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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Received January 14, 2021
Accepted November 21, 2021

https://doi.org/10.14456/tjvm.2022.11

Feline lymphatic filariasis is caused by Brugia pahangi and B. malayi, which are tropical and subtropical mosquito-borne parasitic zoonoses in the Nematoda phylum, Chromodorea class and Rhabditida order and are considered public health concerns (Dahmana and Medianikkov, 2020; Kaikunthod et al., 2020). Mosquito vectors have been identified to potentially transmit the disease, including Armigeres subalbatus (Tan et al., 2011; Muslim et al., 2013) and Mansonia spp. (Apithawasorn 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), PCR-restriction fragment length polymorphism (PCR-RFLP) analysis (Nuchprayoon et al., 2003; 2005; 2006), and quantitative real-time PCR (qPCR) (Areekit et al., 2009; 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). 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 (Kankanopas 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) 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; Kaikunthod 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 Saththaram, 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 Phuththaram, Hom Kret, Khlong Om Tai 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 = Z²P(1-P)/d², 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, Tutlingen, 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 °C until analysis.
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′-TGATTGGTGTTTGGTAA-3′) and COI-int-R (5′-ATAAGTACGATCATCAATAC-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 40 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 3730xl 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).

**Table 1**

<table>
<thead>
<tr>
<th>Location</th>
<th>Subdistrict/Region</th>
<th>Number of Infections</th>
<th>Infection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samphran district</td>
<td></td>
<td>3</td>
<td>21.43%</td>
</tr>
<tr>
<td></td>
<td>Bang Chang subd.</td>
<td>3</td>
<td>21.43%</td>
</tr>
<tr>
<td></td>
<td>Yai Cha subd.</td>
<td>3</td>
<td>21.43%</td>
</tr>
<tr>
<td></td>
<td>Song Khanong subd.</td>
<td>2</td>
<td>14.29%</td>
</tr>
</tbody>
</table>

**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 1000x 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.

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

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 a 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), 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) (Kamyngkird 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; Ciucu 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, previously 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) 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; 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 MK250775 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 (MT733234) 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) 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.

Acknowledgements

This study was funded by the Faculty of Veterinary Science, Mahidol University, Thailand. The authors are grateful to Pimkwan Sawanggai for sample collection and laboratory duties. We also thank the technicians of the Laboratory Unit of Prasat Arthorn Animal Hospital and The Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, for their support.

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