Original Article

Surveillance of zoonotic *Brugia pahangi* in monastery cats, Samphran district, Nakhon Pathom, Thailand

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Abstract

The mosquito-borne filarial nematode parasites *Brugia pahangi* and *B. malayi* have been proposed as potential zoonotic pathogens, with dogs and cats being the main reservoir hosts. The monasteries where both stray and semi-domesticated animals live often serve as areas where humans are exposed to these reservoirs. In Thailand, the prevalence of *B. pahangi*, particularly in infected monastery cats, is underreported. In this study, we investigated *B. pahangi* prevalence in cats at 24 temples in the Samphran district, Nakhon Pathom province, central Thailand. In total, 196 blood samples were collected from the cats and examined using the hematocrit capillary tube technique. Then, modified Wright–Giemsa staining was used to morphologically identify microfilaria at the genus level. We then used the polymerase chain reaction (PCR) to target the mitochondrial cytochrome oxidase I (*COI*) gene and perform sequencing to confirm the species level of the filarial nematode parasite. The results were highly similar to *B. pahangi COI* (100%). A phylogenetic analysis was then conducted using MEGA X software. The *B. pahangi* infection prevalence at the study sites was 4.08% (8/196). Most infected cats exhibited no clinical signs and gender and monastery location were non-risk factors for infection. Our study indicated that molecular techniques and phylogenetic analyses are useful tools for identifying filarial parasites and understanding their evolution. Importantly, going forward, our findings will help improve the diagnosis, treatment, control and prevention of zoonotic filarial nematode parasite infections.

Keywords: *Brugia pahangi*, microfilaria, monastery, COI, cats, Thailand

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Introduction

Feline lymphatic filariasis is caused by *Brugia pahangi* and *B. malayi*, which are tropical and sub-tropical mosquito-borne parasitic zoonoses in the Nematoda phylum, Chromadorea class and Rhabditida order and are considered public health concerns (Dahmana and Mediannikov, 2020; Kaikuntod *et al.,* 2020). Mosquito vectors have been identified to potentially transmit the disease, including *Armigeres subalbatus* (Tan *et al.,* 2011; Muslim *et al.,* 2013[a]) and *Mansonia* spp. (Apiwathnasorn *et al.,* 2006; Chang, 2002). At preliminary diagnostic stage, circulatory-based microfilariae (mf) in animal reservoir hosts, such as cats and dogs, are used to confirm infection (Ambily *et al.,* 2011). However, standard methods such as thin whole blood smear or buffy coat smear staining and the hematocrit capillary tube technique cannot adequately distinguish *Brugia* spp. Thus, the acid phosphatase method (Chalifoux and Hunt, 1971; Ambily *et al.,* 2011) and/or molecular techniques targeting the cytochrome oxidase I (*COI*) gene and the internal transcribed spacer (*ITS*) region, such as polymerase chain reaction (PCR) cloning and sequencing (Casiraghi *et al.,* 2001; Areekit *et al.,* 2009[a]), PCR-restriction fragment length polymorphism (PCR-RFLP) analysis (Nuchprayoon *et al.,* 2003; 2005; 2006), and quantitative real-time PCR (qPCR) (Areekit *et al.,* 2009[b]; Thanchomnang *et al.,* 2010; Wongkamchai *et al.,* 2013) have been developed for species-level identification, especially in regions where concurrent *Brugia* spp. infections occur.

A primary cause of human lymphatic filariasis or elephantiasis is *B. malayi* (WHO, 2009) and this filaria parasite can infect felid animals (Chansiri *et al.,* 2002; Areekit *et al.,* 2009[c]). In previous research, blood mf levels in reservoir domestic cats in Surat Thani and Narathiwat provinces, southern Thailand, were examined, revealing 53.30% (8/15) and 18.40% (7/38) of animals were infected, respectively (Chansiri *et al.,* 2002). This data suggests that cats can act as potential reservoir hosts (Kanjanopas *et al.,* 2001). Recently, zoonotic *B. pahangi* infection studies were conducted in Malaysia and Thailand, with infected humans showing symptoms such as recurrent descending lymphangitis, acute descending lymphangitis, lower limb cellulitis (Tan *et al.,* 2011), ocular infection including eye redness with slight pain and mild fever (Muslim *et al.,* 2013[b]) and small nodules in the breast (Thongpiya *et al.,* 2021). In Thailand, *B. pahangi* infections from dogs and cats are extensively distributed throughout the country (Satjawongvanit *et al.,* 2019; Kaikuntod *et al.,* 2020; Jitsamai *et al.,* 2021; Loymek *et al.,* 2021), whereas *B. malayi* infections are primarily reported in southern regions (Chansiri *et al.,* 2002; Nuchprayoon *et al.,* 2003; 2006; Sarasombath *et al.,* 2019). However, there is a dearth of information in the literature on the prevalence of *B. pahangi* in feral, semi-domesticated and domesticated cats in Thailand; therefore, to address this knowledge gap, substantial research efforts are required to characterize infections in reservoir hosts.

Monastery cats are outdoor or semi-outdoor animals living in temple areas and are maintained by monks, nuns and visitors. Therefore, these animals have very close contact with humans and carry newly emerging zoonotic diseases (Koompapong *et al.,* 2014; Phoosangwalthong *et al.,* 2018). Moreover, blood mf levels in *B. malayi*-infected cats have been correlated with the circadian movement of mosquito vectors, especially at night for mosquito–reservoir hosts or mosquito–human interaction (Chansiri *et al.,* 2002). Therefore, our objective was to investigate zoonotic *B. pahangi* prevalence in monastery cats in Samphran district, Nakhon Pathom province, Thailand. Our results may be used for the strategic management of zoonotic parasitic diseases for control and preventative measures.

