation	Kitten	1	54	0.459	0.057-3.700	0.464
	Young adult	8	182	2.088	0.435-10.028	0.358
	Mature adult	1	26	1.073	0.131-8.804	0.948
Sex	Male	7	157	1.620	0.410-6.403	0.491
	Female	3	109	Reference		
Breed	DSH	7	188	0.931	0.234-3.698	0.919
	Other breeds	3	75	Reference		
Household	Multiple	6	171	0.725	0.176-2.988	0.656
	Single	3	62	Reference		
Outdoor access	Outdoor access	5	108	1.296	0.339-4.956	0.704
	Indoor only	4	112	Reference		
FVRCP vaccine	At least 1	6	152	0.684	0.165-2.834	0.601
	1-2	3	26	3.423	0.806-14.530	0.095
	3 or annual	3	126	0.310	0.075-1.273	0.104
Rabies vaccine	Yes	6	111	1.649	0.323-8.419	0.548
	No	2	61	Reference		
Non-core vaccine	Yes	3	43	2.205	0.507-9.594	0.292
	No	5	158	Reference		
FeLV vaccine	Yes	3	43	2.205	0.507-9.594	0.292
	No	5	158	Reference		
Ectoparasitic prevention	Used to	6	107	1.813	0.441-7.448	0.409
	Not regular	5	66	2.449	0.566-10.602	0.231
	Regular	1	41	0.497	0.060-4.086	0.515
Temperature	Continuous	-	-	0.864	0.534-1.400	0.553

Table 18 Univariable analysis of any *Bartonella* infection in GROUP A (continued)

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
FeLV infection	Yes	3	19	4.000	0.924-17.322	0.064
	No	6	152	Reference		
FIV infection	Yes	1	14	1.402	0.163-12.029	0.758
	No	8	157	Reference		
Hematocrit	Continuous	-	-	0.986	0.898-1.083	0.774
Anemia	Yes	2	69	0.688	0.123-3.865	0.671
	No	4	95	Reference		
Sick	Sick	5	163	0.638	0.180-2.258	0.486
	Apparently healthy	5	104	Reference		
Non-specific signs without obvious causes among sick cats	Yes	3	42	4.214	0.680-26.102	0.122
	No	2	118	Reference		

Table 19 Univariable analysis of any *Bartonella* infection in GROUP B

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	1.121	0.843-1.490	0.433
Sex	Male	6	61	0.639	0.192-2.124	0.465
	Female	6	39	Reference		
Hematocrit	Continuous	-	-	1.006	0.917-1.104	0.894
Anemia	Anemia	2	21	0.752	0.153-3.699	0.726
	Non-anemia	10	79	Reference		
Total protein	Continuous	-	-	0.725	0.283-1.859	0.503
Sick	Sick	2	19	0.737	0.136-3.992	0.723
	Apparently healthy	6	42	Reference		

Table 20 Univariable analysis of any *Bartonella* infection in all samples

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	0.972	0.826-1.143	0.729
Generation	Kitten	1	54	0.285	0.037-2.169	0.226
	Young adult	17	258	1.713	0.563-5.213	0.343
	Mature adult	3	35	1.557	0.437-5.550	0.495

Table 20 Univariable analysis of any *Bartonella* infection in all samples (continued)

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Sex	Male	13	218	0.965	0.409-2.353	0.965
	Female	9	148	Reference		
Breed	DSH	16	263	0.986	0.375-2.592	0.978
	Others	6	100	Reference		
Hematocrit	Continuous	-	-	1.017	0.955-1.082	0.600
Anemia	Anemia	4	90	0.552	0.177-1.727	0.308
	Non-anemia	14	174	Reference		
Sick	Sick	7	182	0.510	0.193-1.350	0.175
	Apparently healthy	11	146	Reference		