Materials and Methods

Study sites: The study protocol (No.MUVS-2020-05-11) was approved by the Faculty of Veterinary Science–Animal Care and Use Committee, Mahidol University, Thailand. In April 2014, 196 blood samples (approximately 500 µL) were randomly collected from cats at 24 monasteries (Mongkhon Chindaram [Rai Khing], Tha Phut, Nakhon Chuenchum, Phren Phet, Pridaram [Yai Som], Wang Nam Khao, Chindaram, Rat Sattharam, Bang Chang Nua, Song Khanong, Tha Kham, Don Wai, Choeng Len, Bang Chang Tai, Thian Dat, Decha Nusorn, San Phet, Suan Mak, Dong Ket, Samphran, Mahasawat Nak Phuttharam, Hom Kret, Khlong Om Yai and Om Yai) in the Samphran district, Nakhon Pathom province, Thailand (geographical information: 13.724167, 100.216667) (Figure 1).

Sample collection and microscopic examination: Male and female cats between 6 months and 10 years were included. The sample size was calculated using the formula, $n = Z^2P(1-P)/d^2$, where $Z = 1.96$, $P = 0.5$, and $d = 0.07$. Blood samples were drawn from cephalic, saphenous or jugular veins and preserved in ethylenediaminetetraacetic acid (EDTA) tubes for blood smear analysis. Woo's technique or the hematocrit capillary tube technique (Woo, 1969) was applied using a microcapillary tube, using approximately 60 µL of whole blood. Following this, bloods were centrifuged in a Haematokrit 20 instrument (Hettich, Tuttlingen, Germany) at 200 × *g* for 10 mins. The movement of mf above the buffy coat layer (leukocyte-enriched fraction) was examined using light microscopy (Zeiss, Oberkochen, Germany) at 100× magnification. Thin buffy coat blood smears were prepared and stained using Wright–Giemsa solution, with mf and associated morphology analyzed (we focused on the mf sheath and two terminal nuclei at the tail tip) at the genus level using microscopy at 100×, 400× and 1000× magnifications.

Buffy coat DNA preparation: All bloods were DNA extracted, with mf species levels determined using molecular analyses. The buffy coat was separated from whole blood by centrifugation at 10,000 × *g* for 10 mins. DNA was isolated from buffy coat using the FlexiGene DNA kits (Qiagen, Hilden, Germany) according to manufacturer's instructions. DNA was stored at –20 ℃ until analysis.

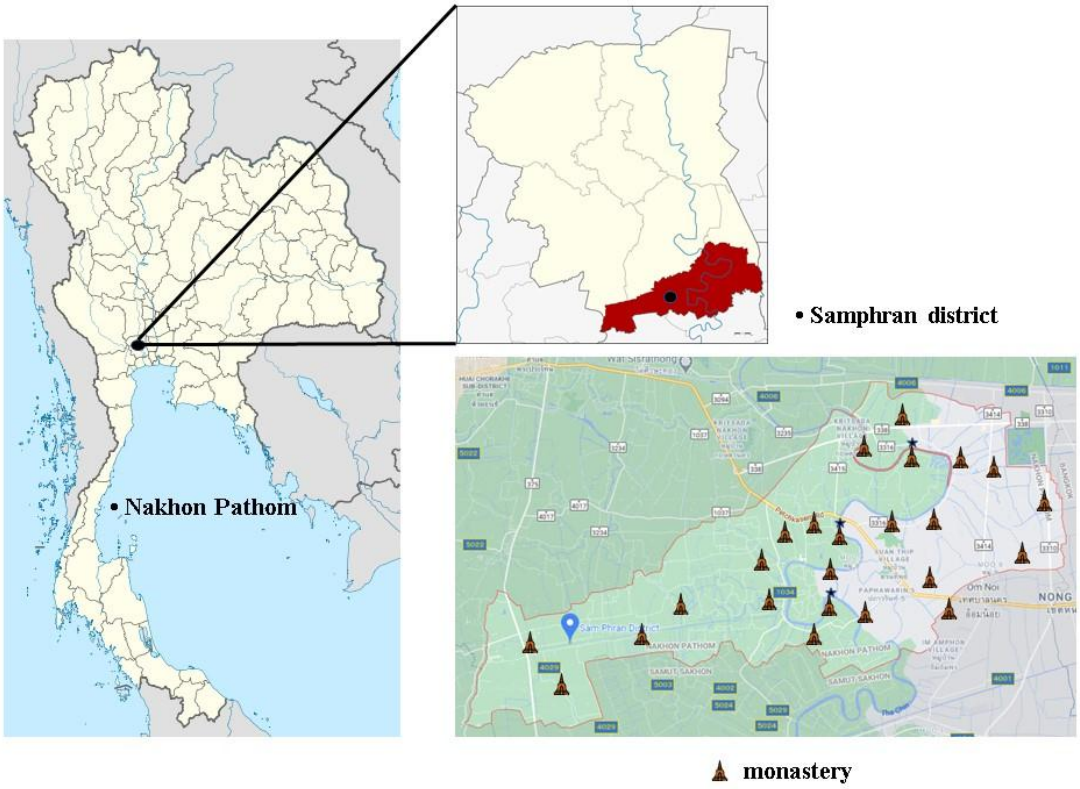

Figure 1 The geographical distribution of samples and positive monasteries depicted by stars.

Filarial parasites mitochondrial cytochrome oxidase I amplification: Blood DNA was used as a template to amplify the majority of the *COI* gene from filaria species (Casiraghi *et al.*, 2001). The purified primers, COI-int-F (5′-TGATTGGTGGTTTTGGTAA-3′) and COI-int-R (5′-ATAAGTACGAGTATCAATATC-3′) (Macrogen laboratory, Seoul, South Korea), were used to amplify approximately 690 base pairs (bp) of *COI*. The following components were added to a PCR tube (Axygen Scientific, Union City, CA, USA): 25 µL *Taq* polymerase TopTaq Master Mix (Qiagen); 5.0 µL CoralLoad Concentrate (Qiagen); 1.0 µL each of forward and reverse primers (10 µM); 5 µL DNA template (50 ng/µL) measured by Nanodrop One (Thermo Fisher Scientific, MA, USA); and molecular biology-grade water (Qiagen) to 50 µL. Amplifications were conducted in a T100™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). Thermocycling conditions were as follows: one 3 min step at 94 ℃ followed by 40 cycles of 45 s at 94℃, 45 s at 52℃, and 90 s at 72℃ with a final extension step of 7 mins at 72℃. Amplicons were electrophoresed on 2.0% agarose gels, stained with GelRed™ (Biotium, Hayward, CA, USA) and visualized using a c150 UV transilluminator (Azure Biosystems, Dublin, CA, USA).

β-actin amplification: To verify DNA quality, β-actin amplifications were conducted using 12.5 µL *Taq* polymerase TopTaq Master Mix (Qiagen), 2.5 µL CoralLoad Concentrate (Qiagen), 0.5 µL forward primer 10 µM (β-actin-F: 5′ - CGGGACCTGACTGACTACC-3′), 0.5 µL reverse primer (10 µM) (β-actin-R: 5′ - CCTTAATGTCACGCACGATTTCC-3′), 2 µL DNA template (50 ng/µL) and molecular biology-grade water (Qiagen) to 25 µL. Amplifications were conducted in the same instrument as for *COI*. Thermocycling conditions were as follows: one 2 mins at 94℃ followed by 35 cycles of 45 s at 94℃, 30 s at 60℃, and 45 s at 72℃ with a final extension step of 5 mins at 72℃. The PCR products (80 bp) were examined, stained and visualized as described.

DNA sequencing: Amplicons (690 bp) were gel extracted and purified at Macrogen laboratory. Bidirectional sequencing of PCR products was also performed at Macrogen laboratory. DNA sequences then were analyzed using fluorescent dye-terminator sequencing using an ABI 3730*xl* sequencer platform (Applied Biosystems, Foster City, CA, USA). Sequencing was performed using filaria *COI*-specific primers, COI-int-F and COI-int-R.

Bioinformatics and phylogenetic analyses**:** *B. pahangi COI* sequence results were analyzed using several bioinformatics programs. Sequencing results were compared with those available in GenBank using the Basic Local Alignment Search Tool (http: / / blast. ncbi.nlm.nih.gov/Blast.cgi). Evolutionary history was deduced using the neighbor-joining method (Saitou and Nei, 1987). The optimal tree was developed, and the percentage of replicate trees, where associated taxa were clustered together using the bootstrap test (1,000 replicates), was shown next to branches (Felsenstein *et al.*, 1985). The tree was drawn to scale, with branch lengths within the same units as those of the evolutionary distances being used to infer the phylogenetic tree. Evolutionary distances were calculated using the Kimura two-parameter method (Kimura, 1980) and were located in the units of the

number of base substitutions per site. This analysis constituted 19 nucleotide sequences. The codon positions included 1st + 2nd + 3rd + noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion). In total, there were 620 positions in the final dataset. Evolutionary analyses were conducted using MEGA X software (Kumar *et al.*, 2018).

Statistical analysis: Statistical relationships between positive results for *B. pahangi* infection, gender and monastery locations were analyzed using multivariable logistic regression, odds ratio (OR), 95 confidence interval (95% CIs), 95% CIs of ORs and *p*-values using SPSS 18 for Windows (SPSS Inc., United States). Statistical significance was accepted at a *p*-value of < 0.05.

Nucleotide accession numbers: *B. pahangi COI* nucleotide sequence data is available from GenBank using the accession numbers: MZ149926–MZ149933.

Results

Three study temples, namely, Choeng Len monastery (Bang Chang subdistrict), Decha Nusorn monastery (Yai Cha subdistrict) and Song Khanong monastery (Song Khanong subdistrict), showed positive results for *B. pahangi*. These monasteries are located beside the Nakhon Chaisri (Tha Chin) river, canals and cultivated lands which could support variable mosquito vector biodiversity (Figure 1).

Buffy coat thin blood smear staining combined with mf detection using microscopy confirmed all positive samples belonged to the *Brugia* genus; a microfilarial sheath and two terminal nuclei were confirmed in samples (Figure 2). Importantly, Woo's technique showed negative result for all samples. Using buffy coat thin blood smears and PCR techniques, we identified the same result that 4.08% (8/196; 95% CI: 1.78%–7.88%) of monastery cats in Samphran district, Nakhon Pathom, were positive for *Brugia* spp. infection. Male cats comprised 62.50% (5/8) of the infected population, while female cats accounted for 37.50% (3/8). The infection rate among male cats was 4.95% (5/101; 95% CI: 1.63%–11.18%), whereas in female cats, this was 3.16% (3/95; 95% CI: 0.66%–8.95%). No significant associations were identified between the infection rate and the gender (p = 0.53) (Table 1). In addition, infections were detected in cats in 3 of the 14 subdistricts (21.43%) in Samphran district: Bang Chang and Yai Cha subdistricts had the same high proportion of blood mf-infected cats (3/14; 21.43%, 95% CI: 4.66%–50.80%), followed by Song Khanong subdistrict (2/14; 14.29%, 95% CI: 1.78%–42.81%). No significant associations were observed between the infection rate and subdistricts ($p > 0.05$) (Table 1).