Table 21 Univariable analysis of *B. henselae* infection in GROUP A

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	0.816	0.545-1.221	0.323
Sex	Male	5	159	3.491	0.402-30.288	0.257
	Female	1	111	Reference		
Breed	DSH	5	190	2.026	0.233-17.629	0.522
	Other breeds	1	77	Reference		
Household	Multiple	4	173	1.480	0.162-13.489	0.728
	Single	1	64	Reference		
Outdoor access	Outdoor access	3	110	1.555	0.255-9.483	0.633
	Indoor only	2	114	Reference		
FVRCP vaccine	At least 1	2	156	0.222	0.036-1.367	0.105
	1-2	1	28	1.607	0.173-14.904	0.676
	3 or annual	1	128	0.156	0.017-1.423	0.100
Rabies vaccine	Yes	3	114	1.632	0.166-16.019	0.674
	No	1	62	Reference		
FeLV vaccine	Yes	2	44	2.424	0.393-14.963	0.340
	No	3	160	Reference		
Ectoparasitic prevention	Used to	3	110	1.336	0.219-8.164	0.754
	Not regular	2	69	1.420	0.195-10.328	0.729
	Regular	1	41	1.018	0.111-9.355	0.987
Temperature	Continuous	-	-	0.765	0.428-1.368	0.367

Table 21 Univariable analysis of *B. henselae* infection in GROUP A (continued)

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
FeLV infection	Yes	2	20	5.167	0.813-32.820	0.082
	No	3	155	Reference		
Hematocrit	Continuous	-	-	1.032	0.908-1.172	0.630
Sick	Sick	3	165	0.642	0.127-3.242	0.592
	Apparently healthy	3	106	Reference		

Table 22 Univariable analysis of *B. henselae* infection in GROUP B

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	1.051	0.764-1.446	0.758
Generation	Mature adult	2	9	2.476	0.445-13.777	0.300
	Other generation	7	78	Reference		
Sex	Male	6	61	1.008	0.268-3.795	0.990
	Female	4	41	Reference		
Breed	DSH	7	77	0.758	0.182-3.152	0.703
	Others	3	25	Reference		
Hematocrit	Continuous	-	-	1.012	0.915-1.120	0.817
Anemia	Anemia	1	22	0.404	0.049-3.363	0.402
	Non-anemia	9	80	Reference		
Total protein	Continuous	-	-	0.740	0.267-2.048	0.562
Sick	Sick	1	20	0.350	0.039-3.105	0.346
	Apparently healthy	6	42	Reference		

Table 23 Univariable analysis of *B. henselae* infection in all samples

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Age	Continuous	-	-	0.948	0.775-1.159	0.602
Generation	Young adult	13	262	2.630	0.583-11.853	0.208
	Mature adult	2	36	1.419	0.308-6.539	0.654
Sex	Male	11	220	1.520	0.518-4.463	0.446
	Female	5	152	Reference		
Breed	DSH	12	267	1.146	0.361-3.635	0.817
	Others	4	102	Reference		

Table 23 Univariable analysis of *B. henselae* infection in all samples (continued)

Variables	Category	PCR positive	PCR negative	Odds ratio	95% CI	*p-value*
Hematocrit	Continuous	-	-	1.041	0.967-1.121	0.287
Anemia	Anemia	1	93	0.145	0.019-1.124	0.065
	Non-anemia	13	175	Reference		
Sick	Sick	4	185	0.356	0.107-1.177	0.091
	Apparently healthy	9	148	Reference		

CHAPTER V DISCUSSION

5.1. Prevalence of feline hemoplasma infection

In our study, the prevalence of feline hemoplasmosis in GROUP A, GROUP B, and all samples are 18.5%, 10.7%, and 16.3%, respectively. In Thailand, the prevalence of client-owned cats has been reported to be 22.9% and 27.7% (Assarasakorn et al., 2012; Kaewmongkol et al., 2020). Additionally, cats which are semi-domesticated (such as cats in the monastery or temple) have hemoplasma prevalence of 36.9% and 38.06% (Do et al., 2020; Do et al., 2021a). In our study, the prevalence of client-owned cats is lower than the previous studies. However, the more recent blood samples yield more positivity in hemoplasma detection than the previously obtained blood samples. The prevalence of the disease may differ based on the study population as well as the period of collection and presence of various risk factors of the sample cats.