The mean number of detected circulating mf in thin buffy coat blood smears was 3.25 (range = 2–5, median = 3, mode = 3). The approximate number of mf/mL of whole blood was calculated from 60 µL of whole blood using the hematocrit capillary tube technique. Positive samples contained 33.33–83.33 mf/mL (mean = 54.16 mf/mL). The motility of mf above the buffy coat layer could not be observed.

We identified 690 bp amplicons from eight positive *Brugia* spp. samples using targeted *COI* PCR. Bidirectional DNA sequencing results demonstrated all were *Brugia* spp. *COI* sequences shared 98%–100% identities with Thai *B. pahangi COI* sequences deposited in relevant databases (MK250755, MK250743, MK250722, MK250710, MK250730, MK250729, MK250726, MK250754, MK250752, MK250732, MK250711, MK250709, MK250707, KP60172, MT027204, MT027202, and AP017680). Phylogenetic analyses indicated that *B. pahangi* identified in our study was closely related to *B. pahangi* in dogs from the Bangkok Metropolitan area (MK250754 and MK250755) and *B. pahangi* in a Malaysian individual (EF406112) (Figure 3).

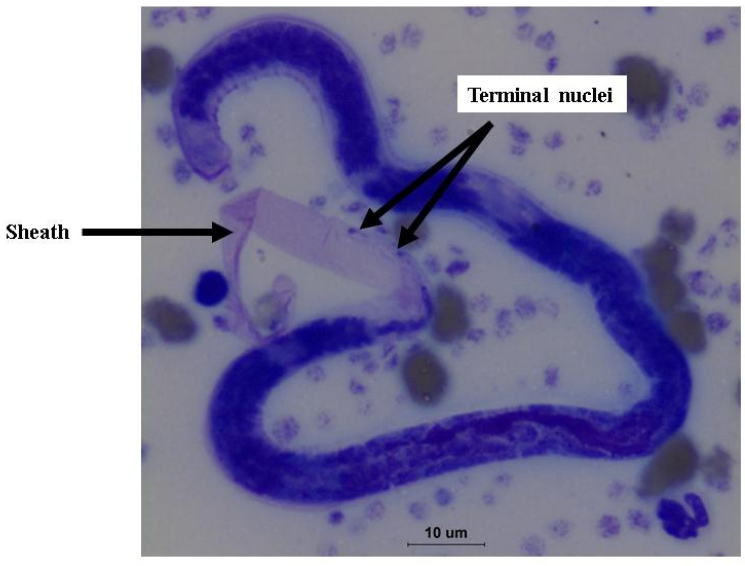

Figure 2 The microfilarial stage of *B. pahangi* using Wright–Giemsa staining, showing the tail portion. The presence of a sheath and two terminal nuclei–distinctly separated from the other nuclei in the tail–are shown in 1000× magnification of the Wright–Giemsa stained thin buffy coat smear. Scale bar = 10 µm.

Table 1 *Brugia pahangi*-positive samples and odds ratio data for filaria infections according to gender and monastery location.

Variable	Number	Positive (%)	95% CI of proportion	OR	95% CI of OR	*p*-value
Gender						
Male	101	5 (4.95)	1.63–11.18	1		
Female	95	3 (3.16)	0.66–8.95	0.63	0.14–2.70	0.53
Location (subdistricts)						
Bang Chang	14	3 (21.43)	4.66–50.80	1		
Yai Cha	14	3 (21.43)	4.66–50.80	1	0.16–6.08	1.00
Song Khanong	14	2 (14.29)	1.78–42.81	0.61	0.08–4.37	0.62
Rai Khing	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Krathum Lom	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Khlong Chinda	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Talat Chinda	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Khlong Mai	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Tha Kham	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Bang Krathuek	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Ban Mai	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Samphran	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Hom Kret	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Om Yai	14	0 (0.00)	0.00–23.16	0.79	0.60–1.03	1.00
Total	**196**	**8 (4.08)**	**1.78–7.88**			