Likewise, *'C.* M. haemominutum' is the most prevalent species in our study followed by *M. haemofelis*. All the prevalence studies of feline hemoplasmosis in Thailand report in the same trend of species detection. We do not detect any '*C.* M. turicensis' from the positive-hemoplasma samples. '*C.* M. turicensis' can be identified among cats in Thailand despite the lowest prevalence of 0.9% (Do et al., 2020; Kaewmongkol et al., 2020). There are 4 remaining hemoplasma samples which have the PCR band size around 170 base pairs that we do not submit for the DNA sequencing; therefore, the infection of this species may be missed in our study.

All submitted samples for DNA sequencing which have the 190-bp PCR product size are *'C.* M. haemominutum'. This band size is expected to distinguish '*C.* M. haemominutum' from other hemoplasma species, as reported in a previous study (Jensen et al., 2001). In addition, all 170-bp samples are identified as *M. haemofelis*.

5.2 Risk factor analysis for feline hemoplasma infection

We found that male cats were significantly at greater risk to be infected with both any hemoplasma species and '*C.* M. haemominutum'. The odds ratios of 2.856 for any hemoplasma species infection and 2.597 for '*C.* M. haemominutum' infection are similar to the previous studies which reveal the odds ratio mostly between 2 to 4 for any hemoplasma species infection and 1.94 to 4.84 for '*C.* M. haemominutum' infection (Willi et al., 2006a; Roura et al., 2010; Tanahara et al., 2010; Lobetti and Lappin, 2012; Jenkins et al., 2013; Ghazisaeedi et al., 2014; Santis et al., 2014; Silaghi et al., 2014; Duarte et al., 2015; Bergmann et al., 2016; Vergara et al., 2016; Hwang et al., 2017; Ravagnan et al., 2017; Hwang et al., 2018; Makino et al., 2018; Sarvani et al., 2018; Latrofa et al., 2020; Demkin and Kazakov, 2021; Do et al., 2021a). Male cats are also prone to be more aggressive which cat fights and biting usually occur.

Domestic Shorthair is also considered a significant risk factor in our studies with the odds ratio of 2.856 for any hemoplasma species infection and 3.721 for '*C.* M. haemominutum' infection. Unlike these cats, purebred cats may have higher living environments which prevent the transmission of the disease.

Kitten is a significant protective factor for feline hemoplasmosis for both any hemoplasma and '*C.* M. haemominutum' infection. Numerous studies report that cats younger than 1 year have significantly lower risk to be infected with hemoplasma (Ravagnan et al., 2017; Do et al., 2020; Salim et al., 2020; Do et al., 2021a). As kittens are a protective factor, cats which are older become at greater risk to be infected with hemoplasmas. Age is a significant risk factor of any hemoplasma species or '*C.* M. haemominutum' infection from univariable but not multivariable logistic analysis in our study. Additionally, univariable logistic analysis shows that mature adult is a risk factor for '*C.* M. haemominutum' infection in GROUP A, as well as senior cat is a risk factor for '*C.* M. haemominutum' infection in all sample cats.

There are reports for both mature adult and senior cats as significant risk factors (Tanahara et al., 2010; Rosenqvist et al., 2016; Vergara et al., 2016; Ravagnan et al., 2017; Do et al., 2020; Salim et al., 2020; Do et al., 2021a). These cats may become chronically infected with hemoplasmosis as well as suffering from other diseases; thus, they have a higher chance to be detected with hemoplasma.

In this study, cats with sickness or presence of clinical signs are at greater risk of being infected with '*C.* M. haemominutum'. Numerous studies report that sickness as a significant risk factor as well (Ural et al., 2009; Attipa et al., 2017; Sarvani et al., 2018; Alanazi et al., 2021). We can imply that this species causes clinical signs in cats, or maybe a co-infection of primary disease. For any hemoplasma species, sickness is a significant risk factor in univariable analysis, but not in multivariable analysis.