CI = confidence interval
OR = odd ratio

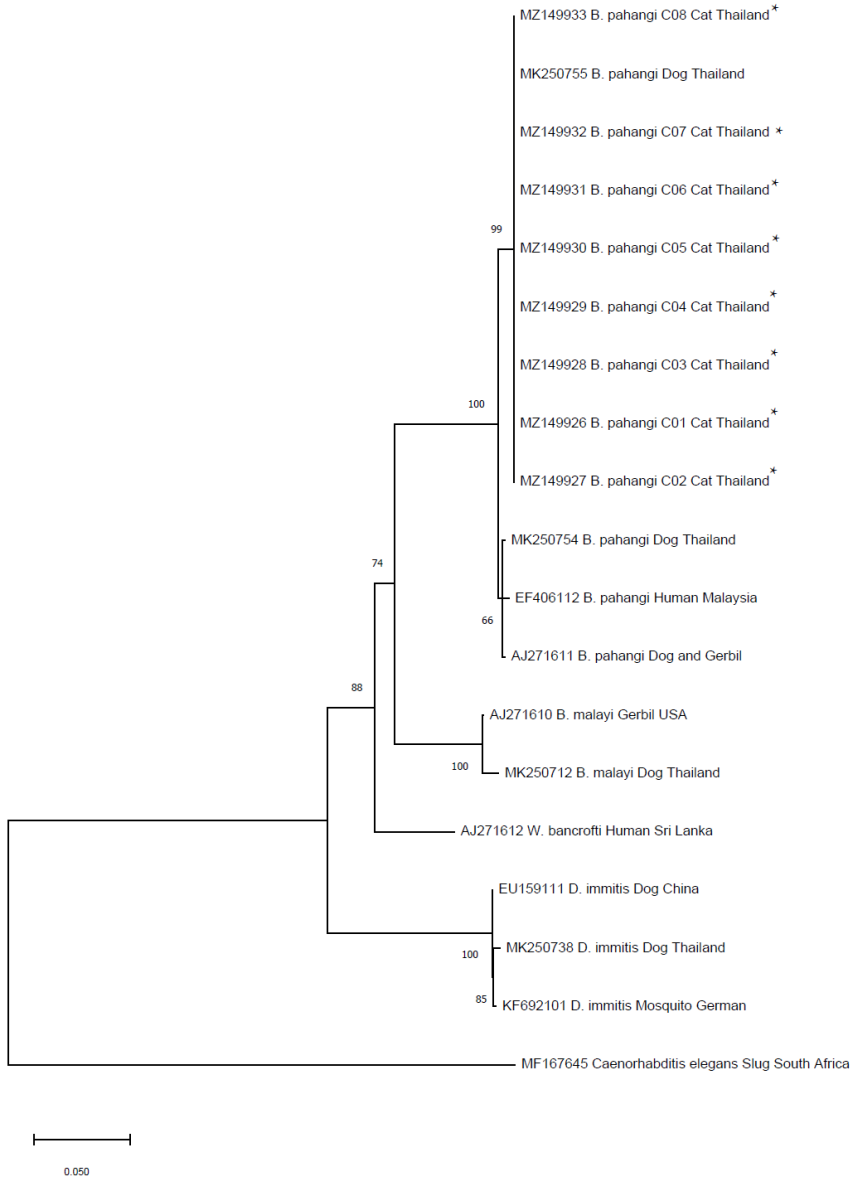

Figure 3 Phylogenetic tree analysis of *B. pahangi* based on nucleotide sequences from a 629 bp fragment of *COI* using the neighbor-joining method. Sequences from this study are marked with stars.

Discussion

Currently, there is dearth of literature on *B. pahangi* infection prevalence in feral or semi-domesticated cats, especially monastery cats in Thailand. A previous examination of 219 monastery cats in Bangkok using microscopy and acid phosphatase activity methods identified 25.3% *B. pahangi*-positive mf (Chungpivat and Sucharit, 1993). In another study investigating blood mf levels in domestic cats in the Prasang district, Surat Thani, southern Thailand, using microscopy and PCR-RFLP analyses, it was reported that 7.7% (4/52) of cats were infected with *B. pahangi* (Nuchprayoon *et al.,* 2006). Furthermore, our study data contrasted with those of a *B. pahangi* study in Selangor, Malaysia, where domestic cats exhibited 35% infection (14/40) rates but no infections in stray animals (Al-Abd *et al.,* 2015). The infections identified in semi-domesticated or monastery cats in our study may be related to different mosquito vectors in these areas (Witoonsatian *et al.,* 2011; Muslim *et al.,* 2013[b]), with some of the cats even staying indoors; they may have been infected during the day via daytime-biting mosquitoes, such as *Aedes aegypti* (Lindsay and Denham, 1986). Additionally, we identified more infected males than females but this gender difference was not statistically significant ($p = 0.53$). In contrast, Al-Abd *et al.* (2015) reported that female cats infected with *B. pahangi* exhibited a higher infection rate (71.43%: 10/14) than did male cats, similar to *Dirofilaria immitis* infection rates (30.36%; 17/56) (Kamyingkird *et al.,* 2017) but no significant differences were observed in these studies. Interestingly, the outdoor behavior of male cats in our study may have increased their chance of vector-borne exposure and subsequent infection as described by Walker Vergara *et al.,* (2016).

We observed no mf motility above the buffy coat layer as mean mf counts were low, 54.16 mf/mL. A previous study of cats naturally infected with *B. pahangi* used experimental ivermectin treatments, with the untreated control group showing a mean ± standard deviation (SD) blood mf count of 1,990 ± 1,059.53 mf/mL over 9 months (Taweethavonsawat and Chungpivat, 2013). In addition, the experimental infection of various number of third-stage larvae (L3); 100, 300 and 500 L3s of *B. pahangi* induced in cats, showed that mf density means ± SD were 501 ± 551 mf/mL, 600 ± 475 mf/mL, and 3,678 ± 2,722 mf/mL, respectively (Junhom *et al.,* 2006). Variations in blood circulating nocturnal subperiodic *B. malayi* mf levels were shown to depend on blood collection times (Sivanandam and Dondero, 1972; Chansiri *et al.,* 2002), whereas in our study, we collected whole blood samples during the day as *B. pahangi* exhibited no periodicity (Chungpivat and Sucharit, 1993). However, our low mf density data may be related to cat immune responses against blood mf levels (Medeiros *et al.,* 1996).

Our study was limited in terms of the mf diagnostic technique as we did not perform the modified Knott's test, which is the gold standard approach (Knott, 1939; Ciuca *et al.,* 2020). This technique requires at least 1 mL of EDTA-treated blood to concentrate mf levels and increase sensitivity; however, we only collected approximately 500 µL blood as monastery cats were extremely aggressive during blood collection. However, the buffy coat smear method for canine dirofilariasis screening was not statistically different from the modified Knott's test (Marcos *et al.,* 2016). The stained buffy coat smear without staining for blood circulating mf may be used as alternative techniques to the modified Knott's test (Marcos *et al.,* 2016; Chagas *et al.,* 2020).