Retroviral testing is only performed in GROUP A. Cats with presence of FIV antibody by test kit are significantly likely to be infected with either *'C.* M. haemominutum' or any hemoplasma species. The odds ratios for *'C.* M. haemominutum' or any hemoplasma infection are 11.476 and 8.824, respectively. FIV infection shares the same route of transmission via biting or fighting and from blood transfusion, as well. Additionally, cats with FIV infection may be immunosuppressive which may not be able to clear the hemoplasma infection.

FeLV infection is also considered significant in univariable analysis, but not in multivariable analysis. There are 2 studies which report FeLV infection as a significant risk factor (Tanahara et al., 2010; Díaz-Regañón et al., 2018). Even though FeLV infection is not likely to share the same route of transmission; however, cats may be immunosuppressive and cannot clear the hemoplasma injection, similarly to FIV infection.

The data of outdoor access is only available for GROUP A. Outdoor access is identified as a significant risk factor for any hemoplasma infection and *'C.* M. haemominutum' infection in univariable but not multivariable logistic regression. Cats with outdoor access are prone to contact with other cats and get infected via various routes of transmission.

Anemia is found to be a significant risk factor for any hemoplasma infection and *'C.* M. haemominutum' infection in univariable but not multivariable logistic regression. Hemoplasmas reside on the red blood cells and cause the hemolysis of the cells. However, these may be based on the virulence of the hemoplasma species. Previous studies identify *M. haemofelis* as the most virulent species (Lobetti and Tasker, 2004; Gentilini et al., 2009; Lobetti and Lappin, 2012). On the other hand, '*C.* M. haemominutum' is also reported to be able to cause anemia in cats but may not be significant (Lobetti and Tasker, 2004; Rosenqvist et al., 2016).

5.3 Prevalence of feline *Bartonella* infection

In this study, cats with *Bartonella* spp. infection are detected in the prevalence of 3.4% in GROUP A, 10.7% in GROUP B, and 5.4% among all samples. In Thailand, the infection rates of detection by conventional PCR are from 0% to 17.0% in client-owned cats (Inoue et al., 2009; Assarasakorn et al., 2012; Saengsawang et al., 2021). Additionally, the prevalence of *Bartonella* spp. infection is reported to be 2.8% in stray cat population, and 9.4% in mixed cat population (Srisanyong et al., 2016; Saengsawang et al., 2021). The prevalence of infected cats in our study is within the range of previously reported prevalence in Thailand.

We also reveal that *B. henselae* is the most prevalent *Bartonella* spp. (5.4% in all cats), similar to other studies which report the prevalence between 5.7-11.1% (Assarasakorn et al., 2012; Srisanyong et al., 2016). The second most prevalent species is *B. clarridgeiae* with the prevalence between 0.7-5.9% (Maruyama et al.,

2001; Inoue et al., 2009; Assarasakorn et al., 2012; Srisanyong et al., 2016). In our study, only one sample from 6 samples which show the PCR band size around 150-160 base pairs is submitted to the DNA sequencing and shows to be *B. clarridgeiae*. We do not amplify *B. koehlerae* which may be missed because it has a similar PCR size as *B. clarridgeiae*. Additionally, *B. vinsonii* subsp. *berkhoffii* has not been amplified in our study as well. These latter 2 species of feline *Bartonella* are rare but have previously been reported in Thailand (Assarasakorn et al., 2012; Srisanyong et al., 2016; Zarea et al., 2022).

5.4 Risk factor analysis for feline *Bartonella* infection

None of the factors in the study are significant which may be explained by several reasons. First, the number of PCR-positive samples for *Bartonella* spp. infection are considered low which may not meet the statistical significance. Second, some factors are only present in either PCR-positive or PCR-negative group which cannot be further analyzed for odds ratio e.g., *Bartonella*-positive cats are only present in non-flea infestation group, or none of the *Bartonella*-positive cats is hyperthermic. Lastly, the number of cats with presence of possible risk factors are low such as 42 cats are reported to be regular ectoparasitic prevention, or only 10 cats have flea infestation.

5.5 Prevalence of feline *Anaplasma* and *Ehrlichia* infection

This is the first prevalence study of feline anaplasmosis and ehrlichiosis in Thailand. However, we do not amplify any species of those infections. There is only one report of feline anaplasmosis in Thailand, which the cat is infected with *A. platys* (Salakij et al., 2012). Furthermore, we interviewed the attending veterinarians and veterinary technicians about feline anaplasmosis, and they responded that they were used to diagnose the cats with possible anaplasmosis which the laboratory reported the inclusion of *A. platys*-like organisms in platelet from blood smear (Personal

communication). Despite the reports of *A. platys* around the world, the situation of this infection in Thailand may be rare. We suspect that the bacteremic load of this species is quite low, and possibly lower than the diagnostic sensitivity of our assay. Unfortunately, none of the confirmed cases with *A. platys* infection is available to test, and we can only obtain the sample from dogs to be used as the positive control for the PCR. However, the prevalence of 0% is possible for feline anaplasmosis and ehrlichiosis, and numerous studies do not amplify these infections in their feline populations worldwide. Further studies are required to assess the diagnostic sensitivity of conventional PCR to detect *Anaplasma* and *Ehrlichia* in cats. When the suspected cases are required to confirm the infection of these pathogens in cats, nested PCR or real-time PCR may be preferred due to the higher diagnostic sensitivity of the assays.

5.6 Prevalence of feline *Babesia* and *Cytauxzoon* infection

In Thailand, the prevalence of feline babesiosis in stray cats has been reported in 2 studies, but there is no study in the client-owned cats. In addition, feline cytauxzoonosis has never been identified by molecular detection in Thailand. Our study is the first study to determine the prevalence of *Babesia* spp. among client-owned cats as well as emphasize on the possibility to detect feline cytauxzoonosis from the PCR assay which is used to detect *Babesia*. None of the cats in our study are positive for the PCR for *Babesia* and *Cytauxzoon*. The result may be explained that prevalence of *Babesia* is extremely low in the study population. We found similar results to multiple studies in other countries that did not detect any cat with feline babesiosis (Criado-Fornelio et al., 2009; Jikuya et al., 2017; Mylonakis et al., 2018; Oliveira et al., 2018; Alanazi et al., 2021). Moreover, the feline babesiosis which is sporadically reported have not been detected in more recent studies from some countries such as Italy and Spain (Criado-Fornelio et al., 2003; Pennisi et al.,

2007; Ortuño et al., 2008; Tabar et al., 2008; Spada et al., 2014c; Persichetti et al., 2016; Persichetti et al., 2018; Morganti et al., 2019; Latrofa et al., 2020; Álvarez-Fernández et al., 2022). Additionally, the studies from Brazil reported the differences in prevalence which they detected feline babesiosis in some studies, but not in the others (Mendes-de-Almeida et al., 2004; André et al., 2014; André et al., 2015; Braga et al., 2016; Malheiros et al., 2016; Oliveira et al., 2018; Pedrassani et al., 2019). Similar to conventional PCR for the Anaplasmataceae, the diagnostic sensitivity of the PCR assay may not be high enough to detect the low levels of parasitemia. We used the canine sample as positive control, but we did not detect *Babesia* infection in feline blood in the assay. Therefore, the evaluation of assay sensitivity may be required in order to improve the sensitivity of the PCR assay.

5.7 Recommendation of screening for feline blood-borne pathogens in blood donors

From this study, we suggest that feline blood donors should be screened for hemotropic mycoplasma and *Bartonella* infection before blood collection. In Thailand, prevalence of feline hemoplasma and *Bartonella* infection are approximately between 10.7-38% and 0-17% regardless to sample population, respectively. Other pathogens in the study including *Anaplasma*, *Ehrlichia*, *Babesia*, and *Cytauxzoon* may not be tested prior to blood donation due to very low prevalence or non-existence.