The COI-int-F and COI-int-R primer set used in this study acted as universal primers with the potential to amplify *COI* from of 11 different species of blood and tissue filariae, including *B. malayi*, *B. pahangi*, *D. immitis*, *D. repens*, *Wuchereria bancrofti* and *Onchocerca* spp. (Casiraghi *et al.,* 2001). The PCR-RFLP method targeting the *ITS1* and *ITS2* discriminated between *B. pahangi* and *B. malayi* species (Nuchprayoon *et al.,* 2003) and *ITS1* was also used to distinguish between various blood filariae (Nuchprayoon *et al.,* 2005; 2006). However, when compared with the *ITS1*-PCR method, the *COI* PCR approach was more prevalent for *B. pahangi* infection in dogs (Satjawongvanit *et al.,* 2019). Additionally, previous evidence indicated false-positive and negative results for the *ITS1*- and *ITS2*-PCR methods when discriminating liver and intestinal fluke egg DNA samples. Furthermore, the specificity and sensitivity of *ITS1* and *ITS2*, which are used for diagnostic procedures, should be considered (Sato *et al.,* 2009). Moreover, in areas where concurrent *B. pahangi*, *B. malayi* (Areekit *et al.,* 2009[a]) and *D. immitis* mixed infections occur in cats (Nonsaithong *et al.,* 2018), such as southern Thailand, acid phosphatase activity and qPCR approaches, which differentiate using melting temperatures, could benefit mixed blood mf diagnoses (Areekit *et al.,* 2009[b]; Wongkamchai *et al.,* 2013; Nonsaithong *et al.,* 2018).

Our phylogenetic *COI* analysis confirmed *B. pahangi COI*, which showed the highest 100% homology to Thailand *B. pahangi* mf of infected dogs; accession number MK2507755 and 99% homology to MK250710, MK250722, MK250726, MK250729, MK250730, MK250743 and MK250755 and other closely branch; MK250707, MK250709, MK250711, MK250732, MK250752, and MK250754 (Satjawongvanit *et al.,* 2019). In addition, *B. pahangi COI* sequence (EF406112) from an infected Malaysian individual showed 98% homology. The *ITS1* sequence (MT732324) from Thai infected patients (Thongpiya *et al.,* 2021) was closely (99%) related to *ITS1* (MK250800) from a dog infected with *B. pahangi* (Satjawongvanit *et al.,* 2019). In addition, we did not obtain the intraspecies variation of *B. pahangi* samples which contrast to the previous study of *ITS2* sequences which presented some intraspecific variation. The *B. pahangi ITS* region especially *ITS2* demonstrated intraspecific variation compared with *ITS1* (Areekit *et al.,* 2009[c]) and *COI* for this study.

Information regarding *COI* sequence submitted by Casiraghi *et al.* (2001), was obtained from *B. pahangi*-infected dogs or gerbils and is available at the United States National Institutes of Health–National Institute of Allergy and Infectious Diseases (NIH-NIAID) Filariasis Research Reagent Resource Center (FR3), formerly known as TRS Laboratories Inc. (Michalski *et al.,* 2011). In addition, other *COI* sequences deposited in GenBank were prepared from Thai-specific *B.*

pahangi mf from an infected dog (Satjawongvanit *et al.*, 2019). According to our phylogenetic tree, two branches of *B. pahangi COI* (MK250754 and MK250755) exist in dogs. However, all *COI* sequences from this study were closely related to MK250755; moreover, Satjawongvanit *et al.* (2019), did not provide district or subdistrict information on their dog samples, which may be related to the disease transmission by mosquito vectors to cats in our study. Surprisingly, *COI* PCR, and not *ITS1*-PCR, could identify *B. malayi*-infected dogs in Bangkok, central Thailand (Satjawongvanit *et al.*, 2019). To the best of our knowledge, we reported the first *COI* sequence data for *B. pahangi* mf in infected monastery cats in Thailand. Active monitoring of both *B. pahangi* and *B. malayi* should be conducted in these areas.

Our study was also limited by the following: we did not investigate infective stages of *B. pahangi* borne by mosquito vectors in our study areas. Also, thin blood smear examinations from humans were not explored. In terms of *B. pahangi* being a potential vector-borne zoonotic parasite, surveilling reservoir hosts such as cats, dogs and mosquito vectors at the national level could help control and prevent disease. Therefore, veterinary practitioners should recommend that animal owners and keepers participate in injectable (Taweethavonsawat and Chungpivat, 2013) or spot-on regimen preventative programs (Sarasombath *et al.*, 2019) to curb disease transmission.

In conclusion, zoontic *B. pahangi* blood mf levels in monastery cats living in Samphran district, Nakhon Pathom, Thailand, were examined. Mitochondrial *COI* sequencing data helped us to conduct phylogenetic tree analyses. The active monitoring of disease in reservoir hosts alongside mosquito vector distribution in endemic areas should be conducted to ensure strategic planning actions toward parasitic zoonosis control and prevention at a national level.

Conflict of interest: The authors declare that they have no conflicts of interest.

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References

Al-Abd NM, Nor ZM, Kassim M, Mansor M, Al-Adhroey AH, Ngui R and Sivanandam S 2015. Prevalence of filarial parasites in domestic and stray cats in Selangor State, Malaysia. Asian Pac J Trop Med. 8: 705-709.

Ambily VR, Pillai UN, Arun R, Pramod S and Jayakumar KM 2011. Detection of human filarial parasite *Brugia malayi* in dogs by histochemical staining and molecular techniques. Vet Parasitol. 181: 210–214.

Apiwathnasorn C, Samung Y, Prummongkol S, Asavanich A, Komalamisra N and Mccall P 2006. Bionomics studies of *Mansonia* mosquitoes inhabiting the peat swamp forest. Southeast Asian J Trop Med Public Health. 37: 272–278.

Areekit S, Khuchareontaworn S, Kanjanavas P, Sriyapai T, Pakpitchareon A, Khawsak P and Chansiri K 2009[a]. Molecular genetics analysis for co-infection of *Brugia malayi* and *Brugia pahangi* in cat reservoirs based on internal transcribed spacer region 1. Southeast Asian J Trop Med Public Health. 40: 30–34.

Areekit S, Kanjanavas P, Pakpitchareon A, Khawsak P, Khuchareontaworn S, Sriyaphai T and Chansiri K 2009[b]. High resolution melting real-time PCR for rapid discrimination between *Brugia malayi* and *Brugia pahangi*. J Med Assoc Thai. 92: S24–S28.

Areekit S, Singhaphan P, Khuchareontaworn S, Kanjanavas P, Sriyaphai T, Pakpitchareon A, Khawsak P, and Chansiri K 2009[c]. Intraspecies variation of *Brugia* spp. in cat reservoirs using complete ITS sequences. Parasitol Res. 104: 1465–1469.

Casiraghi M, Anderson TJ, Bandi C, Bazzocchi C and Genchi C 2001. A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. Parasitology 122: 93-103.

Chagas C, Binkienė R, Ilgūnas M, Iezhova T and Valkiūnas G 2020. The buffy coat method: a tool for detection of blood parasites without staining procedures. Parasit Vectors. 13: 104.

Chalifoux L and Hunt RD 1971. Histochemical differentiation of *Dirofilaria immitis* and *Dipetalonema reconditum*. J Am Vet Med Assoc. 158: 601–605.

Chang MS 2002. Operational issues in the control of the vectors of *Brugia*. Ann Trop Med Parasitol. 96: S71–S76.

Chansiri K, Tejangkura T, Kwaosak P, Sarataphan N, Phantana S and Sukhumsirichart W 2002. PCR based method for identification of zoonostic *Brugia malayi* microfilariae in domestic cats. Mol Cell Probes. 16: 129–135.

Chungpivat S and Sucharit S 1993. Microfilariae in cats in Bangkok. Thai J Vet Med. 23: 75-87.

Ciuca L, Roman C, Prisco F, Miron L, Acatrinei D, Paciello O, Maurelli MP, Vismarra A, Cringoli G, and Rinaldi L 2020. First report of *Dirofilaria repens* infection in a microfilaraemic cat from Romania. Vet Parasitol Reg Stud Reports. 22: 100497.

Dahmana H and Mediannikov O 2020. Mosquito-borne diseases emergence/resurgence and how to effectively control it biologically. Pathogens 9: 310.

Felsenstein J 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 39: 783–791.

Jitsamai W, Piromkij P, Kamkong P, Chungpivat S and Taweethavonsawat P 2021. Seasonal distribution and environmental parameters associated with *Brugia pahangi* and *Dirofilaria immitis* in naturally infected dogs in Bangkok and vicinity, Thailand. Sci Rep. 11: 4594.

Junhom W, Chungpivat S and Viseshakul N 2006. The observation of microfilarial rate and density in cats inoculated with increasing numbers of *Brugia pahangi* infective larvae. Southeast Asian J Trop Med Public Health. 37: 40–42.

Kaikuntod M, Arjkumpa O, Kladkempetch D, Fukumoto S, Thongkorn K, Boonyapakorn C, Punyapornwithaya V and Tiwananthagorn S 2020. Geographic Spatial Distribution Patterns of *Dirofilaria immitis* and *Brugia pahangi* infection in community dogs in Chiang Mai, Thailand. Animals (Basel). 11: 33.

Kamyingkird K, Junsiri W, Chimnoi W, Kengradomkij C, Saengow S, Sangchuto K, Kajeerum W, Pangjai D, Nimsuphan B, Inpankeaw T and Jittapalapong S 2017. Prevalence and risk factors associated with *Dirofilaria immitis* infection in dogs and cats in Songkhla and Satun provinces, Thailand. Agric Nat Resour. 51: 299-302.

Kanjanopas K, Choochote W, Jitpakdi A, Suvannadabba S, Loymak S, Chungpivat S and Nithiuthai S 2001. *Brugia malayi* in a naturally infected cat from Narathiwat Province, southern Thailand. Southeast Asian J Trop Med Public Health. 32: 585–587.

Kimura M 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 16: 111-120.

Knott J 1939. A method for making microfilarial surveys on day blood. Trans R Soc Trop Med Hyg. 33: 191-196.

Koompapong K, Mori H, Thammasonthijarern N, Prasertbun R, Pintong AR, Popruk S, Rojekittikhun W, Chaisiri K, Sukthana Y and Mahittikorn A 2014. Molecular identification of *Cryptosporidium* spp. in seagulls, pigeons, dogs, and cats in Thailand. Parasite 21: 52.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 35: 1547–1549.

Lindsay SW and Denham DA 1986. The ability of *Aedes aegypti* mosquitoes to survive and transmit infective larvae of *Brugia pahangi* over successive blood meals. J Helminthol. 60: 159-168.

Loymek S, Phuakrod A, Zaelai K, Sripumkhai W, Vongjaroensanti P and Wongkamchai S 2021. Investigation on the prevalence of canine microfilaremia in Thailand using a novel microfluidic device in combination with real-time PCR. Vet Sci. 8: 39.

Marcos R, Pereira C, Santos M, Luzzago C, Lauzi S, Maia JP, Faustino A and Puente-Payo P 2016. Buffy coat smear or Knott's test: which to choose for canine microfilaria screening in field studies?. Vet Clin Pathol. 45: 201–205.

Medeiros F, Baldwin CI, and Denham DA 1996. *Brugia pahangi* in cats: the passive transfer of anti-microfilarial immunity from immune to non-immune cats. Parasite Immunol. 18: 79–86.