Feline blood donors must be apparently healthy, and any cat with the presence of illness should be excluded. However, in this study, some of the apparently healthy cats are still be detected with hemoplasma and *Bartonella* DNA in their blood. The prevalence of any hemoplasma and *Bartonella* infection in these cats are 10.8% (17/157) and 7.0% (11/157), respectively. Additionally, feline blood donors should be young adults (age between 1-7 years). We find that older cats are

at greater risk of being infected with any hemoplasma from univariable logistic analysis. In the study, cats with the age between 1-7 years have infection rate of 16.7% (46/275) to any hemoplasma species and 6.2% (17/275) to any *Bartonella* species. Therefore, every feline blood donor candidate should be screened for hemoplasma and *Bartonella* infection.

After selecting blood donors based on signalment and presence of clinical signs, these cats must have blood tests, and any cat with abnormality in blood profile especially anemia must be excluded to be blood donor. Nevertheless, these cats without anemia may be carriers of hemoplasma and *Bartonella* as well. In our study, non-anemic cats are found to be infected with any hemoplasma and *Bartonella* as the prevalence of 11.7% (22/188) and 7.4% (14/188), respectively. In addition, FIV and FeLV infection are found to be the risk factor for feline hemoplasmosis; however, those pathogens can be readily detected by available test kits.

Considering all possible risk factors of feline hemoplasmosis in our study, feline blood donor candidates which may have the lowest possibility to be detected with hemoplasma are those cats which are female, purebred, and have no history of outdoor access. However, as stated above, these cats must be apparently healthy, young adults, and have no laboratory abnormality as well. In addition, there are other factors to consider for being a blood donor such as blood types and blood compatibility.

In blood bank situation, blood products are obtained from feline blood donors, and these can be stored until use. Therefore, blood-borne pathogens in blood products can be detected by PCR, and the results will be available before blood transfusion. However, in an emergency scenario, blood product is collected from feline blood donor, and most of the time, immediately transfused into feline

blood recipient in which PCR results of blood-borne pathogens may not be available at the time of transfusion. Unless these feline blood donors are tested regularly for feline blood-borne pathogens, blood products should be allocated for PCR tests of blood-borne pathogens to assess the possibility of transfusing infectious agents to blood recipients.

5.8 Limitations of the study

The first limitation of our study is that we cannot include all cats which are suitable to be blood donor candidates. Our exclusion criteria are only those cats with the history of antimicrobial use or receiving blood product. Due to time limitations, we enrolled numerous cats which may not be appropriate as blood donors in the study such as those with the presence of clinical signs or being either kitten or senior cat. Another limitation is that we could not submit all PCR-positive samples to DNA sequencing due to cost restriction. Unfortunately, some of the possible species might be missing such as '*C.* M. turicensis' or *B. koehlerae*. Finally, feline blood samples of cases in which are confirmed to be infected with either *Anaplasma*, *Ehrlichia*, *Babesia*, or *Cytauxzoon* are unavailable to test for our PCR assay. Additionally, there is no data on the assay sensitivity of PCR for piroplasm. Due to scarce prevalence in nature, our study is likely to reveal true prevalence of the infection; however, if there is a highly suspected case, determination of assay test or another PCR assay such as nested PCR or real-time PCR may be helpful to increase the sensitivity of detection in this case.

5.9 Summary and conclusion

Client-owned cats are commonly infected with hemoplasma and *Bartonella* spp. In contrast, other blood-borne infection including *Anaplasma*, *Ehrlichia*, *Babesia*, and *Cytauxzoon* is not detected in any of the client-owned cats. The major risk factors of hemoplasma infection include male sex, Domestic Shorthair, sickness, and

FIV infection. In contrast, kitten is considered as a protective factor. Other possible risk factors for hemoplasmosis are outdoor access, anemia, FeLV infection, and senior or mature cats. Risk factors regarding *Bartonella* infection are not identified in the study. When selecting cats to be blood donor, those which are female, purebred, and indoor only are preferable if possible. The current study advocates for screening hemoplasma and *Bartonella* spp. in all feline blood donors prior to blood transfusion to prevent transmitting these blood-borne pathogens to feline blood recipients.

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