Michalski ML, Griffiths KG, Williams SA, Kaplan RM and Moorhead AR 2011. The NIH-NIAID Filariasis Research Reagent Resource Center. PLoS Negl Trop Dis. 5: e1261.

Muslim A, Fong MY, Mahmud R, Lau YL and Sivanandam S 2013[a]. *Armigeres subalbatus* incriminated as a vector of zoonotic *Brugia pahangi* filariasis in suburban Kuala Lumpur, Peninsular Malaysia. Parasit Vectors 6: 219.

Muslim A, Fong MY, Mahmud R and Sivanandam S 2013[b]. Vector and reservoir host of a case of human *Brugia pahangi* infection in Selangor, peninsular Malaysia. Trop Biomed. 30: 727-730.

Nonsaithong D, Yotmek S, Yotmek S, Nochote H, Wongkamchai S, Roytrakul S and Lek-Uthai U 2018. High resolution melting real-time PCR detect and identify filarial parasites in domestic cats. Asian Pac J Trop Med. 11: 682-687.

Nuchprayoon S, Sangprakarn S, Junpee A, Nithiuthai S, Chungpivat S and Poovorawan Y 2003. Differentiation of *Brugia malayi* and *Brugia pahangi* by PCR-RFLP of ITS1 and ITS2. Southeast Asian J Trop Med Public Health. 34: 67–73.

Nuchprayoon S, Junpee A, Poovorawan Y and Scott AL 2005. Detection and differentiation of filarial parasites by universal primers and polymerase chain reaction-restriction fragment length polymorphism analysis. Am J Trop Med Hyg. 73: 895–900.

Nuchprayoon S, Junpee A, Nithiuthai S, Chungpivat S, Suvannadabba S and Poovorawan Y 2006. Detection of filarial parasites in domestic cats by PCR-RFLP of ITS1. Vet Parasitol. 140: 366-372.

Phoosangwalthong P, Hii SF, Kamyingkird K, Kengradomkij C, Pinyopanuwat N, Chimnoi W, Traub RJ and Inpankaew T 2018. Cats as potential mammalian reservoirs for *Rickettsia* sp. genotype RF2125 in Bangkok, Thailand. Vet Parasitol Reg Stud Reports. 13: 188–192.

Saitou N and Nei M 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 4: 406–425.

Sarasombath PT, Thammapalo S, Loymek S, Angkanasinsiri A, Priyavoravong P and Wongkamchai S 2019. First study of topical selamectin efficacy for treating cats naturally infected with *Brugia malayi* and *Brugia pahangi* under field conditions. Parasitol Res. 118: 1289–1297.

Satjawongvanit H, Phumee A, Tiawsirisup S, Sungpradit S, Brownell N, Siriyasatien P and Preativatanyou K 2019. Molecular analysis of canine filaria and its *Wolbachia* endosymbionts in domestic dogs collected from two animal university hospitals in Bangkok metropolitan region, Thailand. Pathogens. 8: 114.

Sato M, Thaenkham U, Dekumyoy P and Waikagul J 2009. Discrimination of *O. viverrini*, *C. sinensis*, *H. pumilio* and *H. taichui* using nuclear DNA-based PCR targeting ribosomal DNA ITS regions. Acta Trop. 109: 81–83.

Sivanandam S and Dondero TJ Jr 1972. Differentiation between periodic and subperiodic *Brugia malayi* and *Brugia pahangi* on the basis of microfilarial sheath-casting in vitro. Ann Trop Med Parasitol. 66: 487-496.

Tan LH, Fong MY, Mahmud R, Muslim A, Lau YL and Kamarulzaman A 2011. Zoonotic *Brugia pahangi*

filariasis in a suburbia of Kuala Lumpur City, Malaysia. Parasitol Int. 60: 111–113.

Taweethavonsawat P and Chungpivat S 2013. Successful treatment of *Brugia pahangi* in naturally infected cats with ivermectin. Korean J Parasitol. 51: 759–761.

Thanchomnang T, Intapan PM, Chungpivat S, Lulitanond V and Maleewong W. 2010. Differential detection of *Brugia malayi* and *Brugia pahangi* by real-time fluorescence resonance energy transfer PCR and its evaluation for diagnosis of *B. pahangi*-infected dogs. Parasitol Res. 106: 621–625.

Thongpiya J, Sa-Nguanraksa D, Samarnthai N and Sarasombath PT 2021. Filariasis of the breast caused by *Brugia pahangi*: A concomitant finding with invasive ductal carcinoma. Parasitol Int. 80: 102203.

Walker Vergara R, Morera Galleguillos F, Gómez Jaramillo M, Pereira Almosny NR, Arauna Martínez P, Grob Behne P, Acosta-Jamett G and Müller A 2016. Prevalence, risk factor analysis, and hematological findings of hemoplasma infection in domestic cats from Valdivia, Southern Chile. Comp Immunol Microbiol Infect Dis. 46: 20–26.

WHO 2009. Global programme to eliminate lymphatic filariasis. Wkly Epidemiol Rec. 84: 437–444.

Witoonsatian K, Sinwat N, Jam-on R, Thiangtum K and Songserm T 2011. Detection of West Nile virus in mosquitoes in Nakhon-pathom and Phetchaburi province, Thailand. Thai J Vet Med. 41: 377–381.

Wongkamchai S, Monkong N, Mahannol P, Taweethavonsawat P, Loymak S and Foongladda S 2013. Rapid detection and identification of *Brugia malayi*, *B. pahangi*, and *Dirofilaria immitis* by high-resolution melting assay. Vector Borne Zoonotic Dis. 13: 31–36.

Woo PT 1969. The haematocrit centrifuge for the detection of trypanosomes in blood. Can J Zool. 47: 921-923.